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Dedication

This thesis is dedicated to all the people who helped me from near or far i will never forget your support and beleive in me .

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No matter how much i say thank you it's not enough but really

Thank you all ^^

The one who give you the smile

Safia

^_^ SB ^_^ Fighting

Dedication

Al hamd li ALLAH, for giving us a chance to complet this work.

This thesis is dedicated to all the people who helped me from near or far.

First of all a special thanks to my mom ***Rachida*** For her support in good and bad times she always there for me ,you are the best .

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To all my friends each by his name.

I love you all.

Yours

Samia

Table of contents

Table of contents

Table of contents

Material and methods

Results and discussion

Liste of abbreviation

DPPH : DiPhenyl PicrylHydrazyl.

G : Group.

GI : GastroIntestinal.

IP : IntraPeritoneal.

KC : Kupffer Cell.

Na_2Co_3 : Sodium Carbonate.

NaCl : Sodium Chloride.

NK : Natural killer.

PALS : PeriArteriolar Lymphoid Sheath.

RBC : Red Blood Cells.

RES : ReticuloEndothelial System.

Liste of figures

Liste of figures

Liste of tables

Liste of tables

Introduction

Introduction

The immune system is the most complex biological systems in the body. At the time of infection immune system go under the attack of a large number of viruses, bacteria and fungi. **(Singh, 2011).**

The immune system comprises two functional divisions; the innate immune system consists of cellular components, soluble factors, physical barriers and the reticulo-endothelial system (R.E.S), and the adaptive immune system which produces a specific reaction and immunologic memory to each pathogen and comprises cellular components and soluble factors. **(Goldsby et al., 2000).**

An important role in the cellular immunity is played by reticuloendothelial system which consists of the phagocytic cells such as monocytes and macrophages that kill the invading organism by phagocytosis which mainly comprise of phagocytic cells whose function is to ensure elimination of senescent cells, pathogenic microorganisms and immune complex from blood and tissues and participate in inflammation. In this way they contribute to non-specific immunity. These cells also participate in specific immunity by way of antigen presentation and cytokine secretions. **(Brannon and Blanchette, 2004**).

In order to perform phagocytic function, cells of reticuloendothelial system must be transformed to the active state. This specific ability is significantly suppressed by the action of physiological and pathological factors in nature. However, it is possible to influence this ability using certain immunomodulating agents. **(Aribi et al., 2013).**

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions. (**Rinki and Mishra, 2011).**

Medicinal plants are a rich source of substances which are claimed to induce paraimmunity, which is the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions. **(Sainis et al., 1997).**

The medicinal plants are used as immunomodulatory effect to provide alternative potential to conventional chemotherapy for a variety of diseases, especially in relation to host defense mechanism. The use of plant product like polysaccharides, lectines, peptides, flavonoids and tannins has been the immune response or immune system in various *in –vitro* modals. **(Singh et al., 2011).**

A large number of the plants and their isolated constituents have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomudulatory effects. **(Huffman, 2003)**(**Salem and Hossain, 2000).**

The medicinal plant which we have used in this research is *Curcuma Longa*.

Curcuma Longa, has long been recognized as a very promising antioxidant drug because of its many beneficial properties, including anti-inflammatory, anticancer and anti-carcinogenic activities. **(Bharat et al., 2006)(Khushwant et al., 2013).**

Curcuma Longa is particularly appealing as a therapeutic agent because of its low toxicity, although its intracellular bioavailability is quite low. **(Aggarwal and Sung, 2009).**

Pharmacological studies have confirmed that *Curcuma Longa* have several biological effects including antioxidant and immunomodulatory activity. **(Radha et al., 2005) (Antony et al., 1999).**

The present investigation was undertaken to evaluate the immunostimulatory effect of *Curcuma Longa* extract using phagocytic responses by carbon clearance test *in vitro* experimental model.

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

The aim of this reseach is to focus on this objective :

- Determination of immunostimulant potential of *Curcuma Longa* extract on the phagocytic activity which measure by the carbon clearance rate test.

Bibliographic part

I. Immune system

I.1. Definition

The immune system consists of a number of organs and several different cell types. (**Greenwood, 2005).** And it is a wonderful collaboration between cells and proteins that work together to provide defense against infection.The coordinated reaction of the immune system against infections and other foreign substances is known as the immune response. **(Richard et al., 2015).**

The immune system can recognize and remember millions of different enemies, and it can produce secretions and cells to match up with and wipe out nearly all of them. **(Widmaier et al., 2013).**

I.2. Types of Immune system

All components of the immune system interact with each other, it is typical to consider two broad categories of immune responses: the innate immune system and the adaptive immune system. **(Richard et al., 2015).**

I.2.1. Innate immunity

Innate immunity is mediated by Cells and molecules such as phagocytes and complement which can make rapid responses that may eradicate the infection**. (Figure 01) (Gordon and Austyn., 2012).**

The innate immune system plays a critical role in the generation of an efficient and effective acquired immune response. Cytokines produced by the innate immune system signal that infectious agents are present and influence the type of acquired immune response that develops. **(Greenwood, 2005).**

The four major components of innate immunity are:

- Epithelial barriers of the skin.
- Phagocytic leukocytes (neutrophils and macrophages).
- The natural killer (NK) cell.
- Plasma proteins of complement system.

(Silverthornet al., 2007).

The innate immune response is able to prevent and control many infections. However, many pathogenic microbes have evolved to overcome innate immune defenses, and to protect ourselves against these infections we have to call in the more powerful mechanisms of adaptive immunity**. (Widmaier et al., 2013).**

I.2.2. Adaptive immunity

Adaptive immune responses comprise the second category. These responses involve T-cells and B-cells, two cell types that require "training" or education to learn not to attack our own cells. **(Richard et al., 2015).**

The fundamental functional and developmental characteristics of lymphocytes are responsible for the specificity, division of labor, memory, diversity and tolerance of the adaptive response. **(Tak et al., 2014).**

The advantages of the adaptive responses are their long-lived memory and the ability to adapt to new germs. **(Figure 01). (Richard et al., 2015).**

I.3. Reticuloendothelial system

I.3.1. Definition

The reticulo-endothelial system consists of the phagocytic cells such as monocytes and macrophages whose function is to engulf microbes, immune complex from blood and tissues and participate in inflammation. This way they contribute to non-specific immunity. These cells also participate in specific immunity by way of antigen presentation and cytokine secretions. **(Brannon and Blanchette, 2004).**

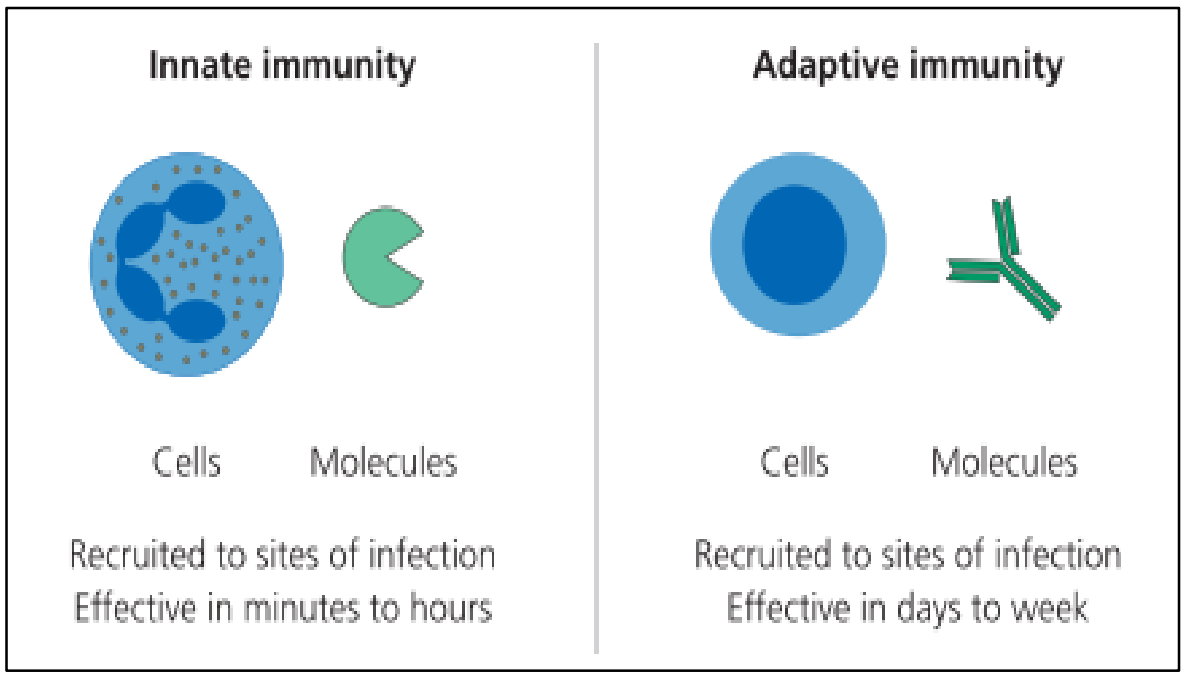

Figure 01 : Mechanisms of defence against infection.

(Gordon and Austyn, 2012).

I.4. Phagocytes

I.4.1. Definition

Phagocytes are specialized cells of the immune system whose primary function is to ingest and kill microorganisms. These cells, like the others in the immune system, develop from primitive stem cells in the bone marrow. When mature, they migrate to all tissues of the body but are especially prominent in the bloodstream, spleen, liver, lymph nodes, and lungs. **(Blaese et al., 2014).**

I.4.2.The discovery of phagocytes and phagocytosis

Elie Metchnikoff was a Russian embryologist whose seminal work on phagocytosis and 'physiological inflammation' was inspired by the observation of these processes in a simple metazoan. Metchnikoff observed the accumulation of cells he termed phagocytes around a rose thorn that he used to induce an inflammatory insult in the larva. This observation was the nidus from which his theories of cellular immunity were generated and for which he won a Nobel Prize in 1908. Importantly, his work emphasized the value of comparative embryology and established the validity of studying phagocytic engulfment in model organisms. **(Stuar and Ezekowitz, 2008).**

I.4.3. Types of phagocytes

There are several different types of phagocytic cells.

- **Mononuclear phagocytes**

The mononuclear phagocytic system consists of monocytes circulating in the blood and macrophages in the tissue. Monocytes are phagocytes that circulate in the blood, they remain in circulation for 8h.When monocytes migrate into tissues, they develop into macrophages. **(Figure 02)**. **(Singh et al., 2008).**

Macrophages have the capability to digest both exogenous (whole microorganism and insoluble particles) and endogenous antigens. **(Figure 03). (Singh and Sukh, 2008).**

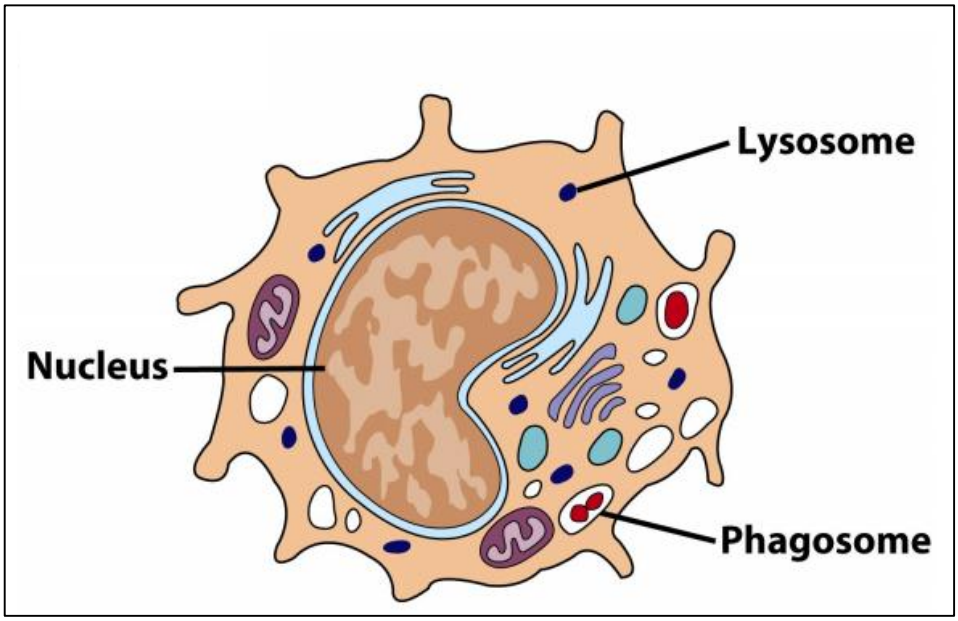

Figure 02 : Structure of a monocyte **(Goldsby et al., 2007).**

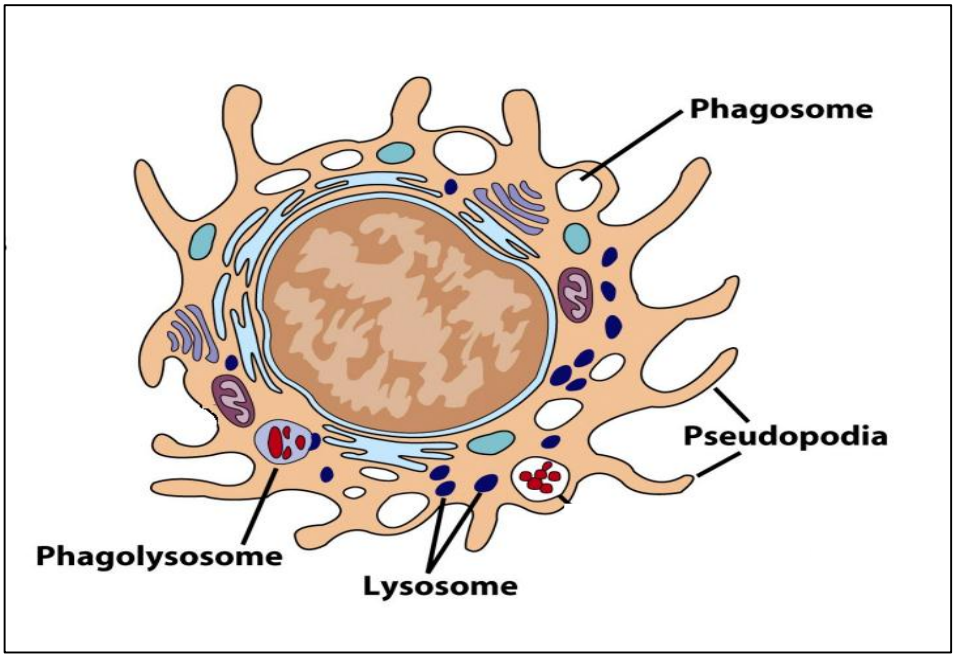

Figure 03 : Structure of a macrophage. **(Goldsby et al., 2007).**

➢ Granulocytes

Granulocyte cells are those that contain membrane bound granules in their cytoplasm. These granules contain enzymes capable of killing microorganisms and destroying debris ingested by the process of phagocytosis. There are three types of granulocytic cells neutrophils, eosinophils and basophils. These cells are characterized by the presence of irregular segmented nuclei, specific granules and fully differentiated cells. **(Abraham et al., 2006).**

- **Neutrophils (polymorphonuclear leukocytes or polymorphs).** They are the most common of the leukocytes and represents about 55- 70% of all the circulating leukocytes. They remain in circulation in the peripheral blood for about 7- 10 h, after their production in the bone marrow. Then they migrate to their tissues where they have the life span of few days. They are involved as a first line of defence against invading microorganisms and are important in inflammation and at sites of injury or wounds. **(Figure 04). (Nairn and Helbert, 2002).**
- **Eosinophils** They have a bilobed nucleus and a granulated cytoplasm and represents 1- 5 % of all the circulating leukocytes. They become more plentiful in circulation and in relevant tissues in allergic and parasitic diseases and their functions can be divided into effects on parasites and the inflammatory process**. (Figure 05). (Silverthorn et al., 2007).**
- **Basophils** These represent only about 1% of the leukocytes. They have very large, irregular basophilic granules. Basophils are circulating cells and possess surface FC receptors with a high affinity for IgE**. (Figure 06). (Singh and Sukh, 2008).**

Phagocytic cells serve a number of critical functions in the body's defense against infection. They have the ability to leave the bloodstream and move into the tissues to the site of infection. Once at the site of infection, they ingest the invading microorganisms. Ingestion of microorganisms by phagocytic cells is made easier when the microorganisms are coated with either antibody or complement or both. Once the phagocytic cell has engulfed or ingested the microorganism, it initiates a series of chemical reactions within the cell which result in the death of the microorganism. **(Blaese et al., 2014).**

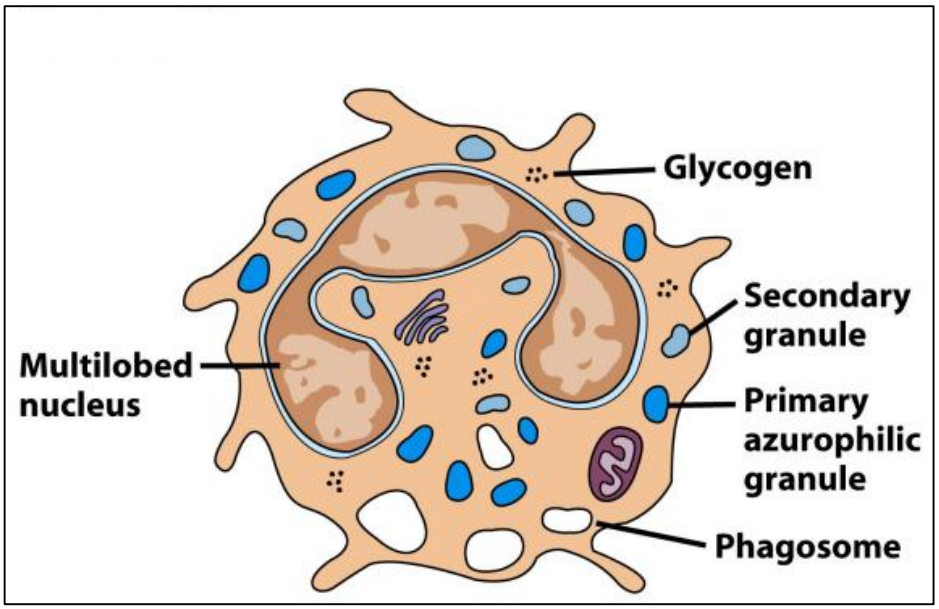

Figure 04 : Structure of a neutrophil. **(Goldsby et al., 2007).**

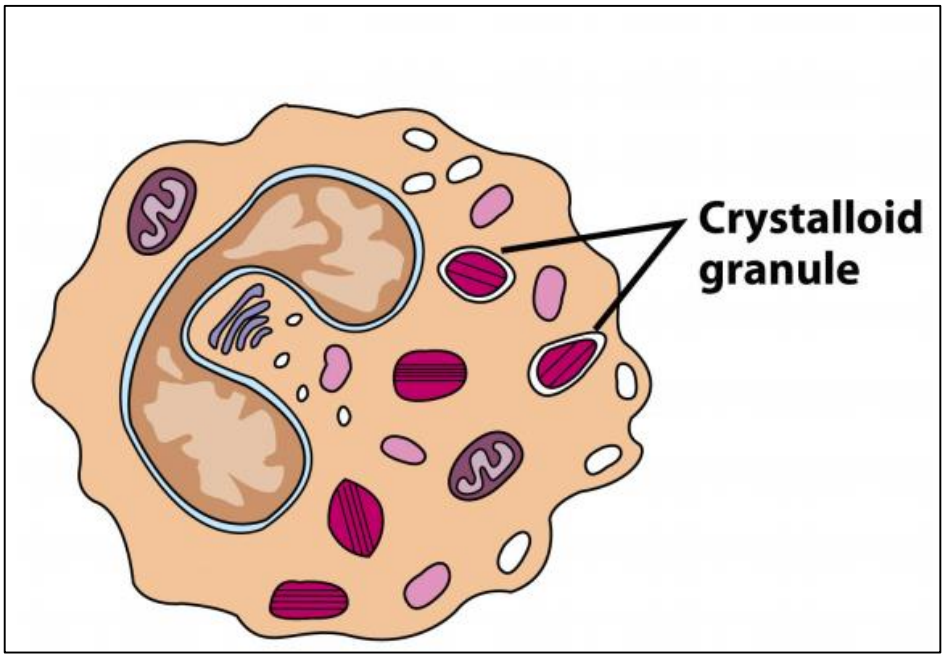

Figure 05 : Structure of a eosinophil. **(Goldsby et al., 2007).**

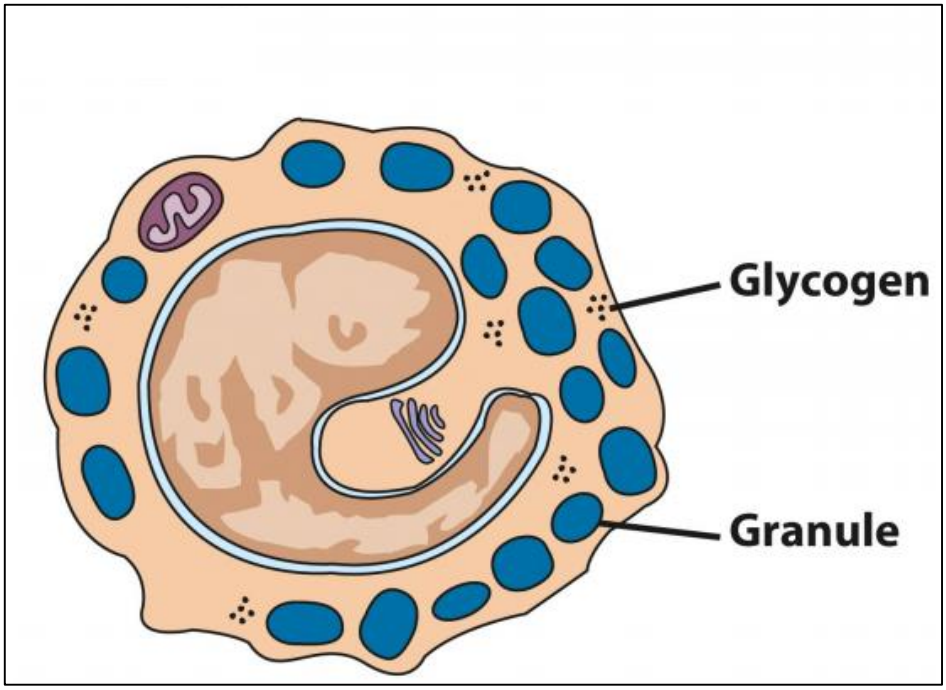

Figure 06 : Structure of a basophil. **(Goldsby et al., 2007).**

I.4.4. Phagocytosis

Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called chemotaxis. The next step in phagocytosis is adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called pseudopodia, to extend around the attached material .Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a phagosome, which then enters the endocytic processing pathway. In this pathway, a phagosome moves toward the cell interior, where it fuses with a lysosome to form a phagolysosome. Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called exocytosis **(Figure 07). (Goldsby et al., 2007).**

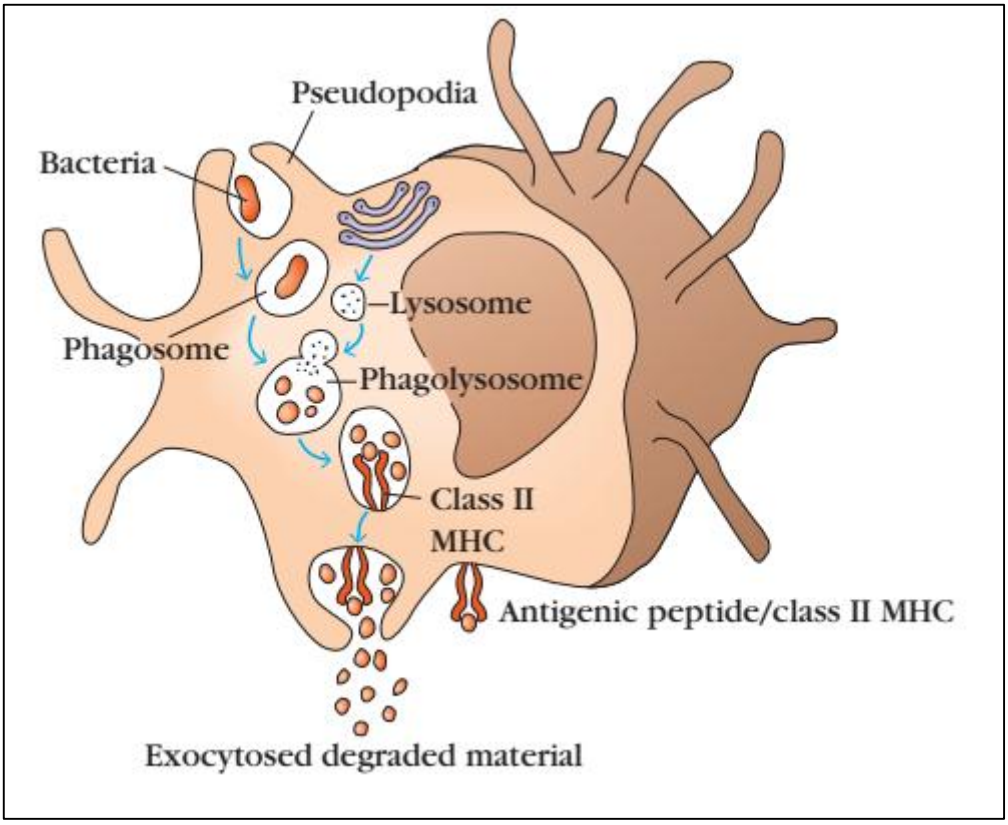

Figure 07 : Phagocytosis and processing of exogenous antigen by macrophages.

(Goldsby et al., 2007).

II. Immun system Organs

The organs of the immune system are positioned throughout the body. **(Widmaier et al., 2013).**

II.1. Liver

II.1.1. Defintion

The liver is the largest and heaviest glandular organ in the body. In health the liver has a deep burgundy colour (reflecting its rich vascularity), a smooth surface and a soft to firm and floppy consistency. **(Marieb, 2008).**

II.1.2. Structure

The liver is largest organ in the human body. It is incompleteley separated into lobes that are covered on their external surfaces by a thin connective tissue capsule. The liver is composed of several cell types that not only interact with each other but also are adapted to perform specific functions. The principal cell type is the hepatic parenchymal cell, generally referred to as the hepatocyte, which accounts for 60% of the total cell population and 80% of the volume of the organ. Hepatocytes are organized into plates or laminae that are interconnected to form a continuous three-dimensional lattice **(Figure 08).** Between the plates of hepatocytes are spaces occupied by hepatic sinusoids the large bore fenestrated capillaries of the liver that nourish each parenchymal cell on several sides.**(Prygiel, 2012).**

The sinusoidal space, and the non-parenchymal cells associated with sinusoids, comprises the majority of the remaining liver volume. The non-parenchymal cells include sinusoidal endothelial cells, perisinusoidal stellate cells, and intraluminal kupffer cells. An interconnecting network of minute intercellular channels forms bile canaliculi,which course between adjacent hepatocytes and receive bile secreted from hepatocytes.From the canaliculi the bile then drains through short bile ductules (cholangioles,which are partially lined by cuboidal epithelial cells) to bile ducts. **(Abraham et al., 2006).**

Hepatocytes execute most of the functions generally associated with the liver. They extract and process nutrients and other materials from the blood, and they produce both exocrine qnd endocrine secretions. **(Thomas et al, 2012).**

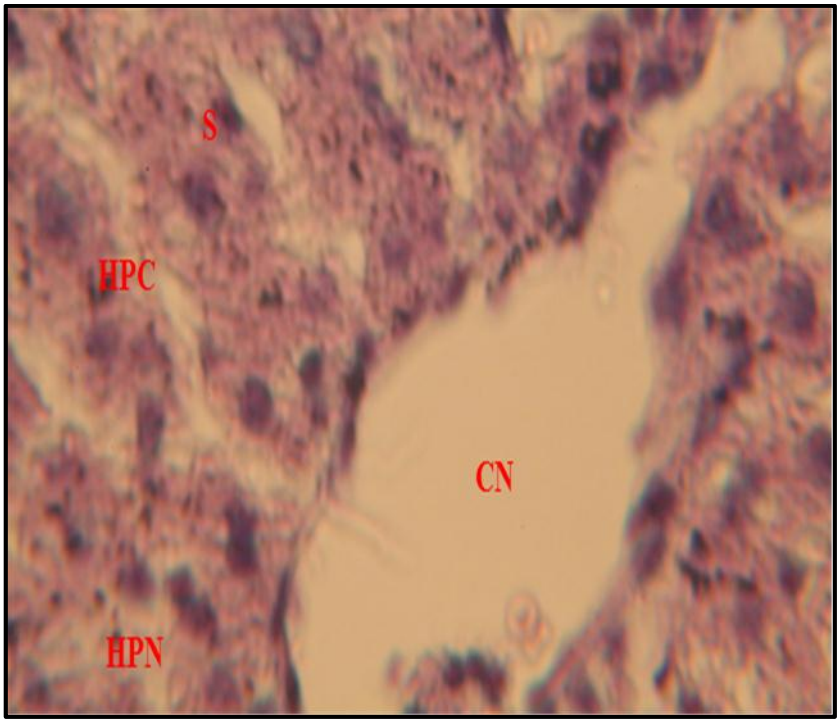

Figure 08: Section of liver (x 400).

HPC. Hepatocyte Cell S. Sinusoid

HPN. Hepatocyte Nuclei CV. Central Vein

(Zerizer and Naimi, 2004).

II.1.3. Vascularization

The liver is unusual in that it has a double blood supply; the right and left hepatic arteries carry oxygenated blood to the liver, and the portal vein carries venous blood from the GI tract to the liver. (**Figure 09) (Thomas et al, 2012).**

The venous blood from the gastrointestinal (GI) tract drains into the superior and inferior mesenteric veins; these two vessels are then joined by the splenic vein just posterior to the neck of the pancreas to form the portal vein. This then splits to form the right and left branches, each supplying about half of the liver. **(Thomas et al, 2012).**

On entering the liver, the blood drains into the hepatic sinusoids, where it is screened by specialised macrophages (KC) to remove any pathogens that manage to get past the gastrointestinal (GI) defences. The plasma is filtered through the endothelial lining of the sinusoids and bathes the hepatocytes; these cells contain vast numbers of enzymes capable of braking down and metabolising most of what has been absorbed. **(Prygiel, 2012).**

The portal venous blood contains all of the products of digestion absorbed from the gastrointestinal (GI) tract, so all useful and non-useful products are processed in the liver before being either released back into the hepatic veins which join the inferior vena cava just inferior to the diaphragm, or stored in the liver for later use. **(Prygiel, 2012).**

II.1.4. Liver function

The liver plays a role in digestion, sugar and fat metabolism, and the body's immune defense. It processes almost everything a person eats, breathes, or absorbs through the skin. About 90% of the body's nutrients pass through the liver from the intestines. The liver converts food into energy, stores nutrients, and produces blood proteins. The liver also acts as a filter to remove harmful substances from the blood. In the developing fetus, blood cells are produced in the liver**. (Hasan et al., 2016).**

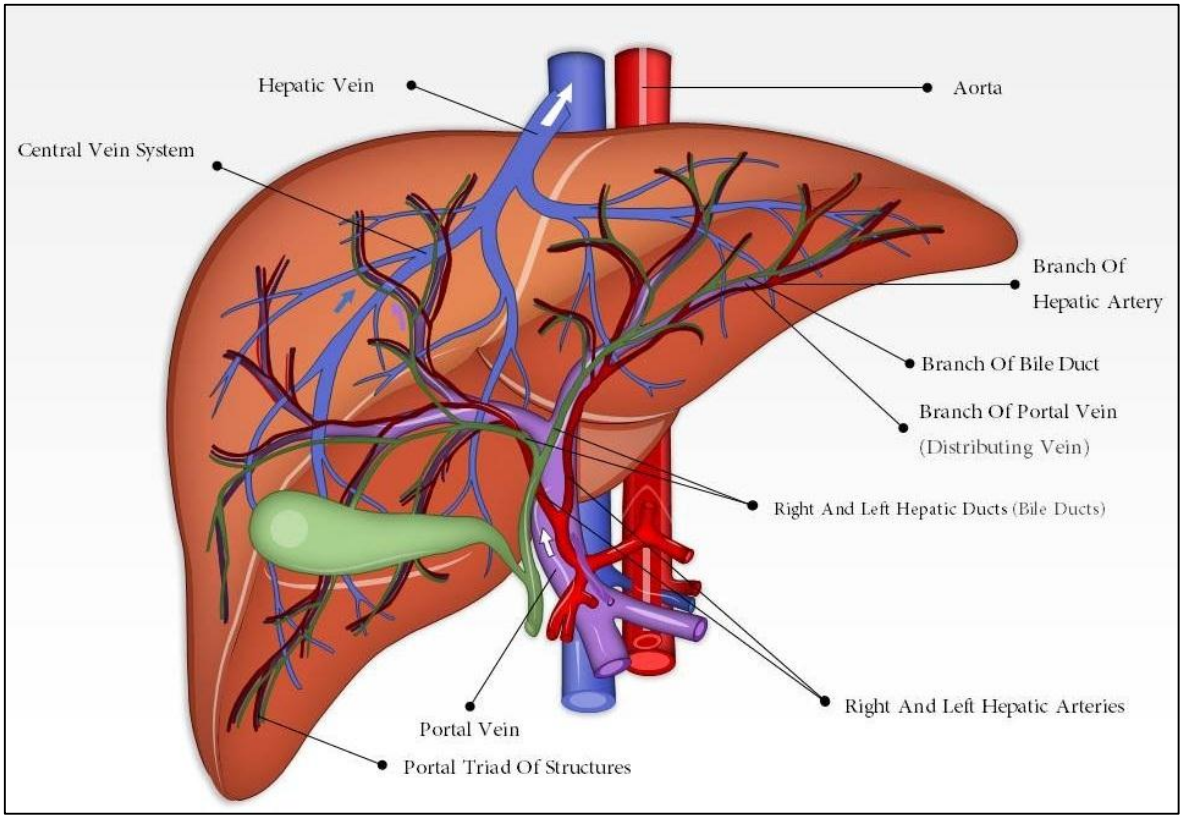

Figure 09 : Liver Vascularization

(www.pixhder.com)

II.2. Spleen

II.2.1. Definition

Spleen is the largest lymphoid organ in the human body and it has a fundamental role as destruction of red blood cells and as actor in the immune response. **(Tarantino et al., 2013).**

The spleen is a collection of T-lymphocytes, B-lymphocytes and monocytes. It serves to filter the blood and provides a site for organisms and cells of the immune system to interact. **(Richard et al., 2015).**

II.2.2. Structure of spleen

The spleen, situated in the left side of the abdominal cavity, is a large, ovoid secondary lymphoid organ that plays a major role in mounting immune responses to antigens in the bloodstream. The spleen specializes in filtering blood and trapping blood-borne antigens; thus, it is particularly important in the response to systemic infections. **(Stevens and Lowe, 2006).**

The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure. The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone **(Figure 10 A and B). (Goldsby et al., 2007).**

The splenic red pulp consists of a network of sinusoids populated by red blood cells, macrophages, and some lymphocytes. It is the site where old and defective red blood cells are destroyed and removed; many of the macrophages within the red pulp contain engulfed red blood cells or iron-containing pigments from degraded hemoglobin. It is also the site where pathogens first gain access to the lymphoid-rich regions of the spleen, known as the white pulp. **(Owen et al., 2013).**

The splenic white pulp surrounds the branches of the splenic artery, and consists of the periarteriolar lymphoid sheath (PALS) populated by T lymphocytes as well as B-cell follicles. **(Owen et al., 2013).**

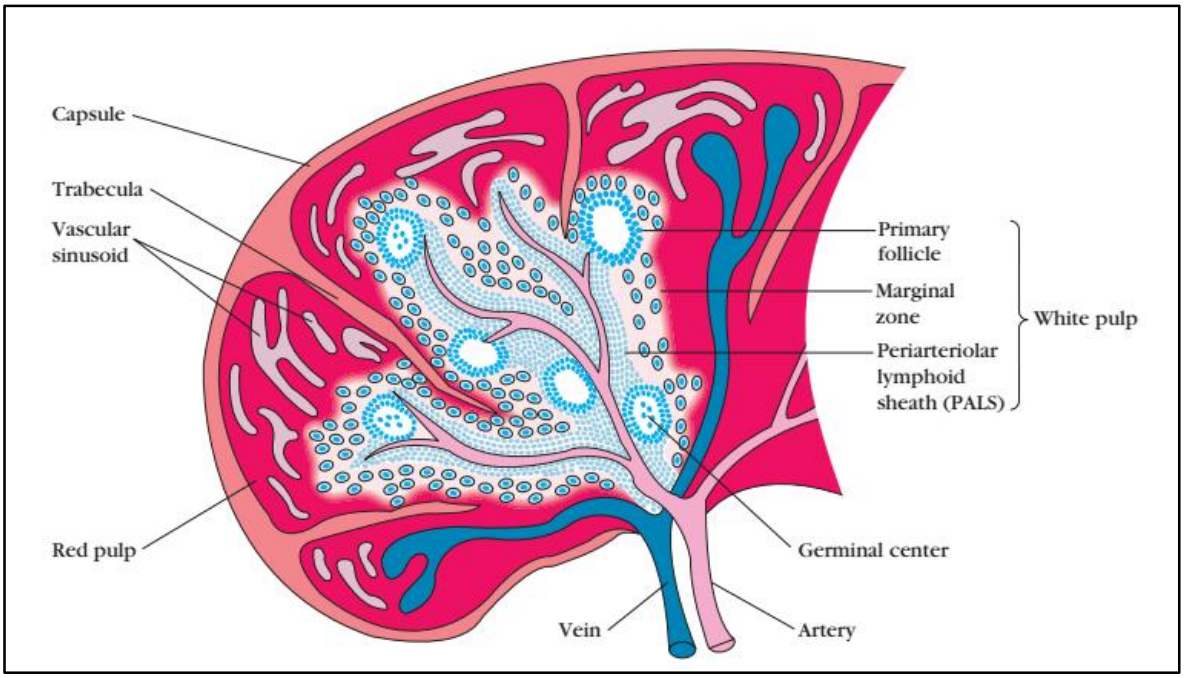

Figure 10 A: Structure of the spleen. **(Goldsby et al., 2007).**

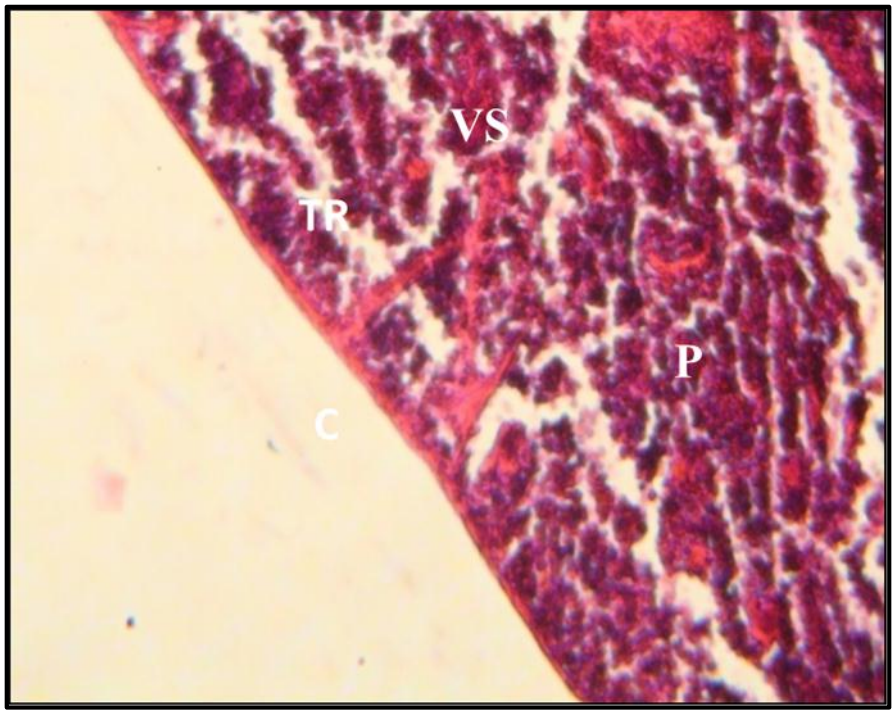

Figure 10 B : Histological section of the spleen (x100)

Coloration hematoxylin eosin (G×100) (G×1000)

VS: Venus Sinus, P: Parenchyma (cords of Billroth), TR: Trabeculae

(Benmebarek, 2014).

II.2.3. Vascularization

The splenic artery supplies blood to the spleen. This artery is the largest branch of the celiac trunk and reaches the spleen's hilum by passing through the splenorenal ligament. It divides into multiple branches at the hilum. It divides into straight vessels called penicillin, ellipsoids, and arterial capillaries in the spleen. The splenic circulation is adapted for the separation and storage of the red blood cells. The spleen has superior and inferior vascular segments based on the blood supply. The two segments are separated by an avascular plane. Its terminal branches aside, the splenic artery also gives off branches to the pancreas, short gastric branches, and the left gastroomental (gastroepiploic) artery. **(Figure 11). (Ashwin, 2014). (Stevens and Lowe, 2006).**

II.2.4. Spleen function

➢ Phagocytosis

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 red blood cells (RBCs), other blood cells, and microorganisms, thereby filtering the blood. Phagocytosis of circulating antigens initiates the humoral and cellular immune responses. **(Nairn and Helbert, 2002).**

➢ Hematopoiesis

The spleen is an important hematopoietic organ during fetal life; lymphopoiesis continues throughout life. The manufactured lymphocytes take part in immune responses of the body. In the adult spleen, hematopoiesis can restart in certain diseases such as chronic myeloid leukemia and myelosclerosis. **(Ashwin, 2014).**

➢ Storage of red blood cells (RBC)

The red blood cells (RBC) are stored in the spleen. Approximately 8% of the circulating red blood cells (RBC) are present within the spleen. **(Ashwin, 2014).**

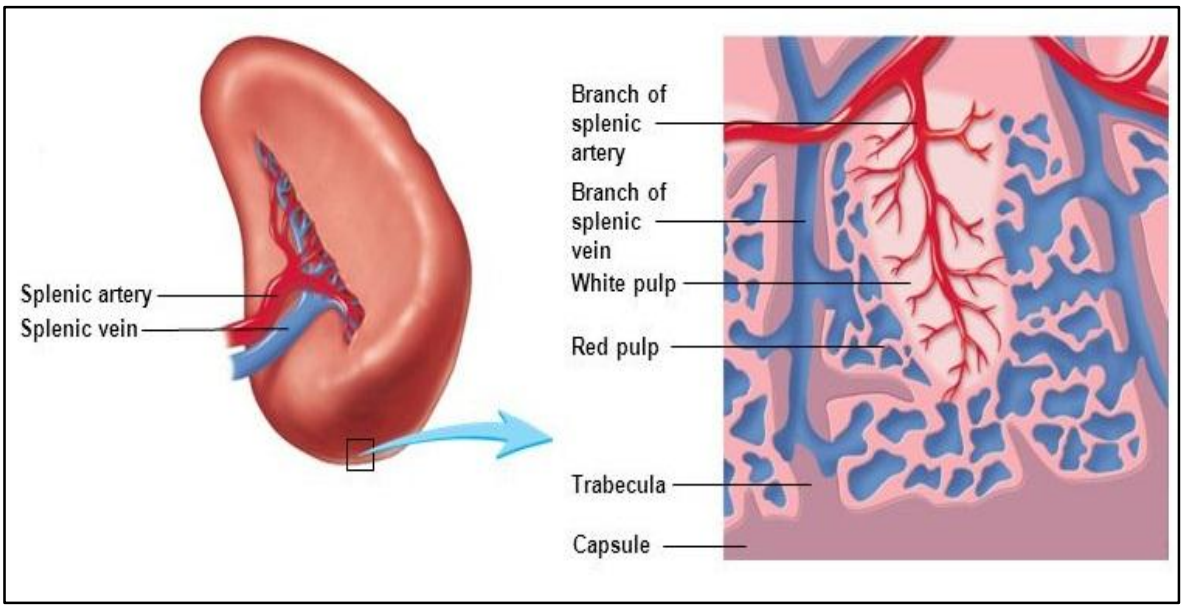

Figure 11: Hilum of the spleen along with anatomy of the splenic artery (a) and the splenic vein (v). **(Www.Slideplayer.com).**

III. Medicinal plants

Natural products are believed to be important source of new chemical substances which have potential therapeutic effects. Medicinal plants, one of the important sources, are extensively investigated both *in vitro* and *in vivo* to examine their potential activities **(Jalaiah, 2014)**. It has been estimated that less than 1- 10% of the large diversity of 250000- 500000 plant species on the earth have been studied chemically and pharmacologically for their medicinal properties. **(Farnsworth and Soejarto, 1991) (Verpoorte, 2000).**

Numerous medicinal plants extracts and products continue to be useful in the prevention and control of a wide range of antioxidant disorders. For example, the extract of *Curcuma Longa* has been shown to possess immunostimulatory effect. **(Sengupta et al., 2011). (Chandrasekaran et al., 2013).**

- ***Curcuma Longa***

III.1. Description

Curcuma Longa in arabic كركم , is a rhizomatous herbaceous plant of the ginger family , Zingiberaceae and contains 49 genera and 1400 species. **(Ravindran et al., 2007)**. It is native to the tropical regions of south and southeastern asia and requires temperatures between 20 °C and 30 °C to grow and a considerable amount of rainfall to thrive. **(Kumar And Sakhya, 2012).**

The plant has a composition of various Nutritional components **(Table 01).**

Table 01 : Nutritional composition of *Curcuma Longa* **(Sasikumar, 2012).**

Constituent	Quantity per 100 g
Water (g)	6.0
Food energy (Kcal)	390
Protein (g)	8.5
Fat (g)	8.9
Carbohydrate (g)	69.9
Calcium (g)	0.2
Phosphorous (mg)	260
Sodium (mg)	30
Potassium (mg)	2000
Iron (g)	47.5
Thiamine (mg)	0.09
Riboflavin (mg)	0.19
Niacin (mg)	4.8
Ascorbic acid (mg)	50

III.2. Botanical classification

Curcuma Longa
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Scientific classification
Kingdom : Plantae **Division :** Magnoliophyta **Class:** Liliopsida **Subclass :** Zingiberidae **Order :** Zingiberales **Family :** Zingiberaceae **Genus :** *Curcuma* **Species :** *Curcuma Longa* **Scientific name :** *Curcuma Longa*
Various names
Arabic : كركم (kurkum) **English :** *Curcuma Longa* **French :** *Curcuma*

III.3. Therapeutic properties

Curcuma Longa has at least 6000 years of documented history of its use as medicine **(Handra et al., 2013)** And it is one of the most widely used spices across the globe and is a great source of many health benefits. (**Figure 12). (Kumar And Sakhya, 2012)**;

Curcuma longa, one of the major spices containing natural antioxidants **(Subramanian et al., 1994)(Ruby et al., 1995)**, and is reported to possess various medicinal properties, immunomodulatory **(Antony et al., 1999**), anti-tumor **(Rao et al., 1995)**, and anti-inflammatory activities **(Ammon et al., 1993).**

In other studies of *Curcuma Longa* proved that it has many pharmacological properties including antiarthritic **(Polassa et al., 1992),** antimutagenic **(Polassa et al., 1992),** antibacterial, **(Negi et al., 1999),** antifungal **(Afaq et al., 2002),** antiviral **(Mazumder et al., 1995)**, alzheimers disease **(Travis, 2001).**

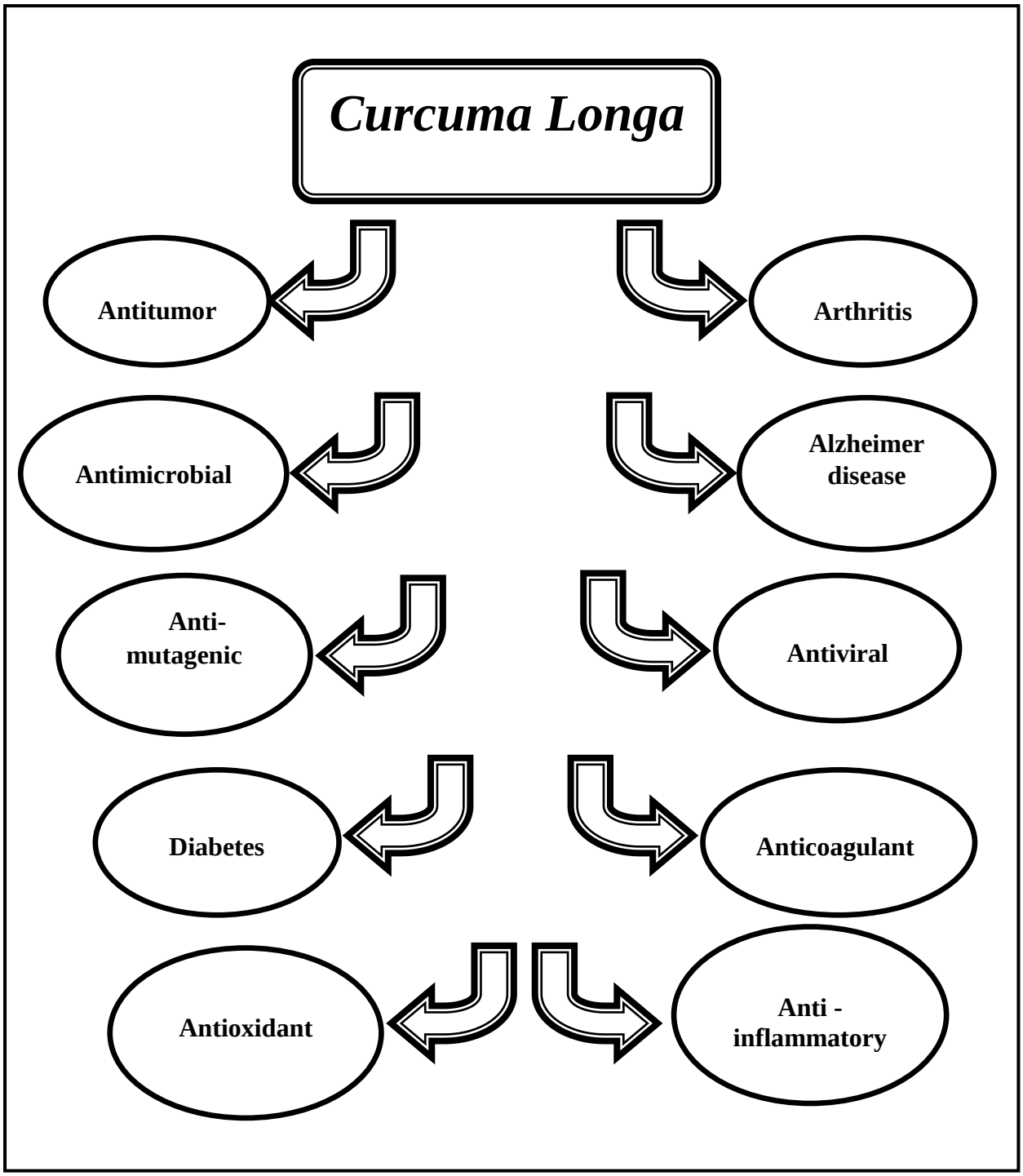

Figure 12 : Medicinal properties of *Curcuma Longa.*

(Radha et al., 2005).

Materials and Methods

Materials and Methods

1. Materials

1.1. Animals

Adult male Albino mice (2- 2.5 month old) from central pharmacy Institute, Constantine, Algeria, weighing between 28 and 35g were used for determination of the phagocytic activity of *Curcuma Longa*.

The animals were housed in polypropylene cages with soft wood and free access to water and diet every day. they were maintained in the animal house under standard laboratory conditions of temperature and humidity.

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 carbon ink.

1.5. Equipements

- Balance (0,01), Agitator, Dissection kit, Spectrophotometer, Tubes.

2. Methods

2.1. Treatment of mice

This study was performed on 28 males mice in four expermental groups, animals were divided into four groups, consisting of five mice in GI, GII, GIII and GIV.

Group I (control) was given 0,9% Nacl (0,5 ml/mouse i.p.), Groups II-III and IV were administered by i.p injection with different concentrations of *Curcuma Longa* extract (50, 100 and 150 mg/kg) respectively **(Table 02).**

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.

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, then 14 drops of blood samples were added to 4ml of 0,1% sodium carbonate solution to lyse the erythrocytes.

2.3. Dissection

The animals were sacrificed and the liver and spleen dissected and weighed immediately in the wet state.

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

$$K = \frac{\mathbf{Ln\ OD1 - Ln\ OD2}}{\mathbf{t2 - t1}}$$

$$t\ ½ = \frac{\mathbf{0.693}}{\boldsymbol{K}}$$

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

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

- **Statistical analysis**

Results were analyzed for differences between the groups across dietary treatments by one–way ANOVA test and Tukey's multiple comparison tests (SPSS version 20).

Table 02 : Shows the treatment of mice

Experimental groups	Number of mice	Treatment	Dose	Duration of the experiment
Group I (control)	5	0,9%Nacl	0.5 ml/5mice	48h
Group II	5	Plant	50 mg/kg	48h
Group III	5	Plant	100 mg/kg	48h
Group IV	5	Plant	150 mg/kg	48h

Results and Discussion

1. Results

1.1. Immunomodulatory activity of *Curcuma Longa*

The immunomodulatory activity of the extract of *Curcuma Longa* was evaluated by carbon clearance rate test in mice.

➢ Phagocytic activity :

As shown in the figure 13, The phagocytic index rate of the groups GI (0.050 ± 0.025), GII (0.042 ± 0.021), GIII (0.057 ± 0.056) and GIV (0.079 ± 0.051). Statistical analysis of the rate of phagocytic activity is increased in groups III and IV but not significant ($P > 0.05$). This indicates that *Curcuma Longa* extract enhanced the phagocytic activity by stimulating the reticuloendothelial system in a dose dependant manner. **(Table 03).**

➢ Carbone clearance rate (half time) :

As shown in Figure 14, The carbone clearance rate of the groups GI (17.24 ± 7.31), GII (24.15 ± 15.73), GIII (29.26 ± 17.54) and GIV (12.53 ± 7.12). The half-time of colloidal carbon was highly significantly ($P < 0.05$) faster at 48h, after the administration of *Curcuma Longa* extract between groups and the clearance rate was decreased GIV when it is compared to the control group (GI). This indicates that the extract reduces the elimination time of carbon particles from blood and affirms that *Curcuma Longa* extract enhanced the phagocytic activity. **(Table 03).**

➢ Corrected phagocytic index (α)

The figure 15 confirm the phagocytic activity of *Curcuma Longa* including the carbon clearance from the animals blood versus the weight of their active organs (spleen and liver), Statistical analysis of the rate of corrected phagocytic activity is increased in groups III and IV but not significant ($P > 0.05$). **(Table 04).**

Table 03: Effect of *Curcuma Longa* extract 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	$t_{1/2}$ min	$t_{1/2,}$ min average
GI (Control)	5	0.5 ml	0.085 0.031 0.035 0.075 0.026	0.050 ± 0.025	8.15 22.35 19.80 9.24 26.65	17.24 ± 7.31
GII	3 2	50mg/kg	0.044 0.015 0.066 - -	0.042 ± 0.021	15.75 46.20 10.50 - -	24.15 ± 15.73
GIII	3 2	100mg/kg	0.019 0.136 0.015 - -	0.057 ± 0.056	36.474 5.096 46.200 - -	29.26 ± 17.54
GIV	4 1	150mg/kg	0.064 0.029 0.062 0.164 -	0.079 ± 0.051	10.828 23.897 11.177 4.226 -	12.53 ± 7.12

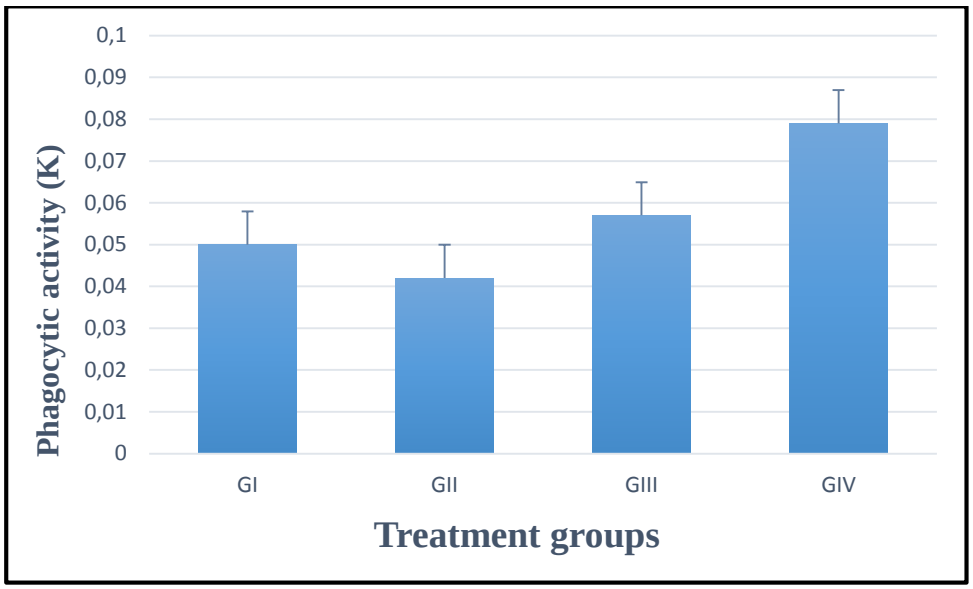

Figure 13 : Effect of *Curcuma Longa* on phagocytic activity

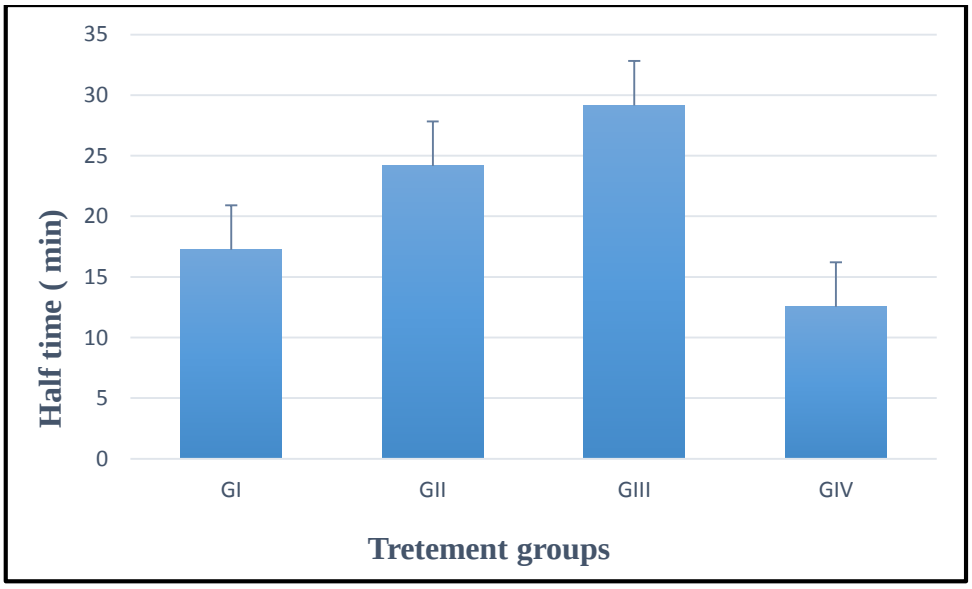

Figure 14 : Effect of *Curcuma Longa* extract on half time of carbon in blood.

Table 04: Effect of *Curcuma Longa* extract on phagocytic activity in mice shown on active organ (liver and spleen) and mice wieght.

Groups	Number of mice	Dose	Weight	Liver weight	Spleen weight	α	α average
GI	5	0.5 ml	40.02 34.54 28.66 33.69 31.02	2.08 1.93 1.88 1.44 1.36	0.12 0.43 0.33 0.32 0.18	7.998 4.598 4.242 8.073 5.967	6.176 ± 1.626
GII	3 2	50 mg/kg	36.56 36.66 35.84 - -	1.65 2.19 2.16 - -	0.15 0.14 0.15 - -	7.171 3.880 6.270 - -	5.774 ± 1.388
GIII	3 2	100 mg /kg	34.90 34.80 36.00 - -	1.77 1.89 1.42 - -	0.10 0.13 0.17 - -	4.980 8.859 5.584 - -	6.474 ± 1.704
GIV	4 1	150 mg/kg	31.94 37.60 35.75 35.64 -	2.40 1.55 1.85 1.88 -	0.29 0.14 0.14 0.28 -	4.749 6.835 7.110 9.108 -	6.951 ± 1.544

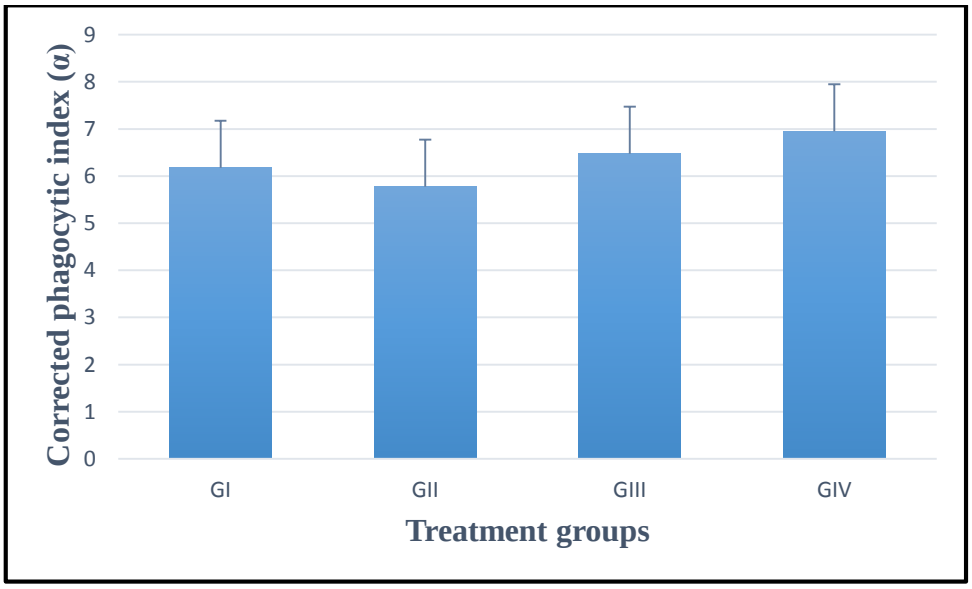

Figure 15 Effect of *Curcuma Longa* extract on corrected phagocytic activity (α).

II. Discussion

The immune system is a well-organized and well regulated system and any disturbance may lead to the development of immune diseases. Many herbs and other substances are used by cultures around the world to nourish and support immunity and protect us from a multitude of disease causing microorganism. In the immune system, phagocytosis is a major mechanism by which certain living cells, collectively called as phagocytes, ingest or engulf microorganisms, malignant cells, inorganic particles and tissue debris. **(Punit et al., 2014).**

The phagocytic activity was investigated by the phagocytic function of the reticuloendothelial system which is known to be important in the removal and destruction of pathogenic organisms from the tissues and blood. **(Kehili et al., 2014)**. The active ingredients in the *Curcuma Longa* are the tetrahydrocurcuminoids, curcumin, demethoxy curcumin and bisdemethoycutcumin. **(Chainani, 2003).**

In this study we observed that the animals administered with the crude extract of *Curcuma Longa* stimulates the phagocytic index at different concentrations. That could be explained according to the observations of **(Antony et al., 1999)** who indicated that the immunostimulatory activity due to the curcumin which is the active ingredient extracted from *Curcuma Longa.* Also **(Antony et al., 1999)** proved that *curcuma Longa* extract which administrated to Balblc mice was found to increase the total red blood cells count due to its immunostimulatory activites of curcumin.

Curcumin has been shown to be a potent immunomodulatory agent that can modulate the activation of T cells, B cells, macrophages, neutrophiles, natural killes cells and dentritic cells **(Allam, 2009).**

A study conducted at the Assam University by (**Sengupta et al., 2011).** has proven that *Curcuma Longa* have a immunostimulatory effect .

Our results agree with those of **(Boulahrouf et al., 2013)**, **(Benmebarek et al., 2013)** and **(Aribi et al., 2013).** who reported that the administration of *Nocardiopsis dassonvillei, S.mialhesi* and *Argania Spinosa* respectively in mice are increased the phagocytic index at different concentrations.

In other study of **(Benmebarek et al., 2014)**. reported that *Sachys ocymastrum* extract enhanced the phagocytic activity at low concentration (50 mg/kg) by stimulating the

reticulum endothelial and then at high concentrations of 100 and 500 mg/kg the extract decreased the phagocytic activity, in a dose dependant manner by inhibiting the reticulum endothelial.

The reticuloendothelial system (R.E.S) is considered as the first line of defense and functionally recognized by its ability to scavenge debris or other foreign matter. The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen which are considered to be the main organs in where endothelial cells and macrophages eliminate undesirable substances from circulation is measured by reticuloendothelial phagocytic activity. (**Nassar et al., 2015).**

In the present study pre-treatment of animals with *Curcuma Longa* powder in various doses showed an augmentation in the phagocytic function by exhibiting a dose-related increase in the clearance rate of carbon particles by cells of the reticuloendothelial system (R.E.S). So, the powder can influence the mechanism of phagocytosis, which result in significant increase in the phagocytic index with carbon clearance test.

Curcuma Longa powder at concentrations of 150 mg/kg increased the phgocytic index, corrected α and decreased the rate of carbon clearance this immunomodulatory effect of *Curcuma Longa* could be attributed to its interesting chemical composition.

Conclusion

Conclusion

The present experimental findings demonstrate that *Curcuma Longa* possesses an immunostimulatory activites at concentration of 150 mg/kg.

Additional studies are required to explore the beneficial therapeutic implication of *Curcuma Longa* for the treatment of immune related diseases.

Based on the present results, our future work and perspectives can evaluate many topics:

- Identification and purification of bioactive molecules from *Curcuma Longa.*
- We will treat the mice and rats with the bioactive molecules during different periods.
- Determination of antioxydants enzymes.
- Antioxidant activity of *Curcuma longa* using DPPH assay.

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Summary

Summary

The role of phagocytic cells on the reticuloendothelial system (R.E.S) in the immune response against infection disease is known for along time and confirmed recently by a quantitative methods of activity exploration of reticuloendothelial system (R.E.S) by using the extracts of medicinal plants *in vivo and in vitro.*

In the present study, we evaluated the *in vivo* the effect of immunomodulatory potential of *Curcuma Longa* on the phagocytic activity which was evaluated using the Carbon Clearance Assay.

The results showed that the dose of 150mg /kg of *Curcuma Longa* is increased the phagocytic index (K), corrected phagocytic index α and a diminution of the carbone clearance rate ($t_{½}$).

For this reason,we considered this extract of the medicinal plant *Curcuma Longa* as a natural source for immunostimmulatory activity gainst pathogens.

Key words: *Curcuma Longa,* phagocytic index, carbone clearance rate, corrected phagocytic index α, immunostimmulatory activity, Liver, Spleen.

ملخص

إن دور الخلايا البالعة المكونة للجهاز الشبكي البطاني في الاستجابة المناعية ضد الانتانات المرضية معروف منذ فترة زمنية طويلة و قد أثبتت حاليا بواسطة طرق فحص كمية لنشاط الجهاز الشبكي البطاني بواسطة استخدام مستخلصات النباتات الطبية عند الجردان او الفئران او خارج العضوية.

في هذه الدراسة تم تقدير النشاط المناعي لمستخلص النبتة الطبية كركم (*Curcuma Longa*) على نشاط الخلايا البلعمية والتي قدرت بطريقة معدل ازالة الكربون.

النتائج المتحصلة عليها بينت بان الجرعة 150مغ/كغ المحقونة عند الفئران أدت إلى زيادة في ارتفاع نشاط الخلايا البلعمية والنشاط البلعمي المصحح الفا مع انخفاض في دورة حياة الكربون.

لهذا السبب، اعتبر مستخلص النبتة الطبية كركم (Curcuma *Longa*) كمصدر طبيعي يستخدم في تنبيه النشاط المناعي ضد الأجسام الممرضة.

الكلمات المفتاحية: كركم (Curcuma *Longa*)، نشاط الخلايا البلعمية، نصف دورة حياة الكربون، النشاط البلعمي المصحح الفا، الكبد، الطحال.

Résumé

Le rôle des cellules phagocytaire du système réticulo-endothélial (S.R.E) dans la réponse immunitaire contre les maladies infectieuses connu depuis longtemps a été récemment confirmé par l'emploi des méthodes quantitatives d'exploration de l'activité du Système réticulo-endothélial (S.R.E) par l'utilisation des extraits des plantes médicinales *in vivo* et *in vitro.*

Dans la présente étude, nous avons évalué *in vivo* l'effet de l'activité immunomodulatrice de *Curcuma Longa* qui a été réalisée a l'aide du test de l'épuration sanguine d'une dose de carbone colloïdal.

Les résultats obtenus ont montré que les la dose de 150mg/kg de *Curcuma Longa* a augmenté l'activité phagocytaire du système réticulo-endothélial (S.R.E), une activation de l'index phagocytaire corrigé α et diminution du taux de la clairance de carbone ($t_{1/2}$).

Pour cette raison, nous avons considéré l'extrait de la plante médicale *Curcuma Longa* comme une source naturelle dans l'activité immunostimulante contre les pathogènes.

Mots clés : *Curcuma Longa*, index phagocytaire, taux de la clairance de carbone, index phagocytaire corrigé α, l'activité immunostimulante, foie, rate.

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. Preparation of plant

Preparation of different doses :

1. Curcuma Longa : 50 mg

NaCl : 10 ml

Dissolve 50 mg of *Curcuma Longa* in 10 ml NaCl.

2. Curcuma Longa : 100 mg

NaCl : 10 ml

Dissolve 100 mg of *Curcuma Longa* in 10 ml NaCl.

3. *Curcuma Longa* : 150 mg

NaCl : 10 ml

Dissolve 150 mg of *Curcuma Longa* in 10 ml NaCl.

II- Calculation of doses

1. Plant dose

0.05 g ⟶ 1000 g

X g ⟶ Mice's Weight/g

2. Injected volume

0.05 g ⟶ 10 ml NaCl

X ⟶ Y

3. INK dose

0.1 ml ⟶ 10 g

X ⟶ Mice's Weight/g

Academic year : 2015/2016

Presented by : BENLAHOUES SAFIA

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The effect of *Curcuma Longa* on phagocytic activity

Thesis submitted for the degree of Master in Immuno-Oncology

Summary

The role of phagocytic cells on the reticuloendothelial system (R.E.S) in the immune response against infection disease is known for along time and confirmed recently by a quantitative methods of activity exploration of reticuloendothelial system (R.E.S) by using the extracts of medicinal plants *in vivo and in vitro.*

In the present study, we evaluated the *in vivo* the effect of immunomodulatory potential of *Curcuma Longa* on the phagocytic activity which was evaluated using the Carbon Clearance Assay.

The results showed that the dose of 150mg /kg of *Curcuma Longa* is increased the phagocytic index (K), corrected phagocytic index α and a diminution of the carbon clearance rate ($t_{½}$).

For this reason, we considered this extract of the medicinal plant *Curcuma Longa* as a natural source for immunostimmulatory activity against pathogens.

Key words: *Curcuma Longa*, phagocytic index, carbon clearance rate, corrected phagocytic index α, immunostimmulatory activity, Liver, Spleen.

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