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The benefit of royal jelly on reticuloendothelial system and on reduced glutathione

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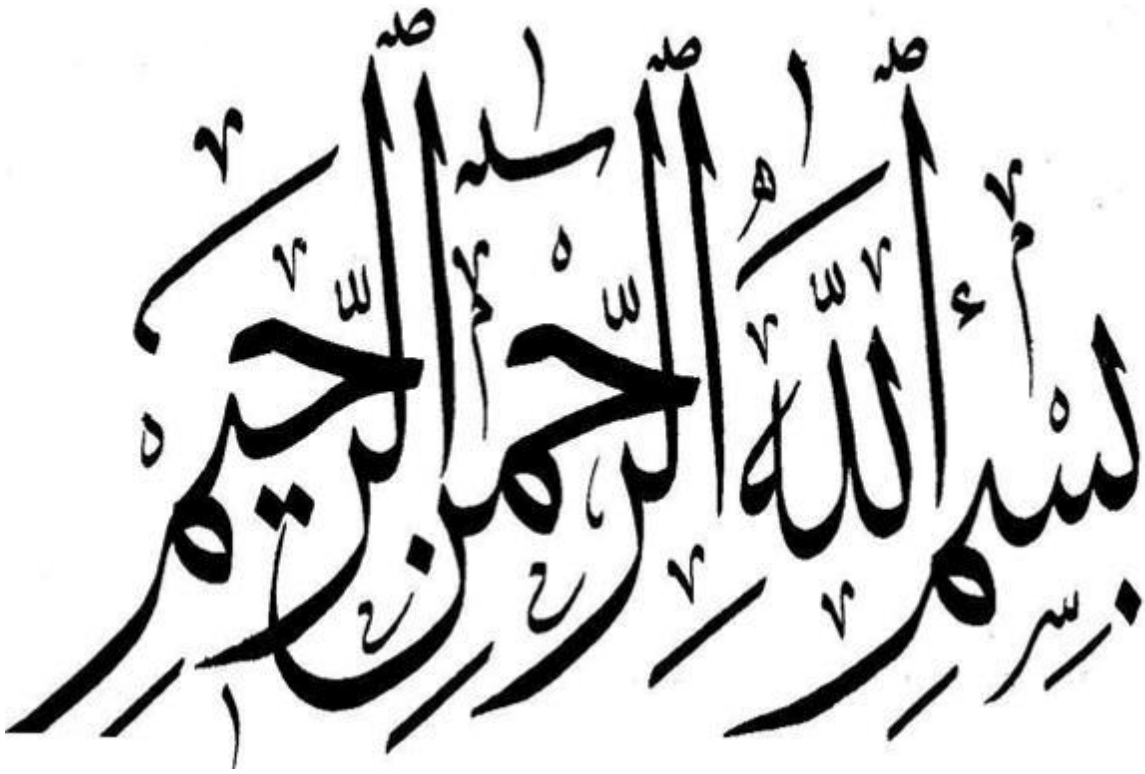
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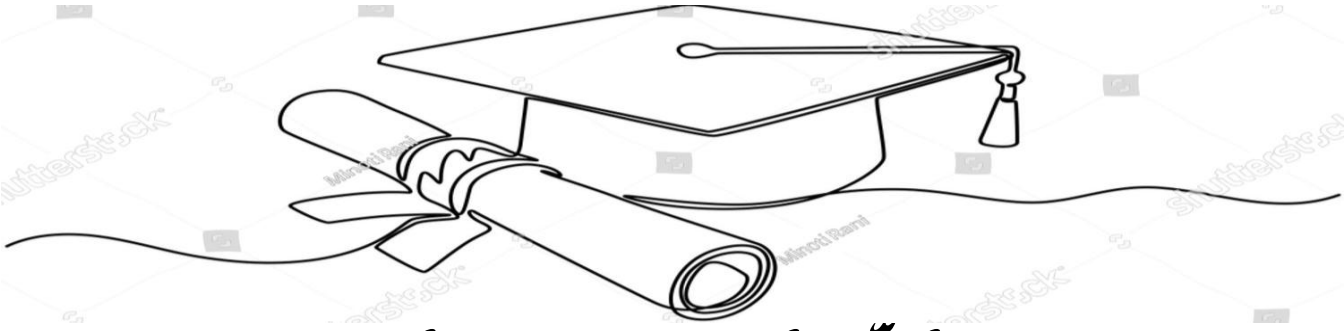
Dedication



بِسْمِ ٱللَّهِ ٱلرَّحْمَـٰنِ ٱلرَّحِيمِ

(يَرْفَعِ ٱللَّهُ ٱلَّذِينَ آمَنُوا مِنكُمْ وَٱلَّذِينَ أُوتُوا ٱلْعِلْمَ دَرَجَٰتٍ)

(وَأَنْ لَيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَىٰ، وَأَنَّ سَعْيَهُ سَوْفَ يُرَىٰ)

الحمد لله الذي يسر البدايات، وأكمل النهايات، وبلغنا الغايات، الحمد لله الذي ما تم جهدٌ إلا بعونه، وما خُتم سعيٌ إلا بفضله،الحمد لله الذي بلغني هذا اليوم، بعد رحلةٍ طويلةٍ من الجهد والاجتهاد،وسهر الليالي، بعد فشلٍ وخيبات، ومحاولاتٍ لا تنتهي.

أُهدي هذا النجاح بكل حب وامتنان :

ولجي سمية

إلى من أحمل اسمه بكل فخر،إلى **أبي صالح** ، فخر عمري وسندي في كل خطواتي... شكراً لك على الدعم الذي لا يُقدّر بثمن، وعلى وجودك الثابت في كل مراحل حياتي. وإلى **أمي زهرة** ، نبع الحنان والدعاء... يا من كان لصبرك ودعائك الأثر الأكبر في هذا الإنجاز، لكِ كل الحب والامتنان.

إ لى إخوتي صبرينة و أسامة و علي ، شركاء الروح والدرب أنتم القوة التي أستند إليها، والضحكة التي خفّفت عني كثيراً.**وإلى أصدقائي**، من جعلوا الطريق ألطف، وشاركوا التعب قبل الفرح، وجودكم في حياتي مكسب لا يُعوّض .

إلى نفسي شكرًا لكل لحظة صبر، لكل مرة قاومت فيها التعب، ولكل مرة قررت أن أستمر رغم كل شيء.كنتِ قوية، وها أنتِ تصلين... فاستحقّي الفخر.

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CD3 Cluster of differentiation 3

CD4 Cluster of differentiation 4

CD8 Cluster of differentiation 8

i.p intra peritoneal

IL-1 Interleukine -1

IL-6 Interleukine -6

MPS Mononuclear phagocyte system

PMNs Polymorphonuclear leukocytes

RES Reticuloendothelial system

Na_2Co_3 Sodium carbonate

NaCl Sodium chloride

TNF Tumor necrosis factor

TCR T cell receptor

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Introduction

Introduction

The immune system is one of the most incredible systems of the human body. It is a very intelligent and tightly regulated system that saves the human body from pathogenic invaders and even keeps a precise memory of the pathogen to arm the host from any further encounter **(Zoghi et al., 2023).**

The immune system has two fundamental lines of defense; innate immunity and adaptive immunity. 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. Adaptive immunity, on the other hand, is antigen-dependent and antigen-specific; it has the capacity for memory, which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen **(Marshall et al., 2018).**

Honey bee products, including propolis, royal jelly, honey, bee venom, and bee pollen, or their bioactive components such as polyphenols, show promising therapeutic potential in regulating inflammatory mediator production. Their biological properties as anti-inflammatory, immunostimulant, antioxidant, anti-apoptotic, and antimicrobial agents have led to further clinical studies **(hesham et al., 2022).**

Royal jelly (RJ) is a highly nutritious natural product with great potential for use in medicine, cosmetics, and as a health-promoting food. This bee product is a mixture of important compounds, such as proteins, vitamins, lipids, minerals, hormones, neurotransmitters, flavonoids, and polyphenols, that underlie the remarkable biological and therapeutic activities of RJ **(nada et al., 2024).**

Both preclinical and clinical studies have reported that RJ improves the immune function such as wound healing, and also decreases the severity of chronic diseases including diabetes and cardiovascular disorders **(Rajesh et al., 2024).**

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) **(nacer et al., 2015).**

The clearance rate is expressed as the half-life period of the carbon in the blood (t1/2, min). These are calculated by means of the following equations **(shah et al., 2008).**

Glutathione (gamma-glutamyl-cysteinyl-glycine : GSH) is the most abundant low-molecular-weight thiol, and GSH/glutathione disulfide is the major redox couple in animal cells, glutathione plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events (including

gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation **(Wu et al., 2004).**

The aim of this research is to focus on these objectives:

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

Bibligraphic 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 "nons elf." 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 for exemple 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 iscritical 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).**

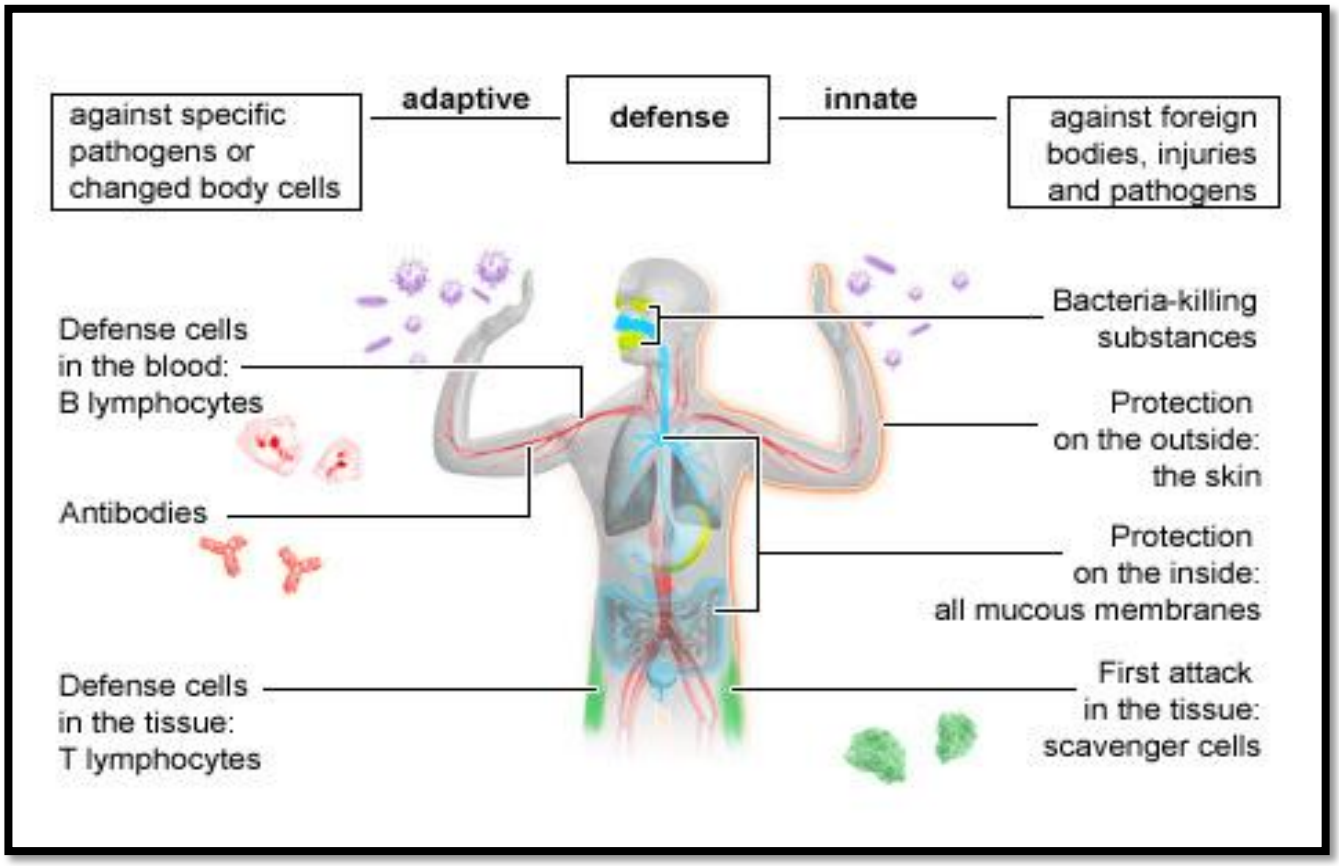


Figure 01. Scheme represents innate and adaptive immune **(Namdeo, 2021).**

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 lymphatique system **(Charles et al., 2001).**

4.1. Dendritic cell

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 and sample 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).**

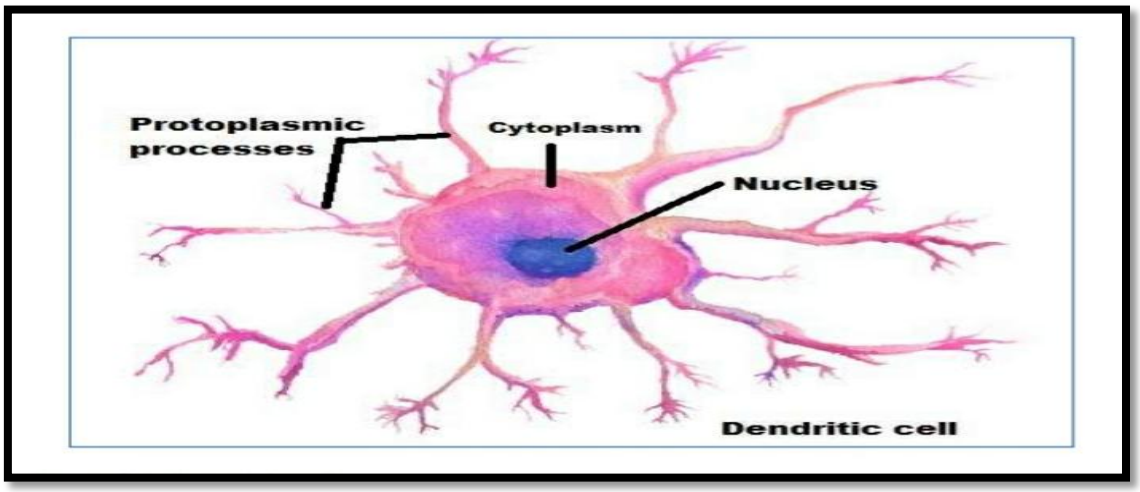


Figure 02. Structure of dendritic cells **(Mehraj et al., 2021).**

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) 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).**

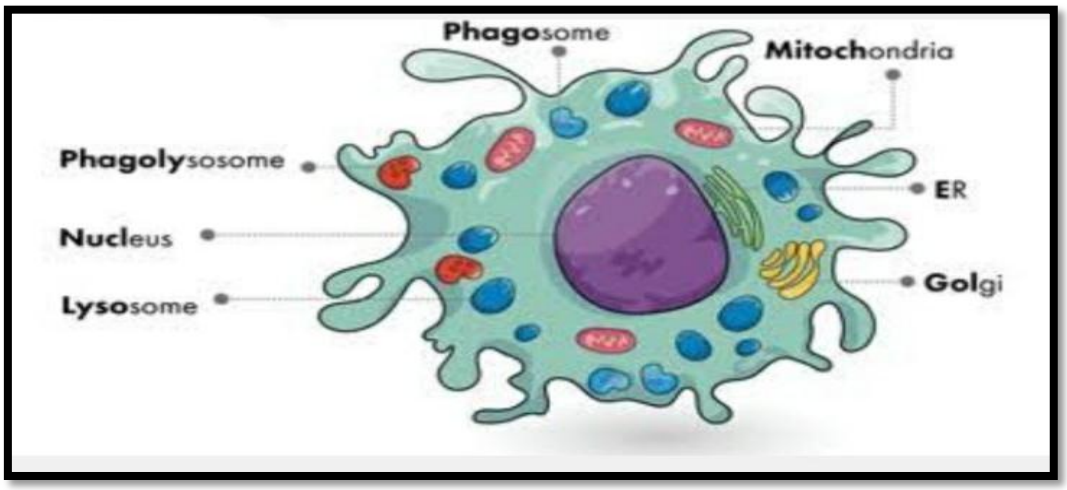


Figure 03. Structure of macrophage (1).

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).**

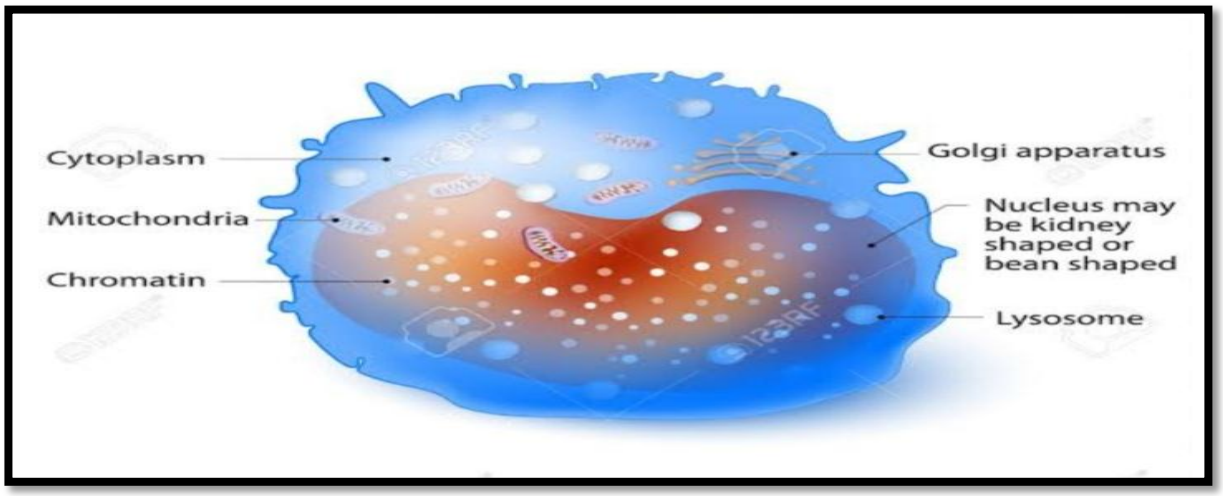


Figure 04. Structure of monocytes (2).

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).**

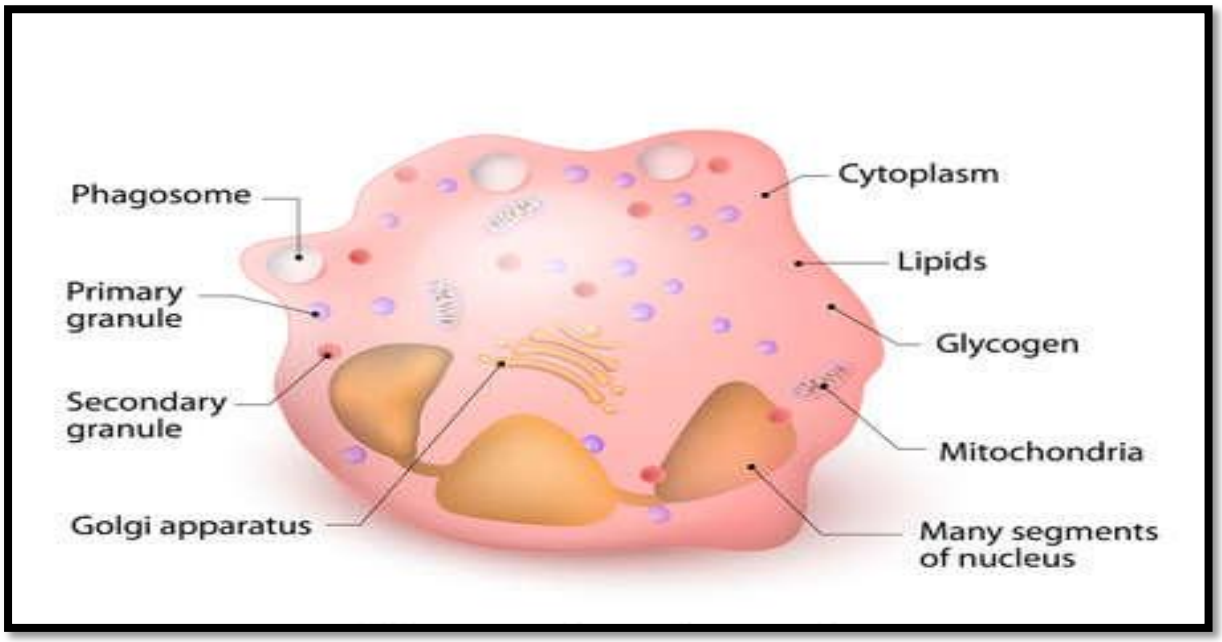


Figure 05. Structure of neutrophil (3).

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. 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).**

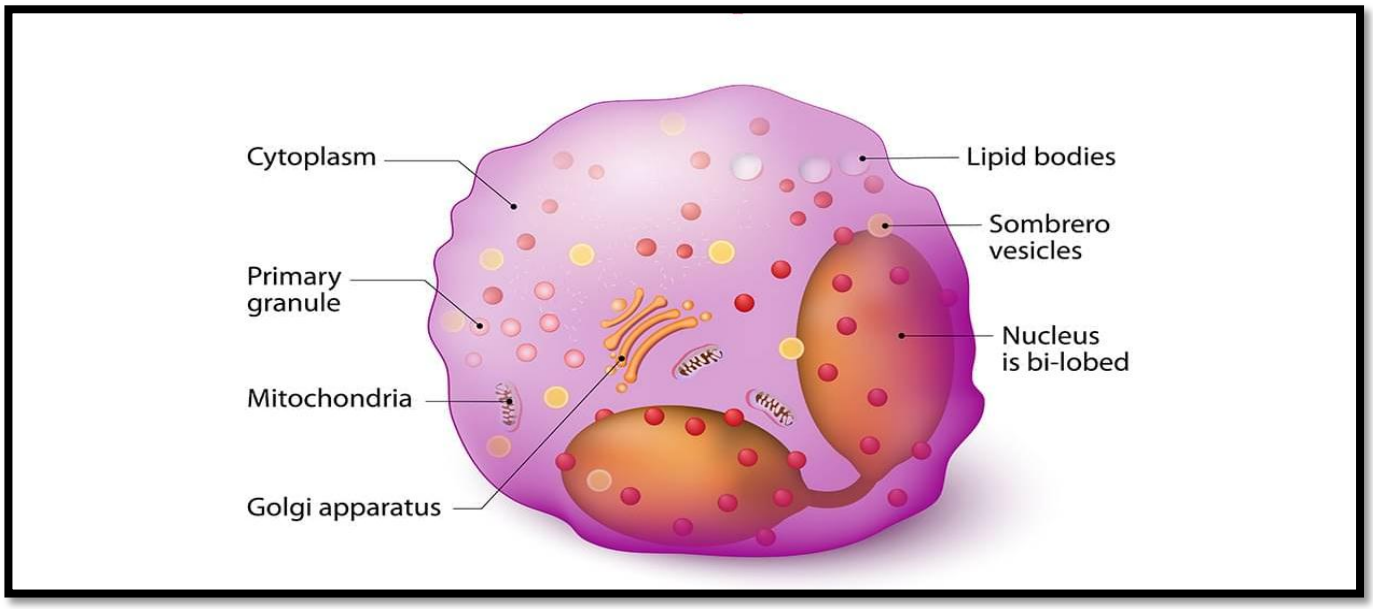


Figure 06. Structure of eosinophil (4).

4.4.3. 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)** and **(Knol and Olszewski, 2011)** and **(Chirumbolo, 2012)** and **(Pellefigues et al., 2018)** and **(Chirumbolo et al., 2018) (Figure 07).**

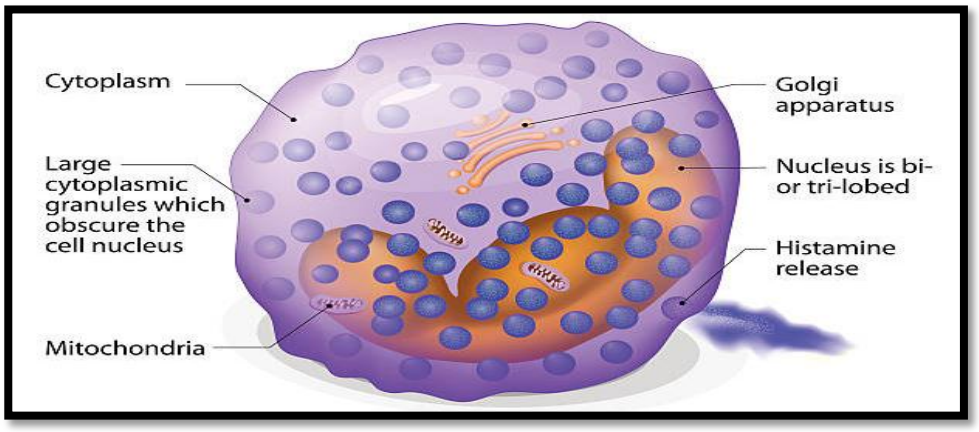


Figure 07. Structure of basophils (5).

4.5. Natural killer cells

NK (natural killer) cells comprise 10%-15% of peripheral blood mononuclear cells and have morphology of large, granular lymphocytes with the central role of killing the virus-infected and malignantly transformed cells, without prior sensitization. NK cells participate in hematopoiesis regulation, reproduction processes, as well as in numerous immune system reactions in vivo **(Jurisić, 2006).** that can be activated to mediate significant levels of cytotoxic activity and produce high levels of certain cytokines and chemokines. NK cells respond to and are important in defense against a number of different infectious agents **(Biron,C.A et al., 1999).** NK cells characterized by CD3-, CD56+, and CD16+ markers and employ two mechanisms for destruction of malignant cells. The first cytotoxic mechanism is spontaneous and major histocompatibility antigen independent process, while the second mechanism is antibody dependent cellular cytotoxicity (ADCC) **(Jurisić, 2006) (Figure 08).**

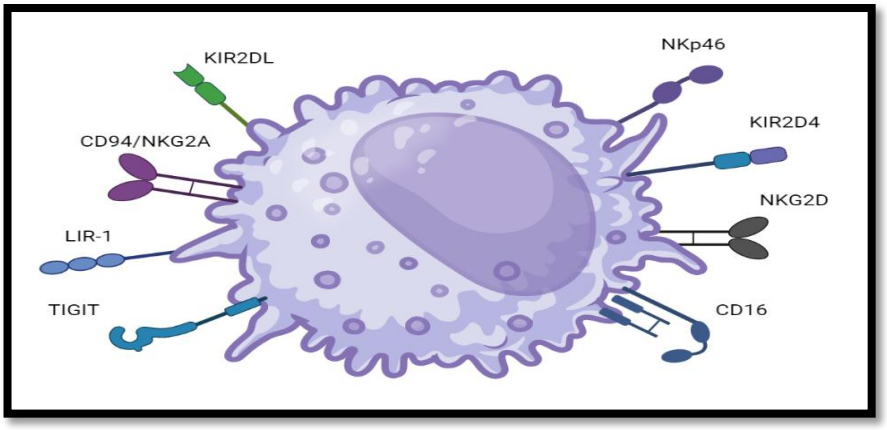


Figure08. Structure of natural killer (6).

I.5.Adaptative 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).**

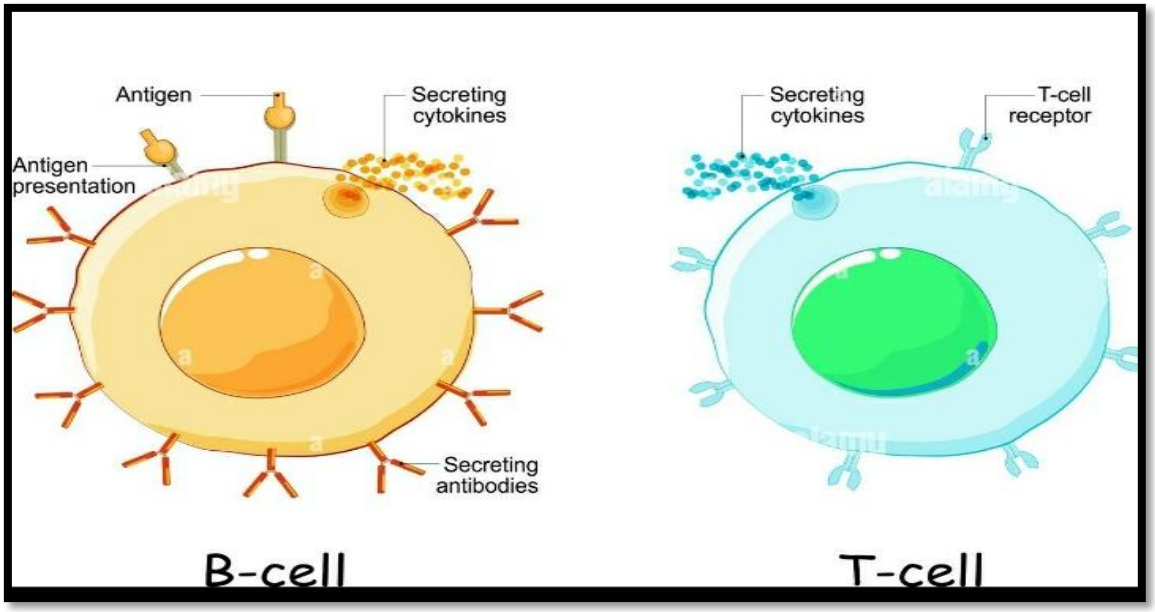


Figure 09. Structure of B_ and T_cells (7).

I.5.Phagocytes

I.4.1 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.4.2. Phagocytosis

Phagocytosis is a basic process for nutrition in unicellular organisms, and it is also found in almost all cell types of multicellular organisms. However, only a specialized group of cells called professional phagocytes **(Rabinovitch, 1995).** Accomplish phagocytosis with high efficiency. Macrophages, neutrophils, monocytes, dendritic cells, and osteoclasts are among these dedicated cells. Professional phagocytes are responsible of removing microorganisms and of presenting antigens to lymphocytes in order to activate an adaptive immune response. Fibroblasts, epithelial cells, and endothelial cells can also accomplish phagocytosis with low-efficiency and are thus described as non-professional phagocytes. These cells cannot ingest microorganisms, but are important in eliminating dead cells and maintaining homeostasis **(Gordon, 2016)**. Phagocytosis is the process of sensing and taking in particles larger than 0.5 μm. The particle is internalized into a distinctive organelle, the phagosome **(Levin et al., 2016)**. The process of phagocytosis involves several phases:

At the beginning of phagocytosis, specific molecules or opsonins on the surface of the invading pathogen bind to the receptors on the cell surface of the phagocyte. This initiates receptor clustering, triggering phagocytosis.

At this point, the cell membrane modifies itself, extending and closing itself around the target, then finally breaking off to create a phagosome, a vesicle created from the cell's membrane that contains the engulfed target. The phagosome interacts with the late endoscopes and lysosomes to create a phagolysosome, and within this vesicle, the engulfed contents are broken down via lysosomal hydrolases **(Rosales and Uribe-Querol, 2020) (Figure 10).**

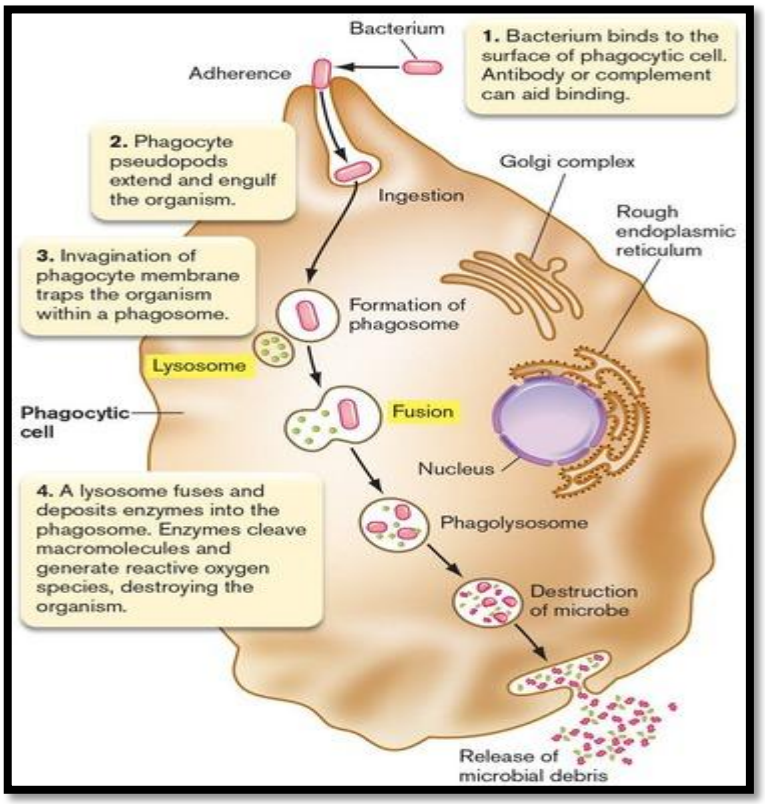


Figure 10. Steps of phagocytosis (8).

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 **(Moore and Dalley, 2006).**

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 711 on 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 anteriorly and superiorly. 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 are visible **(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).** also explained that the hepatic vein, he also explained that the blood processed by the liver exicts 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).**

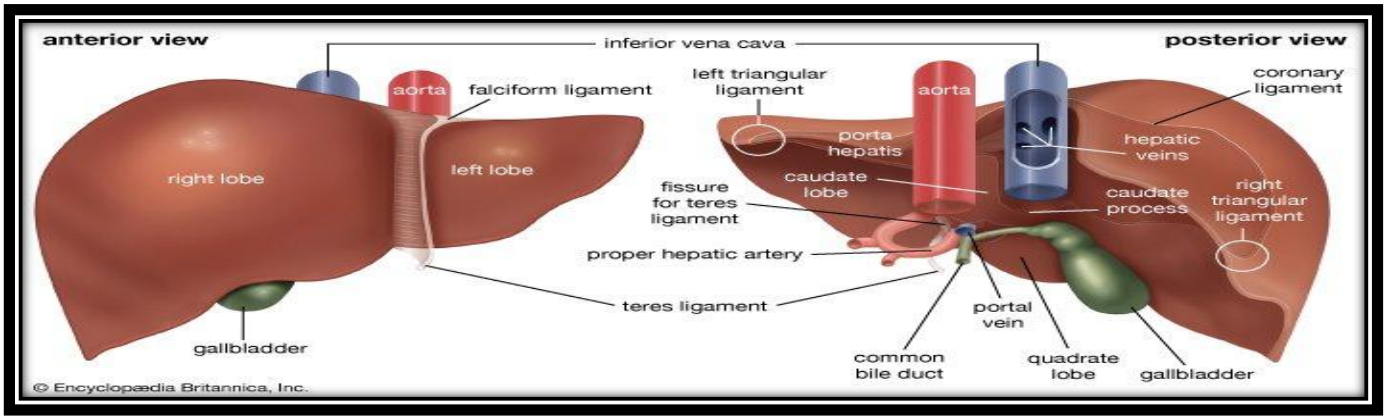


Figure 11. Structure of liver **(Ozougwu, 2017).**

.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 int he perisinusoidal space, between a sinusoid and a hepatocyte Additionally, intrahepatic lymphocytes are often present in the sinusoidal lumen **(Kmiec, 200112) (Figure 12) .**

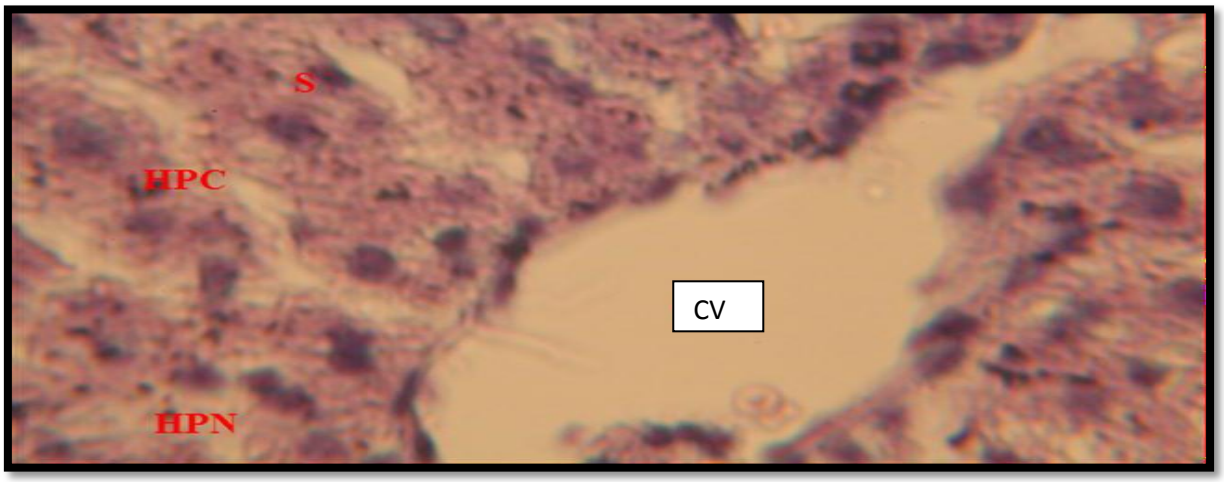


Figure 12. Section of liver (x 400).

HPC. Hepatocyte Cell S. Sinusoid, HPN. Hepatocyte Nuclei CV. Central Vein.

(Zerizer and Naimi, 2004*).*

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 hepaticartery, which contributes 25% to 30% of the blood supply, and the portal vein, which isresponsible 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 hepaticvenous system **(Hilscher et al., 2019) (Figure 13).**

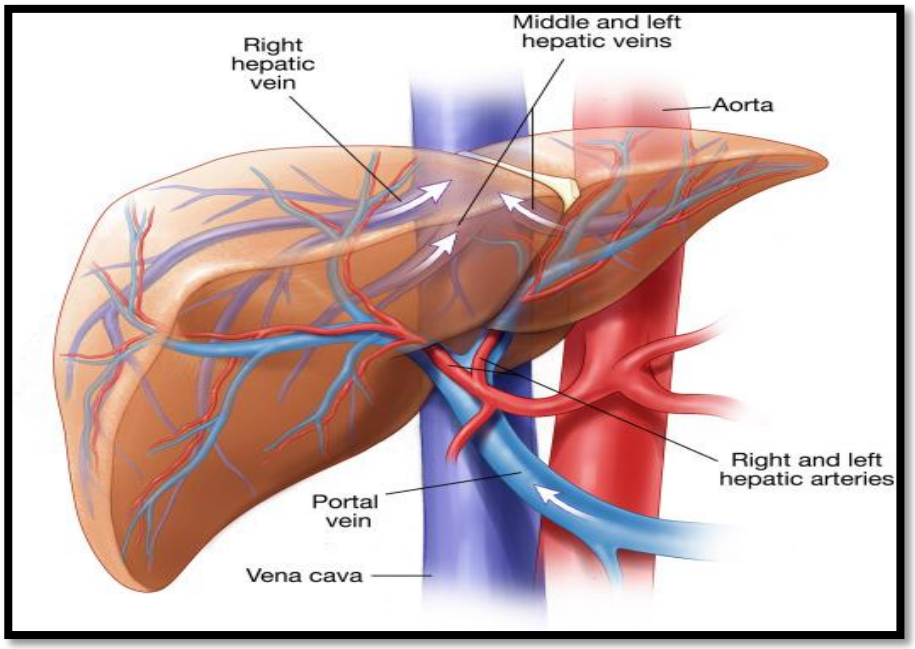


Figure 13. Liver vascularisation **(Hilscher et al., 2019).**

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 ➢ metabolises hormones
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 (about 3.94 to 4.72 in) 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.2.3 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, capillar-Ies, 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, Nonfiltering 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)** and **(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).**

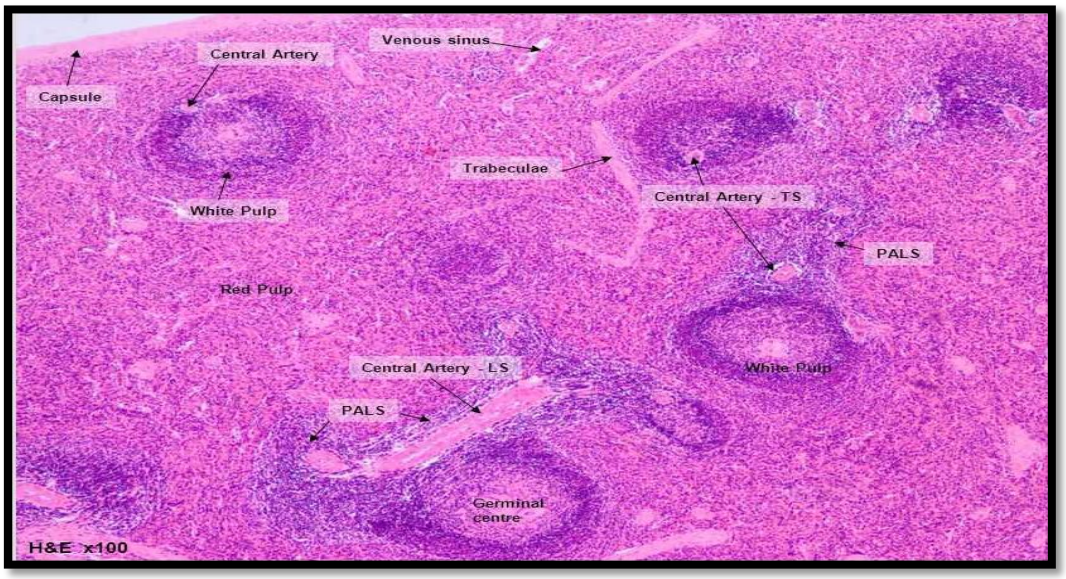


Figure 14. Visceral surface of the spleen **(Meghan and Myutan, 2024).**

II.2.4 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.

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 isna 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.2.5 Spleen function

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).**

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).**

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 blood stream **(Theurl et al., 2016)**

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. Natural compounds

Since ancient times, mankind has taken advantage of the benefits associated with beehive products, such as honey, propolis, bee pollen, beeswax, bee venom, and royal jelly, and distinct cultures have been using them to treat and prevent diseases **(Ripari et al., 2022).**

Because of their specific biochemical profile, all of these bee-derived products are highly bioavailable **(Bobis et al., 2017).**

It is represents a very important nutraceutical, functional food, and nutritional supplement **(Collazoet al., 2021).** The RJ that can efficiently complement a healthy diet. Furthermore, it can also be useful in the diets of various animal species **(Han et al., 2018).**

- Royal jelly

III.1. Description

Royal jelly, a sticky, jelly-like matter, is excreted from the glands (hypopharyngeal and mandibular) of bees. This secreted substance is used to feed queen bees and larvae. The color of this nutrient ranges from light cream to dark yellow. Royal jelly is acidic and has a pungent taste. Royal jelly impacts the organism in many ways. Studies have shown positive effects on blood parameters **(Taşdoğan et al., 2020).**

From 1-3 days-old larvae of all the honey bee castes (queen, worker, and drone) are fed with royal jelly. RJ is a yellowish-white gelatinous substance made from proteins, carbohydrates, lipids, and vitamins produced by secretory cells in the glandular acini **(bark and Kim, 2019).** Royal jelly secretion with worker bees of the apis mellifera species **(fontana et al., 2004)(Figures 15,16 and 17).**

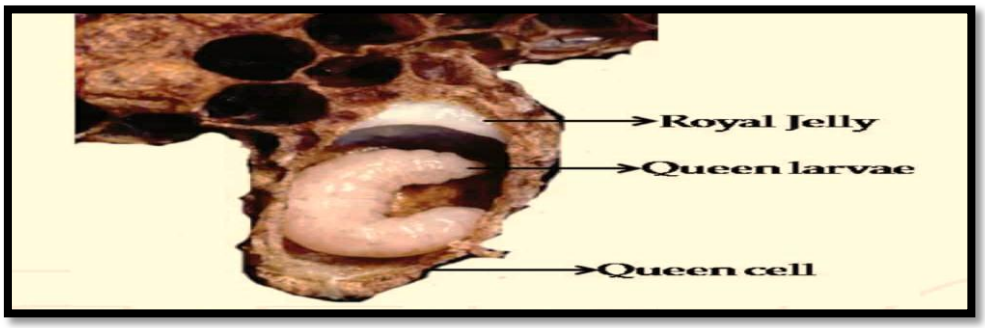
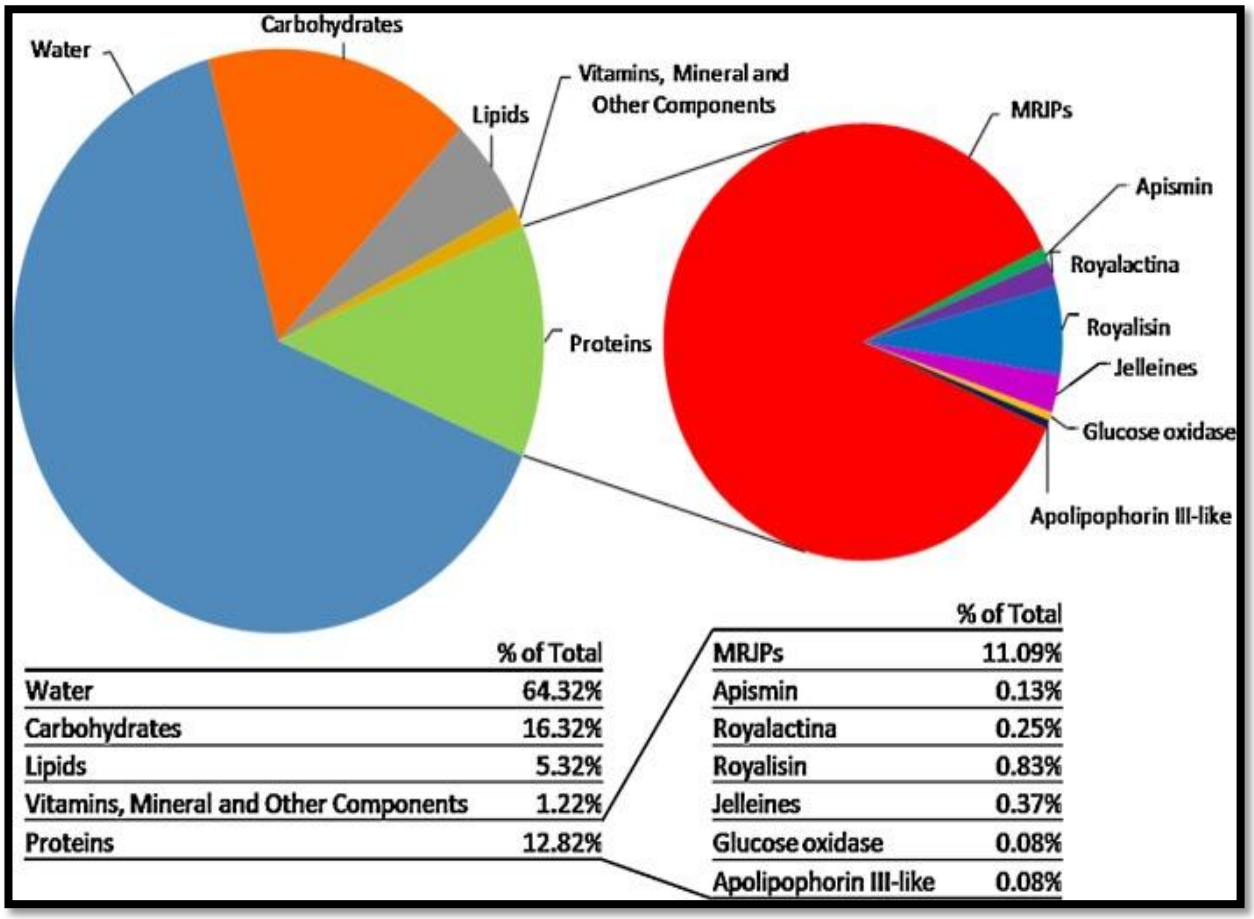


Figure 15. Queen cell with queen larvae of A. Mellifera and royal jelly.

(Takasusuki et al., 2016).

Figure16. Werker bee apis mellifera (9).



	% of Total
Water	64.32%
Carbohydrates	16.32%
Lipids	5.32%
Vitamins, Mineral and Other Components	1.22%
Proteins	12.82%

	% of Total
MRJPs	11.09%
Apismin	0.13%
Royalactina	0.25%
Royalisin	0.83%
Jelleines	0.37%
Glucose oxidase	0.08%
Apolipophorin III-like	0.08%

Figure 17. Mean composition of royal jelly **(Franti et al., 2016).**

III.2. Therapeutic properties

Royal jelly as the most valued bee product, is not only one of the most attractive functional foods; it can also be used in medicine as a medicinal product. In many countries, it is recommended in pediatrics to geriatric medicine, especially in relation to nutrition and cosmetics. Due to the highly valuable nutritive composition, the consummation of RJ is constantly growing. It can be consumed in different forms, either native or as a functional component of different food products. According to some sources, the annual production of RJ in China, the world's largest producer and exporter, is over 4000 tons, which represents more than 90% of the total production on a global level **(Ahmad and Campos, 2020).**

III.3. The role of royal jelly

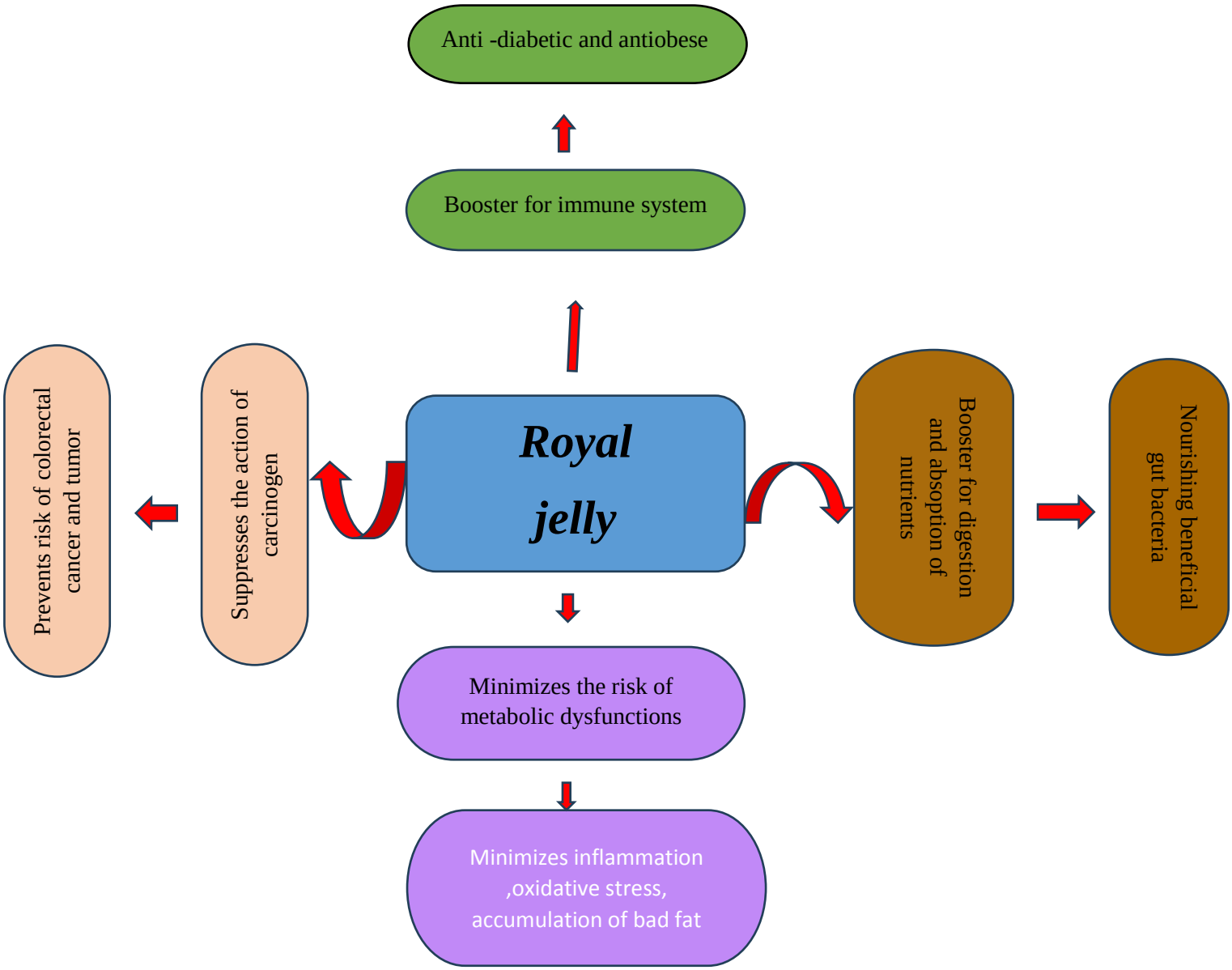


Figure18. Role of royal jelly **(Kumar et al., 2024)**

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 frères Mentouri-Constantine1 (Algeria). weighing between 23 and 34,6g, were used for determination of the phagocytic activity of royal jelly.

Figure 19 . Adult male Albino *Mus Musculus.*

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.

1.4. Chemicals

Chloroforme, Distilled water, NaCl 0.9%, Na_2Co_3, gelatin, black Chinese ink, 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 , NaCl , coomassie Brilliant Blue G-250.

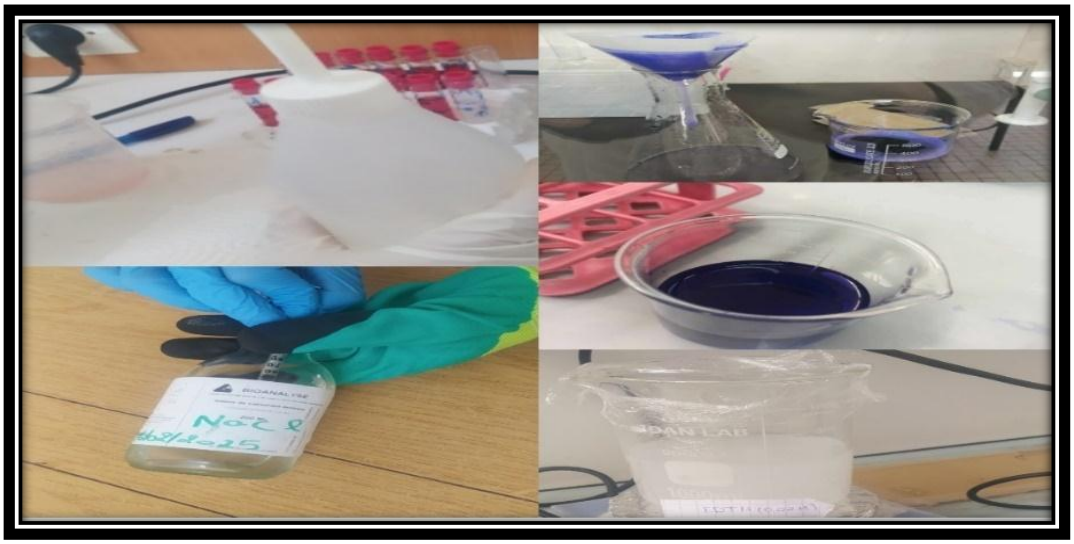


Figure 20. chemicals used.

1.5. Equipments

Precision weighing balances (readability 0.01g), dissection kit, Spectrophotometer, test tubes, heating magnetic stirrer, pH meter, fiter paper, centrifuge, vortex mixer, Eppendorf tubes, small bottles, oven, capillary tubes, insulin syringe, refrigerator.

Figure 21. materials used during experimental work.

2.1. Treatment of mice

The 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 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(R).

Group I (control) was given 0,9% Nacl (0,5 ml/mouse i.p.), Groups II was administered by i.p injection with a concentrations of royal jelly (250mg/kg).

After 48h of ip injection of the treatment, the mice were administered with carbon ink suspension at a dose of (0.1ml/10g) through the tail vein.

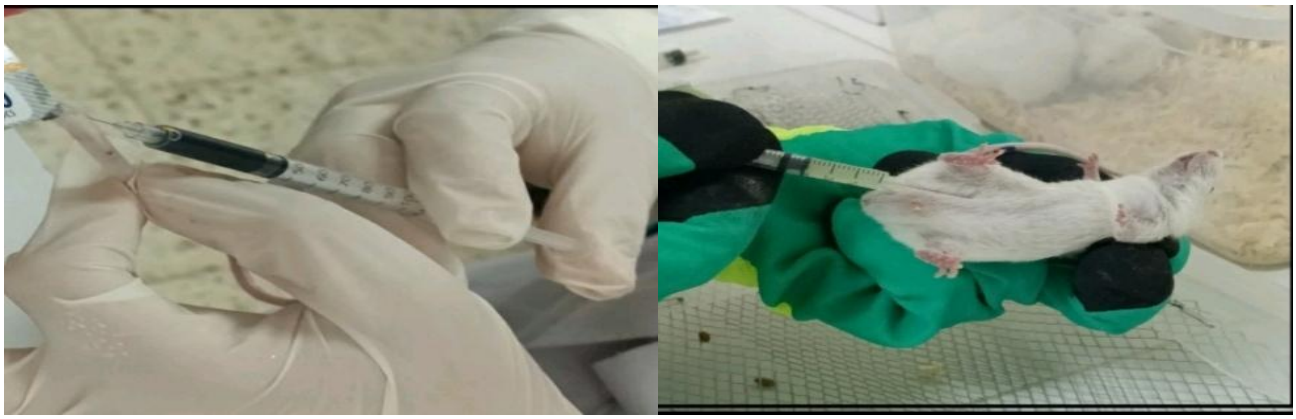


Figure 22. Intra peritoneal injection and intravenous injection.

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 23. Retro-orbital blood collection.

2.3. Dissection

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

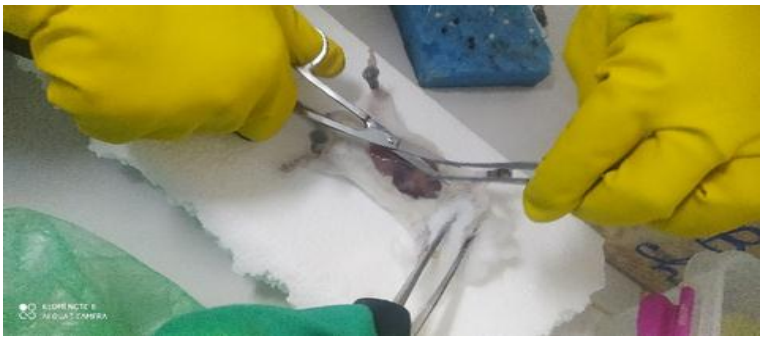


Figure 24. Dissection of mice.

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 were conducted to evaluate differences between two groups subjected to treatment by royal jelly 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 at4°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%) and was allowed stand in the freezer for 15 min. Following centrifugation at 1000 tours/mn) 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:

$$\textbf{GSH (mmol/ mg protein)} = \frac{\boldsymbol{Do \times 1 \times 1{,}525}}{\boldsymbol{13100 \times 0{,}8 \times 0{,}5 \times \times mg\ proteine}}$$

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.

. The protein concentration in the test samples is determined from the calibration graph **(Figure 25).**

Figure 25. materials and solutions used in protein determination and glutathione.

Results and Discussion

I. Results

1.1. Immunomostimulatory activity of *royal jelly*

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

- **Phagocytic activity**

As shown in the figure 13, The phagocytic index is increased but not significantly between group GI (0,016 ±0,002) and GII (0,0195 ± 0,007) (P > 0.05) **(Table 02).**

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

As shown in Figure 14, the carbon clearance rate of the groups was GI (42,86± 7,63) and GII (40,74±13,98), The half-time of colloidal carbon was faster but not significantly in group II when it is compared to the group I (P > 0.05), after 48 h of the administration of royal jelly. **(*Table 02*).**

- **Percentage change**

The percentage of the phagocytic activity group treated with royal jelly was 38,76% **(*Table 03*)**

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

The corrected phagocytic index ***α*** is increased in group GII when it is compared to GI but not significantly (P > 0.05) **(Table 04)**

Table 02. Effect of royal jelly *on* phagocytic activity in mice and the half time (**T ½ min).**

	Number of mice	**dose**	**k**	**K Average**	**T ½ min**	**T ½ min average**
G1 (control)	6	0,9% Nacl	0,018 0,016 0,019 0,012	0,016±0,002	38,5 41,25 35,53 55,44	42,86±7,63

GII (Royal jelly)	6	250mg/kg	0,021 0,012 0,013 0,031	0,0195±0,007	32,68 57,75 50,21 22,35	40,74 ± 13,98

Table 03. The percentage change of phagocytic activity.

Mice number	Percentage	Average %
1	14,28	38,76
2	33,33	
3	46,15	
4	61 ,29	

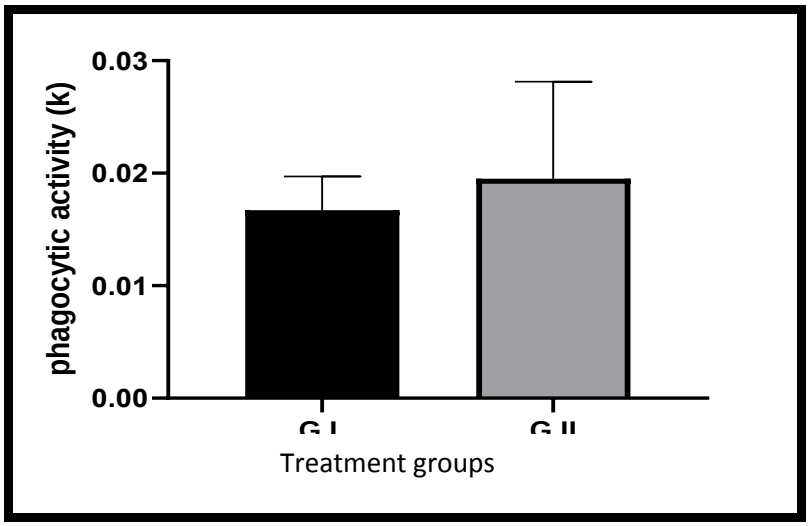


Figure 26. Effect of royal jelly on phagocytic activity.

GI: group control treated with Nacl , GII: group treated with royal jelly

P≥ 0,05.

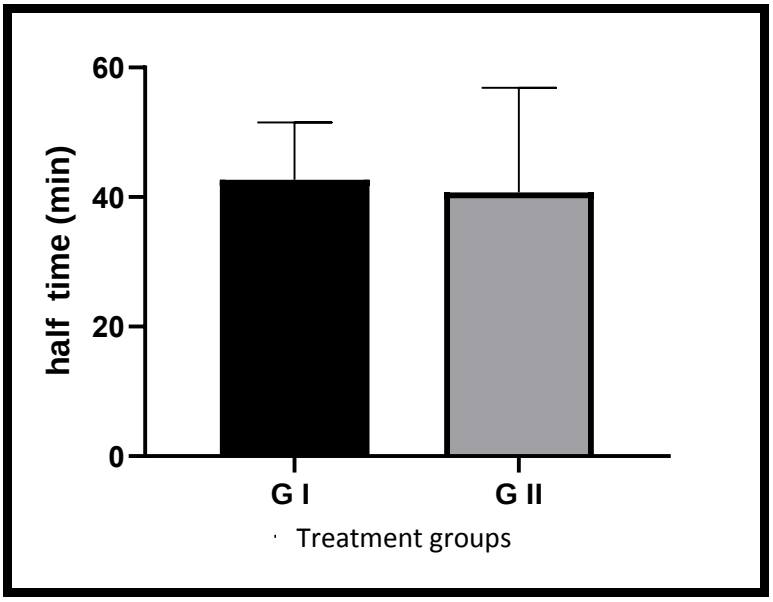


Figure 27. Effect of royal jelly extract on half time of carbon in blood.

GI: group control treated with Nacl , GII: group treated with royal jelly

P≥ 0,05.

Table 04. Effect of royal jelly extract on phagocytic activity in mice shown on active organ (liver and spleen) mice weight (n=4).

Groups	Liver weight (g)	Spleen weight (g)	Ab (5 min)	Ab (10 min)	α	α average
G I (control)	1,53 0,95 1,46 1,52	0,12 0,05 0,13 0,25	0,086 0,162 0,079 0,119	0,132 0,110 0,124 0,159	5,47 5,888 6,245 4,141	5,43±0,79
G II (royal jelly)	1,68 1,37 1,30	0,22 0,16 0,12	0,091 0,048 0,210	0,122 0,066 0,102	4,228 5,835 6,589	6,078±1,24

	0,87	0,10	0,094	0,108	7,66	

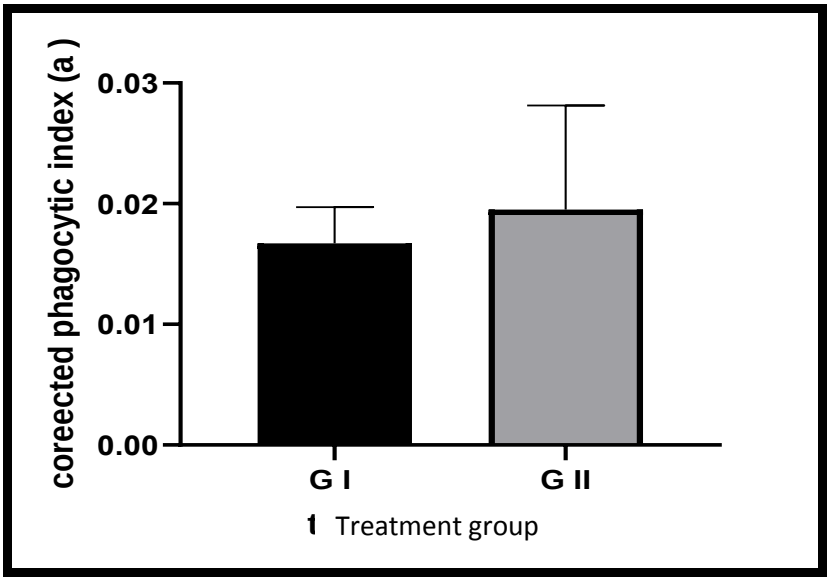


Figure 28. Effect of royal jelly extract on corrected phagocytic activity (α).

GI: group control treated with Nacl , GII: group treated with royal jelly

P≥ 0,05.

- **GSH Test**

The data in figure 28 showed that, the concentration of reduced glutathion (GSH) is decreased in group II when it is compared to the GI but not significantly (P > 0.05). The values in the group GI was (2,56±1,17) and in the group II was (1,44± 0,29).

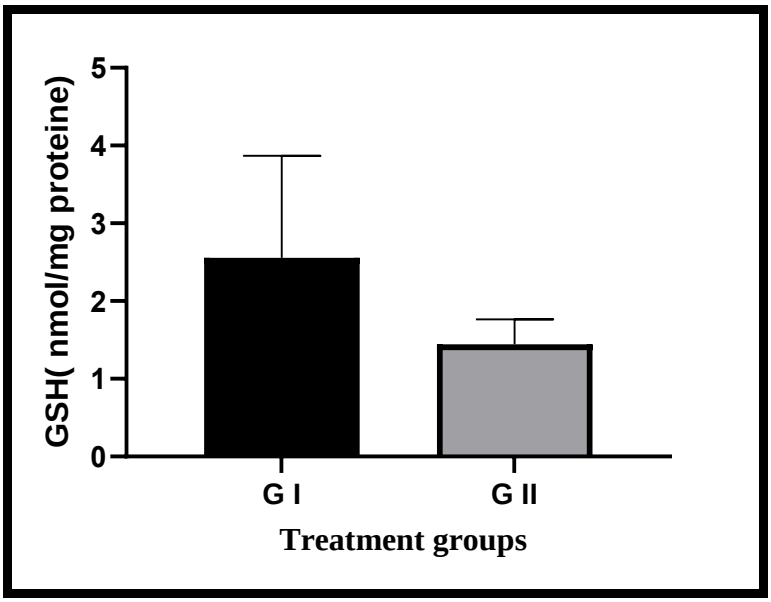


Figure 29. Effect of royal jelly extract on GSH in mice.

GI: group control treated with Nacl , GII: group treated with royal jelly

P≥ 0,05.

II. Discussion

The immune system is a prominent defense institution in the human body. It is comprised of immune organs, immune cells, and molecules. It prevents the invasion of pathogenic genes through the immune response and it is essential in maintaining physiological balance **(Xiao et al., 2022)** and **(Ahmed et al., 2022).**

Natural products have long served as sources of therapeutic drugs and have been used to treat various pathophysiological conditions, including inflammation, cancer, viral infections, immunological disorders, and metabolic diseases **(Yuanet al., 2016).** These products contain multiple components with unexpected biological properties and often show synergistic effects in traditional medicine **(Kiyohara et al., 2004).** Drugs have been developed from medicinal plants and their derivatives **(Newman et al., 2012).** Additionally, the use of medicinal plant extracts as prescription drugs has gradually increased in developed countries **(Yatoo et al., 2017).** Royal jelly could motivated the immune function by stimulating macrophages and decreasing inflammatory factors in a mouse body **(Bron et al., 2017).**

Phagocytic activity is a critical biological activity through which previous reports indicated that major royal jelly proteins could regulate the host and can protect itself from infectious and non-infectious environmental particles and remove unwanted host cells in order to maintain tissue homeostasis. Phagocytosis is an ancient, conserved process that is apparent in all,multicellular organisms **(Platt and Fineran, 2015).**

In this study we have observed that the animals administered with the *royal jelly* stimulates the phagocytic activity with a concentration of (250 mg/kg), this result is agrees with **Crenguta et al. (2011)** who reported that, the royal jelly has immunomodulatory and antialergic activity, antibiotic and wound healing effect, anti inflammatory and antitumoral action. Also this results align with those of **Dehnnet et al. (2022)** who reported that the immunomodulatory activity is increased in animals treated with *Cyphostemma adenocaule*. **Zhang et al. (2003)** showed that ,the J9311 formulation which is a mixture of royal Jelly, propolis, and Chinese medicinal herbs could increased the phagocytic index significantly.

Kehili and Zerizer (2024) obtained that the combination of *Phoenix dactylifera* (date fruit) and *Trigonella foenum-graecum* (fenugreek seeds) increased the phagocytic activity in mice.

Kassahun et al. (2024) demonstrated that, the administered dose of royal jelly increases the carbon clearence rate very highly significantly by stimulating the reticuloendothelial system activity in mice.

The reticuloendothelial system (RES) acts as the body's first line of defense and is primarily recognized for its ability to scavenge and clear debris, foreign particles, and unwanted substances. The phagocytic activity of the RES is evaluated by measuring the clearance rate of carbon particles from the bloodstream. This process is mainly carried out by sessile intravascular phagocytes, which are specialized cells located predominantly in the liver and spleen. These organs play a crucial role in filtering the blood, where endothelial cells and macrophages work together to eliminate harmful materials from circulation, maintaining the body's immune surveillance and detoxification functions **(Baas et al., 1994).**

Our result is agrees with the result obtained by **Nasser et al. (2015)** and **Wang et al. (2023),** who obtained that the corrected phagocytic index α increased with *Salvia verbenaca* and the phagocytic activity increased with the treatment of royal jelly respectively.

We have obtained from this study the percentage of phagocytic activity reached to 38,76 % which confirmed that royal jelly stimulates the immune system by the activation of the reticulo endothelial system. **Abebe et al. (2022)**, detected a percentage of phagocytic activity of *Cyphostemma adenocaule* extract around 70%.

Royal jelly is stimulated the phagcytic index, correction α, and a reduction in the carbon clearance rate. This is due to the bioactive compounds such as 10-HDA (10-hydroxy-2decenoic acid), phenols and flavonoids **(Guo et al., 2021).**

Glutathione (GSH) is a tripeptide molecule comprising cysteine, glycine, and glutamate. GSH is an important antioxidant found extensively throughout the body and synthesized in the cytosol. GSH neutralizes reactive oxygen species (ROS), reactive nitrogen species (RNS), and electrophiles directly via enzymatic reactions. It also plays a role in maintaining redox homeostasis, regulating cellular events, and detoxifying xenobiotics **(Dwivedi et al., 2020).**

In our study we have obtained that the concentration of GSH is decreased in group treated with royal jelly this findings is not agree with the result obtained by **Aksoy and Alper, (2019)** who explained that

GSH is decreased in erythrocyte, liver and kidney in the mice treated with malathion (an organophosphate insecticide) when compared to the control and royal jelly groups.

Bouamama et al., (2021) reported that RJ could increased the intracellular GSH in aged human PBMCS. **Zhang et al. (2017)** established a positive effect of royal jelly on breast cancer in mice when he administered it orally and also **Hayashi et al. (2017)** found that there is a correction in hyperglycemia and obesity in mice.

Conclusion

Conclusion

The findings of this study suggest that royal jelly, administered at a dose of 250 ml/kg, stimulates the phagocytic activity, reduced the half time of the carbon clearance in the blood and helps the secretion of GSH from the liver to the blood to defense against free radicals formed during the phagocytosis. RJ is a rich with bioactive compounds such as phenols, flavonoids.

Future research efforts will focus on:

- identification and isolation of the active compounds from royal jelly;
- investigating the long-term impact of royal jelly treatment across different animal models and exposure durations;
- assessing its influence on oxidative biomarkers and antioxidant enzyme activities.

References

References

1. Abraham S.N and St John A.L. (2010). Mast cell-orchestrated immunity to pathogens. Nature Reviews Immunology. 10 :440–452.
2. Aksoy L and Alper Y. (2019). The effects of royal jelly on oxidative stress and toxicity in tissues induced by malathion, an organophosphate insecticide. Journal of the Hellenic Veterinary Medical Society, 70(2) :1517–1524.
3. Ayenew K.D., Ayalew A., Wondmkun Y.T., Tsige A.W., Wolde A., Sewale Y., Belihu G.D., Fekade E., Habteweld H.A. (2024). In-*vivo* immunomodulatory activities of essential oils of *Artemisia abyssinica* and *Lepidium sativum* in mice. ImmunoTargets and Therapy, 13 : 15–27.
4. Biron C.A., Nguyen K.B., Pein G.C.,Cousens L.P and Salazar -Mather T.P. (1999). Natural killer cells in antiviral defense: Function and regulation by innate cytokines. Annual review of immunologie .17 : 189-220.
5. Blumgart L. H and Belghiti J. (2007). Surgery of the liver, biliary tract, and pancreas. Saunders Elsevier.3rd ed :3–30.
6. Bobis O., Bonta V., Varadi A., Strant M., Dezmirean D. (2017). Bee products and oxidative stress: Bioavailability of their functional constituents. Modern Applications in Bioequivalence and Bioavailability, 1, 555565.
7. Bonilla F.A and Oettgen H.C. (2010). Adaptive immunity. Journal of Allergy clinical immunology. 125(2) : S33–40.
8. Bouamama S., Merzouk H., Latrech H., Charif N and Bouamama A. (2021). Royal jelly alleviates the detrimental effects of aging on immune functions by enhancing the in *vitro* cellular proliferation, cytokines, and nitric oxide release in aged human PBMCS. Journal of food biochemistry, 45(2).
9. Clark M.R., Mandal M, Ochiai K and Singh H. (2014). Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signalling. Nature Reviews Immunology. 14(2) :69-80.
10. Cooper, M.D. (2015). The early history of B cells. Nature Reviews Immunology. ,15:191–197.
11. Cota A. M and Midwinter M. J. (2015). The immune system. Anaesthesia and Intensive Care Medicine,16(7) : 353–355.
12. Dalmo RA, Ingebrigsten K, Bogwald J. (1997).Non-specific defense mechanisms in fish, with particular reference to the reticulo-endothelial system (RES). Journal of Fish Diseases. ;20:241-73.

References

13. DixonG.L., Newton, P.J., Chain, B.M., Katz, D., Andersen, S.R., Wong, S., van der Ley, P., Klein, N., and Callard, R.E. (2001). Dendritic cell activation and cytokine production induced by Group B Neisseria meningitidis : interleukin-12 production depends on lipopolysaccharide expression in intact bacteria. Infection and Immunity 69 : 4351-4357.
14. Drake R.L., Vogl A.W., and Mitchell A.W. (2015). Gray's Anatomy for Students (3rd ed.). Philadelphia : Churchill Livingstone: 346–355.
15. Dwivedi D., Megha K., Mishra R., and Mandal, P. K. (2020). Glutathione in brain: Overview of its conformations, functions, biochemical characteristics, quantitation and potential therapeutic role in brain disorders. Neurochemical Research, 45(7) : 1461–1480.
16. El-Seedi H.R., Eid N., Abd El-Wahed A.A., Rateb M.E., Afifi H.S., Algethami A.F., Zhao C., Al Naggar Y., Alsharif S.M., Tahir H.E., Xu B., Wang K., Khalifa S.A.M. (2022). Honey bee products: Preclinical and clinical studies of their anti-inflammatory and immunomodulatory properties. Frontiers in Nutrition, 8, 761267.
17. Fontana R., Mendes M. A., De Souza B. M., Konno K., César, L. M. M., Malaspina, O., and Palma, M. S. (2004). Jelleines: A family of antimicrobial peptides from the Royal Jelly of honeybees (Apis mellifera). Peptides, 25(6) :919–928.
18. Fratini F., Cilia G., Mancini S and Felicioli, A. (2016). Royal jelly : An ancient remedy with remarkable antibacterial properties. Microbiological Research, 192, 130–141.
19. Fulkerson P.C and Rothenberg M.E. (2013). Targeting eosinophils in allergy, inflammation and beyond. Nature Reviews Drug Discovery. 12 :117–29.
20. Gent L, Blackie P. (2017). The spleen. British Journal of Anaesthesia Education. 17 (6) :214–220.
21. Gillian D. Smith A .and Brown, P. (2006). Hepatic microcirculation and immune surveillance. Journal of Hepatology, 45(3) : 456–468.
22. Gordon S. (2016). Phagocytosis : An immunobiologic process. Immunity USA , 44(3) : 463–475.
23. Gu H., Song I.B., Han H.J., Lee N.Y., Cha J.Y., Son Y.K., Kwon J. (2018). Anti- inflammatory and immune-enhancing effects of enzyme-treated royal jelly. Journal of Applied Biological Chemistry, 61: 227–233.
24. Guyton A. C. and Hall J. E. (2006). Textbook of medical physiology (11th ed.). Saunders : Philadelphia, PA.
25. Hayashi K., Watadani,R., Okano Y., Tanimura K., Kotoh J., Sasaki, D., Matsumoto K., and Maeda A. (2017). Royal jelly improves hyperglycemia in obese/diabetic KK-Ay mice. Journal of Veterinary Medical Science. 79(2) :299–307.

References

26. Hilscher M. B. and Kamath,P. S. (2019). The liver in circulatory disturbances. Clinics in Liver Disease, 23(2) : 209–220.
27. Jurisić V. (2006). Srpski arhiv za celokupno lekarstvo, 134(1-2) :71–76.
28. Kaile L. (2024). Core Concepts in Biology : Immunologie (2nd ed.). USA : 1–283.
29. Kazuki S. (2023). Macrophages: Phagocytosis, Antigen Presentation, and Activation of Immunity . Edited by Athari S. SH. and Nasab E . M. Phagocytosis - Main Key of Immune System . University of Medical Sciences , Iran.
30. Kehili H.E., Zerizer S. (2024). Immune-stimulating and antioxidant properties of a traditional Algerian plant combination : Date fruit (*Phoenix dactylifera*) and Fenugreek seeds (*Trigonella foenum-graecum*). The North African Journal of Food and Nutrition Research, 8(18) : 253–261.
31. Khodadadi L., Cheng Q., Radbruch A and Hiepe F. (2019). The Maintenance of Memory Plasma Cells. Frontiers in Immunology 10 :721.
32. Kmieć Z. (2001). Introduction — Morphology of the liver lobule. In Cooperation of liver cells in health and disease . Advances in Anatomy, Embryology and Cell Biology.161 : iii–xiii.
33. Kumar R., Thakur A., Kumar S., Hajam Y.A. (2024). Royal jelly a promising therapeutic intervention and functional food supplement : A systematic review. Heliyon, 10: 37138.
34. Lautt W. and Greenway C.V. (1987). Conceptual review of the hepatic vascular bed. Hepatology, 7(5) :952–963.
35. Levin R., Grinstein S and Canton J. (2016). The life cycle of phagosomes : Formation, maturation, and resolution. Immunological Reviews, 273(1) : 156–179.
36. Lewis S.M., Williams A., and Eisenbarth S.C. (2019). Structure and function of the immune system in the spleen. Science Immunology.4(33).
37. Lung K., and Lui F. (2023). Anatomy, Abdomen and Pelvis: Arteries. Publishing ; Treasure Island.
38. Maghsoudlou A., Mahoonak A.S., Mohebodini H., Toldra F. (2019). Royal jelly : Chemistry, storage and bioactivities. Journal of Apicultural Science, 63(1) :17–40.
39. Marshall J.S., Upton J.E.M., Vliagoftis H., Hildebrand K.J., Byrne A and Watson W. (2024). Introduction to immunology and immune disorders. Allergy, Asthma & Clinical Immunology .20(3) :69.
40. Marshall J. S., Warrington R, Watson W and Harold L.k. (2018). An introduction to immunology and immunopathology. Allergy Asthma Clinical Immunology. 14 (2), 49.
41. Mayadas T. N., Cullere X., Lowell C. A. (2014). The multifaceted functions of neutrophils. Annual Review of Pathology. 9, 181–218.

References

42. Mebius R.E., and Kraal G. (2005). Structure and function of the spleen. Nature Reviews Immunology. 5(8) : 606–616.
43. Mehraj U., Safura N., Syed S.H.Sheikh BA .Bhat B.A., Hina Q., Mir M.A. Cells of the immune system in Mir M A. (2021) Basics and fundamentals of immunology. Nova Science publishers,Inc.New York. 33-40.
44. Meghan C., and Myutan K. (2024). Anatomy of the pancreas and spleen. Basic Science. Published by Elsevier. 1–5.
45. Miller, J.F.A. P. (2020). The function of the thymus and its impact on modern medicine. Science 369 : 2429.
46. Miyata Y., and Sakai H. (2018). Anti-cancer and protective effects of royal jelly for therapy-induced toxicities in malignancies. International Journal of Molecular Sciences, 19(10). 3270.
47. Moore K.L., Dalley A.F. II, and Agur A.M.R. (2014). Clinically Oriented Anatomy (7th ed.). Lippincott Williams and Wilkins ; Abdomen .263–265.
48. Mosser D.M., Hamidzadeh K and Goncalves R. (2020). macrophages and the maintenance of homeostasis. cellular and molecular immunology. 1-9.
49. Mousset C.M., Hobo W., Woestenenk R., Preijers F., Dolstra H and van der Waart A.B. (2019). Comprehensive phenotyping of T cells using flow cytometry. Cytometry A. 95(6) : 647-654.
50. Murphy K.M., Travers P., Walport M. (2007). Janeway's immunobiology. 7th ed. New York: Garland Science.
51. Namdeo P. (2021). A Review on Herbal Immunity Booster and Nutrition – To Fight against COVID-19. (04) : 1226-1237.
52. Nassar M., Zerizer S., Kabouche Z and Kabouche A. (2015). Antioxidant and the immunomodulatory activities exhibited by three plants from Lamiaceae family. International Journal of Pharmacy and Pharmaceutical Sciences, 7(9) : 331–334.
53. Nauseef W. M., Borregaard N. (2014). Neutrophils at work. Nature Immunology. 15 : 602– 611.
54. Oršolić N., Jazvinščak Jembrek M. (2024). Royal jelly : Biological action and health benefits. International Journal of Molecular Sciences.25 : 6023.
55. Özdikici M. (2018). The relationship between splenic length in healthy children. Journal of Ultrasonography. 18(72) : 5–8.
56. Ozougwu J. C. (2017). Physiology of the liver. International Journal of Research in Pharmacy and Biosciences. 4(8) :13–24.
57. Paraskevas F. (2014). Lymphocytes and lymphatic organs. In : Greer J.P. (Ed.), Wintrobe's Clinical Hematology (13th ed.). Philadelphia : Wolters Kluwer Health/Lippincott Williams and Wilkins.

References

58. Parkin J and Cohen B. (2001). An overview of the immune system. Lancet.357(9270) : 1777–1789.
59. Patel A.A., Zhang Y., Fullerton J.N., Boelen L., Rongvaux A., Maini A.A., Bigley V., Flavell R.A., Gilroy D.W., Asquith B., Macallan D and Yona S. (2017). The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. Journal of Experimental Medicine. 214(7) : 1913-1923.
60. Petty A.J and Yang Y. (2019). Tumor-associated macrophages in hematologic malignancies : new insights and targeted therapies. Cells. 8(12) :1526.
61. Pieper K., Grimbacher B and Eibel H. (2013). B-cell biology and development. Journal of Allergy and Clinical Immunology. 131(4) : 959-71.
62. Porembka M.R., Doyle M., and Chapman W.C. (2000). Disorders of the spleen. In : Greer J.P. (Ed.), Wintrobe's Clinical Hematology (13th ed.). Philadelphia : Wolters Kluwer Health/Lippincott Williams and Wilkins.
63. Prinyakupt J and Pluempitiwiriyawej C. (2015). Segmentation of white blood cells and comparison of cell morphology by linear and naïve Bayes classifiers. Biomed eng online. 14 :63.
64. Rabinovitch M. (1995). Professional and non-professional phagocytes : An introduction. Trends in Cell Biology, 5(3) : 85–87.
65. Rehaume L.M and Hancock R.E. (2008). Neutrophil-derived defensins as modulators of innate immune function. Critical Reviews in Immunology. 185–200.
66. Ruvolo-Takasusuki M.C., Pozza A., Oliveira A ., Parpinelli R ., Costa M.F., Faquinello P and Arnaut de Toledo V. (2016). Improvement and selection of honeybees assisted by molecular markers. 10.5772/62426.
67. Sam-Yello WeT.Y. (2021). Exercise 6 : Clearance of Bacteria from Mouse Blood by the Reticuloendothelial System (RES). In : Immunology : Overview and Laboratory Manual. Springer, Cham.
68. Scapini P., Cassatella M. A. (2014). Social networking of human neutrophils within the immune system. Blood 124 : 710–719.
69. Shah A.S., Wakade A.S., Juvekar A.R. (2008). Immunomodulatory activity of methanolic extract of *Murraya koenigii* (L) Spreng leaves. Indian Journal of Experimental Biology, 46(7) : 505–509.
70. Standring S. (2021). Gray's Anatomy : The Anatomical Basis of Clinical Practice (42nd ed.).
71. Steiniger B.S., Wilhelmi V., Berthold M., Guthe M., and Lobachev O. (2018). Locating human splenic capillary sheaths in virtual reality. Sci rep, 8(1). 15720.

References

72. Stuart AE, Habshaw IA, Davidson A. Phagocytes *in vitro*. In : Handbook of Experimental Immunology. Weter DM. 2nd ed. Blackwell Sciences Publication ; 1973.
73. Taşdoğan, A. M., Vural, M., Özdal, M., & Pancar, Z. (2020). Effect of royal jelly supplementation on aerobic power output and anaerobic power output. Progress in Nutrition, 22(1) : 281–287.
74. Uribe-Querol E and Rosales C. (2020). Phagocytosis : Our current understanding of a universal biological process. Frontiers in Immunology, 11, 1066.
75. Wang W., Li X., Li D., Pan F., Fang X., Peng W., and Tian W. (2023). Effects of major royal jelly proteins on the immune response and gut microbiota composition in cyclophosphamide-treated mice. nutrients, 15(4). 974.
76. Weis W.A., Ripari N., Conte F.L., Honorio M.S., Sartori A.A., Matucci R.H., Sforcin J.M. (2022). An overview about apitherapy and its clinical applications. Phytomedicine, 2. 100239.
77. Wilkins B.S., and Wright D.H. (2000). Illustrated Pathology of the Spleen. United Kingdom : Cambridge University Press .13–16.
78. Wu G., Fang Y.-Z., Yang S., Lupton J.R., and Turner N.D. (2004). Glutathione metabolism and its implications for health. The Journal of Nutrition, 134(3) : 489–492.
79. Zerizer S. and Naimi D. (2004). Homocysteine : An independent risk factor in athergenic Process. Egyptian Pharmaceutical Journal (3) : 110- 114.
80. Zerizer S., AlHajri F., Aras R., Benosmane M. T., Siari E., and Khelfi S. (2023). Catalase activity and immunostimulatory of hot water and vitamin C. 13(1) : 267–274.
81. Zhang X., Yu Y., Sun P., Fan Z., Zhang W., Feng C. (2019). Royal jelly peptides : Potential inhibitors of β-secretase in N2a/APP695swe cells. Scientific Reports. 9(1), 168.
82. Zhang S., Shao Q., Geng H., and Su S. (2017). The effect of royal jelly on the growth of breast cancer in mice. Oncology Letters, 14(6) : 7615–7621.
83. Zhang Q., Wu Z., Miao X., and Zhou J. (2003). Effects of propolis capsules on phagocytic function of macrophage and their acute toxicity. Journal of Fujian Agriculture and Forestry University (Natural Science Edition). 32 (4) : 500–502.
84. Zoghi S., Masoumi F and Rezaei N. (2023). The immune system. In N. Rezaei (Ed.), Clinical immunology. Academic Press. 1–46.

Web sites :

1. **https://images.app.goo.gl/VhW5Vs87AUg24ccK8**
2. **https://images.app.goo.gl/7vQnxq3iRdZvu9x89**
3. **https://images.app.goo.gl/JmHkN44EzLSkErm38**

References

4. **https://images.app.goo.gl/dWCFUEyrH3ueAvFb7**
5. https://images.app.goo.gl/HwU4NL5NpewJZtsaA
6. https://images.app.goo.gl/jP6C3tQqTYiFQkLN6
7. **https://o.quizlet.com/kAyAmUBdt47gJgh6v6jpKg.jpg**
8. https://www.miel-direct.fr/famille-abeilles-apis-mellifera

Summary

Summary

It has long been established that the macrophages that make up the endothelial system play a major role in the immune response against various inflammatory diseases that affect the body.

In this experiment, the immunostimulatory activity of royal jelly extract on macrophage activity and antioxidant were evaluated.

It was shown that a dose of 250 mg/kg of royal jelly injected into mice led to an increase the phagocytic index (K), corrected phagocytic index α and a diminution of the carbon clearance rate t½) and GSH levels.

We concluded that, royal jelly is a natural extract capable of stimulating the body's immune activity against pathogens.

Key words: royal Jelly , phagocytic index, carbon clearance rate, corrected phagocytic index α, GSH .

Résumé

Il est établi depuis longtemps que les macrophages, qui composent le système endothélial, jouent un rôle majeur dans la réponse immunitaire contre diverses maladies inflammatoires affectant l'organisme.

Dans cette expérience, l'activité immunostimulatrice d'un extrait de gelée royale sur l'activité des macrophages et la concentration du glutathion réduit ont été évaluées.

Il a été démontré qu'une dose de 250 mg/kg de gelée royale injectée à des souris entraînait une augmentation de l'indice phagocytaire (K), un index phagocytaire corrigé α et une diminution de la vitesse de clairance du carbone (t½) et la concentration du GSH.

Nous avons conclu que la gelée royale est un extrait naturel capable de stimuler l'activité immunitaire de l'organisme contre les agents pathogènes.

Mots clés : gelée royale, index phagocytaire, taux de clairance du carbone, index phagocytaire α corrigé, GSH.

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ملخص

لقد ثبت منذ زمن طويل أن الخلايا البلعمية، التي تُشكل الجهاز البطاني، تلعب دورًا رئيسيًا في الاستجابة المناعية ضد مختلف الأمراض الالتهابية التي تصيب الجسم.

في هذه التجربة، تم تقييم النشاط المُعدّل للمناعة لمستخلص غذاء ملكات النحل على نشاط الخلايا البلعمية وانخفاض تركيز الجلوتاثيون.

وقد تبيّن أن حقن الفئران بجرعة 250 ملغم/كغم من غذاء ملكات النحل أدى إلى زيادة في مؤشر البلعمة (K)، ومؤشر البلعمة المصحح ألفا، وانخفاض في معدل تصفية الكربون (½t) وتركيز الجلوتاثيون.

واستنتجنا بأن غذاء ملكات النحل مستخلص طبيعي قادر على تحفيز النشاط المناعي للجسم ضد مسببات الأمراض.

الكلمات المفتاحية: غذاء ملكات النحل، مؤشر البلعمة، معدل تصفية الكربون، مؤشر البلعمة المصحح ألفا، الجلوتاثيون.

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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

Ethanol **:** 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)

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

pH= 9.6 (modified by HCl).

9. sulphosalicylic acid solution preparation

Sulphosalic 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. Royal Jelly 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

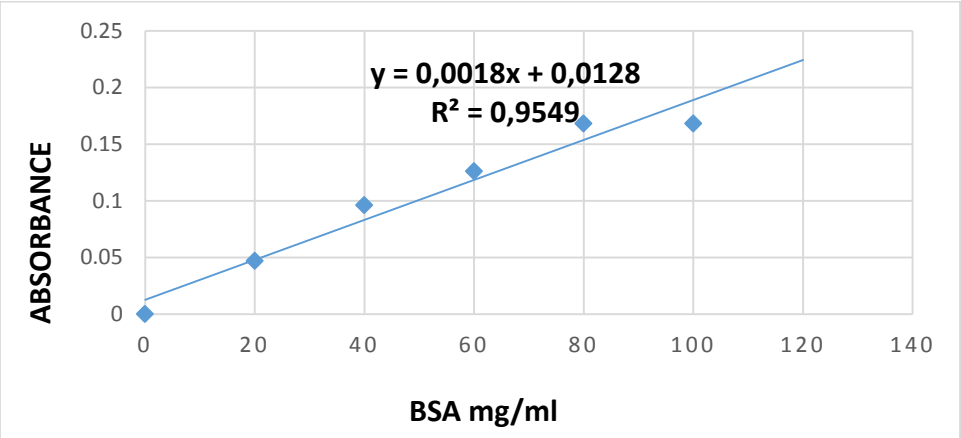

Figure 30. Calibration graph of bovine serum albumin.

Année universitaire : 2024-2025	**Présenté par :** Ouldji Soumeia Madaci Anfal Maazouzi Asma

The benefit of royal jelly on reticuloendothelial system and on reduced glutathione

Mémoire pour l'obtention du diplôme de Master II en Immunologie

Summary

It has long been established that the macrophages that make up the endothelial system play a major role in the immune response against various inflammatory diseases that affect the body.

In this experiment, the immunostimulatory activity of royal jelly extract on macrophage activity and antioxidant were evaluated.

It was shown that a dose of 250 mg/kg of royal jelly injected into mice led to an increase the phagocytic index (K), corrected phagocytic index α and a diminution of the carbon clearance rate t½) and GSH levels.

We concluded that, royal jelly is a natural extract capable of stimulating the body's immune activity against pathogens.

Key words: royal Jelly, phagocytic index, carbon clearance rate, corrected phagocytic, index α GSH.

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