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The effect of propolis on phagocytic and antioxidant activities

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Dedication

To my beloved **mother** — who stayed awake so I could rest, who prayed for my success, and was always my refuge and pillar of strength…

To **my father** — who planted courage in my heart and instilled confidence in my soul…

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BELBAZ Khaoula

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List of abbreviations

CD3 Cluster of differentiation 3

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GSH Glutathione reduced

IgE Immunoglobulin E

LPS Lipopolysaccharide

MPS Mononuclear phagocyte system

NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells

PALS Periarterial lymphoid sheath

PMN Polymorphonuclear leukocyte

PMNs Polymorphonuclear leukocytes

RES The reticuloendothelial system

ROS Reactive oxygen species

TAMs Tumor associated macrophages

TCR T cell receptor

TGF-β Transforming growth factor β

TLR Toll-like receptors

TME Tumor microenvironment

TNF Tumor necrosis factor

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Introduction

Introduction

The immune system is relatively complex; thus, any factors influencing the functions of the immune system may also influence other systems in the human body, such as the nervous system, endocrine system, metabolism etc. **(Isbill et al., 2020) and (Pelvan et al., 2022).** The immune response is either innate, nonspecific, adaptive acquired, or highly specific.

The innate response, often our first defense against anything foreign, defends the body against a pathogen **(Justiz vaillant et al., 2025).**

The adaptive acquired immune response uses the ability of specific lymphocytes and their products, such as immunoglobulins and cytokines, to generate a response against the invading microbe **(Denson, 2013), (Arce-Sillas et al., 2016)** and **(Lawrence et al., 2016).**

The reticuloendothelial system (RES) is a key part of the immune system, consisting of phagocytic cells like monocytes and tissue macrophages. These cells, found in reticular connective tissue, are responsible for engulfing and destroying foreign substances, dead cells, and abnormal tissues **(Ortner, 2003)**, The RES plays a crucial role in maintaining the body's defense and homeostasis. Abnormalities in these cells or their mechanisms can lead to disease **(Derelanko and Auletta, 2014).**

The RES is the best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defense. The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen and from the blood stream is a measure of reticulo-endothelial phagocytic activity **(Stuart et al., 1973).**

Immunomodulation encompasses all therapeutic interventions aimed at modifying the immune response. Augmentation of the immune response is desirable to prevent infection in states of immunodeficiency **(Gea-Banacloche, 2006).**

Medicinal plants serve as a primary source of numerous pharmacological agents, offering therapeutic solutions for a broad spectrum of illnesses. Since ancient times, natural products derived from plants have been widely utilized for both the treatment and prevention of diseases **(Alkahtani et al., 2022)**. The phytochemicals used as plant-derived immunostimulant drugs include polyphenols, flavonoids, diterpenoids, alkaloids, and phytoestrogens **(Jantan et al.,2015)** and **(Pal and Nayak ,2021).** Medicinal plants are employed for a variety of purposes, such as antibacterial, anti-inflammatory, antioxidant, antifungal, antihelmintic, anticancer treatments, as well as for managing cardiovascular disease and immunomodulatory **(Harun et**

al.,2020). Instead of commercial drugs, numerous illnesses can alternatively be treated through immunomodulation using medicinal plants **(Bachheti et al., 2023).** Immunomodulators are compounds that catalyze immune reactions **(Sharma et al., 2015).**

The natural extract utilized in this study is propolis (bee's glue) has attracted considerable scientific interest over the past few decades due to its diverse biological and pharmacological activities (**Al-Hariri,2011)**. It is a complex natural product with well-documented antioxidant, anti-inflammatory, antibacterial, and antiviral properties and immunomodulatory **(Magnavacca et al., 2022)**. Chemically, propolis is rich in polyphenolic compounds, particularly flavonoids, cinnamic acids, and esters **(Anjum et al., 2019)**. Historically, it has been used by various cultures as a dietary supplement and traditional remedy for enhancing health and treating diseases **(Tolba et al., 2013)** and **(Popova et al., 2019)**. Biologically, propolis is a resinous substance collected by bees from plant sources surrounding the hive, which they use to build, maintain, and protect their colony **(Azizah et al., 2022).** Recent scientific studies have identified propolis as one of the most promising agents for immunomodulation **(Al-Hariri, 2019).**

The phagocytic activity is expressed by the phagocytic index (K) reflects the total activity of the RES in contact with the bloodstream and by corrected phagocytic index (α) measures RES activity per unit weight of active organs (liver and spleen), carbon clearance half-time(T1/2) represents the time required for the carbon concentration in the blood to decrease by half **(Kehili and Zerizer, 2024).**

In the present study, our objectives were to:

- determination of phagocytic activity;
- determination of corrected and half time;
- calculation of percentage change ;
- determination of GSH.

Bibliographic part

I. Immune system

I.1. Definition

The immune system is an interactive network of lymphoid organs, cells, humoral factors, and cytokines. It is a mechanism that allows a living organism to discriminate between "self" and "non self." The normal function of the immune system in recognising, repelling, and eradicating pathogens and other foreign molecules **(Parkin et al., 2001)**. It also has memory, allowing a quicker and heightened response on subsequent exposure **(Cota and Midwinter, 2015).**

I .2. Types of immune system

The body has a collection of physical barriers to prevent infection, but once these are overcome, we rely on our immune systems to protect us against a wide variety of infections. The complex mechanisms through which this is achieved are grouped into two lines of defense called the « innate » and « adaptive » immune systems **(Marshall et al., 2024).**

I.2.1 Innate immune

Innate immunity is the first immunological mechanism for fighting against an intruding pathogen. It is a rapid immune response, initiated within minutes or hours after aggression, that has no immunologic memory **(Marshall et al., 2018).**

Innate immunity can be viewed as comprising four types of defensive barriers: anatomic (skin and mucous membrane), physiologic (temperature, low pH and chemical mediators), endocytic and phagocytic, and inflammatory. The important function of innate immunity is the rapid recruitment of immune cells to sites of infection and inflammation through the production of cytokines and chemokines (small proteins involved in cell–cell communication and recruitment). Cytokine production during innate immunity mobilizes many defense mechanisms throughout the body while also activating local cellular responses to infection or injury such as IL-1, IL-6 and TNF **(Murphy et al., 2007) and (Turvey and Broide, 2010).**

I.2.2 Adaptive immune

The adaptive immune system, also known as the acquired immune system or, more rarely, as the specific immune system, is a subsystem of the overall immune system that is composed of highly specialized, systemic cells and processes that eliminate pathogens or prevent their growth **(Kaile, 2024).**

The development of adaptive immunity is aided by the actions of the innate immune system, and is critical when innate immunity is ineffective in eliminating infectious agents. The primary

functions of the adaptive immune response are: the recognition of specific "non-self" antigens, distinguishing them from "self" antigens; the generation of pathogen-specific immunologic effector pathways that eliminate specific pathogens or pathogen-infected cells; and the development of an immunologic memory that can quickly eliminate a specific pathogen should subsequent infections occur **(Bonilla and Oettgen, 2010).**

It is much slower to respond to threats an infections and also takes days or even weeks to become established much longer than the innate response; however, adaptive immunity is more specific to pathogens. In fact, without information from the innate immune system, the adaptive response could not be mobilized **(Kaile et al., 2024).**

The core cellular players in adaptive immunity are lymphocytes, specifically T cells and B cells **(Cooper, 2015)** and **(Miller, 2020) (Figure 01).**

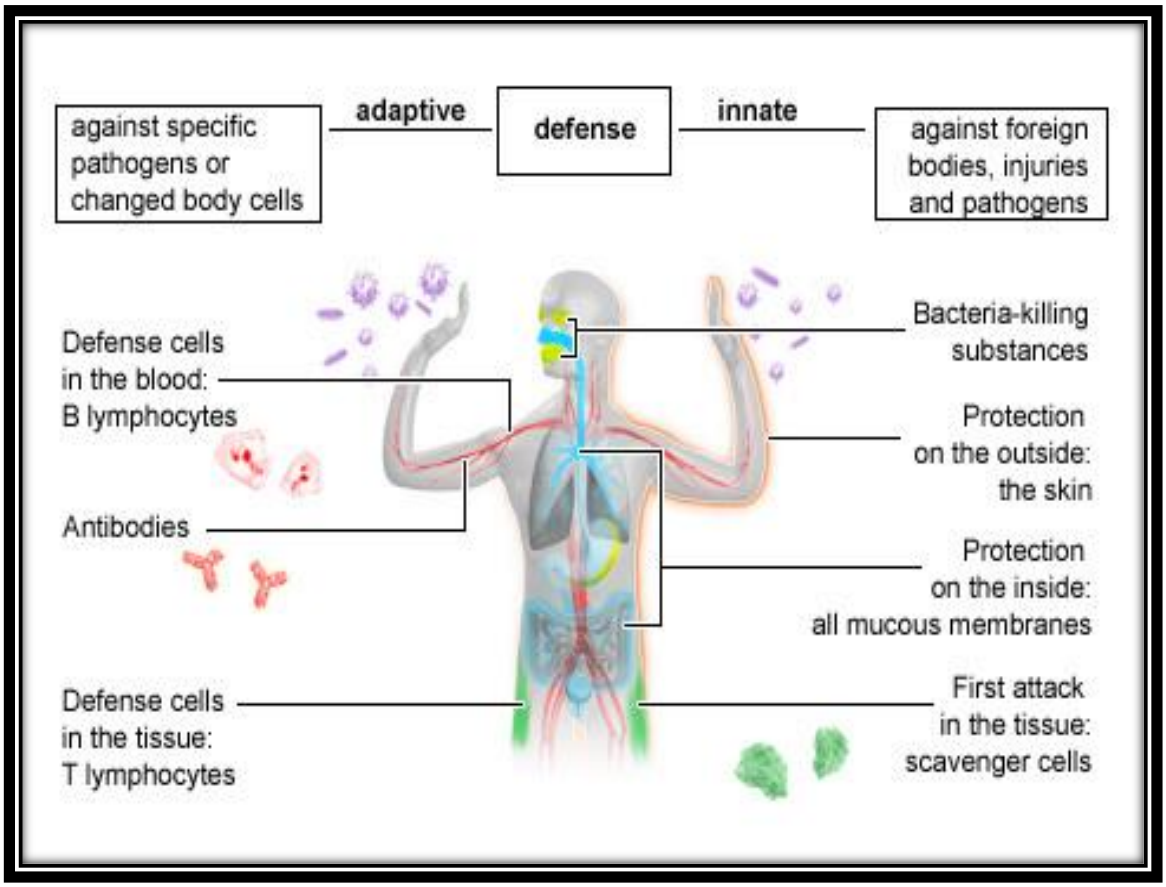


Figure 01. Scheme represents innate and adaptive immune **(Cologne, 2006).**

I.3. Reticuloendothelial system

I.3.1 Definition

The reticuloendothelial system (RES), also known as the mononuclear phagocyte system (MPS), is responsible for the phagocytosis of foreign particles, including bacteria, dead cells, and antigen-antibody complexes from the blood, and participate in inflammatory responses **(Sam-Yellowe, 2021).**

I.4. Innate immune cells

The cells of the immune system originate in the bone marrow, where many of them also mature. They then migrate to guard the peripheral tissues, circulating in the blood and in a specialized system of vessels called the lymphatic system **(Charles et al., 2001).**

4.1.Dendritic cells

Dendritic cells are the primary antigen-presenting cells in the immune system, serving as the link between innate and adaptive immune systems. Similar to macrophages, they can migrate the tissues, actively extending protrusions and forming macropinosomes immature. dendritic cells can become activated following exposure to inflammatory signals including bacterial LPS or phagocytosis of bacteria **(Dixon et al., 2001) (Figure 02).**

Figure 02. Structure of dendritic cell **(Murphy et al., 2016).**

4.2. Macrophages

A hallmark of macrophages is their phagocytic capabilities supporting the clearance of intruders and priming immune responses. However, macrophages also enable other essential homeostatic functions such as metabolism **(Mosser et al., 2020).** Macrophages are highly involved in tumor development and progression as tumor associated macrophages (TAMs) secrete factors inducing immune tolerance towards tumor cells. They are therefore of great interest in immuno-oncology and are central to investigations evaluating the impact of therapy on the tumor microenvironment (TME) **(Petty et al.,2019).**

Two main types of macrophages are described based on their stimuli induced polarization—M1 and M2. M1 macrophages are pro-inflammatory, promote an oxidative state with reactive oxygen species (ROS) production, and secrete inflammatory cytokines and chemokines, M2 macrophages are considered immunosuppressive and secrete anti-inflammatory factors such as IL-10 and TGF-β **(Petty et al.,2019) (Figure 03).**

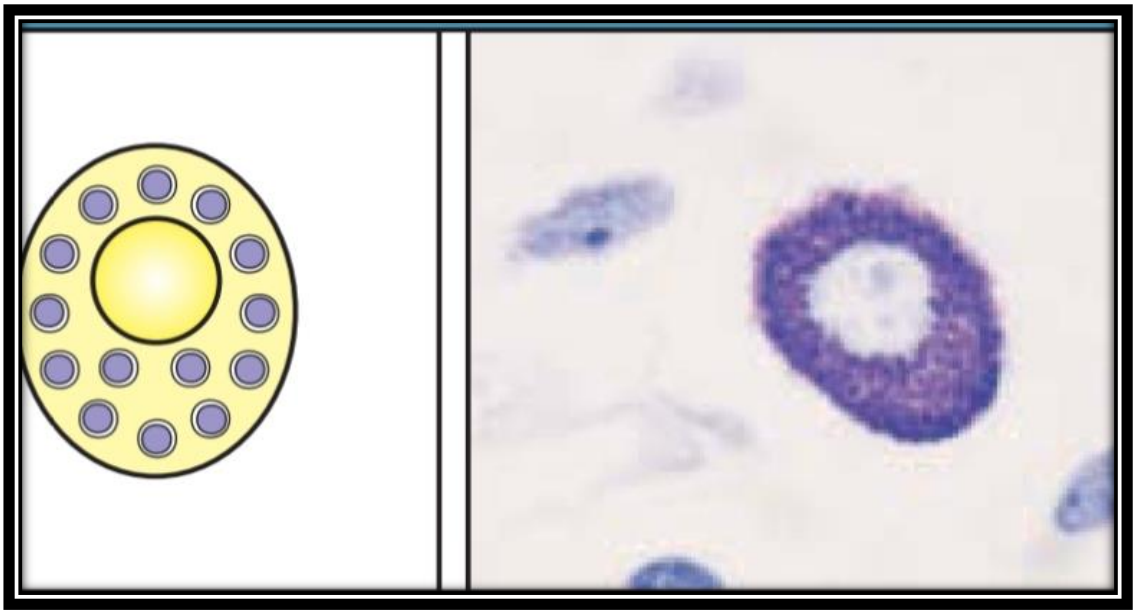


Figure 03. Structure of macrophage **(Murphy et al., 2016**).

4.3. Monocytes

The monocytes are white blood cells that derive from the bone marrow. It is part of the innate immune response and functions to regulate cellular homeostasis, especially in the setting of infection and inflammation **(yáñez et al., 2017)**. They account for approximately 5% of circulating nucleated cells in normal adult blood **(prinyakupt and Pluempitiwiriyawej, 2015).** The half-life of circulating monocytes is approximately one to three days **(Patel et al., 2017).**

It has two distinct roles, they regularly patrol the body for microbial cells and orchestrate an immune response in times of infection and inflammation **(yáñez et al., 2017) (Figure 04).**

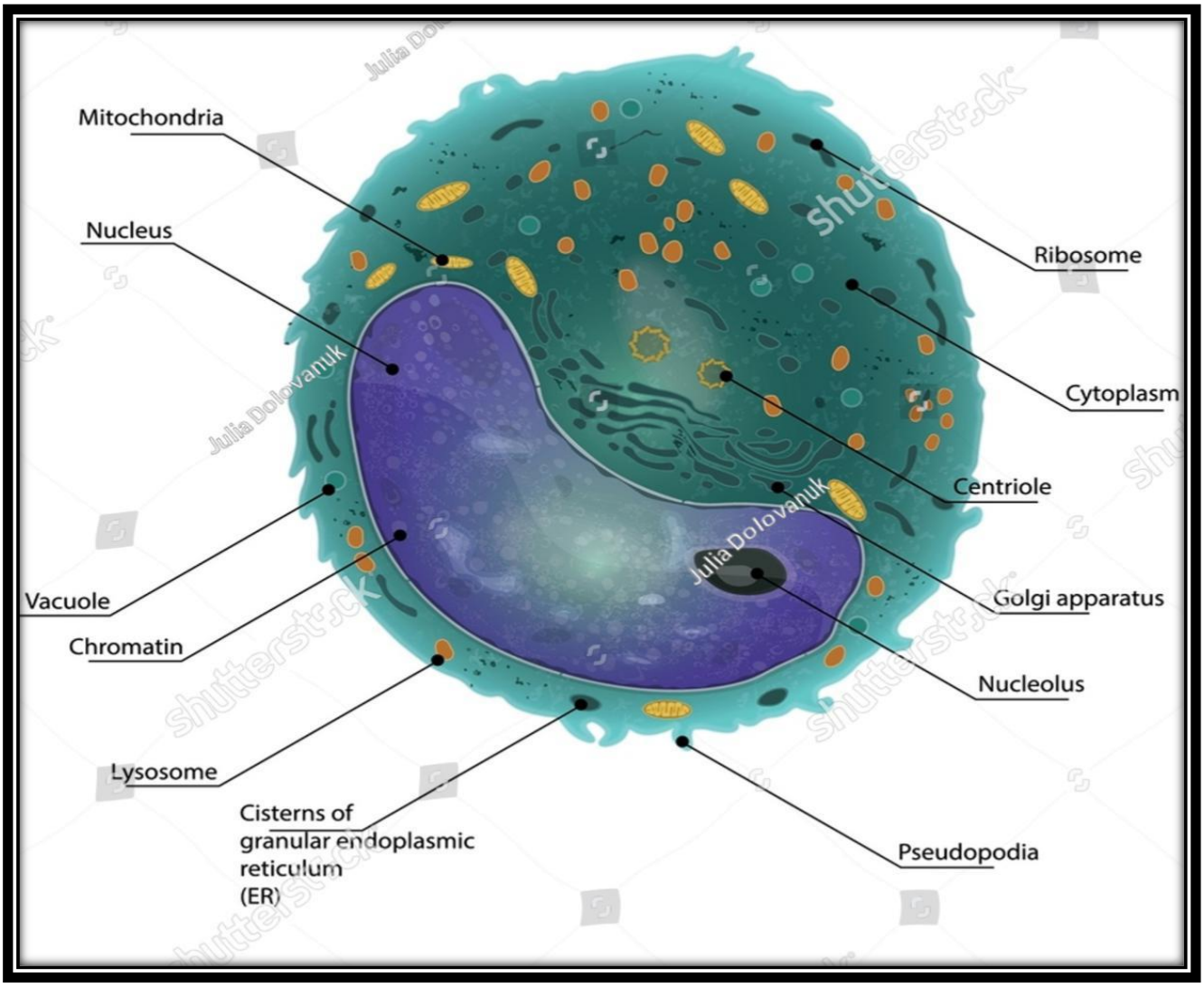


Figure 04. Structure of monocytes (1).

4.4. Granulocytes

The granulocytes are effector cells that predominate during the early or acute phase of the innate immune response. The main function of these cells is to identify, ingest, and destroy microbial pathogens through receptors, oxidative mechanisms, and enzymes including lysozyme, collagenase, and elastase, etc. This group of cells is composed of neutrophils, eosinophils, basophils and mast cells **(Nauseef, 2007)** and **(Rehaume and Hancock, 2008).**

4.4.1. Neutrophils

The neutrophils cells known as polymorphonuclear (PMN) leukocytes, are the most abundant cell type in human blood. They are produced in the bone marrow in large numbers, ~1011 cell per day. Under homeostatic conditions, neutrophils enter the circulation, migrate to tissues, where they complete their functions, and finally are eliminated by macrophages, all in the lapse of a day. Neutrophils are important effector cells in the innate arm of the immune system **(Mayadas et al., 2014).**

They constantly patrol the organism for signs of microbial infections, and when found, these cells quickly respond to trap and kill the invading pathogens. Three main antimicrobial functions are recognized for neutrophils: phagocytosis, degranulation, and the release of nuclear material in the form of neutrophil extracellular traps **(Nauseef and Borregaard, 2014) and (Scapini and Cassatella, 2014) (Figure 05).**

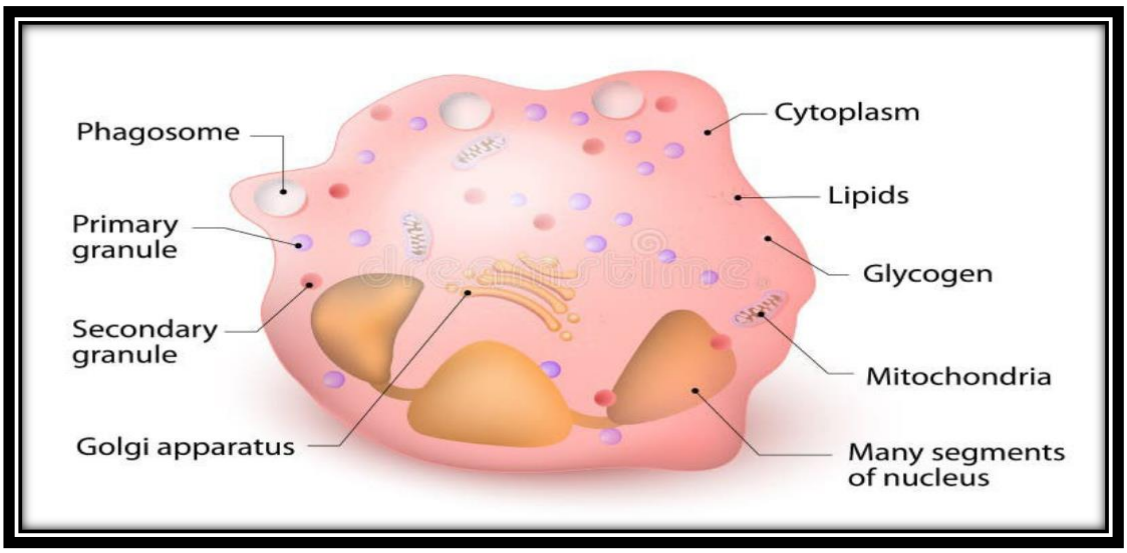


Figure 05. Structure of **neutrophil** (2).

4.4.2. Eosinophils

These granulocytes are present in the respiratory, gastrointestinal, and urinary tract, and they are less abundant than neutrophils. Their effector function is mediated by degranulation and release of histamine, cationic proteins, major basic protein, sulfatases, and chemotactic factors

such as leukotrienes and prostaglandins. The degranulation process is mediated by the IgE or other chemotactic factors, including the IL-5 **(Fulkerson and Rothenberg, 2013).**

The main function of these cells is to destroy microbial pathogens, mainly parasites, but they also play an important role in the allergic processes together with mast cells **(Fulkerson and Rothenberg, 2013) (Figure 06).**

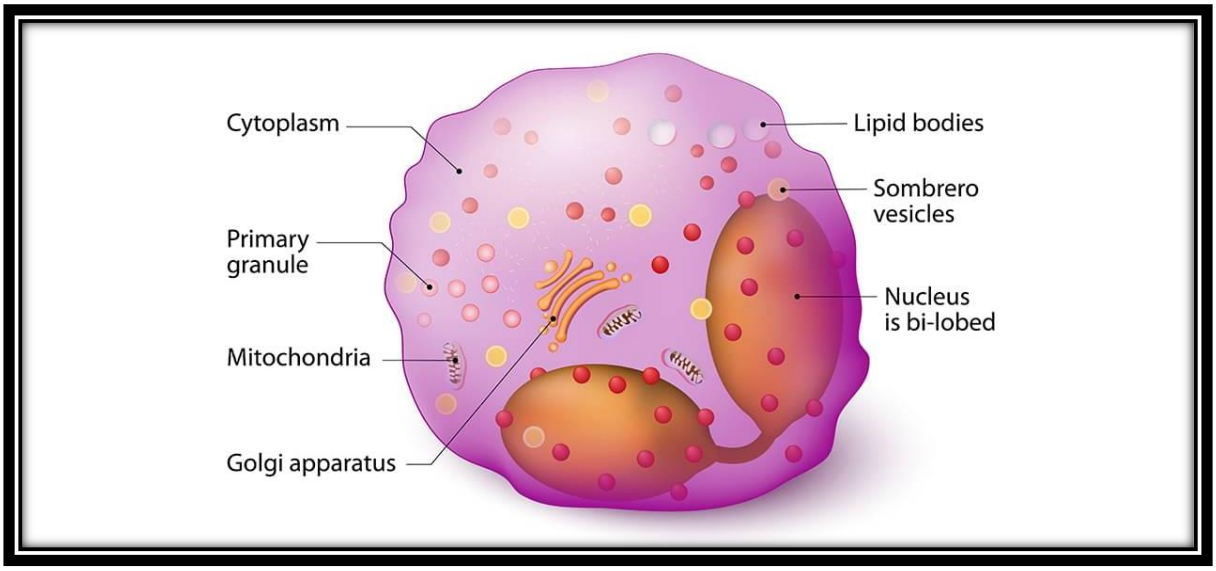


Figure 06. Structure of eosinophil (3)

4.4.3. Basophils

Basophils are potent effector cells of the innate immune system, and they have both beneficial and detrimental functions for the host. They are mainly implicated in pro-inflammatory responses to allergens but can also contribute to protection against pathogens **(Voehringer, 2013).** These cells are not phagocytic in nature and have several receptors including IgE receptors. The proportion of basophils in circulation is lower than the proportion of other granulocytes **(Varadaradjalou et al., 2003)** and **(Abraham and John, 2010)**. Basophils are spherical cells 5–10 μm in diameter with polylobed nuclei containing condensed chromatin. Furthermore, basophil-derived IL-4 enhances eosinophil migration and the differentiation of monocytes into M2 type macrophages, **(Dvorak, 1994)**, **(Knol and Olszewski, 2011)**, **(Chirumbolo, 2012) (Pellefigues et al., 2018)** and **(Chirumbolo et al., 2018) (Figure 07).**

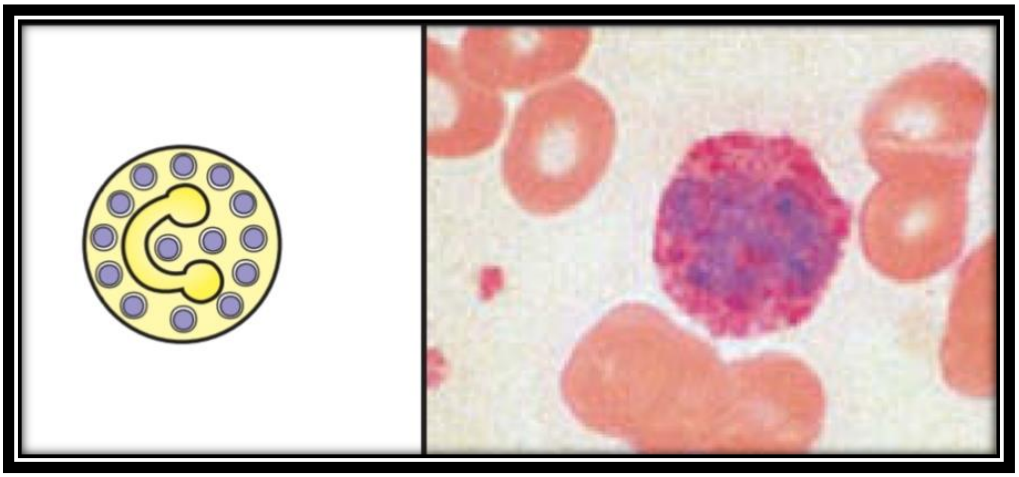


Figure07 . Structure of basophil (**Murphy et al., 2016**).

4.5. Natural killer cells

Natural killer cells (NK) are classically considered to be innate immune effector lymphocytes due to their lack of antigen-specific receptors. However, more recently, they are known to contribute to both arms of the immune system through their regulatory functions exerted by cytotoxicity and cytokine production **(Vivier et al., 2011)**. These cells, accounting for approximately 10% of human peripheral blood (PB) lymphocytes, primarily develop in the bone marrow and other secondary lymphoid tissues including the tonsils and spleen. NK cells predominantly circulate in human peripheral blood with some tissue-specific subsets residing, at least temporarily, within the liver, intestine, spleen and bone marrow. They can also be found in the uterus during pregnancy, in the lungs and the skin **(Grégoire et al., 2006) and (Vivier et al., 2011).** Generally, circulating NK cells can be subdivided according to the surface expression of CD56 and CD16 **(Sivori et al., 2019).** These subsets exhibit major functional differences in their cytotoxicity, cytokine production, and homing capabilities and their functions are governed by activating, co-stimulatory and inhibitory receptors. Generally, CD56 bright NK cells are predominantly found in tissues and have poor cytolytic activity, while CD56 dim NK cells are found in human peripheral blood, have stronger cytolytic activity and express CD16, the molecule responsible for initiating antibody dependent cell-mediated cytotoxicity (ADCC**) (Sivori et al., 2019) (Figure 08).**

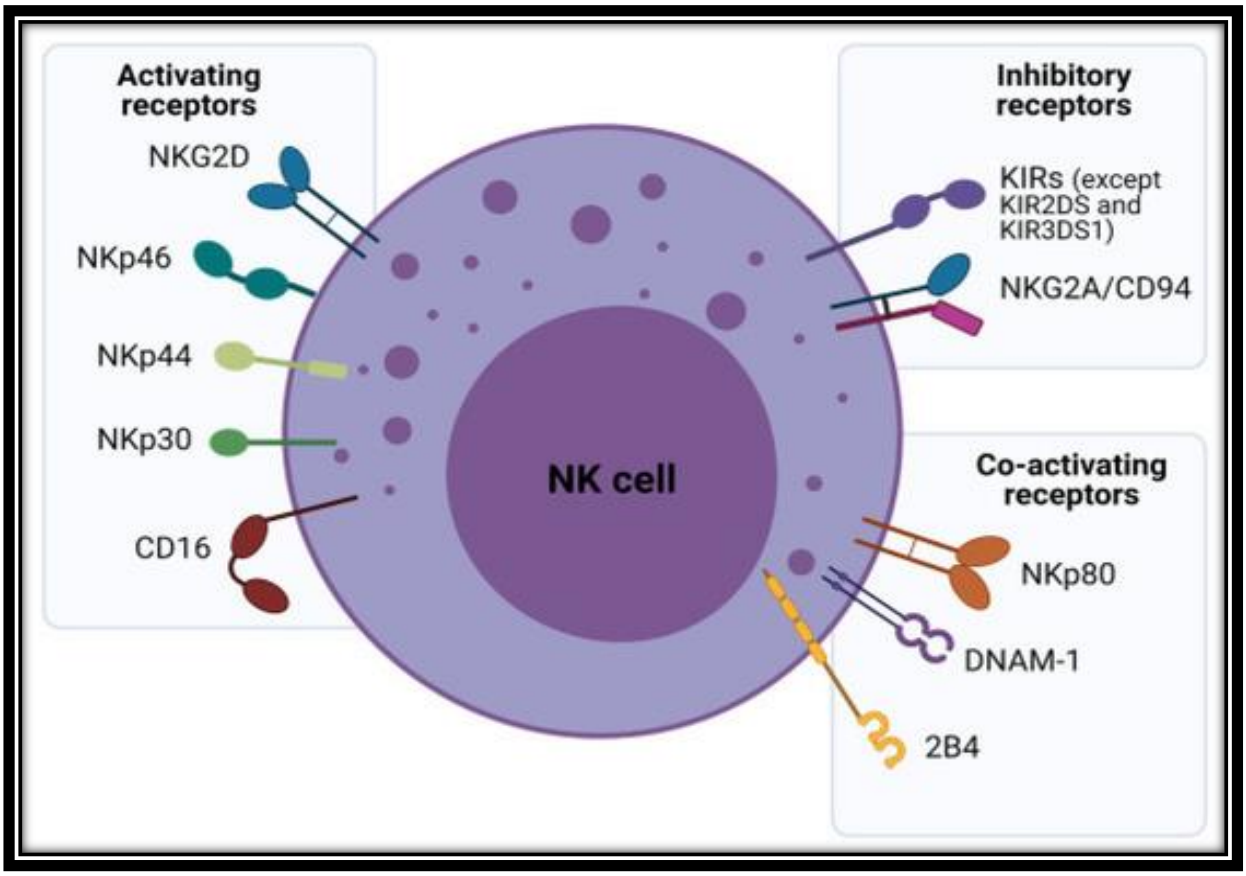


Figure 08. Structure of NK cell (**Rasa et al., 2021).**

I.5.Adaptive immune cells

5.1.T lymphocytes

T lymphocytes or T cells originate in the bone marrow and travel to the thymus for their maturation. Characteristic markers of T cells include CD3 and T cell receptor (TCR). They are later categorized by the expression of other surface molecules CD4 and CD8 **(Mousset et al., 2019) Figure 09.**

5.2. B lymphocytes

B lymphocytes cells (bursa-derived cells) are essential components of adaptive immune response, primarily responsible for humoral immunity in mammals **(Pieper et al., 2013)**. B-cell development originates from hematopoietic stem cells and involves several stages of early differentiation, progressing through maturation, antigen interaction, and antibody synthesis **(Clark et al., 2014).** Through this process, B cells acquire two essential features of adaptive immunity—the ability to distinguish between self and non-self (recognizing foreign antigens

rather than self-antigens) and the ability to form a memory of previous antigen encounters. This memory allows for a more effective and rapid response upon subsequent interactions with the same antigens **(Khodadadi et al., 2019) (Figure 09).**

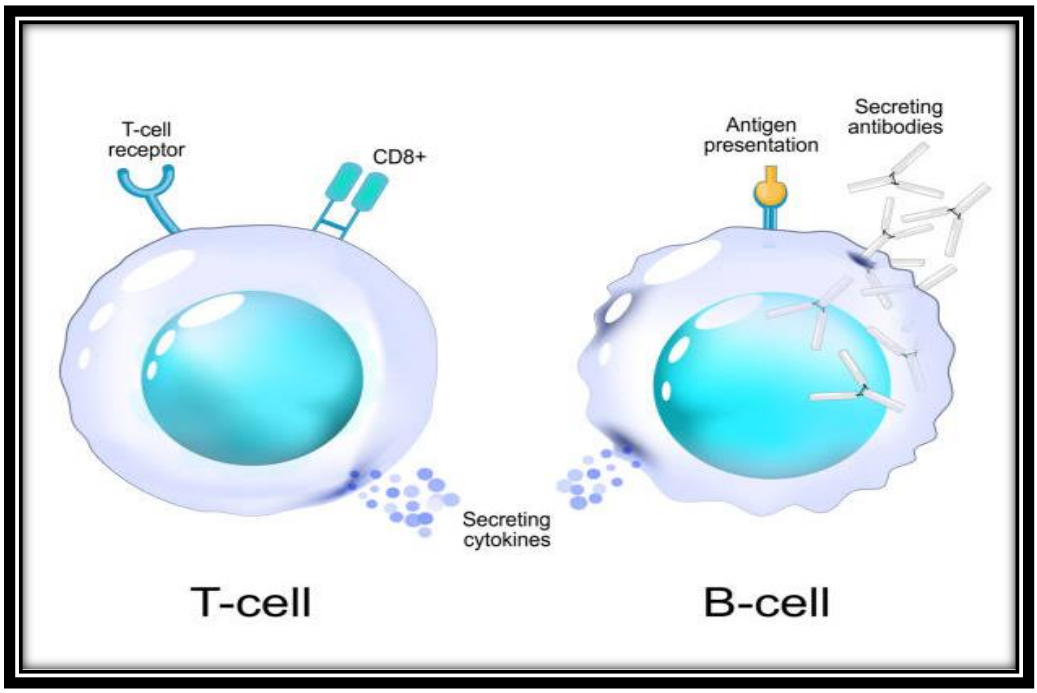


Figure 09. Structure of T lymphocyte and B lymphocyte (4).

I.6.Phagocytes

I.6.1 Definition

Macrophages are phagocytes and one of the white blood cells discovered by Ilya I. Mechnikov in1892. They engulf and digest foreign substances like pathogens and conduct antigen presentation, mature from haematopoietic stem cells in bone marrow, moving into blood vessels and become monocytes, and differentiate into macrophages in the tissue. Macrophages have intracellular granules called lysosome accumulating digestive enzymes. Their life span is several months and proliferates by cell division **(Kazuki, 2023)**.

I.6.2. Phagocytosis

1. Binding (Recognition of pathogens)

The first step involves the recognition and binding of pathogens to specific receptors on the surface of phagocytic cells (like macrophages and neutrophils)**.**

These receptors include:

- TLRs (Toll-like receptors): Recognize microbial molecules like LPS (TLR4), flagellin (TLR5), and nucleic acids (TLR3, TLR7, TLR9).
- Mannose receptor / Dectin-1: Bind fungal components.
- Fcγ receptors (FcγRs): Recognize opsonized microbes (coated with antibodies).
- Scavenger receptors (SR-A, MARCO): Bind a wide range of microbial products Integrins: Recognize apoptotic cells (**Charles et al., 2001).**

2. Signal transduction (Activation of intracellular pathways)

After ligand binding, receptor activation triggers intracellular signaling cascades. For example:

- TLRs activate adaptor proteins (e.g., MyD88), leading to the activation of NF-κB.
- Small GTPases such as Rac1 and Cdc42 are activated and are essential for actin remodeling.
- These signals coordinate cytoskeletal changes, transcriptional activation, and priming of the cell for engulfment **(Gordon, 2016).**

3. Pseudopodia extension

Signal transduction activates actin polymerization via:

- PAK (p21-activated kinase).
- FAK (Focal adhesion kinase).
- Arp2/3 complex: Drives actin branching and membrane protrusion. These structures form pseudopodia, which extend around the microbe in a zipper-like motion, engulfing it into a vesicle **(Underhill and Goodridge, 2012).**

4. **Internalisation (Phagosome formation)**

The plasma membrane completely surrounds the pathogen, forming an intracellular vesicle called the phagosome.

The phagosome separates from the membrane and moves into the cytoplasm, where it will undergo maturation **(Flannagan et al.,2012)** and **(Underhill and Goodridge., 2012).**

5. **Lytic degradation (Phagolysosome formation)**

The phagosome fuses with a lysosome to form a phagolysosome inside:

- acidification occurs (via proton pumps).
- hydrolytic enzymes (e.g., proteases, nucleases) degrade the microbe.
- reactive oxygen species (ROS) and nitric oxide (NO) may also be used to kill the microbe **(Charles et al., 2001) and (Vieira, 2002).**

6.Gene Expression (Activation of inflammatory genes)

While phagocytosis occurs, transcription factors like NF-κB translocate to the nucleus and will induce:

- cytokine expression (e.g., TNF-α, IL-1, IL-6 ;
- chemokine release;
- antigen presentation molecules (e.g., MHC class II) **(Figure 10).**

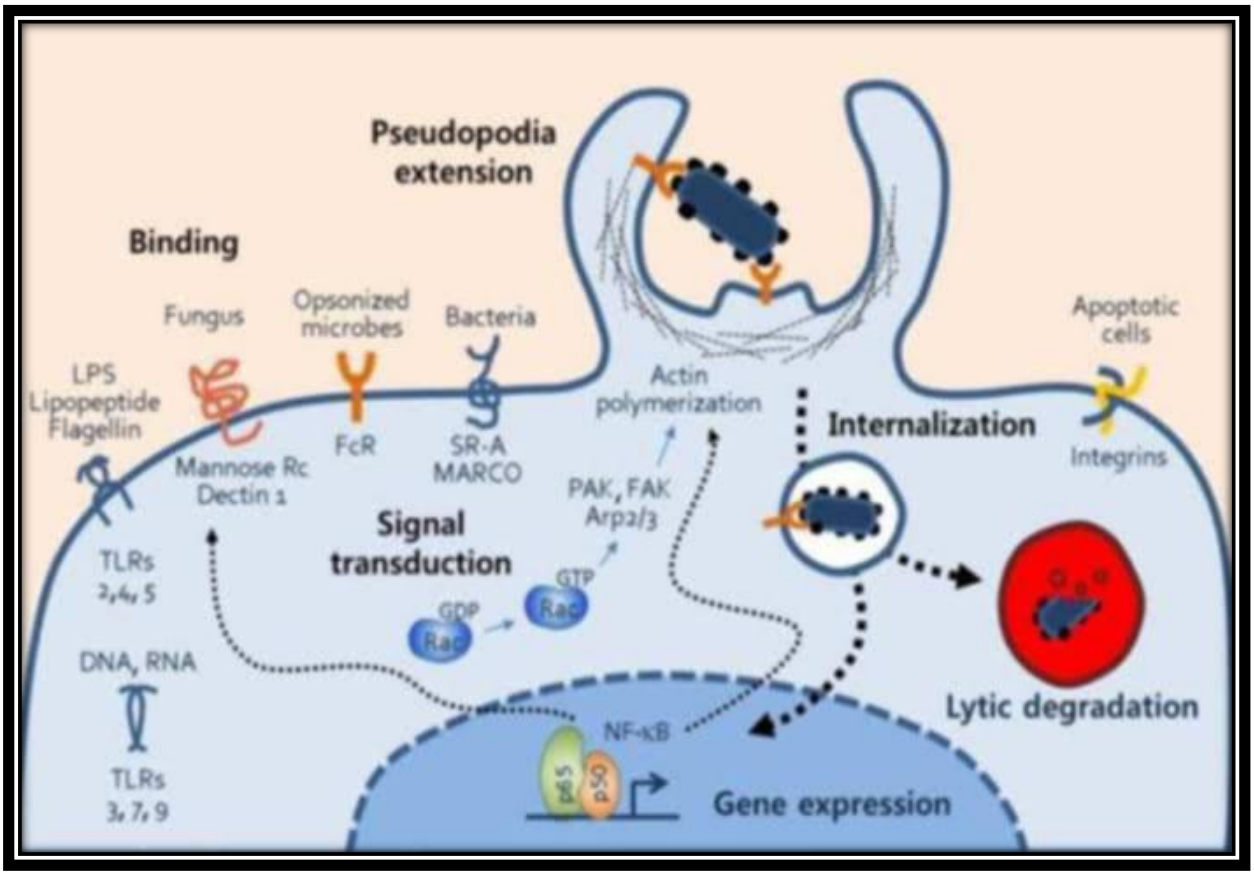


Figure 10. Steps of phagocytosis **(Sun et al., 2023).**

II. Immune system organs

II.1. Liver

II.1.1. Definition

The liver is the largest visceral organ in mammals. In man its weight amounts is 1.5 kg; about 30% of the hepatic volume is made up by blood **(Lautt, 1987)** and accounts for approximately 2.5% of adult body weight **(Ozougwu, 2017).**

II.1.2. General description of the liver

The liver lies mainly in the right upper quadrant of the abdomen, where it is hidden and protected by the thoracic cage and diaphragm. The normal liver lies deep to the ribs 7 on 11 the right side and crosses the midline towards the left nipple.

The surface of the liver is smooth and dome shaped, where it is related to the concavity of the inferior surface of the diaphragm **(Ozougwu, 2017).**

The liver is divided into 4 lobes (right, left, caudate, and quadrate). The right and left lobes are the largest, while the caudate and quadrate are smaller and located posteriorly. Two ligaments are visible anterior and superior surfaces. The falciform ligament separates the right and left lobes. Inferior to the falciform ligament is the round ligament, which protrudes from the liver slightly. Also visible anteriorly on the most inferior portion of the right lobe is the gallbladder. Posteriorly, many more interesting structures can be observed **(Ozougwu, 2017).**

The caudate lobe is located superiorly, approximately between the right and left lobes. Adjacent to the caudate lobe is the sulcus for the inferior vena cava. Just inferior to the caudate lobe is the porta hepatis, where the hepatic artery and hepatic portal vein enter the liver. The portal vein carries nutrient laden blood from the digestive system. Inferior to the porta hepatis is the bile duct which leads back to the gallbladder **(Ozougwu, 2017),** he also explained that the blood processed by the liver exits into the hepatic vein, is found inferior and adjacent to the sulcus for the inferior vena cava. The liver is held on place by a system of mesenteries posteriorly. And is also attached to the diaphragm via the falciform ligament. Additionally, most of the liver is covered by visceral peritoneum **(Ozougwu, 2017)** (**Figure 11).**

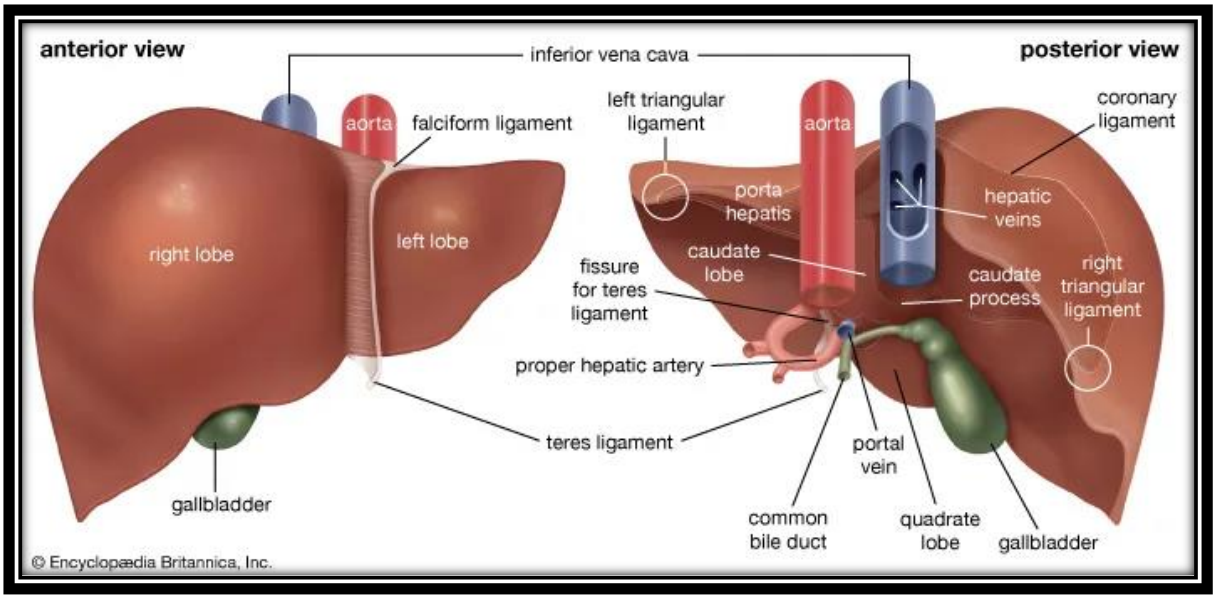


Figure11 . Structure of liver (**Ozougwu, 2017).**

II.1.3. Histology

the study of microscopic anatomy, shows two major types of liver cell: parenchymal cells and nonparenchymal cells. About 70–85% of the liver volume is occupied by parenchymal hepatocytes. Nonparenchymal cells constitute 40% of the total number of liver cells but only 6.5% of its volume **(Kmiec, 2001).** The liver sinusoids are lined with two types of cell, sinusoidal endothelial cells, and phagocytic Kupffer cells **(Gillian et al., 2006).** Hepatic stellate cells are nonparenchymal cells found in the perisinusoidal space, between a sinusoid and a hepatocyte Additionally, intrahepatic lymphocytes are often present in the sinusoidal lumen.**(Kmiec, 2001)(Figure 12).**

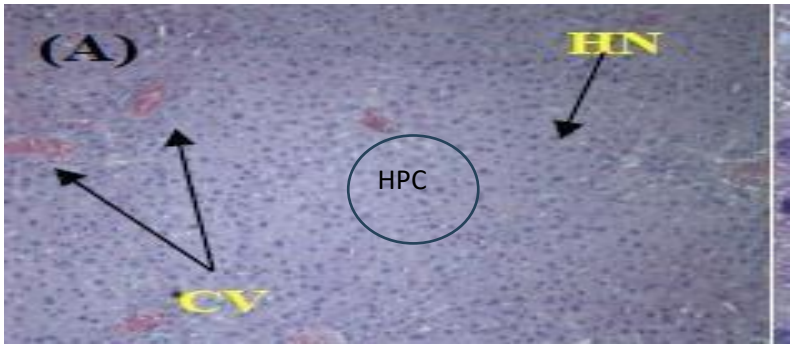


Figure12. Section of liver in mice (x 100) (**Baghriche, 2024)**

CV: Central vein, HN: Hepatocyte nuclei, HPC: Hepatocyte cells

II.1.4. Vascularisation

The liver is a very vascular organ and at rest receives up to 25% of total cardiac output, more than any other organ. Its dual blood supply is uniquely divided between the hepatic artery, which contributes 25% to 30% of the blood supply, and the portal vein, which is responsible for the remaining 70% to 75%. The arterial and portal blood ultimately mixes within the hepatic sinusoids before draining into the systemic circulation via the hepatic venous system (**Hilscher et al., 2019) (Figure 13).**

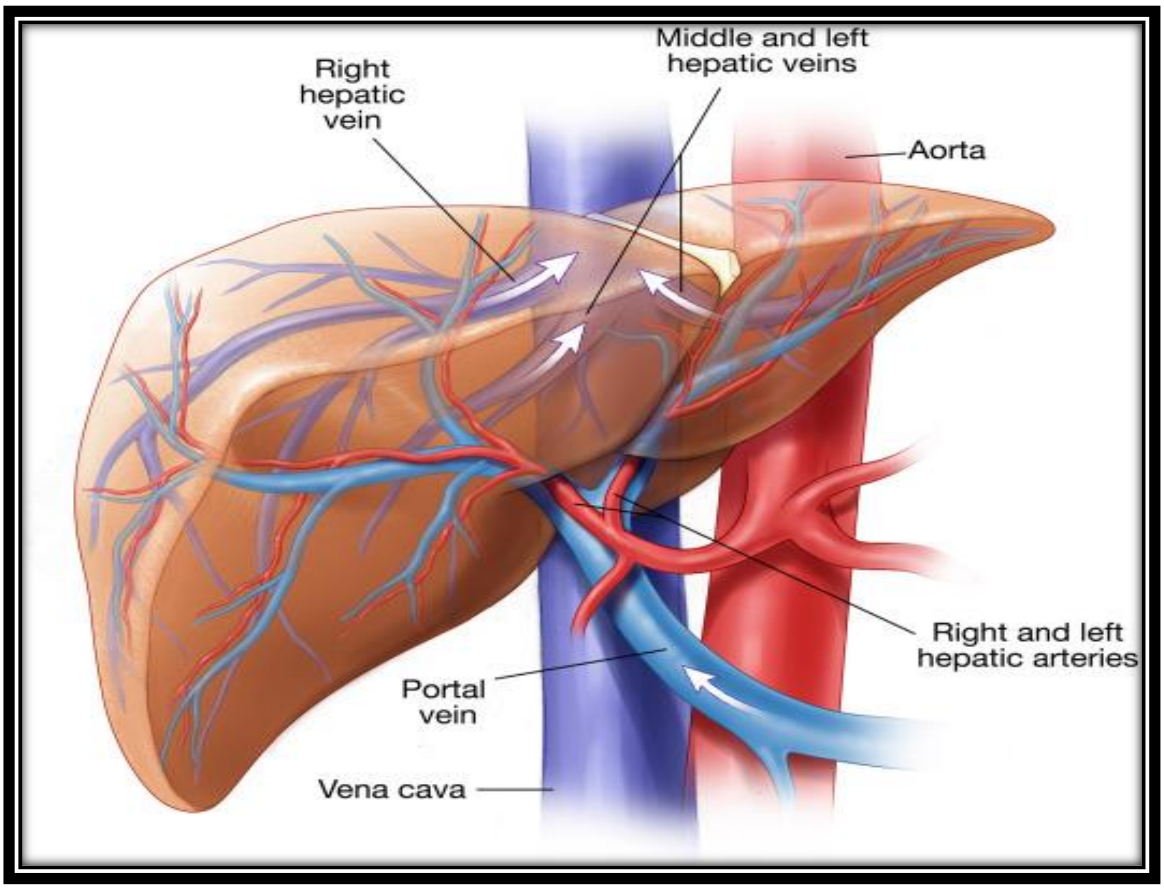


Figure 13. Liver vascularisation. **(Hilscher et al., 2019).**

II.1.5. Liver function

The liver has numerous functions best grouped into secretion of bile, metabolism of bilirubin, vascular and hematologic functions, metabolism of nutrients, metabolic detoxification, storage of minerals and vitamins **(Table 01).**

Table 01: Summary of major functions of the liver **(Ozougwu, 2017).**

Secretion of bile
Metabolism of bilirubin
Vascular and hematologic functions ➢ Important blood reservoir
Metabolism of nutrients ➢ Fat - fatty acid oxidation, synthesis of cholesterol/lipoproteins and production of ketoacids ➢ Protein – Amino acid production, turnover of proteins ➢ Carbohydrate – converts galactose/fructose to glucose, gluconeogenesis and contains 100g of glycogen for release
Metabolic detoxification ➢ Toxins ➢ Hormones ➢ Drugs
Storage of minerals and vitamins ➢ Iron ➢ Copper ➢ Vitamins ADEKB12 ➢ Glycogen
Endocrine functions ➢ Activation of vitamin D ➢ Conversion of thyroxine (T4) to T3 ➢ Secretes angiotensinogen ➢ Hormone metabolism
Immunological/ protective functions Reticuloendothelial component ➢ Filters the portal blood from bacteria ➢ Important in antigen presentation

- Phagocytosis via kupffer cells
- Removes haemolysis products

Inactivation of toxins and drugs

- Phase I (oxidation, reduction and hydrolysis)
- Phase II (conjugation/ cytochrome P450 system)

II.2. Spleen

II.2.1. Definition

The spleen is the largest organ of the lymphatic system positioned between the fundus of the stomach and the diaphragm in the left hypochondriac region of the abdominal cavity, relatively below the left costal margin between the ninth and 11th ribs. The spleen is spongy and appears reddish purple on account of it being densely vascularized. A healthy spleen is usually not palpable in most individuals. It is encased in a weak outer connective tissue capsule which allows for protection and also the expansion of the organ and is subdivided into many smaller internal sections termed lobules **(Steinger et al., 2018)** and **(Lung and Lui, 2023).**

The spleen has an anterior and posterior segment and rests on the upper pole of the left kidney and tail of the pancreas. The spleen has 3 distinct borders: superior, inferior, and intermediate. The superior border of the spleen has a notch on the anterior end. The spleen has 2 surfaces, the visceral and diaphragmatic. It is roughly the size of an individual's fist, measuring about 10 cm to 12 cm and weighing about 150 g to 200 g (about 5.29 oz to 7.05 oz) **(Steinger et al., 2018)** and **(Lung and Lui, 2023).**

It plays a key role in immunological defense, metabolism, and maintenance of circulating blood elements **(Gent and Blackie, 2017), (Özdikici, 2018) and (Standring, 2021).**

II.2.2 Spleen histology

The human spleen is composed of red and white pulp, which are separated by a thin marginal zone the red pulp makes up approximately 75% of the spleen and is predominantly composed of splenic cords, capillaries, and venous sinuses, which express endothelial markers (e.g., clotting factor VIII), within loose reticular tissue. This richly vascular, specialized portion of the spleen Enables it to function as a filter of blood. The white pulp consists of lymphoid follicles (mostly B Lymphocytes) and the periarterial lymphoid sheath (PALS) (mostly T

lymphocytes). These, along with the lymphoid, non-filtering red pulp (both B and T lymphocytes), are responsible for the spleen's immunologic function. Although comprising only a minority of the overall mass, this lymphoid compartment plays an important role in The early immunologic response against blood-borne Antigens and is the compartment primarily responsible for splenic involvement with lymphoproliferative disorders **(Mebius and Kraal, 2005), (Paraskevas, 2014) and (Porembka and Doyle, 2014)**. The spleen's lymphoid cells express characteristic cluster designation (CD) and other markers that confer specific immunophenotypes to various regions of the spleen **(Meghan and Myutan, 2024) (Figure 14).**

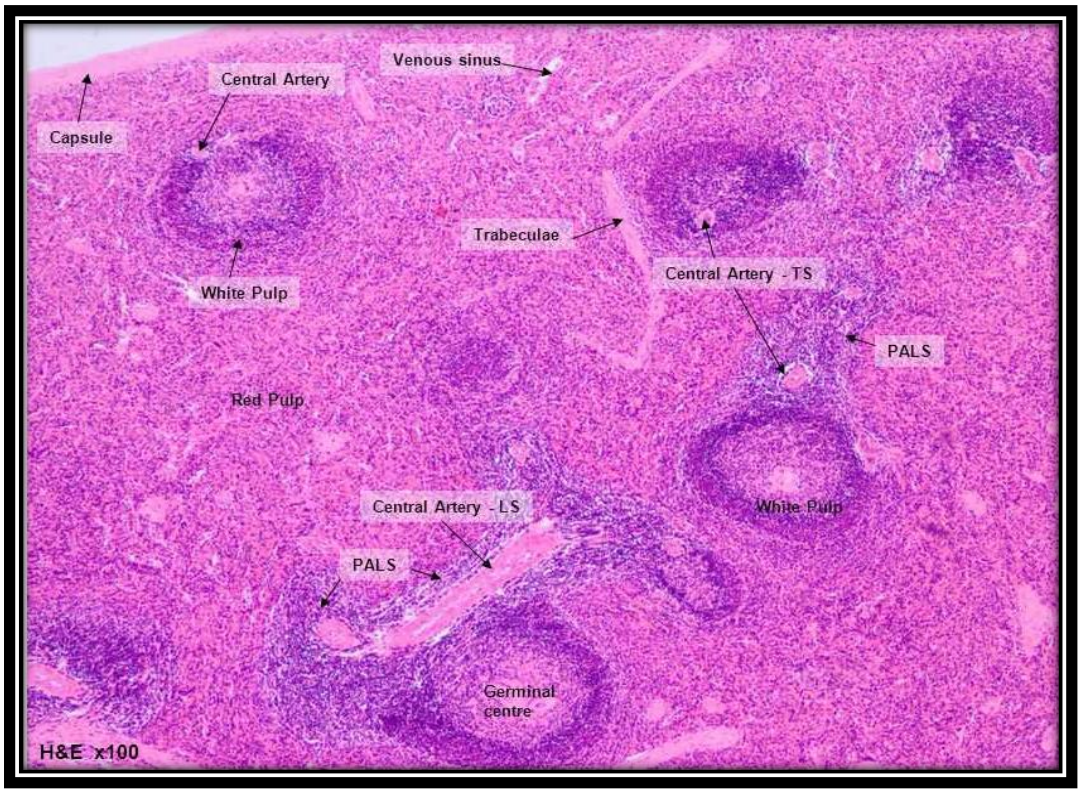


Figure 14. Visceral surface of the spleen **(Meghan and Myutan, 2024).**

II.2.3 Vascularisation

The main arterial supply of the spleen is the splenic artery. It courses to the left, along the superior border of the pancreas, posterior to the omental bursa, and anterior to the left kidney. It divides into five or more branches that enter the hilum of the spleen, within the splenorenal ligament. As the splenic artery passes the superior border of the pancreas, it gives off small arterial branches that supply the pancreatic neck, tail, and body. It also provides the short gastric arteries that supply the fundus of the stomach, and left gastro-omental artery which courses

along the greater curvature of the stomach. The splenic vessels do not anastomose, resulting in 2 or 3 distinct vascular segments. Between these segments are avascular planes that can be used to minimize blood loss during subtotal splenectomies **(Moore et al., 2014)** and **(Darke et al., 2015).**

The main vascular drainage of the spleen occurs through the splenic vein. Tributaries include the short gastric veins from the fundus and greater curvature of the stomach, the left gastro-omental vein from the greater curvature of the stomach, pancreatic veins that drain the pancreatic body and tail, and the inferior mesenteric vein. It courses to the right and lies inferior to the splenic artery, passing through the splenorenal ligament. The inferior mesenteric vein unites with the splenic vein, as it courses posterior to the pancreatic body and tail. Then, the splenic vein joins the superior mesenteric vein to form the hepatic portal vein, posterior to the neck of the pancreas **(Moore et al., 2014)** and **(Darke et al., 2015).**

There is a thin fibrous capsule surrounds the spleen where the connective tissue trabeculae extend into the splenic parenchyma to the hilum, including the branches of the splenic artery and vein **(Wilkins and Wright, 2000).**

II.2.4 Spleen Function

A- **Immune responses**

The spleen plays a pivotal role in both innate and adaptive immunity. It filters blood-borne pathogens, antigens, and debris, initiating immune responses. The white pulp of the spleen, which functions as a secondary lymphoid organ, is organized into T-cell zones (periarteriolar lymphoid sheaths) and B-cell follicles. Upon antigenic stimulation, B cells in the marginal zone and follicles proliferate to form germinal centers, producing high-affinity antibodies through somatic hypermutation and class switching. Marginal zone B cells capture antigens via complement receptors and present them to T cells, facilitating both T cell-dependent and independent immune responses. Additionally, natural killer T cells in the spleen recognize lipid antigens and secrete cytokines that amplify adaptive immune responses **(Lewis et al., 2019).**

One of the spleen's most important functions is phagocytosis. The spleen is a component of the reticuloendothelial system. The splenic phagocytes include reticular cells, free macrophages of the red pulp, and modified reticular cells of the ellipsoids. Phagocytes in the spleen remove debris, old and effete RBCs, other blood cells, and microorganisms, thereby filtering the blood. Phagocytosis of circulating antigens initiates the humoral and cellular immune responses. The spleen's architecture facilitates this process by allowing blood to percolate through the red pulp

cords, where macrophages scrutinize RBCs for deformability and biochemical markers such as CD47 **(Lewis et al., 2019).**

B-Hematopoiesis

The spleen plays a significant role during fetal development, particularly in the third trimester. By the late second trimester, hematopoiesis shifts to the bone marrow. In healthy adults, the spleen generally does not participate in hematopoiesis. However, in certain pathological conditions where the bone marrow fails to produce blood cells (such as myelofibrosis) or cannot meet production demands (like in chronic hemolytic anemia), extramedullary hematopoiesis in the spleen increases. The cells produced in such conditions are often more immature compared to those from the bone marrow **(Connell et al., 2013).**

C-Iron metabolism

Splenic macrophages in red pulp are specialized to recycle iron from the breakdown of senescent and damaged red blood cells **(Ganz, 2016).** Macrophages can either store ingested iron in the cytoplasm or export it via ferritin into the bloodstream **(Theurl et al., 2016).**

D-Filtration

Erythrocytes and platelets is filtrating occurs via splenic cords in the red pulp. Young, flexible red blood cells pass through the epithelial cells of the splenic cords and continue through blood flow. On the other hand, older, larger, and deformed red blood cells are trapped by the splenic cords and phagocytosed by macrophages waiting on the reticulum and sinus endothelium **(Barnhat and Lusher, 1979).**

III. Propolis

III.1. Definition

The Greek word propolis means *pro*, for or in defense, and *polis*, the city, that is "defense of the hive**" (Salatino et al., 2005)**. Another name of propolis is bee glue **(Wagh, 2013).** In arabic called العكبر **(Bankova et al., 2018).**

Propolis, a resinous substance produced by honeybees from various plant sources, has been used for thousands of years in traditional medicine for several purposes all over the world. The precise composition of propolis varies according to plant source, seasons harvesting, geography, type of bee flora, climate changes **(Hossain et al., 2022).**

It composed of about 50% resin, 30% wax, 10% essential oil, 5% pollen, and 5% other substances including a diversity of minerals (calcium, copper, iodine, iron, magnesium, manganese, potassium, sodium, and zinc), vitamins (B1, B2, B6, C, E, D, and pro-vitamin A), poly- and oligo-saccharides, and phenolic compounds (i.e., flavonoids, aromatic acids, and esters, etc) **(Silva-Carvalho et al., 2015)(Figure 15).** It also contains some enzymes as glucose-6-phosphatase, dehydrogenase, adenosine triphosphate and acid phosphatase **(Yilmaz et al., 2003)** and **(Kurek-Górecka et al., 2014).**

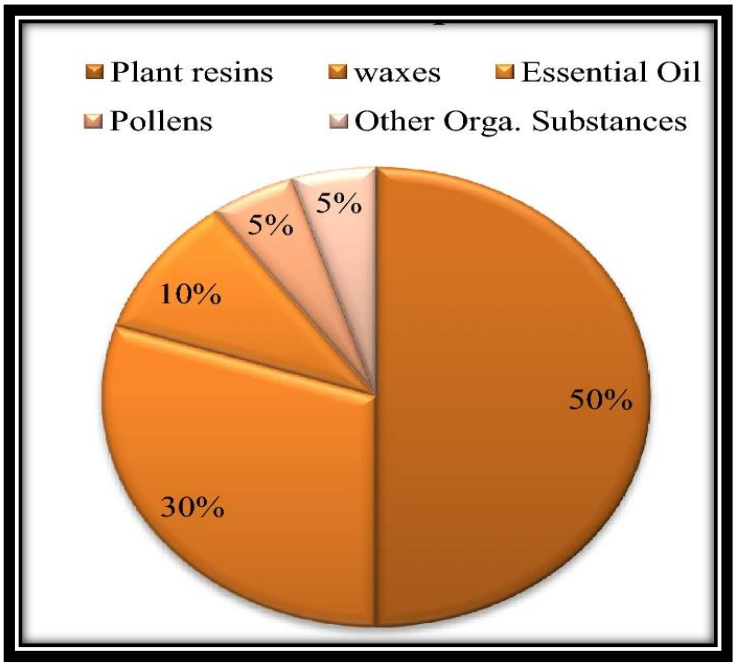


Figure 15. Chemical composition (%) of propolis **(Hossain et al., 2022).**

III.2. Therapeutic activity

Propolis has a great effect on human health and is used for various purposes. It is used as an antibacterial, antifungal, anti-inflammatory, antiviral, anesthetic, antioxidant **(Boukraâ and Sulaiman, 2009)** and **(Omar et al., 2017)**, propolis also treat various chronic diseases, particularly autoimmune diseases, diabetes, burns, wounds, gynecological problems, and

laryngological, dermatological, neurodegenerative, gastrointestinal, and respiratory tract-related diseases, cardiovascular disorders, antimicrobial, anticancer and antioxidant activities, and COVID-19 **(Zullkiflee et al., 2022).(Figure15) .**

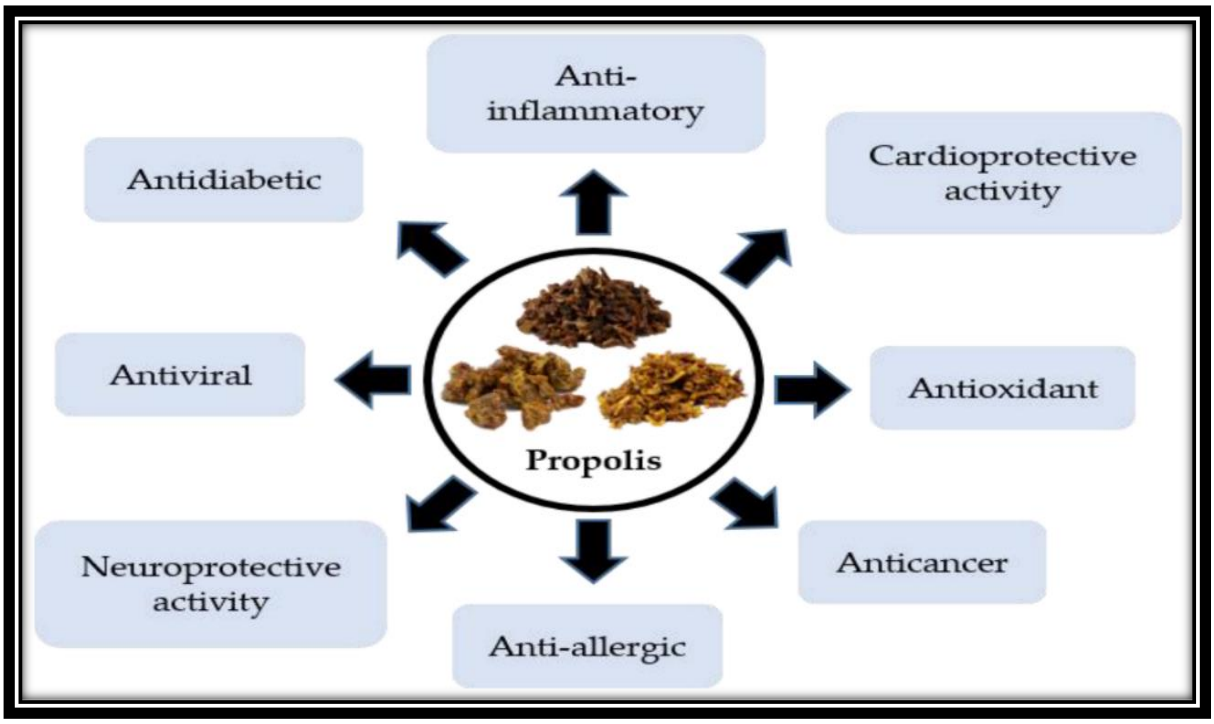


Figure 16. Biological and therapeutic properties of propolis. **(Zullkiflee et al., 2022).**

III.3. Types of propolis and their origins

Referring to literature data, there are several types of propolis, depending on its botanical and geographical origin and chemical composition. The most common is poplar, European, Brazilian, and pacific propolis. Poplar type of propolis occurs in Europe, North America, and non-tropical regions of Asia. It is made from various species of poplars **(Bankova et al. 2005).** And according to the color there are:

Red propolis (*Clusia* propolis), green propolis and two further subtypes of poplar propolis blue and orange**,** yellow propolis, black propolis, brown propolis, Two further subtypes of poplar propolis blue and orange **(Weinstein et al. 2005), (Fer nandes-Silva et al., 2013), (Morlock et al., 2014), (Ristivojević et al., 2015) and (Ruffato et al., 2017).**

Figure 17. Types of propolis **(Ghallab et al., 2025).**

Table 02. types of propolis and their origins **(Ghallab et al., 2025).**

Propolis type	***Geographic origin***	***Plant main sources***	***Main chemical constituents***
Poplar propolis	Europe, North America and non-tropical regions of Asia	*Populus* spp. most often *P. nigra L.*	Flavones, flavonols, flavanones, dihydroflavonols, phenolic acids and their esters
Brazilian propolis	Brazil	*Baccharis* spp. predominantly *B. dracunculifolia DC*	Prenylated p-coumaric acids and diterpenic acids
Mediterranean propolis	Greece, Sicily, Cyprus and in some Croatian Adriatic islands	conifer species of *Cupressaceae* family	Diterpenes
Pacific propolis	Pacific regions such as Japan (Okinawa) and Taiwan	*Macaranga* spp.	C-prenylflavanones
Canarian propolis	Canary island	Unknown	Furofuran lignans
Russian propolis	Russia	*Betula verrucosa*	phenolic, glycerides flavones and flavonols

Materials and Methods

Materials and Methods

1. Materials

1.1. Animals

Adult male Albino *Mus Musculus* mice (2- 2.5-month-old) from the Animal house at University Constantine1 frères- Mentouri (Algeria), weighing between 23 and 34,6g were used for determination of the phagocytic activity of Propolis (**Figure 18).**

Figure 18. Male Albino *Mus Musculus* mice.

1.2. Blood samples

Blood samples were collected from retro-orbital vein by using glass capillaries and collected into dry tubes with Na_2Co_3.

1.3. Dissected organs

The animals were sacrificed and the liver and spleen dissected for the calculation of corrected α.

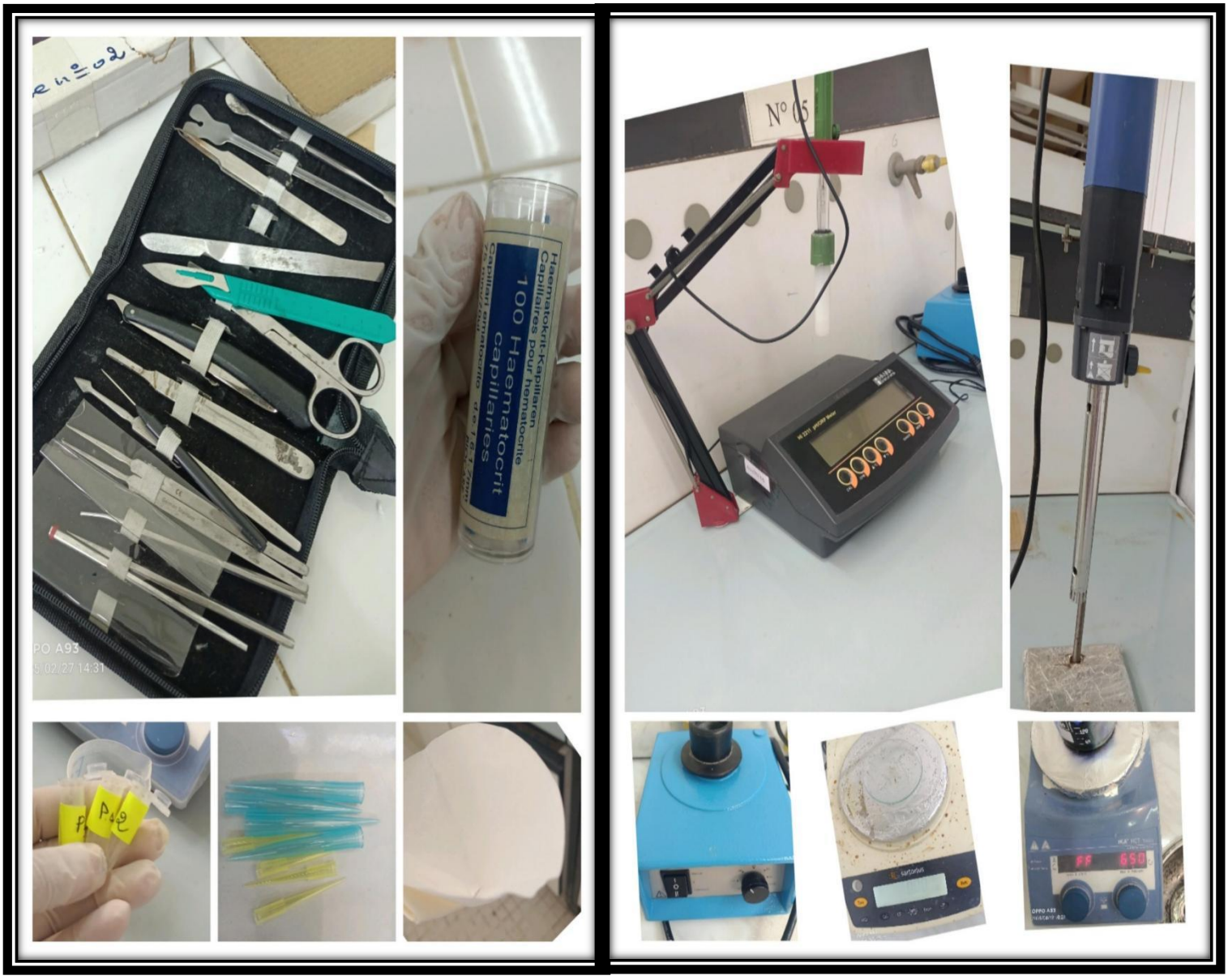
1.4. Chemicals

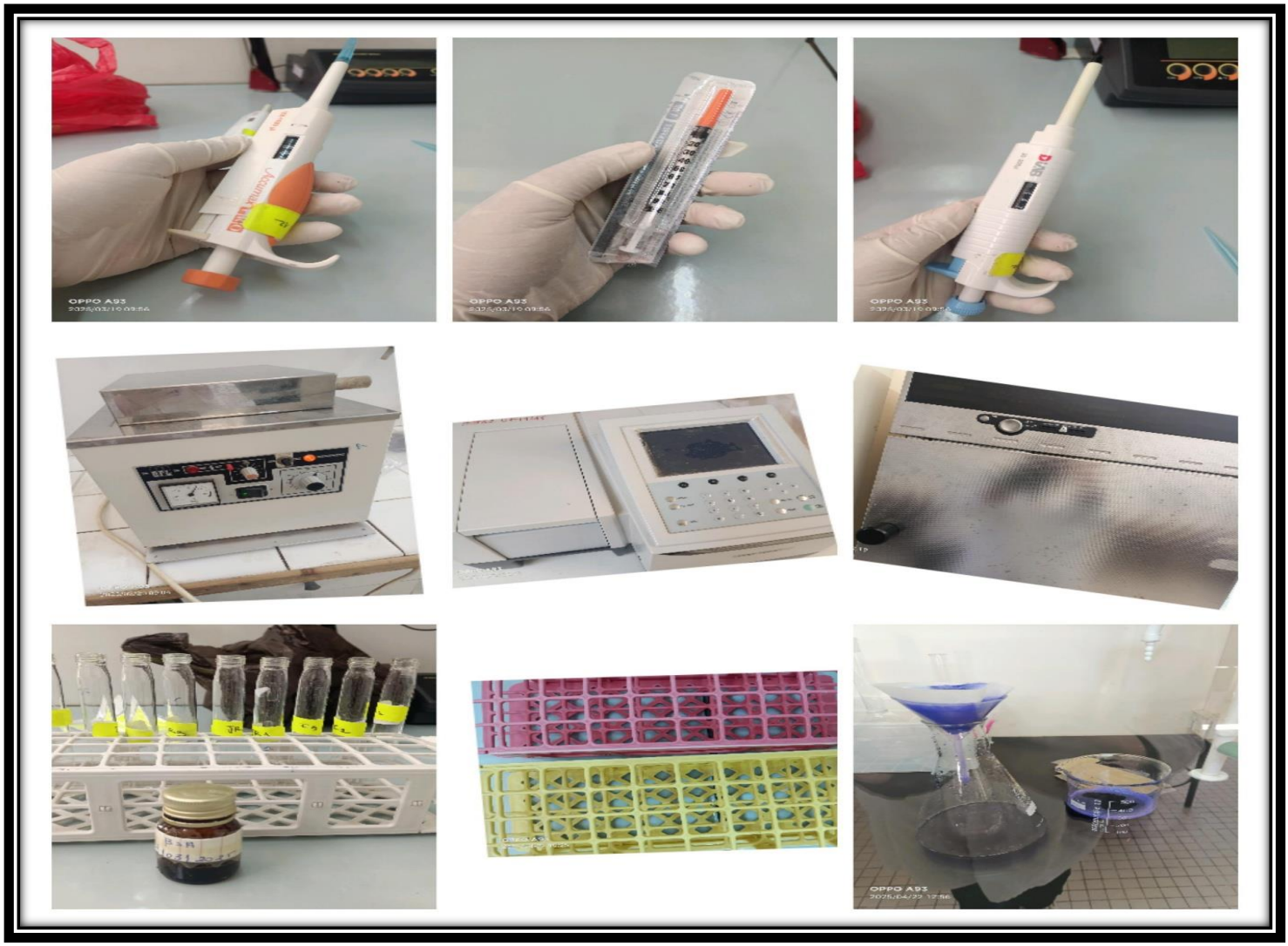
Chloroform, distilled water, NaCl, Na_2Co_3, gelatin, ink solution, dithiobis-2-nitrobenzoic acid (DTNB), sulfosalicylic acid (0.01M), bovine serum albumin (BSA), phosphoric acid

(85%), tris ethylene di-amino tetra acetic acid (EDTA, 0.02M), ethanol, HCl, NaOH, Coomassie brilliant blue G-250.

1.5. Equipment

Precision weighing balances (readability 0.01g), dissection kit, spectrophotometer, test tubes, heating magnetic stirrer, pH meter, filter paper, centrifuge, vortex mixer, Eppendorf tubes, capillary tubes, insulin syringe, incubator **(Figures 19).**





Figures 19. Laboratory equipment.

2. Methods

2.1. Treatment of mice

This study was carried out on a group of 14 mice. After obtaining the animals, they were separated and housed in plastic cages covered with wire mesh, with a layer of sawdust placed at the bottom of each cage, and replenished daily, they were placed under standard laboratory conditions of temperature, humidity, and free access to water and diet every day. Animals were acclimated to laboratory conditions for 12 days prior to the experiment.

Animals were divided into two groups of similar mean body weights consisting of seven mice in GI(C), and GII(P).

Group I (control) was given 0,9% Nacl (0,5 ml/mouse i.p.), Groups II was administered by i.p injection with a concentration of propolis (500mg/kg).

After 48h of i.p injection of the treatment, the mice were administered with carbon ink suspension at a dose of (0.1ml/10g) through the tail vein as shown in the **(Figure 20)**.

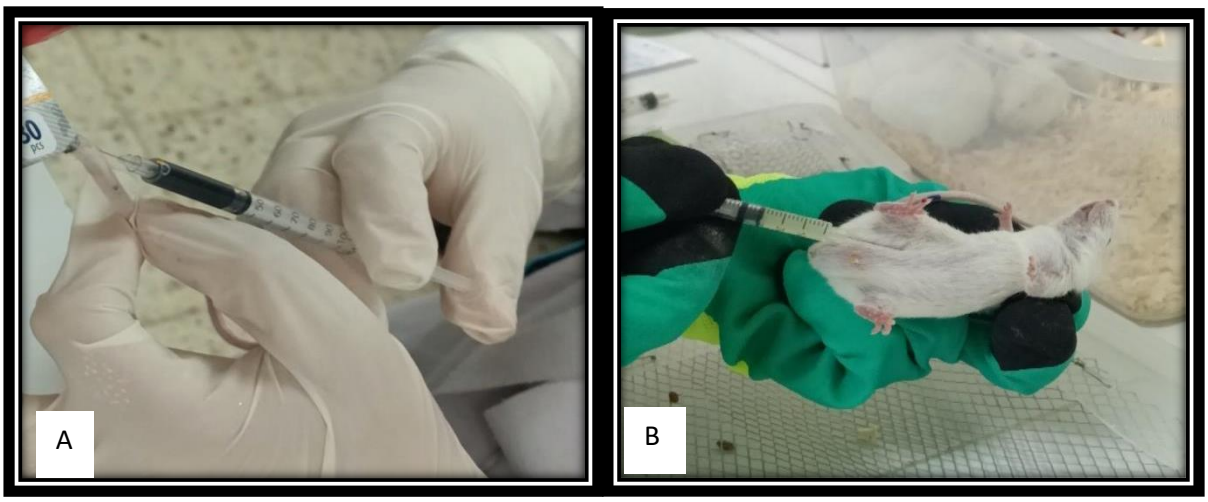


Figure 20. A : intravenous injection. B: Intraperitoneal injection

2.2. Blood samples

Blood samples were collected from retro-orbital vein by using glass capillaries at an interval of 5 min and 15 min after the injection of ink solution, 14 drops of blood samples were added to 4ml of 0,1% sodium carbonate solution to lyse the erythrocytes **(Figure 21).**

Figure 21. Retro-orbital blood collection.

2.3. Dissection

The animals were sacrificed and the liver dissected and weighed immediately in the wet state. the liver and spleen are stored in the freezer without rinsing them with a saline solution for the dosage of the antioxidant (GSH) **(Figure 22).**

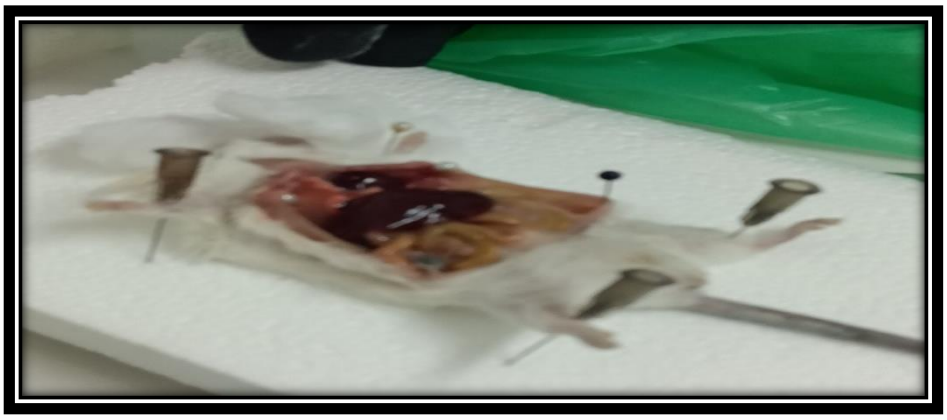


Figure 22. Dissection of mice.

2.4. Phagocytic activity

The phagocytic activity is expressed by the phagocytic index K which measures all the reticuloendothelial system function in the contact with the circulating blood and by

corrected phagocytic index α which expresses this activity by unit of active weight organs: liver and spleen.

The clearance rate is expressed as the half-life period of the carbon in the blood ($t_{1/2}$, min) These are calculated by means of the following equations:

$$K = \frac{\log OD1 - \log OD2}{T2 - T1}$$

$$t\,½ = \frac{0,693}{K}$$

$$\alpha = \sqrt[3]{k} \times \frac{Body\ weight\ of\ animal}{Liver\ weight + spleen\ weight}$$

$$\%\ Change = \frac{K\ treatment - K\ control}{K\ treatment} \times 100$$

Where OD1 and OD2 are the optical densities at times t1 and t2 respectively.

2.5. Statistical analysis

Statistical analysis was conducted to evaluate differences between two groups subjected to different treatments using a T test (PRISM, 10). Significance levels were set at $p < 0.05$ which indicating statistical significance

3.Determination of antioxidant

3.1. Homogenate preparation

0,4 g of the liver was homogenized in 1600µl of EDTA. Then the homogenates were centrifuged at 9000g for 15min at 4°C. The supernatant was kept in the freezer et -20°C until the determination of proteins and reduced glutathione concentrations.

3.2. Glutathione reduced measurement

Liver homogenate sample (0.8 ml) was deproteinized with (0.2ml) of 5-sulfosalicylic acid solution (0.25%), shake the tubes and was allowed stand on ice for 15 min. Following

Liver homogenate sample (0.8 ml) was deproteinized with (0.2ml) of 5-sulfosalicylic acid solution (0.25%) and was allowed stand in the freezer for 15 min. Following centrifugation at 1000 tours/min) during 5 minutes to remove the precipitated protein. (0.5ml) of supernatant

was mixed with 1 ml Tris/EDTA buffer (pH 9.6) and (0.025 ml) of DTNB-reagent (0.01M 5,5'dithiobis-2- nitrobenzoic acid and left at room temperature for 5 min. Then the absorption was measured at 412 nm using a spectrophotometer (SHIMADZU UV-1280) against the blank reaction.

This is calculated by equation:

$$\mathbf{GSH(mmol/l/mg\ proteine)} = \frac{\mathbf{DO \times 1 \times 1,525}}{\mathbf{13100 \times 0,8 \times 0,5 \times mg\ proteine}}$$

3.3. Protein determination

We have measured the protein concentration by the method of Bradford (1976), using bovine serum albumin as a standard. Where the absorbance is proportional to the protein present in the solution.

A 100µl liver homogenate sample was mixed with 5000µL of Bradford reagent and left for 5 min. Then the absorbance was measured at 595 nm using a spectrophotometer in comparison with blank reaction.

1. Results

1.1. Immunostimulatory and antioxidants activities of propolis

The immunostimulatory activity of the extract of propolis was evaluated by carbon clearance rate test in mice.

- ✓ **Phagocytic activity**

As shown in the figure 23, The phagocytic index rate of the groups GI (**0,017±0,003**), GII (**0,039±0,028**). The phagocytic activity is increased in groups II when it is compared to the group I but not significantly ($P > 0.05$) **(Table 03).**

- ✓ **Carbon clearance rate (half time)**

As shown in Figure 24, The Carbon clearance rate of the groups GI **(63,23±49,924), GII (55,098± 33,21)**. The values of half-time are decreased in GII when it is compared to the control group (GI) **(Table 03).**

- ✓ **Corrected phagocytic index (α)**

The figure 25 show an elevation of corrected α in group (GII) (6,270± 1,69) but not significantly when it is compared to the group (GI) (5,795± 1,66) ($P > 0.05$) **(Table 04).**

- ✓ **GSH test**

The data in figure 26 showed that, the concentration of glutathione (GSH) is increased between the two groups but not significantly ($P > 0.05$). The value in the group GI was (1,55 nmole/l ± 0,38) and in the group II was (2,09 nmole/l ±0,49) (Figure 26).

- ✓ **Pourcentage change of phagocytic activity**

The data showed that the percentage of the phagocytic activity reached to 105% (**Table 04**).

Table 03. Effect of propolis on phagocytic activity in mice and the clearance rate is expressed as the half-life period of the carbon in the blood.

groups	Number of mice	dose	k	K average	T ½ min	T1/2 min average
GI (control)	6	0,5ml/mouse	0,018 0,016 0,019 0,023 0,012	0,017±0,003	38,5 43,31 36,47 173,25 30,13 57,75	63,23±49,92
G II	6	(propolis 500 mg/Kg)	0,013 0,049 0,090 0,018 0,026	0,039±0,028	53,30 14,14 99 38,5 99 26,65	55,09± 33,21

Table 04. Percentage change of phagocytic activity

Mice Number	Pourcentage Change %	Average %
1	40	105
2	222	
3	165,29	
4	95,67	
5	28,57	
6	81,25	

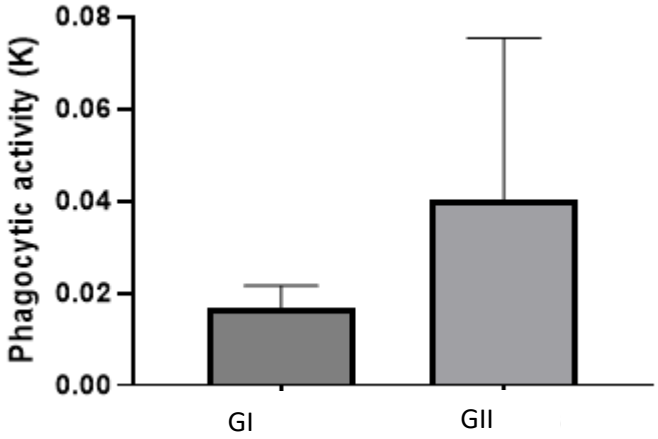


Figure 23. Effect of propolis on phagocytic activity (K).

GI: group control treated with Nacl , GII: group treated with propolis

P≥ 0,05.

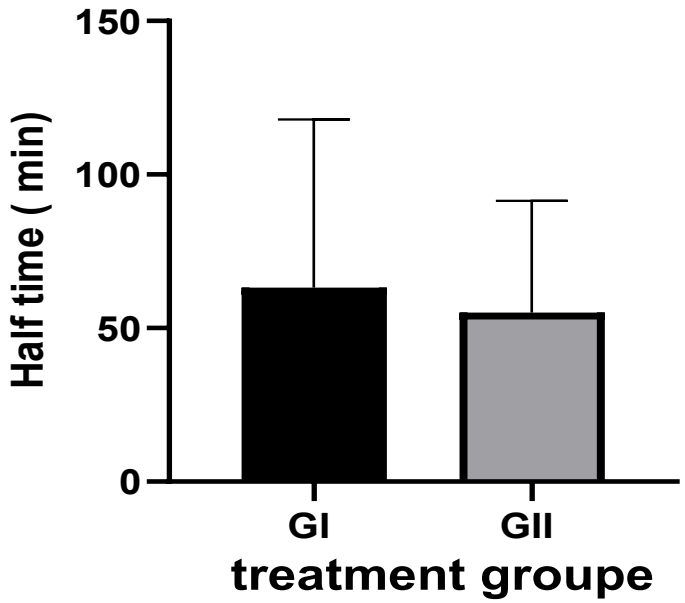


Figure 24. Effect of propolis on half time of carbon in blood.

GI: group control treated with Nacl, GII: group treated with propolis

P≥ 0,05.

Table 05. Effect of Propolis extract on phagocytic activity in mice shown on active organs (liver and spleen) and mice weight.

Groups	Mice number	Liver weight (g)	Spleen weight (g)	OD 1 (5min)	OD 2 (15min)	α	Average
Group I (control)	C1	1,53g	0,12g	0,086	0,132	5,54	5,795± 1,662
	C2	0,95g	0,05g	0,162	0,110	5,99	
	C3	1,52g	0,25g	0,079	0,124	4,62	
	C4	1,44g	0,11g	0,090	0,099	3,53	
	C5	1,46g	0,13g	0,095	0,164	6,15	
	C6	1,52g	0,25g	0,119	0,159	8, 94	
Group II (propolis)	P1	1,58g	0,16g	0,054	0,168	8,117	6,270± 1,696
	P2	0,99g	0,08g	0,112	0,094	5,971	
	P3	1,34g	0,17g	0,026	0,210	9,793	
	P4	1,25g	0,16g	0,038	0,058	5,910	
	P5	1,13g	0,18g	0,091	0,077	4,847	
	P6	1,55g	0,16g	0,212	0,116	5,647	

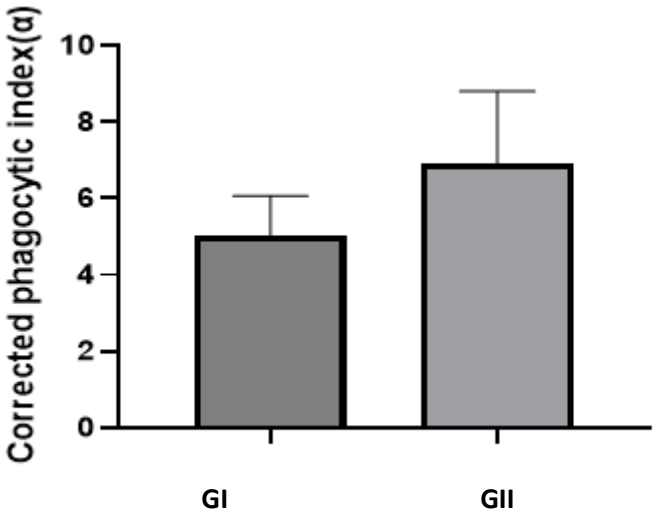


Figure 25. Effect of Propolis on corrected (α).

GI: group control treated with Nacl , GII: group treated with propolis P≥ 0,05.

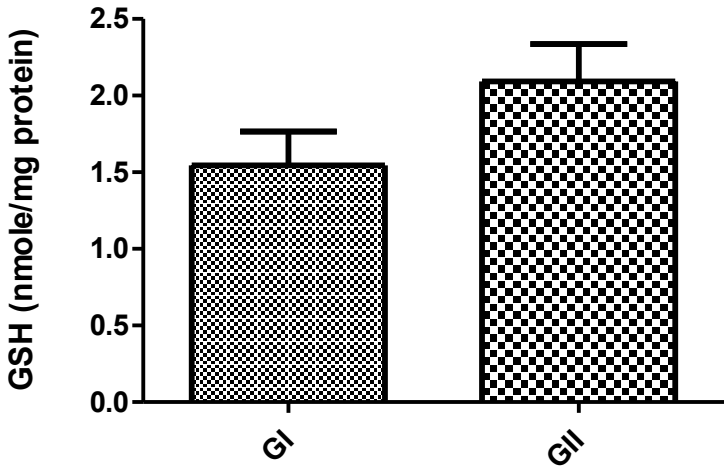


Figure 26. Effect of propolis on GSH.

GI: group control treated with Nacl , GII: group treated with propolis

P≥ 0,05.

Human immune system is an important and very complex system that helps to fight against different microorganisms as well as act as immunomodulators **(Baxter, 2007)** and **(Coico et al., 2015)**. From ancient times, natural products or what is called allopathic drugs are commonly applied to boost the immune system **(Maggini et al.,2018)** and **(Kumar et al.,2022)**.

In immune system phagocytosis is a fundamental process for the ingestion and elimination of microbial pathogens and apoptotic cells. All types of cells can perform phagocytosis, but specialized cells called professional phagocytes **(Uribe -Querol and Rosales, 2020).**

The phagocytic activity was investigated by the phagocytic function of the reticuloendothelial system is clearing particulate substances, such as bacteria, and altered endogenous materials, such as fibrin aggregates **(Benmebarek et al., 2013).**

Propolis contains several chemical bioactive compounds as polyphenols (flavonoid aglycones, phenolic aldehydes, phenolic acids, alcohols and their esters and ketones), steroids terpenoids, amino acids **(Eyng et al., 2015).** Vitamins (A, C, D, E and B1, B2, B6, niacin and folate) and some micro and macro minerals like calcium, iron, copper, zinc, magnesium, manganese, nickel and cobalt **(Zabaiouet al.,2017)**. It also contains some enzymes as glucose-6-phosphatase, dehydrogenase, adenosine triphosphate and acid phosphatase **(Yilmaz et al., 2003)** and **(Kurek-Górecka et al., 2014).**

In our research we have obtained that the phagocytic activity is increased with treatment of propolis which could be explained according to the observations of **Dimove et al. (1992)**, the water-soluble derivative of propolis enhanced phagocytic activity, which contributed to stimulating phagocytosis and also immunostimulatory activity.

Also **Gao et al. (2014)** proved, that the Brazilian green propolis in aged mice indicating a positive effect on innate immunity.

Fan et al. (2014) indicated that, the microemulsion formulation of propolis flavonoids (M-PF) significantly improved immune responses, enhanced spleen and thymus function, and boosted cytokine (IFN gamma, IL2) production (IgM and IgG), macrophage activity and immune modulation.

In another study by **Kędzia and Hołderna kędzi (2019)**, who indicated that the propolis extracts and their components increased the agglutination, phagocytosis and antibody production.

The high content of flavonoids, polyphenols, saponin, steroids, proteins, carbohydrates, and phenols in the developed propolis tablets might be responsible for their notable increase in immunostimulatory activity **(Kapar et al.,2024).**

Our results agree with the work of **Zhussupoba et al. (2022)**, who obtained that the phagocytic activity is increased in animals treated with plant extracts from *Salvia deserta Schang* and *Salvia sclarea L.* Also, **kehili and zerizer. (2024)**, who reported that the phagocytic activity is increased in animals treated with traditional Algerian plant combination of date fruit (*Phoenix dactylifera)* and Fenugreek seeds (*Trigonella foenum-graecum*).

Tao et al. (2014), demonstrated that flavonoids from propolis encapsulated in liposomes significantly increased the phagocytic index and stimulated cytokine release (IFN-γ, IL-1β, IL-6), indicating enhanced immune function. and this is consistent with our results. In study by Indian propolis significantly increased the phagocytic index by stimulating the reticuloendothelial system, indicating enhanced immune function and a faster clearance rate of carbon particles from the blood study of **Kapar et al. (2024).**

Our research results showed that the corrected clearance rate (half time) is decreased in the group treated with propolis , this result is agrees with the work of **Aribi et al. (2013)** who reported that the administration *of Argania Spinosa* decreased carbon clearance in the mice.

The results of **Mustafiah et al. (2011)**, reported that when the doses of propolis decreased, the average of phagocytosis index increased. However, as the dose increased, the results became non-significantly and at higher doses, it acted as an immunosuppressant rather than an immunostimulant.

In this experiment, GSH levels were relatively high, which is consistent with the findings of the study by **Mujica et al. (2019)**, where he confirmed that propolis treatment increased the glutathione (GSH) and GSH disulfide (GSSG) ratio, reduced the pro- inflammatory TNF-α , and upregulated the anti -inflammatory lL-10 levels of the treated tissue. Also, **Nazarı- Bonab et al (2023)** reported that the GSH levels are increased in patients with chronic diseases treated with doses between 160 mg/day to 1500 mg/day of propolis.

We have obtained from this study the percentage of phagocytic activity reached to 105% which confirmed that propolis stimulates the immune system by the activation of the reticulo

endothelial system. This result is agrees with the work of **Abebe et al. (2022)**, who detected a percentage of phagocytic activity of *Cyphostemma adenocaule* extract around 70%.

Conclusion

conclusion

Propolis is not merely a dietary supplement but also shows promise as an adjuvant therapy for conditions associated with impaired phagocytosis and immune modulation. Although the current findings did not demonstrate statistically significant differences in phagocytic activity or glutathione levels, the results suggest that propolis may play a substantial role in enhancing immune responses.

Nevertheless, further investigations are strongly recommended to elucidate the underlying mechanisms of its action, optimize dosage strategies, and evaluate its effects under diverse experimental conditions.

In the future, our research needs more studies to:

1. Evaluation of the toxicity of propolis;
2. Determination of the phagocytic activity with different concentration;
3. Evaluation of the two methods of treatment by oral and intraperitoneal injection.

Summary

Summary

The immune system plays a crucial and complex role in defending the human body, and any dysfunction within it can lead to autoimmune diseases. Researchers have explored natural resources to modulate and enhance immune function. Among these natural substances is propolis, a resinous compound rich in phenolic and polyphenolic compounds known for their immunomodulatory and antioxidant properties.

This study aimed to investigate the immunological effects of propolis extract by assessing phagocytic activity and glutathione levels in mice. A single dose of propolis was administered to a test group of mice and compared to a control group.

The results showed that the dose of 500 mg/kg of propolis is increased the phagocytic index (K), corrected phagocytic index α, GSH and a diminution of the carbon clearance rate ($t_{½}$).

We concluded that the propolis could stimulate immune function and it can be used as a natural product for different diseases except for people who have allergies from it.

Keywords: phagocytic activity, phagocytic index α, propolis, phagocytic activity percentage, GSH

Résume

Le système immunitaire joue un rôle crucial et complexe dans la défense du corps humain, et tout dysfonctionnement peut entraîner des maladies auto-immunes. Les chercheurs explorent les ressources naturelles pour moduler et renforcer la fonction immunitaire. Parmi ces substances naturelles figure la propolis, un composé résineux riche en composés phénoliques et polyphénoliques, connu pour ses propriétés immunomodulatrices et antioxydantes.

Cette étude a visé à évaluer les effets immunologiques de l'extrait de propolis en mesurant l'activité phagocytaire et les niveaux de glutathion chez les souris. Une dose unique de propolis a été administrée à un groupe test, comparé à un groupe témoin.

Les résultats ont montré qu'une dose de 500 mg/kg de propolis a augmenté l'indice phagocytaire (K), l'indice phagocytaire corrigé (α), le test de glutathion (GSH), et a entraîné une diminution du taux de clairance du carbone (t½).

Nous concluons que la propolis peut stimuler la fonction immunitaire et peut être utilisée comme produit naturel pour traiter différentes maladies, sauf chez les personnes allergiques à cette substance.

Mots-clefs : activité phagocytaire, indice phagocytaire α, propolis, pourcentage de l'activité phagocytaire, GSH

ملخص

يلعب الجهاز المناعي دورًا اساسيا ومعقدًا في حماية جسم الإنسان، و عند جدوث أي خلل في هدا الجهاز قد يؤدي إلى أمراض المناعة الذاتية.، استكشف الباحثون مركبات طبيعية التي تقوم بتقوية وظيفة المناعة' من بين هذه المواد الطبيعية، البروبوليس، وهو مركب راتنجي غني بالمركبات الفينولية والبوليفينولية، التي تعمل كمضادات للأكسدة و التي لها القدرة على تنبيه الجهاز المناعي.

يهدف هذا البحث إلى دراسة التاثير المناعي لمستخلص البروبوليس من خلال تقييم النشاط البلعمي ومستويات الجلوتاثيون لدى الفئران. أُعطيت جرعة واحدة من البروبوليس لمجموعة من الفئران و التي قورنت مع الشاهد.

أظهرت النتائج أن جرعة ٥٠٠ ملغم/كغم من البروبوليس قد زادت من مؤشر البلعمة ' مؤشر البلعمة المصحح' الجوثاثيون و انخفاض في تسريع معدل تصفية الكربون.

من خلال هده النتائج المتحصلة عليها يمكننا ان نستنتج بان البروبوليس له القدرة على تنبيه الجهاز المناعي و يمكن استخدامه كمركب طبيعي في امراض عديدة ماعدا الأشخاص الذين يعانون من الحساسية لهدا المركب.

الكلمات المفتاحية: النشاط البلعمي، مؤشر البلعمة المصحح ، البروبوليس، النسبة المئوية للنشاط البلعمي' الجلوثاثيون

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Annex

Annex

I- Solution preparation

1. NaCl Preparation (Sodium chloride)

NaCl: 0.9 g

Distilled water: 100 ml

Dissolve 0.9 g of NaCl in 100 ml of distilled water.

2. Na_2Co_3 preparation (sodium carbonate)

Na_2Co_3: 0.4 g

Distilled water: 400 ml

Dissolve 0.4 g of Na_2Co_3 in 400 ml of distilled water.

3. Gelatin preparation

Gelatin: 3g

Distilled water: 100 ml

Dissolve 3 g of gelatin in 100 ml of distilled water.

4. INK solution preparation

NaCl: 4 ml

Gelatin: 4 ml

Indian ink: 3 ml

Mix them all together.

5. BSA solution preparation

BSA: 0,01g

Distilled water: 10 ml

Dissolve 0,01 g of BSA in 10 ml of distilled water.

6. DTNB (0,01M) solution preparation

DTNB: 0,05g

Methanol**:** 125ml

Dissolve 0,05 g of DTNB in 125 ml of Ethanol

7. EDTA (0,02M) solution preparation

EDTA: 3,72g

Distilled water: 500ml

Dissolve 3,72g of EDTA in 500 ml of distilled water.

8. Tris (0,4M) EDTA (0,02M) (pH= 9.6).

Tris: 6,06g

EDTA: 0,93g

Distilled water: 125ml

Dissolve 6,06g of Tris and 0,96g of EDTA in 125 ml of distilled water

9. sulphosalicylic acid solution preparation

sulphosalicylic acid: 0,25g

Distilled water: 100 ml

Dissolve 0,25g of sulphosalicylic acid in 100 ml of distilled water

10. Bradford solution preparation

Coomassie Brilliant Blue G-250: 0,05 g

Ethanol**:** 25ml (96%)

Phosphoric acid: 50ml

Distilled water: 425ml

Dissolve 0,05g of Coomassie brilliant blue G-250 in 25 ml of ethanol and mix them two hours, then add 50ml of phosphoric acid and 425ml of distilled water.

II- Calculation of doses

1. Propolis dose

0.05g ⟶ 1000 g

X g ⟶ Mice's weight/g

2. Injected volume

0.05g ⟶ 10 ml NaCl

X ⟶ Y

3. INK dose

0.1ml ⟶ 10 g

X ml ⟶ Mice's weight/g

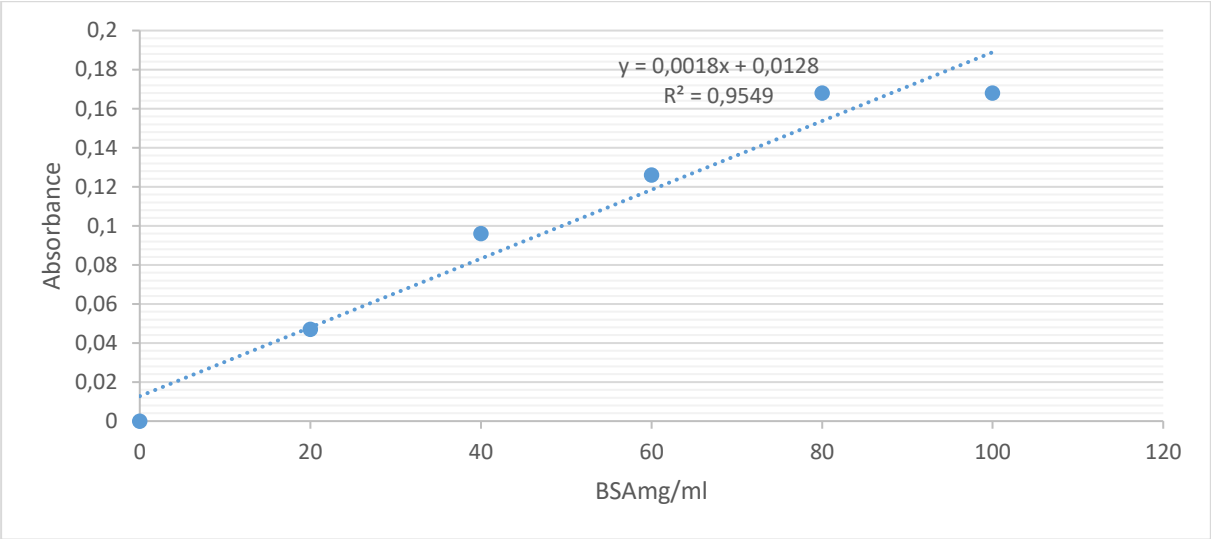

Table 06. Bovine serum albumin reaction.

Tube	**1**	**2**	**3**	**4**	**5**	**6**
Distilled water (µl)	**100**	**80**	**60**	**40**	**20**	**0**
BSA (µl)	**0**	**20**	**40**	**60**	**80**	**100**
Bradford(ml)	**5**	**5**	**5**	**5**	**5**	**5**
Absorbance	**0**	**0.047**	**0.096**	**0.126**	**0.168**	**0.168**

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The effect of propolis on phagocytic and antioxidant activities

Mémoire pour l'obtention du diplôme de Master en Immunologie Moléculaire et Cellulaire

Summary

The immune system plays a crucial and complex role in defending the human body, and any dysfunction within it can lead to autoimmune diseases. Researchers have explored natural resources to modulate and enhance immune function. Among these natural substances is propolis, a resinous compound rich in phenolic and polyphenolic compounds known for their immunomodulatory and antioxidant properties.

This study aimed to investigate the immunological effects of propolis extract by assessing phagocytic activity and glutathione levels in mice. A single dose of propolis was administered to a test group of mice and compared to a control group.

The results showed that the dose of 500 mg/kg of propolis is increased the phagocytic index (K), corrected phagocytic index α, GSH and a diminution of the carbon clearance rate ($t_{½}$).

We concluded that the propolis could stimulate immune function and we can used as a natural product for different diseases except for people who have allergies from it.

Mots-clefs: Phagocytic activity, GSH, phagocytic index α, propolis, percentage change

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