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MÉMOIRE

Presented by

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En vue de l'obtention du

MASTER' S DIPLOMA

In Nutrition & Pathology

THEME

CONTRIBUTION TO THE STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF OIL BY-PRODUCTS

Submitted on 09 June 2022, to a Jury of the following members:

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Dedications

God for being my strength from the beginning till the end.
Without him I wouldn't be where I am today.

Myself for hanging on when it got tough and didn't look like what I pictured or planned.

Family and Friends
for their never-ending support when the journey got tough, they pushed me through with their words of encouragement and helping hand in various tasks concerning this work.

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LIST OF ABBREVIATIONS

MAMPs: Microbial associated molecular patterns

PAMPs: Pathogen associated molecular patterns

IIS: Innate immune system

PRRs: Patterns recognition receptors

TNF: Tumor necrosis factor

IL : Inter –leucines

LB : Lymphocytes B

LT : Lymphocytes T

IOC: International olives council

USA: United States of America

MADRP: Ministry Of Agriculture Rural Development and Fisheries

NPAD: National program for Agricultural Development

OMWW: Olive Mill Waste Water

PC: Phenolic compounds

EGCG: Epigallocatechin-3-gallate

EPN: Eosinophilic Polynuclear

IG: Immunoglobulins

M1: Macrophage 1

M2: Macrophage 2

SAI: Steroid anti-inflammatory drugs (SAI)

COX; Cyclooxygenase

INOS: Inducible form of nitric oxide synthase

NADPH Oxidase: Nicotinamide adenine dinucleotide phosphate oxidase

IE: Inflammatory edema

AC: Active congestion

EVR: Exudative vascular reaction

IFG: Inflammatory foyer granuloma

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ملخص

الالتهاب هو آلية دفاعية لجسم ضد العدوى أو أي خطر يحدث داخل الجسم أو خارجه، ويتم ذلك لاستعادة الجسم لعمله الطبيعي والصحي. والهدف من هذه الدراسة هو تحديد الخصائص المضادة لالتهابات لمخلفات مطحنة الزيتون بسبب محتواها العالي من المنهجية المستخدمة هي التقييم التاريخي المرضي لتطور و/أو تثبيط المركبات الفينولية. كانت الالتهاب على أساس دوري في فئران ويستار. كشفت النتائج عن نشاط مثير للاهتمام مضاد للالتهابات اعتمادًا على الجرعات المختلفة المستخدمة

الكلمات المفتاحية: المنتجات الثانوية لزيتون

الكلمات المفتاحية :

النشاط المضاد لالتهاب - الالتهاب - المنتجات الثانوية لزيتون

RESUME

L'inflammation est le mécanisme de défense de l'organisme contre les infections ou tout danger survenant à l'intérieur ou à l'extérieur du corps, ceci est fait pour restaurer le corps à son fonctionnement normal et sain. Le but de cette étude est de déterminer les propriétés anti-inflammatoires des déchets des moulins à olives en raison de leur teneur élevée en composés phénoliques. La méthodologie entreprise et une évaluation histo-pathologique de l'évolution et/ou de l'inhibition de l'inflammation de façon pariodique chez de rats Wistar. Les résultats ont révélé une activité antiinflammatoire interessante en fonction des différentes doses utilisées.

Mots clés : Sous produits oléicoles de l'olivier- Inflammation – Activité anti-inflammatoire.

ABSTRACT

Inflammation is the body's defence mechanism against infection or any danger occurring inside or outside the body, this is done to restore the body to its normal, healthy functioning. The aim of this study is to determine the anti-inflammatory properties of olive mill waste due to its high content of phenolic compounds. The methodology used was a histo-pathological evaluation of the development and/or inhibition of inflammation on a pariodic basis in Wistar rats. The results revealed interesting anti-inflammatory activity depending on the different doses used.

Keywords: Olive by-products – Inflammation - Anti- inflammatory.

CHAPTER I:

BIBLIOGRAPHIC SYNTHESIS

1. Generalities on olives

The olive tree (Olea europaea L. subsp. europaea var. europaea) is regarded as a pillar of Mediterranean civilization Because of its numerous historical and contemporary uses as well as its pervasiveness in traditional agrosystems (Loumou and Giourga, 2003). Originally, olive agriculture was limited to the Mediterranean region, to quote the historian Fernand Braudel Where the olive tree ends, the Mediterranean also stops, Nonetheless at this time, the dissemination of olive growing has spread to several non-traditional producer nations, including Argentina, Australia, Chile, China, and the United States (Diez et al., 2015) . Globally, olive farming took up more than 10 million hectares by 2017, with more than 90% of the land being in the Mediterranean region (FAO, 2018). The olive tree is a large, branching, evergreen tree that grows to a height of 20 to 30 feet. Its angular branches and opposite leaves make a symmetrical head (Bitting, 2022). The leaves are lanceolate, whole, deeply green above, and pale horsy underside. They have a dry, leathery texture. The flowers are tiny, start-shaped, creamy white with yellow centers, axillary in compact racemes, and have a mildly pleasant odor. The fruit, a fleshy pendulous drupe about the size of a pigeon's egg, is fairly common. It is oval or globular in shape, gradually becomes yellow and red until maturing into a glossy purplish-black or black color when fully ripe. (Bitting, 2022). The two main components of olive fruit are water and oil. After a growing period of 25 weeks, the fruit starts to ripen. The fruit then grows to its full size while retaining its natural green skin tone. After that, anthocyanin pigments gradually take the role of chlorophyll pigments in the olive skin, giving it its distinctive purple hue. The chemical makeup of the fruits also reflects this shift in appearance (Bitting, 2022). It is true that an accumulation of fatty acids, primarily oleic acid, takes place and is what gives the oil its acidity (Gunstone, 2011) . An objectively ideal harvest period occurs when lipogenesis finishes, which is a milestone that denotes the peak quality status of olive fruits. Hence, the quality of olive fruits can be determined by objective factors including fat content, moisture, and free acidity (Barranco et al ., 2017) , These factors can develop at various rates in various parts of a field as long as the ripening process occurs, resulting in a quality indicator heterogeneity that can be handled utilizing precision agriculture approaches (IOC, 2019).

2. Extraction process of olive oil

Fresh olives are collected mechanically and used to make olive oil (Beltrán et al ., 2021) Traditional oil mills and a more recent extraction method known as continuous mills, both of which have two or three phases, are the two major methods used to extract olive oil (Domingues et al., 2021 , Gullón et al ., 2020) . The goal of each of the aforementioned procedures is to separate the fruit's liquid oil phase from its other components (Goldsmith et al., 2014).

Olives should also be prepared as soon as possible after being harvested to prevent oxidation and maintain their freshness (Souilem et al., 2017).

2.1. Methods of extraction of olive oil

2.1.1. Traditional method

Regarding the traditional press technique, olive fruits that have been freed from leaves are cleaned, ground with mill stones, and malaxed into a paste that contains both solids (core debris, epidermis, cell walls, etc.) and liquids (oil and vegetation water contained in the cells of olives). Afterwards, these are spread out on circular mats (Goldsmith et al., 2014), The mixture of oil and water is then placed into a container and pressure is applied using a hydraulic piston press to acquire the solid fraction (known as pomace) before the oil and water are eventually separated by gravity and collected through decantation (Gullón et al., 2020). The oldest method of obtaining olive oil is the pressing method (Khdair et al., 2015).

2.1.2. Continuous method

According to Domingues et al ., (2021; and Hocaoglu et al ., (2018), the advancement of centrifuge technology during the past ten years referring to discontinuous pressing systems which have been mostly replaced by continuous systems . Based on the type of decanter utilized and the degree of the separation phase, continuous separation systems can be categorized into two-phase and three-phase systems (Hocaoglu et al.,2018). After the washing, crushing, and mixing processes, the mechanical extraction of the oil is mostly carried out continuously utilizing a decanter and a centrifuge process. Due to the decanter centrifuge's inclusion of a rotational bowl and a screw conveyor, large amounts of olives can be processed quickly (Souilem et al., 201).

In three phases of the three-phase process (insoluble particles, oil phase, and aqueous phase) are separated based on their densities when extra hot water is supplied to wash the oil (Souilem et al., 2017, Cheremisinoff et al., 1995). First, the liquid phases (oil phase and aqueous phase) are

subjected to vertical centrifugation to separate the olive oil from the olive mill wastewater after the solid wastes (insoluble solids) are separated from the other two phases in the decanter (Souilem et al ., 2017)

3. Characterization of olive co - products

3.1. Physicochemical characteristics of liquid olive by-product

The liquid olive by-product have an acidic pH with values ranging from 3.6 to 5.08 and a percentage. Humidity from 84.83 to 94.83%. They usually have a high salinity due to the addition of salt. To promote the preservation of olives (Tsioulpas et al., 2002). Their chemical composition varies depending on the stage of olive ripening, the process Extraction, climate conditions and variety of olives (Sayadi et al., 1993).

3.2.Composition of of liquid olive by-product

The main compounds of the margins are: water (83.2%), organic substances (15%) and mineral substances (1,8%) in the table below (Sansoucy, 1984).

Table 1: Chemical composition of Olive Mill Waste Water

(Sansoucy, 1984)

Composes	**Rate in %**
Water	8,3-88%
Organic matter	10,5-15%
Mineral matter	1,5%-2,4%
Total of nitrogen matter	1,25%-2,4%
Fatty matter	0,03%-1%
Polyphenols	1,0%-1,5%

4. Polyphenols in plants

Natural biophenols are a large class of molecules (over 8000 have been identified so far) that are only found in the plant kingdom. These molecules have one or more aromatic rings carrying one or more hydroxyl groups, and they also exhibit remarkable antioxidant power. Plants produce these molecules as secondary metabolites to defend themselves from bacteria, fungi, and insects (phytoalexins) (Stefani and Rigacci, 2014). Non-flavonoids and flavonoids are both types of plant

polyphenols. Flavonoids are further broken down into flavonols, flavononols, flavones, anthocyanins, procyanidins, phenolic acids, stilbenes, and tannins depending on the number of hydroxyls in the molecule and the type and position of other substituents. (Bravo, L , 1993) , phenols regularly studied are Curcumin (Ranalli et al 2009), a phenolic acid present in Curcuma longa Linn's (family Zingiberaceae) rhizome and a component of curry, epigallocatechin-3-gallate (EGCG), a flavanol present in green tea, resveratrol (3,5,4′-trihydroxy-trans-stilbene), a stilbene present in grapes and red wine, quercetin and myricetin, flavonol Numerous other plant phenols or their metabolic byproducts have also been studied, but the outcomes have been mixed. These include tannic, ellagic, ferulic, nordihydroguaiaretic, and caffeic acids, as well as morin, rutin, apigenin, baicalein, kaempferol, fisetin, luteolin, rottlerin, malvidin, and silibinin. (Ranalli et al., 2009).

4.1. Polyphenols in olives

The overwhelming majority of phenolic compound families in olives are represented in the table below (Cuffaro et al., 2023 ; Perinelli et al 2022; Zhao et al ., 2021; Gendrisch et al., 2021; Annunziata et al ., 2021; Cui et al ., 2020), (Table 1).

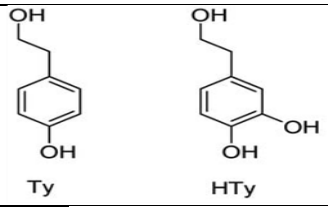
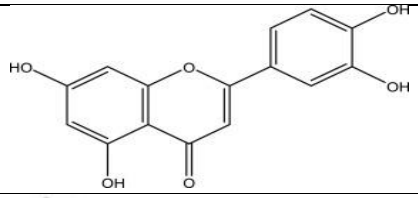
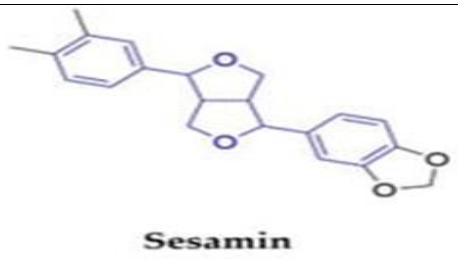
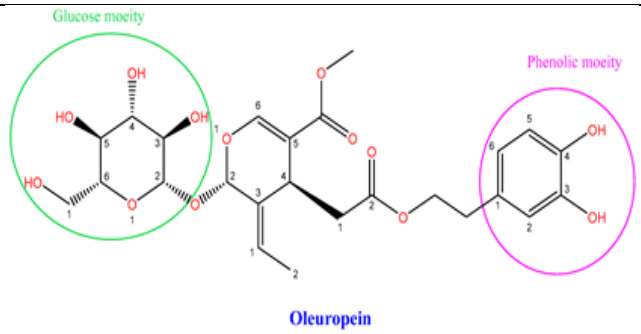
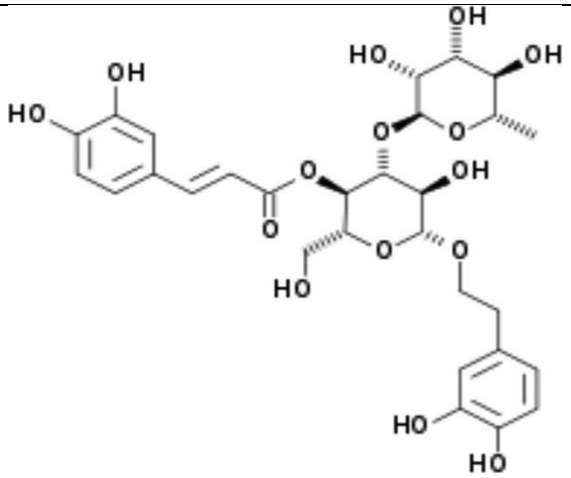
4.2. Biological activities of bioactive components of olives

The main biological activities of olive bioactive compounds are summarised in table 2-3 **(**Cuffaro et al., 2023 ; Rodríguez-López et al 2020 ;Vuolo et al., 2019 ; De Torres et al 2018 ; De Souza, 2017).

5. Inflammation

The Latin term inflamer, which means to light on fire, is where the word inflammation is derived. An inflammation is the body's reaction of regional and systematic response mechanisms in the face of any external or endogenous vascular tissue aggressions. Because we live in a microbial world and are partially microbial, inflammation occurs frequently. These conditions guarantee numerous interactions with microbial stimuli in the setting of tissue damage. Inflammation is most frequently brought on by the interaction of these two categories of stimuli in both time and space (Nathan, 2002). Inflammation often protects against infection spread, followed by resolution the return of the afflicted tissues to their regular structural and functional state. An inflammation is result of immune responses namely innate and adaptive immune response (Figure 1).

Table 1: Structures of polyphenols in olives (Perinelli et al 2022; Zhao et al ., 2021)

Phenolic alcohols	Hydroxytyrosol Tyrosol	
Flavanoids	Luteolin	
Lignans	Sesamin	
Secoiridoid	Oleuropein	
Cinnamic acid	Verbascoside	

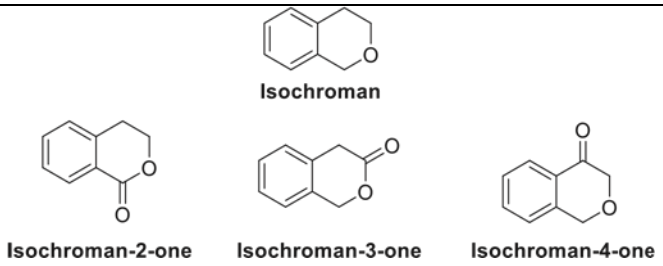
Isochromans	Heterocyclic compounds with Anti-Oxidant, Anti-Tumor Anti-microbial	

Table 2 : Biological activities of bioactive components of olives **(**Hara et al., 2023; Cuffaro et al., 2023 ; Rodríguez-López et al 2020 ; Vuolo et al., 2019)

Name	**Definition**	**Biological activity**
Phenolic alcohols - Oleocanthal - Hydroxytyrosol -Tyrosol	An aromatic hydrocarbon group is joined to a hydroxyl group.	Anti-viral, Anti-inflammatory Neuro-protection, Cardio protection Functional nutrition
- Lignans	Are defined chemically by the accumulation of aromatic aldehydes .	Anti-inflammatory Anti-viral
Flavonoids	Chemically, flavonoids are composed of two benzene rings connected by three linear carbon chains. Apigenin and luteolin in free form.	Anti-inflammatory, Anti-viral Anti-oxidant , Anti-microbial
Phenolic acids -Hydroxycinnamic -Hydroxybenzoic	Two primary families	Anti-fungal Antioxidant
Secoiridoids	Secoiridoids are phenolic compounds present in high amounts in olive oil in comparison to other plant species.	Anti-oxidant Anti-cancer Anti-diabetic Neuroprotective
Hydroxyisocromans	Hydroxyisocromans is formed from combining benzaldehyde and vanillin with hydroxytyrosol.	Anti-inflammatory Neuro-protection

Table 3: Presentation of diverse activities of polyphenols in olives, olive mill waste water and pomaces

Biological activity	**Effects**	**References**
Functional Nutrition	Nutraceutical Properties	Cuffaro et al. (2023)
Anti-cancerous activity	Reduce the proliferation of tumoral cells	Benedetto et al. (2022) Grubić and Ćurko-Cofek (2022) Bucciantini et al. (2021 Briguglio et al. (2020)
Anti-inflammatory activity	Modulation of inflammations	Hara et al. (2023) Bucciantini et al. (2021)
Anti-neurodegenerative activity	Reduce neuro-inflammation	Ahmad et al. (2022) Hano et al. (2020)
Anti-microbial activity	Capacity to reduce microbial activity	Chojnacka et al. (2021) Borjan et al. (2020)
Cardio-protective activity	Inhibition of factors of risk of cardiac diseases	Alfaddagh et al. (2020)
Anti-oxidant activity	Trapping free radicals	Silenzi et al. (2020) Stagos (2019)
Anti-diabetic activity	Regulate glycemic levels	Sun et al. (2020)

Innate immune response is the host's first line of defense it is quick and effective, because it does not need an adaptive environment to function as the adaptive system requires (Libby, 2007). The IIS It recognizes a wide variety of molecular patterns known Microbial associated molecular patterns(MAMPs) which can also be referred to as pathogen-associated molecular patterns(PAMPs), which are frequently present on pathogens but distinct from mammals (Newton, 2012). These molecular patterns are known to trigger or activate the inflammation process, In IIR the inflammation process begins with a detection of a pathogen infiltration or cell injury by the pattern recognition receptors (PRRs) which are intracellular or surface expressed and are used by the resident tissue cells macrophage, fibroblast, mast cells, dendritic cells including the circulation monocytes and neutrophils (Newton, 2012). Then, after oligomerization and complex assembly, activated PRRs start signaling cascades that cause the .release of substances that aid in leukocyte recruitment to the area this process leads to vascular alterations Histamine, prostaglandins, and nitric oxide all have a vasodilatory effect on the vascular smooth muscle which leads to swelling Although inflammatory mediators such as histamine and leukotrienes act on endothelial cells to raise vascular permeability and allow plasma proteins and leukocytes to escape the circulation, blood flow is increased and circulating leukocytes are brought into the body (Newton, 2012).

Cytokines include Leukocyte extravasation is encouraged by tumor necrosis factor (TNF) and interleukin 1 (IL1), which raise the concentrations of leukocyte adhesion molecules on endothelial cells. At the site of infection or damage, activated innate immune cells such as dendritic cells, macrophages, and neutrophils phagocytose foreign substances and host detritus. They also emit cytokines that influence the slower, lymphocyte-mediated adaptive immune response (Figure 1), (Newton, 2012).

The adaptive immune response is made up of the second set of reactions. Due to the adaptive system's tiny cell population and cellular specialization for each individual pathogen, to build up a large enough population to mount a successful defense against the bacterium or the poison, the responding cells must multiply after coming into contact with the antigen. As a result, the innate reaction to host defense usually manifests before the adaptive response (Holmskov et al., 2003) The production of long-lived cells that remain in what appears to be a dormant state but are capable of re-expressing effector activities is a crucial aspect of the adaptive response, quickly following additional contact with their unique antigen.

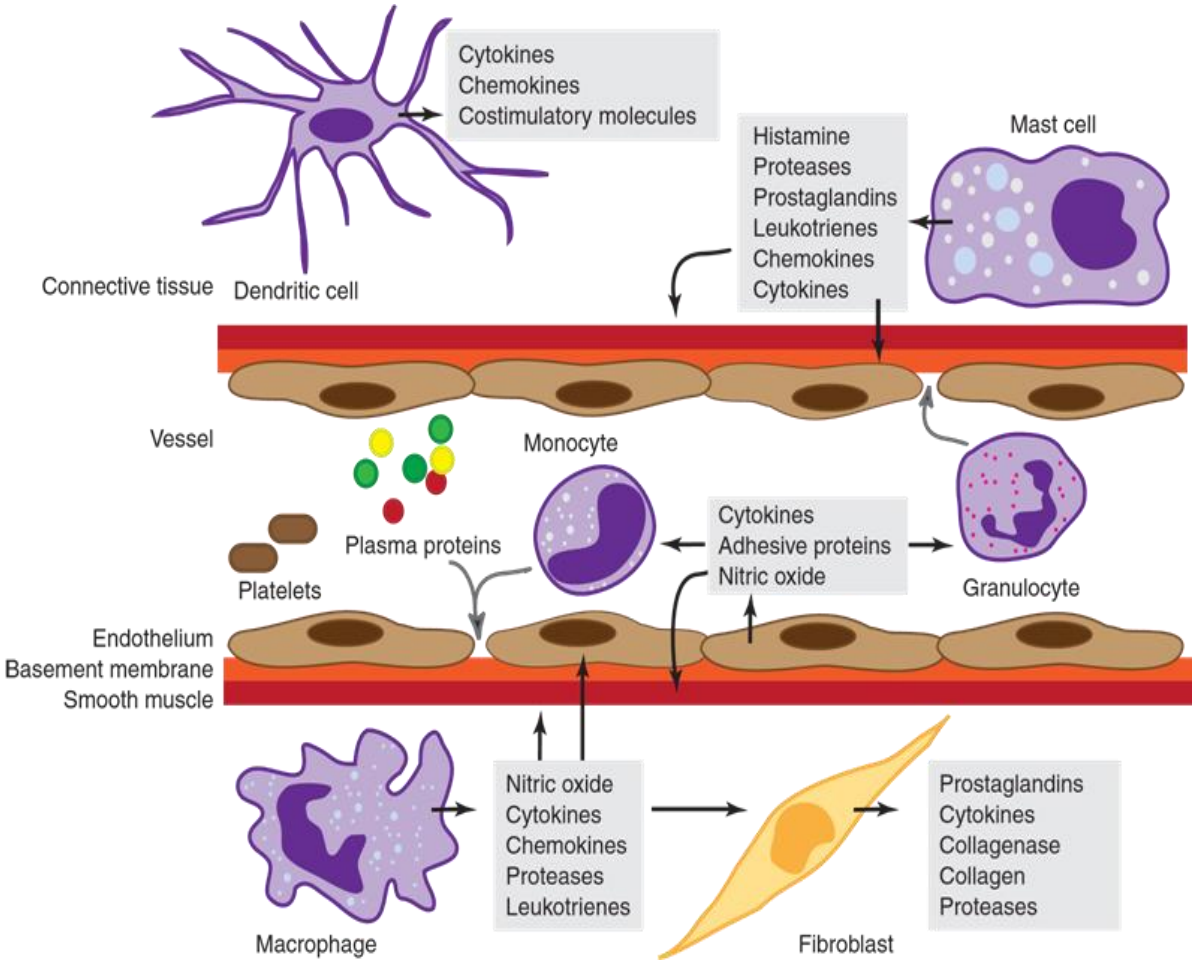


Figure 1: Cells and inflammatory response mediators. Inflammation is mediated by molecules generated from plasma proteins and cells in reaction to infections or tissue damage. These molecules also promote vascular alterations, leukocyte migration, and activation. Neutrophils, basophils, and eosinophils (Newton, 2012).

As a result, the adaptive response has the capacity to develop immunological memory, enabling it to significantly improve the host's ability to respond to particular viruses or poisons when they are encountered again, even decades after the initial sensitizing encounter (Holmskov et al., 2003).

5.1. Types of inflammations

- ✓ **Acute inflammation-**

Acute inflammation can be brought on by noxious substances, microbial invasion, or tissue injury caused by trauma. For example, acute pneumonia or cellulitis both have symptoms that start out quickly, get worse quickly, and linger for a few days (Pahwa, 2021). Acute inflammation is diagnosed by 4 clinical symptoms redness, swelling, pain and heat.

- ✓ **Subacute inflammation**

Is a type of inflammation that is between acute and chronic inflammation (Pahwa, 2021).

- ✓ **Chronic inflammation**

The phrase "chronic inflammation" also refers to a sluggish, long-term inflammation that lasts for months or years at a time. The severity and outcomes of chronic inflammation typically depend on the origin of the injury and the body's capacity to heal and reverse the harm. There are 2 types of chronic inflammation (Pahwa, 2021).

a) Non-specific proliferative: This condition is characterized by the presence of non-specific granulation tissue created by the infiltration of mononuclear cells (lymphocytes, macrophages, plasma cells), as well as the proliferation of fibroblasts, connective tissue, vessels, and epithelial cells, such as in an abscess in the lung or a nasal polyp that has an inflammatory appearance similar to a polyp (Pahwa, 2021).

b) A specific kind of chronic inflammation known as granulomatous inflammation is characterized by the presence of distinct nodular lesions or granulomas that are produced by an accumulation of activated macrophages or its descendant cell, epithelioid cells, which are typically encircled by lymphocytes (Pahwa, 2021) . Aschoff, Reed-Sternberg, and tumor giant cells are just a few examples of the Langhans or gigantic cells that are frequently formed when the macrophages or epithelioid cells within the granulomas consolidate. The two are as follows: (Pahwa, 2021).

c) I foreign body granuloma, such as silicosis, are granulomas caused by an immunological reaction to a foreign body or T-cell.II Infectious granuloma, such as those caused by leprosy and tuberculosis, are those that develop as a result of a prolonged infection. (Pahwa, 2021).

5.2. Types of inflammatory cells

➲ There are many molecular activation states of macrophages. Depending on the cytokines or signals that activate them, they are frequently categorized as pro-inflammatory (M1) or anti-inflammatory (M2) cells (Mills et al., 2000). In cases of chronic inflammation, the dysfunction of macrophage transformation from the M1 phenotype (inflammatory) to the M2 phenotype (anti-inflammatory) is the primary cause of severe chronic inflammation. Due to the variables associated to angiogenesis and anti-inflammatory cytokines they release, M2 macrophages are essential for the revascularization of wounds. In conclusion macrophage do participate or has a role as an anti-inflammatory agent.

- ➲ Endothelial cells : Vascular permeability, lymphocyte adhesion to the vascular endothelium (intracellular adhesion molecules)
- ➲ Mastocytes and Basophilic Polynuclear GNP: Allergy mediating cells (Asthma, Allergies)
- ➲ Eosinophilic Polynuclear EPN: Particular role of capture of Antigens -Antibody complexes
- ➲ Lymphocytes
    - Lymphocytes B: Transform into plasma cells that secrete immunoglobulins (Ig) - Humoral immunity.
    - Lymphocytes T: Direct cytotoxicity - Cellular immunity.

6. Factors that trigger inflammations

Inflammations are triggered by both endogenous and exogenous factors

6.1. Endogenous factors

- Auto-immune diseases example lupus.
- Infections, invasions by bacteria, viruses, microbes.

6.2. Exogenous factors

- Toxins, Radiation, Chemicals …
- Trauma : Injuries, Insect bites, Burns…

7. Anti-inflammatory properties of plant origin

Since ancient times, plants have been used extensively in the treatment of human illnesses. They produce a variety of chemicals that have biological effects as a defense against disease attack and environmental stress. These tiny chemical compounds have a variety of biological functions and

are produced by secondary metabolism. Anti-inflammatory functions are highlighted among the several functionalities (Virshette et al ., 2019; Locatelli et al ., 2016) , According to (Caporaso et al ., 2017; Bonetti et al ., 2016) table olives and olive oil are the principal products from olive trees. The polyphenol chemicals included in olive oil, which exhibit antioxidant activity, are among the substances that make it a crucial component of a human diet. Tyrosol, oleuropein, caffeic acid, vanillic acid, and hydroxytyrosol are some of the most significant phenols found in olive oil (Caporaso et al., 2017; Bonetti et al., 2016). The extraction method for olive oil has been enhanced over the years in an effort to boost production and enhance quality. Due to the significant amount of waste (OMWW) produced during the manufacture of oil, which is phytotoxic due to its high fat, lipid, and polyphenol content. (Turano et al 2002) several studies have highlighted that hydroxytyrosol, the most abundant biophenol in OMWW acts as a free radical-scavenger and metal-chelator (Karković et al., 2019) , protects against oxidative damage (Rodríguez-Ramiro et al ., 2011; Grasso et al ., 2007), inhibits the NADPH oxidase (Maiuri et al ., 2005) , the inducible form of nitric oxide synthase (iNOS), and the proinflammatory enzymes such as 5-lipoxygenase and cyclooxygenase (Scoditti et al ., 2012) , decreasing the production of nitric oxide, leukotrienes, and prostaglandins. Furthermore, tumor necrosis factor (TNF) and other proinflammatory mediators can be released under the control of hydroxytyrosol (Fuccelli et al., 2018, Richard et al., 2011).

CHAPTER II:

MATERIAL & METHODS

MATERIALS AND METHODS

1. Characteristics of the target rat samples

The 25 rats Wistar were adult around 10 weeks with weights scaling between 170g-240g. The rats were divided into five lots, individually identified on the tail by coloured markers (Table 1).

Table: Weight of rats in each batch

Controls (g)	Standards (g)	Dose 1(g)	Dose 2(g)	Dose 3 (g)
172	198	211	185	221
170	225	177	183	185
193	209	184	196	196
188	243	201	179	210
190	202	179	184	197

2. Preparation of extract

3. Feeding

4. *In vivo* Experimetation

The animals were distributed into five groups of 5 rats;

5. Measurement of edema

6. Histopathological study

6.1. Experimental procedure

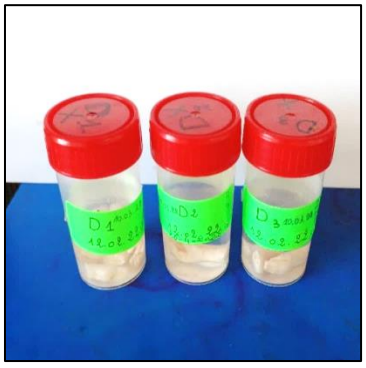


Photo 1: Samples of rat tissue

Photo 2: Decalcification process

Photo 3: Process of cutting Of paw rat

Photo 4: Cassettes

Photo 5: Embedded Tissue

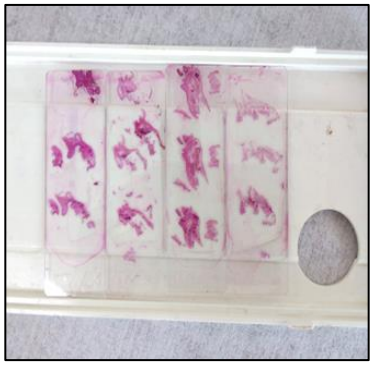
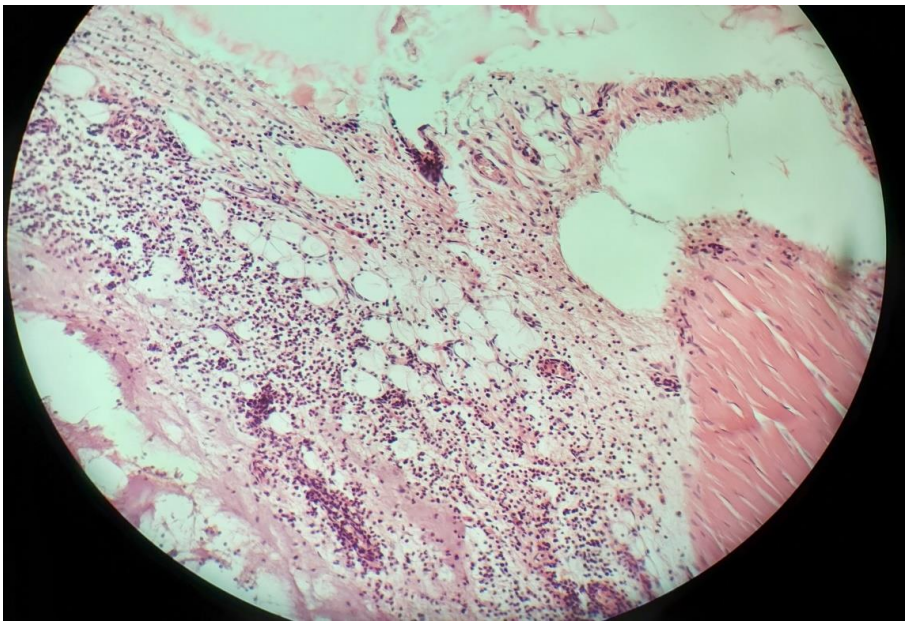


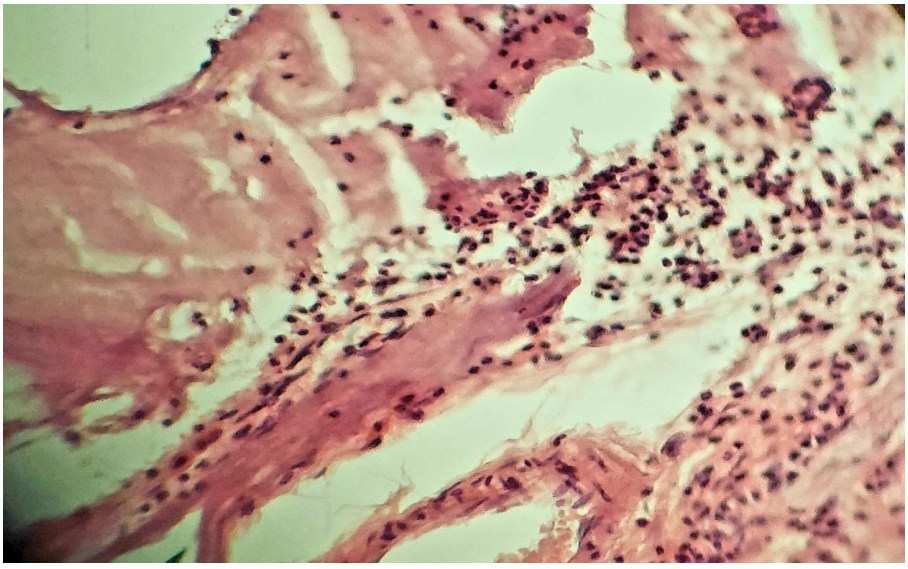
Photo 6: Stained Slides

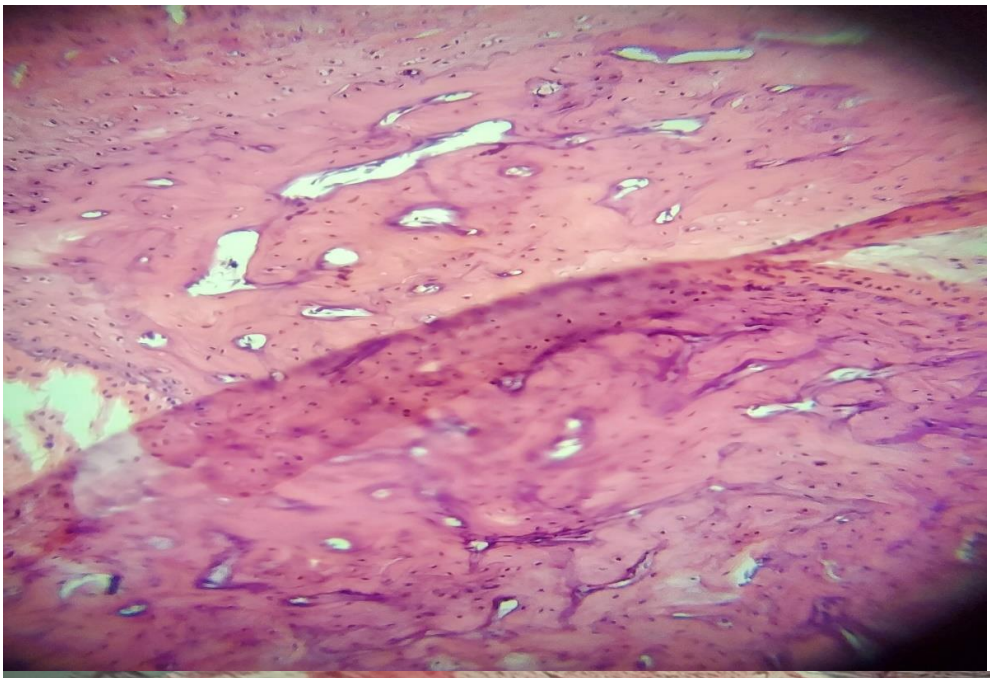
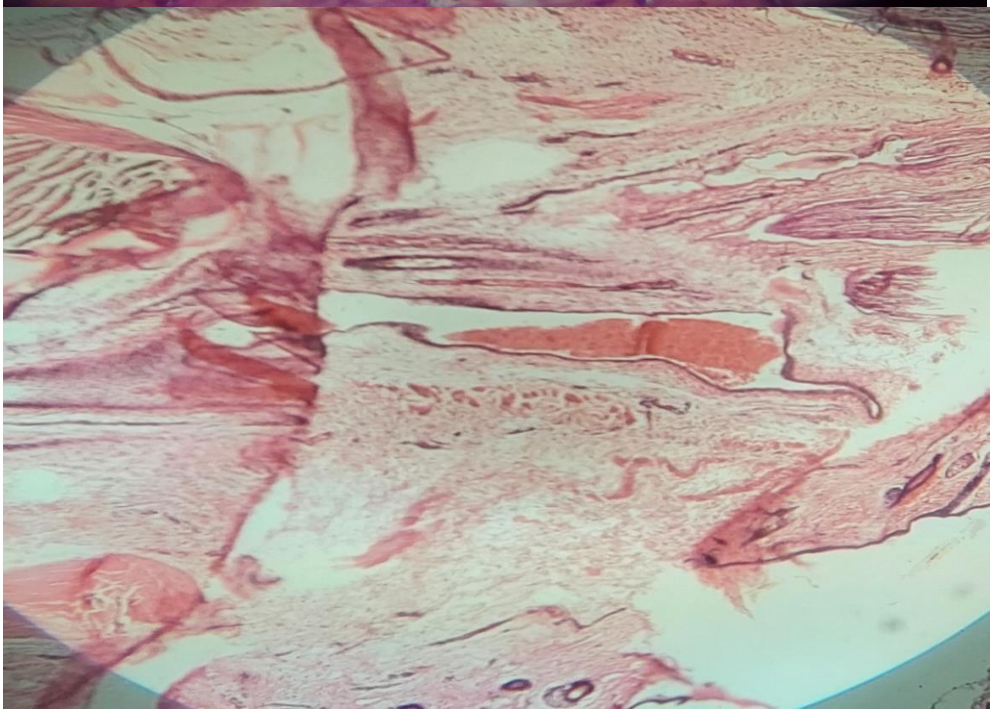
CHAPTER III:

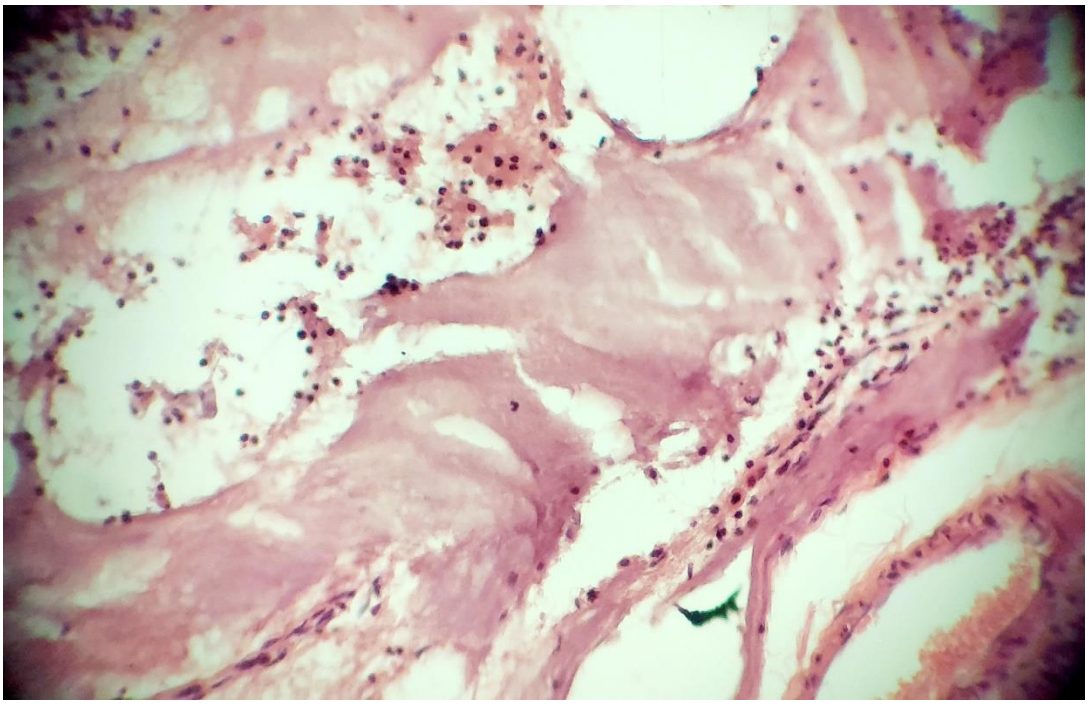
RESULTS AND INTERPRETATION

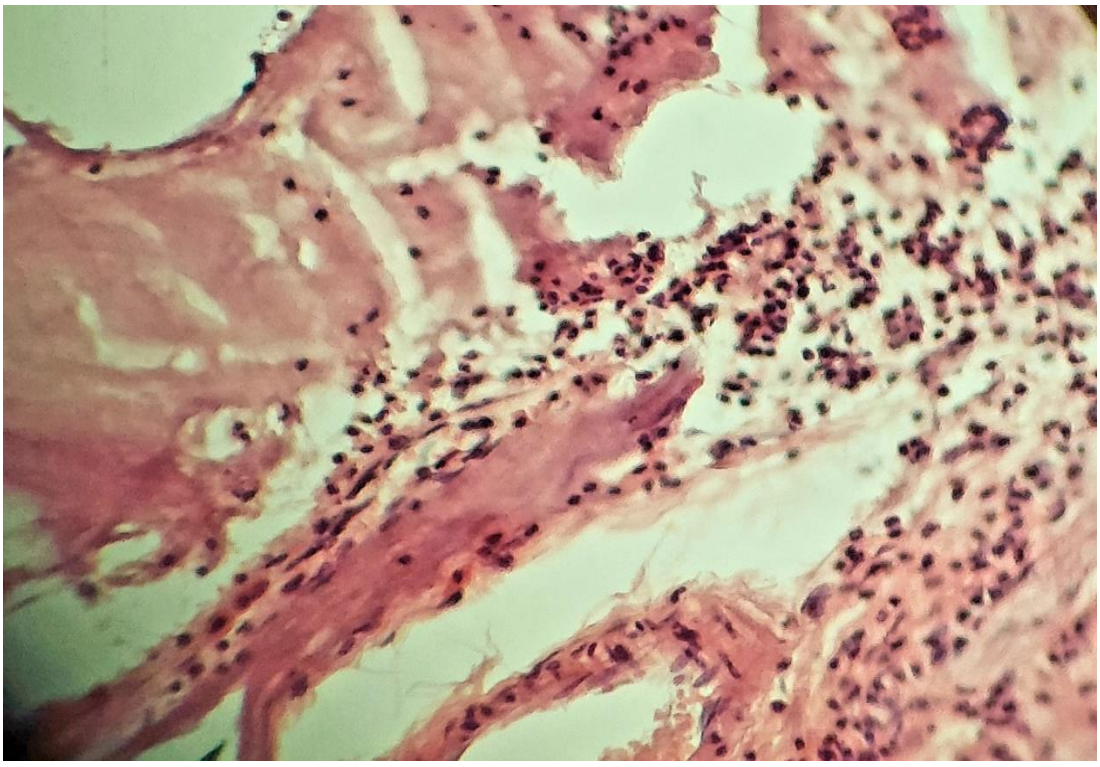
Results and Interpretation

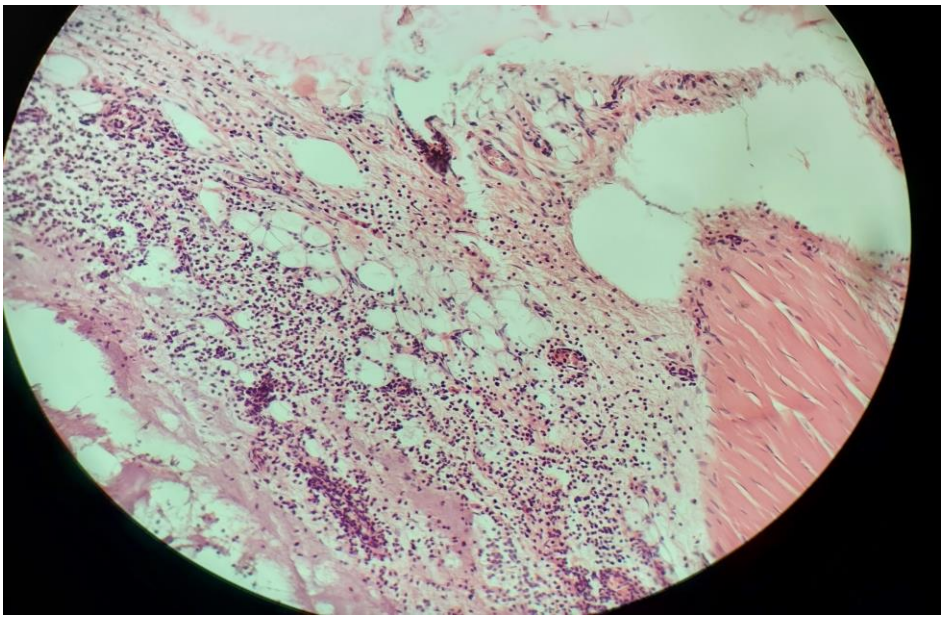


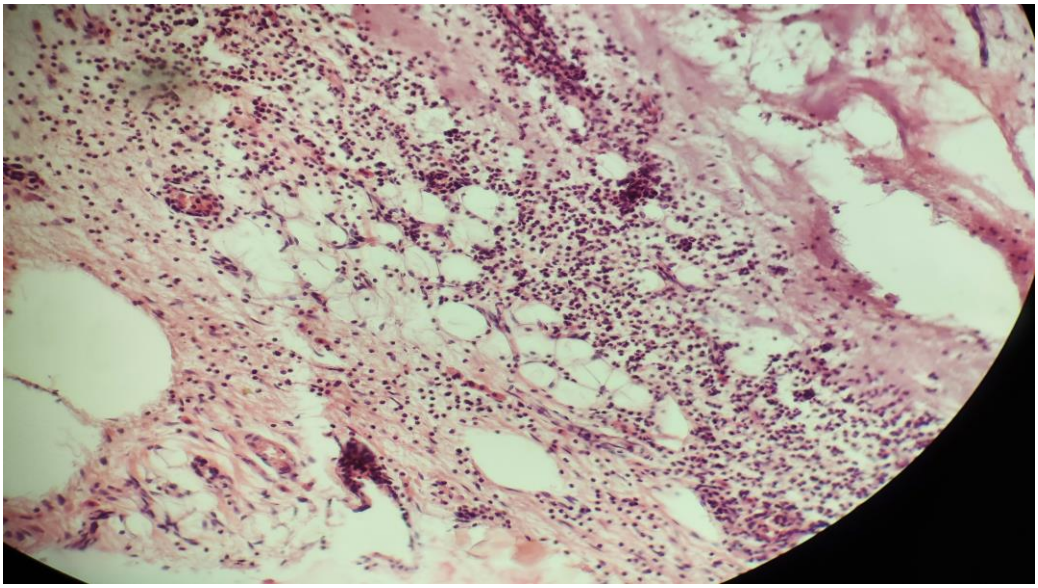


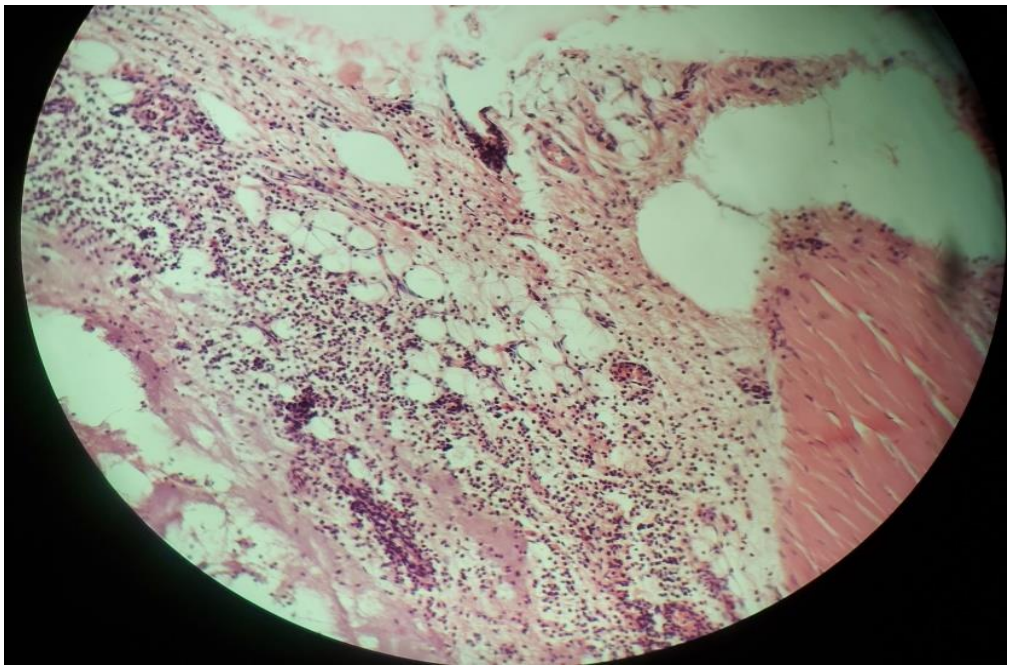
1. 


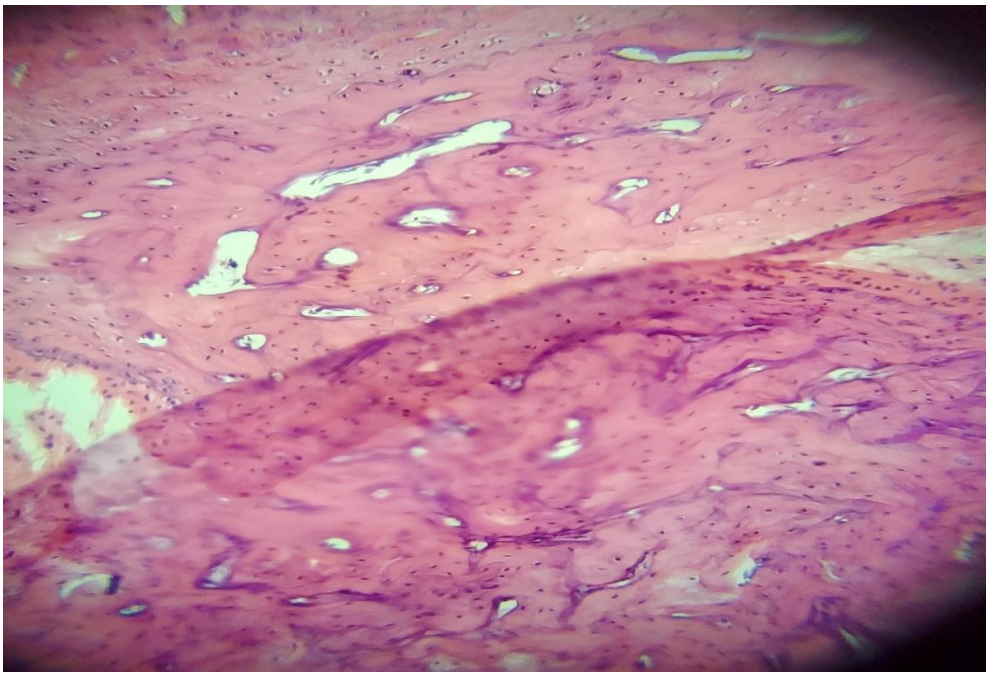












Discussion

The principal objective of the study was to explore the histopathological effect of our extracts in induced edema in Wistar rat (Guo et al., 2022 ; Albarakati, 2022 ; Salem et al., 2021) in order to gather information on certain properties of the extract which, if adapted to humans, could be used in the development of a new anti-inflammatory drug.

In the present study, oral administrations of extract in three of body weight for the anti-inflammatory activity, the acute toxicity do not produce any significant physical and behavioral changes and no death is recorded in the extract of. during of the acute oral toxicity testing.

The study concerned the treated, control and standard (the diclofenac sodium, substance belonging to the family of non-steroidal anti-inflammatory drugs (NSAIDs) which reduce inflammation, pain and fever (Vidal, 2014) groups. The enhancing effect of the extract was apparent as earl after injection, with an attractive result, particularly for dose 2 of the extract compared to standards and controls. This clear reduction in edema was histologically comparable to that caused by treatment . The histological sections confirmed the results obtained in the previous study. (Photo 1).

The standard samples revealed in muscle tissues a moderate inflammation with reasonable low lymphocytic infiltration and edema, and a peri-vascular inflammation-infiltration compared to control group. The same aspect is showed in the white adipose tissue highlighting vessel congestions and inflammatory cells and a peri-vascular inflammation-infiltration. The sebaceous glands were also affected by lymphocytic infiltration. The cartilage showed no significant inflammatory symptoms (Photo 2).

The histological sections related to dose 1 i-e /kg CW showed symptoms are similar to those in standard samples. Indeed, it underlined an acute inflammation in muscles, white adipose tissues and sweat glands, a lymphocytic infiltration with polynuclear lymphocytes and edema, and a peri-vascular inflammation-infiltration compared to standard and control groups (Photo 2).

In the same context, the microscopic examination of the use of dose i-e /kg CW and dose i-e /kg CW , highlighted significant recovery compared to standard and control groups, with disappearing signs of inflammation, reduced inflammatory edema and absence of lymphocytic infiltration in muscles and adipose tissues (Photo 3). These results are comparable to those obtained by Cappelli et al., (2021).

CHAPTER V:

CONCLUSION AND PERSPECTIVES

Conclusion and perspectives

The conclusion drawn from this study is that the by-product extract non-acutely toxic, has an anti-inflammatory effect . This effect was pertinent at the dose of /kg CW of extract reflecting a complete recovery of paw edema in Wistar rats, with full healing for doses of extract. These results These results herald a new era of research into the development inflammatory diseases, in particular with a lower risk of side effects, including those of NSAIDs.

Additional research into sub-acute and chronic toxicity test *in vivo* and *in vitro* cytotoxicity activity of the extract , in determining the safety profile of drugs should also be achieved in the future.

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ANNEX

ملخص

الالتهاب هو آلية دفاعية لجسم ضد العدوى أو أي خطر يحدث داخل الجسم أو خارجه، ويتم ذلك لاستعادة الجسم لعمله الطبيعي والصحي. والهدف من هذه الدراسة هو تحديد الخصائص المضادة للالتهابات لمخلفات مطحنة الزيتون بسبب محتواها العالي من كانت المنهجية المستخدمة هي التقييم التاريخي المرضي لتطور و/أو تثبيط المركبات الفينولية. الالتهاب على أساس دوري في فئران ويستار. كشفت النتائج عن نشاط مثير للاهتمام مضاد للالتهابات اعتمادًا على الجرعات المختلفة المستخدمة

النشاط المضاد للالتهاب - الالتهاب -الكلمات المفتاحية : المنتجات الثانوية للزيتون

ABSTRACT

Inflammation is the body's defence mechanism against infection or any danger occurring inside or outside the body, this is done to restore the body to its normal, healthy functioning. The aim of this study is to determine the anti-inflammatory properties of olive mill waste due to its high content of phenolic compounds. The methodology used was a histo-pathological evaluation of the development and/or inhibition of inflammation on a pariodic basis in Wistar rats. The results revealed interesting anti-inflammatory activity depending on the different doses used.

Keywords: Olive by-products – Inflammation - Anti- inflammatory.

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RESUME

L’inflammation est le mécanisme de défense de l’organisme contre les infections ou tout danger survenant à l’intérieur ou à l’extérieur du corps, ceci est fait pour restaurer le corps à son fonctionnement normal et sain. Le but de cette étude est de déterminer les propriétés anti-inflammatoires des déchets des moulins à olives en raison de leur teneur élevée en composés phénoliques. La méthodologie entreprise et une évaluation histo-pathologique de l’évolution et/ou de l’inhibition de l’inflammation de façon pariodique chez de rats Wistar. Les résultats ont révélé une activité antiinflammatoire interessante en fonction des différentes doses utilisées.

Mots clés : Sous produits oléicoles de l’olivier- Inflammation – Activité anti-inflammatoire.