CURRENT ADVANCES IN NATURAL SCIENCES II

EDITORS

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Dear Readers

The second volume of the book "Natural Sciences" has been released to facilitate researchers access to a diverse array of information in this field. In this volume, we have placed special emphasis on expanding the topics compared to the first volume.

Scientific activities deepen our understanding of various problems and their solutions. By consolidating different scientific studies, we can accelerate society's access to knowledge. In this regard, books serve as invaluable resources for the community.

"Natural Sciences" features articles that illuminate the methods currently employed in this field and address contemporary topics. The natural sciences are foundational to human existence; our curiosity about nature drives us to interpret it, leading to the evolution of these disciplines. This exploration also provides answers to numerous societal questions.

This book presents the findings of researchers who contribute their studies to science, driven by their curiosity and inquiries about nature and the environment.

We extend our heartfelt gratitude to all the researchers who supported the preparation of this book. We hope that the compiled articles will prove valuable to the scientific community.

Editors

CHAPTER 1

ENHANCEMENT OF PHASE CHANGE MATERIALS WITH NANOMATERIALS

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INTRODUCTION

Substances known as phase change materials (PCMs) have the remarkable ability to absorb or emit substantial amounts of thermal energy during solid/liquid or liquid/solid phase transitions.

Their popularity has been increasing in numerous fields owing to their outstanding capability to store, absorb, release, and deposit heat during phase transitions. PCMs are mainly utilized in thermal energy storage systems since they offer effective solutions for energy savings in various applications (Huang et al., 2021).

PCMs serve numerous purposes in various fields. In the construction sector, they are included into building components such as concretes, bricks, or plasters in order to provide energy saving (Shah et al., 2022). In textile sector, their usage can provide smart or technical functions to garments (Iqbal et al., 2019). They are also used in solar energy systems, refrigerators, and air conditioning units to provide cost-free cooling from ambient air (Ali, 2022).

Combining PCMs with energy systems, especially with building components, has become a promising approach for increasing energy efficiency and energy saving. Being able to absorb or emit substantial amounts of thermal energy during phase changes at virtually constant temperatures, PCMs help buildings preserve their indoor temperatures. This capability of them minimizes the reliance on conventional heating, ventilation, and air conditioning (HVAC) system. Given that these systems are responsible for a large portion of energy consumption in buildings around the world, estimated at 38-40% (Peng, 2024; Xi et al., 2022), it becomes more clear how much potential PCMs hold.

A number of the recent studies have underlined on the potential of PCMs to improve energy efficiency in buildings. It has been often emphasized that the use of PCMs can shift peak electrical loads to off-peak periods, which reduces the stress on the electrical grid besides providing cost savings for consumers (Yousefi et al., 2023).

The effectiveness of PCMs in energy savings is further supported by their high energy storage density and the capacity to maintain virtually constant temperatures during phase transitions. This is

particularly useful in minimizing temperature changes in buildings as they can cause energy waste (Cui et al., 2017). A study in the UAE found that PCM-incorporated houses could achieve energy consumption reductions of around 19% (Sovetova et al., 2019). Another study conducted in Taiwan noted that the use of PCMs in buildings could reduce electricity costs, energy consumption, and carbon emissions by 60,272 tons, 32% and 118,411 kWh per year, respectively (Peng and Lo, 2024). Such results emphasize the importance of PCM integration in thermal energy storage systems. Other than conventional PCMs, new types of PCMs such as shape-stabilized PCMs (SSPCMs) and composite materials, have also been proven to amend the performance and applicability of PCMs in various energy systems. These innovative approaches contribute to improved thermal conductivity and stability, which make them suitable for various applications, including solar thermal energy storage and HVAC systems (Guo at al., 2022; Nguyen, 2023; Sun et al., 2022).

Another innovative approach in PCM technology is the use of PCMs in conjunction with nanomaterials. Nanomaterials are used with PCMs to enhance their thermal properties and overcome several limitations. The primary reason for the addition of nanomaterials into PCMs is to improve their thermal conductivity. PCMs typically have poor thermal conductivity, which limits their heat transfer efficiency (Sundaramahalingam et al., 2021). Inclusion of conductive nanomaterials into PCMs are considerably capable of increasing thermal conductivity so that faster charging/discharging rates could be achieved (Nazari et al., 2021; Parameshwaran and Kalaiselvam, 2015a). This enhancement is explained by the formation of a densely-packed network of thermal interfaces and phonon-like heat transfer mechanisms (Parameshwaran and Kalaiselvam, 2015b).

On the other hand, while nanomaterials improve thermal conductivity, they can also curtail the latent heat of PCMs. However, this trade-off often results in an overall acceleration of melting and solidification processes, which is beneficial for many applications (Nazari et al., 2021). Moreover, nanomaterials can also help overcoming the other PCM limitations such as leakage of PCM liquid,

phase segregation, and supercooling (Amberkar and Mahanwar, 2022; Sundaramahalingam et al., 2021; Tao et al., 2020).

There are many studies on the use of nanomaterials with PCMs in literature. One of these studies showed that the inclusion of graphene nanoplatelets (GNPs) are able to increase the thermal conductivity of polymer-based nanocomposites (Lin et al., 2018; Zhu et al., 2017). Similarly, carbon nanotubes (CNTs) can increase the mechanical strength and thermal stability of nanocomposites, which is very important for preserving structural integrity under thermal cycling conditions (Jin et al., 2014; Wang, 2024). Recent studies have also explored the optical properties of nano-enhanced PCMs. One of them revealed that the inclusion of hybrid nanoparticles can improve the optical absorptivity of PCMs, which is beneficial for solar energy harvesting (Bhutto, 2023; Pandey, 2023). In addition to improving thermal and optical properties, nanomaterials can also mechanically reinforce the PCMs, which contributes to their durability and longevity in real-world applications. For example, the addition of cellulose nanofibers or other natural fibers can improve the mechanical strength of polymer-based PCMs so that they become more suitable for structural applications (Chowdhury et al., 2016; Hai et al., 2019). Additionally, usage of biodegradable polymers combined with nanomaterials aligns with the growing demand for sustainable materials, which provides an eco-friendly alternative to traditional petroleum-based polymers (Lin et al., 2018; Nayani et al., 2013).

Besides understanding the effects of thermal and mechanical properties, it is also crucial to understand the fundamental mechanisms governing the interactions between nanomaterials and PCM matrix. This knowledge is essential for the rational design of future materials to meet the rising demands for energy efficiency and sustainability in various applications, such as construction, electronics, and thermal energy storage systems (Lu et al., 2013).

PCMs

Classification of PCMs

The primary classifications of PCMs include organic, inorganic, and eutectic phase change materials as shown in Figure 1. Each category has distinct advantages and disadvantages that affects their applications in thermal energy storage, building materials, and other technologies.

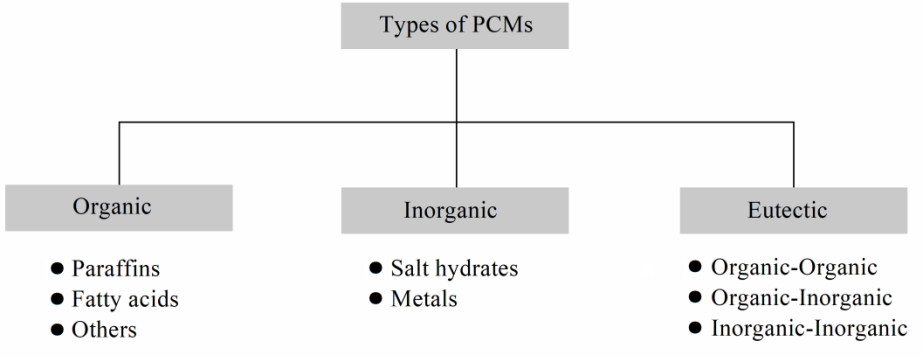

Figure 1. Types of PCMs

Organic PCMs

Organic PCMs typically include paraffins and fatty acids. They possess high latent heat capacities, stability, non-toxicity, and non-corrosiveness (Kenisarin, 2014). These materials are particularly useful in applications such as building materials, textiles, and food packaging (Png et al., 2022). However, they exhibit several disadvantageous properties limiting their widespread use. One of the these disadvantages is their flammability, which poses a significant fire risk in applications such as building materials, battery thermal management, and protective insulations (Pielichowska et al., 2023). Moreover, they usually suffer from low thermal conductivity, which is typically less than 0.5 W/(m·K), and unstable shape during their melting phase, which restricts their practical use (Xue et al., 2021). Leakage during phase transition is another advantage of organic PCMs. To overcome these limitations, researchers have developed various strategies. Micro and

nano encapsulation are one the promising solutions to prevent leakage and rise thermal conductivity (Wang et al., 2023). Moreover, to enhance fire safety, inclusion of flame retardants in SS-PCMs and encapsulated PCMs, chemical transformations, and surface coating are promising approaches (Png et al., 2022). In addition, the development of composite PCMs using 3D filler networks has shown potential in improving thermal conductivity and encapsulation ability while maintaining high phase change enthalpy (Xue et al., 2021). These innovative approaches are very important for increasing the number of application areas of organic PCMs.

Inorganic PCMs

Inorganic PCMs, such as salt hydrates, generally offer higher thermal conductivity and higher energy storage density compared to organic materials. Since they can store more energy per unit mass, they are more suitable for applications demanding high thermal performance (Zhang et al., 2011). On the other hand, they are more prone to issues such as supercooling, phase separation, and corrosiveness, which can adversely impact their durability and service life (Zhang et al., 2011).

Inorganic PCMs are particularly effective at temperatures below 120°C (Man et al., 2023). They mainly consist of salt hydrates, salt solutions, and metals and provide several advantages over organic PCMs (Canela-Xandri et al., 2023; Mohamed et al., 2017). Their thermal conductivity and storage capacity are higher compared to organic PCMs (Mohamed et al., 2017). In addition, they have a wide range of melting and solidifying temperatures so that they could be utilized for various applications in solar engineering, building materials, heat pumps, and textiles (Lin et al., 2018).

Inorganic PCMs, especially molten salts and metals, are suitable for high-temperature applications such as concentrated solar power generation and industrial waste heat recovery (Lin et al., 2018). However, several challenges limit their widespread use. These

challenges are mainly high supercooling, phase separation, leakage, low thermal conductivity (in some cases), and instability (Man et al., 2023). Moreover, they may be corrosive, which is another important limitation of inorganic PCMs (Mohamed et al., 2017). To overcome or minimize these issues, several solutions have been proposed, such as the use of nucleating agents, thickeners, eutectic mixtures, porous materials, and micro/macro/nano-encapsulation methods (Man et al., 2023; Wang et al., 2023).

Eutectic PCMs

Eutectic PCMs are the mixture of at least two different materials that solidify and melt at a single temperature. They could be made of organic/organic, inorganic/inorganic or organic/inorganic substances. They can be tailored to obtain the desired melting point and have the advantages of combined beneficial properties of their constituents. However, since determining the suitable ratio of the constituents may be quite complex, their wide use manufacturing and application is limited.

Eutectic PCMs achieve melting enthalpies of 200 - 215 J/g, which makes them effective thermal regulators (Kalidasan et al., 2023). When incorporated into asphalt binders, they can improve specific heat capacity, resulting in significant temperature lags and differences (Dai et al., 2023). However, they also present some challenges. Their recrystallization can be prevented by the molten asphalt binder matrix, which results in reductions in both melting temperature and enthalpy (Dai et al., 2023).

For inorganic salt hydrate-based eutectics, although they exhibit reduced supercooling compared to pure PCMs, supercooling and corrosivity can reduce the service life (Kalidasan et al., 2023; Man et al., 2023).

Limitations of PCMs

Despite their promising capabilities, several issues and limitations hinder PCMs' widespread utilization and effectiveness. One of the major limitations of PCMs is related to their thermal stability and cycling performance. Many PCMs degrade over time, especially after going through repeated thermal cycles. Certain organic PCMs can experience significant mass loss and thermal degradation after multiple heating and cooling cycles (Shobo et al., 2018). This degradation could reduce the phase change enthalpy and therefore affect the performance of thermal energy systems (Rathod, 2018). Additionally, the thermal stability of PCMs can vary significantly depending on their chemical composition and structural properties; consequently, it is necessary to select and optimize them carefully for specific applications (Qiu et al., 2011).

Another critical issue is the potential for leakage during phase transition. As PCMs transition from solid to liquid states, risk of leakage increases. This risk should be carefully considered particularly for the applications where the materials are embedded within building structures or thermal storage systems (Zhang et al., 2018). Because leakage not only results in material loss but can also cause contamination of surrounding environments (Ni et al., 2014). The encapsulation of PCMs has been proposed as a solution to prevent leakage; however, not all encapsulation methods work effectively as desired; and also, microencapsulation is not considered to be a low-cost approach (Zhao and Zhang, 2011).

The thermal conductivity of PCMs is another significant limitation. Many PCMs, particularly organic materials, have low thermal conductivity, which can impede their ability to convey heat efficiently during the phase change process (White et al., 2024). Since low thermal conductivity leads to slower response times in thermal management systems, PCM struggles with regulating temperature fluctuations and exhibits low efficiency. To address this issue, inclusion

of thermally-conductivity fillers into PCM could be an effective approach to enhance the thermal performance (Wang et al., 2011). However, this approach usually involves complex production processes and may introduce additional costs.

Phase change temperature range of PCMs is also critical to their applicability. Several PCMs exhibit narrow phase change temperature ranges, which makes them unsuitable for the particular thermal needs of various applications. In building applications, for example, a PCM works best with those whose phase change temperature range closely coincides with desired indoor temperature range; hence optimum energy savings. The biggest challenge remains the appropriate identification or engineering of suitable phase change temperature PCMs without losing other properties-as high latent heat capacity and thermal stability.

Besides, another area of concern is environment. Many PCMs are formulated to be eco-friendly; however, certain synthetic PCMs pose ecological hazards on manufacturing and disposal. For example, some manufacturers employing toxic chemicals in the production could cause environmental pollution if not properly handled (Teamah, 2024). Furthermore, the end-of-life disposal of PCMs must be considered thoroughly to avoid environmental issues.

Apart from these issues, the inclusion of PCMs into existing systems may also cause practical problems. Therefore, before integrating PCMs into materials, material compatibility and structural integrity of the final product should be considered thoroughly (Lukic et al., 2012).

External factors, such as humidity and temperature changes, may have an impact on the performance of PCMs, which makes their utilization more complicated in real-world practices (Salih et al., 2023). Accordingly, it is necessary to extensively test and validate PCMs so as to confirm that their performance is as expected under various environmental conditions.

NANOMATERIALS

Nanomaterials can be defined as materials whose at least one dimension is in the nanoscale range from 1 to 100 nanometers. This ultra-small size range endows them various different physical and chemical properties distinguishing them from their bulk equivalents. These special characteristics stem from their high surface area-to-volume ratio, quantum effects, and dominance of surface phenomena at the nanoscale. These qualities make them fairly beneficial in different fields such as medicine, electronics, energy, and environment.

Special Characteristics of Nanomaterials

High Surface Area

As nanomaterials exhibit a greater surface area than their equivalents, their reactivity towards and interaction with the surroundings are enhanced. This makes it a more critical parameter in their applications, that is, catalysis, drug delivery, and sensor development (Gavali et al., 2023).

Quantum Effects

At nanoscale, quantum mechanical effects become non-negligible, which can lead to the emergence of unique optical, electronic, and magnetic properties. For instance, quantum dots show size-dependent fluorescence and can be utilized in medical imaging and sensing applications (Li et al., 2019).

Improved Mechanical Properties

Many nanomaterials, such as CNTs and graphene, showcase outstanding mechanical strength and flexibility. These properties make them excellent candidates for reinforcing materials in composites, particularly for ultra-light structures with high mechanical properties (Nasir et al, 2018).

Tunable Properties

Specific synthesis procedures can tune the custom properties of nanomaterials so that they can satisfy specific demands. This tunability is critical for the development of next-generation multi-functional materials with diverse potential uses (Prakash, 2016).

Types of Nanomaterials

Nanomaterials can be divided into several classes based on their dimension and compositions as shown in Figure 2 and Figure 3.

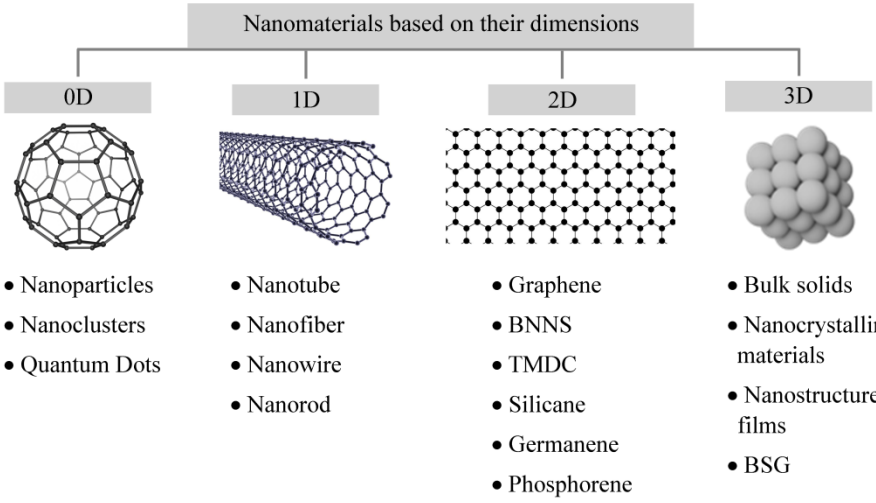

Figure 2. Types of PCMs Classification of nanomaterials based on their dimensions. Abbreviations are Boron Nitride Nano Sheets (BNNS) ; Monolayer Transition Metal Dichalcogenides (TMDCs); Basil Seed Gum (BSG).

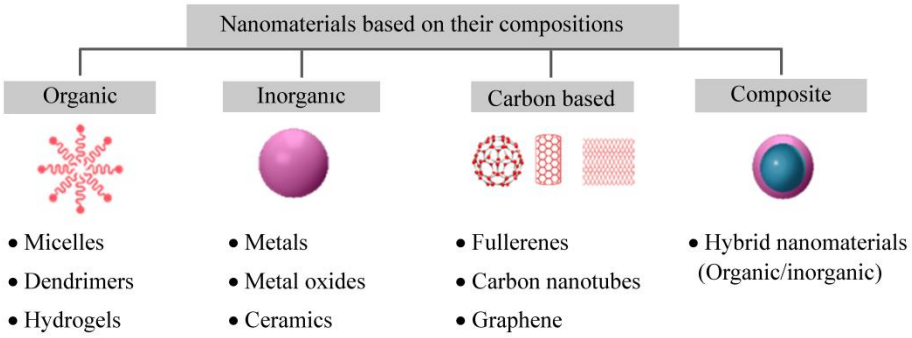

Figure 3. Classification of nanomaterials based on their compositions

i) Nanoparticles: They are solid particles in the nanoscale range. They can be made of metals such as Au or Ag; oxides such as ZnO or TiO_2, or polymers. These materials are commonly used in drug delivery, imaging, and as catalysts (Alkilany et al., 2012).

ii) Nanotubes: CNTs and other types of nanotubes are cylindrical structures displaying outstanding mechanical, electrical, and thermal properties. CNTs have particularly been drawing attention owing to their superior strength and electrical conductivity. These unique qualities make them suitable for applications in electronics and materials science (Nasir et al., 2018).

iii) Nanoplates and Nanosheets: This category covers graphene other 2D nanomaterials. As these materials display distinguished electronic and optical properties, they are likely to be used in flexible electronics, sensors, and energy storage (Tan et al., 2017).

iv) Quantum Dots: These are semi-conductor nanoparticles displaying size-dependent optical properties as a result of quantum confinement. They have found applications in monitors, solar cells, and biological imaging owing to their tunable fluorescence (Li et al., 2019).

v) Nanocomposites: These materials are composed of a matrix (mostly polymer) embedded with nanoparticles or nanofillers. They have been designed to improve mechanical, thermal, and barrier

properties for packaging, coatings, and structural applications (Prakash, 2016).

vi) Nanofibers: These are defined as fibers that range from a few nanometers to nearly a micrometer in diameter. They are usually made by electrospinning method. As they have high surface area and porosity, they can be utilized in filtration, tissue engineering, and as reinforcement in composites (Lin, 2011).

vii) Nanorods and Nanospheres: These are specific shapes of nanoparticles exhibiting superior properties depending on their geometry. One of them is gold nanorod drawing attention due to its plasmonic properties proven to be useful particularly in photothermal therapy and sensing applications (Alkilany et al., 2012; An et al., 2021).

THE INTEGRATION METHODS OF NANOMATERIALS IN PCMs

Integrating nanomaterials into PCMs via various impregnation techniques is a budding field of study seeking to improve the thermal conductivity and energy storage capabilities of the materials involved. There are five prominent integration methods (Said et al., 2024);

- Direct mixing,
- Ultrasonication
- Vacuum impregnation,
- Nanocapsulation,
- Sol-gel synthesis,
- Electrospinning.

Each of these methods can offer advantages and pose certain challenges that can be adjusted to specific applications in thermal energy storage.

Direct mixing

Direct mixing is one of the simplest and most straightforward methods for nanomaterial-PCMs integration. In this method nanomaterials are physically blended with PCMs (Vinayaka Ram et al., 2020). The effectiveness of direct mixing is dominated by the uniform distribution of nanoparticles within PCM matrix. Distribution qualities of the particles are strongly dependent on particle size, concentration, and the viscosity of the PCM. However, achieving homogenous dispersion could be challenging due to the agglomeration tendency of nanomaterials, which overshadow the benefits of the desired integration (Said et al., 2024).

Ultrasonication

To avoid or minimize the agglomeration of nanoparticles in PCM matrices, ultrasonication method could be used. In this method, high-frequency sound waves are utilized to disperse nanomaterials and break down agglomerates within the PCM and thus a uniform dispersion could be achieved (Said et al., 2024). The application of ultrasonic waves produces cavitation bubbles in the liquid, which collapse and create localized high temperatures and pressures, thus allowing the dispersion of nanoparticles. This method is especially convenient for improving the interaction between the nanomaterials and the PCM, which highly contributes to improved thermal performance.

Vacuum Impregnation

Vacuum impregnation is a more sophisticated method that enhances the penetration of nanomaterials into the PCM matrix. This technique involves applying a vacuum to remove air from the PCM, allowing for better infiltration of nanoparticles. Jiao et al. (2012) utilized vacuum impregnation to create composite PCMs based on lauric acid/stearic acid binary eutectic and expanded perlite, resulting in composite PCMs with high latent heat and suitable melting temperature

for building applications. The vacuum environment helps to minimize the formation of air pockets, ensuring a more homogeneous distribution of nanomaterials. This method is particularly advantageous for creating shape-stabilized PCMs, as it allows for the effective encapsulation of the PCM within a porous matrix, preventing leakage during phase transitions. Similarly, n-hexadecane was incorporated into polymeric frameworks containing oleic acid-modified ZnO nanoparticles using solvent-assisted vacuum impregnation, leading to enhanced thermal storage capability and improved heat conduction rates (Mert and Mert, 2024).

Nanoencapsulation

Encapsulation entails coating the PCM with a layer of nanomaterials, which can enhance thermal stability and restrict leakage during phase changes (Said et al., 2024). This method can be especially beneficial and safer for applications in which high thermal efficiency is required. Encapsulation not only protects the PCM but also allows for controlled release of thermal energy. The material of the shell or capsule must possess thermal stability and compatibility with the PCM; therefore, selection of encapsulating material is crucial to achieve the desired efficiency and safety.

Sol-Gel Synthesis

The term *Sol-gel synthesis* refers to the transition of a solution (sol) into a solid (gel) phase, during which the formation of nanostructured materials take place. This process is used as a versatile synthesis procedure for the formation of nanomaterials and their incorporation into PCMs. To date, sol-gel methods have been successfully employed to prepare PCM nanocapsules and inorganic shape-stabilized PCMs (SS-PCMs). For instance, nanocapsules composed of palmitic acid (PA) core in SiO_2 capsules were successfully synthesized via the sol-gel method by adjusting pH values

between 11-12 (Latibari et al., 2013). The process thus finely resulted in uniformly-distributed spherical nanocapsules with thermal conductivity and stability immensely high compared to pure PA.

Electrospinning

Electrospinning is a method used for producing nano-scale fibers from polymer solutions in order to create form-stable PCMs. In this method a polymer solution is subjected to a high voltage, which results in the formation of ultra-fine fibers that can encapsulate the PCM. The formed fibrous network is not also used as a carrier or capsulation medium for PCM but also enhances the thermal conductivity and mechanical properties of the PCM while also preventing leakage during phase transitions (Suárez-García et al., 2023).

RECENT STUDIES ON NANO-ENHANCED PCMs

A wide range of nanomaterials have been utilized with PCMs chiefly to enhance their thermal performance. These nanomaterials are essentially used as a solution to tangle up all the inherent deficiencies of PCMs, particularly their low thermal conductivity.

Table 1, Table 2, Table 3, and Table 4 provide a summary of the studies conducted between 2015 and 2024 on the use of PCMs with various nanomaterials such as graphene and graphene-based nano-materials, expanded graphite, carbon nanotubes, seramic and metallic nanoparticles, respectively.

Table1. Utilization of PCMs with graphene and graphene-based nanomaterials

PCM	Key results	Ref
Paraffin	1 wt.% graphene increases ΔH_m from135 to 162 J/g	(Liu and Rao, 2017)
Paraffin	1.5 wt.% graphene/CNT (7:3) improves TC by 124%.	(Zou et al., 2018)
Beewax	0.3 wt.% graphene increased the ΔH_m by 22%.	(Amin et al., 2017)
PA/PPy	1.6 wt.% graphene improved TC by more than 34.3 % but decreased ΔH_m from 298 to 151 J/g	(Silakhori et al., 2015)

PEG	0.2 vol% of acidic functionalized graphene improved the ΔH_m capacity by 24%.	(Selvaraj and Krishnan, 2022)
1-octadecanol	5 wt.% of graphene improved the TC by 18 times. ΔH_m was nearly 225.3 J g^{-1} and good shape stability were achieved.	(Yang et al., 2018)
PA/HDPE	4 wt.% of GNP increased the TC by more than 250% but reduced the ΔH_m from 211 to 164 J/g.	(Silakhori et al., 2015)
AA/SB (48/52 wt.%-Eutectic)	0.5 wt.% of GNP improved the TC. The supercooling of the PCM decreased from 17.7 ^oC to 3.6 ^oC.	(Seki, et al., 2015)
Tetradeconal	10 wt.% of graphene aerogel improved the TC by 394% but reduced the ΔH_m from 200 t0 177 J/g.	(Mu and Li, 2018)
1-octadecanol	12 wt.% of high density graphene/rGO hybrid aerogel Improved TC by 16 times.	(Yang et al., 2016)
SA (75%)	5 wt.% of graphene/nanocellulose (20:5) increased the TC by 261%.	(Yufeng and Yanghua, 2021)

*TC: Thermal conductivity, T_m: Melting temperature; ΔH_m=Melting enthalpy; PA: Palmitic acid; AA:Adipic acid; SB: Sebacic acid; PEG: Polyethylene glycol; PP: Polypropylene; HDPE: High-density polyethylene; PPy: Polypyrole; rGO: Reduced-graphene oxide.

Table 2. Utilization of PCMs with expanded graphite (EG)

PCM	Key results	Ref
RT44HC	25 and 35 wt.% of EG improved the TC of PCM by 20–60 times.	(Ling et al., 2015)
Paraffin	5, 10, 15 wt.% of EG improved the TC by 4, 6 and 6.5 times, respectively.	(Raza et al., 2016)
SAT	Wıth the addition of 8% urea + 8% EG, a T_m of 47.84 °C, ΔH_m of 223.1 kJ·$kg^{-1,}$ low supercooling degree of 1.54 °C, and TC of 2.076 W·m^{-1}·K^{-1} were achieved.	(Fu et al., 2018)
SA	12 wt.% of EG improved the TC by 19.179 times. The ΔH_m was 163.35 J g^{-1}m and T_m was 67.08 °C.	(Ao et al., 2022)
Paraffin	TC in the parallel direction improved by 70 times.	(Wang et al., 2020)
Nonadecane (ND)	Activated carbon (AC)-EG (90:10) /ND composite with wt75% of ND exhibited a T_m and ΔH_m of 29.84 °C and 173.11 J/g., respectively The TC of the composite improved	(Hekimoğlu et al., 2023)

	by 3.81 times.	
OM37	7 wt.% of EG increased the TC by 114.4%. PCM with 7 wt.% of EG heated 25.9% faster and cooled 19.2% faster compared to neat PCM.	(Rathore and Shukla, 2021)
Hexadecane	The composite with 75 wt.% of hexadecane/15 wt.% EG exhibited a ΔH_m of 189.84 J/g.	(Chriaa et al., 2021)

* TC: Thermal conductivity, T_m: Melting temperature; ΔH_m=Melting enthalpy; SA: Stearic acid; EG: Expanded graphite; SAT: Sodium acetate trihydrate

Table 3. Utilization of PCMs with carbon nanotubes (CNTs)

PCM	Key results	Ref
Lithium Carbonate/Potassium Carbonate (Eutectic)	2,5 wt.% of SWCNTs improved the TC by 56,98%.	(Tao et al., 2015)
PEG/diatomite	2 wt.%. of SWCNTs increased the TC by 2.8% 60 wt.% of PEG composite can maintain its original form without leakage after 200 melt/freeze cycles.	(Qian et al., 2017)
PA + copper foam	5 wt.% of CNT improved the TC of the composite, made of 28.59g PA and 18.53g copper foam, by 1.82 times comparing to the composite without CNT.	(Cong et al., 2021)
Paraffin	MWCNT/graphene (3:7 mass ratio) increased the TC by 31.8%, 55.4% and 124% as against graphene-based composite PCM, MWCNT-based composite PCM and pure PCM, respectively.	(Zou et al., 2018)
CA/SA eutectic	1, 3 and 5 wt.%. of CNT improved the TC by 83.3, 125.0 and 258.3%, respectively.	(Sarı et al., 2018)
Paraffin	5 wt.% of ultra-long MWCNT improved the TC and ΔH_m by 37% and 6.3%, respectively. Low supercooling temperature as 2.4 °C was achieved.	(Kuziel et al., 2021)
Paraffin	CNTs modified by ethylene grafting or silicon coating. The TC is enhanced by 84.6% .	(Li et al., 2022)
Lauryl alcohol	3 wt.% of MWCNT enhanced the TC by nearly 41%, 77.8%, and 74.6% at 10 °C, 30 °C, and 40 °C, respectively. The composite with 3 wt.% MWCNT was stable after 100 thermal cycles.	(Chinnasamy and Cho, 2022)

PlusICE	1.0 wt.% of non-functionalized MWCNT and functionalized MWCNT enhanced the TC by 109.5% and 150.7%, respectively.	(Fikri et al., 2022)

* TC: Thermal conductivity, T_m: Melting temperature; ΔH_m=Melting enthalpy; SWCNT: Single-walled carbon nanotube; MWCNT: Multi-walled carbon nanotube; PEG: Polyethylene glycol; PA: Palmitic acid; CA: Capric acid; SA: Stearic acid.

Table 4. Utilization of PCMs with ceramic and metallic nanoparticles

PCM	Key results	Ref
Paraffin	10 and 20 wt.% of nanomagnetite (Fe_3O_4) enhanced the TC by 48% and 60%, respectively	(Şahan et al., 2015)
Phenol-water mixture	4 wt.% of α-Al_2O_3 increased the TC by nearly 93.5%.	(Mishra et al., 2018)
Paraffin	20, 30, 40, 50, 60, 70, 80 wt.% of nanoclay containing paraffin/nanoclay composites were prepared. Among them, composite with 60 wt.%,of nanoclay exhibited no leakage.	(Alkhazaleh and Kandola, 2017)
Paraffin	5 wt.% of Fe_3O_4 increased the ΔH_m by 20.67%.	(Amin et al., 2018)
	10 wt.% of CuO increased the ΔH_m by 78.89%.	
	5 wt.% of TiO_2 increased ΔH_m by 7.5%.	
	5 wt.% of ZnO increased ΔH_m by 20.17%.	
Paraffin wax	0.5, 1, 2 wt.% of SiO_2 increased the TC by 12.78%, 22.78%, and 33.34%, respectively. However, the ΔH_m was decreased with the increasing mass% of the nanoparticles	(Manoj Kumar et al., 2020)
LA/PA eutectic	5 wt.% of SiO_2 increased the TC by 54.4%.	(Baskar et al., 2022)
n-octadecane	Red clay (RC)/expanded perlite (EP) (80.5%) and expanded vermiculite (EV) (59%) composite was prepared. The maximum temperature dropped by up to 1.6 °C during the phase change of RC/EP-SSPCMs.	(Wi et al., 2020)
Paraffin	1.0 vol% of nanosilica displayed thermal consistency even after 100 thermal cycles.	(Saravanakumar et al., 2022)
SA	12 wt.% boron nitride increased the TC by 2.82 times. Excellent cycling and thermal stability were observed.	(Ao, et al., 2023)
Paraffin wax	h-BNNSs enhanced the TC by 12-times.	(Yang et al., 2016)

PEG	Boron nitride grafted with PVA improved the TC by 286%.	(Wie and Kim, 2021)
Carnauba wax	30, 40, 50 wt.% of nanoclay montmorillonite increased the ΔH_m 107.9 ± 1.7 J/g, 95.0 ± 2.5 J/g, and 69.5 ± 3.7 J/g, respectively.	(Brychka et al., 2024)
PEG	7.2 wt.% of Ag nano particles improved the TC PEG/diatomite composite by 127%.	(Li et al., 2015)
PEG	Nano-Ag was added for surface-modification. Navel orange peel-based porous carbon was used as carrier. Relative enthalpy efficiency increased from 104.8 % to 107.2 %.	(Xiao et al., 2022)
Paraffin	0.05 and 0.1 wt.% of Ag nano particles lowered the phase change temperatures. As the content of Ag-nanoparticles increased, the ability of thermal energy storage increased as well.	(Pradeep, et al., 2021)
Paraffin	Ag-nanoparticles/EG inclusion rose TC to 2.987 W/(mK). ΔH_m and T_m were 130.5 J/g, and 46.3 °C, respectively.	(He et al., 2024)
Paraffin wax	0, 0.5, 1.0, 1.5, and 2.0 w.t% of CuO nanoparticles notably increased the TC with the increasing CuO content.	(Manoj Kumar et al., 2020)
PA/SA eutectic (64:36)	0.3, 0.8, 1.5 and 3 wt.% of CuO nanoparticles increased the TC by 19%, 34.23%, 55.24%, and 118.87%, respectively.	(Agrawal et al., 2022)
LA/CA (53:47)	TiO and CuO nanoparticles increased the TC of by 17.56%.	(Sarafoji et al., 2022)
PS-PEG	PbO/BN/ PS-PEG (60.90/13/26.10 wt.%) reached TC of 18.874 W/(mK).	(Ortaç et al., 2023)

* TC: Thermal conductivity, T_m: Melting temperature; ΔH_m=Melting enthalpy; EG: Expanded graphite; PA: Palmitic acid; SA: Stearic acid; h-BNNS: Hexagonal noron nitride nano sheet; PVA: Polyvinyl alcohol; PS-PEG DM: Poly (Styrene-block-Ethylene Glycol Di Methyl Methacrylate).

CONCLUSIONS

Even though energy storage has dominated research, thermal energy storage with PCM has advanced significantly in recent years. As advancements are made to overcome the drawbacks of inorganic PCMs' poor phase change performance and chemical/thermal stability, as well as the limitations of organic PCMs' poor thermal conductivity, flammability, and leakage, the restriction of PCMs keeps getting smaller. Therefore, it is important to follow the latest developments in the research field and keep the information up to date. This review has provided such an overview. The final conclusions derived from the reviewed literature are listed below.

- Research focuses on organic, inorganic and eutectic solid-liquid PCMs as well as common problems encountered with them, such as poor thermal performance.
- To address issues with phase segregation, flammability, leakage, and inadequate heat transfer, microencapsulation or nano-sized materials is used.
- Researchers have proposed fully biodegradable PCMs, also referred to as "greener" phase change materials, as an alternative to PCMs due to their toxicity and polluting nature.
- Because nanomaterial dispersion is delicate, care should be taken when choosing which nanomaterials to utilize and what weight percentages to employ; according to the reviewed literature, 10 weight percent and less is typical.
- As the weight ratio of added nanoparticles increases, thermal conductivity also increases. Although they tend to be more expensive, smaller-diameter nanoparticles produce greater results and are lighter.
- The use of nanoparticles reduces supercooling, flammability, and phase segregation; additionally, the phase transition temperatures of PCM can change after the inclusion process.
- Agglomeration is brought on by excess weight percentage, which also occasionally lowers and occasionally raises the latent heat capacity. To determine whether the rise in latent heat capacity can be controllably regulated, more research is still needed.
- Advanced equipment is used in the preparation and characterization of nano-encapsulated PCMs.
- Nano-encapsulated PCMs have potential properties that can lead to energy savings and increased system efficiency for thermal energy storage.

We expect that high-performance nano-enhanced PCMs technology will play a critical and wide-ranging role in the future of sustainable and renewable energy in thermal storage applications, such as electronic device cooling, waste heat management, solar energy storage, industrial heat storage, and release processes.

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CHAPTER 2

ADAPTATION AND PLASTICITY IN PLANTS

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INTRODUCTION

Plant functions occur through the adaptation of their leaves to dynamic structure and their ability to adapt to environmental conditions. This adaptation occurs thanks to plants' flexibility and adaptability (González and Oyama, 2005).

Plants use adaptation mechanisms to survive. Leaves are organs through which plants interact with their environment and perform essential functions such as photosynthesis. The dynamic structure of leaves includes their ability to react to various environmental factors. These adaptations may include features such as the ability of leaves to change shape, controlling water loss through stomata, and making the best use of sunlight. These adaptations help plants adapt to different climates and environmental conditions.

Plasticity in plants refers to the plant's ability to adapt to environmental changes. Plants show plasticity in different ways so that they can adapt to environmental conditions. Plasticity in plants occurs through morphological and physiological changes.

Plants show movement and behavioral characteristics in order to survive. These behaviors are geotropism, phototropism, chemotropism, and hydrotropism. These adaptation mechanisms exhibited by plants are of great importance in increasing their lifespan, enabling them to perform photosynthesis to produce oxygen, which is a life activity, and preserving the natural balance in nature.

-Geotropism: It is the movement of the leaves and stems of the plant with gravity and touch. While some plants do not react to insect contact or low rainfall, some do. But they bend their leaves to protect themselves against high rainfall (Dean and Smith, 1978).

-Phototropism: It is the adaptation of plants to light. Some plants change their phenotype in response to infrared light, thus preparing them for the shade they will encounter later (Mullen et al., 2006; Novoplansky, 2016; Creux and Harmer, 2019).

-Chemotropism: It is the adaptation mechanism of plants against chemical substances.

-Hydrotropism: It is the adaptation mechanism of plants to water. Plants need water to survive. Plant roots move to reach and hold water. Thus, they have developed their adaptations to survive.

Living things exhibit significant differences in terms of many parameters such as their physiological characteristics, lifespan, maturation processes, reproductive methods, reproductive potential, dissemination strategies and the persistence of reproductive/propagation organs. This diversity is related to the ability of living things to react in a wide range to the environment they live in and changes in the environment, and reveals different adaptation strategies of living things (Booth et al., 2010; Fabian and Flatt, 2012).

ADAPTATION

The ability of a living life form to survive and reproduce in specific environmental environments is called adaptation (Allard, 1988). Adaptation, which is necessary for both cultured and natural habitat species, requires that vital activities be compatible with climatic and edaphic factors. Thus, plants are least affected by adverse environmental conditions (Roberts et al., 1993).

Genetic adaptation represents the change in the genetic structure of plant communities in response to environmental changes. With adaptations, plants adapt to environmental conditions more effectively and continue their generation. This is very important for the ability of plants to adapt to natural selection and environmental pressures.

The most important indicator of the presence and distribution of plant species that have evolved with geographical regions or environmental factors in specific habitats are the adaptation processes of plants with edaphic factors and climatic conditions. However, it is not always easy to identify the genetic origins of this adaptation phase. The natural factors to which plant communities react are often quite complex and only sometimes recognized. A large genome may be effective in helping plants adapt to specific environmental conditions. These genomes play a critical role in plant adaptation to climate, soil

and other environmental factors. During the adaptation process, various genes working together enable the plant to respond effectively to environmental pressures to survive and gain reproductive advantage. This genetic diversity may contribute to the plant adapting more effectively to changing environmental conditions by increasing its adaptation abilities and resilience.

Plants react to differences in environmental conditions in two ways. (i) Physiological differences (may be called modifications): these changes refer to changes in the external appearance or behavior of the organism. It may result from environmental influences as well as genetic factors and may reflect the adaptation of the organism. These phenotypic changes, also called modifications, reflect the complexity of environmental interactions as well as genetic variation. (ii) Evolution or genetic changes that occur over generations: these changes show how genetic variation in populations evolves over time. Evolutionary processes lead to diversification and adaptations of organisms through genotypic changes.

Physiological change is explained as the period during which the gene sequences that constitute genetic information are exposed to differences in changing environmental conditions. This variability occurs when the genes that make up the genotype are affected by various environmental factors. The organism's phenotype is determined as a result of environmental interactions combined with genetic factors (Bradshaw, 1965).

Aquatic plants adapt to survive. It has large air spaces. These air spaces allow plants to stay on the water surface. There are stomata on the leaf surfaces, and they enable gas exchange. Aquatic plants have thin cuticles because they are not likely to lose water. Aquatic plants in swamps carry out gas exchange by bringing their roots to the surface.

Genetic Adaptation of the Plant to Climate Conditions

It is difficult to understand the adaptation strategies of plant communities due to specific reasons. These:

i. In many cases, the climatic conditions of the environment to which the organism is trying to adapt are either unclear or may be quite complex. Therefore, genetic variations and phenotypic adaptations may reflect efforts of organisms to adapt to changing environmental conditions. Adaptations play an important role in organisms' efforts to adapt to environmental complexities.
ii. The environmental conditions that populations are exposed to throughout their life cycle may cause genetic variations and adaptations to differ according to their developmental stages. This can lead to the emergence of diverse adaptation strategies among individuals within a population and the evolution of diverse responses to environmental pressures at different developmental stages.
iii. Genomic systems control the reactions of populations to certain climatic factors. These systems include mechanisms such as gene expression, hormonal regulation, and metabolic processes for organisms to adapt to environmental changes. Physiological and genetic systems determine the organism's ability to adapt to climatic conditions and influence the resilience of populations to various environmental stresses.
iv. The similar gene and reaction strategy provides adaptation to many variables. The dominant variable exerts its influence on plant populations. For example, the amount of rainfall in the period before the reproduction or development of plants is an important environmental factor that ensures productivity in plant populations (Allard et al., 1992).
v. Seasonal diversity is also very important in the adaptation of plants. Plants have developed various strategies to adapt to seasonal environmental changes. These adaptation mechanisms include processes such as flowering, fruit formation, leaf drop and dormancy. Plants adapt to environmental changes by regulating their growth and

reproduction strategies according to the seasons (Roberts et al., 1993).

vi. Adaptation and photoperiodism are also closely related. Plants show the ability to regulate their flowering and reproduction processes through photoperiodism, depending on environmental light conditions. This adaptation mechanism allows plants to respond to day length or shortness and bloom and produce seeds at the appropriate time. With their photoperiod requirements, plants have adapted and spread to different ecologies on Earth, and different species of the same genera are distributed in different geographies (Erskine et al., 1994).

vii. Adaptation diversifies the life strategies of plants and animals in extreme climatic conditions. Living things survive this stressful process through their ability to adapt to various physiological changes. In these cases, two results regarding genetic adaptation emerge.

*In the adaptive mechanism, genetic differences of populations occur in connection with natural factors. Different genotypes can better adapt to various environmental conditions, so populations can be more resilient to environmental changes.

*When the selection pressure is high, those with high adaptability within the populations can continue their lives, while those with low adaptability cannot continue their lives in a certain environment, with certain events, and fall out of the population. This pressure strengthens the effect of the natural selection process and allows species to adapt more effectively to environmental changes. Adaptations seen in individuals under high selection pressure affect the evolution of species by causing certain genetic variations to increase or decrease within the population. Thus, living things must be able to adapt to constantly changing

conditions in order to remain in harmony with their environment.

Genetic Adaptation of the Plant to Soil Conditions

The soil/substrate factor plays a critical role in the adaptation of plants. Heavy metals, which are among the stress factors, cause genetic diversity within populations in terms of their ability to adapt. The ability of plants to adapt to a particular habitat depends on the genetic capacity of the population to adapt to adverse conditions and the ability to adapt effectively. These adaptations enable plants to adapt to specific soil conditions and survive in these environments. Families with high adaptation to heavy metals were identified as Poaceae, Brassicaceae and Caryophyllaceae. Studies have reported that *Agrostis*, *Anthoxanthum*, *Deschampsia*, *Festuca*, *Holcus*, *Mimulus*, and *Silene* species have adapted to high concentrations of heavy metals (Al-Hiyaly et al., 1990; Meharg et al., 1993).

It has been observed that there are differences in the characteristic features of plant communities in the lands where external interventions are made. These interventions can lead to adaptation pressures by affecting plants' growth conditions. For example, it has been observed that changes occur in the characteristic features of plant communities (seed and fruit structure, etc.) after a certain period of time when lime is applied to the soil (Van Tienderen and Van der Toorn, 1991a; Van Tienderen and Van der Toorn, 1991b). Plants can adapt to the nutrients found in the soil and other environmental factors. These adaptations trigger intergenerational genetic change by affecting the genetic expression of plants (Aarssen and Burton, 1990).

The pH level in the soil is one of the important factors affecting the adaptation of plants. While neutral soils generally provide suitable conditions for many plant species, some plant populations prefer acidic or basic soils (Royo et al., 1993). Another basic factor that ensures the spread and survival of populations is salinity. Salt levels affect water uptake and nutrient absorption of plants (Shannon, 1985). The

characteristic feature of most plants is resistance to salinity. It is very important to find plants with advanced genetic adaptation abilities to salinity and to increase plant productivity in places with salinity problems (McNeilly, 1990; Al-Khatib et al., 1993).

Adaptation of Plants to Drought Stress

Drought stress causes various physiological, biochemical and molecular responses in plants. These responses enable plants to develop adaptation mechanisms against water deficiency. These adaptations vary depending on plant populations, genetic characteristics, drought severity and extent, plant growth, lifespan, plant parts such as roots, stems and leaves, and cell types (Bray, 1997).

Adaptation of plants to drought includes the capacity to withstand drought processes (Gürel and Avcıoğlu, 2001; Mundree et al., 2002), the ability of plants to save water, minimize water loss and use water more effectively.

The first adaptive action that plants show when faced with water stress is to regulate the opening and closing of their stomata. In order to minimize water loss, plants either shrink their stomata or close them completely. This mechanism is related to the effort of plants to preserve water and minimize water loss (Osakabe et al., 2014). Closing the stomata helps the plant save water and use its water more effectively. For this reason, plants regulate water use by quickly closing their stomata. As the stomata close, there is a decrease in the amount of carbon dioxide taken from the leaf surface, resulting in a decrease in the amount of photosynthesis (Chaves et al., 2003).

Photosynthesis rate decreases with drought stress, causing the formation of elements such as reactive oxygen derivatives and singlet oxygen, which are natural by-products of cell metabolism on the plant and play a key role in the signal transduction mechanism. Reactive oxygen derivatives are important in the adaptation of plant populations to drought (Anjum et al., 2011; Bhargava and Sawant, 2013; Cabello et al., 2014).

Adaptation of Plants in the Mediterranean Basin

The Mediterranean Basin has a climate structure characterized by hot, dry summers and mild, rainy winters. Under these ecological conditions, plants have developed special adaptation mechanisms. Features such as upright leaves and small surface area, deep root systems, ability to retain water, salt tolerance and fire adaptation enable plants to survive in the harsh conditions specific to the Mediterranean climate.

The distribution of plant species and their adaptation to their distribution areas occur under the influence of their growing conditions, especially their genetic abilities.

As a general rule, stressful environments are places where plant adaptation is most intense. For example, while trees in humid forests have a ladder-like perforation at the end of their water transmission pipes, trees and shrubs in the Mediterranean Basin generally have a simpler and more effective perforation plate in water transmission. In order to increase safety in water transmission, the grouping rate in water transmission pipes is higher in trees and shrubs in arid regions (Baas and Schweingruber, 1987). In the Mediterranean Basin, different plants have formed a community that can live together by developing similar or different abilities against habitat conditions and fires.

PLASTICITY (FLEXIBILITY)

Plasticity can be defined as differences that occur depending on the environmental effects in the environment in which the genotype is located.

The ability to adapt to changes that is, ‘noisy plasticity’, generally functions as a balanced system against the differences in nature. However, adaptation and difference here should not be seen as opposites (Via, 1994).

Since the genetically determined environmental response ability occurs together with the effect of natural selection, variability is actually considered an adaptation mechanism (Emery et al., 1994).

It is known that stomatal movements play an important role in reducing water loss in plants in arid conditions. Research has shown that stomatal density and pore openings change in plants under the influence of drought. This indicates that the plasticity of stomata shows high variability depending on environmental conditions (Tobiessen and Kana, 1974; Wang et al., 2016).

The distribution and movements of stomata may change under the influence of not only drought but also stress factors such as temperature (high and low), high winds, salinity, and rootstocks other than those grown in a specific way in a particular ecosystem or culture. These various environmental factors influence the ability of plants to open and close their stomata, allowing them to control water loss and adapt (Buckley et al., 2020).

Phenotypic plasticity is the genetic potential of an organism, the ability to develop various phenotypes depending on environmental conditions. Three hypotheses regarding phenotypic plasticity have been presented.

- The flexibility of species in different environmental conditions is also different from each other. This allows plants to respond more effectively to changing environmental conditions by varying characteristics such as the density and size of stomata.
- Phenotypic plasticity is also different for organisms with distant kinship ties. Evolutionary distance can influence genetic differences and adaptation traits, resulting in different phenotypic responses to similar environmental pressures.
- More phenotypic plasticity is often seen in organisms that show little change in their genetic characteristics. This may be due to limited genetic diversity, allowing them to respond more flexibly to environmental changes through phenotypic changes.

Reproductive power and reproductive cost are the main factors affecting phenotypic plasticity. The role of phenotypic plasticity in vegetative reproduction and generative reproduction capacity, adaptation of plant communities to natural life, and population

formation is very effective and shows that it is of great ecological importance. These characteristics affect the ability of plants to adapt to environmental changes and determine their ecological role within the ecosystem.

Females in herbaceous plant populations are larger than males, while in woody dioecious plants females are smaller than males. Generally, increasing plant size increases the rate of sexual reproduction and decreases the rate of vegetative reproduction. This refers to various adaptations in the life cycle and reproductive strategies of plants. In certain plants, sexual reproduction is generally more common in the presence of high populations, while vegetative reproduction may be more dominant in the presence of low populations. In these plants, the substances formed as a result of photosynthesis are generally transported to the organs on the soil surface during vegetative development, while they can be directed to the organs underground during generative development. The adaptations shown may reflect strategies to adapt to changes in population density and environmental conditions. This situation is called “top senescence”. Top senescence is an important strategy, especially for geophyte plants. This strategy allows plants to adapt to the environmental conditions in which they live and to use nutrients efficiently and cost-effectively. Geophyte plants can adapt to population density and environmental changes thanks to their ability to accumulate energy in underground organs. This represents an example of adaptation based on plants’ long-term growth strategies.

Plantago lanceolata can be considered a successful weed due to its ability to adapt to a variety of environmental conditions. The wide distribution and adaptability of this plant has resulted in a wide phenotypic variation. Therefore, many studies carried out on *Plantago lanceolata* are aim at obtain a deeper understanding of the plant’s adaptation mechanisms and plasticity (Young and Schmitt, 1995; Marshall et al., 2019).

Phenotypic plasticity refers to the response of an organism to environmental conditions through changes in its structure. It has long been known that the growth habit of *Plantago lanceolata* is highly variable. Many vegetative and reproductive characteristics of this plant change in parallel with environmental changes. This may reflect the phenotypic flexibility of *P. lanceolata* and its capacity to adapt to various environmental conditions (Van Tienderen and Van Hinsberg, 1996).

Trees are plant communities that quickly adapt to different ecological conditions and have high natural plasticity. For this reason, they show various reactions to environmental stress conditions, especially factors such as drought and frost, as well as different ecological conditions, management styles and local conditions. In the context of major stress factors such as environmental pollution, the adaptation and resistance states of tree species show obvious differences in different ecoregions, depending on their genetic structure and species characteristics. This indicates that tree species have developed various adaptation strategies specific to different environmental pressures, and their adaptation capacity is linked to their genetic diversity.

Seeds and seedlings with superior genetic characteristics should be used to ensure the long-term success of forest establishment and afforestation efforts, especially in periods when costs are high and risks are minimized. It is important to know genetic variations to determine the genetic quality of the seedling. The population and the trees in the population represent the source of generatively or vegetatively produced material. The resistance of resistant plants to biotic and abiotic damage is directly linked to intraspecific genetic diversity, which affects their ability to adapt to changing climatic conditions (Filiz et al., 2011; Güney et al., 2014; Koç, 2022). Leaf morphology plays an important role not only in heat dissipation but also in photosynthesis and light uptake. Trees in the forest are located in different layers within the ecosystem because they are exposed to different light intensities. This difference causes leaf morphology to

have various shapes and sizes. Plants in different layers of the forest develop various adaptations to optimize light use and fulfil their roles in the ecosystem (López de Heredia et al., 2009).

CONCLUSION

Plasticity is a fundamental trait that allows plants to respond quickly and effectively to changing environmental conditions. This adaptability supports ecosystem diversity by enabling plants to survive successfully under different growth conditions. Adaptation in plants involves traits that are passed down from generation to generation through genetic changes, but plasticity refers to the ability of a plant to change its phenotype according to environmental conditions. While plants achieve long-term environmental adaptation through traits inherited through genetic changes, plasticity can temporarily change a plant's phenotype by responding to sudden environmental changes. Adaptation is often associated with evolutionary processes. Plasticity acts as a more flexible and rapid adaptation mechanism.

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CHAPTER 3

BIOCHEMICAL EFFECTS OF CARDIOPLEGIA SOLUTIONS APPLIED DURING CPB ON THE MYOCARDIUM

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INTRODUCTION

The heart is one of the most important organs for many organisms, including humans. In the 1800s, doctors thought the heart was an untouchable organ, but in the 1900s, with the development of technology, significant breakthroughs in heart surgery occurred. Innovations in imaging technology, depending on the more detailed examination of the structure of the heart, have led to the development of important methods in line with the needs of heart surgery (Hossain and Anisuzzaman, 2023; Fosch-Villaronga et al., 2023).

In this book chapter, the effects of cardioplegia used during cardiopulmonary bypass, one of the important methods used in cardiac surgery, on the myocardium will be discussed.

CARDIOPULMONARY BYPASS

At the beginning of the 20th century, surgeons gained momentum in their work on the heart. Although the two world wars slowed down the pace of heart surgery, important developments occurred after these wars. Dr. Henry Souttar developed a treatment for mitral stenosis after World War I, which enabled the correction of the mitral valve. After World War II, 'Influenza occlusion surface hypothermia' and 'Controlled cross circulation' emerged as two new heart surgery techniques invented by heart surgeons in Canada and North America. Due to the cold climate conditions in these regions, patients' bodies were hypothermed in ice-filled tubs and prepared for tissue restoration work. After the hypothermic body was taken to the operating table, heart surgeries were performed quickly and then the body was placed in a tub full of warm water to rewarm. Thanks to these studies, it was concluded that hypothermia protected sensitive tissues such as nerve tissue (Hossain and Anisuzzaman 2023).

While these developments were taking place in the world, another important development in cardiac surgery was the development of the heart-lung machine by John Gibbon (Figure 1). The basis of the heart-lung machine is the transfer of blood from the body from the vein to a device where it can be oxygenated, and the oxygenated blood is

collected again with the help of a pump and transferred to the artery (Demirkılıç, 2015).

J.W. Kirklin improved the heart-lung machine designed by Gibbon and named it the Mayo-Gibbon heart-lung machine (Figure 2). The success that Gibbon and other surgeons achieved with Gibbon's machine was also achieved with the Mayo-Gibbon machine. Thus, the preference for heart-lung machines in open heart surgeries has increased (Demirkılıç, 2015).

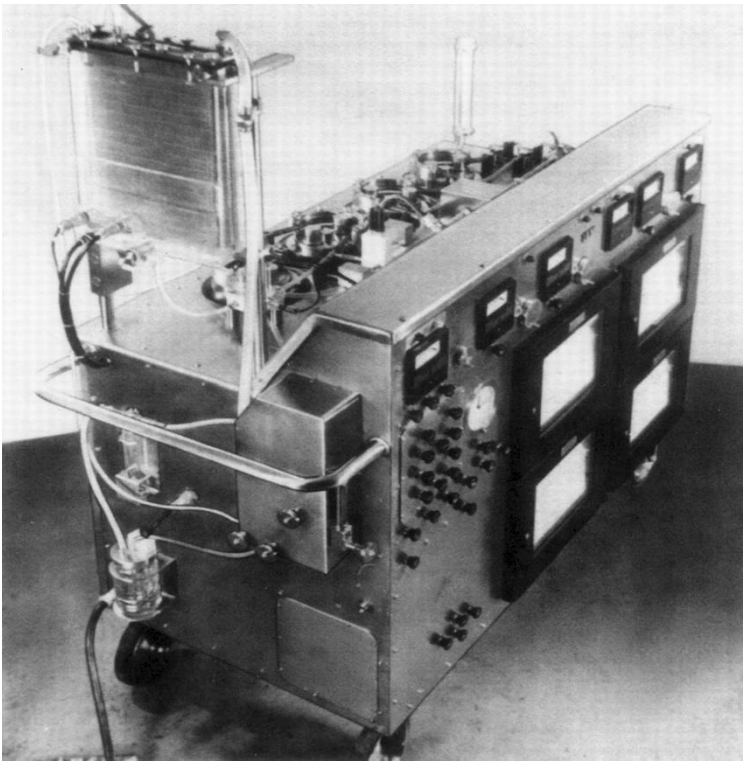

Figure 1. John Gibbon-IBM Co-Designed First Heart-Lung Machine

Figure 2. The Mayo-Gibbon machine

The development of the heart-lung machine has enabled the application of extracorporeal circulation in cardiac surgery. Cardiopulmonary bypass is based on the principle of temporarily stopping the patient's heart and lungs (Ghosh et al., 2014).

One of the basic requirements of the heart-lung machine is anticoagulant substances. The substance used for this is heparin. Heparin was discovered by a medical student, Jay McLean, in 1915 and it was included in the literature that it could be used as an anticoagulant thanks to the successes obtained from animal experiments (Demirkılıç, 2015).

In open heart surgery, the heart-lung machine must also drain the blood inside the heart after the heart and lungs are stopped. The device also consists of an oxygenator that takes over the lungs and a pump that takes over the heart's function. With the rapid development of technology, blood flow rate, blood flow rate, blood temperature, blood gas and blood electrolyte values in today's heart-lung machines are monitored, followed and adjusted as desired during open heart surgeries (Figure 3) (Kocakulak, 2001).

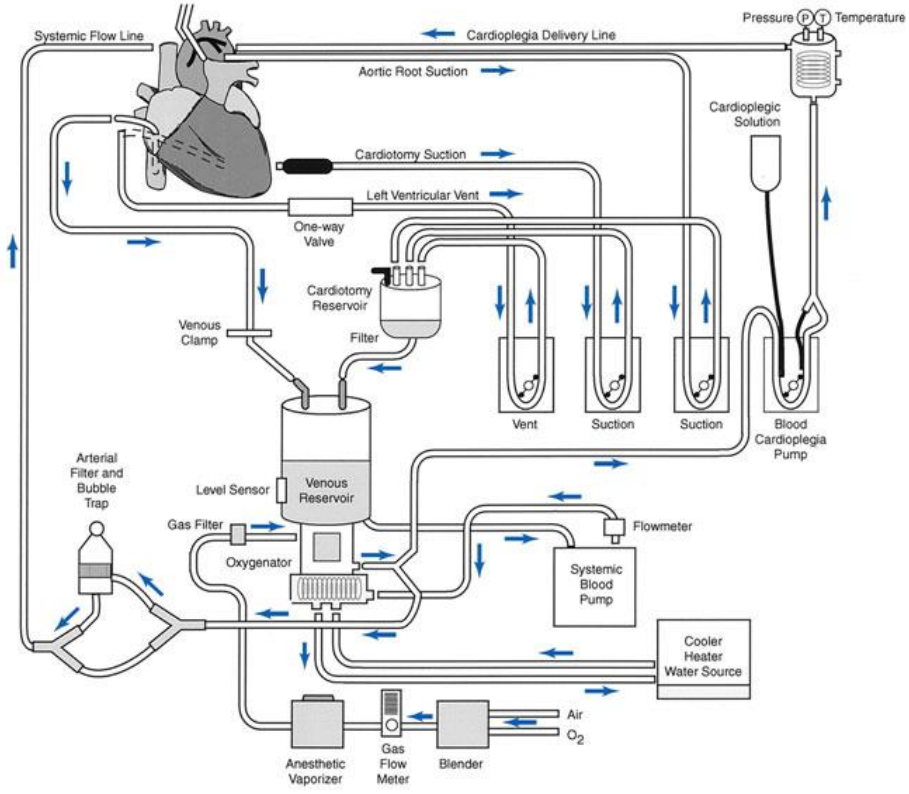

Figure 3. Cardiopulmonary Bypass Circulation

CARDIOPLEGIC SOLUTIONS

In surgical operations that need to be performed on the heart, inside the heart, or on the major arteries and veins connected to the heart, the heart must be stopped. By stopping the heart, a bloodless and static area is provided for the surgeon to perform the operation. Various

methods have been developed to stop the heart in a controlled manner during the operation and to protect the myocardium during this process. Since the beginning of open-heart surgery, researchers have been developing methods to stop the heart in a controlled manner during the operation and to protect the myocardium during this process. These methods are listed as whole-body hypothermia, local cardiac hypothermia, and cardioplegia solutions according to their historical process (Raman et al., 2001).

Cardioplegia is a condition in which the heart rhythm is deliberately stopped during surgery, providing a still, bloodless area for heart repair. Cardioplegia, a potassium-rich (usually) solution, causes rapid cardiac arrest and sudden electromechanical calm (Raman et al., 2001).

Different types of cardioplegia

Throughout the history of cardiac surgery, different types and techniques of cardioplegia have been applied. Cardioplegic solutions are divided into two main groups: blood and crystalloid. In addition, cardioplegia can be cold (4°C), isothermic (at the same temperature as the patient) and normothermic (35-37°C). Cold cardioplegic solutions are usually administered intermittently, while normothermic solutions can be administered as a continuous infusion. For optimal myocardial protection during cardiac surgery, a policy of choosing a cardioplegic method on a patient basis and combining existing methods should be adopted rather than using a standard method for all patients (Yang and He, 2005).

Preparation and application methods of cardioplegia

Cardioplegia solutions with high potassium content are used to provide diastolic arrest. The potassium concentration used varies according to clinical protocols, but is usually between 8-20 mEq/L.

Diastolic arrest is achieved by administering cardioplegia solution from the aortic root after cross-clamping the aorta. Giving cardioplegia in this way is called antegrade cardioplegia application, while giving cardioplegia through the coronary sinus is defined as

retrograde application. Antegrade application can be done non-selectively from the aortic root or selectively from the coronary ostia (Rigattieri et al., 2000).

Diastolic arrest should be achieved by administering cardioplegia immediately after cross-clamping the aorta. Cardioplegia solutions are divided into three groups according to their content: blood, blood + crystalloid (mixture in a ratio of 3:1 or 4:1) and crystalloid. In routine practice, 20-30 mL/kg antegrade cardioplegia is given for the purpose of stopping the heart (cardioplegia induction). The potassium concentration in the induction solution should be adjusted to 20-30 mEq/L. Antegrade cardioplegia is maintained as 10 mL/kg by continuous or intermittent infusion. The potassium content in the maintenance cardioplegia solution is generally kept low (10 mEq/L). Antegrade cardioplegia pressure should generally be between 60-100 mmHg. It is important to give cardioplegia at high pressure, especially in patients with severe proximal coronary artery lesions. The heart should usually stop within 30-60 seconds. In cases of delayed or non-delayed cardiac arrest; Inadequate cardioplegia pressure, inadequate potassium in cardioplegia, blood leakage into the coronary system due to incomplete cross-clamping, aortic valve insufficiency and coronary arteries being fed by collateral branches originating from the systemic circulation should be considered. Although topical cold is applied in many centers to reduce myocardial oxygen consumption during cardioplegia application, it is not a standard method. Cardioplegia can be administered continuously from the aortic root or coronary ostia or intermittently every 20 minutes (Zhou et al., 2022).

Retrograde cardioplegia is performed with a balloon catheter placed in the coronary sinus. Transesophageal echocardiography is an important examination in determining the position of the placed catheter. In many centers, cardiac arrest is provided with antegrade cardioplegia following cross-kemp application, while the continuation of cardiac arrest is provided with continuous or intermittent retrograde cardioplegia. Retrograde cardioplegia is especially important in hypertrophic hearts. The delivery rate is 200-400 mL/min and coronary sinus pressure should be kept between 30-50 mmHg. If cardiac arrest is

provided with retrograde cardioplegia, the duration of cardiac arrest is longer than antegrade cardioplegia (2-4 minutes) (Rigattieri et al., 2000).

The most important disadvantage of retrograde cardioplegia is that the right ventricle cannot be well protected. Patients with left ventricular hypertrophy are more susceptible to myocardial ischemia after cross-clamping and to reperfusion events after cross-clamping than patients without LV hypertrophy due to increased myocardial workload and oxygen demand. It has been reported that giving warm blood cardioplegia (terminal hotshot cardioplegia) to these patients just before cross-clamping reduces ischemia-reperfusion injury (Zhou, 2022).

BIOCHEMICAL PROCESSES OCCURRING IN THE MYOCARDIUM AFTER CARDIOPLEGIA

Ischemia causes rapid metabolic deterioration in the myocardium. High-energy phosphate stores are rapidly depleted and approximately 50% of ATP reserves are lost within the first 10 minutes of ischemia. Decreased myocardial contractility occurs in the following minutes. Irreversible myocardial damage occurs within half an hour in normothermic ischemia (Magovern et al., 1982). The subendocardial muscle of the heart is the most sensitive part to ischemic damage. Blood flow in the subendocardial part of the left ventricle occurs only in diastole (Chitwood et al., 1979).

Cardioplegic solutions minimize cardiac contraction, thus causing ischemia because of interruption of blood flow to the myocardium. After surgical procedures are completed, blood flow to the myocardium resumes with the resumption of cardiac contraction. An increase in the production of reactive oxygen species (ROS) occurs due to reperfusion. Disruption in the electron transport system due to ischemia causes disruption of aerobic respiration in the mitochondria and disruption of ROS production in the mitochondria (Leszek et al., 2020).

ISCHEMIA REPERFUSION INJURY

Despite effective cardioplegia, myocardial ischemia during surgery and subsequent reperfusion can cause significant damage. Reperfusion injury is characterized by oxidative stress, inflammation, and calcium overload, which can lead to myocardial stunning, fatal reperfusion injury, and arrhythmias (Kurihara and Sakai, 1985).

Reperfusion injuries and prevention methods

Understanding myocardial protection requires a grasp of various anatomical and physiological concepts. A basic principle is to stop the heart from suppressing energy needs while maintaining cellular integrity. This approach provides a bloodless, immobilized space and reduces the risk of air embolism while opening the left side of the heart and was the basis for the development of the first cardioplegic solution. The potassium-rich solution is administered directly into the coronary arteries to induce cardiac arrest (Leszek et al., 2020).

During intraoperative myocardial ischemia, tissue hypoxia after aortic cross-clamping leads to acidosis and lactate accumulation within minutes as adenosine triphosphate (ATP) is consumed faster than it can be produced in the mitochondria. This proton accumulation activates the $^{Na+/H+}$ exchanger and then the $^{Na+/Ca2+}$ exchanger, leading to intracellular $^{Ca2+}$ accumulation. If ischemia continues untreated, it disrupts cell membranes and causes leakage of intracellular components (Chitwood, 1979).

Mitochondrial protection is vital because mitochondria provide ATP to cardiomyocytes and play a central role in activating cell death pathways. The mitochondrial permeability transition pore (mPTP) is a non-selective channel in the inner mitochondrial membrane that remains closed during ischemia-induced acidosis and provides some protection. However, if the mPTP opens, it leads to depolarization, uncoupling of oxidative phosphorylation, intracellular ATP depletion and cell death. Protecting the heart during ischemia focuses on preventing the opening of mPTP and promoting activation of the mitochondrial ATP-dependent potassium channel. This activates 2 primary protective pathways: The reperfusion injury rescue kinase

pathway activated through G-protein-coupled receptors and the survivor-activating factor-enhancer pathway operating through tumor necrosis factor-alpha receptors (Chitwood, 1979).

Myocardial reperfusion injury occurs upon restoration of blood flow to the myocardium and presents several challenges. Myocardial stunning refers to reversible mechanical impairment of cardiac muscle function, whereas fatal reperfusion injury is primarily driven by reactive oxygen species, intracellular calcium overload and inflammatory processes. Reperfusion arrhythmias, which can usually be treated with interventions such as defibrillation, and the no-reflow phenomenon, in which perfusion cannot be restored in a previously ischemic area despite optimal revascularization, also pose significant risks (Rezkalla and Kloner, 2005).

The original physiological basis of cardioplegia was based on the need to protect the myocardium during cardiac surgery by creating a state of reversible cardiac arrest, thus minimizing myocardial energy consumption and preventing ischemic damage. This concept arose from the understanding that the heart requires a continuous supply of oxygen to function and that any interruption in blood flow can lead to tissue damage, especially during the complex and time-consuming procedures of open-heart surgery (Sugimoto et al., 1995).

Understanding the interaction between potassium and the myocyte action potential played an important role in the development of cardioplegia. The Nernst Equation, formulated by Walther Hermann Nernst in 1881, played an important role in this development. In the case of myocardial protection, the following can be calculated: equilibrium potential (resting potential) of any membrane (myocyte membrane), given the concentration of any ion (electrolyte K) on either side of the membrane. (Myocyte Action Potential) The equation is:

- $E = 61{,}5 \log 10 (C_1 / C_2)$

This equation, where E is the resting potential, C1 is the K+ concentration outside the membrane and C2 is the K+ concentration inside the membrane, allows calculation of the equilibrium potential across the myocyte membrane. Typically, when extracellular potassium is 4 mmol/L, the resting membrane potential is approximately -90 mV.

However, if extracellular potassium increases to 20 mmol/L, the resting potential shifts to approximately -50 mV. This shift in membrane potential effectively "traps" the myocyte in a state where the resting membrane potential is -50 mV and prevents the onset of phase 0 of the cardiac action potential, since a threshold of -70 mV is required for the opening of sodium channels. This mechanism ensures that the heart remains arrested, providing a bloodless and immobilized space necessary for surgical precision and reducing the risk of complications such as air embolism during surgery on the left side of the heart (Bouchard et al., 1995).

Extracellular and intracellular electrolyte composition is vital to the effectiveness of cardioplegia. For example, potassium is typically 144 mmol/L intracellular and 4 mmol/L extracellular, sodium is 10 to 15 mmol/L intracellular and 144 mmol/L extracellular, and calcium is 1 mmol/L or less intracellular and 4 mmol/L extracellular. These concentrations are critical in maintaining the action potential of myocytes and manipulating them with cardioplegic solutions allows the heart to stop in a controlled manner during surgery. Overall, the physiological basis for cardioplegia was the need to protect the heart from ischemic damage during surgery, to keep the myocardium viable throughout the procedure, and to improve the overall outcomes of cardiac surgery (Ismail and Semien, 2021).

Histological studies revealed that ischemia/reperfusion (I/R) injury can cause damage similar to 24 hours of permanent coronary occlusion in just 30 to 60 minutes. This led to the realization that reperfusion itself contributes significantly to myocardial damage and led to the coining of the term ischemia-reperfusion injury. Although the full extent of I/R injury is beyond the scope of this discussion, 2 relevant elements are calcium overload and the calcium paradox (Ismail and Semien, 2021).

When the first cardioplegic solutions were used, researchers observed cases of abnormal heart failure, persistent fibrillation and widespread necrosis. Calcium overload was identified as a potential cause of this damage. Calcium-free solutions were proposed; however, these also caused similar damage during reperfusion with physiologic

calcium levels, a phenomenon known as the calcium paradox. Several theories have been proposed to explain this, one of which has been widely accepted and demonstrated. Notably, the observed effects of calcium processing problems preceded a full understanding of the underlying mechanisms (Ismail and Semien, 2021).

Cardioplegic solutions, although protective, were found to cause some damage, leading researchers to carefully examine their beneficial and harmful effects. A critical study by Follette et al and Dr. Gerald D Buckberg examined myocardial oxygen consumption in different situations: beating hearts decompressed on cardiopulmonary bypass (CPB), fibrillating hearts and electromechanically stopped hearts were compared to normal hearts with a consumption rate of 10 mL/100 g/min.

The following Table shows the Myocardial Oxygen Consumption of the Heart in Different Situations:

Temperature	**Beating empty heart**	**Fibrillating heart**	**Arrested heart**
37 °C	6 mL/100 g/min	6.5 mL/100 g/min	1 mL/100 g/min
32 °C	5 mL/100 g/min	4 mL/100 g/min	<1 mL/100 g/min
28 °C	4 mL/100 g/min	3 mL/100 g/min	0.5 mL/100 g/min
22 °C	3 mL/100 g/min	2 mL/100 g/min	<0.5 mL/100 g/min

The results of this study revealed that electromechanical arrest is the most effective method of reducing myocardial oxygen consumption, regardless of temperature. A beating heart, especially at normothermia, reduces oxygen consumption more effectively than a decompressed beating heart (Ismail and Semien, 2021).

In his 1981 book, Preservation of the Ischemic Myocardium: Cardioplegia, DJ Hearse detailed the elements of myocardial protection and divided them into 3 main components:

1) electromechanical arrest, 2) hypothermia, and 3) additional protective factors to neutralize the deleterious effects of the cardioplegic solution and hypothermia. These additional protective

agents include magnesium citrate to neutralize calcium overload, tromethamine or bicarbonate to neutralize acidosis, mannitol or albumin to neutralize cellular edema, local anesthetics such as procaine to neutralize membrane instability, and amino acids or glucose to replenish energy substrates and nutrient stores.

As cardioplegia composition and methods of administration have evolved, each iteration has revealed new flaws and led to further innovations. While some techniques have become more popular, such as blood cardioplegia over crystalloid cardioplegia or extracellular over intracellular solutions, none have been completely abandoned. For example, retrograde cardioplegia is preferred in severe aortic insufficiency or in tight proximal coronary stenosis (Ismail and Semien, 2021).

Initially, 2.5% potassium citrate cardioplegic solution was used to stop the heart after decompressing with a heart-lung machine. This solution was infused into the aortic root until complete arrest was achieved, with no fixed amount specified. The safe duration of arrest was limited to 30 minutes, after which re-dosing was mandatory. At the end of the operation, reperfusion was initiated using blood, calcium chloride, adrenaline and neostigmine and supported by occasional cardiac massage. While the heart was constantly stopping and recovering, ventricular fibrillation was almost universal and required defibrillation. Researchers began to notice persistent fibrillation, poor contractility and widespread myocardial necrosis. Various theories emerged to explain these poor outcomes, some attributing them to the citrate solution, others to the method of reperfusion (Hausenloy et al., 2015).

CONCLUSION

Although critical for successful cardiac surgery, myocardial protection strategies are associated with a variety of potential complications that affect patient outcomes. These complications can result from myocardial protection methods and surgical procedures. The main complications are:

- *Myocardial ischemia and reperfusion injury*: Despite effective cardioplegia, myocardial ischemia during reperfusion during and after surgery can cause significant damage. Reperfusion injury is characterized by oxidative stress, inflammation and calcium overload, which can lead to myocardial stunning, fatal reperfusion injury and arrhythmias (Ismail and Semien, 2021).

- *Calcium overload and the calcium paradox*: Cardioplegic solutions, especially those with high potassium concentrations, can cause calcium overload. This occurs when calcium levels rise within the cell, leading to cell damage and dysfunction. The calcium paradox refers to the phenomenon that calcium-free cardioplegic solution followed by reperfusion with normal calcium levels can cause severe myocardial damage (Ismail and Semien, 2021).

- *Electrolyte imbalance*: Cardioplegic solutions should be carefully balanced to avoid electrolyte imbalances. Excess potassium levels can affect myocardial depolarization and repolarization, while imbalances in other electrolytes such as calcium and sodium can disrupt normal cardiac function.

- *Inadequate myocardial protection*: Incomplete or ineffective delivery of cardioplegic solutions may result in areas of the myocardium not being adequately protected. This is particularly difficult in cases of severe coronary artery disease or complex anatomy where retrograde cardioplegia may not adequately cover the right ventricle (Ismail and Semien, 2021).

- *Complications of cooling*: Hypothermia used to reduce myocardial oxygen consumption can cause complications such as coagulopathy, arrhythmias and problems associated with systemic hypothermia. Hypothermia is contraindicated in patients with cryoglobulinemia due to the risk of widespread intravascular clumping (Ismail and Semien, 2021).

- *Cardioplegia solution toxicity*: Prolonged exposure to cardioplegic solutions can be toxic to myocardial cells. If not managed appropriately, the components of these solutions, including preservatives and additives, can have deleterious effects on the myocardium (Ismail and Semien, 2021).

- *Hemodynamic instability during surgery*: The use of cardioplegia and CPB can lead to hemodynamic instability, including hypotension or arrhythmias, which can complicate the surgical procedure and affect patient recovery (Ismail and Semien, 2021).

- *Risk of infection:* The use of cardioplegia increases the risk of infection, especially in a setting with multiple intravenous lines and access points. Appropriate sterile techniques and monitoring are essential to minimize this risk (Hausenloy et al., 2015).

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CHAPTER 4

DIVERSITY AND BIOCHEMICAL CHARACTERISTICS OF ANTHOCYANINS

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INTRODUCTION

Anthocyanins are known as natural pigments produced in plants with their colors ranging from red, purple, and blue and are also known as water-soluble phenolic compounds that provide typical coloration in plants (Saito et al., 2013). These molecules are responsible for the color of many fruits and vegetables (Wu et al., 2023). When anthocyanidins are combined with sugars, they form anthocyanins (Xue et al., 2024). The general structure of anthocyanins consists of a heterocyclic ring (C) that contains oxygen and two phenyl rings (A and B) that are attached. As the number of OH groups in the B ring increases, color change is observed (Figure 1) (Castañeda-Ovando et al., 2009). Anthocyanins are safe and non-toxic flavonoids (Wang et al., 2014). According to previous studies, thousands of plant species contain anthocyanins (Xue et al., 2024). More than 700 anthocyanin molecules have been reported in the plant kingdom (Liu et al., 2018). Pelargonidin, delphinidin, peonidin, malvidin, petunidin and cyanidin are the most common molecules (Table 1 and Figure 2) (Saha et al., 2020). Some edible anthocyanins and their sources are shown in Table 2 (Zhang et al., 2019).

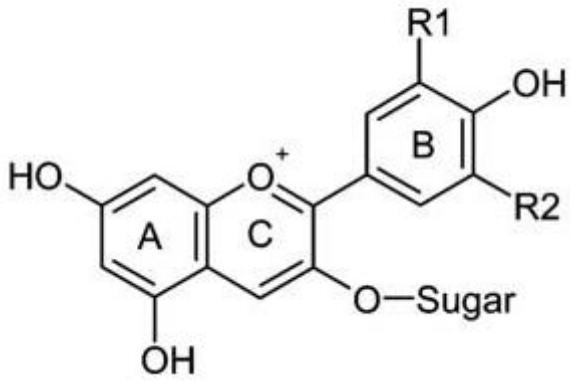

Figure 1. The general structure of anthocyanins (de Sousa Moraes et al., 2019).

Table 1. Major anthocyanidins in nature (Zhao et al., 2017)

Anthocyanin	R1	R2	Distribution in foods %
Cyanidin (Cy)	OH	H	50
Delphinidin (Dp)	OH	OH	12
Pelargonidin (Pg)	H	H	12
Peonidin (Pn)	OCH_3	H	12
Malvidin (Mv)	OCH_3	OCH_3	7
Petunidin (Pt)	OCH_3	OH	7

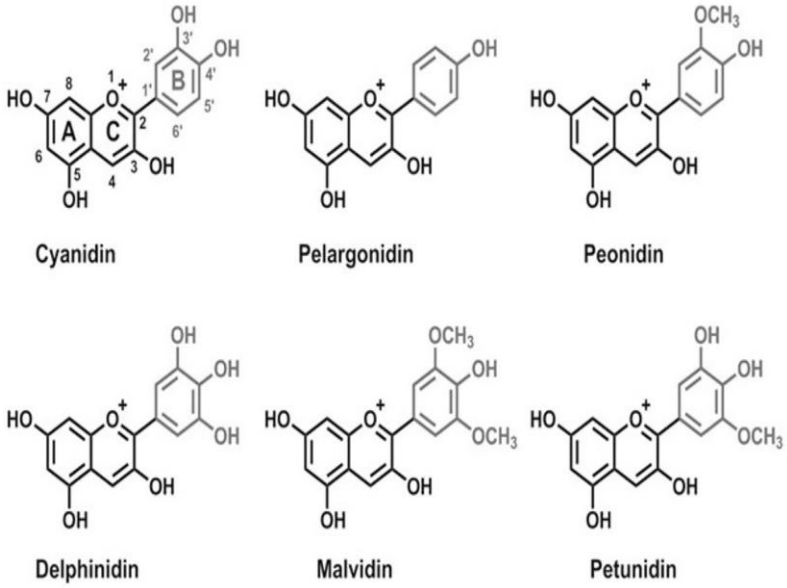

Figure 2. Chemical structure of main anthocyanins (Salehi et al., 2020)

Table 2. Some edible anthocyanins and their sources (Zhang et al., 2019)

Food type	Chemical name of anthocyanin
Apple: (*Malus pumila* L.)	Cy-3-galactoside
Apricot: (*Prunus armeniaca* L.)	Cy-3-rutinoside
Cherry: (*Prunus avium* L.)	Cy-3-glucoside, Cy-3-rutinoside

Food type	**Chemical name of anthocyanin**
Red orange: (*Citrus sinensis* L.)	Cy-3-glucoside
Peach: (*Prunus persica* L.)	Cy-3-glucoside
Strawberry: (*Fragaria* x *ananasa* Duch.)	Pg-3-glucoside, Cy-3-glucoside
Grape: (*Vitis vinifera* L.)	Complex composition consisting of: Cy Dp, Pt, Pn y Mv mono or di-glucosylated, and acylated with p-coumaric acid
Mulberry: (*Morus alba* L.)	Cy-3-glucoside, Cy-3-rutinoside, Cy-3-galactoside, Dp-3-rutinoside, Cy-3-(6″-rhaminosyl) glucoside
Grosella: (*Ribes rubrum* L.)	Cy-3-xylosylrutinoside, Cy-3-glucosylrutinoside, Cy-3-sambubioside, Cy-3-rutinoside, Cy-3-glucoside
Red cabbage: (*Brassica oleracea* L. var. *capitata* . f. *rubra*)	Cy-3-diglucoside-5-glucoside, Cy-3-(pcoumaroyl)-diglucoside-5-glucoside, Cy-3 (sinapoyl)diglucoside-5-glucoside
Ginger: (*Zingiber officinale* Roscoe)	Cy-3-glucoside, Pn-3-rutinoside
Black rice: (*Oryza sativa* L.)	Cy-3-glucoside, Pn-3-glucoside
Purple corn: (*Zea mays* L.)	Cy-3-gluc´osido, Pg-3-gluc´osido, Pn-3-gluc´osido, Cy-3-malonilgluc´osido y Cy-3-dimalonilgluc´osido

Food type	Chemical name of anthocyanin
Blueberry (Vaccinium corymbosum L.) and (Vaccinium angustifolium Ait.)	3-galactoside and 3-arabinoside from Dp, Mv and Pt. 3-galactoside from different anthocyanins and acylated anthocyanins 3-acetylglucoside and 3-acetylgalactoside from Mv.
Purple carrot (Daucus carota L.)	3-(xylosyl)-glucosyl-galactoside, 3-(xylosyl), (sinapoyl)-glucosyl-galactoside, 3-(xylosyl), (feruloyl)-glycosyl-galactoside and 3-(xylosyl) (coumaroyl)-glucosyl-galactoside from Cy.

Anthocyanins can be used as food colorants because they provide a variety of colors (Li and Ahammed, 2023). They can also be used as reagents for the detection of toxic substances (Wardani et al., 2024).

Anthocyanins can be degraded due to the presence of unstable phenolic hydroxyl groups in their structures. Many factors such as light, pH, temperature, oxygen and metal ions can affect the stability of anthocyanins (Eker et al., 2019). Among these effects, pH is the most important. The second factor is temperature. Therefore, temperature is one of the most important parameters in the food industry. Low temperatures are more suitable for maintaining the structural stability of anthocyanins. It is known that these molecules have strong stability in the temperature range of 2–4 °C. Light is very important in the growth process of plants. Light plays an important role in the biosynthesis of anthocyanins in the plant structure (Xue et al., 2024). It has been reported that light quality, light intensity, and light duration can affect anthocyanin biosynthesis and stability in plants. The anthocyanin pathway remains inactive under low light conditions in red-leaf lettuce. When light concentration increases, genes involved in anthocyanin synthesis are also positively affected. For this reason, it was reported that strong light activates the structural and regulatory genes of

anthocyanin (Li and Ahammed., 2023). There are unsaturated bonds in anthocyanin molecular structures. Oxygen can react with these bonds. The degradation process of anthocyanins is accelerated by the oxidative mechanism and the effect of oxidases (Delgado-Vargas and Paredes-Lopez, 2002). Oxygen in high humidity can reduce the stability of anthocyanin molecules by increasing oxidation (Xue et al., 2024). Anthocyanins help plants cope with abiotic stress by scavenging reactive oxygen species and reducing oxidative stress (Li and Ahammed, 2023). The structure of anthocyanins can be deteriorated in the gastrointestinal tract. Therefore, it is important to stabilize these molecules and facilitate their cellular absorption. This problem can be overcome by microencapsulation (Xue et al., 2024). Anthocyanins must be released slowly to fulfill their bioactive roles. Controlled release and appropriate timing are required for this (McGhie and Walton, 2007). It was also reported that the bioavailability of anthocyanins increases with the encapsulation of anthocyanins with microcapsules (Xue et al., 2024). It was reported that encapsulated anthocyanins have higher antioxidant and antibacterial activities than unencapsulated anthocyanins (Ma, 2017). To increase the preservation of anthocyanins, microencapsulation of the Aronia melanocarpa plant was performed and it was found that anthocyanin loss in gastric juice was reduced (Chen et al., 2023). After anthocyanins are taken into the body, they are absorbed by a number of enzymes. It has been reported that they subsequently undergo glucuronide, sulfate and methylation by different enzymes (Agull´o et al., 2020). The majority of anthocyanins reach the large intestine during the digestion and absorption process. These molecules are broken down into smaller molecules such as gallic acid and vanillic acid in the colon. This transformation supports health and may delay aging (Chen et al., 2025). Anthocyanins accelerate the growth of beneficial bacteria and inhibit the growth of harmful bacteria (Molan et al., 2014). Gallic acid, an anthocyanin metabolite, has been reported to reduce oxidative stress levels, reduce memory loss, and improve brain tissue aging in elderly mice. When dried blueberry powder was given to older adults with cognitive impairment, positive improvements in memory were observed. Findings have been reported

that anthocyanins in grape seeds improve cognitive function (Chen et al., 2025). Anthocyanin-rich cherry juice has been reported to improve language fluency and memory abilities in older adults with mild dementia (Kent et al., 2017). Anthocyanins from the plant species Ribes meyeri have been reported to improve memory and reduce neuronal loss in aging mice. Anthocyanins obtained from blueberries and Vaccinium myrtillus have been shown to improve neuronal damage in mice with Alzheimer's disease. Anthocyanins obtained from black currants have been reported to improve memory impairment and emotional abnormalities in mice. It has been reported that when mice with Parkinson's disease were treated with cyanidin-3-O-glucoside anthocyanin, it could reduce intestinal barrier damage by improving intestinal microbiota (Chen et al., 2025). Cardiovascular diseases are often caused by factors such as hypertension, atherosclerosis, and endothelial dysfunction (Donato et al., 2018). Many studies report that dietary intake of anthocyanins can reduce cardiovascular risk factors (Chen et al., 2025). Anthocyanins can prevent atherosclerosis in coronary arteries and reduce myocardial damage by stimulating the production of glutathione, which provides various protective effects to heart cells (Salehi et al., 2020). The Chinese Dietary Reference List reported the positive effects of 50 mg/day anthocyanin intake on health. It was observed that higher anthocyanin intake reduces the risk of coronary artery disease by 25% and hypertension by 10% (Chen et al., 2025). It was also reported that haskap fruit, which is rich in anthocyanins, significantly reduces hypertension and heart rate (Bell and Williams, 2019). When anthocyanins interact with the intestinal microbiota in the colon, it was observed that they reduce cardiovascular diseases by regulating the intestinal flora (Chen et al., 2025). In animals with heart failure, findings show that anthocyanin-loaded corn starch hydrogels can improve histological heart function (Hanafy, 2021). Cell cycle disorders and mitochondrial dysfunction that occur during the aging process can lead to cancer formation. It was reported previously that anthocyanins can significantly inhibit tumor growth in cancer cells and reduce cancer formation. Black raspberry anthocyanins were shown to play important roles in preventing colorectal cancer by

regulating the composition of intestinal microbiota. Numerous studies report that blueberry anthocyanins can prevent tumor growth, colorectal cancer (Chen et al., 2025), and colon cancer (Lavefve et al., 2020) by restoring the balance of intestinal microbiota. Anthocyanins were reported to have effects such as reducing triglycerides, total cholesterol, and insulin resistance in health problems such as diabetes and obesity (Kang, 2023). Blackberry anthocyanin content was shown to inhibit triglyceride and reactive oxygen species significantly (Chen et al., 2025). In a previous study, anthocyanins obtained from grape skins were given to male obese C57BL6/J mice fed a high-fat diet. As a result, it was reported that anthocyanins significantly reduced the concentration of leptin, the appetite hormone, and reduced body mass index (Fan et al., 2019). Another study was conducted on mice fed anthocyanins obtained from 2 g/kg purple corn for 12 weeks and it was reported that anthocyanins reduced obesity, increased brown fat tissue, and reduced white fat tissue, which causes obesity (Granados-Balbuena et al., 2024). In a large-scale study conducted between 1986 and 2011, the effect of anthocyanin intake on obesity was examined and it was reported that flavonoid intake, including anthocyanins, reduces weight gain over time (Bertoia et al., 2016). The harms of synthetic color pigments are quite serious. In this context, it has been reported that the use of anthocyanins comes to the fore because they are safe and not harmful (Xue et al., 2024). For this reason, anthocyanins are used widely in the food, cosmetics and pharmaceutical fields (Alappat and Alappat, 2020). Anthocyanins also provide significant advantages for solid-state colorimetric analytical sensors with their natural abundance, non-toxicity, low-hazard waste production, affordable price and environmental friendliness. Renal failure is detected by analyzing the level of urea released from the kidneys into the blood and urine. In a previous study, a colorimetric sensor containing anthocyanins was successfully used to diagnose urea levels in real time (Wardani et al., 2024). Anthocyanins have functions such as preventing UV damage, improving biological stresses and participating in physiological processes (Gamage et al., 2022). Anthocyanins have antidiabetic, antioxidant, anti-inflammation, antiaging, lipid-lowering, anticancer

and neuroprotective effects (Escalante-Aburto et al., 2023). Anthocyanin consumption was shown to reduce inflammatory markers in the colon caused by a high-fat diet in obese mice (Neyrinck et al., 2013) and that it shows neuroprotective effects by preventing neuronal cell death (Primatanti and Jawi, 2019). In a study conducted on rats, it was reported that the administration of active anthocyanin to the brain suppressed oxidative stress and changes in dopamine in the brain (Ghosh et al., 2007). Anthocyanins were shown to regulate many signaling pathways (Scarano et al., 2018). It was reported that cyanidin can reduce inflammation by inhibiting the signaling of the proinflammatory cytokine interleukin-17A (Liu et al., 2017). It was mixed with anthocyanin-rich Malva parviflora leaves with different concentrations of yogurt to examine its inhibitory impact on acetic acid-induced ulcerative colitis in rats and it was found that free radicals could be scavenged and antioxidant defense enzymes could be improved (El-Naggar et al., 2020). Hydrogelated anthocyanins were found to have better stability than non-hydrogelated ones (Guo et al., 2018). The success of the anthocyanin-loaded hydrogel cut from blueberries in wound healing is credited with accelerating regeneration (Zhang et al., 2020). The cyanidin-3-O-glucoside molecule has the functions of reducing endoplasmic reticulum stress and regulating sphingolipid signaling (Chen et al., 2025). Anthocyanins and their metabolites strengthen the intestinal barrier function and provide resistance to pathogen invasion (Jaskiw et al., 2019). The absorption rate of anthocyanins is related to their chemical structure, molecular weight, and dietary components. Studies show that the absorption rate of anthocyanins decreases in high-sugar diets. The small intestine is the main site of absorption of anthocyanins, showing absorption by passive diffusion or active transport, and the absorption rate is about 5%. Approximately 90-95% of anthocyanins reach the colon, where they are degraded by intestinal microbiota (Chen et al., 2025). Anthocyanins might have effects on the protection of the circulatory and nervous systems. It has also positive effects on the oral-intestinal microbiota (Salehi et al., 2020). Many studies report various benefits of enriching foods with anthocyanins (Naibaho et al., 2022). A previous study

reported that purple tomatoes have a high antioxidant content. It was reported that anthocyanins in tomatoes extend the life span of mice (Butelli et al., 2008). Toxic heavy metal ions are released into the natural environment because of intensive industrialization and other environmental factors and cause serious heavy metal pollution in soils. Heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni), mercury (Hg), cobalt (Co), and arsenic (As) not only pollute the environment, but also pose a threat to all life forms in the environment, including humans. It was reported that anthocyanins might alleviate stress or toxicity caused by heavy metals (Li and Ahammed, 2023). As a result, anthocyanins have positive effects such as antidiabetic, anti-inflammatory, antimicrobial, anticancer, antiobesity, and cardiovascular diseases (Algarra et al., 2014; Mizgier et al., 2016; Khoo et al., 2017). We think that we can be healthier by using these biomolecules in our daily lives.

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CHAPTER 5

EPIGENETIC MECHANISMS IN PLANTS

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INTRODUCTION

Researchers conducted from past to present have revealed that there are many facts in plants that can be explained by mechanisms beyond genetics, where known genetic analyses are insufficient. It has been proposed, particularly in the last 20 years, that epigenetics is a major factor in the majority of molecular controls in plants. Epigenomic hereditary changes resulting from chemical modifications to the DNA sequence, modifications to histone and non-histone proteins, modifications to chromatin structure, or a combination of both, are becoming more significant nowadays with the development of new methodologies and technological advancements, in addition to genetic inheritance.

Although epigenetics is known to have been introduced as the concept of epigenesis in ancient Greek times by philosophers such as Aristotle, Democritus and Leucippus to explain the development of a new living being, its emergence as a field of science covers a short period of time, approximately the last 20 years (Tsaftaris et al., 2008). Epigenetics, which has been used by different scientists with different meanings, is defined by molecular biologists as hereditary changes in gene function without DNA sequence change (Akhter et al., 2021). Today, the term epigenetics is defined as the study of inherited variations in gene expression that are not caused by changes in the DNA sequence. The main target molecules of epigenetics are DNA, RNA and chromatin (nucleosomes) (Karaca et al., 2019). Inherited features other than the sequence in these molecules are of interest to epigenetics. The use of episome, epigenomics, epigenomics, epiallel, EpiRIL (epigenetic recombinant inbred lines) concepts together with epigenetics has become widespread in recent years. Epialleles refer to alleles that, without sequence difference, constitute regular and heritable phenotypic or physiological variation arising from the spatial location of DNA, RNA, chromatin (histone) and nucleus. The presence of epialleles is the main difference that distinguishes epigenetics from modifications (Sudan et al., 2018). Epigenetics has made a great impact among scientists working in clinical medicine, pharmacy, forensic medicine, plant and animal production (agriculture) and basic sciences.

The main reason for this is that epigenetics has demonstrated that all physiological and phenotypic events in the cell are not simply encoded in the DNA sequence, as stated in the main rule of molecular biology. The concepts of epigenetics and epialleles explain how cells with exactly the same DNA sequence can have independent functions in different organs of the organism, how, for example, lymphocytes in humans can stably maintain their phenotype through cell division, how it is determined which of the XX chromosomes with almost identical DNA sequences in the same nucleoplasm is inactivated, It emerged after modern molecular biological approaches and techniques failed to unravel phenomena such as how identical twins with the same genetic material can have different susceptibilities to diseases, how two cells with the same genetic material can develop differently from each other, and why the leaves of a plant can be different from each other (Skinner et al., 2010).

In the living world, since plants cannot change the environment in which they exist in nature and have to cope with the variable and often unfavorable climatic conditions in which they exist, it is thought that thanks to their epigenome and epigenetic mechanisms, plants can make significant changes in gene activity and successfully survive in unpredictable environments and continue their generations by inheriting these epigenetic properties (Hemenway and Gehring, 2023). Epigenetic variation and regulation is now known to be the result of enzyme-assisted chemical modifications of DNA and chromatin structure. This suggests that understanding the control (e.g. inhibition) of epigenetic mechanisms can play an important role in diagnosing and treating epigenetic regulation in medicine, improving yield and quality in agriculture, and unraveling cellular phenomena in basic science. In this review, we aim to introduce epigenetic mechanisms that play a key role in gene expression in plants and to provide information on the application areas of epigenetics in agricultural production (Bellard et al., 2012).

Molecular Epigenetic Mechanisms

In the functioning of the epigenetic mechanism, also called epigenesis, a new phenotype or cellular physiological event occurs in the nucleus or organelle genomes as a result of the generation of an environmental and/or internal signal, the perception and response of this signal by the cell or tissue, chemical modifications on the DNA sequence, especially cytosine, modifications in histone and non-histone proteins and changes in chromatin structure or both (DNA sequence modification and histone modifications) (Akimoto et al., 2007; Hegarty et al., 2011). Some epigenetic variations differ between organisms. The functioning of the epigenetic mechanism, which is more effective in eukaryotes than in prokaryotes, also differs in the plant and animal kingdom (Li et al., 2013). In eukaryotes, epigenetic mechanisms generally include adenine and cytosine methylation modifications at the DNA level, adenine deamination and other RNA editing events at the RNA level, and acetylation and deacetylation of histone and non-histone proteins in chromatin structure, SUMOylation, ubiquitination, ADP-ribosylation, ADP-ribosylation, proline isomerization and deiminization, glycosylation or citrullination, either alone or in combination, changes the spatial position of DNA in nucleus and reorganize chromatin (Abdulraheem et al., 2024) (Figure 1).

DNA-Based Control Mechanism: "DNA Methylation"

Methylation is known to be a major source of epigenetic variation in eukaryotic organisms. DNA methylation levels that are too high (hypermethylation) or too low (hypomethylation) affect many genetic functions, including DNA replication and repair, gene transposition and transcription, cell differentiation and gene silencing, imprinting, biodefense, transgene expression, and foreign gene expression in the cell (Zhang et al., 2018). In research, cytosine methylation is used in many organisms because of its effect on embryonic and prenatal development, cancer, bacterial host defense, transgene silencing, hormone regulation, biotic and abiotic stress, genome folding and speciation, heterosis and imprinting (Law and Jacobsen, 2010).

In chemistry, methylation is the attachment or transfer of a methyl (CH3) group to a chemical compound. In biochemistry, it specifically refers to the replacement of a hydrogen atom with a methyl group. Methylation is one of the few known covalent modifications on the DNA molecule and occurs specifically at carbon 5 of Cytosine. Methylation causes the formation of primidindimers and is also involved in repression of transcription, restriction of transposon movement, genomic imprinting, X chromosome inactivation, tissue and organ specific gene expression.

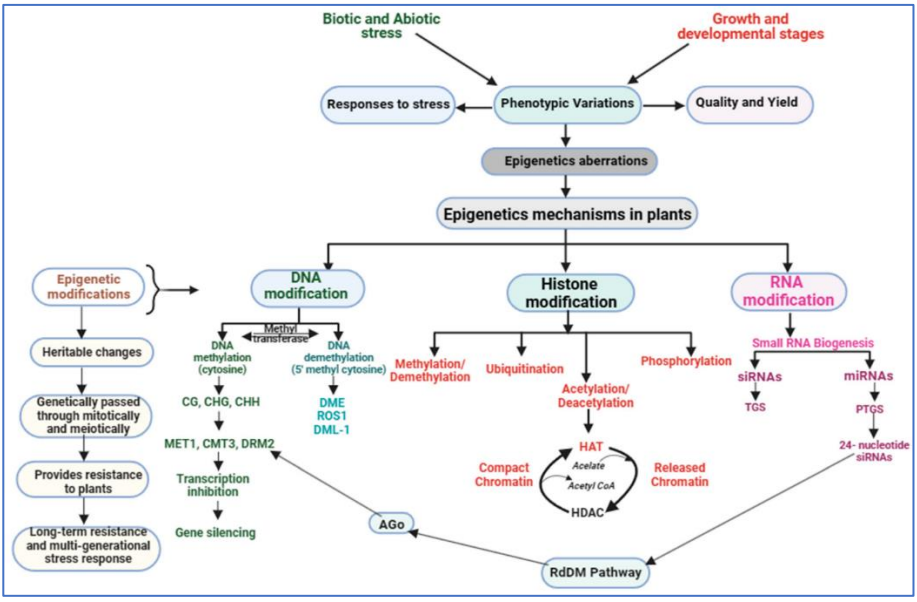

Figure 1. Plant epigenetic mechanisms (Abdulraheem et al., 2024)

DNA methylation in living genomes is carried out by different types and classes of DNA methyltransferases that use the S-adenosylmethionine (SAM) molecule as a substrate and can convert the adenine or cytosine nucleotide to 6-methyladenine (m^6A), 4-methylcytosine (m^4C) and 5-methylcytosine (m^5C). DNMT1, DNMT3a and DNMT3b, as well as DNMT2 and DNMT3L, are known to be involved in the establishment and maintenance of the DNA methylation pattern. Adenine nucleotide methylation is first reported to occur at nitrogen six of the first adenine in the TGATCA sequence in plants.

There are studies indicating that this type of methylation is more common in mRNA and other RNA types. However, the number of studies on the epigenetic effects of adenine methylation in DNA is limited. Methyl group addition (transfer) occurs to C-N in m^6A and m^4C and to C-C in m^5C. Nuclear DNA (nDNA) methylation is a specific feature of plant genomes and in some cases can be species, tissue, organ, organelle and developmental stage specific. In plants, nucleotide methylation is known to be prevalent in the conversion of cytosine to 5-methylcytosine (m^5C) and adenine to N6-methyladenine (m^6A). Methylation is carried out by a large number of different methyltransferase enzymes, sometimes referred to as epigenetic enzymes. In plant genomes, 5-methylcytosine (m^5C) is referred to as the fifth base besides A, T, G and C due to the high frequency of methylation, especially at the cytosine base. Methylation is more efficient in CG rich sequences. These CG-rich regions are called CG domains. CG enrichment is usually seen in the transcription initiation sites of housekeeping genes involved in cell function or in the promoters of these sites (60%), but less in differentiated tissues and different methylation patterns are seen in tumor cells. Today, the terms CHH and CHG particles are used more frequently along with CG particles (Kravets et al., 2010).

Demethylation is as important as cytosine methylation. Demethylation of the methyl group on cytosine is carried out by translocation enzymes of the Ten-eleven translocation family (TET). Demethylation can be active or passive or both. Passive DNA demethylation is usually carried out during replication by the DNMT1 enzyme on the newly synthesized DNA helix, while active DNA demethylation is carried out by the TET enzyme after oxidation with removal of 5-meC. The eleven-eleven translocation family consists of the TET1, TET2 and TET3 proteins, which are usually active at CHG sites and convert 5-meC first to 5-HmC and then to 5-formyl cytosine (5-fC) and 5-carboxycytosine (5-caC) through their hydrolyzase activity. 5-fC can be directly converted to cytosine by base cut repair (BER) with Thymine DNA glycolase (TDG) (Chen et al., 2010).

Histone Modification

Histones are the major protein components of chromatin and, in eukaryotes, each nucleosome structure is composed of a main histone octamer consisting of Histone 2A (H2A), Histone 2B (H2B), Histone 3 and Histone 4 (H4), each containing two copies, and a 147 bp long DNA surrounding it. When viewed with an electron microscope, the nucleosome structures are arranged side by side like "beads on a string", where the "bead" represents the nucleosomes and the "thread" represents the DNA (Mahrez et al., 2016). Each nucleosome structure is linked to each other in packaging by binding proteins called histones. Histone proteins are not simply "DNA packaging" proteins, but also regulators of chromatin dynamics. Modifications of the N-terminal tails of histones are known to alter chromatin function and play important roles in many biological activities, including gene regulation, DNA repair and chromosome condensation. Post-transcriptional changes are made to histones to change how they interact with nuclear proteins and DNA. The following modifications are observed in the tails of histones: *i)* Acetylation (which is associated with transcription activation, telomere repression, and DNA repair); *ii)* Methylation (association with transcription repression and gene regulation); *iii)* Phosphorylation (association with DNA repair and mitosis); *iv)* Ubiquitylation (in transcription activation); *v)* SUMOylation (association with transcription repression and gene regulation); *vi)* ADP-Ribosylation (DNA repair and cellular signaling); *vii)* Isomerization (Transcription); *viii)* Deimination (Citrullination); and *ix)* Glycosylation are all significant; these modifications are reversible and mediated by a particular enzyme (Okada et al., 2005) (Figure 2). Acetylation and deacetylation act on basic histone proteins. Histone Acetyl Transferase (HAT), a multi-unit protein complex, reduces the positive charge of histones by adding an acetyl group (negative charge) to the amino acid lysine in histones, thereby reducing DNA-histone binding (affinity). Acetylated chromatin reduces (unwinds) from a 30 nm fiber structure to a 10 nm fiber structure and opens the promoter to transcription factor proteins. Deacetylation works in the opposite direction. Histone deacetylase removes the acetyl group and the 10 nm fiber structure

becomes a 30 nm fiber structure, deacetylation then represses transcription (Berger, 2007).

In histone phosphorylation, H1 phosphorylation distinguishes H1 from other histones and leads to transcription. In histone ubiquitination, ubiquitin proteins (Ub), which are usually 76 amino acids long and 8.6 kDa in size, are less abundant in condensed chromatin (heterochromatin) and more abundant in less condensed chromatin (euchromatin). ADP-ribosylation is the addition of one or more ADP-ribose moieties to a protein. It is a reversible post-translational modification involved in many cellular processes, including cell signaling, DNA repair, gene regulation and apoptosis. Histones are conserved eukaryotic proteins. Therefore, the proteins that make up nucleosomes are largely similar in all eukaryotes. However, several histone variants can be found in eukaryotic cells. These structures can substitute for standard histones to form alternative nucleosomes. These nucleosomes form specific regions on chromosomes or provide specific functions to nucleosomes. Many histones are synthesized in the S phase of the cell cycle and enter nucleosomes in the synthesized DNA helices immediately downstream of the replication fork. In contrast, many histone variants are synthesized during interphase. For example, H2Az is a variant of H2A distributed in all eukaryotic cells and is associated with transcribed regions of DNA (Sui et al., 2012). The H2Az histone variant prevents the formation of a repressive chromatin structure of the nucleosome, shaping a chromatin structure more favorable to transcription and more readily accessible.

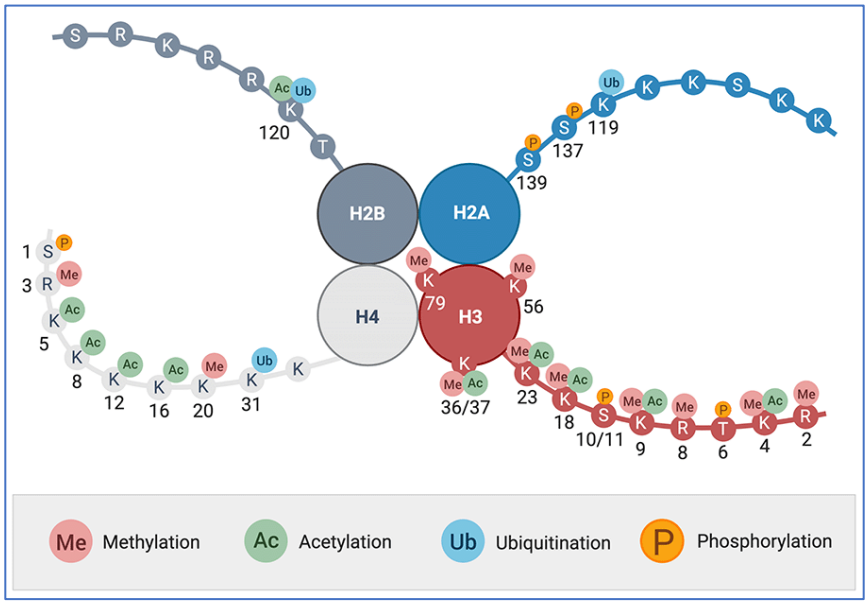

Figure 2. Post-translational modifications of histones H2A, H2B, H3 and H4 (Agarwal et al., 2020)

Chromatin modifications

It is now known that gene expression is affected by the position of a gene on a chromosome, the position of the chromosome in the nucleus, the position of the nucleus in the cell, the position of the cell in the tissue, the position of the tissue in the organ and the position of the organ in the organism (Gong et al., 2009). Eukaryotic genomes are spatially organized in the nucleus through chromosome folding, interchromosomal contacts and interaction with nuclear structures, leading to the idea that the organization of the genome inside the nucleus has been shaped and maintained by evolutionary processes and probably serves an adaptive function (Li, 2002). Both DNA-dependent proteins and changes in chromatin structure affect the positioning of genes and larger domains within the nucleus. The spatial positioning of individual genes relative to stable nuclear cues can be controlled by cis-acting DNA elements that recruit sequence-specific DNA-dependent proteins. This suggests that the space organization of the genome may be genetically encoded by binding sites for DNA-binding proteins and may also involve changes in chromatin structure, potentially through

non-genetic mechanisms (Eichten et al., 2014). The location of chromosomes within the nucleus also correlates with cell type; gene-rich chromosomes that are copied at a higher rate tend to be located towards the center of the nucleus, while gene-poor chromosomes tend to be located towards the nuclear periphery.

Chromatin modifications are often associated with spatial position and in some cases are required for normal spatial organization of the genome. Since transcription factors and chromatin exchanges influence each other and have indirect effects, it is yet to be determined whether, in most cases, one of these mechanisms alone is sufficient for spatial positioning. Furthermore, the function of transcription factors can be regulated by post-translational modifications such as acetylation and SUMOylation. Binding sites for sequence-specific DNA-dependent proteins such as transcription factors are known to function as genetic information influencing chromosome folding and spatial positioning, and non-genetic chromatin modifications such as histone methylation and variable histones also play important roles in controlling the positioning of individual genes and chromosomal domains within the nucleus (Bricker, 2017).

Genetic and Epigenetic Variation

It is a unifying theme in biology that the visible characteristics of organisms are controlled by the sequence of nucleotides found in their genome. Another cornerstone of modern biology is the random shuffling of hereditary information on chromosomes without environmental influences, especially with phenotypic consequences (Quintana-Murci, 2016). In current biological thinking, these elements are being tested by studies in the field of epigenetics. The association of RNAi with chromatin and DNA methylation-based mechanisms mediates a semi-independent epigenetic inheritance system between genetic control and the environment. In plants, epigenetic status can be inherited through the transmission of epialleles across generations. These epigenetic alleles can be considered as new sources of polymorphism and new phenotypes can be generated. Heritable phenotypic variation in populations is fundamental for selection and

breeding. In particular, the importance of methylated epialleles in plant breeding can be determined by *i)* detecting variation in methylation pattern among individuals within selected populations, *ii)* the degree of methylation pattern affecting phenotype, *iii)* assessing the heritability of methylation variants associated with superior phenotypes (Underwood et al., 2017).

Improving Plant Stress Tolerance

It is known that environmental biotic and abiotic stress conditions cause epigenetic changes in plants during growth. The accumulation of oxygen radicals that can damage macromolecules under stress conditions such as drought, salinity, high light, and heavy metals is a known mechanism (Yaish, 2013). Various studies have shown that under stress conditions, enzymes such as DNA methyltransferase and DNA glycosylase are affected, resulting in decreased or increased DNA methylation, resulting in different results depending on the stress conditions (Figure 3). Additionally, modifications like histone methylation and acetylation are decreased, enzymes like histone methyltransferase, histone acetylase, and histone deacetylase are altered, and stress circumstances generate alterations like early or late blooming and absence of fertilization. The identification of miR389 as a CSD1 and CSD2 superoxidase dismutase suppressor in an Arobidopsis investigation demonstrated the regulatory function of microRNAs (miRNAs) in the stress response of defense genes. Under oxidative stress circumstances, miR389 expression is decreased. This downregulation under oxidative stress conditions has been shown to be important for the posttranscriptional induction of CSD1 and CSD2 expression. This has shown that resistance can be improved under oxidative stress conditions via miRNA (Kumar et al., 2018; Samantara et al., 2021).

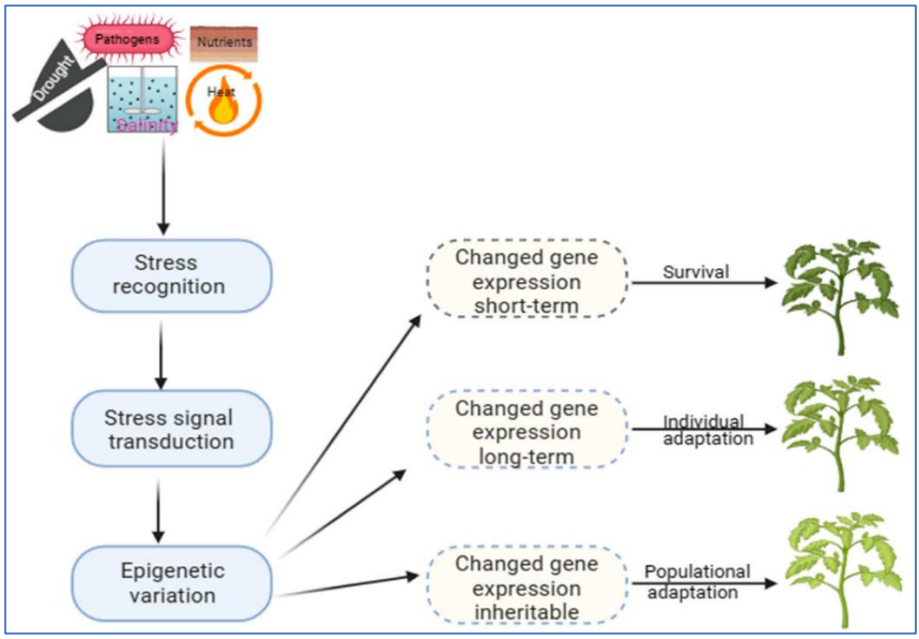

Figure 3. Epigenetic processes and mechanisms of plant adaptation to stress (Abdulraheem et al., 2024)

In addition, repair studies conducted on damaged regions in the plant genome can cause genetic and epigenetic changes. Studies have shown that epigenetic regulation is important in determining cell fate in the root epidermis. The results obtained regarding the germination of Arabidopsis seedlings after treatment with Trichostatin A (TSA), a histone deacetylase (HDACs) inhibitor, promoted hair cell formation. It was determined that hyperacetylation of H3 and H4 core histones in CAPRICE, GLABRA2 and WER was increased with TSA application (Ali et al., 2022). This is evidence that an environmental effect causes epigenetic changes. A better understanding of these mechanisms will help breeders in finding a more effective way for resistance to stress (Figure 4).

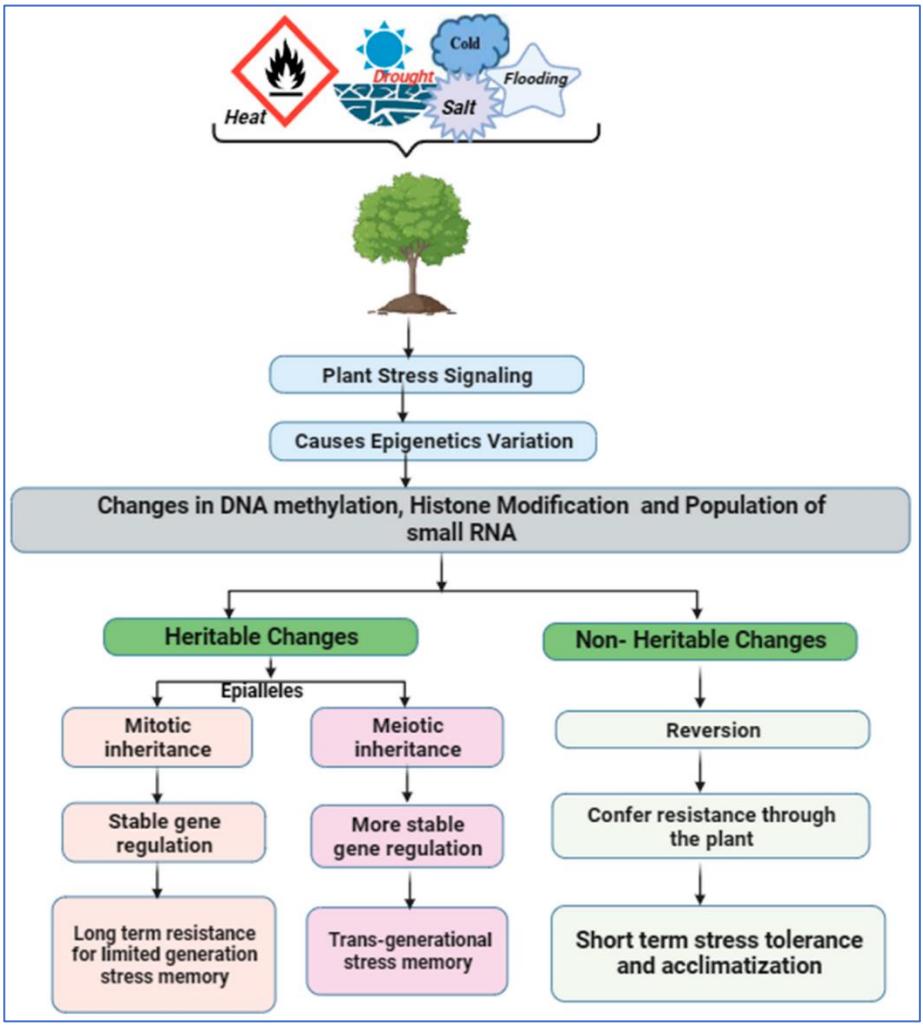

Figure 4. Plant Stress Tolerance and Epigenetic Variation Responses (Abdulraheem et al., 2024)

Genomic Imprinting

Epigenetics is the subject of epigenetics, which is the change in gene expression that is not caused by changes in the DNA sequence, but is genetic. Imprinted genes are epigenic genes. Imprinted genes: are expressed monoallelically. This comes from either the maternal or paternal genome. In imprinted gene imprinting, the gene is not transcribed because of the region it is located in (Bartolomei et al., 2020; Batista and Köhler, 2020). This is because that region is

specifically repressed by repressors. These genes located in heterochromatin regions are imprinting genes. For example, telomeres and centromere regions are heterochromatin regions, if genes are in these regions, their transcription is suppressed. The histone deacetylase enzyme complex can generally suppress a gene by promoting heterochromatin structure. The spreading of the heterochromatin region can be stopped by methylating histone proteins, such as the tail of the H3 protein, with histone methyltransferase enzyme (Hauser et al., 2011). In another example, due to parental genomic imprinting in the endosperm, the regulation of methyltransferase 1 enzyme activity between female and male gametes and the activity of the demethylation enzyme demethylase 1 enzyme can switch to an asymmetrical order, and this situation can cause a global imbalance in DNA methylation of inherited maternal and paternal alleles in the endosperm (Jullien and Berger, 2010).

RNAi Technology

RNAi technology has been widely used as a tool for the analysis of gene function. The most common way to achieve success in RNAi is to use a dsRNA and a transgene producing hairpin RNA (hp RNA). In plants, the double-stranded RNA method has been shown in the past to be a good method for silencing the trigger gene (Sioud, 2021). However, the RNAi method induced by hairpin RNA is much more effective than the classical gene silencing method, antisense RNA. Constructs carrying tissue-specific promoters have been successfully used for silencing guide RNA. For example, seed-specific genes have been effectively silenced using napin and lectin promoters. RNAi technology has been used as a tool for crop development. For example, zein proteins, which are the primary proteins of corn endosperm and are divided into four classes as a, b, g, and d, are proteins that cause low lysine content in the seed. A mutant obtained with the RNAi technique produced corn seeds rich in lysine content (Seroussi et al., 2022).

Epigenetic Control of Vernalization and Flowering Time

Vegetative and generative development in angiosperms is under the control of many internal and external factors. Flowering is promoted by expression of the FT gene, a flowering activator gene. Another gene, FLC protein (flowering locus C), represses the expression of the FT gene. In other words, when FLC proteins are abundant, plants do not flower. In the cold state, the genes encoding the FLC protein are silenced. When the environment warms up again, FLC expression is not expressed unchanged. This situation is generally called vernalization. In the vernalization process, FLC being active causes the plant to remain in a vegetative state. It causes FLC to switch from the vegetative period before cold weather, for example in autumn, to the generative period after cold weather. The FLC gene is epigenetically silenced after vernalization (Heo et al., 2013).

Vernalization is under epigenetic control. Vernalization and flowering in plants occur by readjusting the epegonum. Flowering locus C (FLC) encodes MADS box transcription factors. FLC suppresses FT, one of the main effect genes in flowering. Vernalization silences the FLC gene. Silence of the FLC gene occurs as vernalization. Chromatin modification and histone variants gain importance in silencing the FLC gene. Silencing occurs with acetylation in the promoter and intron H3 histone of the FLC gene before vernalization, and with H3K27me and H3K9me methylations after vernalization. H4 acetylation, methylation on Lys-4 and-36 on H3 and also the addition of the H2A.Z variant are important in the activation of the FLC gene. Studies have shown that the expression of the vernalization insensitive 3 (VERNALIZATION INSENSITIVE3) gene, which is part of the VRN2 PRC2 complex, increases with the extension of cold application (Figure 5). The VRN2 PRC2 complex and other histone modification systems regulate the histones of the FLC gene. H3K4 methylation and H4 acetylation, H3K9me and H3K27me3 modifications occur in the FLC gene. LHP1 (ORC1-like) combines with FLC and plays a role in silencing during cold application. Thus, the FT gene is expressed and flowering is initiated (Tamada et al., 2009; Heo and Sung, 2011). In plants, there are high levels of epigenetic regulation in the endosperm

and pollen vegetative nuclei. The transformation of heterochromatin to euchromatin in endosperm nuclei is seen as an epigenetic mark. Transposons in pollen vegetative nuclei reduce methylation levels. Epigenetic regulation in plants is regulated during the reproductive period and RNA-supported DNA methylation pathways are used. In non-generative cells, siRNA is predicted to be effective in regulation.

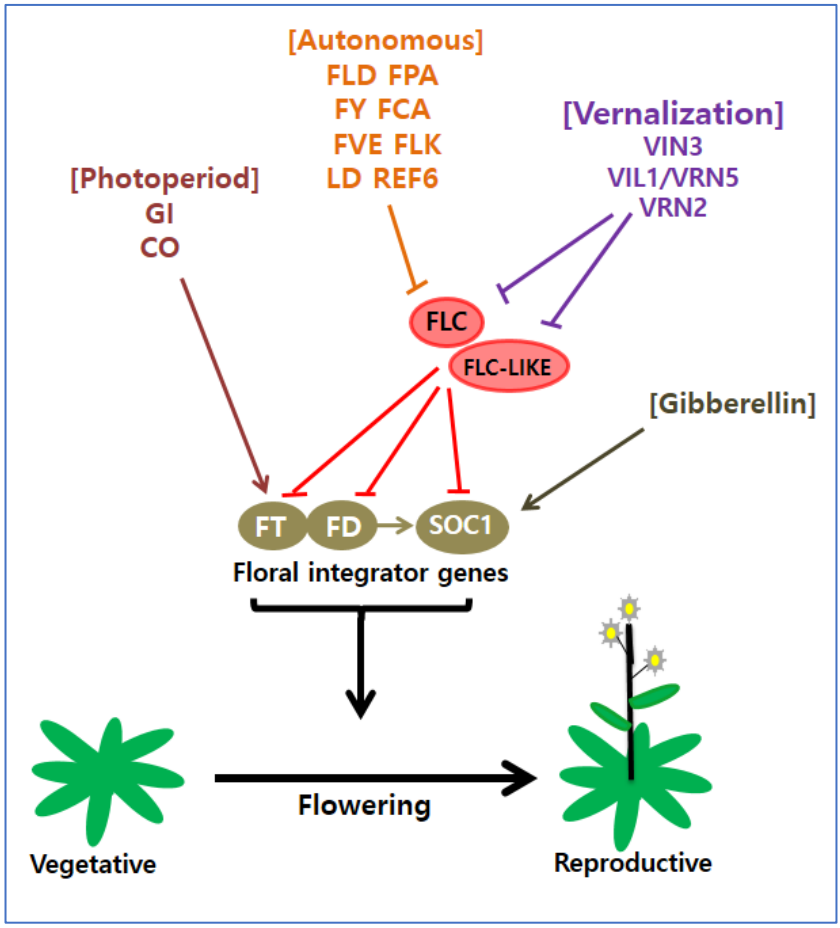

Figure 5. Vernalization requires epigenetic silencing of *FLC* (Kim and Sung, 2014)

Results

Plant physiological features are known to be influenced by both environmental changes and genome reorganization, an idea that is now strongly supported by experimental evidence. Plant breeding can be

guided by the efficient utilization of both genetic and epigenomic sources of diversity. Plant breeding has mostly relied on selective breeding during the majority of the last century, utilizing both artificial and natural selection. But the genomic revolution has brought forth a number of significant breakthroughs that have expanded the possibilities for breeders, including sequence information and sequence comparison, the identification of single nucleotide differences and the comprehension of the significance of nucleotide changes, transcription factors and other regulatory elements, and regulatory genes. These days, breeders will be able to use epigenomic markers just as effectively as genomic markers in plant breeding studies, given that chromatin modification can occur in any DNA-protein binding site and result in the formation of a new target molecule that may carry hereditary traits.

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CHAPTER 6

CHEMICAL STRUCTURE AND BIOCHEMICAL CHARACTERISTICS OF FLAVANONES

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INTRODUCTION

It is known that flavonoid molecules are plant-derived polyphenols (Jain et al., 2011; Kumar and Pandey, 2013; Panche et al., 2016) found as aglycones, glycosides, or methylated derivatives. They give different color tones to the flowers, fruits, and leaves of plants (Di Carlo et al., 1999; Jain et al., 2014). Flavonoid family (i.e., flavones, flavonols, flavanonols, flavanones, anthocyanins, and isoflavonoids) are generally the most researched natural biological molecules (Figure 1). It is already known that these molecules regulate the physiopathological processes related to infection, inflammation, cancer, oxidative stress, and metabolic and neurological diseases in metabolism (Chen et al., 2023).

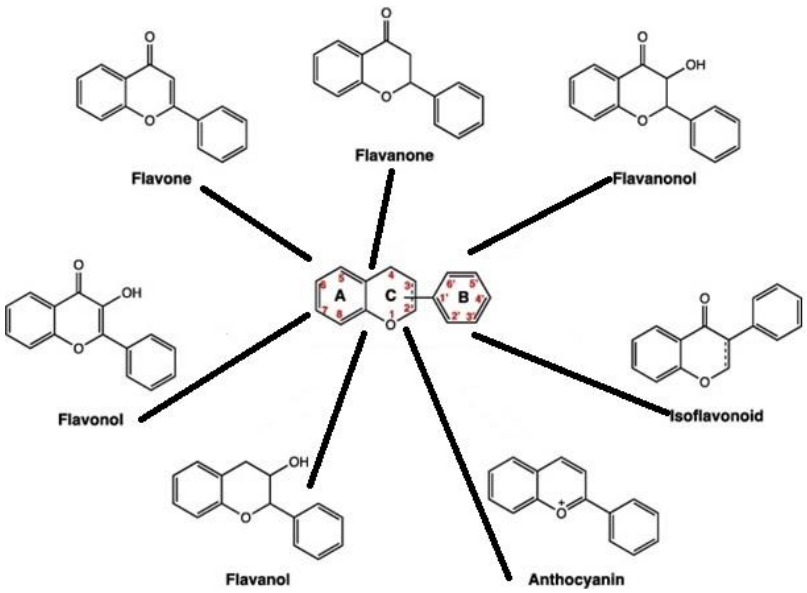


Figure 1. The general skeleton of natural bioflavonoids and their most important subgroups (La Monica et al., 2024).

Flavanone molecules are found in many citrus fruits (Di Majo et al., 2005). When Figure 1 is examined, it is seen that the C-ring of flavanones does not contain a double bond, and these molecules are also known as dihydroflavones **(**Schmidt, 1999). The biosynthesis of flavanones involves the conversion of chalcone to flavanones as a

reaction mediated by chalcone isomerase (Sherman et al., 2023). The physiological and pharmacological impacts of flavanones are shown to have positive health impacts (Barreca et al., 2017).

The most widely known Flavanones are the molecules naringin, naringenin, hesperetin, and hesperidin (Krysa et al., 2022).

NARINGIN

Naringin is also known as the flavanone responsible for the bitter taste of grapefruit (Madrigal-Santill´an et al., 2014). (Figure 2) Naringin is broken down by the enzymes naringenase and -l-rhamnosidase to obtain prunin and rhamnose. Prunin is decomposed into naringenin by the enzyme β-D-glucosidase, and the resulting naringin is hydrolyzed by naringinase and absorbed in the digestive system (Kanaze et al., 2007).

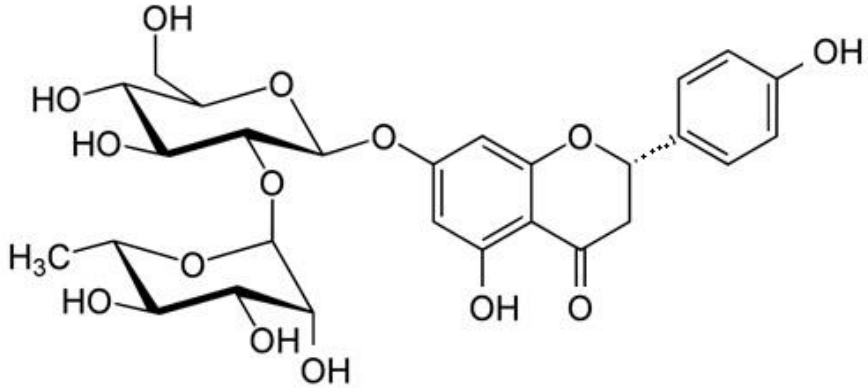


Figure 2. Chemical structure of Naringin (Anonymous, 2024a).

Inflammation can occur as a result of diseases in metabolism (Müller, 2018). Naringin can prevent inflammation as a result of its antioxidant effect. It also plays a role in preventing the loss of cellular homeostasis and TNF-α-induced inflammation (Chen et al., 2022).

It was reported that naringin increases GSH-Px and SOD activities while significantly decreasing TNF-α, IL-1β, hydroxyproline content, and MDA levels in rats with bleomycin (BLM)-induced acute lung inflammation and fibrosis. In light of this, naringin appears to

have roles in reducing inflammatory cytokine levels and preventing the formation of oxygen-free radicals (Turgut et al., 2016). Naringin increases IL-17 levels. It also shows anti-inflammatory and apoptotic activity by reducing hepatic p53 and caspase-3 expression (Hassan et al., 2021). It also has characteristics that protect cells from free radical damage (Jagetia et al., 2004). It was reported in many previous studies that the naringin gene is converted into human urine and plasma after oral intake of grapefruit juice or pure naringin (Ishii et al., 1997). In a previous study, a decrease was detected in plasma total cholesterol and Low-Density Lipoprotein (LDL) levels with the intake of 400 mg capsule naringin per day for 8 weeks. It was also reported that it increases the antioxidant defense of the body as it causes an increase in the activity of SOD and CAT, which are known as antioxidant enzymes (Jung et al., 2003). It was also reported that cognitive decline was improved in rats after 16 weeks of 100-200 mg/kg naringin injection into streptozotocin-induced diabetes (Maity et al., 2017). Naringin was detected in trachea, lung and plasma after a single oral administration to rats (Najmanov et al., 2020; Zeng et al., 2020). Naringin also decreases oxidative stress, inflammation, and apoptosis and was reported to reduce the toxic impacts of clinical drugs (Peng et al., 2024). It also improves memory deficits and cognitive dysfunction by exhibiting neuroprotective impacts (Goyal et al., 2022). Naringin inhibits the TLR4/NF-κB signaling pathway in rats and improves cognitive dysfunction and histopathological damage in rats (Dai et al., 2023).Naringin, which has important therapeutic effects in anxiety and depression, also has antidepressant and anxiolytic impacts (Hern´andez-V´azquez et al., 2022). Cardiovascular diseases are known as heart or vascular diseases. There are many factors that cause cardiovascular diseases (Libby et al., 2019; Ma et al., 2022). It was reported in previous studies that naringin flavanone helps lipolysis, decreases cholesterol and triglyceride, regulates fatty acid β-oxidation, and has important roles in preventing atherosclerosis progression (Pengnet et al., 2019; George et al., 2021). In a previous study investigating the effects of naringin on hypertensive patients, it was found that although there was a significant decrease in systolic blood

pressure, diastolic blood pressure was reduced more effectively in patients receiving high doses of naringin (Reshef et al., 2005). It was also found that naringin inhibits inflammation and can significantly improve intestinal damage caused by ulcerative colitis and sepsis by regulating the homeostasis of intestinal flora (Fid´elix et al., 2020).

NARINGENIN

The molecular formula of naringenin is $C_{15}H_{12}O_5$ (Joshi et al., 2018) and has a simple flavonoid skeleton that consists of 3 rings (Kumar and Pandey, 2013). Chemically, it is called 4′,5,7-trihydroxyflavone (Figure 3) with a molar of 272.3 and its melting point is 251°C (Gattuso et al., 2007). It is found in solid form under natural conditions and is soluble in organic solvents known as ethanol, ether, dimethylformamide, and dimethyl sulfoxide (Den Hartogh and Tsiani, 2019). Naringenin is obtained from the hydrolysis of glycone forms of flavanone such as naringin or narirutin (Madrigal-Santill´an et el., 2014).

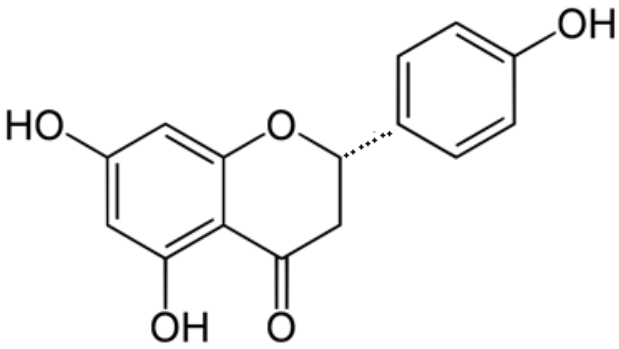


Figure 3. Chemical structure of naringenin (Anonymous, 2024b).

Naringenin is found especially in grapefruit, blood orange, lemon, grape and orange (Den Hartogh and Tsiani, 2019) and this molecule has many pharmacological properties in metabolism (Tajlor, 1992). After entering the metabolism, this molecule reaches the tissues as a result of several enzymatic reactions. Finally, its metabolites are excreted from the body through the bile and urinary systems (Figure 4)

(Cai et al., 2023). With its antiatherogenic, antidepressant, anti-inflammatory, anticancer, antimicrobial, antimutagenic, antioxidant, and antiproliferative impacts, naringenin has pharmacological activity. It was reported that this molecule has antispasmodic, choleretic (Veloso et al., 2021) and expectorant (Dong et al., 2022) impacts, especially in diabetes, cancer, and cardiovascular diseases (Patel et al., 2018). Naringenin helps neutralize the impacts of oxidative stress and nerve growth factor incompatibility (Salehi et al., 2019). It was shown that naringenin supplementation alleviates symptoms of diabetic neuropathy (Kabir et al., 2021). It was also reported that women with Type II DM received daily naringenin supplementation (150 mg, three times daily for 8 weeks) and reduced body weight and insulin resistance (Murugesan et al., 2020). Intestinal α-glucosidase activity was inhibited significantly by naringenin supplementation in rats fed a high-fat diet and streptozotocin-induced diabetes. It was also reported to delay carbohydrate absorption in rats with Type II DM and lead to a significant decrease in postprandial blood sugar levels (Priscilla et al., 2014). Naringenin was widely employed to study the etiology of streptozotocin-induced diabetes. It has been reported that naringenin significantly reduces the high nitric oxide and inflammatory cytokine levels of diabetic rats (Annadurai et al., 2013). It was reported that oral administration of 100 mg/kg of naringenin to diabetic rats for 15 days reduced blood glucose levels and regulated body weight (Singh et al., 2018). It was reported that insulin and glucose levels improved and body weight returned to normal when gestational diabetic rats were fed naringenin at a dose of 50 mg/kg body weight for 20 days (Xing et al., 2016). In many studies on naringenin, it has been reported that it significantly reduces the risk of renal failure and hyperlipidemia (Hua et al., 2021), has anti-inflammatory and anti-infectious properties in autoimmune inflammatory diseases (Tutunchi et al., 2020), and reduces inflammation and hyperproliferation of the Wistar albino rat colon (Rehman et al., 2018).It was shown that naringenin can increase glucose-stimulated insulin secretion in pancreatic endoderm cells obtained from rats (Stabrauskiene et al., 2022). Naringenin was seen to be more effective in insulin secretion than the impacts of other

flavonoids such as caffeic acid and quercetin (Hartogh et al., 2019). It was determined that naringenin has an anti-estrogenic effect and prevents the growth of cancer cells with endogenous or exogenous ERα when applied together with 17β-estradiol (Bulzomi et al., 2010). It was also reported that naringenin has positive impacts on muscle functions (Pellegrini et al., 2014). When the anti-inflammatory impacts of naringenin were examined, it was reported that it could reduce inflammation by changing proinflammatory mediators released by different inflammatory cells (Yeung et al., 2018). In a study that was conducted on humans, it was reported that it was well tolerated as a result of single-dose administration of 150, 300, 600, and 900 mg of naringenin. It was also reported that its metabolites were detected in the circulation and were eliminated from the body within 24 hours (Rebello et al., 2020). It was observed in rats with Alzheimer's Disease that naringenin improved neurodegeneration and cognitive performance with its neuroprotective impacts (Ghofrani et al., 2015). Naringenin and vitamin C together were reported to significantly increase insulin levels and reduce oxidative stress in diabetic rats (Punithavathi et al., 2008).

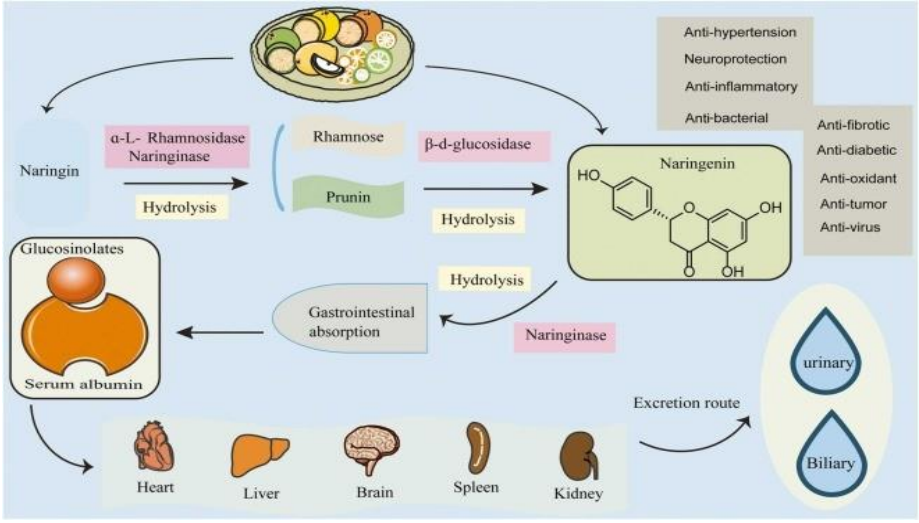
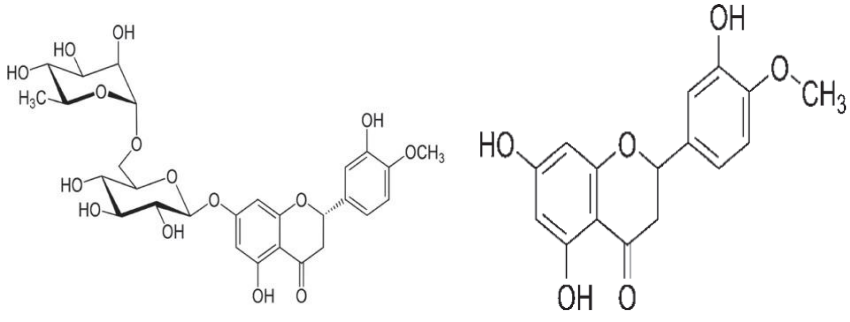


Figure 4. Digestion, absorption, and excretion of Naringenin (Cai et al., 2023).

HESPERIDIN AND HESPERETIN

The chemical name of hesperidin ($C_{28}H_{34}O_{15}$) is 4'-methoxy-3',5,7-trihydroxyflavanone-7-rhamnoglucoside, and the chemical name of hesperetin ($C_{16}H_{14}O_6$) is 4'-methoxy-3',5,7-trihydroxyflavanone (Alissa and Ferns, 2017). Figure 5 **(**Ji et al., 2024**)**.



Hesperidin Hesperetin

Figure 5. The chemical structure of Hesperidin and Hesperetin (Atoki et al., 2023).

Citrus fruits (e.g., lemon and orange) are rich in hesperidin, which is also found in mint, *Hypericum perforatum* (St. John's Wort), and *Salvia miltiorrhiza* (red sage) (Roberts et al., 2013). The hesperidin molecule has antioxidant characteristics with the main effects of reducing the activities of intracellular superoxide, singlet oxygen, and hydroxyl radicals (Azhar et al., 2023). These molecules have anti-inflammatory, antioxidant, antitumor and antimicrobial properties (Ji et al., 2024). The impacts of hesperidin and hesperetin on exercise performance have been investigated so far and it was reported that these flavanones optimize oxygen and nutrient delivery to muscles, improve anaerobic performance, and help muscle recovery in athletes (Sthijns et al., 2018). Hesperidin was also reported to help suppress blood-retinal destruction, increase retinal thickness, and reduce blood glucose (Shi et al., 2012). Hesperidin improves lipid and sugar metabolism disorders caused by high-fat diets and increases energy expenditure by promoting fat breakdown **(**Ji et al., 2024). Hesperidin increases p53 expression in many types of cancer (Xia et al., 2018). Hesperidin and hesperetin were reported to enhance host defense by

reducing bacterial DNA synthesis, motility, and membrane permeability (Havsteen, 2002).

Both hesperidin and hesperetin have antifungal activity against *Botrytis cinerea*, *Trichoderma cinerea*, and *Aspergillus fumigatus* species (Islam and Ahsan, 1997). These molecules may play a role in preventing osteoporosis by supporting bone cell metabolism. They have also been shown to be novel therapeutic agents in the treatment of neurodegenerative disorders such as Alzheimer's Disease (Ji et al., 2024). Hesperidin has several potential therapeutic impacts in acute kidney injury (Sahu et al., 2013). It has also been reported to protect against acrylamide-induced nephrotoxicity and DNA damage in rats (Elhelaly et al., 2019). When the effects of orange juice rich in flavanone on neurological responses were examined in a previous study, it was reported that 24 healthy middle-aged individuals improved their cognitive status 2 and 6 hours after consuming hesperidin (Matsuzaki et al., 2022). Hesperidin flavanone has positive impacts in protecting against digestive system diseases **(**Ji et al., 2024). Animal studies reported that hesperidin has anti-inflammatory, anti-hypercholesterolemic, anti-hypertensive, and antioxidant characteristics. It was observed that hesperidin (400 mg/day) reduced total cholesterol and Low-Density Lipoprotein (LDL) levels in rats. In adult humans, hesperidin supplementation was reported to reduce serum triglycerides (TG), total cholesterol (TC) and LDL, blood glucose levels, and free fatty acid levels (Mahmoud et al., 2019). As well as the flavanones mentioned above, flavanones such as pinocembrin, alpinetin, and neohesperidin are also common. Pinocembrin is obtained from the *Eucalyptus pressiana* plant and was reported to have antifibrotic, anti-inflammatory, anticancer, and antioxidant impacts (Rasul et al., 2013). Alpinetin is a natural flavanone obtained from the *Alpinia intermedia* plant and was shown to reduce the levels of TNF-α, IL-1β, and IL-6 in inflammation significantly (Gul et al., 2022). It was also reported to have significant impacts in mouse models of lung injury, which is a promising potential for therapeutic applications (Huo et al., 2012). As a flavanone consisting of Hesperetin and the disaccharide neohesperidoside,

Neohesperidin is a *Citrus changshanensis* fruit (Zhang et al., 2012). Neohesperidin was reported to have antifibrotic impacts in mice and the potential to reduce established pulmonary fibrosis (Guo et al., 2019). There are thousands of polyphenolic molecules in plants and the number of these molecules has increased from past to present. In the future, the detection of these molecules will be done more and will lead to the discovery of new molecules as a result of the development of analytical devices. It is considered that these molecules, whose structures were discovered and whose pharmacological activities were determined and clarified, can play a role in the treatment of many diseases.

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CHAPTER 7

STRIGOLACTONES AS PLANT GROWTH REGULATORS

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INTRODUCTION

Strigolactones (SLs) are a group of chemical compounds produced by plant roots and, are a class of next-generation plant hormones that play important roles in various aspects of plant growth, including shoot branching, root architecture, regulation of plant development, signaling and establishment of mycorrhizal relationships (Selwal et al., 2023). Studies have identified that strigolactones are responsible for three different physiological processes: First, they promote the germination of parasitic organisms growing on the roots of the host plant, such as *Striga lutea* and other plants of the *Striga* genus. Secondly, strigolactones are fundamental for plant recognition by symbiotic fungi, especially arbuscular mycorrhizal fungi, as they establish a mutualistic relationship with these plants and, provide phosphate and other soil nutrients. Third, strigolactones have been identified as branching-inhibitory hormones in plants; when present, these compounds arrest the branching mechanism in plants by preventing excessive bud growth at stem terminals (Umehara et al., 2015).

Discovery of Strigolactones

Strigolactones were first isolated from the roots of cotton plants in 1966 and were isolated from plant root exudates as germination stimulants for root parasitic plants of the family Orobanchaceae, including broomrapes (*Orobanche* and *Phelipanche* spp.) and *Alectra* spp., and, were therefore considered harmful to producing plants. Later, their role as indispensable chemical signals for root colonization by symbiotic arbuscular mycorrhizal fungi was revealed and SLs were later recognized as beneficial plant metabolites. However, their role in the germination of other organisms was determined much later (Cook et al., 1966).

Studies with the witch hazel, *Striga lutea*, had shown that root extracts from host plants were required for the parasitic seed to begin germination, clearly demonstrating that a substance produced in the roots stimulated this process. The isolation of strigolactones led to a series of tests that proved that this compound was the molecule needed

to initiate germination of *Striga* species. It was later found that similar compounds had the same effect. These compounds are sorgolactone and alectrol, and since both belong to the characteristic lactone group, they were classified as strigolactones (Xie et al., 2010). To initiate the germination of parasitic plants, 5 ppm of strigolactones in the environment is sufficient (Arıkan and Karaman, 2021).

Chemical Structures and Biosynthesis

The biosynthesis of strigolactones has been described in many plant species, including *Arabidopsis thaliana*, rice (*Oryza sativa*), petunia (*Petunia hybrid*), pea (*Pisum sativum*), tomato (*Solanum lycopersicum*) and chrysanthemum (*Dendranthema grandiflorum*) (Koltai and Kapulnik, 2011). Matusova et al., (2005), revealed that the biosynthesis of strigolactones is related to the carotenoid pathway. Strigolactones consist of carotenoids containing 40 carbon atoms in the terpene class (Borghi et al., 2016; Smith, 2014).

Carotenoids are also precursors of abscisic acid, which controls the response of plants to environmental stress. The biosynthesis of strigolactones occurs in two separate compartments, the plastid and the cytosol. The production of carlactone, the precursor of strigolactones, occurs in plastids. Carotenoid cleavage dioxygenase (CCD, CCD7, CCD8) and D27 enzymes take part in the synthesis steps from carotenoid to carlactone. SL formation occurs at the end of the breakdown of carlactone in the cytoplasm (Borghi et al., 2016) (Figure 1).

SL has a tricyclic lactone (ABC ring) and a methylbutenolide ring (D ring), and these two moieties are connected by an enol ether bond (Seto et al., 2012). All natural strigolactones identified so far have common C and D rings, which are thought to be responsible for biological activity (Yoneyama et al., 2008).

Based on their chemical structure, strigolactones can be divided into two groups: canonical and non-canonical SLs. Canonical SLs contain the butenolide D ring linked to the ABC ring system via an enol ether bridge, this group contains most SLs characterized to date.

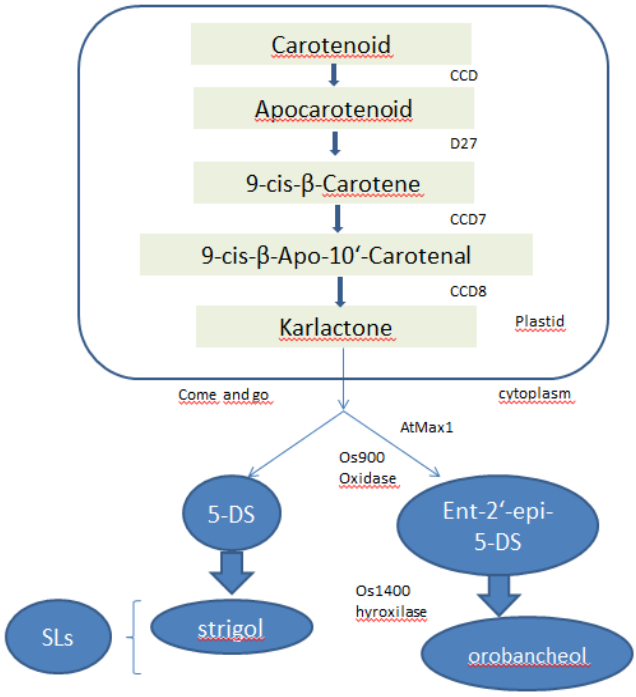

Figure 1. Biosynthetic pathway of SLs: formation of strigol and orobanchole (Borghi et al., 2016)

Canonical SLs are divided into 2 types, strigol and orobanchol type SLs, according to the stereochemistry of the C ring (Xie et al., 2019). On the contrary, non-canonical SLs do not contain the ABC ring system but the enol ether-D ring structure, that is, although the D-ring is not changed, one or more of the ABC-rings are absent (Yoneyama et al., 2018). Examples of non-canonical SLs include aveanol (Kim et al., 2014), heliolactone (Ueno et al., 2014), zealacktone (Charnikhova et al., 2017), zeapyranolactone (Charnikhova et al., 2017) and lotuslactone (Xie et al., 2019).

SL species, namely 5-deoxystrigol, sorgomol and more recently zealactone and zeapyranolactone, have been discovered in maize root exudates. Similarly, a variety of structurally distinct SLs, including strigol, sorgolactone, 5-deoxystrigol and sorgomol, have been identified in the root exudates of *Sorghum*. To date, at least 25 different strigolactones have been identified (Yoneyama et al., 2010) (Figure 2).

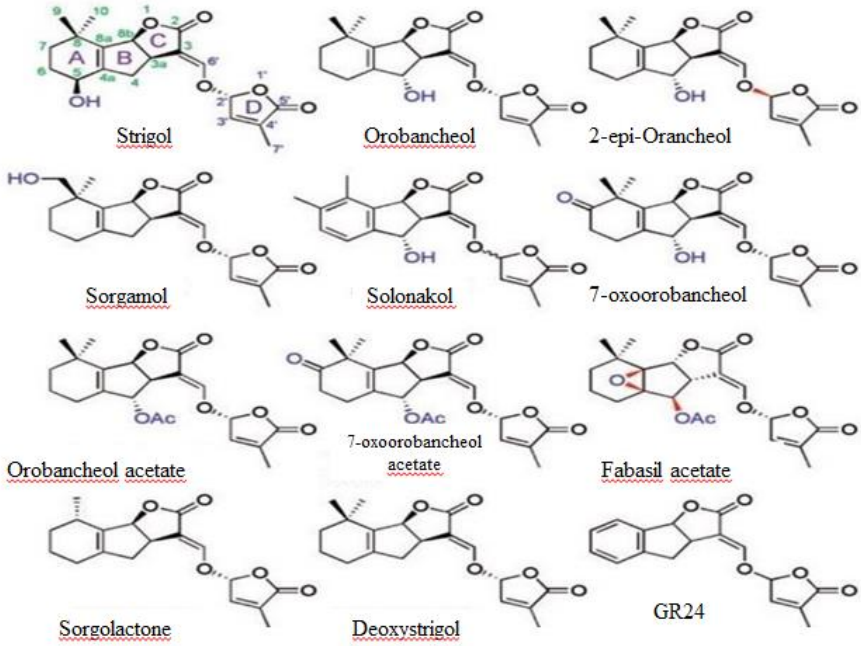

Figure 2. Structures of some natural strigolactones and the synthetic analog GR24 (Yoneyama et al., 2010).

Synthesis of natural SLs is difficult, time-consuming and expensive, making them unsuitable for agricultural applications. Naturally occurring SLs have a structure on the multigram scale and are too complex for synthesis. Synthetic SLs are preferred because they have a simpler structure than natural SLs and maintain their bioactivity. The synthetic analogue of SL, GR24, was named after the first scientist who synthesized it, Gerry Roseberry (Cardinale et al., 2018). GR24 was produced on a multigram scale as a mixture of stereoisomers (containing both 5-deoxystrigol and 4-deoxyorobanchol types) (Vurro et al., 2016). Enantiomers of these two stereoisomers (GR245DS with GR24ent-5DS or GR244DO with GR24ent-4DO) are mixed in equal amounts to obtain racemic GR24 (rac-GR24). Synthetic GR24 is used as a germination stimulant in biological studies and as a model compound for SLs in testing the physiological effects of hormones (Zwanenburg and Blanco-Ania, 2018).

Due to the multifunctional molecular role of SLs, the synthesis of simple SL inhibitors and the comparison of inhibitors with SLs play an important role both in basic research and agricultural applications. Since fluridone inhibits the biosynthesis of all carotenoids and carotenoid-derived metabolites, it was thought that it could also be an inhibitor of SL biosynthesis. However, since fluridone causes photodestruction of chlorophyll and fatal damage to cells, it is not an ideal inhibitor to study the biological roles of SLs in plants (Nakamura and Asami 2014). Instead, the chemical named TIS13 (2,2-dimethyl-7-phenoxy-4-(1H-1,2,4-triazol-1-yl) heptan-3-ol) was first synthesized as SL biosynthesis inhibitors. Although SL levels decreased in experiments with this chemical, some abnormal phenotypes not related to inhibition of SL biosynthesis were observed.

Many TIS13 derivatives have been synthesized to reduce side effects and better results have been obtained. The most widely used one among them is reported as TIS108 (6-phenoxy-1-phenyl-2-(1H-1,2,4-triazol-1-yl) hexan-1-one) (Ito et al., 2013).

Roles in Plant Development

While branching in plants is genetically determined, the behavior of axillary buds can also change in response to environmental changes (Xie et al., 2010). Shoot branching is regulated by the interaction of various factors and hormonal signals. Auxin, which plays an active role in branching, regulates the expression of SL and cytokinin biosynthesis genes. It is stated that SLs inhibit branching in auxin-defective plants and auxin signaling mutants (Koltai, 2013). The discovery of SLs as inhibitors of shoot branching has revealed an important dimension to the biological activity of the hormone (Marzec, 2016). In situations of limited nutrient uptake, plants direct limited nutrient resources to existing shoots by minimizing new shoot production (Xie et al., 2010). SLs suppress the formation of lateral roots while promoting the elongation of primary roots and root hairs (Koltai, 2015). In lateral roots, SLs may affect auxin flow by controlling PIN proteins, through which auxin regulates the formation of lateral roots. SL application interferes with PIN auxin efflux transporters in roots and leads to a

decrease in PIN1-GFP intensity in the lateral root primordium, thereby suppressing lateral root formation by altering the auxin concentration required for lateral root formation (Brewer et al., 2009a). Root hairs absorb water and nutrients from the soil and help establish symbiotic interactions between rhizobium and legumes.

SLs accelerate leaf senescence, promote internode elongation, and also control leaf shape. Leaf senescence is actively promoted in a nutrient-poor soil environment, and nutrients are transported from older leaves to young tissues and seeds (Yamada and Umehara, 2015). Delayed senescence has been reported in SL-deficient and SL-insensitive mutants. SLs also regulate darkness-induced leaf senescence (Ueda and Kusaba, 2015a).

Effect in Case of Nutrient Deficiency in the Plant

The synthesis rate of SLs in plants is highly sensitive to some mineral levels in the soil, and SL levels have been reported to increase significantly under low phosphate and nitrogen conditions (Umehara et al., 2010). Under low phosphate conditions, increased levels of SLs suppress branching, increase lateral root formation, and increase root hair density; mutants defective in the SL pathway are less responsive to low phosphate (Brewer et al., 2013). Apart from the role of SLs in the management of phosphate homeostasis, SLs are also known as a possible pathway to regulate plant growth in response to nitrogen demand. Nitrogen deficiency accelerates the decrease in chlorophyll content caused by leaf senescence. GR24 applied under nitrogen deficient conditions suppressed the decreases in plant weight and chlorophyll content indicative of nitrogen deficiency.

Effects against Biotic and Abiotic Stress in Plants

Recent studies have shown that SLs may act as positive regulators of abiotic stresses in plants such as drought, salinity, light and cold stress, as well as their roles in inhibiting shoot branching in response to nutrient deficiency, modulating root architecture and promoting leaf senescence (Ha et al., 2014). In Arabidopsis, it has been observed that the response to drought and salinity is mediated by SL.

As a result of studies conducted with SL biosynthesis (max3 and max4) and response (max2) mutants, it was found that plants were sensitive to drought and salt stress because SL was absent in all mutants examined (Ha et al., 2014).

It has been shown that GR24-applied grapes have reduced negative effects, including oxidase-induced damage in photosidation in plants under drought conditions, and GR24 also regulates the amount of chlorophyll and the levels of different hormones (Min et al., 2019). Researchers concluded that applying GR24 could be an effective strategy to improve drought tolerance of grapevine seedlings. SLs play a role in regulating root and root hair development by alleviating the negative effects of nutrients and heavy metals, including calcium, nitrogen, iron, sulfur, phosphorus and aluminum. When seeds are exposed to various abiotic stress conditions such as high temperature, limited light or osmotic stress for a long time, their germination ability decreases even if the plant becomes suitable for germination (Toh et al., 2012).

Protective Effect against Plant Diseases

SLs are reported to increase plant resistance to specific pathogens (Marzec, 2016). Leaves of SL-defective ccd8 tomato (*Solanum lycopersicum*) mutants are highly susceptible to infections by *Botrytis cinerea* and *Alternaria alternate* (Torres-Vera et al., 2014). Studies have reported that SLs increase plant defense against the damaging pathogenic fungus *Magnaporthe oryzae* in rice (Nasir et al., 2019). The study with mutant *A.thaliana* plants shows the positive role of SL in plant resistance against infection by bacteria such as *Rhodococcus fascians*, *Pectobacterium carotovorum* and *Pseudomonas syringae* (Mishra et al., 2017).

Relationship between Strigolactones, Plant Hormones and Growth Regulators

Phytohormones play a central role in increasing plant tolerance to environmental stresses that negatively affect plant productivity and threaten future food security (Pozo et al., 2015). Following the

discovery that SLs play a role in the inhibition of axillary bud growth, it was reported that their effects on root architecture, secondary growth, hypocotyl elongation and seed germination occur as a result of their interaction with other hormones (Cheng et al., 2013a).

Strigolactones-Auxin

Several studies have revealed cross-talk between auxin and SLs. It is suggested that SLs act as auxin-mediated secondary messengers in the bud to suppress plant growth (Brewer et al., 2009b). SLs restrict the polar transport of auxin systemically and locally, causing auxin to accumulate in buds to growth-inhibitory levels to suppress lateral shoot branching, suggesting that SLs act synergistically with auxin. It has been reported that the control of shoot branching in polar auxin transport is regulated by SLs, that SLs reduce the basal transport of auxin, and that SLs increase the competition between two branches on the stem in the presence of auxin (Yamada et al., 2014).

Strigolactones-Cytokinin

Cytokinins play a role in important activities such as promoting shoot branching, preventing leaf senescence, and supporting meristematic activity (Rameau et al., 2019). It has been reported that cytokinin and SL synthesis are altered in different physiological processes (Jiang et al., 2016). Cytokines and SLs act antagonistically in bud activation and shoot branching, independently in adventitious rooting, but synergistically in regulating lateral root development (Dun et al., 2012). Both hormones regulate the expression of the other's biosynthesis genes (Ferguson and Beveridge, 2009). SL-associated genes have been shown to mediate cytokine biosynthesis and root export, but there is no information on the effect of cytokinin on SL transport (Omoarelojie et al., 2019). While GR24 promotes an increase in meristem cell number and size, CK promotes cell differentiation and hence reduces meristem size (Ioio et al., 2008).

Strigolactones-Ethylene

Ethylene, both in SL and gaseous forms, plays an active role in various plant growth and developmental processes, including seed germination, leaf senescence, root hair elongation, and hypocotyl growth (Cheng et al., 2013b). Studies determining the relationship between ethylene, auxin and strigolactones have shown that auxin and ethylene positively affect root hair elongation. While SL and ethylene are effective in regulating root hair growth in Arabidopsis, it has been reported that ethylene must be synthesized in order for SLs to exert their effect on root hair growth, although ethylene has an epistatic effect on SLs (suppressing each other's effects). The requirement of the ethylene pathway for the root hair response to SLs suggests that ethylene creates a cross-talk link between the SL and auxin pathways (Kapulnik et al., 2011).

During light treatments, SLs inhibit hypocotyl elongation by increasing the expression of HY5 (Long Hypocotyl5) (Jia et al., 2014). In contrast, ethylene promotes hypocotyl elongation by increasing HY5 degradation via COP1 (Constitutive Photomorphogenesis 1) (Yu et al., 2013). These indicate opposing roles of these two hormones in regulating hypocotyl growth (Wani et al., 2020). SLs activate relevant signals mediated by ethylene during leaf senescence (Ueda and Kusaba, 2015b). SLs have been shown to induce ethylene biosynthesis in seeds of the parasitic plant Striga, leading to their germination. Therefore, the effect of SLs on plants is to affect ethylene biosynthesis (Sugimoto et al., 2003). SLs adjust the balance between auxin and ethylene signaling pathways to activate different developmental programs in response to soil phosphate deficiency, thereby controlling their own biosynthesis through a positive feedback loop (Koltai and Beveridge, 2013).

Strigolakton- Absisik asit

Abscisic acid (ABA) has an important role in the regulation of seed development, dormancy and stress tolerance. Studies have shown that ABA plays a role in the regulation of SL biosynthesis (López-Ráez et al., 2010). In the study conducted in *Arabidopsis*, SLs were found to positively regulate seed germination by counteracting the inhibitory

effect of ABA during thermo-inhibition (Zhang et al., 2013). In a study conducted on grapes with GR24 and ABA application, it was reported that anthocyanin accumulation was delayed when both hormones were applied together, there was no or very little difference in anthocyanin accumulation in fruits treated with GR24 alone compared to the control group, and SL has the potential to be used instead of ABA in the ripening of grape fruits (Ferrero et al., 2018).

The functions of ABA, CK and SL in the regulation of stomatal closure and leaf senescence, two traits closely related to stress response and adaptation, are highlighted. ABA and SL promote leaf senescence, while CK delays leaf senescence. On the other hand, ABA and SL act as positive regulators of stomatal closure and hence stress response, while CK acts as a negative regulator of the same process (Ha et al., 2014).

Exogenous ABA can increase SL accumulation, especially under stress conditions. In addition, SL and ABA are critical for regulating plant defense against salt stress and establishing symbiotic relationships between host plants and AMF (Ren et al., 2018).

Various studies have shown that the relationship between SL and ABA in the induction of stress tolerance in plants is complex. Studies have reported that there is a connection between the SL and ABA pathways and that the D27 gene plays an important role in the synthesis of ABA and SL in rice, and that this gene may be a promising pathway for improving drought in crops (Haider et al., 2018).

Strigolactones-Gibberellic Acid

Gibberellic acid (GA) is the hormone responsible for breaking seed dormancy, promoting seed development and root growth, mitotic division in leaves of some plants, and plant and flower growth (Rameau et al., 2019). Recent studies suggest that GA and SLs may act together in plant development processes (Marzec, 2017).

Deficiency in SL biosynthesis or signaling leads to decreased GA content and weakened GA response, which in turn reduces shoot length by decreasing transcription levels of genes involved in cell division and cell elongation (Zou et al., 2019). Researchers seeking to uncover the

communication between SL and GA have shown that SLs act synergistically with GA in regulating seed germination in Arabidopsis (Toh et al., 2012). In another study, 10 mM GA_3 application reduced the expression of SL-biosynthesis genes in rice for 15, 30 and 60 min, while a lower GA_3 concentration (50 nM) reduced the expression of SL-biosynthesis genes for up to 24 h (Marzec, 2017).

In general, the developmental functions of SL and GA are largely opposite (Rameau et al., 2019), with SL signaling acting antagonistically rather than in concert with GA signaling in regulating shoot branching in the woody plant *Jatropha curcas* (Wallner et al., 2016).

Strigolactone-Brassinosteroid

Although the communication between SL and brassinosteroid (BR) signaling pathways has been discovered in recent years, the relationship between these two hormones has not yet been fully elucidated. In studies conducted, researchers have shown that both SLs and BRs have a positive effect on nodule initiation in pea, but their effects on nodulation have not been proven (Foo et al., 2014).

One of the most important signaling components discovered in Arabidopsis is MAX2, which inhibits branching of lateral shoots. Application of exogenous SL triggered the degradation of the BR transcription factor mediated by MAX2 and consequently inhibited shoot branching (Beveridge and Kyozuka, 2010; Peres et al., 2019).

As a result of another study, they revealed the genetic and molecular mechanisms by which SL and BR signaling pathways antagonistically regulate branching in rice (Faizan et al., 2020; Wang et al., 2013). To date, very little data have been obtained on the SL and BR signaling pathway. Advances in the investigation of this new class of phytohormones will further clarify the hormonal communication between SL and BR.

Strigolactone-Jasmonic Acid

Although there is not enough data on the processes carried out by SL and Jasmonic Acid (JA) together, it has been observed that both

hormones reveal common responses in similar developmental processes such as mesocotyl elongation, aging, and plant-microorganism interactions (Omoarelojie et al., 2019). Researchers investigating how SLs are involved in plant defense responses conducted a study to determine whether the combined application of SL and other plant hormones (SA, JA, ABA) increased the resistance and susceptibility of plants against *Botrytis cinerea* and *Alternaria alternata*, two foliar fungal pathogens that have a devastating effect on tomatoes. Phosphorus deficiency was induced in SL-deficient mutant tomatoes and wild-type tomato seedlings (cv. *Craigella*), and the wild-type plant seedlings were induced to secrete SL, and then the two pathogens were added to both groups. A decrease in the content of defense-related hormones jasmonic acid, salicylic acid and abscisic acid was detected, indicating that hormone homeostasis was altered in the mutant plant. They indicated that SL plays a role in the regulation of plant defenses through its interactions with other defense-related hormones, especially through the jasmonic acid signaling pathway (Torres-Vera et al., 2014).

Studies to identify the undetermined role of SL in drought tolerance and essential oil yield of *Dracocephalum kotschyi* and the possible interaction between methyl jasmonate (MeJA) and SL revealed a significant relationship between MeJA and SL in improving drought resistance and optimizing essential oil production of *D. kotschyi* (Shirani Bidabadi and Sharifi, 2021).

Strigolactone-Salicylic Acid

Salicylic acid (SA), a phenolic compound, affects seed germination, seedling establishment, cell growth, respiration, stomatal closure, nodulation and fruit yield in legumes (Hernández-Ruiz and Arnao, 2018). SA is an important signaling molecule that regulates plant responses to stress as well as defense against pathogens (Singh and Usha 2003). Many studies report the protective effects of SA on plants against salinity (Azooz et al., 2011), drought (El Tayeb and Ahmed, 2010), high temperature (Khan et al., 2013) and heavy metal stress (Chavoushi et al., 2019). In studies on SA and SL, exogenous applications of GR24 caused SA accumulation, while SA

concentrations were decreased in SL-deficient MAX2 mutants, suggesting that SLs are involved in plant defenses by inducing SA production (Rozpądek et al., 2018).

In wheat, combined foliar application of SL and SA resulted in lower electrolyte leakage, higher relative leaf water content and enhanced antioxidant enzyme activities during drought stress. They found that the exogenous application of SL and SA by spraying method increased the stomatal conductance, photosynthesis and transpiration rate of wheat plants, as well as the proline and soluble sugar content. It was observed that drought tolerance increased in wheat where SL and SA were applied together, and there was an interaction between SA and SL in drought stress (Sedaghat et al., 2017, 2020).

Strigolactone-Karrikin

Karrikins are small butenolide molecules that have the capacity to promote germination and enhance seedling establishment (Yao and Waters 2020). Karrikins are highly active, seed germination-inducing and regrowth-stimulating molecules found in the smoke of burned vegetation after wildfires (Nelson et al., 2011). Karrikins are plant growth regulators that are formed from the synthesis pathway of SL, but have physiologically different functions (Waters et al., 2012). SLs are synthesized by plant tissues and transported between tissues or infiltrated into the rhizosphere as components of root exudates. Karrikins are a component of plant smoke resulting from the combustion of plant materials (Omoarelojie et al., 2019).

In *Arabidopsis*, SL and karrikin receptors, D14 and KAI2, are reported to regulate drought resistance by regulating abscisic acid response, anthocyanin accumulation, stomatal closure, cell membrane integrity and cuticle formation. In their studies to find the effects of receptors on root development in *Arabidopsis*, the researchers found that the KAI2 signaling pathway controls root curvature, root hair density and root hair length, and together with the D14 signaling pathway, it also regulates lateral root density (Swarbreck et al., 2019).

It has been reported that abiotic stresses such as salinity or osmotic stress can change karrikins from a positive germination

regulator to an inhibitor (Wang et al., 2018). It has been proven that karrikins can play a positive role in enhancing seedling growth of wild plants (Daws et al., 2007) and medicinal plants (Mousavi et al., 2016). Therefore, the relatively new smoke application technology is a technique that can be used in germination in both conventional and organic agriculture (Akeel et al., 2019).

Plants respond to a range of abiotic stimuli in nature, including anatomical, cellular, morphological and physiological changes. The ability of plants to adapt to different environments is greatly influenced by phytohormones that mediate growth, development and nutrient partitioning. These signaling molecules are biosynthesized by the plant and are generally known as plant growth regulators.

Strigolactones (SLs) are a unique class of phytohormones that have an ecological role in overcoming multiple stress conditions. SLs are initially found in germinating seeds of parasitic weeds, biosynthesized from precursor carotenoids, and perform widespread physiological functions in bud outgrowth, shoot branching, nodulation, and photomorphogenesis. SLs in turn induce hyphal branching of arbuscular mycorrhizal fungi in germinating spores. Recently, SLs have attracted the attention of researchers due to their roles in molecular and physiological adaptations in response to abiotic stresses.

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CHAPTER 8

NUTRITION IN OPILIONES

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INTRODUCTION

Opiliones is the third largest order among arachnids, after Acari (mites and ticks) and Araneae. They are components of many terrestrial ecosystems. They are distributed in almost all ecosystems of the World. Opiliones species exhibit narrow distributions and cases of endemism are common within the group. These characteristics lead to an abundance of species diversity (Giribet and Sharma, 2015).

Opiliones are well adapted to terrestrial life. They are distributed in large areas from sea level to the peaks of mountains. There are approximately 6,660 species in the world. Species, differ greatly in their morphology, ecology and behaviour. The balance between energy intake and expenditure in animals is critical for their survival and reproductive success. Opiliones species exhibit an enormous diversity of lifestyles and foraging strategies in terrestrial ecosystems. They have traditionally been described as predators or carnivores, but are thought to be largely dependent on arthropods for food (Nentwig, 1987; Nyffeler et al., 1994; Acosta and Machado, 2007; Foelix, 2011). According to recent studies, they feed on various arthropods, fruits, seeds, pollen, fungi, lichens, algae and plant material (Acosta and Machado, 2007; Nyffeler et al., 2023; Nyffeler et al., 2016). Unlike other arachnids, they do not have a predatory feeding regime. They are mostly considered herbivor or detritivores. Opiliones usually consume organic material found on the ground, such as dead plant and animal material, leaves, fruit remains. Some species may also consume live insects. However, this behavior is rare and this group of them are called facultative predators (Castanho and Rocha, 2005; Benson and Chartier, 2010; Wolff et al., 2016).

The diet of opiliones is generally foraging rather than predatory. They are arachnids that feed on solid food. They do not show coxisternal nutrition like scorpions or spiders. They take food directly. They can bite through the solid tissue of fungi, seeds and fruits and break them into small pieces. Carnivorous opiliones are poor at catching prey compared to other arachnids. This is because they do not have venom glands or webs that make them more powerful in their open habitat. They also do not exhibit trapping behavior. Therefore,

they have to find their prey by travelingaround. This is why calorie counting is so important for their survival. The foraging behavior of them are completely focused on roving feeding (Simpson and Raubenheimer, 2012; Nyffeler et al., 2016).

On the other hand, the feeding habits of them are different between species. In particular, different species have a different diet depending on their habitat and available resources. The feeding strategies of them species may vary with factors such as habitat, morphological structure, resource utilization and competition. Therefore, a detailed study of habitat components may be necessary to determine the feeding habits of each Opiliones species.

Figure 1. Opiliones Habitus (Çorak Öcal and Yiğit Kayhan 2020)

Consumption of Plant Materials

Plant materials constitute the main food source of Opiliones. There are records of consumption of pollen, fruits, seeds, mushrooms, mosses and lichens. They consume organic matter, especially decayed leaves, fruit residues and other plant material found on the ground. This is often referred to as a vegetarian diet and may constitute the main food source of Opiliones (Acosta and Machado, 2007).

The Opiliones' process of consuming plant materials works like this:

They detect the presence of plant material using their second legs, pedipalps and sensory hairs to locate food sources. By nature, they actively wander around where these materials are present, i.e. on the ground or in the leaf litter. Opiliones select from a variety of plant material what is suitable as a food source. They usually prefer the rotten and soft ones, because they are easier to digest.

Mushroom consumption by opiliones is widespread and has been known for a long time. Mushroom consumption has been identified on five continents (Europe, Asia, Central America, South America and North America). The family Sclerosomatidae consists mainly of species that consume small forest fungi (families Marasmiaceae and Mycenaceae). In addition, Cladonychiidae, Cosmetidae, Globipedidae, Nemastomatidae, Nomoclastidae, Phalangiidae, Sclerosomatidae were also found to feed on fungal material. Mushroom consumption by opiliones has been found to be especially common in hot regions such as Southeast Asia. The main reason for this is that tropical climate and rich vegetation have increased fungal diversity in these regions (Nyffeler et al., 2023).

In a study on the consumption of fruit material by opiliones, Cosmetidae, Globipedidae, Gonyleptidae, Nemastomatidae, Neopilionidae, Phalangiidae, Sclerosomatidae families were found to feed on fruit and fruit waste (Acosta and Machado, 2007), while Gonyleptidae, Phalangiidae, Sclerosomatidae families were reported to feed on seeds and fruits (Acosta and Machado, 2007). Fruit pulp consumption by opiliones is also widespread, with records of Leiobunum feeding on *Rubus* spp. berries and other lipid-poor fruits in the Holarctic region. In Neotropical forests, harvesters in the families Gonyleptidae and Cosmetidae feed on the lipid-poor pith of fallen fruits (Nyffeler et al., 2016). Fruit pulp consumption by opiliones has been reported from six continents (Oceania, North America, Central America and South America, Europe, Asia) and observed in seven families (Nemastomatidae, Phalangiidae, Cosmetidae, Globipedidae, Gonyleptidae, Neopilionidae and Sclerosomatidae). In the Holarctic

region, opiliones in the genus Leiobunum have been repeatedly observed feeding on the fruits of Rubus spp. (Edgar 1971; Halaj and Cady, 2000; Shardlow, 2013). In Neotropical forests, the families Gonyleptidae and Cosmetidae have been found to feed on the pulp of fallen fruits (Chelini et al., 1996; Acosta and Machado, 2007; Machado and Pizo, 2000; Pagoti et al., 2019).

Gonyleptidae and Cosmetidae species have also been observed feeding directly on ripe fruit on trees and shrubs. There are also records of opiliones consuming fruit pulp in laboratory studies (Nyffeler et al., 2023). In the Holarctic region, *Lacinius dentiger* (Koch, 1847) feeds on apples and pears, various species of neopilionids feed on apples; in the Neotropical region, *Heteromitobates discolor* (Sorensen, 1884) and *Mischonyx squalidus* Bertkau, 1880 species feed on bananas (Mitov, 1988; Costa et al., 2016; Segovia et al., 2019; Dias et al., 2020). *Opisthoplatus prospicuus* (Holmberg, 1876) preferred cucumber; *Discocyrtanus pertenuis* (Mello-Leitao, 1935) preferred pear; *Promitobates ornatus* (Mello-Leitao, 1922) and *Acanthopachylus aculeatus* (Kirby, 1819) preferred papaya as food (Capocasale and Bruno-Trezza, 1964; Fernandes et al., 2017; Willemart, 2001).

In 2007, Acosta and Machado compiled a list of various food items consumed by opiliones, including reports on the consumption of seeds, fungi, pollen, fruits, pollen, algae and lichens. Nearly a decade later, Nyffeler et al., (2016) published a review of plant eating by spiders. They found that opiliones feed on a wide variety of fungi and plant material in addition to their usual arthropod prey. However, the number of reports on the consumption of plant material by opiliones is very limited (Acosta and Machado, 2007).

Recently, new evidence has emerged indicating a 'vegetarian diet' (i.e., the consumption of plants and fungi) in opiliones (Eastburn, 2017; Del-Claro et al., 2017; Lietzenmayer and Wagner, 2017; Pagoti et al., 2019; Nahas et al., 2017; Hyodo et al., 2019). The term “vegetarian” is used in relation to carnivores that occasionally prefer plant or fungal food as an alternative to prey Despite the increasing record of plant and fungal materials in the diet of opiliones, many questions about their

vegetarian diet remain unanswered (Wackers and Fadamiro, 2005; Beckman and Hurd, 2003; Meehan et al., 2009).

Fruits can be categorized as high quality or low quality based on their caloric content. High-quality fruits generally have 40-60% water content, are rich in lipids and have a high caloric value. In contrast, fruits considered to be of low quality are less nutritious and have a watery content. These fruits are rich in carbohydrates, some of which are present as sugars. Such fruits (e.g. banana ,apple, strawberry, cucumber, coconut, elderberry, orange, papaya, plum, watermelon, pear, and pineapple) are a poor source of energy. Most of the reported cases of opiliones feeding on fruit pulp relate to low-quality fruit. This prompts the question of whether there are nutritional advantages to consuming low-calorie fruits. (Johnson et al., 1985).

Detritivore Nutrition

Detritivore communities regulate the decomposition of harmful resources in almost all natural systems. Opiliones play an important role in the decomposition and digestion of dead organic matter. They break down and digest decayed plant material and other organic residues. In this way, they contribute to nutrient cycling in ecosystems. They are also largely involved in the opportunistic scavenging of animal remains (Sankey, 1949). *Phalangium opilio* has been found to feed on bee and moth carcasses discarded by *Misumena vatia* crab spiders (Morse, 2001).

The detritivorous feeding activities of opiliones support and stabilise the cycle of organic matter in ecosystems. The breakdown and digestion of dead organic matter completes the release of nutrients and the cycling of minerals necessary for plant growth. Furthermore, this feeding regime of them supports biodiversity in the ecosystem, improving the habitats of different organisms. Therefore, the detritivorous feeding activities of Opiliones are important for the energy cycle in the ecosystem (Bartrons at al., 2015).

Predator Nutrition

Some Opiliones species may also consume other harvestmen, small snails, spiders, millipedes, earwigs, mites, flies, collembola, aphids, leaf hoppers or dead insects, although this behaviour is rare. However, this behaviour is generally uncommon and is considered an exception to the feeding regime of opiliones (Hillyard and Sankey, 1989). Opiliones that engage in this feeding opiliones are termed facultative predators. Some herbivore taxa such as Ischyropsalididae and Trogulidae are thought to be exclusively carnivorous (Acosta and Machado, 2007; Nyffeler and Symondson, 2001). For example, some large Opiliones species can prey on small insects or other arthropods. Some species are known to be skillful predators (Castanho and Rocha, 2005; Wolff et al., 2016; Benson and Chartier, 2010). Members of the Trogulidae family eat snails alive and then use the remaining shell for oviposition (Martens, 1978; Pabst, 1953; Komposch, 1992). However, most opiliones species with known diets utilize a wide variety of foodstuffs, primarily invertebrates (Acosta and Machado, 2007). It has been reported that the food consumed by species of the family Triaenonychidae in New Zealand consists mainly of insects and other small arthropods (Powell et al., 2021).

Opiliones are a ubiquitous but largely overlooked group of facultative predators. They form an important component of arthropod communities in woodland and agricultural areas. Research also suggests that opiliones, especially *Phalangium opilio*, may be important biocontrol agents for Coleoptera, Lepidoptera and Homoptera in crop communities. Despite their ubiquity and proposed importance in agricultural systems, detailed information on the feeding ecology of opiliones is lacking (Bishop, 1949; Todd, 1949, 1950; Phillipson, 1960a; Bristowe, 1949).

Result and Discussion

They are not usually associated with nutrition by scientists. They have no venom glands. They cannot set traps for hunting. For this reason, opiliones have adapted to passive feeding for their survival. The vast majority of opiliones feed by consuming plant material or

dead organic matter. Therefore, predatory feeding should not be considered as the general feeding strategy of Opiliones, but as a rare exception.

Passive feeding in opiliones refers to the process by which the organism obtains nutrients from sources in its environment without expending energy to actively hunt or prey on the food source. They usually consume plant material or dead organic matter, taking it from food sources in their environment. In the process of passive feeding, They do not pursue or hunt their prey, instead their nutrients usually come naturally from sources found in their environment. For example, rotten leaves, fruit residues or other organic material are potential food sources for them. Opiliones obtain their nutrients by breaking down and digesting these organic materials. However, they do not show any active hunting or predation behaviour in this process. Instead, they obtain their nutrients by utilising the food sources in their environment.

Passive feeding saves energy and allows Opiliones to access food sources with less energy expenditure. Furthermore, the strategy of obtaining food from natural resources in their environment has enabled Opiliones to adopt a quiet and secretive lifestyle, often in nature. This behaviour has provided opiliones with evolutionary advantages, such as protection from predators and easy access to food.

As a result, it has been reported that they feed mostly on fruit pulp, seeds and seed appendages, while some opiliones taxa are exclusively predatory. Consequently, the dietary habits of opiliones remain largely unclear.

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CHAPTER 9

BRASSINOSTEROIDS AS PLANT GROWTH REGULATORS

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INTRODUCTION

Organic or inorganic substances that are synthesized in the plant structure and regulate growth, development and vital activities in cells and tissues are called plant growth regulators (PGRs), in other words, phytohormones. These regulatory substances are synthesized in the relevant regions of plants; they can affect growth, development, and other physiological processes either independently or interactively. They can be active in the tissue where they occur and can be transported to the relevant region of the plant by the transport system, where they can also exhibit their activity (Kumlay and Eryiğit, 2011, Sezgin and Kahya, 2018).

Phytohormones are divided into three groups: growth regulating, development promoting and suppressive (inhibitory). In addition to the classical five, namely Auxin, Cytokine, Abscisic Acid, Gibberellins, and Ethylene, which are from the group of growth-promoting hormones, the sixth group, Brassinosteroids, is the sixth group of plant hormones in the steroid structure that can be synthesized endogenously by plants. Steroid hormones are essential for embryonic development and adult homeostasis in animals (Evans, 1988). They also play a role in the structure of hormones in plants. Plants produce many steroids and sterols for this purpose (Geuns, 1978; Jones and Roddick, 1988).

Brassinosteroids (BR) are plant steroids that promote growth (Müssig, 2005). The growth-promoting properties of some pollen extracts have accelerated the research on BRs. One of the pioneering studies was carried out by Mitchell et.al (1970), who observed that an extract obtained from *Brassica napus* (rapeseed) pollen caused elongation in bean internodes. It was found that extracts obtained from pollen of approximately 60 different plants promoted growth. The researchers named these compounds brassins because the active compound used in the studies was obtained from plants of the Brassica genus.

In studies by Grove et al., (1979) to determine the chemical structure of brassins, they obtained 4 mg of crystals from 40 kg of pollen and named the compound they obtained brassinolide (BL). The studies that followed the isolation of brassinolide suggested that this

compound was involved in plant growth and development. Brassinosteroids were recognised as a new class of plant hormones (Bajguz, 2000, Bajguz and Tretyn, 2003, Clouse and Sasse, 1998, Li and Chory, 1999, Michelini et al., 2004, Müssig and Altmann, 1999, Rao et al., 2002, Vardhini and Rao, 2002). In 1982, castasterone (CS), a structurally steroidal substance with apparent growth-promoting properties, was discovered (Yokota et al., 1982).

Types of Brassinosteroids

Grove et al., (1979) isolated brassinolide [(22R, 23R, 245)-2α, 3α, 22, 23-tetrahydroxy-24-methyl-β-homo-7-oxa-5α-choleston-6-one)] from rapeseed. Brassinosteroids are hydroxylated derivatives of cholestane. Structural variations of brassinosteroids are obtained with different groups on the A and B rings of the C17 side chain. Different Brassinosteroids are classified as C27, C28, and C29 Brassinosteroids based on the side chain length (number of Cs in the chain) (Bajguz, 2007). Brassinosteroids including C27, C28 and C29 steroids have 5α-cholestan structures with similar side chains (Sakurai, 1999).

C27 Brassinosteroids do not have an alkyl group as in the C24 position and are produced from cholesterol (Sürgun et al., 2012).

C28 type Brassinosteroid compounds are the most common form found in plants (Sakurai, 1999). C28 Brassinosteroids have a methylene, α-methyl, or β-methyl group in the C24 state and can be derived from 24-methylene cholesterol, campesterol, or 24-epi campesterol.

C29 Brassinosteroids consist of an ethyl or α-ethyl group at C24 and are derived from isofucosterol, or sitosterol (Yokota, 1997).

In studies, Brassinosteroids found in nature are numbered consecutively and named BR1, BR2, BR3, BR4…BRn is based on the abbreviation brassinolide. Not all Brassinosteroids are always biologically active. Brassinolide (BL), 24-epibrassinolide (24-epiBL), and 28-homobrassinolide (28-homoBL) are the active brassinosteroids commonly used in studies (Rao et al., 2002) (Figure 1).

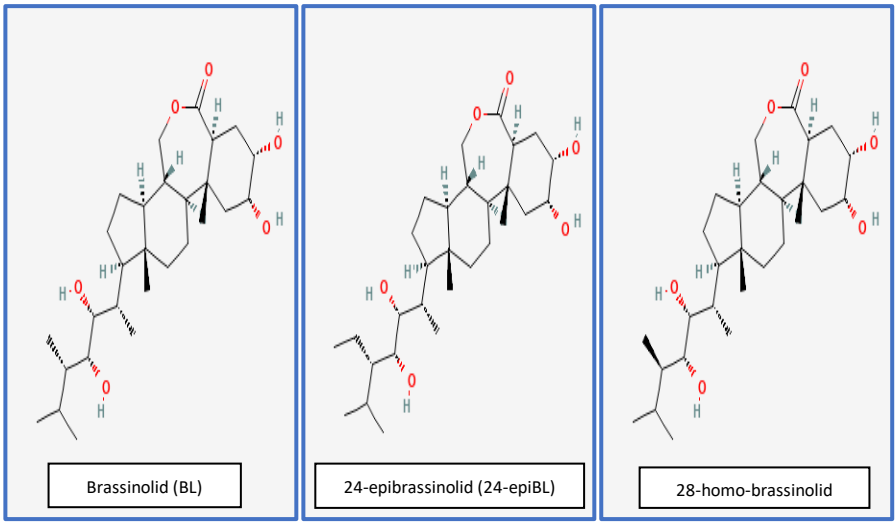

Figure 1. Active brassinosteroids

Plants Containing Brassinosteroids

Brassinosteroids are found in 3 families of gymnosperms, 15 in monocots, and 16 in dicots, totaling 31 families of angiosperms. It has also been determined that they are found in algae and ferns. Brassinosteroids, which can be biosynthesized in all structures of the plant, can generally be found in large quantities in parts such as seeds, pollen, leaves, stems, roots, and flowers (Ankudo, 2004). Endogenous brassinosteroid levels may vary according to plant tissues. Brassinosteroids are found at the highest levels in pollen and seed structures, while they can be found at different levels in shoots, anthers, flower buds, fruits, and cambial regions (Zullo and Adam, 2002).

In studies conducted to determine endogenous Brassinosteroid levels in plant tissues, the highest concentration belongs to Brassinolide obtained from *Brassica napus* and *Vicia faba* pollens at 10-1 nmol/g fresh weight. The lowest level belongs to homocastasterone obtained from immature seeds and shoots of Chinese cabbage (*Brassica campestris* var. *pekinensis*) at 10-7 nmol/g fresh weight (Clouse, 2003).

The main source of Brassinolides in plants is found in immature pollen and seeds at levels of 1-10 ng/g. It is found at the lowest levels in leaves at levels of 0.01-0.1 ng/g. In the study carried out on mature

seeds of *Arabidopsis thaliana*; 3.9x10-3 nmol/g Brassinolide (BL), 9.5x10-4 nmol/g castasterone, 3x10-3 nmol/g thiasterol, 3.5x10-3 nmol/g 6-deox -socastasterone, 2.1x10-3 nmol/g 6-deoxotifasterol, 1.2x10-3 nmol/g 6-deoxo-theasterone were determined (Ankudo, 2004).

Synthesis of Brassinosteroids in Plants

Genes responsible for brassinosteroid synthesis and synthesis were first detected in studies conducted in *Arabidopsis*, rice, and tomato. Brassinosteroids; Campesterol is synthesized from sitosterol and cholesterol structures. In the cell membrane; while campesterol and sitosterol are abundant, cholesterol is less abundant. Three sterols in the cell exist in many metabolite structures, but very few of them are biologically active (Savaldi et al., 2006). Metabolic studies have shown that there are two different parallel pathways from campesterol to castasterone, namely early and late C-6 oxidation (Figure 2) (Divi and Krishna, 2009).

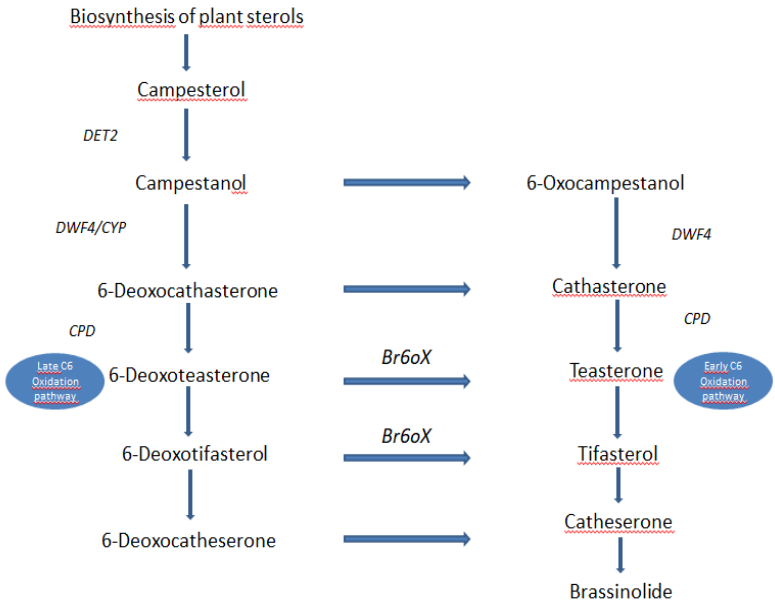

Figure 2. Synthesis of Brassinosteroids (Divi and Krishna, 2009)

In the last step, it is converted to brassinolide by lactonization of the B ring attached to castasterone. The oxidation steps in the synthesis of brassinosteroids are carried out by cytochrome P450 monooxygenase enzyme. C-22, C-23 hydroxylation and C-6 oxidation reactions act as regulators for synthesizing brassinosteroids (Divi and Krishna, 2009).

When the expression of brassinosteroid synthesis genes was examined, the expression of BR6ox and DWF4 genes was highest in shoot tips and fruits, indicating that they are synthesized intensively in young and developing regions, depending on the endogenous Brassinosteroid levels in these organs (Bajguz, 2007; Divi and Krishna, 2009).

Brassinosteroid Synthesis Inhibitors

The determination of brassinosteroid synthesis steps has led to studies on inhibitors that suppress this process. The effects of brassinosteroids on plant development, photomorphogenesis, vascular differentiation, and disease resistance have become more understandable (Clouse, 2003). The first study on brassinosteroid inhibitors was published in 1999. Brassinosteroid synthesis inhibitor Brassinozol (Brz) reduces brassinosteroid concentration in plant cells. Brassinazole binds to the DWARF4 steroid hydroxylase enzyme blocks the hydroxylation of side chains in brassinosteroid synthesis and inhibits brassinosteroid synthesis. Uniconazole and different triazole compounds are gibberellic acid synthesis inhibitors and inhibit brassinosteroid synthesis. Fenarimol, paclobutrazol, propiconazole, triadimefon, Brz2001, Brz220, Brz22012, and DPPM4 are also brassinosteroid synthesis inhibitors (Asami et al., 2003).

Brassinosteroids in plant structure; Brassinozole was used to understand its role in functions such as xylem development and photomorphogenesis. In the study carried out for this purpose; For its role in the differentiation of plastids, brassinozole was applied to Arabidopsis thaliana at a concentration of 0.1 to 2 μM, and the seedlings growing in the dark; It has a stunted, hypocotyl structure, elongated cotyledons, and leaves, and has morphological characteristics

similar to plants exposed to light during normal development. In the control group plants in dark conditions, leaf primordia were not developed. In the experimental group plants; hypocotyl elongation and leaf primordia development were obtained in those applied with the lowest brassinozole concentration level, and plants with short hypocotyls and full leaf development were obtained in those applied with the highest brassinozole concentration. At the end of the studies; It has been understood that brassinosteroids are important in the mechanism of plastid differentiation and photomorphogenesis in the plant cell (Asami et al., 2003).

Brassinozol was applied to cress (*Lepidium sativum*) plants to determine its function in vascular development of plants. At the end of the study; 5 days after the application, the development of primary phloem and xylem tissues in *Lepidium sativum* hypocotyls was normal, while 40 days later, the secondary xylem regions of the plants in the experimental group were smaller than the control plants (Asami et al., 2003).

To understand the role of Brassinosteroids in the defense mechanisms of plants, the study conducted by Asami et al., (2003) showed that Brz2001 plays a role in increasing resistance to cold stress by increasing ethylene biosynthesis. It was also stated that it reduces the negative effects of powdery mildew caused by *Oidium* sp., the fungal pathogen of tobacco wild fire disease (*Pseudomonas syringae* pv. *tabaci*).

It has been determined that brassinosteroid synthesis inhibitors play a role in plant development by delaying internode elongation, and leaf development, as well as leaf and flower formation, and development (Asami et al., 2003).

Transport of Brassinosteroids in Plants

In the study conducted by Savaldi et al., (2006) on the transport of brassinosteroids in the plant system, it was understood that the direction of transport in the exogenous application of 24-epiBL was from the root to the stem. It was found that when radioactive (^{14}C) labeled 24-epiBL was applied to the roots of cucumber, tomato and

wheat plants, the radioactivity was transported to the stem region. Arabidopsis mutants that do not synthesize brassinosteroids were induced to revert to the wild-type phenotype in vitro in the presence of brassinolide-containing agar, and the leaf petioles and roots of wild-type Arabidopsis thaliana plants elongated due to brassinolide.

When ^{14}C-24-epiBL was applied to the surface of cucumber leaves, it was found to be rapidly absorbed by the leaf, but slowly transported out of the leaf. 6% of the applied ^{14}C-24-epiBL was transported to young leaves. This study supported the conclusion that exogenous Brassinosteroid applications are transported from roots to stem and leaves, but not to the remaining plant organs. It is thought that 24-epiBL taken up by roots moves into the stem structure within the xylem. Since substance transport in the xylem is unidirectional, it is thought that 24-epiBL exogenously applied to leaves is transported from the leaf to other leaves only by phloem. Although it is thought that exogenous 24-epiBL is transported from the root to the stem, it has been understood that the transport of endogenous Brassinosteroids from the root to the stem is different. When the distribution of brassinosteroids and metabolites in tissues was examined, it was determined that the metabolites were present in all plant tissues, although their amounts were different in different organs. In studies conducted on *Arabidopsis*, pea and tomato, early transmitter compounds were found to be more abundant in the root, while late transmitters such as castasterone were found to be more abundant in the shoot than in the root. It has been understood that in the synthesis of brassinosteroids, enzymes are expressed in tissues, but their levels vary from tissue to tissue (Savaldi et al., 2006).

Signaling of Brassinosteroids

The importance of brassinosteroid applications in normal plant development and growth has been understood in genetic studies on plant mutants (Hayat et al., 2007). As a result of the research; It was understood that the signal transduction model of Brassinosteroids in plant cells is that Brassinolide on the cell surface is perceived by the leucine-rich repeat-LRRs receptor-like kinase (RLK) BRI1

(Brassinosteroid Insensitive 1), which is a homo-oligomer structure localized to the plasma membrane (Savaldi et al., 2006)(Figure 3).

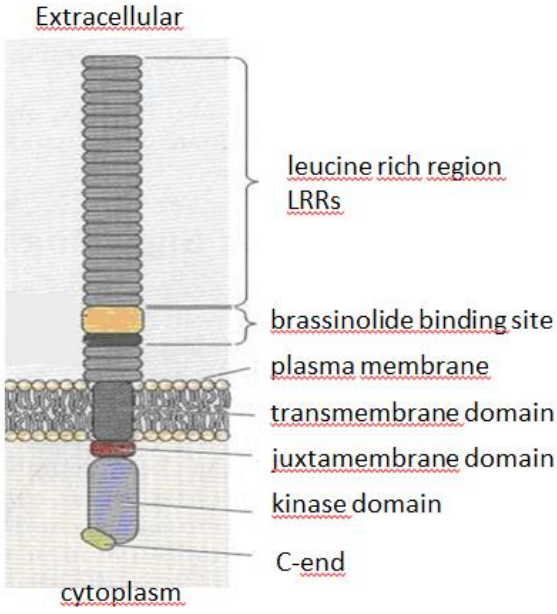

Figure 3. Brassinosteroid transmission model (Savaldi et al., 2006)

When Brassinosteroid is present in the cell environment; signal transmission begins with the activation of BRI1/BAK1, and phosphatase BSU1 (bri1- suppressor-1) positively regulates BZR1 and BES1 by dephosphorylation. Active dephosphorylation of BES1 and BZR1 forms results in transcription of Brassinosteroid-induced genes (BES1), and repression of Brassinosteroid synthesis genes (BZR1). Another known component of brassinosteroid signaling is the negative regulator BIN2 (Brassinosteroid Insensitive 2), which negatively affects the transcription factors BZR1 (brassinazole-resistant 1) and BES1 (bri1-EMS-suppressor 1) by binding to them via a phosphorylation bond (Divi and Krishna 2009, Savaldi et al. 2006). BRI1, which is a brassinosteroid (+) receptor, binds to BAK1 and becomes active, while inactivating BIN2. With the inactivation of BIN2, BSU1 becomes active, and with this activation, it also activates

BZR1 and BES1. Activated BZR1 causes inhibition of Brassinosteroid synthesis genes, while BES1, together with transcription factors, affects the expression of Brassinosteroid-induced genes. In the absence of BR (-), BIN2 becomes active and causes inactivation of BZR1 and BES1. In the inactive state, BZR1 and BES1 are either degraded by the proteasome pathway or bind to 14-3-3 proteins. The interaction of BIN2 and BSU1 with BRI1 and how Brassinosteroids conduct signal transduction are still unknown (Divi and Krishna, 2009) (Figure 4).

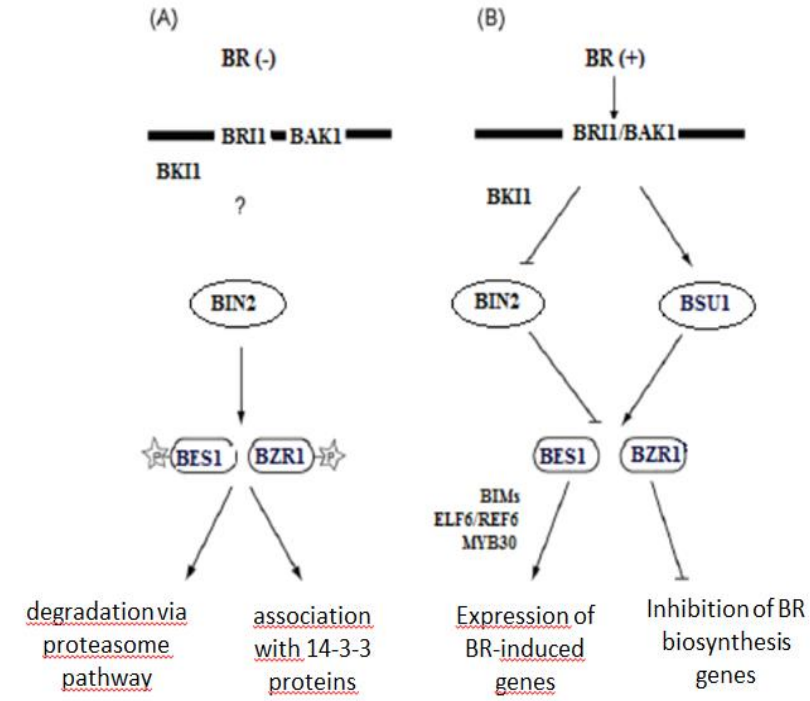

Figure 4. Signal transduction pathway in the presence (a) and absence (b) of brassinosteroid (Divi and Krishna, 2009)

Synthesis and Signaling Genes of Brassinosteroid

To understand the role of brassinosteroids in plant development, molecular genetic studies were carried out with the Brassinosteroid non-synthesizing (BR-deficient) and Brassinosteroid-insensitive (BR-insensitive) mutants of the *Arabidopsis* (Divi et al., 2010). Mutants that cannot synthesize brassinosteroids are gene mutations that code for

biosynthetic enzymes. They are converted to wild-type phenotypes with hormone application. Mutants that do not respond positively to hormone application are mutants that show the same phenotype but cannot be reversed by hormone application. In mutants that do not synthesize brassinosteroids and do not respond to hormones, phenotypic changes such as decreased cell size and intercellular spaces, dark green color, dwarfism, rosette structure, delayed flowering time and reduced fertility were observed (Divi and Krishna, 2009).

While studies on the physiological effects of Brassinosteroids on plant growth and development continued in the 1970s, studies in the 1990s to obtain *det2* and *cpd* mutants that do not synthesize brassinosteroids became the first genetic evidence showing the importance of Brassinosteroids in plant development.

Understanding the molecular structures of *det2* and *cpd* proteins, and the sequence and functional similarities of *det2* protein with steroid 5α-reductase and *cpd* protein with steroid hydroxylase, have shown the function of the proteins in steroid metabolism.

Exogenous applications of *det2* and *cpd* mutants according to the dose level showed the roles of *det2* and *cpd* in brassinosteroid synthesis in the phenotype of wild type mutants (Clouse, 1996; Dhaubhadel et al., 1999; Krishna, 2003). The genes responsible for the signaling and synthesis of brassinosteroids in studies conducted in different plants are shown in Table 1 (Divi and Krishna, 2009).

Table 1. Summary table of Brassinosteroid synthesis and signaling genes with modified functions (Divi and Krishna, 2009)

	Gene	Description of the gene	Genetic modification	Plant	Result
BR synthesis	*DWF4*	*A. thaliana* cytochrome *P450* monooxygenase	Ectopic overexpression	*Arabidopsis*	Increase in seed yield
	ZmDWF4	Maize analogue of *DWF4* (ortholog)	Ectopic overexpression	Maize	Increase in the number of seeds, branches and length of the inflorescence stem

	CYP	*C-22 hydroxylase*	High expression in stem, root and leaves	Rice	Increase in photosynthetic efficiency and grain yield
	GhDET2	The cotton counterpart of *det2* (steroid 5α-reductase)	Specific expression in seed coat	Cotton	Increase in fiber yield and quality
	OsDWARF4	Rice analogue of *dwf4*	Inhibition of gene expression (gene knockout)	Rice	Semi-dwarf, upright leaves, increased biomass and grain yield under dense planting conditions, small seeds
BR signal	*OsBRI1*	Rice analog of BRI1 (BR-receptor)	Co-suppression	Rice	Upright leaves, increased yield potential
	AtBAK1	(BR-coreceptor)	Ectopic overexpression	Rice	Semi-dwarf plants
	UZU (HvBRI1)	Barley analog of BRI1 (BR-receptor)	Single nucleotide change (BR-non-responsive mutant)	Barley	Semi-dwarf, increased yield and lodging resistance
	OsGSK1	Rice analog of BIN2 (BRreceptor)(GSK3/SHAGGY- protein kinase-like, negative regulator of BR signaling)	Inhibition of gene expression	Rice	Increase in abiotic stress tolerance
Accepted steroid regulation	*AtHSD*	11-β-Hydroxysterol dehydrogenase	Ectopic overexpression	*Arabidopsis*	Increased growth, yield and salt tolerance, sustained expression of BR-activated genes

Effect of Brassinosteroids Against Abiotic Stress in Plants

Abiotic stress in plants causes morphological, physiological and molecular changes. The positive contribution of Brassinosteroids in terms of plant resistance to abiotic stress factors has been understood through studies (Divi et al., 2010).

24-epiBL was applied endogenously to the leaves of wheat plants grown under salt stress by spraying method, and the effect of 24-epiBL

on growth and nutrient uptake was investigated. After the application, no significant effect was observed on the uptake of nutrients under salt stress. It was understood that it had a positive effect on biomass and growth (Shahbaz and Ashraf, 2007).

Application of 24-epiBL to drought-stressed tomato plants decreased H_2O_2 content and lipid peroxidation, but increased proline, protein content and antioxidative enzyme activities (Behnamnia et al., 2009).

Dhaubhadel et al., (1999) in the study conducted on tomato and *Brassica napus* seedlings in order to understand the effect of 24-epiBL on plants under abiotic stress conditions, it was understood that the experimental groups of 24-epiBL applications were tolerant to lethal temperature compared to the control groups. It has been shown that under heat stress, 24-epiBL treatments in *Brassica napus* seedlings caused higher accumulation of heat shock proteins than untreated seedlings, which may be due to 24-epiBL inducing the expression of HSPs, which are stress proteins (Dhaubhadel et al., 1999).

Against cold stress, 28-homoBL, which was exogenously applied to pumpkin seedlings, had a significant effect on growth and photosynthesis in seedlings compared to the control group (Fariduddin et al., 2011).

In the study conducted to understand the effects of brassinosteroids against multiple abiotic stresses, 24-epiBL application increased drought and cold stress tolerance in Arabidopsis thaliana and Brassica napus. It prevented seed germination inhibition caused by salt stress. Analysis of marker genes showed that 24-epiBL increased the tolerance of plants against multiple abiotic stress conditions (Kagale et al., 2007).

In a study conducted by Xia et al., (2009) to investigate the abiotic stress tolerance of Brassinosteroids in plants, they showed a positive correlation between the photooxidative tolerance, cold stress, and the negative effects of cucumber mosaic virus of endogenously applied Brassinosteroids in the *Cucumis sativus*.

In *Sorghum vulgare*, 28-homoBL and 24-epiBL were applied to seedlings under osmotic stress. As a result of the research, positive

effects were observed on seed germination percentage and seedling development of soluble protein and proline content (Vidya and Rao, 2003).

Effects of Brassinosteroids on Heavy Metal Stress in Plants

Application of 24-epiBL in *Chlorella vulgaris*, in a study investigating the effects of copper, lead, cadmium, and zinc stress on growth and heavy metal accumulation in cells, it was concluded that it had an anti-stress effect on plants contaminated with heavy metals (Bajguz, 2000).

Cadmium stress was applied to 60-day-old seedlings of *Brassica juncea*. In stressed seedlings, the development of 28-homoBL and changes in photosynthesis and antioxidative enzyme levels were investigated. As a result of the research, enzyme levels in roots and above-ground organs increased, and it was stated that the negative toxic effect of cadmium on the plant was improved (Hayat et al. 2007).

In mung bean (*Vigna radiata* L.), 24-epiBL and 28-homoBL were applied together or separately to stressed seedlings in a medium prepared with different dose of aluminum concentrations. While the epiBL and homoBL applied seedlings showed normal development, antioxidant enzyme, and proline levels increased in plants to which epiBL and homoBL were applied together (Ali et al., 2008).

24-epiBL was applied to seedlings of *Raphanus sativus* L. grown under cadmium stress. As a result of the application, cadmium caused a decrease in plant growth activities, while 24-epiBL application to the seeds of the same plant showed a reducing impact on the toxic effect on plant development and the healing effect of 24-epiBL was understood (Anuradha and Rao, 2009).

For salinity stress in bean (*Phaseolus vulgaris*) plants; NaCl and $CdCl_2$ were applied and the effect of 24-epiBL was investigated. At the end of the research; in the control group, a significant decrease was detected in plant development, pigment content and fruit yield. In the group to which 24-epiBL was applied, the toxic effect was detoxified and a positive and statistically significant improvement was observed in the parameters (Rady, 2011).

Different concentrations of cadmium (0, 3, 6, 9, 12 mg/kg) and 10-8 M 28-homoBL/24-epiBL were applied to two different tomato cultivars. In the control group plants, some enzyme activities decreased significantly in both cultivars. In the experimental group where homoBL/epiBL applications were made, there was a significant increase in antioxidant enzyme activities and proline content (Hasan et al., 2011).

0.5, 1.0, and 2.0 mM boron was applied to 6-day-old seedlings of mung bean (*Vigna radiata*) for 7 days. After the application, 10-8 M 28-homoBL was applied to the plants by spray method. At the end of the 21st day, growth and biochemical parameters were investigated. In the control group; boron application reduced growth, water content and photosynthetic activities depending on the dose amount. Lipid peroxidation, electrolyte leakage, proline accumulation and antioxidant enzyme activities were found to increase in direct proportion to the amount of boron. As a result of 28-homoBL application to plants under boron stress; growth, water content, photosynthetic activity, catalase, peroxidase, superoxide dismutase and proline amount were increased. It is thought that brassinosteroids can reduce the effects of ionic stress caused by boron thanks to their anti-stress effect (Yusuf et al., 2011).

Brassinosteroids are in the steroid plant hormone group. Brassinolide is the most active Brassinosteroid type in plant tissues and in exogenous applications to young, developing stem tissues of plants; it has been found to positively contribute to growth by controlling cell elongation and division and also to be effective on cell expansion. Brassinosteroids, when applied in micro/nano amounts to plant tissues, interact with other plant hormones such as auxin, cytokinin, and gibberellin groups, increasing the tolerance of plants to abiotic stress and providing significant increases in product yield. In molecular studies, genes induced by brassinosteroids have been identified and it has been understood that almost all of these identified genes play a direct role in plant growth and development. Although brassinosteroids are understood at the molecular scale, how their signal is transferred from the cell surface to the cytoplasm and how they regulate the

activation of many genes is not fully understood. Thanks to ongoing research, answers to many questions will be found shortly.

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CHAPTER 10

THE CYTOTOXIC EFFECTS OF EMAMECTIN BENZOATE ON THE HUMAN CERVICAL CELL CULTURE

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INTRODUCTION

Pesticides are chemicals used in agricultural practices as well as to control pests in domestic and municipal areas. The chemical structures of pesticides are naturally occurring or chemical-based pesticides. Pesticides in the first group use naturally occurring substances to defend against pests, while pesticides in the second group are synthesized from synthetic chemicals (Oskoei et al. 2024). Pesticides in the first group are biochemical, microbial, and plant-incorporated protective pesticides, while pesticides in the second group are divided into four parts as carbamate, organophosphate, organochlorine, and pyrethrin. Pesticides are also classified according to the type of pest they affect. There are different classes such as insecticides used against insects, fungicides used against fungi, and herbicides used against weeds (Hassaan and El Nemr, 2020).

Avermectins, one of the microbial biopesticides, are a substance produced by *Streptomyces avermilitis*, which lives in soil. Avermectins consist of A and B compounds. These compounds have subtypes 1 and 2, and these subtypes also have varieties as a and b. The a variants of subtypes 1 and 2 as major compounds and the b variants of subtypes 1 and 2 as minor compounds provide the formation of eight different homologous compounds. Abamectin, one of the main substances of this group, consists of a mixture of B1 substances. Pesticides belonging to the avermectin group are used as insecticides (Jansson et al. 1997; El-Saber Batiha et al. 2020).

Emamectin benzoate is a semi-synthetic substance produced from abamectin (Figure 1). Emamectin benzoate is used in vegetable fields in the United States and Japan to control lepidoptera species, and in fish cultures in the United Kingdom, Iceland, Finland, Norway, Spain, Chile, Ireland, and the Faroe Islands to control parasites in Atlantic salmon *Salmo salar* (Yen and Lin 2004). It also uses as a nematocide against root nematodes in pepper fields and its effects are quite high (El-Saber Batiha et al. 2020).

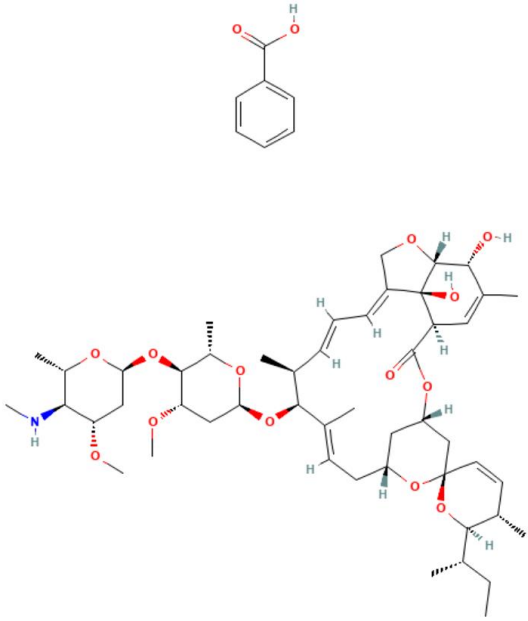

Figure 1. The chemical formula of emamectin benzoate (taken from National Center for Biotechnology Information 2024).

As a result of the intensive use of pesticides in almost every field and the resulting spread of their metabolites into the environment, non-target organisms are also exposed to pesticides (de Almeida Roque 2023). The exposure of humans, one of these organisms, to pesticides is a very worrying situation. According to the UNEP (1993) report, it was reported that pesticides have many effects on humans, such as immune system deficiency, congenital deformities, and cancer. Despite this, studies on the effects of pesticides and their mixtures on humans are still investigated.

Cancer is a disease that causes abnormal cells to grow uncontrollably. Metastasis may occur when this disease occurs in more than one tissue or organ in a living organism (López-López et al. 2023). Cervical cancer, one of the types of cancer, is one of the most common cancers in women. According to WHO, in 2022, approximately 660,000 new cases and 350,000 deaths were reported (Anonymous 2024). There are studies showing the presence of pesticides such as

organochlorine and organophosphate in tissue samples taken from cervical cancer patients (Rodríguez et al. 2017; Liu et al. 2021).

Cell culture studies date back to the last century. The first human cell culture, HeLa, was produced from tissue samples taken from a patient with cervical cancer (Landry et al. 2017) and is one of the most important in vitro models still used today. This study aimed to investigate the cytotoxic effect of the biopesticide emamectin benzoate on humans using HeLa cells.

MATERIALS AND METHODS

Chemicals

The antibiotics used in cell culture studies (penicillin/streptomycin) and Trypsin-EDTA were supplied by Capricon Scientific (Germany). Dulbecco's Modified Eagle Medium (DMEM) (Sartorius, Biological Industries, Germany) was used as cell culture medium. Fetal bovine serum (FBS) (Hyclone, USA) was supplied for cell culture medium. The MTT for cell viability studies was bought from Serva Electrophoresis GmbH (Germany). The insecticide substance examined in the study, emamectin benzoate (95%), was supplied by Hangzhou Xinlong Industrial Co. LTD (China).

Cell Culture

The HeLa was bought from the Republic of Türkiye Ministry of Agriculture and Forestry, Şap Institute, Ankara, Türkiye (Government Registration No: 90061901) (Figure 2). All procedures performed in cell culture were carried out under sterile conditions in a laminar flow chamber. HeLa cell culture was cultured in DMEM including 10% FBS and 1% antibiotics at 37 °C in 5% CO_2. When the cells in the cell flasks were 80%-90% confluent (Figure 3), they were trypsinized using 0.05% Trypsin-EDTA to allow them to grow before proceeding to the next stage of the experiment (Figure 4).

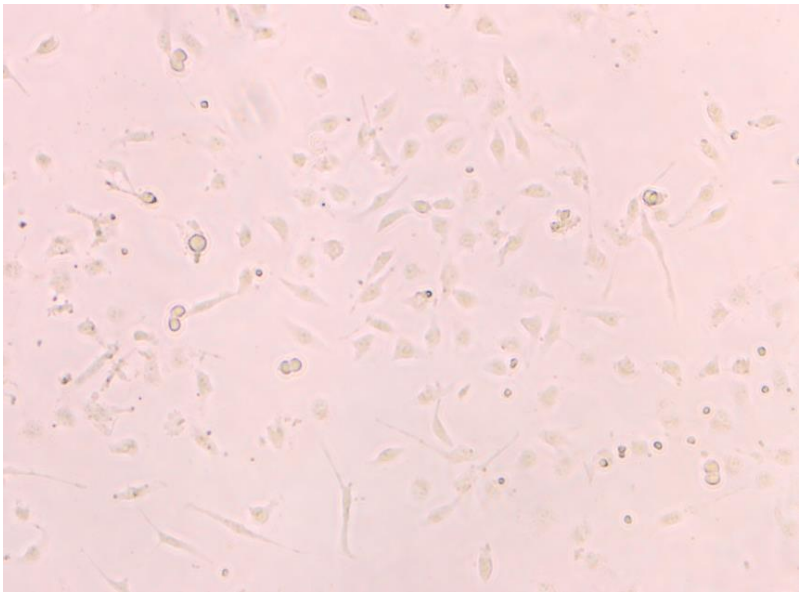

Figure 2. The HeLa cell line (taken from authors)

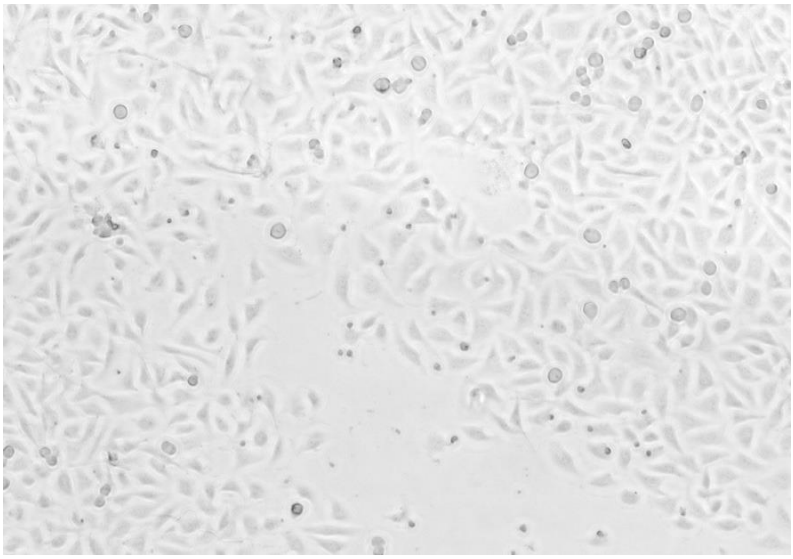

Figure 3. The 80% confluent HeLa cells (taken from authors)

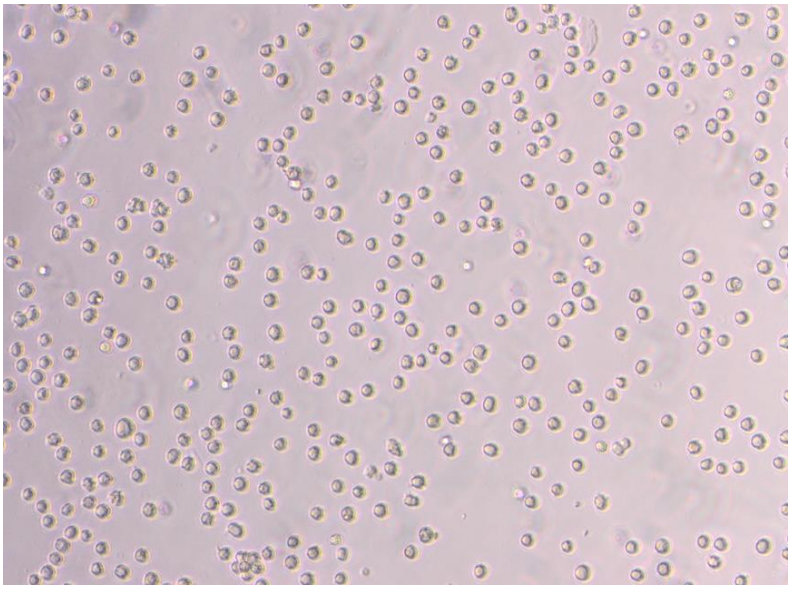

Figure 4. The trypsin-removed HeLa cells (taken from authors)

Cell Culture Exposure

HeLa cells growing in cell culture flasks were lifted with trypsin when they were 80-90% confluent and seeded into 96-well plates at 10^4 cells/well and allowed to adhere for 24 hours. Different concentrations of emamectin benzoate were dissolved in 0.1% DMSO. The cells were exposed to these prepared concentrations for 24 h. This experiment was performed in 3 replicate plates. Each plate contained negative control (cells and DMEM), positive control (cells, 1x triton-X, DMEM) and solvent control (cells, 0.1% DMSO, DMEM).

Cell Viability Assay

After the cells were exposed to the substance, the wells were emptied. 20 µL of MTT (5 mg/mL MTT in PBS) was added to the wells and incubated for 4 hours. Then, 100 µL of DMSO was added to the wells and the plates were read at 540 nm in a microplate reader to calculate the percentage of cell viability. Cell viability was evaluated according to the method of Yurdakök Dikmen (2015). According to the formula of this method, $[1 - (S_{OD} - NC_{OD})/(PC_{OD} - NC_{OD})] * 100$ cell viability was evaluated where S_{OD} means the sample optical density,

PC_{OD} means the positive control optical density, and NC_{OD} means the negative control optical density.

RESULTS AND DISCUSSION

Cell lines used as in vitro systems in the evaluation of pesticide toxicity are one of the important bioindicator models. In this study, we aimed to investigate the toxicity of human cell line HeLa against the avermectin insecticide emamectin benzoate.

In the study, HeLa cells were exposed to emamectin benzoate at concentrations ranging from 1 M to 10^{-8} M for 24 h. Cell viability percentage showed a decrease from high concentration to low concentration (from 78% to 10%) (Figure 5).

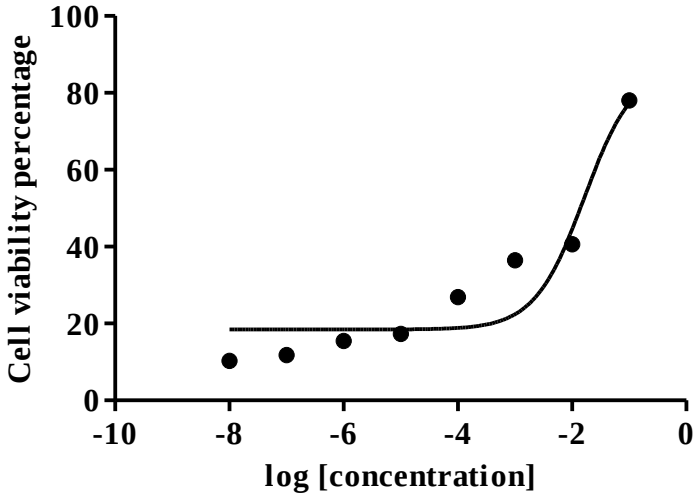

Figure 5. HeLa cell line viability rate in emamectin benzoate application

There are studies on the effects of application concentrations of pesticides in cancer cell culture studies or the effects of active substances and metabolites of pesticides on cell viability. In human hepatoma cell culture, HepG2, a concentration-dependent decrease in cell viability was reported after 24-hour application of difenoconazole, cypermethrin, and triazophos (Wang et al. 2021). In the acute

monocytic leukemia cell culture, THP-1, it was reported that when the active substances of "glyphosate, 2.4-dichlorophenoxyacetic acid, mancozeb, and atrazine and their metabolites aminomethylphosphonic acid, 2.4-dichlorophenol, ethylenethiourea, and desethylatrazine" were applied to the pesticides, there was a decrease in cell viability levels in the metabolite-applied groups after 48 h of exposure compared to the active pesticides, and this decrease was higher after 72 h of exposure (de Almeida Roque 2023). It has been reported that dieldrin application to HeLa cells reduces cell viability and cell size (Sharfi et al. 2023).

In this study, the mean inhibitory concentration (IC_{50}) of emamectin benzoate for 24 h was determined as 0.0164 mM (0.002113 mM - 0.1268 mM for 95% confidence interval) for HeLa cells. The studies investigating the IC_{50} values of pesticides in different cancer cells are given in Table 1. According to this table, the mM concentrations obtained in this study are similar to the literature but also show differences.

Table 1. The cytotoxic values of 24 h exposure of different pesticides on different type of cancer cell lines

Reference	Cell culture	Pesticide	IC_{50}
Wang et al. (2021)	HepG2	Difenoconazole	24.72 ± 4.29 μM
		Cypermethrin	127.88 ± 4.07 μM
		Triazophos	36.99 ± 6.96 μM
Silva et al. (2022)	Caco-2	Imidacloprid	>1000 μM
	HepG2		624 ± 24 μM
	Caco-2	Imazalil	254 ± 3 μM
	HepG2		94 ± 12 μM
Goldar et al. (2024)	HepG2	Chlorpyrifos	180.9 ± 8.3 μM
		Chlorpyrifos - oxon	73.5 ± 5.8 μM
	HK-2	Chlorpyrifos	73.6 ± 7.6 μM
		Chlorpyrifos - oxon	10.6 ± 1.5 μM
This study	HeLa	Emamectin benzoate	0.0164 ± 0.002 mM

CONCLUSION

In this study, the cytotoxic effects of emamectin benzoate, used as a biopesticide, on cervical cancer cell lines were investigated. Although it is a natural compound, it was found that this pesticide reduced cell viability at increasing concentrations and showed high toxicity with the IC value obtained at the mM level.

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CHAPTER 11

THE IMPORTANCE OF THE MEDICINAL MUSHROOM *GANODERMA LUCIDUM*

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INTRODUCTION

With the rapidly growing population, people have been seeking alternative food sources to address emerging food issues. Among these, mushrooms have emerged as an agricultural product with high nutritional value that can be harvested year-round in a controlled environment. Additionally, some mushrooms, which can grow naturally depending on the season and are an important food source for people living in rural areas (Kurt et al., 2019), are commercially valuable as food supplements due to their medicinal properties. Medicinal mushrooms, unlike edible mushrooms, cannot be consumed directly as food but are used in areas such as pharmaceutical applications due to the components they contain.

Both edible and medicinal mushrooms are valuable resources in the food, pharmaceutical, and cosmetic industries due to their nutritional, infection-preventive, stress-relieving, and antioxidant properties. It is widely known that mushrooms have been used as therapeutic agents in traditional medicine throughout history, but making them more bioavailable has become essential considering their benefits for human health (Batra et al., 2013; Sharma et al., 2019).

Furthermore, medicinal mushrooms have attracted considerable research interest due to their biologically active compounds, which include lectins, polysaccharides (β-glucans), polysaccharide-peptides, polysaccharide-protein complexes, lanostanoids, terpenoids, alkaloids, sterols, and phenolic compounds. The potential anticancer, antioxidant, antitumor, anti-inflammatory, antimicrobial, and anti-immunomodulatory properties of these compounds have been extensively studied. Many ongoing studies on medicinal mushrooms have provided evidence for their use in boosting immunity, balancing blood cholesterol, and treating conditions such as cancer, respiratory diseases, insomnia, and anxiety-related stress disorders (Sharma et al., 2019; Liu et al., 1998; Koo et al., 2019; Akihisa et al., 2007).

However, despite all these studies supporting the increased production of these mushrooms, not every mushroom can be cultivated in a controlled environment. Research on this subject continues both in our country and worldwide. In Türkiye, efforts are being made for the cultivation of *Ganoderma lucidum* (Curtis) P. Karst., which is the most widely known and commercially viable species.

This article provides information about *G. lucidum*, one of the most commonly used and cultivated mushroom species globally. It discusses the description, distribution, therapeutic uses, and pharmacological activities of this mushroom.

Reishi Mushroom (*Ganoderma lucidum*) and Its Distribution

Ganoderma lucidum is the most intensively studied species within the genus *Ganoderma* of the *Ganodermataceae* family. Known by different names in various countries, it is called 'Reishi,' meaning 'spiritual strength' in Japan; 'Lingzhi,' meaning 'divine mushroom' in China; and 'Youngzhi,' meaning 'immortality mushroom' in Korea. In these countries, *G. lucidum* has been used in traditional medicine for many years, believed to offer health benefits. However, research in Western countries is relatively recent compared to Asia (El Sheikha, 2022; Seng, 2014; Jiang et al., 2017; Ahmad et al., 2022; Ekiz et al., 2023).

Among medicinal mushrooms, *G. lucidum* offers notable advantages compared to chemical medications. The term '*Ganoderma*' originates from Greek, where 'Ganos' translates to 'shining' and 'derma' means 'skin.' This mushroom was named *Ganoderma* by mycologist Petter Adolf Karsten in 1881 (Karsten, 1881). Before this, it had been classified as *Boletus lucidus* by Curtis in 1781 and subsequently as *Polyporus lucidus* (Curtis) Fr. in 1821 (Karsten, 1881). A revision of the genus Ganoderma in 1889 identified 48 species. Prior to Murrill's research in North America in 1902, most studies had concentrated on European species like G. *lucidum*, *G. resinaceum* Boud. (1890), and *G.*

valesiacum Boud. (1895) (Murril, 1902; Patouillard, 1989; Atkinson, 1908).

Chinese edible and medicinal mushrooms have identified *G. lucidum* as the scientific name for 'Lingzhi.' *Ganoderma lucidum* is a fungus that falls under the family *Polyporaceae* (or *Ganodermaceae*) within the class Basidiomycetes. It typically grows on decaying wood, and various parts of the fungus—including its mycelia, spores, and fruiting body—are consumed and available in different forms such as powders, capsules, tea, and coffee (Bijalwan et al., 2020)

Figure 1. *Ganoderma lucidum* (Curtis) P. Karst. (Bal, 2019)

Advancements in molecular biology have led to changes in the classification of *Ganoderma* species. For instance, *G. lucidum*, which is extensively cultivated and commercially utilized in China, is now classified as a new species known as *G. lingzhi*. Presently, there are 131 recognized species of *Ganoderma* worldwide (Dai et al., 2013). This genus is predominantly found in tropical regions, encompassing areas

in Africa, the Americas, Asia, and subtropical regions, as well as in Europe (Figure 2). (Zhou et al., 2016; Sun et al., 2020).

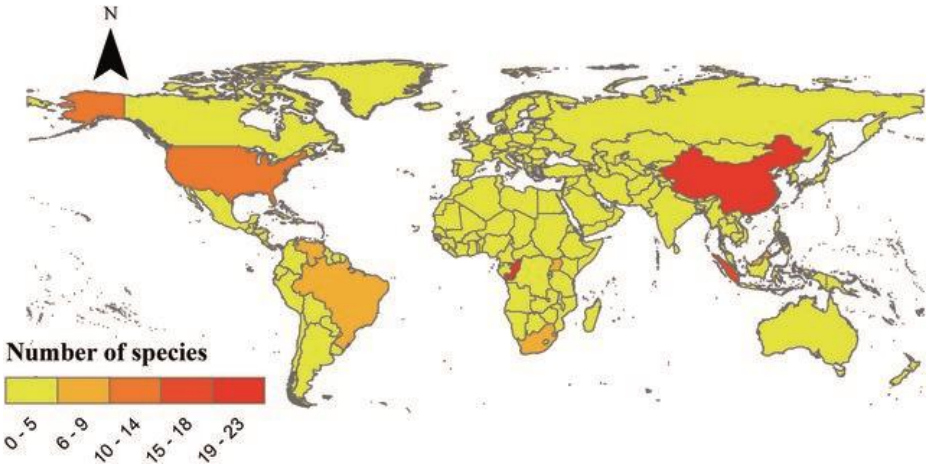

Figure 2. The map showing the global distribution of Ganoderma (Wang et al., 2020)

Development of *Ganoderma lucidum*

Ganoderma lucidum grows in limited amounts in the wild, requiring several months to complete its fruiting cycle. The mushroom typically has a fan-shaped, kidney-shaped, or semicircular structure, displaying colors ranging from dark red to reddish-brown or reddish-black, with yellow or earthy tones more pronounced at the edges. The flesh of the mushroom generally varies in color from yellowish-brown to dark brown (Dong et al., 2021; Ekiz et al., 2023).

Due to its thick, smooth surface and a fruiting body that is corky and hard rather than fleshy, this species is considered inedible (Ćilerdžić, 2011). It is one of the most extensively studied mushrooms, particularly in traditional Chinese medicine (Baby et al., 2015). In recent years, around 23 species within the *Ganoderma* genus have been thoroughly researched for their diverse biological activities, with *G. lucidum* being the most prominent among them. This research highlights the various colors and uses of *G. lucidum* (Ekiz et al., 2023).

Cultivation of *Ganoderma lucidum*

Cultivation of *Ganoderma lucidum* has become crucial due to the inconsistent quality found in nature and the rising demand from industries such as food service, pharmaceuticals, cosmetics, and health products. Active compounds have been extracted from the fruiting bodies, mycelia, and spores of *G. lucidum*. Typically, the fruiting bodies of *G. lucidum* are grown on hardwood logs, stumps, and sawdust (Ćilerdžić et al., 2011, Figure 3).

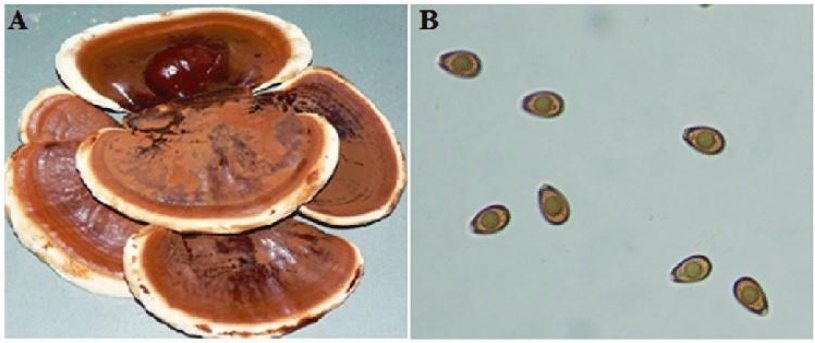

Figure 3. The fruiting bodies (A) and spores of *Ganoderma lucidum* (B) (Karadeniz et al., 2013)

The spores of the mushroom are brown. The optimal temperature requirement for mycelium development is 30-32°C, and the mycelium development period is 25-30 days. During the fruiting stage, an optimal temperature of 30-32°C, 80-85% humidity, light, and ventilation are required. Mushrooms can be harvested in 2-3 cycles, after which the entire cycle is repeated. The total cultivation cycle lasts 120-150 days. Since this mushroom has a woody structure, it can be dried and stored for several months and can be marketed in powdered form. As Reishi mushroom is a plant pathogen, extreme caution must be taken when disposing of the used mushroom substrate. Burning the used substrate may be recommended to prevent its spread to other trees (Anonymous 1, 2024).

The artificial cultivation of *G. lucidum* takes a long time and is sensitive to environmental conditions. Liquid and solid-state fermentation are popular for mycelium production (Zhou et al., 2012). The main cultivation methods for the production of *G. lucidum* (fruiting body and mycelium) are presented in Figure 3.

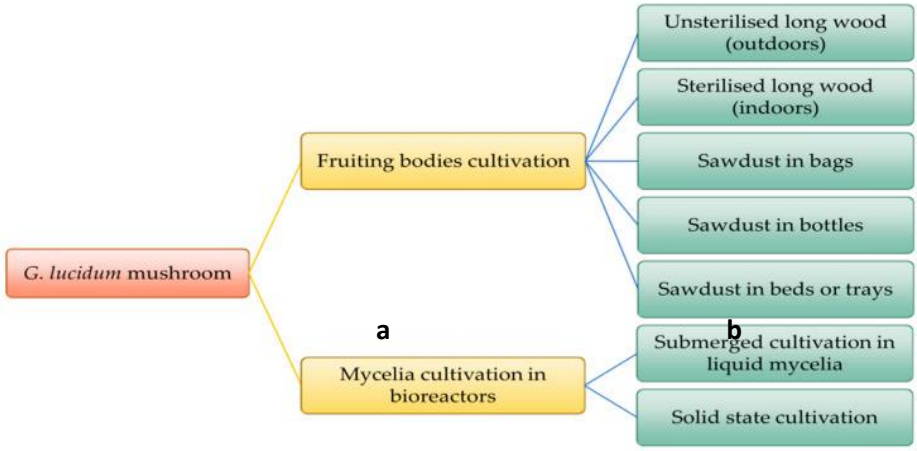

Figure 3. Different cultivation methods for the fruiting body and mycelium of *Ganoderma lucidum* (Boh et al., 2007; Cadar et al., 2023)

Since *G. lucidum* is rarely found in nature, it is cultivated in greenhouses using two methods: on wooden logs for fruiting bodies or in sawdust bags (or large plastic bottles) (Anonymous 1, 2024; Figure 4) (https://www.hayger.com/reishi-mantari-ganoderma-lucidum.html 2024).

In sawdust cultivation, hardwood (oak, beech, hornbeam) sawdust is filled into heat-resistant bags, and the openings of the bags are closed with cotton plugs. After this process, the bags are sterilized in an autoclave at 121 °C for 1.5 hours. Once the temperature of the bags drops to 20-25 °C, mycelium inoculation is carried out in sterilized rooms. The inoculated bags are placed in a mushroom production room, where they are kept in darkness at 25-30 °C to allow for mycelium wrapping. The room should be illuminated with 100-200 lux light to encourage the formation of carpophore in bags that have completed mycelium development (Chen, 2004). The tops of bags

showing primordium are opened, and the room is regularly humidified and ventilated, adjusting the room temperature to 25±2 °C. After primordia are observed, the upper parts of the bags are cut open to promote mushroom development and emergence (Yakupoğlu, 2007; Anonymous 1, 2024).

Figure 4. Cultivation of sawdust in bags (Anonymous 2, 2024, Accessed 2024, https://www.dxnproducts.co.za/what-is-ganoderma-lucidum/)

In the log method, oak logs are cut into 15 cm long and 15 cm in diameter. 6-10 holes are drilled into the logs, and the mycelium is placed into these holes, which are then sealed with beeswax. The inoculated logs are kept in a room at 25 °C and 70% humidity for 6 months (Figure 5). After the mycelium has wrapped around the logs, they are buried in soil enriched with organic matter in a greenhouse, with the temperature set to 25-30 °C and humidity to 70-90%. Direct sunlight should be blocked from entering the greenhouse, and care should be taken for proper ventilation. It is essential to harvest the product at the right time and store the harvested product in a cool, dark, and stable (unchanging) place (Yakupoğlu, 2007; Anonymous 1, 2024).

Figure 5. Production of *Ganoderma lucidum* (Anonymous 3, 2024, Accessed 2024, https://www.fungus-extract.com/product-detail/ganoderma-lucidum-beta-glucan/)

Chemical Structure of *Ganoderma lucidum*

G. lucidum is a significant medicinal mushroom due to its diverse biologically active chemical compounds, which are used to treat various ailments as well as in health tonics, cosmetics, and other applications. Numerous studies have highlighted the therapeutic benefits of this mushroom. Comprising roughly 90% water, the remaining 10% of its dry matter contains a wealth of nutrients: 10-40% protein, 2-8% fat, 3-28% carbohydrates, 3-32% fiber, and 8-10% ash, along with essential minerals like calcium, phosphorus, potassium, copper, iron, zinc, magnesium, and selenium based on fresh weight (Bijalwan et al., 2020).

Chemical Composition of *Ganoderma lucidum*

Researchers have identified over 400 bioactive compounds from different parts of *G. lucidum*, including its fruiting bodies, spores, and mycelium. These compounds comprise polysaccharides, triterpenoids, nucleotides, steroids, fatty acids, and other trace elements (Bijalwan et al., 2020). The mushroom is also rich in bioactive components such as glycoproteins, phenols, terpenoids, steroids, and nucleotide derivatives. The proteins found in *G. lucidum* are notable for providing all essential amino acids, with high levels of lysine and leucine. In addition, the mushroom's relatively low fat content, alongside a greater proportion of polyunsaturated fatty acids, contributes to its health-enhancing properties (Sanodiya et al., 2009).

These bioactive substances are known for their pharmacological benefits, including antibacterial, antiviral, antitumor, immunomodulatory, anti-aging, sleep-promoting, and ulcer-preventing effects. Research suggests that chloroform extracts from *G. lucidum* may reduce the formation of free radicals and prevent their degradation (Bijalwan et al., 2020).

The key active compounds in *G. lucidum* include polysaccharides, triterpenes, and peptidoglycans (Boh et al., 2007). However, the concentration of these compounds can differ between natural and commercial products. A study of 11 commercially available *Ganoderma* products in Hong Kong showed that triterpene content ranged from undetectable levels to 8%, while polysaccharide levels were between 1% and 6%. These variations are likely due to differences in cultivation and production methods among mushroom species and strains (Chang and Buswell, 2008).

Ganoderma lucidum has been shown to have therapeutic effects on a range of medical conditions, including liver inflammation (hepatitis), high cholesterol, diabetes, tumors, immune deficiencies, low white blood cell counts, atherosclerosis, hemorrhoids, chronic fatigue, sleep disorders, dizziness due to nerve weakness, as well as

cancer, bronchitis, and high blood pressure (Bao et al., 2001; Fujita et al., 2005; Gao et al., 2002; Hajjaj et al., 2005; Lu et al., 2003; Sliva, 2003). The medicinal value of this mushroom is believed to stem from its wide variety of bioactive components. The majority of biologically active substances identified in *G. lucidum* belong to two main categories: lanosterol derivatives (including ganoderic acids and similar compounds) and polysaccharides (Cole and Schweikert, 2003; Paterson, 2006). Additionally, some studies have identified peptides and low molecular weight proteins (Sripuan et al., 2003; Sun et al., 2004; Wang and Ng, 2006). Research has highlighted that water-based extracts of *G. lucidum* are particularly effective in suppressing sarcoma development, while nonpolar extracts do not exhibit the same effect (Jones and Janardhanan, 2000; Lu et al., 2003; Trigos and Medellín, 2011).

Over 200 polysaccharides have been isolated from *G. lucidum*, known for their antitumor and immune-boosting properties (Baby et al., 2015; Xia et al., 2014). The primary bioactive polysaccharides in *G. lucidum* include D-glucans with β-1-3 and β-1-6 glycosidic bonds (Sone et al., 1985; Yuen and Gohel, 2005). It is generally understood that the antitumor properties of *Ganoderma* polysaccharides result primarily from their ability to enhance immune system function, rather than through direct destruction of cancer cells (Lin and Zhang, 2004).

A protein named LZ-8, known for its mitogenic properties, has been isolated from the mycelium of *G. lucidum*. This polypeptide is composed of 110 amino acids and has an acetylated amino terminal, with a molecular weight of 12 kDa (Paterson, 2006).

In a study by Sliva (2003), it was demonstrated that the spores or dried fruiting bodies of *G. lucidum* resulted in significant mortality in human breast and prostate cancer cells, suggesting potential for cancer therapy.

El-Mekkawy et al. (1998) explored the anti-HIV effects of various compounds derived from the fruiting bodies of *G. lucidum*.

Their findings showed that Ganoderiol F and ganodermanontriol exhibited anti-HIV activity, whereas ganoderic acid alpha, ganoderic acid B, ganoderiol A and B, ganoderic acid C1, 3 beta-5 alpha-dihydroxy-6 beta-methoxystigmasterol-7,22-dien, and ganoderic acid H showed moderate efficacy. Additionally, Gao et al. (2002) investigated the effects of Lucidenic acid O and lucidenic lactone from *G. lucidum* fruiting bodies on HIV. Alcohol and water extracts of *G. lucidum* were found to lower blood sugar levels in diabetic mice, with Xia et al. (2014) confirming the hypoglycemic properties of polysaccharides from this mushroom, while Lin and Zhang (2014) reported similar results.

Kurtipek et al. (2016) indicated that polysaccharides found in *G. lucidum* exhibit anti-inflammatory effects and suggested that this mushroom could be a promising option for managing inflammatory skin conditions (e.g., dermal sarcoidosis). Furthermore, numerous studies have highlighted the antimicrobial effects of *G. lucidum* against a variety of gram-positive and gram-negative bacteria. This mushroom also shows antifungal, anti-allergic, anti-angiogenic, anti-ulcerogenic, anti-mutagenic, and anti-proliferative activities, along with cardiovascular and hepatoprotective benefits (Bijalwan et al., 2020).

The diverse effects of the components in this mushroom have also been supported by various studies, as summarized in Figure 6 by Wu et al. (2024).

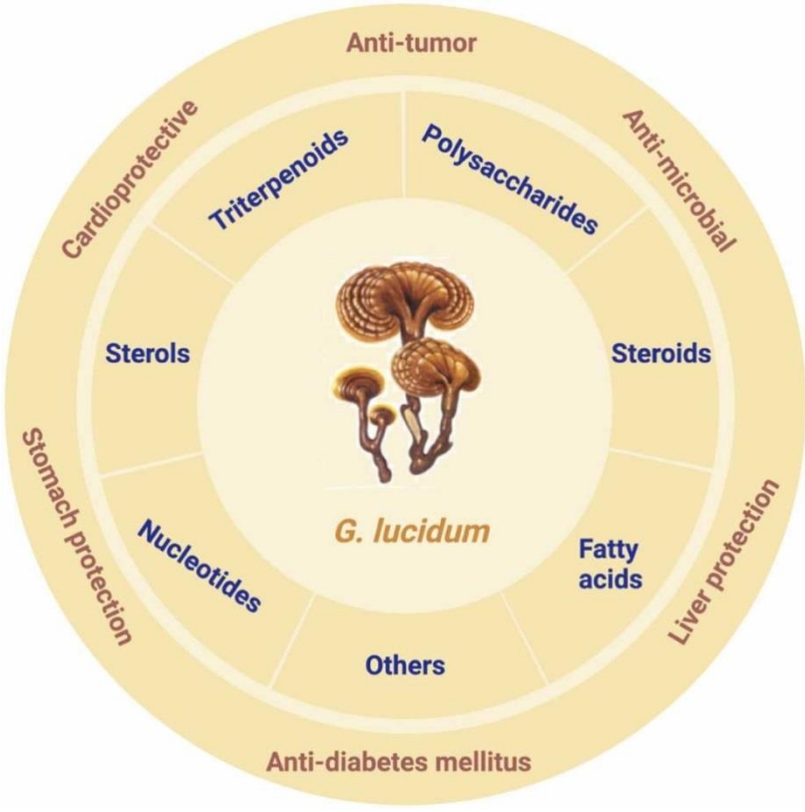

Figure 6: A graph summarizing the effects of *Ganoderma lucidum*'s content on human health (Wu, 2024)

DISCUSSION

Ganoderma lucidum is a renowned herbal remedy in Asia and has gained substantial popularity globally as a nutritional supplement. The demand for *G. lucidum* has led to a rise in patented dietary supplements available on the market, including various formulations of extracts and isolated components offered in the form of capsules, creams, hair tonics, and syrups.

In recent years, the *Ganoderma* genus has been the focus of extensive research aimed at uncovering new therapeutic metabolites. The majority of valuable medicinal compounds identified in these

studies have been lanosterol derivatives (ganoderic acids and related compounds) or polysaccharides. Lanosterol derivatives exhibit significant biological activities, including cytotoxic effects against various cancer cell types, anti-inflammatory and antiviral properties, hepatoprotective effects, and the inhibition of 5α-reductase and cholesterol biosynthesis. Conversely, polysaccharides are recognized for their ability to enhance immune function and act as free radical scavengers. However, as one of the largest genera within the *Ganodermataceae* family, there remains a wealth of topics yet to be explored.

As *G. lucidum* continues to gain traction, numerous studies are being conducted to analyze its composition, cultivation methods, and documented effects. Data suggests it offers various health benefits, including anticancer properties. However, most research has been performed using animal models or cell cultures, with human experimental studies typically being limited in scale and not always aligning with in vitro findings. To determine the validity and significance of the numerous chemical data and reported health claims associated with *G. lucidum*, reliable experimental and clinical data from well-designed human trials is essential. Challenges, such as dosage variability and production quality, complicate this process. There is a need for strategies that focus on quality control procedures to standardize and identify *G. lucidum* preparations, which will aid in elucidating the mechanisms of action and characterizing the active components of this medicinal mushroom (Irkin, 2024).

In the global market, over 90 *G. lucidum* products have been registered and are actively marketed (Irkin, 2024; Lin, 2000). It is estimated that worldwide consumption reaches thousands of tons, with the market experiencing rapid growth. While recent data detailing the overall market value of these products is scarce, various commercial sources reported an estimated total annual market value of $1.628 million in 1995 (Irkin, 2024; Chang and Buswell, 1999).

Although substantial evidence supports the healing potential of certain compounds found in many species of this genus, it does not imply that every *Ganoderma* mushroom is a universal remedy. Firstly, the specific conditions necessary for producing compounds that positively impact human health are still unknown (this is also true for many secondary metabolites), which means they may be absent in certain preparations. Secondly, as Paterson (2006) has pointed out, the toxicology of each mushroom species has received minimal attention. Nonetheless, continued myco-chemical and pharmacological research will likely enhance our understanding of these issues and promote their safer use.

In Türkiye, products imported from these regions have been approved by the Ministry of Health in various formats. Packaged products, including nutritional supplements, teas, coffees, and even creams and toothpaste, are gaining considerable traction in Türkiye, similar to many other markets. Compounds such as beta-glucans, polysaccharides, and triterpenes from the mushroom are being utilized for effective treatment of metabolic diseases. Particularly, these food products, presented as alternative therapies to conventional cancer treatments, show promise for conditions that currently appear challenging to manage (Irkin, 2024).

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DÇ; Investigation SAŞ; Conceptualization, Investigation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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CHAPTER 12

CELLULAR ADAPTATIONS TO NON-LETHAL INJURY

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INTRODUCTION

Adaptation refers to reversible changes in a cell's phenotypic, metabolism or activities in response to environmental changes. Various distinct cell types can survive through adaptation, even in the absence of complete recovery, when faced with naturally occurring or non-lethal, recurrent, or chronic injury. However, the extent of this response varies depending on the specific type of cell, as not all cells are capable of expressing every possible response (Kumar, et al., 2017; Miller and Zachary, 2017; Silbernagl, et al., 2010).

Physiological adaptations typically represent the responses of cells to normal stimuli, such as those induced by hormones or endogenous chemical mediators.

Pathological adaptations refer to responses to stress that allow cells to modify their structure and function, thus avoiding injury (Kumar et al., 2017; Miller and Zachary, 2017; Silbernagl et al., 2010). These adaptations can take several different forms (Figure 1).

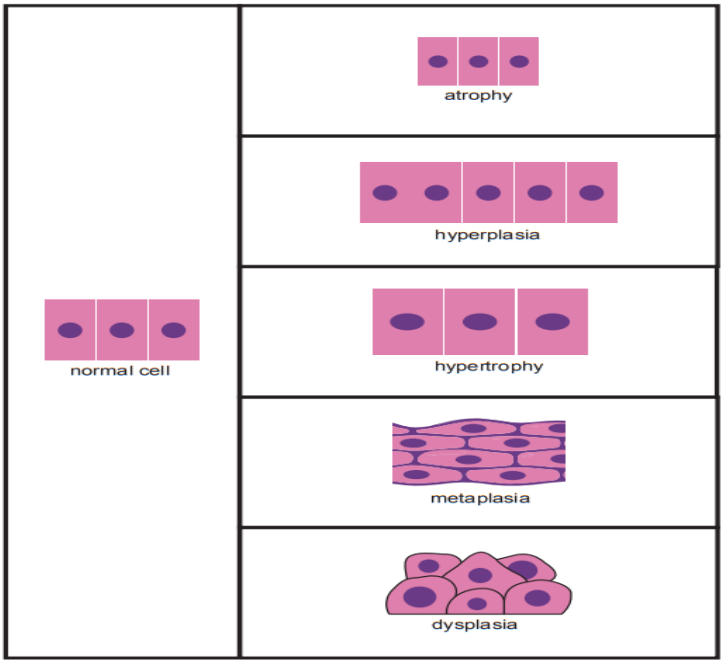

Figure 1. Adaptive changes and dysplasia in epithelial cells (Graphic Artist:Kader Horasan, 2024)

Hypertrophy

Hypertrophy is characterized by an increase in cell size, which subsequently leads to an increase in organ size. The enhancement of tissue mass is primarily attributed to the enlargement of parenchymal cells rather than that of stromal cells or leukocytes. In the process of hypertrophy, no new cells are generated; instead, the cellular volume increases due to the accumulation of structural proteins and organelles. This process differs from cellular swelling, which results from a loss of volume control or enlargement due to the accumulation of endogenous or exogenous substances (Kumar et al., 2017; Miller and Zachary, 2017; Silbernagl et al., 2010).

Hypertrophy is predominantly observed in tissues where cells lack the capacity for replication, such as cardiac and skeletal muscle. Hyperplasia is an adaptive response in cells that can replicate, resulting in increased cell numbers. Hypertrophy and hyperplasia can develop concurrently, leading to organ expansion.

Hypertrophy can be classified as either physiological or pathological. It may arise in response to increased functional demand or stimulation by growth factors and hormones. An illustrative example of physiological hypertrophy is the enlargement of the uterus during pregnancy, which is driven by estrogen-induced smooth muscle hypertrophy and hyperplasia. Conversely, skeletal and cardiac muscle can only undergo hypertrophy in response to heightened functional demand, such as that induced by exercise or elevated blood pressure, due to their limited capacity for cell division (Miller and Zachary, 2017; Riede and Werner, 2004; Kumar et al., 2017; Silbernagl et al., 2010).

Pathological hypertrophy is exemplified by cardiac hypertrophy resulting from hypertension or aortic valve disease. This type of hypertrophy is triggered by mechanical factors, such as stress, along with hormonal and growth factor signals that promote cellular growth. These stimuli activate various cellular proteins and gene pathways, culminating in the increased synthesis of myofilament proteins and, ultimately, more powerful contractions with each heartbeat.

While hypertrophy initially enhances the functional capacity of the muscle, prolonged hypertrophy can lead to decompensation. For instance, the myocardium may undergo fibrous stromal expansion or experience reduced vascular perfusion, resulting in the degeneration of myocardial fibers and, ultimately, heart failure (Hidayat, 2023; Kumar et al., 2017; Miller and Zachary, 2017; Riede and Werner, 2004; Silbernagl et al., 2010; Tsuda and Robinson, 2024).

Hyperplasia

Hyperplasia can occur concurrently with hypertrophy in cell populations capable of replication, in response to increased functional demands. Many epithelial cells, such as hepatocytes, epidermal cells, and intestinal mucosal epithelia, exhibit rapid hyperplasia in response to hormonal stimulation, inflammation, or physical injury. The hyperplasia of glandular epithelium, such as that observed in the thyroid follicular epithelium, may lead to a marked enlargement of the thyroid gland (Hidayat, 2023; King, 2006; Miller and Zachary, 2017; Silbernagl et al., 2010).

Tissues such as striated muscle and the nervous system possess negligible proliferative capacity and generally do not undergo hyperplasia. In contrast, other tissues, including smooth muscle, bone, and cartilage, exhibit moderate proliferative capacity.

Hyperplasia can be classified as either physiological or pathological. In both instances, cellular proliferation is driven by growth factors. Growth factors induce cell proliferation in both cases (Kumar, et al., 2017; Silbernagl et al., 2010).

Two major kinds of physiological hyperplasia are identified:

1. Hormonal hyperplasia is characterized by the growth of glandular epithelium in female breasts throughout pregnancy and adolescence.
2. Compensatory hyperplasia**;** which occurs when a portion of an organ is excised or lost prompting the remaining tissue to undergo growth.

For instance, after partial liver resection, mitotic activity in the residual hepatocytes commences promptly, and within approximately

12 hours, the liver typically returns to its normal weight. Hepatocytes and stromal cells release growth factors that stimulate hyperplasia, and once liver mass is restored, cellular proliferation is inhibited by various growth suppressors (Hidayat, 2023; Miller and Zachary, 2017; Riede and Werner, 2004).

Pathological hyperplasia may arise from excessive hormonal stimulation or aberrant growth factor signaling. An imbalance between estrogen and progesterone can result in endometrial hyperplasia, a prevalent cause of abnormal menstrual bleeding. Similarly, hyperplastic goiter develops as the thyroid gland compensates for iodine deficiency by enlarging in an effort to produce thyroid hormones.

Additionally, fibroblast and endothelial cell hyperplasia is observed during wound healing, where connective tissue cells proliferate in response to injury to facilitate repair of damaged areas. Leukocytes and other cells at the injury site secrete growth factors that promote the hyperplastic process.

Hyperplasia associated with certain viral infections may also be influenced by growth factors encoded by viral genes. For example, papillomaviruses induce the formation of warts on the skin and mucosal surfaces through hyperplastic mechanisms (Silbernagl et al., 2010).

It is crucial to note that hyperplastic processes are typically regulated. If the signals that initiate hyperplasia are removed, the hyperplastic state will subside. Pathological hyperplasias differ from neoplastic processes because, in cancer, the regulatory mechanisms governing cellular growth become deregulated or ineffective.

Nevertheless, pathological hyperplasia may sometimes create a conducive environment for cancer development; individuals with endometrial hyperplasia, for instance, are at an elevated risk of developing endometrial cancer (Hidayat, 2023; Riede and Werner, 2004).

Atrophy

Atrophy refers to a reduction in cell size resulting from the loss of cellular substance. It is distinct from hypoplasia, which describes

tissues or organs that are smaller than normal due to incomplete development. Aplasia denotes the complete absence of a tissue or organ. In atrophic tissues, the shrinkage is attributed to a decrease in either cell size or cell number. When a large number of cells are damaged, the tissue or organ may shrink in size (Silbernagl et al., 2010).

Autophagy and apoptotic cell death contribute to the loss of cell mass in atrophic organs, leading to cell shrinkage or cellular demise, respectively. Histologically, the predominant cells in atrophic tissues appear smaller and exhibit minimal or absent mitotic activity. Ultra-structurally, atrophic cells contain fewer mitochondria and organelles (Hidayat, 2023).

The causes of atrophy are multifaceted and may include reduced workload (e.g., immobilization of a limb to facilitate bone healing), loss of innervation, decreased blood flow, inadequate nutritional intake, loss of endocrine stimulation, pressure atrophy (e.g., compression caused by neoplasms or other masses), and aging.

While some stimuli may be physiological (e.g., loss of hormonal stimulation during menopause), others may be pathological (e.g., denervation). Despite these differences, the underlying cellular alterations exhibit similarities. The overarching goal is to decrease cell size to a level that permits survival, thereby establishing a new equilibrium among cell size, blood flow, and available nutrients or trophic signals (Riede and Werner, 2004).

A combination of a decrease in protein synthesis and an increase in protein breakdown is one of the mechanisms generating atrophy. Because to a drop in metabolic activity, there is a decrease in protein synthesis and an increase in protein breakdown through the ubiquitin-proteasome pathway. In instances of disuse or nutrient deficiency, this pathway targets proteins for degradation within proteasomes. This mechanism is also implicated in cancer-associated cachexia.

Most forms of atrophy are characterized by an increase in autophagic vacuoles, a process through which cells degrade their own components in response to nutrient deprivation (Hidayat, 2023; King,

2006; Miller and Zachary, 2017; Riede and Werner, 2004; Silbernagl et al., 2010).

Metaplasia

Metaplasia is defined as a reversible change wherein one differentiated cell type, whether epithelial or mesenchymal, is replaced by another cell type that is better adapted to withstand adverse environmental conditions. In this form of cellular adaptation, a cell that is sensitive to stress is substituted by a cell type that possesses enhanced capabilities for survival in the altered environment (Silbernagl et al., 2010; King, 2006).

This alteration is thought to be caused by the reprogramming of stem cells to develop along an alternative phenotypic pathway. A classic example of epithelial metaplasia is the substitution of respiratory epithelium with stratified squamous epithelium in smokers. In the trachea and bronchi, the ciliated columnar epithelium is replaced by squamous cells that exhibit greater resilience against the harmful chemicals present in cigarette smoke. However, while the metaplastic squamous epithelium is more resistant, it forfeits critical protective mechanisms, such as mucus secretion and ciliary clearance (Hidayat, 2023).

As metaplastic changes persist or become permanent, the affected epithelium may become predisposed to malignant transformation. Squamous metaplasia is frequently associated with the development of squamous cell carcinoma of the lung. Notable risk factors for metaplasia include smoking, vitamin A deficiency (essential for normal epithelial differentiation), chronic inflammation, hormonal imbalances, and trauma.

Another instance of epithelial metaplasia is the transformation of normal stratified squamous epithelium in the lower esophagus into gastric or intestinal-type columnar epithelium, a condition often linked to chronic gastroesophageal reflux disease (GERD). Likewise, squamous metaplasia may be observed in the urinary bladder epithelium following trauma caused by kidney stones.

Metaplasia can also occur in mesenchymal cells; however, this form is typically a response to pathological changes rather than a genuine adaptive response to stress. For example, new bone formation may occur at sites of soft tissue injury (Hidayat, 2023; Miller and Zachary, 2017; Riede and Werner, 2004; Silbernagl et al., 2010; King, 2006).

Dysplasia

Dysplasia is classified as a form of atypical hyperplasia rather than a true cellular adaptation. Dysplastic changes are predominantly observed in epithelial tissues. The World Health Organization (WHO) defines dysplasia as a lesion characterized by the replacement of a portion of the epithelial thickness with cells exhibiting varying degrees of atypia (Silbernagl et al., 2010).

Dysplasia is generally associated with somatic genetic alterations, which increase the likelihood of cells progressing to malignant tumor formation. It can manifest on mucosal surfaces in response to chronic injuries, such as chemical damage to the bronchi in smokers or viral damage to the uterine cervix (Kumar et al., 2017; Vooijs et al., 2008).

Microscopically, atypical characteristics of dysplastic epithelial cells include hyperchromatic nuclei, increased nuclear size (karyomegaly), abnormal variations in cell size and shape (anisocytosis and poikilocytosis), and an increased number of mitotic figures, which may appear normal but are elevated in frequency (Miller and Zachary, 2017; Kumar et al., 2017)

In the epidermis, dysplasia is characterized by atypical keratinocytes that display abnormal size, shape, and staining properties, along with disorganized polarity. Although dysplasia is considered reversible and does not equate to cancer, it can represent early neoplastic changes. Distinguishing dysplasia cytological from well-differentiated carcinoma can be challenging. When dysplastic changes penetrate the basement membrane, the lesion is referred to as a pre-invasive neoplasm, or carcinoma in situ (Riede and Werner 2004; Becker et al., 2024; Tilakaratne et al., 2019).

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CHAPTER 13

BIRD PELLETS

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INTRODUCTION

Birds, known as Aves, are creatures that belong to the class of flying vertebrates and are generally known for their feathers, light skeletal structures and egg-laying reproduction (BridLife International, 2011).

Features

- Feathers: Feathers that cover the entire body are important in situations such as maintaining body temperature, ease of flight, swimming, courtship behavior and camouflage.
- Flight: Their front legs are shaped like wings. With the exception of flightless birds such as ostriches and penguins, all other species are capable of flight.
- Egg laying: Female birds lay nutrient-rich, hard-shelled eggs. Birds also have specialized behaviors such as incubation and care of the young.
- Nutrition: They have to eat regularly due to their high-speed metabolism, and their beaks have different structures and features modified according to their feeding habits. Different bird species feed on a variety of foods, from insects to seeds, fruits and carnivorous prey (Eduardo et al., 2010).

Groups

- **Birds of prey**: These are birds of prey whose primary food source is usually mammals, reptiles and other small birds. They have keen eyesight and/or hearing. They catch their prey with their strong claws and eat it with the help of their curved and hooked beaks. Many species prefer to hunt live prey, but the food source of species such as vultures is carrion. Birds of prey observed in Turkey are taxonomically divided into 3 different orders Accipitriformes, Falconiformes and Strigiformes. Species such as eagles, buzzards, harriers, falcons, kites, hawks and vultures in the orders Accipitriformes and Falconiformes are known as "diurnal birds of prey", while owl species in the order Strigiformes are known as "nocturnal birds of prey (Figure 1) (Perrins and

Middleton, 1984; Burton, 1989; Fowler et al., 2009; Benzeyen and Okur, 2023).

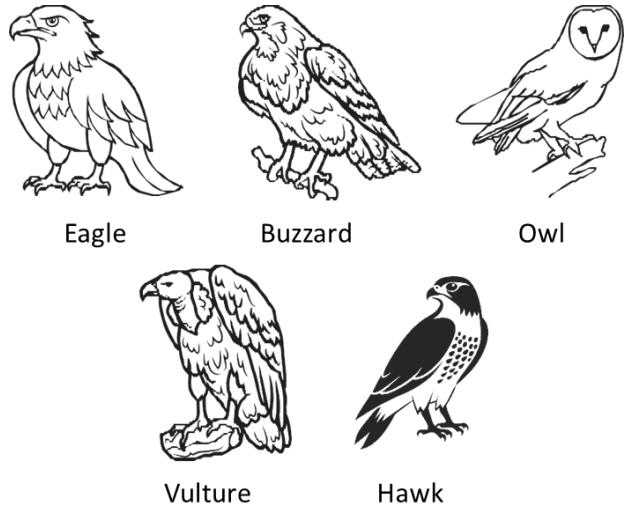

Figure 1: Some species of Raptor birds (illustration editing: Merve Seyfe, 2024)

- **Shorebirds:** They are shorebirds in the Charadriiformes order, most of which live in wetlands. There are species such as auks, avocets, oystercatchers, plovers, sandpipers, snipes, stilts, thick-knees (Figure 2). Members of this group are collectively known as "Shorebirds." Shorebirds migrate long distances and their diet consists of invertebrates, fish or other small animals (McCain, 2015).

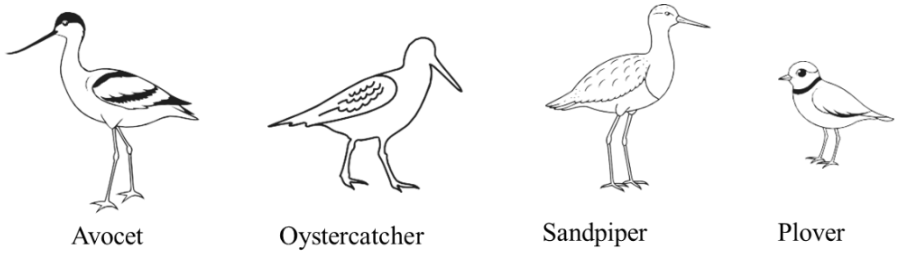

Figure 2: Some species of Shorebirds (illustration editing: Merve Seyfe, 2024)

- **Songbirds:** This group includes birds of the order Passeriformes. Birds in this order constitute more than half of all known bird species (Jønsson and Fjeldså, 2006; Raikow, 2009) (Figure 3). Birds in this group have special vocal structures and their voice boxes are very well developed. This is why they are called "songbirds". They are found in grasslands, woodlands, scrublands, forests, deserts, mountains. In short, they live in all ecosystems except the poles. Members of this order have a variety of feeding habits. Each species has a beak structure depending on its feeding style. There are relatively small species, but the largest species is the raven, weighing over 1.5 kg (Ericson et al., 2003; Chalfoun et al., 2023).

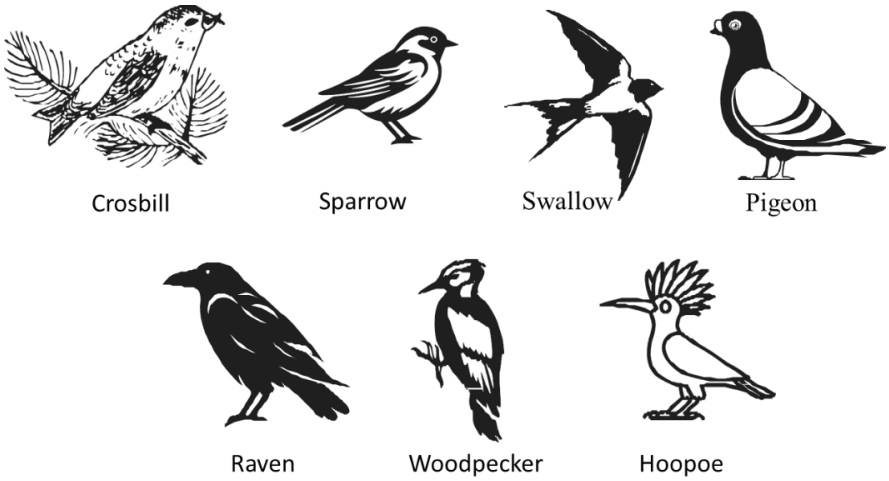

Figure 3: Some species of Songbirds (illustration editing: Merve Seyfe, 2024)

- **Migratory birds**: Birds that spend different seasons in different geographies periodically and regularly are called migratory birds (Somveille et al., 2013). These birds migrate every year during breeding season and winter, while some species make long journeys, others migrate short or medium distances. (Figure 4). In the winter months, due to reasons such as cold weather, unequal seasonal distribution of resources, and daylight hours, it becomes difficult for birds to find food and competition between them increases. They

prefer these regions because the Southern Hemisphere is warmer and rich in nutrients. In the winter months, due to reasons such as cold weather, unequal seasonal distribution of resources, and daylight hours, it becomes difficult for birds to find food and competition between them increases. They prefer these regions because the Southern Hemisphere is warmer and rich in nutrients. (Marra et al., 1998; Conklin et al., 2010; Gunnarsson et al., 2006). We could also think of it as a strategy adopted by birds to take advantage of a seasonal opportunity (Scott, 2020)

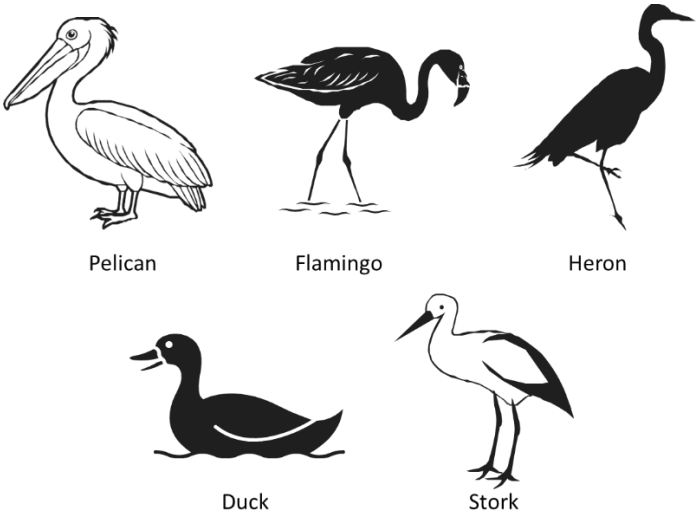

Figure 4: Some species of Migratory birds (illustration editing: Merve Seyfe, 2024)

Migrating birds adopt different migration strategies. Some species fly long distances, while others hop from place to place, refueling along the way. Some species migrate in groups, while others go alone. Males and females of some species migrate separately or to different places (Ketterson and Nolan 1983, Scott, 2020). Water birds, soaring birds and songbirds show migratory behavior that is different from each other. While migrating from Europe to Africa in the fall, songbirds fly over the Mediterranean and the Sahara, soaring species such as storks, hawks and eagles use warm air currents (thermals) to rise. Waterfowl migrate by stopping in wetlands and suitable habitats

on their migration routes (Liechti and Chmaljohann, 2007; Michev et al., 2012; Watson et al., 2018; Kumar and Alam, 2023).

The Role of Birds in the Ecosystem

Birds have many important tasks in the ecosystem. These include maintaining the nutrient cycle, supporting biological decomposition, providing pest control by eating insects, contributing to the fertilization of plants by carrying seeds, making seed separation and acting as ecosystem engineers, and performing many other essential services (Şekercioğlu, 2006, Mariyappan et al., 2023). Ecosystem services directly and indirectly benefit humans through a variety of resources and processes.

Birds are an essential vertebrate group in a multitude of habitats and ecosystems across the globe. They are vital components of the food chain, playing a pivotal role in the food web and the food cycle. Their place in the food chain affects other living things as both prey and predator (Whelan et al., 2008). One of the most well-known examples of ecological balance is the eating of some insects that harm trees in the forest by birds. In this case, birds protect the trees by both feeding themselves and reducing the number of that insect species (Mariyappan et al., 2023). The control of crop-damaging insects by birds can be of great economic value (Şekercioğlu, 2017). In addition, they make an important contribution to the sustainability of the ecosystem by keeping other living populations in balance as hunters. Thanks to these versatile tasks, birds contribute to the balancing of populations (Vishwavidyalaya, 2021).

Birds that feed on seeds and fruits contribute to the reproduction and spread of plants by throwing the plant seeds they eat with their feces in distant places. While they contribute to the fertilization of plants by pollination, they also help diversify the vegetation by spreading seeds (Lunberg and Moberg, 2003, Solomon and Rao, 2006). These functions of birds in the ecosystem are critical for maintaining the balance of nature and increasing biodiversity. Therefore, the protection of birds and the sustainability of their habitats are essential for ecosystem health (Kardaş and Cebe, 2021).

Birds of prey are an important indicator of ecosystem health. Factors such as human pressure, habitat loss, pesticides and climate change are problems that threaten the ecosystem, and the effects of these problems can be quickly observed through raptor populations. Scientific monitoring of populations of birds of prey species makes a significant contribution to the early detection of changes in the ecosystem, taking precautions against environmental problems and developing conservation efforts that include other living species (Kardaş and Cebe, 2021).

Birds have adapted to many ecosystems with their different feeding habits. Here are the common feeding habits:

- *Seeders*: Birds such as finches, sparrows and pigeons feed on seeds, food and fruits.
- *Carnivorous Birds:* Birds of prey such as eagles, hawks and owls feed on small mammals, birds and fish.
- *Feeding on Nectar:* Birds such as hummingbirds feed on nectar and may also eat insects while feeding their young.
- *Feeding on Filtering:* Flamingos and ducks feed in water by filtering out plankton, small organisms and food particles. For example, flamingos feed by dipping their beaks into the water and filtering plankton and other small creatures.
- *Cleaner Birds:* Some species such as crows and vultures feed on carrion, supporting the cycle in the ecosystem.

Each bird species has developed a specific feeding strategy depending on the environment it lives in and its ability to find prey. This dietary diversity increases the survival and reproduction abilities of birds (BridLife International, 2011; Mishra, 2020; Khati and Jaipal, 2022).

The Regurgitate of Pellet in Birds

"Pellet regurgitation" in birds is the process of expelling substances that cannot be digested or consumed by birds. (Figure 5).

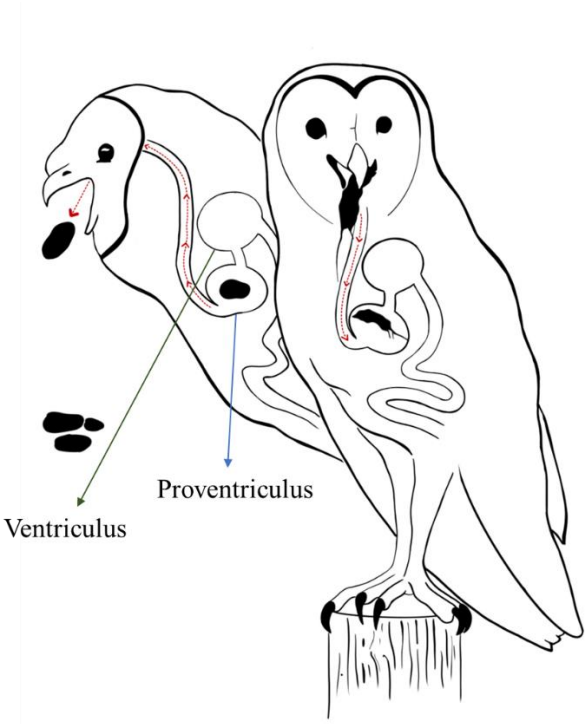

Figure 5: Regurgitate of pellets. (illustration drawing: Şevval Aygün, 2024).

Pellet: To aid in digestion, some bird species expel hard, indigestible remnants of their food—such as bones, feathers, fur, chitinous structures, or seeds—by regurgitating them as compact pellets (Janžekovic ve Klenovšek, 2020) (Figure 6).

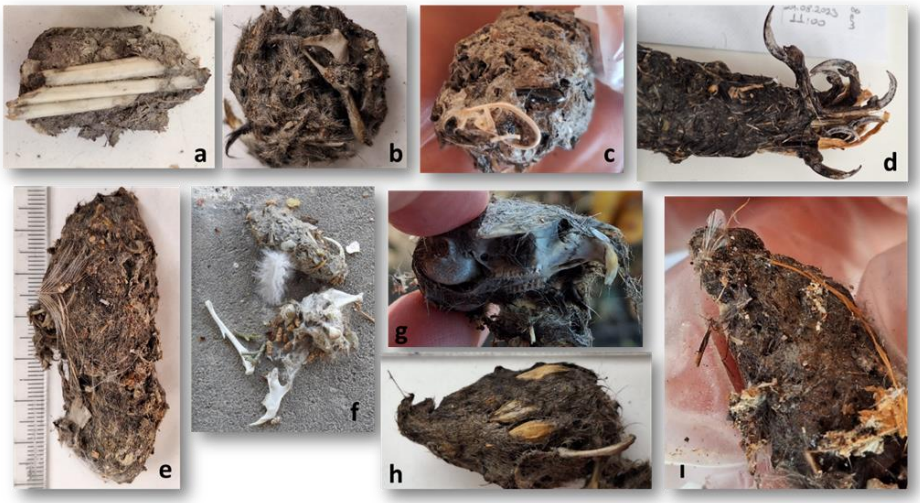

Figure 6: Long-eared Owl pellet samples; a) Bird feather stalk, b) Rodent hip, c) Insect elitra, d) Bird feet, e) Bird feather, f) Rodent jaw, front leg, shoulder blade, g) Rodent skull, h) Seed particles from prey, ı) Insect wing (Source: Melike Seyfe, 2024).

The Importance of Pellet Regurgitation in Birds

Pellet regurgitation is an important process for birds to live a healthy life (Barbosa, 2021). Regurgitating pellets serves the bird's health in another way by "cleaning out" parts of the digestive system, including the esophagus (Souza, 2016).

- *Health Indicator:* Frequent regurgitation of pellets is a sign of a healthy digestive system in birds. It indicates that they are efficiently processing nutrients and fulfilling their body's nutritional requirements (Cooper, 2002).
- *Nutritional Status:* Excreted pellets provide valuable insights into a bird's diet and feeding habits. Researchers can analyze the contents of these pellets to identify the types of food consumed, the species of prey, and overall feeding behavior. This information is crucial for understanding the ecological role of birds and their responses to environmental changes. (Wang et al., 2009; Maciver, 2022).

Pellet Formation Process

Birds lack teeth, so they must either tear their food into small pieces or swallow it whole. During this process, digestive juices—comprising hydrochloric acid, the enzyme pepsin, and mucus—work together to soften the food before it moves into the gizzard. This muscular part of the stomach functions like teeth, grinding the food in conjunction with grit or small stones swallowed by the bird. This mechanical breakdown is crucial for enhancing the effectiveness of the digestive juices, allowing for better nutrient absorption by the body (Arent and Franzen-Klein, 2015).

The gizzard may struggle to digest tough materials like teeth, bones, fur, feathers, insect parts, and certain hard plant materials. Teeth and bones can tear the bird's intestines, while fur, feathers, and plants can lead to blockages, potentially resulting in fatal consequences. To prevent these issues, birds have evolved digestive systems that indigestible materials through regurgitation rather than forcing them through the intestines (Cooper, 2002; Applegate et al., 2017).

Birds of prey and ravens have larger and more tightly packed pellets than many other bird species, making them more noticeable and durable in the environment due to their hard, compact structure (Brown et al., 2003; Sutherland et al., 2004). Pellets of these species are the most commonly found. Owls, being strict carnivores, typically contain indigestible remnants of their prey, such as teeth, bones, fur, and feathers (Yalden, 2003; Glue, 2008). Ravens, on the other hand, are omnivores, feeding on a mix of plant and animal matter, which means their pellets may contain hard, indigestible plant materials, seeds, and small amounts of animal remains (Heinrich, 1999; Boarman and Heinrich, 2020). The presence of bones in owl pellets and plant matter and seeds in raven pellets highlights this nutritional difference (Kristan et al., 2004; Woodman et al., 2005). As an example of pellet formation, when we look at digestion in owls;

- *Swallowing the prey*: The predator swallows its prey, which then passes through the esophagus and into the proventriculus, or glandular stomach (Lynch, 2007; König and Weick, 2008; Yalden, 2009).

- *Digestion*: The process occurs in two stages, involving the proventriculus (glandular stomach) and ventriculus (muscular stomach). The proventriculus, or the first stomach, receives food from the esophagus and begins the digestive process by secreting mucus, hydrochloric acid (HCL), and pepsinogen to soften the food. From there, the food moves into the ventriculus or gizzard, which is lined with thick, muscular layers. The gizzard grinds the food smaller pieces and passes digestible substances to the small intestine for nutrient absorption. Meanwhile, indigestible parts, such as bones and fur, are retained in the gizzard, forming an owl pellet 6 – 10 hours after eating. This pellet is then pushed back into the proventriculus, where it is stored for a few hours before being regurgitated (Ford, 2010).
- *Regurgitation*: Before an owl can consume another meal, it must regurgitate the pellet from its previous meal. This process, which can last from a few seconds to a few minutes, involves the esophagus contracting to expel the pellet. The owl then deposits the pellet beneath its roosting or nesting areas through regurgitation (Leprince et al., 1979).

Bird Species That Regurgitate Pellets

A wide variety of bird species produce pellets, including grebes, herons, cormorants, fulmars, gulls, terns, kingfishers, crows, jays, dippers, shrikes, swallows, bee-eater and most shorebirds (Terres, 1980). While owl pellets are perhaps the most well-known, owls are not the only bird species that regurgitate pellets. All birds of prey—including eagles, buzzards, hawks, falcons, and vultures—exhibit pellet regurgitation behavior as well (Fuller et al., 1979; Medammal et al., 2013; Schubert and Mead, 2019; Hacker et al., 2021; Menzel and Krone, 2021; Taylor et al., 2022; Boxall and Lein, 2024).

Besides birds of prey, many bird species that primary consume hard, indigestible parts of animals or plants also regurgitate pellets (Drewit, 2024). The size of these pellets varies by species: large birds are typically producing pellets that are ~ 2,5 to 5,0 cm long, while

songbirds are about 1,5 cm. These pellets provide an important insight into birds's feeding habits and prey preference (Terres, 1980; Redpath, 2001).

The feces of mammalian predators and the pellets of birds of prey may resemble each other. However, there are differences between them. While the feces of mammalian predator species are long and twisted, in a spiral structure, the pellets of birds of prey are shorter and more convoluted. In addition, mammalian feces have a sharp dent due to the digestion of food residues. Pellets contain indigestible prey parts (bones, feathers, fur, etc.). While mammal droppings can be found wherever the animal wanders, pellets are found under the bird's roosting areas (Hardey et al., 2014).

Finding and examining seabird and waterbird pellets provides valuable information about the diverse diets of these birds across a variety of species. Aquatic birds that live in shallow waters have a variety of diets, each adapted to its own ecological niche (Barrett et al., 2007; Acampora et al., 2017; Perold et al., 2024). For example, grey herons are known to feed on a variety of prey, including fish, small mammals, and amphibians. While white storks primarily consume frogs, grasshoppers and other large insects, kingfishers specialize in catching small fish (Jakubas and Mioduszewska, 2005; Čech and Čech, 2011; Surdo, 2022; Malan and Heimstadt, 2023). The sandpiper, a large waterfowl, is known for its nutritional adaptability and can consume both large prey, such as marine worms and shellfish, and smaller organisms, such as mud snails (Drewit, 2024). The grebe, like the kingfisher, prefers habitats with flowing water such as streams, creeks, and rivers (Luo et al., 2024). Unlike most songbirds, the grebe can swim and even walk underwater, maneuvering with its wings as it searches for aquatic invertebrates among pebbles and rocks (O'Donnel and Fjeldsa, 1997). After feeding, the aquatic grebe deposits pellets, usually made from indigestible parts of its prey, on regularly used rocks (Terrill and Shultz, 2023). These markings provide clues to feeding spots and habits, just as kingfisher droppings mark favorite perches (Peinado and Ortega, 2023; Drewit, 2024). Each bird's diet is closely linked to its habitat and feeding technique, reflected in different body

structures such as bill shape and leg length, which help them effectively catch and process specific prey species (Drewit, 2024). Cormorants and crested cormorants have a fascinating way of processing indigestible material from their diet. They produce mucus-rich pellets that, when dry, harden to a rock-like texture. These pellets, mainly composed of fish bones and marine invertebrate remains, provide valuable insights into the feeding habits of these seabirds (Johnson, 2010; Drewit, 2024).

Grebes are also known to produce pellets daily (Storer,1961; Jehl, 2017). However, observations of this behavior are rare, and only a few pellets have been found. These pellets are usually composed of indigestible materials such as fish bones, aquatic insect exoskeletons, and feathers (Jehl, 2017). Unlike other piscivorous birds, grebes are believed to digest most of the fish bones they consume, which makes their pellets unique. These pellets consist mainly of indigestible plant matter and often, but not also always, feathers (Piersma and Van Eerden, 1989). Sometimes, fish bones and invertebrate remains may also be present. Grebes in the wild, pellet regurgitation typically occurs in water, making it nearly impossible to collect them for study (Marchant and Higgins, 1990). The aquatic environment causes the pellets to break down quickly, complicating detailed research into their diet and pellet formation difficult (O'Donnell, 1982; Wiersma et al., 1995).

Unlike many other waterbirds, ducks generally do not produce pellets because their digestive processes are efficient at breaking down most of what they consume into simpler wastes that are excreted as feces (Trewin and Welsh, 1976). When ducks overeat or consume large pieces of food that cannot be digested, they may regurgitate these pieces as “loose pellets” or larger pellets. This regurgitation is a natural mechanism to remove large or indigestible materials that cannot pass through the digestive system. Thus, ducks can manage excess food by excreting indigestible substances in this way and maintain a balanced diet despite occasionally overeating (Tarshis and Rattner, 1982; Kleyheeg and van Leeuwen, 2015).

Fulmars belong to the order Procellariformes and have a very limited ability to regurgitate indigestible materials, so the garbage and foreign materials they swallow accumulate in their digestive systems. However, many species of seabirds regurgitate indigestible materials such as fish bones, otoliths (inner ear stones of fish), squid beaks and stones as “pellets” or “boluses” and thus prevent their accumulation in their digestive systems (Barrett et al., 2007). Regurgitated pellets are a valuable source of data for seabird dietary studies. These pellets can be collected at bird colonies with minimal disturbance and provide important information about what the birds are eating. Understanding seabird feeding habits is essential for assessing ecosystem health and monitoring the effects of environmental change (Barrett et al., 2007; Acampora et al., 2017). Some example of bird species that regurgitate pellets and pellet samples were given in below:

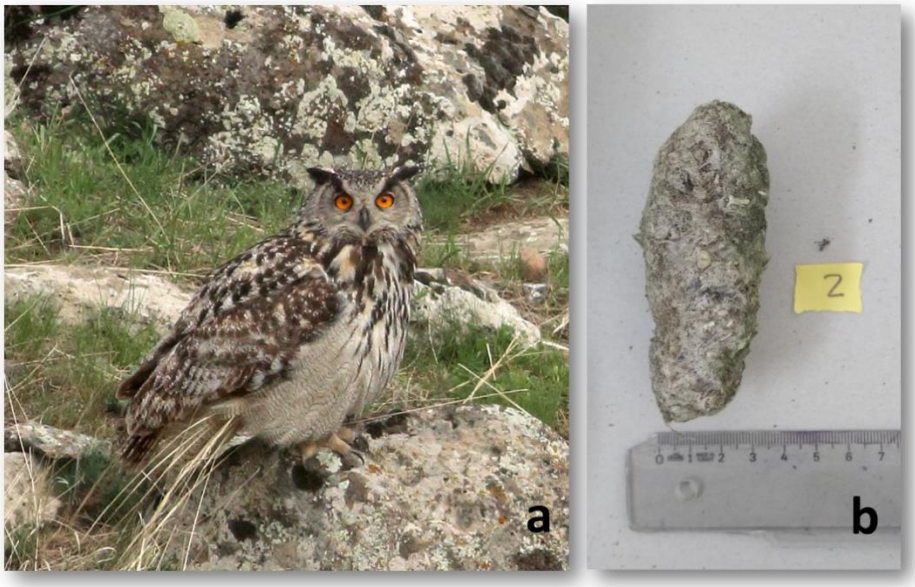

Figure 7: Eagle owl (*Bubo bubo*) and pellet example (source: a,b; Ömral Ünsal Özkoç, 2021)

Figure 8: Long Eared owl (*Asio otus*) and pellet example (source: a,b; Merve Seyfe, 2023)

Figure 9: Imperial eagle (*Aquila heliacal*) and pellet example (source: a,b; Özmen Yeltekin, 2023)

Figure 10: Steppe eagle (*Aquila nipalensis*) and pellet example (source: a: Mehmet Gül, 2024 b; Ömral Ünsal Özkoç, 2024)

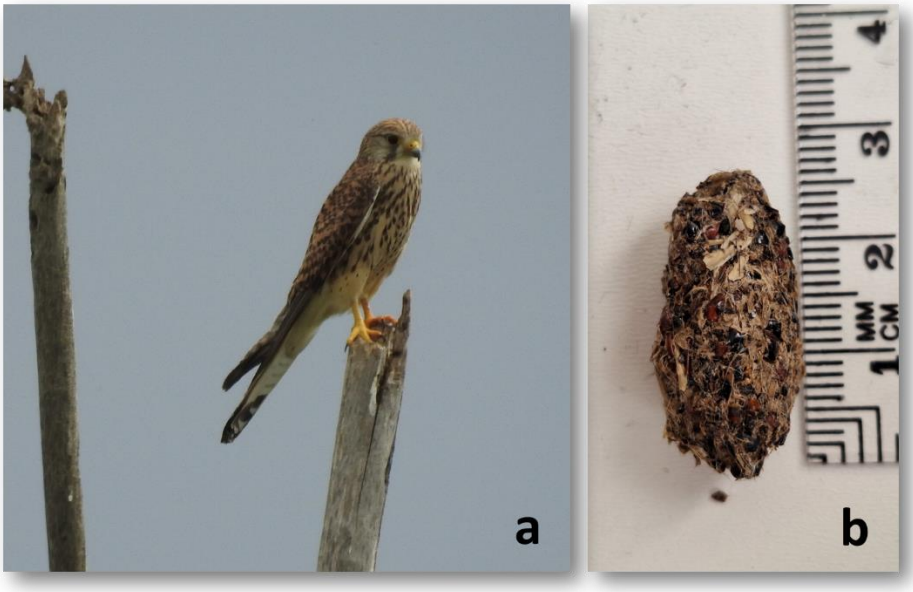

Figure 11: Eurasian Kestrel (*Falco tinnunculus*) and pellet example (source: a,b; Merve Seyfe, 2024)

Figure 12: European Bee-eater (*Merops apiaster*) and pellet example (source: a: Merve Seyfe, 2023, b: Özmen Yeltekin, 2024)

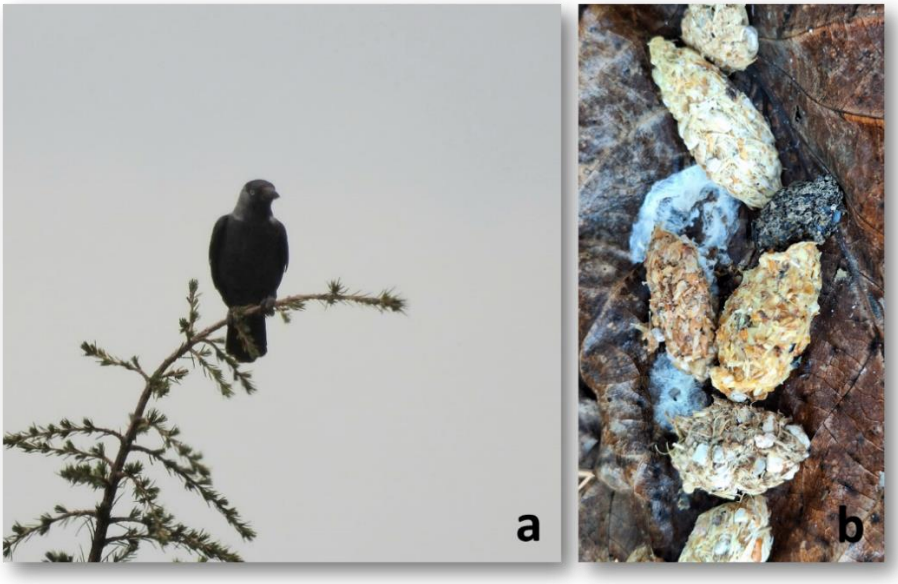

Figure 13: Eurasian Jackdaw (*Corvus monedula*) and pellet example (source: a,b; Merve Seyfe, 2024)

Pellet Studies Conducted in Türkiye

Pellet studies in Türkiye have generally been conducted on pellets of species in the Strigiformes order. In addition to these, there are two studies examining the Lesser Kestrel (*Falco naumanni*) pellets (Figure 14) (Table 1).

Figure 14: Distribution of studies on the pellets of the Lesser kestrel (*Falco naumanni*) and Strigiformes order in Türkiye by province (illustration editing: Merve Seyfe, 2024)

Table 1. Some pellet studies conducted in Türkiye

	Title	Region	Species	References
1	Small mammals in the diet of the Long-eared Owl, *Asio otus*, from Diyarbakır	Diyarbakır	*Asio otus*	Seçkin and Coşkun, 2006
2	Analysis of mammal remains from owl pellets (*Asio otus*) in a suburban area in Beytepe, Ankara	Ankara	*Asio otus*	Bulut et al., 2012
3	Diet of the Long-eared Owl, *Asio otus*, in Central Anatolia (AVES: Strigidae)	Konya	*Asio otus*	Hızal, 2013
4	The Remains of Mammals in the Owl Pellets from Nevşehir Province	Nevşehir	*Athene noctua*, *Tyto alba*, *Bubo bubo*	Kaya and Coşkun, 2014
5	Diet of a nesting pair of Long-eared Owls, *Asio otus*, in an urban environment in southwestern Turkey (Aves: Strigidae)	Denizli	*Asio otus*	Göçer, 2016

	Title	Region	Species	References
6	The Remains of Small Mammals in the Long-eared Owl (*Asio otus*) Pellets from Erzurum Province	Erzurum	*Asio otus*	Kaya and Coşkun, 2017
7	Comparison of Winter Diet of Long-eared Owls *Asio otus* (L., 1758) and Short-eared Owls *Asio flammeus* (Pontoppidan, 1763) (Aves: Strigidae) in Northern Turkey	Amasya	*Asio otus* *Asio flammeus*	Selçuk et al, 2017
8	Small Mammal Remains in Pallid Scops Owl *Otus brucei* (Hume 1872) Pellets	Şanlıurfa	*Otus brucei*	Coşkun et al., 2018
9	Investigation on breeding success, habitat and food preferences of the lesser kestrel (*Falco naumanni*, Fleischer, 1818) in Golbasi (Ankara) region	Ankara	*Falco naumanni*	Avcı, 2018
10	Diet Composition of the Barn Owl *Tyto alba* (Scopoli, 1769) (Strigiformes: Tytonidae) in the Kızılırmak Delta, (Turkey)	Samsun	*Tyto alba*	Selçuk et al., 2018
11	Diet composition of the Long-eared Owl (*Asio otus*) in the Eastern Anatolia (Turkey)	Kars	*Asio otus*	Selçuk et al., 2019
12	Investigation on diet of Long-eared Owl (*Asio otus*) inhabiting Fatih Natural Park (Turkey)	Yozgat	*Asio otus*	Yorulmaz and Arslan, 2019
13	Winter Diet of Eurasian Eagle Owl *Bubo bubo* Near the Yedikır Dam, Amasya (Turkey)	Amasya	*Bubo bubo*	Özkoç and Selçuk, 2021
14	Diet Composition of the Wintering *Asio otus* L. (Strigiformes: Strigidae) in Two Different Habitat Types in Turkey	Edirne-İstanbul-Kars	*Asio otus*	Selçuk et al., 2021a
15	Preliminary data on diet of the Lesser Kestrel (*Falco naumanni* Fleischer) in Aralık, Iğdır province (Eastern Anatolia Region, Turkey)	Iğdır	*Falco naumanni*	Selçuk et al., 2021b

Pellet Extraction Methods

When reviewing pellet studies conducted in Türkiye and other countries over the past 25 years, it was determined that the majority focus on owl pellets from the order Strigiformes. Additionally, globally, pellet studies have been conducted on various species, including the Lesser Kestrel (*Falco naumanni*), Vulture species (*Vultur gryphus, Coragyps atratus, Cathartes aura*), Kingfisher (*Alcedo atthis*), Dipper (*Cinclus cinclus*), Flesh-footed Shearwater (*Ardenna carneipes*), Seagull species (*Larus glaucescens, Larus pacificus*), Great Cormorant (*Phalacrocorax carbo*), Black Legged Kittiwakes (*Rissa tridactyla*) and Northern Fulmars (*Fulmarus glacialis*), with at least one study for each species.

Upon Reviewing the pellet analysis methods used in these studies, it was determined that some did not mention the method used, while others employed various different techniques, leading to a lack of a standardized approach. Studies conducted both in Türkiye and internationally applied multiple methods. One study revealed that in certain methods, the prey items within the pellets were damaged during sorting, while in others, it was challenging to separate the prey remains from fur. These issues resulted in both longer processing times and potential damage to the prey items during analysis.

During the experiments, Yalden's (2009) method of soaking the pellet samples and maintaining them at 60°C for 10 minutes was identified as the most effective approach for sorting the prey remains (Figure 15).

Figure 15: Pellet analysis method; a) Soaking the pellet, b) Waiting in a hot water bath at 60 °C for 10 minutes, c) Removing prey items from wet pellets, d) separated form of fur and prey (Source: Merve Seyfe, 2024).

The most suitable method for processing prey items according to Yalden (2009) was given below:

- Water was poured over the pellet sample placed in a glass beaker to wet it.
- The samples were then heated at 60°C for 10 minutes.
- The pellet became soft and moist, allowing easy handling.
- The fur easily loosened and separated.
- The bones remained hard, intact, and could be separated from the fur without any damage such a melting, deterioration, or breakage.
- The fur did not clump or stick to prey items, making separation smooth.
- The jawbone remained hard and complete, with teeth intact, and no softening or loss of prey items occurred. The hairs separated easily from the prey remains.

The fur could be easily gathered in one place after separation.

This method allowed for efficient pellet analysis, minimizing both processing time and damage to the prey items (Figure 16).

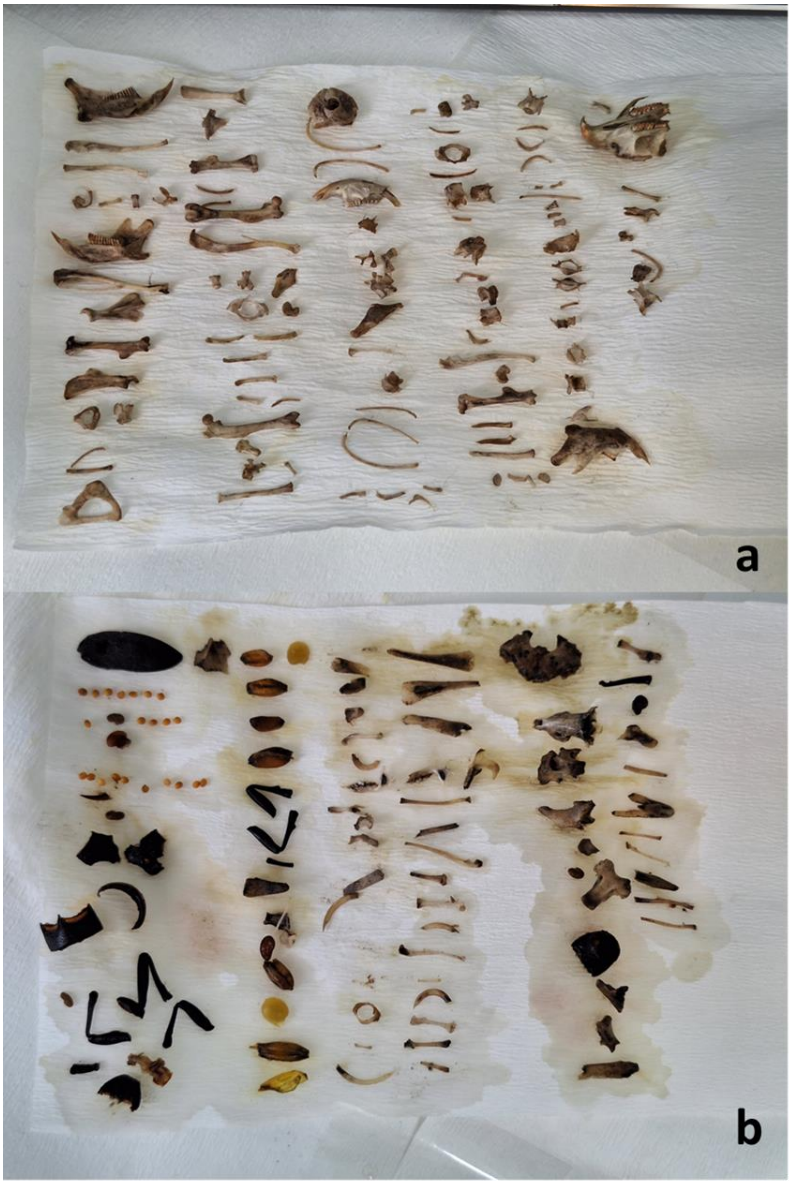

Figure 16: Prey parts separated from the pellet; a) Rodent prey items, b) Insect and insectivor prey items (Source: Merve Seyfe, 2024)

Conclusion

Researchers can gather important information about their feeding habits, preferred prey, and ecological roles by analyzing indigestible debris found in pellets such as fish bones, invertebrate exoskeletons, plant material, and even feathers. This information helps to better understand the interactions between these birds and their habitats and their role in aquatic ecosystems. Despite the difficulties of collecting pellets, especially from species that regurgitate in water, examining these pellets provides a unique window into the complex lives of seabirds and waterbirds (Drewit, 2024).

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CHAPTER 14

DIABETES MELLITUS AND THE USE OF NATURAL PRODUCTS

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INTRODUCTION

Diabetes mellitus (DM) is a well-known metabolic and chronic disease which is the oldest known disorder. Even it is known since ancient times, the types of DM were described first in 1936. The most common reason of DM is the deficiency of insulin secretion but the etiology of insulin secretion impairment can be varied. Making a clear classification in DM is not always possible but type 1 DM which is known as insulin dependent DM is seen lesser than type 2 (non-insulin dependent) DM. There is new evidence about a new type of DM which is named as type 3 DM, the Alzheimer Disease (AD). Studies show us that decreased glucose usage in brain leads to AD. Insulin receptors (IR) do not be found only in pancreatic tissues, they are exist in hippocampus and cerebral cortex too but have no effect on glucose metabolism that's why AD is classified in DM. There is a one more type of DM which is named as gestational diabetes but this type of DM is mostly seen in pregnancy which is accepted as early stage of type 2 DM (Baynest, 2015; Guthrie and Guthrie, 2004; Leszek et al., 2017; Metzger et al., 2010). The main problem in diabetes is hyperglycemia as it is known. The secretion of insulin is not adequate or there is a defect in insulin receptors which causes to insulin resistance. Chronic hyperglycemia leads to chronic other diseases in time. Diabetic patients are prone to heart, kidney, liver failure, eye problems like cataract and atherosclerosis etc. Treatment of DM is not possible but there are ways to take the disease under control. To control type 1 DM mostly insulin injection is necessary but for the treatment of type 2 DM there are several hypoglycemic agents, the most important one of these agents is the metformin (Kaya Kartal, 2021).

TYPE 1 DIABETES MELLITUS

T1DM is an autoimmune disease in humans and genetic factors are involved in its etiology. Especially the HLA gene located in the MHC alleles on the 6. chromosome causes autoimmune diseases in some people and especially attacks the beta cells of the pancreas. However, this is not a genetic factor that occurs alone. HLA genes secrete MHC proteins, and class 2 MHC proteins are responsible for

most of the development of diabetes. In dogs, on the other hand, the DLA gene causes the development of a predisposition to type 1 diabetes, as in humans. It is known that an autosomal recessive locus called insulin dependent diabetes mellitus 1 in BB Rats is closely related to the development of autoimmune diabetes and is also responsible for the phenotype of lymphopenia on chromosome 4 (Cudworth and Woodrow, 1975; Jacob et al., 1992; MacMurray et al., 2002; Poretsky, 2017; Rand et al., 2004). As a result of all these genetic factors, type 1 diabetes is characterized by the inability of the pancreas to produce insulin because of beta cell damage. Markers of immune destruction of the cell include islet cell autoantibodies, insulin autoantibodies, autoantibodies against GAD65 and autoantibodies against tyrosine phosphatase, and these antibodies are highly sensitive in diagnosis (Chiang et al., 2014).

In previous years, the diagnosis of type 1 was made only to newborns and babies, but now it is known that type 1 diabetes can also occur at advanced ages. Each person who is genetically predisposed and has autoantibodies that can cause beta cell damage is a candidate for the diagnosis of type 1 diabetes. In a type of type 1 diabetes called latent autoimmune diabetes in adults (LADA), which occurs at an advanced age, there is usually beta cell damage before it is diagnosed. But ketoacidosis is not observed, and although cell damage persists for many years, it can show symptoms like type 2 diabetes (Naik et al., 2009). In idiopathic type 1 diabetes mellitus, there is an insulin deficiency, but there are no autoantibodies that cause to cell damage. Although this condition is very rare, it is thought that racial predisposition can cause it, and it has been revealed by studies that it is usually seen in people of African and Asian origins (ADA, 2021).

TYPE 2 DIABETES MELLITUS

In this form of diabetes, there is hyperglycemia caused by beta cell damage and the development of resistance to secreted insulin, rather than the inability to secrete insulin. In addition to insulin resistance, deficiency in insulin secretion and excessive secretion of glucagon hormone also accompany hyperglycemia in type 2 diabetes.

Although nutrition and obesity are common causes, many genetic factors can cause the formation of type 2 DM. One of these factors is mutations that occur in insulin receptors, and even if insulin is secreted because of these mutations, insulin cannot perform its task due to receptor damage. There are many mutations that disrupt the insulin receptor. Another genetic factor is an insulin gene mutation, which usually causes excessive secretion of pro-insulin and results with defective insulin production. This causes to reduced effect on the target tissues (Fakruddin, 2019; Flannick et al., 2013).

Insulin production not only reduces the glucose level in the blood, but also prevents the production of free fatty acids, helping VLDLs to move away from the vascular endothelium. Therefore, in the presence of a problem related to the production of insulin or the use of insulin, plasma lipid levels also become unbalanced and glucose intolerance occurs. A prediabetes process occurs before type 2 DM occurs, and during this time, beta cells first try to compensate for the decreased sensitivity to insulin by overworking to keep the glucose level balanced. Although it is managed in this way for a long time, sensitivity to diabetes occurs with the onset of some disruptions in beta cells over time. It is believed that the actual disorder that initiates this event is due to a mutation or defect of insulin receptors (DeFronzo, 1992; DeFronzo, 1997; Warram et al., 1990; Weyer et al., 1999).

Insulin resistance means that insulin supplementation is needed because the effect of secreted insulin decreases. Increased insulin production, especially in the case of hunger, proves the role of defective insulin receptors in resistance. The special signaling pathway that insulin creates while exerting its effect is a very complex structure, but it is very important. When insulin receptors are mentioned, it has been said that beta-subunit provides transmission into the cell. The insulin receptor substrate (IRS) family of proteins found here ensures the continuity of signaling pathways and blood glucose homeostasis. The IRS-1 protein is the first receptor family identified and the most secreted protein. It is also the most researched protein due to IRS-1 gene polymorphisms that cause insulin resistance, especially in type 2 DM. With the disconnection of IRS-1 and insulin receptor (IR),

cytokines and pathways involved in inflammation are activated and pathological developments also occur in different tissues with insulin resistance (Eriksson et al., 1989; Görgişen, 2018; Matthaei et al., 2000).

COMPLICATIONS OF DIABETES

Diabetes is a metabolic disease and brings with it many different ailments. Especially with the deterioration of lipid metabolism, it prepares the ground for arteriosclerosis and, as a result, heart diseases due to an increase in LDL levels. Kidney disorders may occur secondary to urinary excretion of glucose that exceeds the renal threshold over time. In addition, with the introduction of alternative pathways to balance hyperglycemia, eye, brain, entire nervous system, delayed wound healing, liver diseases, etc. many more secondary diseases are also observed. It would be more accurate to think of these complications as acute and chronic complications. Acute complications are caused by suddenly very high hyperglycemia. In general, conditions such as ketoacidosis (usually causes diabetic coma), lactic acidosis, bacterial or fungal infections are the acute complications of DM while chronic complications are organ and tissue deficiencies caused by hyperglycemia over a long period of time. The progression of diabetic complications is especially directly proportional to the values of Glycolyzed hemoglobin (HbA1c), which gives the average blood sugar value for 3 months (NIDDK, 2022; Uludağ, 2010). In Table 1 the acute and chronic complications of diabetes are given as a list.

Table 1. The complications of diabetes grouped as acute and chronic.

Acute Complications	**Chronic Complications**	
Diabetic Ketoacidosis	Retinopathy	Microvascular complication
Lactic acidosis	Nephropathy	
Hyperglycemic hyperosmolar nonketotic stage	Cardiomyopathy	Macrovascular complication
Bacterial or fungal infections	Diabetic foot	

Retinopathy, one of the microvascular complications, is a complication that also occurs in type 1 DM and type 2 DM and usually occurs because of the activation of alternative pathways in hyperglycemia. Histologically, pericyte loss occurs in the eye. This condition is very important for early diagnosis. Because it is a pathognomonic finding of the eye. As a result of pericyte loss, there are consequences such as micro aneurisms in the eye and deterioration of the blood brain barrier. In patients with retinopathy, a collagen accumulation forms in the basement membrane of the eye and can cause blindness over time (Kuwabara and Cogan, 1963; Orlidge and D'Amore, 1987).

There are many roles in the pathogenesis of diabetic nephropathy (DN), one of them is the formation of the polyol pathway and advanced glycation end (AGE) products. In addition, the glycation products of amino acids and lipids (Schiff base formation because of the Maillard reaction) and oxidative stress are of great importance. Especially with hypertension, the kidneys are affected more resulted in disruptions in the renin angiotensin system. It is activated in the Protein Kinase C pathway. Fibronectin causes an increase in the synthesis of type 4 collagen and TGF-beta1. These structures cause to glomeruloscleroses in kidneys (Dyer et al., 1993; Ha and Kim, 1999).

The formation process of cardiovascular diseases is caused by hyperlipidemia, obesity, and hypertension. Every 1% increase in HbA1c increases the heart failure rate by 12%. Diabetic women are predisposed to heart failure more than men since they have more fatty tissue. Increased LDL and decreased HDL rates and increased triglycerides can cause atherosclerosis and, accordingly, a heart attack. There is a direct interest between LDL and heart diseases. When heart problems take shape, there are usually fluctuations in systolic and diastolic blood pressures. It has been found that the blood pressure rate increases by 12% in heart failure, while this rate is 17% in diabetes (Huxley et al., 2006; Turner et al., 1998).

Diabetic foot is a chronic complication of diabetes in which chronic hyperglycemia damages nerve fibers. This damage results such as ulcers, necrosis, gangrene in the feet, and deformities may occur in

the foot too. In addition, toenails can also be affected by this condition, and even nail loss can occur (Levin et al., 1993; Sharad, 2003).

ALTERNATIVE PATHWAYS AND OXIDATIVE STRESS

When talking about glucose metabolism, metabolic pathways that occur at normal glucose levels were mentioned. However, trying to balance the increased glucose with alternative pathways in long-term hyperglycemia brings with it different problems. It is the main cause of chronic diabetic complications (Asnaghi et al., 2003).

The polyol pathway is an important alternative pathway that results in sorbitol accumulation. It is an important cause of microvascular complications due to the inability of pathways that reach glucose saturation to catabolize more glucose. An increase in intracellular sorbitol eventually causes disruptions in signal transmission, and this is how its role in the development of neuropathy begins. An increase in sorbitol usually leads to the depletion of taurine and myoinositol, while the prostaglandin mechanism and nitric oxide synthesis decrease or even deteriorate, especially with the depletion of myoinositol. A decrease in taurine negatively affects Schwan cells, the cyclooxygenase pathway is disrupted, and neurological structures such as ganglia and nerve fibers are disrupted. Glutathione reductase decreases in this pathway. The aldose reductase enzyme is responsible for the formation of sorbitol and NADPH is used in this reaction. The polyol pathway causes oxidative stress and contributes to the activation of many other pathways (Askwith et al., 2009; Cubeddu and Hoffmann, 2002; McFarlane et al., 2001).

Enzymatic and nonenzymatic glycation products also activate the mechanisms of oxidative stress, resulting in various disadvantages and playing a role in the formation of complications. Organic molecules such as proteins and lipids nonenzymatically shape the Maillard reaction and become more stable after creating Schiff bases, which ultimately form Amadori products. The formation of Schiff bases of proteins and Lipids occurs due to the presence of sugars in the environment. It forms a condensation product with the amino group in the protein. The carbonyl groups of the reducing sugars react so chronic

hyperglycemia creates an impossible opportunity for the formation of AGE. The amadori products do not form AGE immediately, it is necessary to take a while. These glycation products are HbA1c and fructosamine, whose place is very important in diabetes. Especially HbA1c ratios are very important in the diagnosis of diabetes. It is preferred to instant glucose measurement because it gives average 3-month blood sugar results, and results of 6.5% and above are considered diabetic (ADA, 1998; Altan et al., 2006; Deedwania, 2000; Kılınç, 2011).

Under physiological conditions, most of the glucose produces energy by participating in the electron transport chain, and superoxide radicals are formed during this process. When hyperglycemia becomes chronic, more superoxide radicals are formed, and oxidative damage is ocurred. Reactive oxygen species form the oxygen radical, and it is assumed that the oxygen radical activates the Nf-kB pathway. Then, the protein kinase C pathway is activated, producing more reactive oxygen species (ROS) and reactive nitrogen species (RNS) and oxidative stress is initiated in this way (Harris, 1993; Johansen et al., 2005; Memişoğulları, 2005).

Oxidative stress begins with the activation of protein kinase-C (PKC). Hyperglycemia increases the ratio of NADH and NAD and diacylglycerol increases too. This arises the macrovascular complications. PKC activation also activates mitogen-activated protein kinase (MAPK). Heat shock proteins and c-JNK kinases leads to atherosclerosis and apoptosis. Antioxidants prevent PKC activation. It induces vascular endothelial growth factor (VEGF) and increases vascularization which then leads to increased endothelial complications (Memişoğulları, 2005; UKPDS, 1998; Vincent et al., 2004).

Another pathway that ensures the balancing of increased glucose is the hexosamine pathway. It is a pathway that supports the insulin resistance mechanism and increases beta cell degradation. Fructose-6-phosphate enters the hexosamine pathway and is converted to glucosamine-6-phosphate by the enzyme fructose-6-phosphate amidotransferase, and then turns to UDP-N-acetylglucosamine. This product is the precursor substance of glycosaminoglycans. UDP-N-

acetylglucosamine is then converted into O-GlcNAc (O-N-acetylglucosamine) and accumulates in the tissue. UDP-N-acetylglucosamine accelerates the posttranslational modifications of proteins (Buse, 2006; Du et al., 2003; Goldstein, 2002).

It has been a known fact for a long time that oxidative stress plays a role in the onset and progression of diabetes, and when the organs where diabetic complications occur are examined, it has been found that oxidative stress increases in these tissues. The primary source of oxidative stress in diabetes is the superoxide radical formed in the electron transport chain. In the continuation, the previously mentioned pathways are involved, oxidative stress continues to occur continuously as long as hyperglycemia cannot be controlled (Altan and Dinçel, 2006; Giacco and Brownlee, 2010).

NATURAL PRODUCTS AND PHENOLIC COMPOUNDS

Phenolic compounds, also known as polyphenols, are substances with an aromatic benzene ring and constitute the biologically active substances of fruits and vegetables. Polyphenols are compounds with antioxidant properties that determine the flavors, colors and even odors of foods (Dragovic-Uzelac et al., 2007). The simplest phenolic compounds are simple phenols and phenolic acids, which contain one phenol group in their structure. Phenolic acids (which contain carboxylic acid) are divided into three types: hydroxycynamic acid, hydroxybenzoic acid and koumarins. The most important polyphenols are studied in two subheadings: flavonoids and tannins (Mamari, 2021).

In obesity and prediabetes, chronically elevated insulin levels lead to increased mitochondrial respiration and, ultimately, increased formation of reactive oxygen species. Increased ROS production increases the neutralization capacity of antioxidant defenses and causes oxidative stress. Long-term oxidative stress disrupts glucose uptake in muscle and adipose tissue and reduces the secretion of insulin from pancreatic beta cells, thereby accelerating the pathological process of Type 2 diabetes mellitus. Creating a diet that increases fruit and vegetable intake is very useful in preventing the occurrence of chronic degenerative diseases. Thus, both obesity is prevented and the risk of

contracting T2DM, which occurs because of obesity, can be reduced. The importance of these fruits and vegetables comes from the polyphenols they contain. The most important of these polyphenols are catechins, resveratrol, lipoic acid, and anthocyanins (Guo et al., 2015).

In a study conducted by Tsuda (2003) et al., purple corn-colored anthocyanin containing high fat diet and only high fat diet were given to C57BL / 6 J mice for 12 weeks. It has been reported that anthocyanin supplementation significantly reduce the body weight gain and the accumulation of fat in white and brown adipose tissue. Anthocyanins can act as free radical scavengers, hydrogen-yielding compounds, single oxygen extinguishers and metal ion chelators, and therefore are highly involved in in vitro analyses of antioxidant properties such as oxygen radical absorption capacity and ferric reducing antioxidant potential.

METFORMIN USE IN TYPE 2 DIABETES

The effect of this drug, which is used to reduce the severity of hyperglycemia, especially in type 2 diabetes, has been known for almost 1000 years. It has been known for many years that the European plant Galega officinalis has a reducing effect on polyuria, and it was only discovered in 1930 that guanidine residues found in plant extracts cause this effect. A drug was developed by synthesizing biguanidines with its current name, and the drug containing biguanidine, which is still known for its antidiabetic effect today, is metformin (Chandalia et al., 2014).

Metformin, a lipophilic agent, acts by binding to mitochondrial membrane phospholipids. It targets hepatocyte, skeletal muscle cells and adipocytes. Adenosine monophosphate (AMP) protein kinase or activated protein kinase is the first enzyme it targets, and these enzymes are effective in glucose and fat metabolism and regulate energy production. It acts as a glucose production reducer when targeting hepatocytes and tries to keep blood glucose levels stable by supporting glycogen synthesis. Since AMP acts through kinase activation, it also has effects such as a decrease in protein synthesis and a decrease in cell survival. It has been proven to regenerate glial cells in

rat studies. The most felt obvious side effect is a disorder in the gastrointestinal tract. It causes diarrhea and stomach disorders. A serious side effect is that it can cause lactic acidosis, and this condition is usually observed in those who develop renal insufficiency (DeFronzo, 2011; Holt et al., 2010; Pijl et al., 2000; Speakman et al., 2012; Via et al., 2010).

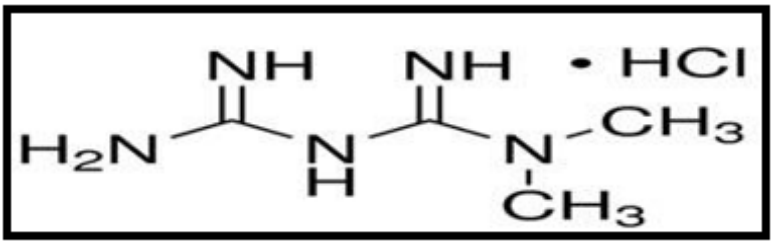

Figure 1. Chemical formulation of metformin

LAUROCERASUS OFFICINALIS (CHERRY LAUREL)

This natural product is a known fruit in the black sea region of Türkiye and is widely used as a folk medicine because of its reducing effect on hyperglycemia. The fact that it has a rich content of phenolic substances makes this fruit a powerful antioxidant and in the study of Kaya Kartal et al (2021) total phenolic content of L. officinalis as gallic acid equivalent was found 3 mg/g. In another study of Ulusoy (2019) fermented cherry laurel extractions were used and total phenolic composition was found 7.73 mg GAE/g. This shows us the difference of contents even in same fruits. In a study, wistar albino rats were inducted with nicotinamide and streptozotocin to cause to type 2 diabetes, and the interaction of metformin and cherry laurel was studied. According to the study the use of metformin alone had a better effect than the combination (Kaya Kartal, 2023). Even the use of natural products alone could have better effects on diabetes, it has been concluded that attention should be paid to the use of natural products in the use of medicines.

RUTIN FLAVONOIDS

Rutin is one of the main flavonols found in 'clingstone' peaches, and the source in which it is most concentrated is buckwheat. Rutin can

increase intracellular ascorbic acid levels, reduce capillary permeability and fragility, reduce oxidants and free radicals it prevents the destruction of bones, also reduces the risk of heart diseases. Rutin administration to diabetic rats reduces food consumption and improves body weight and this may be due to better control of hyperglycemic status in diabetic rats (Gupta et al., 2014; Kamalakkannan et al., 2005). In the study of Kaya Kartal (2023), type 2 diabetic rats treated with metformin and rutin flavonoid combination have a reduced body weight than the untreated diabetic rats. But still the combination use did not have a better effect on glucose levels. A study with high fat diet induced type 2 diabetes could be a better choice for the effect of rutin because of its reducing effect on food consumption.

ALPLHA LIPOIC ACID

Alpha lipoic acid, which has a low molecular weight and contains a thiol group, is synthesized de novo. The synthesis takes place by lipoic acid synthase and in the mitochondria. It is also an important cofactor of the citric acid cycle. This molecule, which has a high reduction property, has a dithiolane ring. Since it is a physiologically synthesizable molecule, its exogenous use and even its use as a drug is quite common. It can inhibit nitric oxide synthase (NOS) and P450. Since it contains a thiol compound, it may also have a prooxidant effect in the presence of radicals such as O2, H2O2 or OH in the environment. Almost all cells convert lipoic acid to dihydroxylipoic acid (DHLA), the antioxidant effect of lipoic acid is not as high as DHLA (Çakatay, 2006; Goralska et al., 2003; Liang and Akaike, 2000).

It is still not fully understood where this effect of lipoic acid, which can reduce increased blood glucose in type 2 diabetes, comes from. An idea put forward about this is that the mild prooxidant effect of the lipoic acid compound has a reducing effect on the reducing stress in diabetes and can lead to adaptation to oxidative stress (Roy et al., 1997). It is believed that it is activated by insulin and thus can act as a hypoglycemic agent. Oxidized lipoic acid stimulates glucose uptake. Oxidative stress eases in short-term lipoic acid applications, while intracellular glutathione levels increase and glucose uptake rate

decreases in long-term applications (Moini et al., 2002; Mottley and Mason, 2001).

Type 2 diabetic wistar rats treated with metformin and lipoic acid combination therapy showed about 188,86±19,86 mg/dL fasting blood glucose levels at the end of the study. The blood glucose levels were still high but the antidiabetic effect of lipoic acid could be due to the reducing effect on the poliol pathway. In the study there was a significant reduction in eye aldose reductase activity measurement. So this could be a sign for delaying the complications of diabetes when lipoic acid is used (Kaya Kartal, 2023).

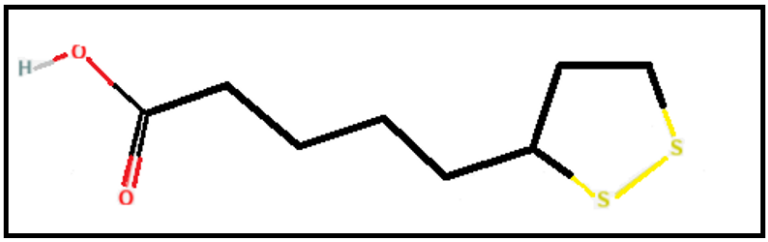

Figure 2. Structure of alpha lipoic acid.

There are too many fruits and vegetables rich in phenolic and flavonoid compounds. Many of them are known with its antioxidant activities and are investigated on several diseases. Phenolic compounds show their antioxidant properties at the same time along with their abilities such as antiallergic, anti-inflammatory, antidiabetic, anticarcinogenic, antimicrobial, antithrombotic etc. The research of the antioxidant content of plants, fruits and other foods has gained a lot of importance in recent years and has started to take the lead in human and animal health (Burak and Çimen, 1999; Scalbert et al., 2005; Sen and Chakraborty, 2011). In table 2 the natural products rich in phenolic composition which can be used as antidiabetic agents are given.

Table 2. Antidiabetic natural products (Karaman and Elgin Cebe, 2016)

Name of natural product	**Family (Genus)**	**Used part of natural product**
Allium cepa L.	Liliaceae	Root and leaf
Allium sativum L.	Liliaceae	Root and leaf
Amygdalus communis L.	Rosaceae	Fruit or seed
Zizyphus jujuba Mill.	Rhamnaceae	Fruit
Vitis vinifera L.	Vitaceae	Seed
Vaccinium myrtillus L.	Ericaceae	Fruit and leaf
Urtica piluliflora L.	Urticaceae	Leaf
Rubus canescens DC.	Rosaceae	Fruit
Rosa canina L.	Rosaceae	Root, fruit and leaf
Olea europaea L.	Oleaceae	Leaf
Helichrysum plicatum DC.	Compositae	Flower
Elaeagnus angustifolia L.	Elaeagnaceae	Leaf

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CHAPTER 15

THE USE OF MEDICATIONS IN LABORATORY ANIMALS

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INTRODUCTION

Studies with experimental animals began during the period when Aristotle and Hippocrates were researching human anatomy and physiology. These studies contributed to the advancement of modern medicine. In the 20th century, experimental animals were used in advances in scientific fields such as pharmacology, toxicology and immunology (Gökmen et al., 2019). In the field of pharmacology, experimental animals are widely used to evaluate the efficacy of new drugs, to test the effects of drugs, and to evaluate the indications of drugs (Fox et al., 1984; Rollin, 1992). Disease models created in experimental animals are used in preclinical drug screening before clinical trials. Experimental animals are considered as important in vivo models in the determination of basic and important pharmacokinetic parameters such as safety and toxic properties as well as efficacy of drugs. Special toxicological properties of drugs such as mutagenicity, carcinogenicity and teratogenicity can be investigated in various animals. These preclinical animal trials consist of specific stages. If the trial drug demonstrates the desired efficacy, the process moves on to the next stages. If a drug is proven to be safe in preclinical trials on animals, it is then administered to small groups of human volunteers.

Approximately 95% of laboratory animals are mice and rats (Dhadde, 2019; Hickman et al., 2017). In particular, Wistar and Sprague-Dawley rats are the most widely used worldwide. Rats are preferred because they have many metabolic characteristics similar to humans, short gestation periods, the ability to produce large numbers of offspring, and relatively good adaptability to laboratory environments (Sengupta 2013). Rats are frequently used in research: in health-related research, in the testing of biomedical products and in the testing of newly developed vaccines and drugs (Williams 1976; Zutphen Van and Beyren 1993). They are particularly preferred for modelling cardiovascular, metabolic and various neurological disorders and for developing therapeutic agents for these diseases (Geçmez et al., 2023; Krinke 2000).

Mice are useful models for studying inflammation, immunity, infections (Polat et al., 2024). Hamsters, which have a lifespan of about

4–5 years, are classified into three groups based on fur type: short-haired, rough-haired, and long-haired. However, only short-haired hamsters are used in scientific studies (Geçmez et al., 2023; Quesenberry and Carpenter 2011). Due to their immunogenetic characteristics, they are particularly used in tumor studies and immunological research because of their cheek pouches. Syrian hamsters, being hibernators, are also used in thermophysiology, circadian rhythm studies, as well as in research on reproduction, teratogenicity, circulation, and dental studies (Whittaker, 2010; Winnicker, and Pritchett-Corning, 2024). Guinea pigs have a strong sense of hearing, as well as robust sensory abilities related to vision and smell (Gültiken, 2010). They are mostly used in the production of serum, vaccines, and other biological materials (Padilla-Carlin et al., 2008; Shomer et al., 2015). Rabbits, which are relatively large and widely used laboratory animals, are ideal for studies that are difficult to perform on smaller experimental animals due to their large size. Their docile nature and lower risk of zoonotic diseases are additional reasons for their preference. In particular, the anatomical and physiological structures of rabbits' respiratory tracts, cardiac conduction systems, and reproductive systems provide convenience for certain fundamental research. Additionally, rabbits have been used in antibody production and are suitable animals for some vaccine studies (Burkholder et al., 2012; Cooper et al., 2021).

Carrying out experimental studies with different animal species requires the choice of the appropriate route of administration and the appropriate drug formula. Before administering drugs to experimental animals, the purpose of drug administration should be clearly defined and it should be remembered that the proper route of administration and formulation are very important in drug development processes. It is well known that the route of administration has a very important influence on the control of the pharmacodynamics and toxicity of pharmacological agents as well as their final absorption, distribution, metabolism and excretion (Turner et al., 2011). Drugs can be administered through various routes. When choosing the route of drug administration, factors such as pharmacological profile of the drug,

frequency and time of administration and animal species should be taken into consideration. Every parenteral route of administration offers advantages and disadvantages depending on the particular objectives of the experiment (Shoyib et al., 2020). Drug administration route should be adjusted according to the type, amount and form of the drug to be administered. It should be remembered that if the technique used for drug administration requires more than a simple needle injection or if the substances administered are known to cause pain, sedation or general anesthesia is usually necessary. These administrations must be performed using aseptic techniques, and the substances should be sterile and apyrogenic (Nebendhal, 2000).

Ip drug administration in rodents is a commonly used route in many in vivo disease model studies. It offers several advantages such as being suitable for chronic treatments, causing low stress in laboratory rodents, allowing the administration of large volumes of solutions, providing rapid and complete absorption, not requiring anaesthesia. However, there is a knowledge deficit about the pharmacokinetics of the administered drugs and the mechanisms by which these agents achieve systemic effects (Morton et al., 2001; Shoyib et al., 2020).

For intraperitoneal administration, the rodent is placed in the supine position and, due to the absence of vital organs, the injection is administered into the left lower quadrant of the abdomen at an angle of 10° (Eldridge et al., 1982). When using this method, substances exceeding a volume of 10 ml/kg should not be administered to rodents, and care must be taken regarding the temperature of the injected substance (Bredberg et al., 1994; Pekow, 2003).

The iv pathway, which typically provides significant bioavailability for a drug, is often not feasible for rodent studies. Therefore, oral, ip, or sc routes are more commonly preferred over the iv route. However, compared to other injection routes, iv administration offers advantages such as controlling the rate of entry into systemic circulation, producing a rapid response, and allowing the achievement of stable plasma concentrations by adjusting the infusion rate. The preferred route of administration for drugs that are difficult to be

absorbed by the digestive system or drugs that cause pain during intramuscular or subcutaneous administration is the intravenous route (Jin et al., 2015). However, in repeated drug administrations, if the same vein is to be used multiple times, it should be considered that injecting substances can damage the vein. The first injection should be made distal to the cardiac centre and if necessary, subsequent injections should be made proximally. The lateral tail vein, vena safena or dorsal metatarsal vein can also be used for iv injections in rats. If the rat is not anaesthetised, a restraint device is required to limit movement and allow access to the tail vein (Nebendhal, 2000).

Im injection is a technique that allows the drug to be administered deep into the muscles, enabling the drug to quickly enter the bloodstream. However, it is generally not preferred in small laboratory animals unless necessary due to their small muscle mass. Non-irritating and non-painful drug solutions and suspensions can be administered via this route. The injection is performed in the area of the quadriceps or gluteal muscles. To minimize the risk of nerve damage, the correct injection site should be chosen. In rabbits, this injection can only be performed on the forelimb and hindlimb muscles. The needle should be injected into the muscle mass at an angle of 45 degrees. The syringe plunger should be checked by retracting the syringe plunger to ensure that no large blood vessels are entered (Ekici 2024; Küçük et al., 2013).

For sc injections in mice, rats, and guinea pigs, the skin on the neck or back is stretched, and a needle is inserted at a 45-60° angle to inject 1-2 ml in mice and approximately 10 ml in rats. In rabbits, this is a simple procedure and is preferred when intravenous access is not possible and fluids need to be administered. Using this method, up to 50 ml of fluid can be injected into rabbits (Küçük et al., 2013).

Enteral administration can be performed orally, by gavage or rectally. This method allows the administration of relatively large quantities of non-sterile agents or substances. When using the oral route, it should be remembered that enzymes in the gastrointestinal microflora may metabolise agents, agents may be degraded by gastric acid, food may affect the rate and sequence of gastric emptying, and time spent in the stomach may significantly affect the rate of agent

absorption. In rats, the small intestine is the primary area for absorption of all drugs after oral administration due to its high surface area and blood circulation (Durgut and Yarsan 2007; Levine, 1970; Nebendahl, 2000). Oral administration can be done by adding the drug to drinking water or food. When administering drugs this way, animals must be monitored to ensure they are consuming the water and food. If a full oral dose is required, oral gavage is the most reliable method. For rectal administration, a small-diameter, soft, flexible tube with smooth edges is used (Ekici, 2024).

Anaesthetic/analgesic agents are widely applied in laboratory animals to alleviate stress and pain caused by surgical manipulations, to ease demanding procedures and to improve postsurgical care (Nuhoğlu and Aksoy, 2019). Analgesic and anesthetic agents are among the main drug groups used in laboratory animals to prevent unnecessary suffering and to improve scientific quality. This group includes multiple pharmacological agents, each with different mechanisms of action, durations of effect, and varying effectiveness in pain relief (Büyükçoban, 2021; Kest et al., 1999). Some of the analgesics used for pain relief are listed in Table 1.

Table 1. Reported commonly used analgesic dosing regimens for rodents and rabbits (Foley et al., 2019; Mook, 2005)

Drug	Mouse	Rat	Guinea pig	Rabbit
Aspirin	100-120 mg/kg PO	100-120 mg/kg PO	87 mg/kg PO	100 mg/kg
Acetaminophen	-	-	-	1 ml elixir in 100 ml drinking water
Buprenorphine	0.05-0.1 mg/kg SC	0.01-0.1 mg/kg SC, IM	0.05 mg/kg SC	0.01-0.05 mg/kg SC
Butorphanol	1-5 mg/kg SC	2 mg/kg SC	-	0.1-0.5 mg/kg SC

Carprofen	2-5 mg/kg SC	2-5 mg/kg SC	2-5 mg/kg SC, IM	1.5 mg/kg PO
Flunixin meglumine	2.5 mg/kg SC	2.5 mg/kg SC	-	1.1 mg/kg SC
Ibuprofen	30-40 mg/kg PO	15 mg/kg PO	-	-
Ketoprofen	2-5 mg/kg SC	2-5 mg/kg SC	-	3 mg/kg IM
Meloxicam	1 mg/kg SC, PO	1 mg/kg SC, PO	0.1-0.3 mg/kg SC, PO	-
Meperidine	10-20 mg/kg	5-10 mg/kg SC	10-20 mg/kg SC	5-10 mg/kg SC 0.2 mg/ml in drinking water
Morphine	2-5 mg/kg SC	2-5 mg/kg SC	2-5 mg/kg SC	2-5 mg/kg SC
Nalbuphine	4-8 mg/kg SC	1-2 mg/kg SC	1-2 mg/kg SC	1-2 mg/kg SC

Veterinarians monitor the health of laboratory animals to gather information about their pathogenic status and to prevent infectious diseases. Early detection of diseases and infections is crucial for preventing infectious diseases in experimental animals, as it significantly affects the reliability of research results. However, unlike other animal species, laboratory animals can experience toxicity from antimicrobials. For example, in guinea pigs and hamsters, penicillin, tetracycline, chloramphenicol, and lincomycin can lead to intestinal diseases that may result in death, or antimicrobial agents may cause local effects (Şanlı and Kaya, 1994). The antimicrobials that can be used in experimental animals are shown in Table 2.

Table 2. Reported commonly used antimicrobials dosing regimens for rodents and rabbits Caution: Most dosages are extra-label. (Ekici, 2024; Seedor et al., 1987)

Drug	Mouse	Rat	Guinea pig	Rabbit
Ampicillin	2-10 mg/100 g PO 50-150 mg/kg SC	50-150 mg/kg SC	-	-
Cephalexin	60 mg/kg PO 15 mg/kg IM	60 mg/kg PO 15 mg/kg IM	50 mg/kg IM	11–22 mg/kg SC
Enrofloxacin	2.5-5mg/kg IM, PO, SC	2.5-5mg/kg IM, PO, SC	0.05–0.2 mg/mL dw 5–15 mg/kg PO, SC, IM	5–10 mg/kg PO, SC, IM 200 mg/L dw
Gentamicin	0.5mg/100 g IM 5-8mg/kg SC	0.5mg/100 g IM 5-8mg/kg SC	2–4 mg/kg SC, IM	1.5–2.5 mg/kg SC, IM, IV
Griseofulvin	25 mg/100g PO	25 mg/100g PO	25 mg/kg, PO	25 mg/kg, PO
Chlortetracycline	25 mg/kg, IM, SC	6-10 mg/kg, IM, SC	-	50 mg/kg PO
Metronidazole	2.5 mg/ml in drinking water	-	25 mg/kg PO	20 mg/kg PO
Neomycin	2 mg/ml in drinking water 50 mg/kg SC	50 mg/kg IM	8 mg/kg PO	30 mg/kg PO
Oxytetracycline	100mg/kg SC	10-20 mg/kg, PO	-	50 mg/kg, PO
Tetracycline	3-5 mg/ml in drinking water 20 mg/kg PO 100 mg/kg SC	100 mg/kg SC	10–40 mg/kg PO	50 mg/kg PO 250–1000 mg/L drinking water
Trimethoprim-sulfadiazin	240 mg/ml çözeltiden 0.5 ml/kg SC	240 mg/ml çözeltiden 0.5 ml/kg SC	15–30 mg/kg PO, SC	30 mg/kg PO, SC, IM
Tylosin	0.2-0.8 mg/100g IM 10 mg/kg SC 10 mg/100 g PO	10 mg/kg SC	10 mg/kg PO, SC	10 mg/kg PO, SC

Lack of vital needs, such as nutrition and care, or stress can lead to a weakened immune system and infectious conditions. Rabbits, in particular, are more sensitive to stress. Rabbits and rodents are sensitive

to unfamiliar environmental stimuli, such as sounds and smells, which can increase their heart and respiratory rates. Therefore, it should be remembered that successful treatment is often not achieved solely by selecting the correct antimicrobial agent (Wheler, 2013). In managing pain, which can be difficult to detect in rodents, analgesics should be used when necessary. There are multiple pharmacological agents available for pain management in research animals. However, we cannot assume that pain can be completely cured in research animals. Therefore, non-pharmacological interventions that can reduce pain, especially during postoperative recovery, are important. Having researchers who are skilled in their field and avoid disturbing the animals, as well as the use of appropriate surgical techniques, can minimize stress in animals and reduce postoperative pain.

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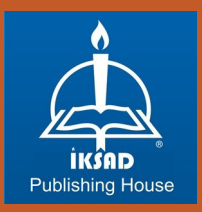

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