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كلية علوم الطبيعة والحياة

Département : de Biologie Animale

قسم: بيولوجيا الحيوان

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Evaluation of the possible anti-inflammatory and protective role of polyphenols from *Centaurea sp.* against diclofenac-induced toxicity in *Wistar* rats

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Présenté par : Bensadallah Amina
Baba Ali Anfel

Le : 12/06/2024

Jury d'évaluation :

Présidente : **Pr. Amrani Amel** (Prof - U Constantine 1 Frères Mentouri)

Encadrant : **Dr. Boubekri Nassima** (MCA - U Constantine 1 Frères Mentouri)

Examinateur : **Dr. Kabouche Sami** (MCA - U Constantine 1 Frères Mentouri)

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Evaluation of the possible anti-inflammatory and protective role of polyphenols from *Centarea sp.* against diclofenac-induced toxicity in *Wistar* rats

Abstract

Inflammation is an essential response provided by the immune system that ensures survival during infection and tissue injury. Diclofenac (DF) is administrated to treat pain, inflammatory disorders, and dysmenorrhea but kidney and liver problems are the main worries of the agent. *Centaurea sp.* is widely used in traditional medicine for its therapeutic properties essentially attributable to natural bioactive compounds. In the present study, we evaluated the possible anti-inflammatory activity using formalin-induced paw edema, and the protective role of polyphenols from *Centaurea sp.* against diclofenac-induced toxicity in *Wistar* rats.

Rats were orally administered *n*-butanol extract (100 mg/kg b.w.) for 5 days and diclofenac was administered on the 4^{th} and 5^{th} day (50mg/kg, ip). Serum transaminases, creatinine, urea, lipid peroxidation (LPO), reduced glutathione (GSH), and glutathione peroxidase (GP_X) were estimated to access liver and kidney damage. A histological study was determined.

The results showed that *n*-butanol extract (100 and 200mg/kg) exhibited a significant reduction of edema in formalin-induced rat paw edema. Significant changes in biochemical indicators (ALT, ASAT, urea, creatinine) and oxidative stress (malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPx)) in the group treated with diclofenac (50 mg/kg). This dysfunction was accompanied by alterations and changes in hepatic and renal architecture. While, these levels were restored to control value in animals treated with plant extract. The regularized levels of LPO, GSH, transaminases, creatinine, urea and GPx activities revealed the antioxidant properties of the extract plant. The histological study showed the hepatoprotective and nephroprotective effect of our extracts against diclofenac-induced toxicity.

These results reveal the promising potential of the extract of *Centaurea sp.* as antioxidant, anti-inflammatory agent, and protectors against the toxicity of anti-inflammatory drugs (DF). Thus, opening new perspectives in the field of medical research and pharmacology for the development of complementary therapies.

Keywords : Diclofenac, *Centaurea sp.*, Hepatotoxicity, Nephrotoxicity, Polyphenols, Antioxidant activity, Anti-inflammatory activity.

Évaluation du rôle anti-inflammatoire et protecteur possible des polyphénols de *Centarea sp.* Contre la toxicité induite par le diclofénac chez les rats *Wistar*

Résumé

Le système immunitaire s'appuie sur l'inflammation comme réponse cruciale pour assurer la survie en cas d'infections et de lésions tissulaires. Le diclofénac (DF) est utilisé pour soulager la douleur, traiter les conditions inflammatoires et gérer la dysménorrhée, mais son impact sur les reins et le foie suscite des inquiétudes. *Centaurea sp.* est utilisé depuis longtemps dans la médecine traditionnelle en raison de ses composés bioactifs naturels, dont on pense qu'ils contribuent à ses propriétés thérapeutiques. Dans notre étude, nous avons évalué l'effet anti-inflammatoire des polyphénols de *Centaurea sp.* en utilisant l'œdème de la patte induit par le formol, ainsi que le rôle protecteur de ces composés contre la toxicité induite par le diclofénac chez les rats *Wistar*.

Ce travail de recherche a porté sur l'évaluation de l'effet protecteur de l'extrait *n*-butanol de la plante *Centaurea sp.* (100mg/kg, par gavage pendant 5 jours) contre la toxicité induite par le diclofénac (50mg/kg, le $4^{ème}$ et le $5^{ème}$ jour par voie intra-péritonéale) chez des rats males de souche *Wistar Albinos*. Les transaminases (AST, ALT), urée, creatinine, la peroxydation lipidique (MDA), le glutathion réduit (GSH) et la glutathion peroxydase (GPX) ont été estimées. Une étude histopathologique a été déterminée.

Les résultats ont montré que l'extrait de *n*-butanol (100 et 200mg/kg) présentait une réduction significative de l'œdème de la patte du rat induit par le formol. Des changements significatifs des indicateurs biochimiques (ALT, ASAT, urée, créatinine) et du stress oxydatif (malondialdéhyde (MDA), glutathion (GSH) et glutathion peroxydase (GPx)) dans le groupe traité avec du diclofénac (50 mg/kg). Ce dysfonctionnement s'est accompagné d'altérations et de modifications de l'architecture hépatique et rénale. En revanche, ces niveaux ont été rétablis à la valeur de contrôle chez les animaux traités avec l'extrait butanolique. La régularisation des taux de LPO, GSH, transaminases, créatinine, urée et des activités GPx a révélé les propriétés antioxydantes de notre extrait. L'étude histologique a montré l'effet hépatoprotecteur et néphroprotecteur l'extraits contre la toxicité induite par le diclofénac.

Ces résultats révèlent le potentiel prometteur de l'extraits de *Centaureas sp.* en tant qu'agents antioxydants, anti-inflammatoire et protecteurs contre la toxicité des médicaments anti-inflammatoires (DF), ouvrant ainsi de nouvelles perspectives dans le domaine de la recherche médicale et de la pharmacologie pour le développement de therapies complémentaires.

Mots-clés : Diclofénac, *Centaurea sp.*, hépatotoxicité, néphrotoxicité, polyphénol, activité antioxydante, activité anti-inflammatoire.

تقييم الدور المحتمل لمضادات الالتهابات والدور الوقائي لعديدات الفينول المستخلص من نبات *Centarea sp.* ضد السمية التي يسببها الديكلوفيناك في فئران *Wistar*

الملخص

الالتهاب هو استجابة أساسية يقدمها الجهاز المناعي الذي يضمن البقاء على قيد الحياة أثناء العدوى وإصابة الأنسجة. يستعمل دواء الديكلوفيناك (DF) لعلاج الألم والاضطرابات الالتهابية وعسر الطمث ولكن مشاكل الكلى والكبد هي من اهم اثاره الجانبية. كما يُستخدم نبات *Centaurea sp.* على نطاق واسع في الطب التقليدي لخصائصه العلاجية التي تُعزى أساسًا إلى المركبات الطبيعية النشطة بيولوجيًا. في هذه الدراسة، قمنا بتقييم النشاط المحتمل المضاد للالتهابات باستخدام الوذمة التي يسببها الفورمول، والدور الوقائي لعديدات الفينول من نبات Centaurea *sp.* ضد السمية التي يسببها الديكلوفيناك في فئران *Wistar Albino*.

أُعطيت الجرذان مستخلص n. butanol عن طريق الفم (100 مغ/كغ من وزن الجسم) لمدة 5 أيام وأُعطيت جرعات الديكلوفيناك في اليوم الرابع والخامس (50 مغ/كغ، حقن تحت السفاق). تم تقدير الإنزيمات الكبدية في المصل والكرياتينين واليوريا وبيروكسيد الدهون (LPO) والجلوتاثيون المختزل (GSH) والجلوتاثيون بيروكسيداز (GPX) لتقييم تلف الكبد والكلى. كما تم اجراء دراسة نسيجية.

أظهرت النتائج أن مستخلص n-butanol(100 و200 ملغ/كغ) قلل بشكل كبير من الوذمة التي يسببها الفورمول في قدم الجرذ. كانت هناك تغيرات كبيرة في المؤشرات الكيميائية الحيوية (ALT، AST، اليوريا، الكرياتينين) والإجهاد التأكسدي (مالونديالديهيد (MDA)، الجلوتاثيون (GSH) والجلوتاثيون بيروكسيداز (GPx) في المجموعة التي عولجت بالديكلوفيناك (50 ملغ/كغ). رافق هذا الخلل الوظيفي تغيرات واختلالات في البنية الهيكلية للانسجة الكبدية والكلوية. ومع ذلك، تمت استعادة هذه المستويات إلى القيمة المرجعية لدى الحيوانات المعالجة بالمستخلص النباتي. وكشف تنظيم مستويات LPO، GSH، وGPx، وأنشطة الترانساميناز، والكرياتينين، واليوريا عن الخصائص المضادة للأكسدة للمستخلصات النباتية. أظهرت الدراسة النسيجية التأثير الوقائي الكبدي والوقاية الكلوي لمستخلصاتنا ضد السمية التي يسببها الديكلوفيناك.

تشير النتائج التي تم الحصول عليها إلى النشاط المضاد للالتهابات والوقائي للجزء متعدد الفينول من نبات *Centaurea sp.* والذي يمكن أن يكون نتيجة لتثبيط توليد أنواع الأكسجين التفاعلية (ROS).

الكلمات المفتاحية: ديكلوفيناك، *Centaurea sp.*، السمية الكبدية، السمية الكلوية، عديدات الفينول ، النشاط المضاد للأكسدة، النشاط المضاد للالتهابات.

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Abbreviations

-A-

AKI: Acute Kidney Injury

ALT: Alanine Aminotransferase

AST/ASAT: Aspartate Aminotransferase

ATP: Adenosine triphosphate

-C-

CAT: Catalase

CC: Collecting Tube

CD: Crohn's Disease

cGMP: Cyclic Guanosine Monophosphate

CKD: Chronic Kidney Disease

COX1: Cyclooxygenase type 1

COX2: Cyclooxygenase type 2

CVD: Cardiovascular Disease

-D-

DF: Diclofenac

DNA: Deoxyribonucleic acid

DTNB: 5,5'-Dithiobis-(2-Nitrobenzoic Acid)

-E-

ETC: Electron Transport Chain

EXT: Extract

-G-

GPx: Glutathione Peroxidase

GRE: Glucocorticoid Response Element

GSH: Glutathione

-H-

H_2O_2: Hydrogen Peroxide

Hmox1: heme oxygenase 1

HNE: 4-hydroxy-nonenal

$HO^{\bullet}$: Hydroxyl Radical

-I-

IBD: Inflammatory Bowel Disease

IFN-y: Interferon Gamma

IL-1: Interleukin 1

IL-10: Interleukin 10

IL-12: Interleukin 12

IL-1β: Interleukin 1β

IL-6: Interleukin 6

IL-8: Interleukin 8

iNOS: Nitric Oxide Synthase

-K-

KCl: Potassium Chloride

-L-

LDH: Lactate Dehydrogenase

LPO: lipoxygenase

LT B4: Leukotriene Receptor B4

LTC4: Leukotriene C_4

LTE4: Leukotriene E4

-M-

MDA: Malondialdehyde

-N-

NaCl: Sodium Chloride

NADH: Nicotinamide Adenine Dinucleotide

***n-B*uOH: *n*-butanol**

NF-κB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells

NO^{-}: Nitric Oxide

NSAIDs: Non-Steroidal Anti-Inflammatory

-O-

O_2^- : Ion Superoxide

OH: Hydroxyl Group

$ONOO^-$: Peroxynitrite

-P-

PAF: Platelet-Activating Factors

Pamps: Pathogen-Associated Molecular Patterns

PCT: Proximal Coiled Tubule

PG: Prostaglandin

PTGS2: Prostaglandin-Endoperoxide Synthase 2

Pufas: Polyunsaturated Fatty Acids

-R-

RA: Rheumatoid Arthritis

Rcls: Reactive Chlorine Species

RNS: Reactive Nitrogen Species
ROS: Reactive Oxygen Species

Rpm: Revolutions Per Minute

RSS: Reactive Sulfur Species

-S-

SIAs: Steroidal Anti-Inflammatories

SOD: Superoxide Dismutase

-T-

TBA: Thiobarbituric Acid

TBARS: Thiobarbituric Acid Reactive Substances

TBS: Tris-Buffered Saline

TCA: NO^- Trichloroacetic Acid

TGO: Oxaloacetate Glutamate Transaminase

TGP: Pyruvate Glutamate Transaminase

TNF-a: Tumor Necrosis Factor a

TNF-α: Tumor Necrosis Factor α

-U-

UGT2B7: UDP Glucuronosyltransferase-2b7

-W-

WHO: World Health Organisation

Introduction

Introduction

Inflammation, an essential tissue response to extrinsic/intrinsic damage, is a very dynamic process in terms of complexity and extension of cellular and metabolic involvement. The inflammatory response aims to eliminate the pathogenic initiator with limited collateral damage of the inflamed tissue, followed by a complex tissue repair to the preinflammation phenotype. Persistent inflammation is a major contributor to the pathogenesis of many musculoskeletal diseases including ageing-related pathologies such as osteoporosis, osteoarthritis, and sarcopenia **(Gallo *et al.*, 2017; Oronsky *et al.*, 2022)**.

Over the last decade, great progress has been made in understanding the physiopathology of inflammation and the involvement of free radicals in its pathogenesis. The reactive oxygen species (ROS) produced from the action of free radicals on molecular oxygen increase abnormally during inflammation, causing an imbalance between the oxidizing molecules and the antioxidant system of the body. This oxidative stress causes inflammatory cascades that damage the cellular components. The humoral and cellular mechanisms of inflammation are numerous and complex. They involve gene regulatory factors such as the nuclear factor-kappa B (NF-κB) and signaling substances synthesized by immune system cells such as cytokines and prostaglandins **(Amri *et al.*, 2018; Favier and Nikovics, 2023)**. Although numerous anti-inflammatory medications are currently in clinical use, including both steroidal and non-steroidal medications, they all have the potential to cause adverse effects. Therefore, continued screening and development of new effective anti-inflammatories without adverse effects is still necessary **(Sun *et al*., 2016)**.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most extensively consumed drugs worldwide. They are widely used in treating pain and processes induced by mild-to-moderate inflammation, exerting anti-inflammatory, analgesic, and antipyretic effects by suppressing prostaglandin (PG) synthesis, which inhibits the cyclooxygenase (COX) enzyme. Nonselective NSAIDs inhibit COX-1 and COX-2. While COX-1 is a constitutive enzyme found in the kidneys, COX-2 is an inducible enzyme that increases in tissues in response to injury and inflammation **(Devillier, 2001; Mendoza-Fernández *et al.*, 2023)**.

Diclofenac (DF) is a member of the nonsteroidal anti-inflammatory drugs (NSAIDs) and is generally taken for pain-relieving in addition to inflammation and fever treatment. Despite its healing benefits, DF is reported as one of the agents that trigger liver and kidney cell injury. The toxic effects of DF and its metabolites (4′, 5-hydroxydiclofenac) have been linked to mitochondrial injury and the disruption of immune-mediated protective systems according to several

investigations and research. Evidence also showed that DF causing cell necrosis is accompanied by a formation of the reactive oxygen species (ROS), as well as the inhibition of the enzymatic and non-enzymatic antioxidant activity in the kidney and liver cells. Thus, research on medicinal plants has increased in recent years, as they are a valuable resource of protective compounds and are used to develop safer and more potent phytochemical drugs **(Simon *et al*., 2020; Abed Al-Kareem *et al*., 2022)**.

Phytochemicals of medicinal plants are the origin of medicines for healing various health disorders and the potential sources of new drug research and development. The most accessible and easily affordable medicines for primary health care in developing countries remain medicinal plant products **(Gumisiriza *et al*., 2019)**. The phytochemistry of plants has the primary advantage of applying plant products as a potential remedy to different ailments. Because of their minimal side effects, plant-based medicines are the primary remedy to treat different human and live-stock ailments and still have a high acceptance level in the community **(Gonfa *et al*., 2023)**.

Plants contain numerous antioxidants that help to confer protection against free radicals associated diseases. The antioxidant compounds are mostly produced in plants in the form of secondary metabolites. Phytochemicals can be literally referred to as 'plant-chemicals.' They are the non-nutritive chemical components of plants that possess numerous health benefits and disease-preventing properties. The nutrients they contain are non-essential, they are not required by the body for sustaining life. These chemicals are produced by plants to sustain life which in turn confer health benefits to humans upon consumption. There are over a thousand known phytochemicals classified as primary or secondary constituents based on their role in plant metabolism **(Mahmoudi *et al*., 2013; Nwozo *et al*., 2023)**.

Polyphenols are major dietary phenolics comprising polyphenols, phenolic acids, and flavonoids. Flavonoids are the most extensively studied group of polyphenols. The major dietary sources of polyphenols are legumes, cereals, nuts, oilseeds, beverages, fruits, and vegetables. The subclasses of phenols include flavones, flavanols and minor flavonoids. They exhibit their antioxidant potential by preventing the decomposition of hydroperoxide into free radicals and by inactivating free radicals. Flavonoids play important roles in preventing diseases associated with oxidative stress **(Awuchi and Twinomuhwezi, 2021)**.

Phytochemicals and their products are important sources of clinical agents including anti-inflammatory activities. Currently, many products obtained from plants, either in the form of extracts are studied for their potential treatment of inflammatory disorders Phytochemicals which

are mainly responsible for anti-inflammatory activities were reported from the family of polyphenols, terpenoids, flavonoids, saponins, and tannins **(Verma, 2016; Sangiovanni and Dell'Agli, 2021)**.

The genus *Centaurea* which belongs to the Asteraceae family contains about 700 species essentially centered in the Mediterranean region **(Talhouk *et al.*, 2008; Susanna and Garcia-Jacas, 2009)**. In Algeria, it is represented by 45 species including 7 in the Sahara. However, many species of this genus have long been used in traditional medicine for the cure of various ailments such as antidiabetics, diuretics, and antirheumatic, as well as for the treatment of cancer and microbial infections. A variety of secondary metabolites have been reported from different species of this genus such as sesquiterpene lactones and flavonoids which are biologically active **(Azzouzi *et al.*, 2016; Sharonova *et al.*, 2021)**.

In consideration of the preceding data, it is of interest to evaluate the possible anti-inflammatory and protective role of polyphenols from *Centaurea sp.* against diclofenac-induced toxicity in *Wistar* rats. To achieve the aforementioned objectives and respond to the problem posed in the present study, we first conducted a bibliographical study. The second part of this thesis outlines the methodology followed, while the third part presents all the results obtained and the discussion.

Chapter I :

Bibliographic synthesis

I. Inflammation

I.1. Definition

The immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation is called inflammation (table1). This term is an age-old, ancestral word, which comes from the Latin *inflammare*, meaning to ignite or burn **(Chen *et al*., 2018; Oronsky *et al*., 2022)**.

More recently, inflammation has been described as a succession of alterations that take place in a living tissue when it is injured, provided that the damage is not significant enough to immediately destroy its structure and vitality. It participates importantly in host defences against infectious agents and injury, as it functions by eliminating injurious stimuli and initiating the recovery process **(Chen *et al*., 2018; Karrat *et al*., 2022)**.

At the tissue level, redness, swelling, heat, pain, and loss of tissue function are the main characteristics of inflammation which result from local immune, vascular and inflammatory cell responses to infection or injury **(Chen *et al*., 2018)**.

I.2. Inflammatory inducers

Inflammation's inducers can be classified into two main groups as follow in the table 1:

Table 1 : inflammatory inducers classification (Hannoodee and Nasuruddin, 2020)

Exogenous inducers		**Endogenous inducers**
Microbial inducers	**Non-Microbial inducers**	Signals released by dead, damaged, malfunctioned, or stressed's tissues
• Pathogen-Associated Molecular Patterns (PAMPs) • Virulence factors restricted to pathogens	• Allergens, • Toxic compounds, • Irritants, • Foreign bodies	

Inflammatory inducers can also be divided into infectious and non-infectious factors :

- **Infectious factors**

Including bacteria, viruses, and other microorganisms.

- **Non-Infectious factors**

This category includes physical injuries such as frostbite, burn, physical injury, foreign bodies, trauma, ionizing radiation, chemical compounds such as glucose, fatty acids, toxins, alcohol, and chemical irritants such as nickel and other trace elements **(Gallo *et al.*, 2017; Hannoodee and Nasuruddin, 2020)**.

I.3. Types of inflammation

Based on the time of the process that responds to the injurious cause, inflammation can be divide into two types**:**

I.3.1. Acute inflammation

The body's immediate response to an aggressive agent is known as acute inflammation. Its duration varies from a few days to a few weeks, depending on the degree of injury. Its main features are the exudation of plasma fluids and proteins (edema) and the migration of leukocytes (primarily neutrophils) from blood vessels to the inflammatory site (injured tissue). Innate immunity will play a role in the direct elimination of the pathogen, but it also enables the triggering of the adaptive response that will help eradicate the hazard. Acute inflammations heal spontaneously or with treatment, but can leave sequelae if tissue destruction is extensive (figure 1) (**Raghavendra *et al.*, 2015; Noack and Kolopp-Sarda, 2018)**.

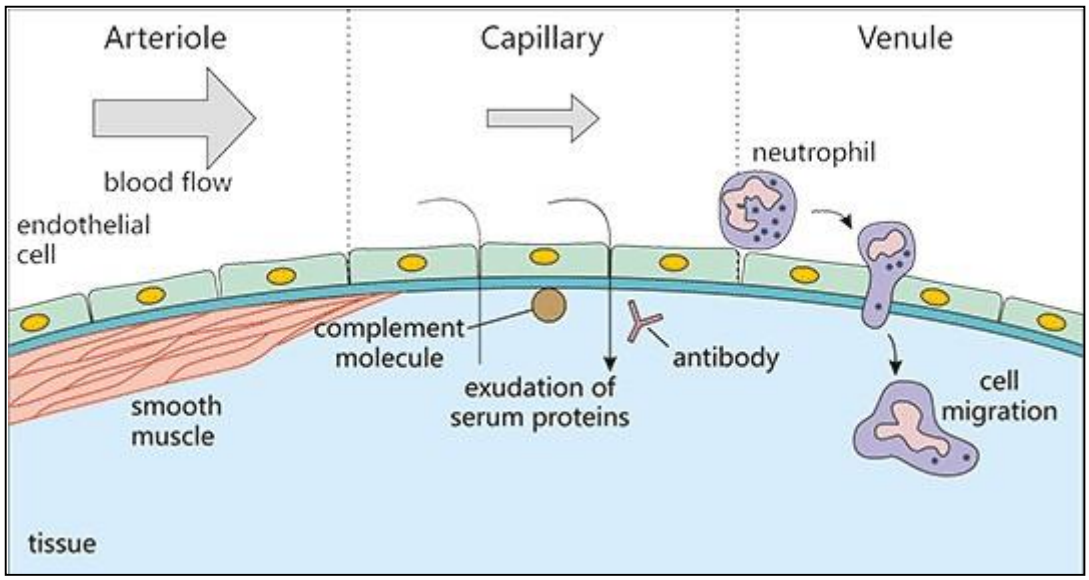


Figure 1: The stages of acute inflammation (Hannoodee and Nasuruddin, 2020)

Acute inflammation evolves through 3 phases :

I.3.1.1. The vascular phase

It is characterized by the activation of platelets and the first soluble mediator. Platelets are activated very rapidly and intervene with coagulation factors in the plasma, closing the gap and limiting pathogen access to the body. Activated platelets also release proteins with strong aggregation and vasoconstriction properties (construction of vessel diameter). The vasoconstrictive effect is very short-lived and helps limit blood leakage. Many soluble mediators present in the blood are activated very rapidly (complement system, kinin system, etc.). Their purpose is to stimulate and mobilize innate immune cells to the site of inflammation. The complement system plays a role in vasodilation, increasing vascular permeability and attracting circulating cells to the damaged site. The kinin system plays a role in vascular permeability, enabling immune cells to reach tissue level, but also in the sensation of pain through their interaction with sensory neurons, which are cells that act as "pain sensors" (figure 2) **(Mathieu and Guimezanes, 2011)**.

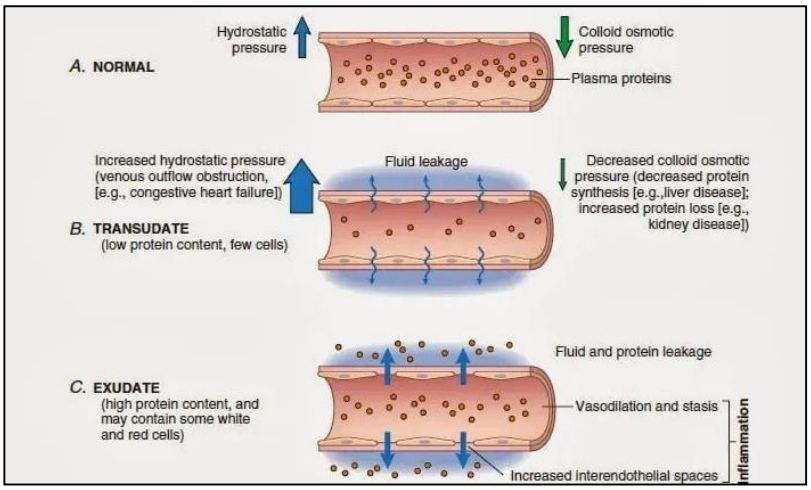


Figure 2: Transudate and exudate formation (Kumar et al., 2007)

I.3.1.2. The cellular phase

Inflammatory cells come from two sources: the blood, such as neutrophils, or the tissue itself, such as phagocytic cells **(Gallo *et al*., 2017)**.

- **Blood cells**

Chemotaxis is the phenomenon responsible for the migration of polymorphonuclear cells, monocytes and lymphocytes toward the lesion site. Neutrophils are present from the first hours

and disappear after 48 hours, by which time monocytes and macrophages have become abundant **(Gallo *et al*., 2017; Li *et al*., 2017)**.

- **Tissue-derived cells**

Histiocytes are tissue-resident macrophages (liver Küpffer cells, lung alveolar macrophages, brain microglia). Mast cells, containing granules rich in histamine and serotonin, also reside in tissues **(Klein-Wieringa *et al*., 2016)**.

I.3.1.3. The resolution and repair phase

Once the aggression has been brought under control, the inflammatory reaction is halted. Macrophages not only clean up cellular debris but also secrete cytokines enabling tissue repair by fibroblasts (collagen) and endothelial cells (neoangiogenesis).

Anti-inflammatory cytokines such as IL-10 gradually replace the inflammatory mediators, inhibiting their secretion and action. Inflammation is now in the resolution phase. Once immune cells are no longer required at the inflammatory site, they will either leave the tissue or die, through loss of survival signals or apoptosis. The mechanisms of acute inflammation are identical whatever the triggering agent **(Noack and Kolopp-Sarda, 2018)**.

I.3.2. Chronic inflammation

The failure of acute inflammation leads to chronic inflammation and numerous pathologies. It is characterized by a prolonged course that can extend over months or even years, with chronic inflammation defined by a duration of more than six weeks **(Heymonet, 2013; Noack and Kolopp-Sarda, 2018).** Chronic inflammation can occur as a result of :

- A failure to eliminate an agent causing acute inflammation : such as infectious organisms that can resist host defenses and remain in the tissue for a prolonged period **(Pahwa *et al*., 2021)**.
- A low-level exposure to a particular irritant or foreign material : which cannot be eliminated by enzymatic degradation or phagocytosis in the body, such as silica dust **(Pahwa *et al*., 2021)**.
- An autoimmune disease : in which the immune system recognizes the body's normal component as a foreign antigen and attacks healthy tissue giving rise to diseases such as rheumatoid arthritis (RA) **(Pahwa *et al*., 2021)**.

- A defect in the cells responsible for mediating inflammation : leading to persistent or recurrent inflammation, such as autoinflammatory disorders **(Pahwa *et al*., 2021)**.

Unlike in acute inflammation, vascular and cellular phases do not follow one another, but coexist throughout the evolution of this inflammation. The cellular infiltrate at the inflammatory site persists, contributing to tissue hyperplasia and destruction. The microenvironment plays a key role in this process. Indeed, the production of cytokines and chemokines will promote the survival and maintenance of cells on the inflammatory site **(Kada, 2018; Noack and Kolopp-Sarda, 2018)**.

The mechanisms and mediators involved in the process of chronic inflammation are similar in different chronic inflammatory diseases such as rheumatoid arthritis (RA), psoriasis or chronic inflammatory bowel disease (IBD) such as Crohn's disease (CD) **(Libby, 2007)**.

I.4.Inflammatory mediators

The inflammatory response is a vital aspect of the tissues' responses to deleterious inflammatory agents. This complex response involves many inflammatory cells (leukocyte cells). In response to the inflammatory process, these cells release specialized substances that mediate the inflammatory process by preventing further tissue damage and ultimately resulting in healing and restoration of tissue function **(Abdulkhaleq *et al*., 2018)**.

I.4.1.Inflammatory cells

The inflammatory site is rapidly filled with cells from the blood (polymorphonuclear cells, monocytes, lymphocytes) and local connective tissue (fibroblasts, endothelial cells, mast cells and resident macrophages) **(Karrat *et al*. 2022)**.

I.4.1.1. Monocytes and macrophages

They are considered the main cells of chronic inflammation. They play a key role in tissue destruction and the maintenance of the inflammatory process. They are particularly involved in amplifying inflammation through the massive release of inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12), chemotactic factors (IL-8), prostaglandins or leukotrienes, which contribute to the recruitment and activation of other immune cells **(Mouffouk *et al*., 2019)**.

I.4.1.2. Eosinophilic Polynuclear Cells

Eosinophilic polynuclear cells account for only 1-3% of leukocytes and release various inflammatory mediators such as PAF and LT B4. There is a wide variety of pro-inflammatory cytokines (IL-1, IL-6, IFN-y, TNF-a) **(Khaddache *et al*., 2017)**.

I.4.1.3. Platelets

During inflammation, blood platelets can produce a multitude of soluble mediators whose role can be pro-thrombotic, regulating the activity of neighboring cells but also pro-inflammatory **(Tariket *et al*., 2019)**.

I.4.1.4. Vascular endothelial cells

The entire cardiovascular system is lined with a monolayer of endothelial cells, regulating all the steps involved in the trans-endothelial transport of leukocytes to the inflammatory site. Endothelial cells can participate in post-inflammatory repair phenomena through the production of matrix proteins and various proteases **(Pober and Sessa, 2007; Davoine and Lacy, 2014)**.

I.4.2. Chemical mediators

Inflammation involves cells (granulocytes, mast cells, macrophages, platelets, fibroblasts, lymphocytes and endothelial cells) and a variety of molecules, including : cytokines, interleukins, nitric oxide, lipid mediators and even oxygen free radicals. The table below (table 2) shows the origin and effect of the most important inflammatory mediators:

Table 2: Cellular origins and effects of the main mediators involved in the development of the inflammatory response (Davoine and Lacy, 2014)

Mediators	Origins	Effects
Histamine	Mast cells, basophils, eosinophils and platelets	Ensures vasodilation Increases vascular permeability, Induces expression of adhesion molecules on vascular endothelium.
Serotonin	Mast cells and platelets	Increases vascular permeability, Dilates capillaries and stimulates smooth muscle contraction.
Prostaglandin	leukocytes	Vasodilation, Reinforces the action of histamine, Increases neuronal sensitivity and is responsible for pain.
Cytokines	Macrophages and lymphocytes	Acting on membrane receptors, they can be pro-inflammatory (IL-1β, IL-6, or TNFα) or anti-inflammatory (IL-10). Involved in tissue repair.
Platelet-activating factors (PAF)	Platelets, neutrophils, monocytes and endothelial cells	Vasodilation, Increases vascular wall adhesiveness, Stimulates platelet aggregation, Induces ROS production and lysosomal enzyme release by neutrophils, eosinophils and macrophages.
Leucotreine	Arachidonic acid	Vasodilatation; Increased vascular permeability, Involved in edema formation, chemotactic properties.

I.4.2.1. Lipid mediators

A variety of biological effects and origins are associated with inflammatory mediators (Table 2). These mediators are produced by macrophages, mast cells and endothelial cells. They are derived from cell membrane phospholipids via phospholipase and contain mainly arachidonic acid metabolites (figure 3) **(Bennett and Gilroy, 2017; Oronsky *et al*., 2022)**.

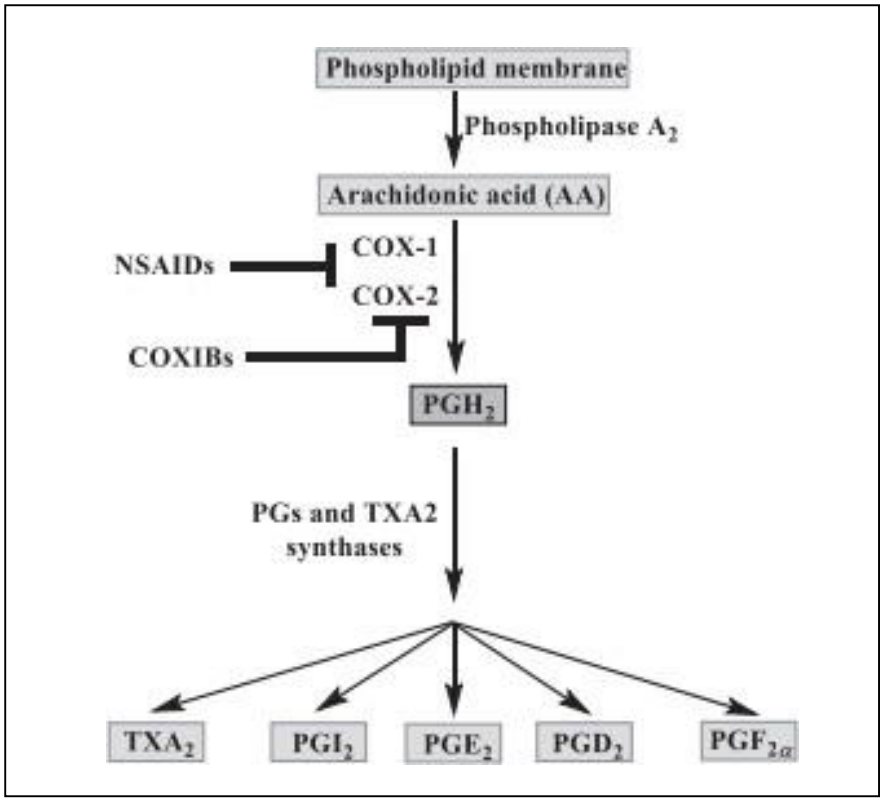


Figure 3 : A diagram of the biochemistry of prostanoids (Ju et al., 2022)

Arachidonic acid is metabolised via two distinct pathways:

a- Lipooxygenase pathway

- **Lipoxygenases** are non-heme dioxygenases that participate in the oxidation of arachidonic acid, resulting in the formation of hydroperoxyacids and leukotrienes (LTB4, LTC4, LTD4 and LTE4), as well as other compounds that exhibit structural and functional similarities to leukotrienes (lipoxins and hepoxylins) (**Kytikova *et al*., 2019**).
- **Leukotrienes**: increase vascular permeability and exert a chemotactic action on neutrophils. In association with prostaglandins, they can trigger all the symptoms characteristic of inflammation. They cause platelet aggregation, as well as bronchoconstriction with bronchial hypersecretion **(Maskrey *et al*., 2011)**.

b- Cyclooxygenase pathway

- **Cyclooxygenase type 1or COX1**: constitutive, present in the stomach, kidney and thrombocytes, it allows the synthesis of prostaglandins in the stomach and kidneys, prostacyclins in the gastric mucosa and endothelial cells and thromboxanes A2 at the platelet level.

- **Cyclooxygenase type 2 or COX2**: COX-2 has 60% homology with COX-1 and is inducible. Several pro-inflammatory cytokines like TNFα, IL-1!, IL-6, as well as growth factors, pathogens (e.g., LPS) are capable of inducing the expression of COX- 2. The COX-2 gene is located on chromosome 1 (PTGS2 gene) and has an NF-κB response element in its promoter as well as other cytokine-dependent response elements such as IL-6 (figure 4) (**Ahmed, 2011; Oronsky *et al*., 2022**).

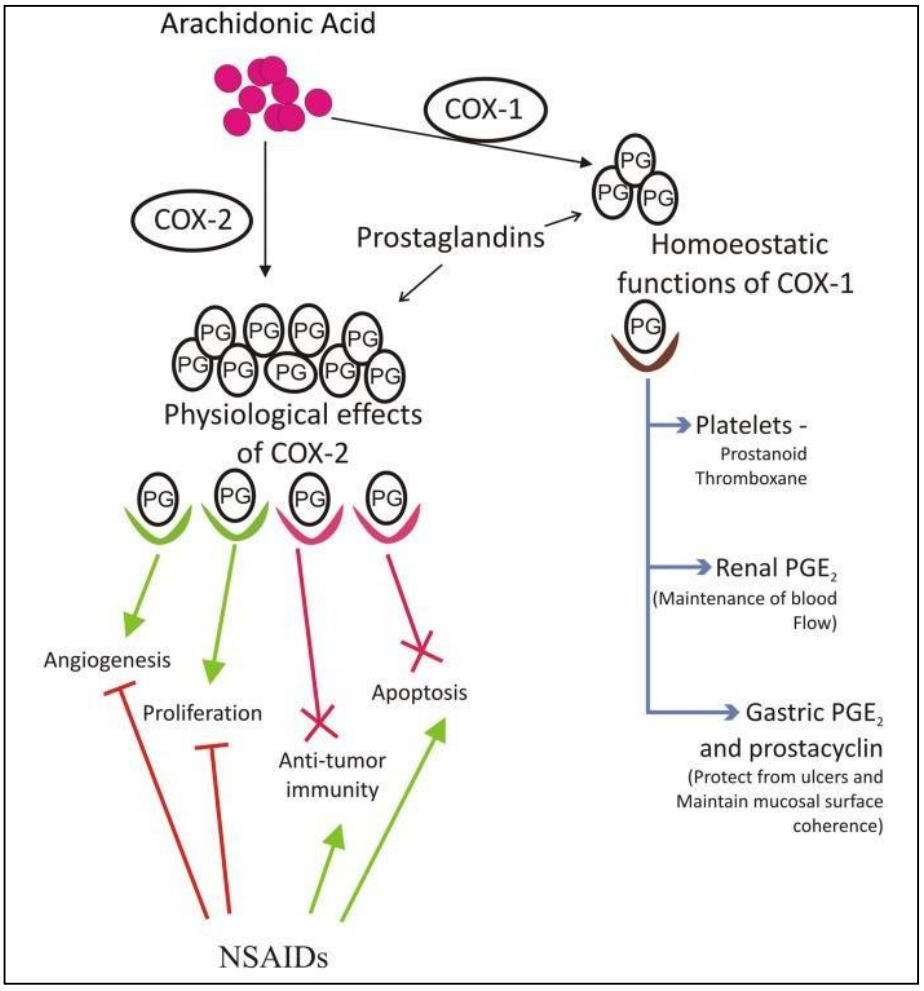


Figure 4: Arachidonic acid is converted to prostaglandins by action of cyclooxygenase 1 and 2 Cox-1 and Cox-2 enzymes (Gandhi et al., 2017)

I.5. Stress and inflammation

Oxidative stress has been implicated in the pathogenesis of many chronic diseases including the inflammatory process. Oxidative stress and inflammation are two closely interrelated and interdependent physiopathological processes and both processes are simultaneously found in many pathological conditions. On one hand, ROS initiate an intracellular signalling cascade that enhances proinflammatory gene expression. On the other hand, inflammatory cells secrete large amounts of ROS and RNS to kill invading pathogens as well as immune mediators (i.e. cytokines and chemokines) leading to induced oxidative stress and tissue injury at the site of inflammation.

Furthermore, Oxidative stress and inflammation are two phenomena that are directly involved in practically all pathologies, especially in ageing and cardiovascular diseases **(Ahmad and Ahsan, 2020; Aleksandrova *et al*., 2021)**.

I.6. Organ-specific inflammatory responses

Inflammation has long been recognized as a major cause of disease. It is estimated that some 15% of human cancers are associated with chronic infection and inflammation. Acute and chronic inflammation-mediated tissue injury is observed in many organ systems, including the heart, pancreas, liver, kidney, lung, brain, intestinal tract, and reproductive system **(Chen *et al.*, 2018; Chhabra *et al*., 2021)**.

I.6.1. Liver

Inflammation in the liver protects this organ from infection and injury, but excessive inflammation may lead to extensive loss of hepatocytes, ischemia-reperfusion injury, metabolic alterations, and eventually permanent hepatic damage. Inflammation can destroy hepatic parenchymal cells, increasing the risk of chronic liver diseases, such as non-alcoholic fatty liver disease or viral hepatitis **(Leitão *et al*., 2017)**.

I.6.2. Kidney

Kidney inflammation contributes to progressive renal injury, which may lead to glomerulonephritis, end-stage renal disease, or acute or chronic kidney disease (CKD). Approximately 10–12% of the population suffers from CKD, and some 50% of elderly patients show signs of kidney dysfunction, which is associated with high morbidity and mortality.

Kidney inflammation is most commonly induced by infection, ischemia/reperfusion, in situ immune-complex formation/deposition, or complement pathway dysregulation. CKD and acute

kidney injury (AKI) are the most severe types of kidney disease. Interstitial inflammation and tubular injury are commonly observed in acute and chronic kidney injury cases. Renal tubular epithelial cells are likely important promoters of kidney inflammation, secreting a variety of inflammatory cytokines in response to both immune and non-immune factors, and leukocyte infiltration depends on the local presence of these cytokines **(Ernandez and Mayadas, 2016)**.

II. Anti-inflammatory drugs

Anti-inflammatory therapy is designed to control the excess of specific tissue reactions and avoid the transformation of the acute phase of inflammation into a chronic phase. It is generally carried out by synthetic molecules of the non-steroidal or steroidal anti-inflammatory type (corticoids). These drugs are widely used, but their side effects are sometimes serious, in particular, their toxicity on the hepatic renal and digestive **(Trabsa, 2015; Moura *et al*., 2018)**.

II.1. Steroidal anti-inflammatories (SIAs)

Steroidal anti-inflammatories or glucocorticoids are synthetic derivatives of cortisone, naturally secreted by the adrenal glands. They are powerful anti-inflammatories with immunomodulatory and anti-allergic properties. They all have a hormonal effect on metabolic regulation (notably carbohydrate, protein and lipid), and put the adrenal glands to rest via a hypothalamic-pituitary braking mechanism **(Heymonet, 2013; De Labry Lima *et al*., 2021)**.

Glucocorticoids can inhibit all phases of the inflammatory reaction, through their direct action on vessels, they reduce the vascular phenomena of inflammation. As well, through their antiproliferative effect on histiocytes-macrophages of all types, lymphocytes, plasma cells, fibroblasts and neutrophils, they inhibit the early and late cellular phenomena of inflammation **(Paglia, 2021)**.

II.1.1. Mechanism of action of SIAs

Corticoids have original mechanisms of action which are essentially genomic (Transcriptional), characterized by the activation (Transactivation) or inhibition (Trans-repression) of numerous target genes. These actions are exerted in numerous cells involved in innate immunity (Macrophages, polynuclears, mast cells), in adaptive immunity (Lymphocytes) but also other cells (Fibroblasts, epithelial and endothelial cells) **(Mekenza and Medjmedj, 2018)**.

Glucocorticoids are carried by the transport proteins transcortin and albumin. They cross cell membranes by diffusion. In the cytoplasm, they bind to a specific receptor belonging to the steroid receptor superfamily. After glucocorticoid binding, the glucocorticoid-receptor complex migrates to the nucleus and acts directly on DNA by binding to specific sequences called GRE (Glucocorticoid response element), thus intervening in the regulation (activation or inhibition) of target gene transcription (figure5) **(Heymonet, 2013)**.

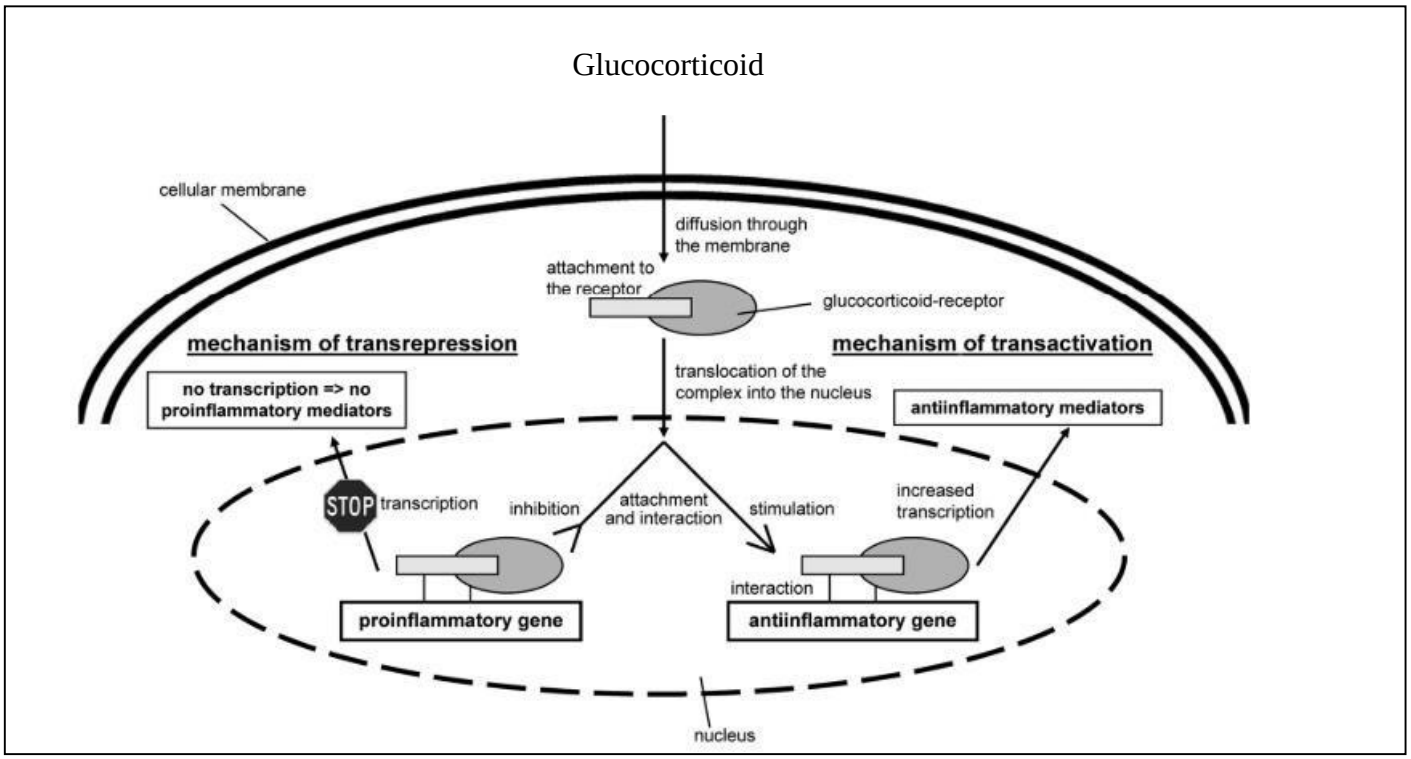


Figure 5: Mechanism of action of glucocorticoids (Zang et al., 2011)

This stimulates the synthesis of a protein, lipocortin, which inhibits phospholipase A2, thereby reducing the release of arachidonic acid from membrane phospholipids and thus the formation of the inflammatory mediators prostaglandins and leukotrienes. Glucocorticoids also reduce the synthesis of a series of proteins important for inflammatory phenomena (interleukins and other cytokines, phospholipase A2, cyclooxygenase 2). Corticoids also regulate cell activation and survival (apoptosis), which explains their cytostatic efficacy in certain hematological malignancies **(Lùllmann *et al*., 1996; Paglia, 2021)**.

- **Unwanted effects**

Even high doses of glucocorticoids have virtually no side effects when administered for short periods. Long-term administration of glucocorticoids leads to a tendency to infection and impaired healing processes. Exaggerated glucocorticoid activity leads to :

- Increased gluconeogenesis and glucose release. Under the action of insulin, glucose is converted into triglycerides and in the event of an insufficient increase in insulin secretion, "steroid diabetes" is observed **(Lùllmann *et al*., 1996)**.

- Increased protein degradation, with skeletal muscle atrophy, osteoporosis, growth impairment in children and skin atrophy. The consequences of cortisol's mineralocorticoid activity are water and sodium retention, increased blood pressure, edema and potassium loss, with the risk of hypokalemia **(Lùllmann *et al*., 1996)**.

II.2. Non-steroidal anti-inflammatory (NSAIDs)

Non-steroidal anti-inflammatory **(**NSAIDs), unlike glucocorticoids, are a group of different synthetic chemical classes with a non-steroidal structure. They are symptomatic drugs capable of opposing the inflammatory process, whatever the cause (mechanical, chemical, infectious, immunological), and are highly effective for pain and inflammation. Due to their properties, this therapeutic class is one of the most widely used in the world (4.5% of drug consumption in industrialized countries) **(Saleh & Buxeraud, 2021; Bhat *et al*., 2024)**.

II.2.1. Mechanism of action

NSAIDs anti-inflammatory effects are primarily achieved through inhibiting prostaglandin production. During inflammation, phospholipase A2 is activated, transforming membrane phospholipids into arachidonic acid, which is then metabolized into prostaglandins (PG) by cyclooxygenase (COX). Non-steroidal anti-inflammatory drugs act on this phase of inflammation by inhibiting cyclooxygenase and consequently prostaglandin synthesis (figure 6).

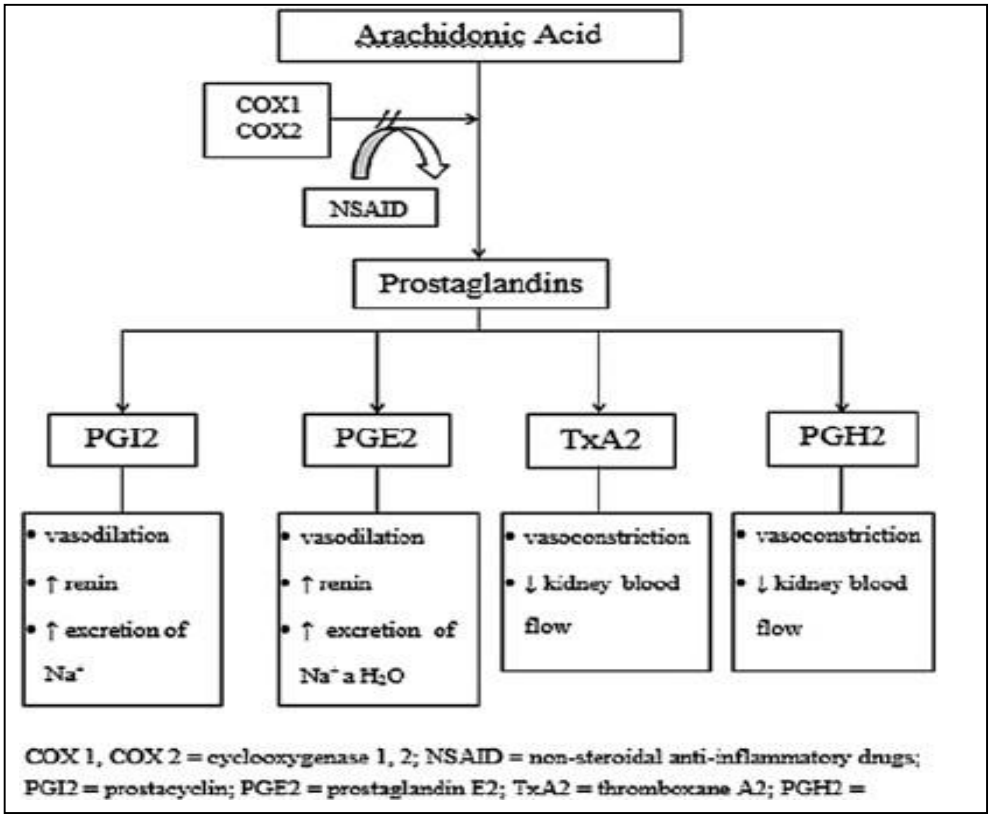


Figure 6: Mechanism of action and effects of non-steroidal anti-inflammatory drugs (Kalafutova, 2014)

NSAIDs inhibit the production of primary prostanoids by blocking arachidonic acid's access to the active site of cyclooxygenases (COX). Since the prostanoids produced by COX-1 appear to play a physiological role (protection of the gastric mucosa, platelet aggregation, vascular homeostasis, maintenance of water-sodium balance), while those produced by COX-2 appear to be involved mainly in the inflammatory response and in some processes associated with cell proliferation, the hypothesis was born that NSAIDs specifically inhibiting COX-2 could theoretically retain the therapeutic properties of NSAIDs while having fewer adverse effects, thanks to the maintenance of physiological prostaglandin production **(Bindu *et al*., 2020)**.

- **Unwanted effects**

With their anti-inflammatory properties, NSAIDs, which are friends in need often become redoubtable enemies (due to their non-specific cytotoxic eff ets), leading to multiple organic pathologies, the most important of them are:

- **Risk of gastric mucosal injury**

In the treatment of chronic inflammatory conditions by NSAIDs, the development of gastric mucosal injury creates a major limitation. The duration and dose of NSAID usage are the major factors that affect the severity of complications **(Bindu *et al*., 2020)**.

- **Risk of cardiovascular disease**

Over the years, a large number of scientific reports have associated NSAIDs with CVD and have observed resistance to antihypertensive treatment, hypertensive relapse, congestive heart failure, arterial thrombotic risk (If taken for a long time or in high doses) **(Gungormez, 2015)**.

- **Risk of renal injury**

NSAIDs carry a significant risk of kidney damage which includes multiple nephrological complications like acute kidney injury (AKI), and chronic kidney disease (CKD) encompassing electrolyte imbalance, glomerulonephritis, fluid retention-induced hypertension, hyponatremia and hyperkalemia, renal papillary necrosis, renal tubular acidosis to name a few **(Bindu *et al*., 2020)**.

- **Risk of hepatotoxicity**

Hepatotoxicity is another serious complication that has been linked to NSAIDs although the incidences are less frequent compared to that of gastrointestinal damage, CVD and renal insufficiency **(Bindu *et al*., 2020)**.

II.3. Herbal anti-inflammatories

Though a number of synthetic anti-inflammatory drugs like steroids, nonsteroidal anti-inflammatory drugs (NSAIDs) and immunosuppressants are well-established for use in inflammatory disorders, their long term use is limited by the associated side effects **(Ghasemian *et al*., 2016; Gupta *et al*., 2021)**.

Therefore, the need of safe, easily available and cost effective treatments of inflammatory disorders led to the exploration of plant based drugs. A number of medicinal plants are being used successfully in the treatment of inflammation since ancient times and are being successfully converted into their convenient, elegant and effective dosage forms for use in the context of modern medicines. Several plant derived chemical constituents such as alkaloids, tannins, flavonoids, terpenoids, glycosides, carotenoids and saponins have been reported to possess anti-inflammatory properties **(Gupta *et al*., 2021)**.

Medicinal plants and their secondary metabolites are progressively used in the treatment of diseases as a complementary medicine.A large number of photochemical compounds are derived

from the plant and fungal kingdoms, with a wide range of biological activities. Some of them have anti-inflammatory properties and specifically target COX-1, -2, lipoxygenase, nitric oxide, phospholipase A2...etc. These molecules are interesting. They are growing to offer benefits over traditional anti-inflammatories with fewer side effects **(Ghasemian *et al.*, 2016; Sami *et al.*, 2021)**.

II.4. Diclofenac

Diclofenac (DF) is one of the most commonly prescribed anti-inflammatory drugs. It is available on the market for uncontrolled use (self-medication), which can entail major risks after long-term use or overdose. First introduced in Europe in 1973, diclofenac is an NSAID belonging to the family of phenylacetic acids, it has an established role in the treatment of acute and chronic pain and acts to decrease inflammation as other class drugs do. It also has analgesic properties and antipyretic effects that are shared by other NSAIDs (figure 7) **(Schmidt *et al.*, 2018)**.

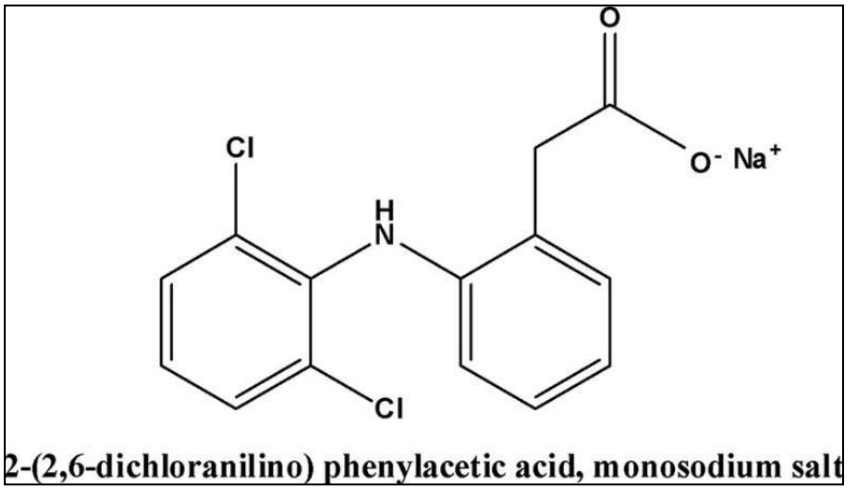


Figure 7: Chemical structure of diclofenac sodium (Sulaiman and Al-Jabari, 2021)

II.4.1. Mechanism of action

Diclofenac acts by inhibiting the activity of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) by inhibiting the synthesis of prostanoids such as prostaglandin-E2 (PGE2), prostacyclins, and thromboxanes, which are vital components of the inflammatory and nociceptive response. It binds to COX-1 and COX-2 and inhibits competitively arachidonic acid. COX-1 and COX-2 are inhibiting relatively equally. During *in vitro* experimentation, evidence suggests that it has selective COX-2 inhibition, about four times that of the inhibition of COX-1.

It is considered one of the most effective inhibitors of the production of PGE2; the primary prostanoids are elevated during an inflammatory response.

Diclofenac's peripheral analgesic effects are attributable to its activity in decreasing the availability of sensitized peripheral pain receptors via down-regulation, which appears to be accomplished by stimulating the L-arginine nitric oxide cGMP pathway via activation of ATP-sensitive potassium channels. Also, evidence suggests that diclofenac also has activity in reducing the previously increased levels of substance P, a known pro-inflammatory neuropeptide with nociceptive activity in the synovial fluid of patients with rheumatoid arthritis (figure 8) **(Alfaro and Davis, 2020)**.

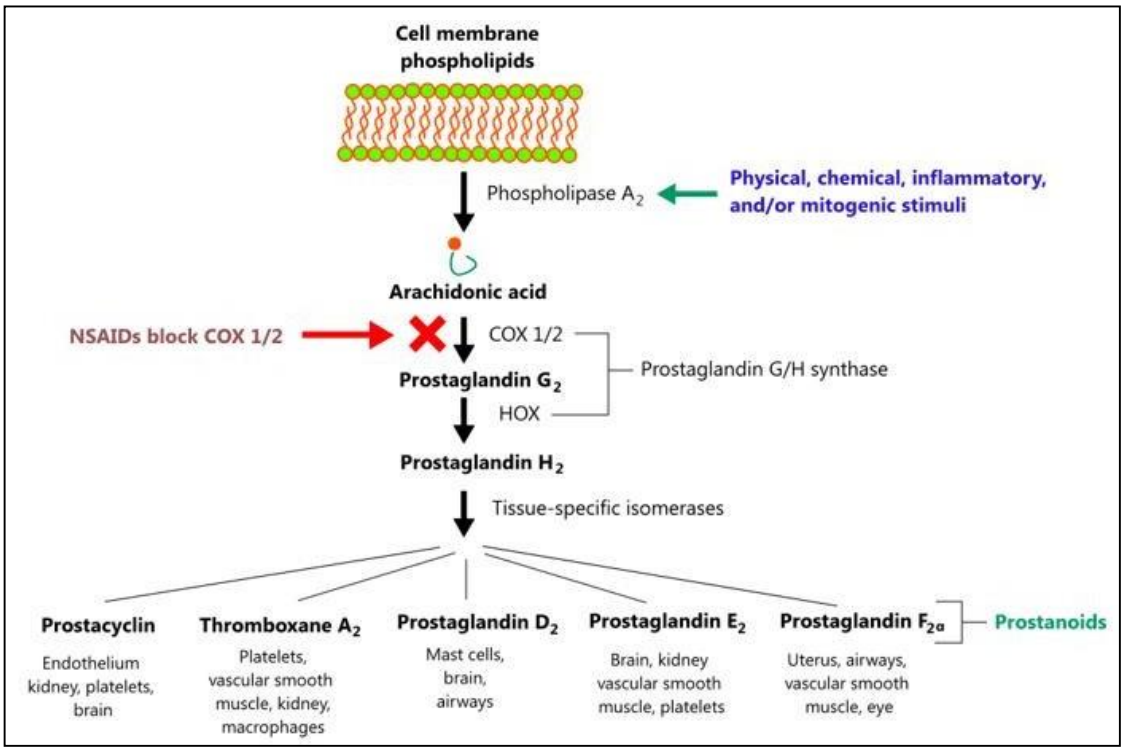


Figure 8: Mechanism of action of NSAIDs including diclofenac (Curtis et al., 2019)

II.4.2. Metabolism

Diclofenac metabolism extensively occurs in the liver, where the conjugation of diclofenac to glucuronic acid takes place. The conjugation to uronic acid is aided by the enzyme UDP glucuronosyltransferase-2b7 (UGT2B7). The resultant metabolite, acyl glucuronide, reacts with the sulfhydryl groups of proteins. Acyl glucuronide can be metabolized into 4-hydroxy diclofenac

acyl glucuronide by enzyme cytochrome P4502C8 (CYP2C8), which forms benzoquinone imine, resulting in the oxidative bioactivation of diclofenac. For the most part, this phenylacetic acid drug metabolizes into 4′hydroxyl metabolite, along with other minor metabolites, viz., 3′ hydroxyl metabolite and 5′hydroxy metabolite. Cytochrome P450 enzyme catalyzes the 4′ and 3′ hydroxylation and cytochrome P450 3A4 catalyzes the formation of the 5′ hydroxyl metabolite. More than 60% of the administered dose of diclofenac is excreted through the urine, whereas the remainder is removed as bile conjugates or metabolites of diclofenac. The concentration of major metabolite, 4′hydroxyl derivative, has been observed at levels around 30% in urine and 20% in bile. As diclofenac has a short half-life of 2 h, repeated doses are required to maintain its plasma concentration to manage certain serious conditions (figure 9) (**Amanullah *et al*., 2022**).

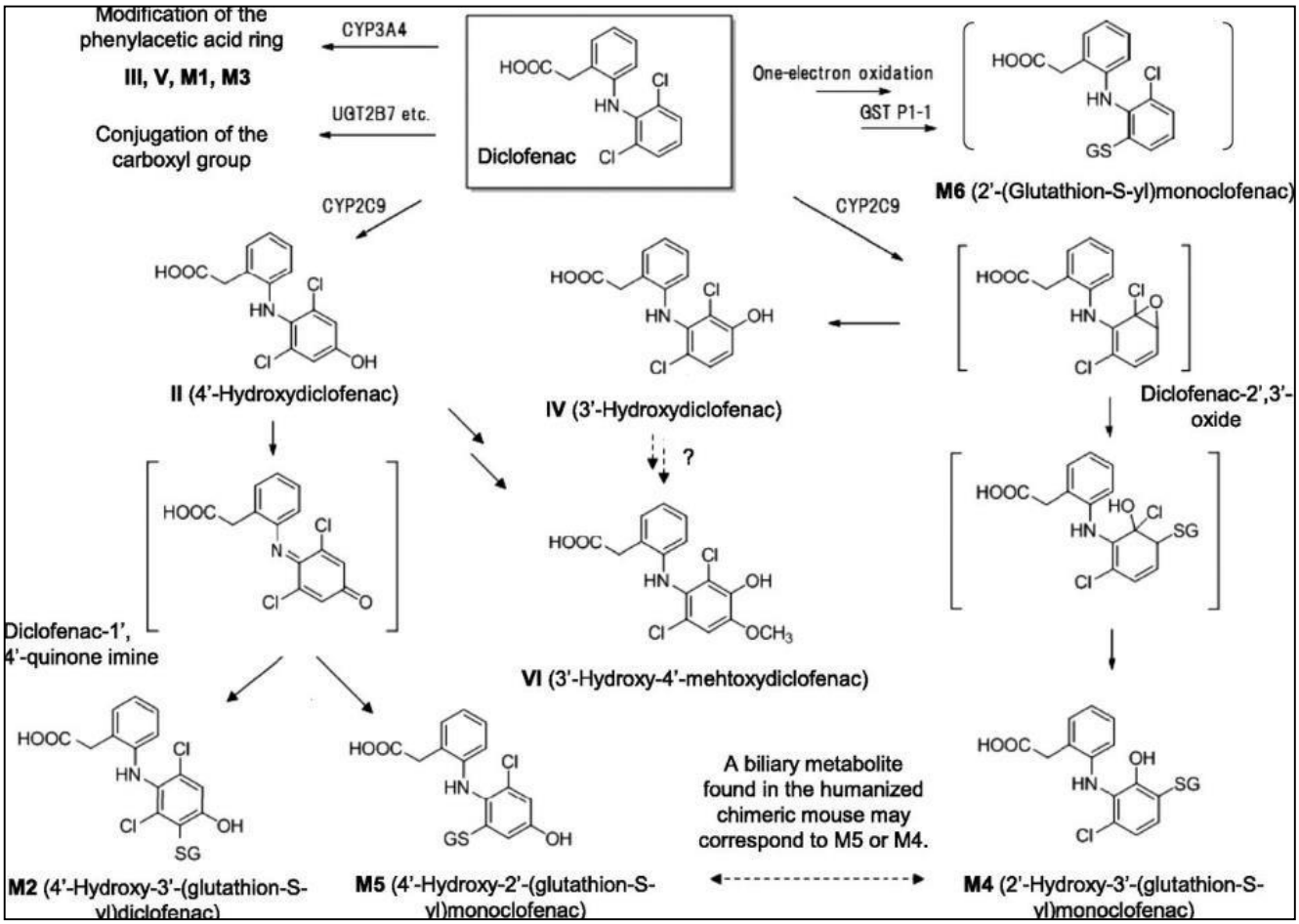


Figure 9: Diclofenac metabolism in humans (Amanullah et al., 2022)

II.4.3. Unwanted effects

The most frequent side effects are:

- Digestive : nausea, vomiting, diarrhea, bloating, constipation, abdominal pain, gastritis, aggravation of chronic inflammatory bowel disease (Crohn's disease, ulcerative colitis). Ulcers of the stomach or duodenum, particularly in the case of high-dose or prolonged treatment, or in the elderly. Exceptional cases of digestive tract hemorrhage (bloody vomiting, black stools or, more often, imperceptible blood loss, leading to anemia).
- Allergic reaction: asthma attack, anaphylactic shock.
- Skin rash, urticaria, itching. Exceptional cases: photosensitization, severe skin allergy (bullous eruptions), an infectious complication in case of chickenpox.
- Hypertension, edema.
- Water retention may aggravate cardiac insufficiency.
- Renal failure, particularly in cases of cardiac insufficiency, dehydration or diuretic treatment.
- Headaches, dizziness. More rarely: drowsiness or insomnia, irritability, tremors. Exceptional: visual disturbances, ringing in the ears.
- Increased transaminases, hepatitis (rare).
- Very rare blood count abnormalities **(Tamazirt, 2017)**.

II.4.4. Diclofenac toxicity

Diclofenac is mainly metabolized and eliminated by the liver and kidneys, that what makes them susceptible to toxicity.

II.4.4.1. Hepatotoxicity

- ***Liver tissue***

The liver is not only the largest organ in the body but also the one playing one of the most important role in the human metabolism as it is in charge of transforming toxic substances in the body. It is a critical organ in the human body responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, and vitamin storage, among other functions. It comprises around 2% of an adult's body weight. The liver is unique due to its dual blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%) **(Kalra *et***

***al*., 2018; Lorente *et al*., 2020)**. 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 to the rear. Two ligaments are visible anteriorly. Superiorly, the falciform ligament separates the right and left lobes. Inferior to the falciform ligament is the round ligament, which protrudes slightly from the liver. The caudate lobe lies above, 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 is the porte hepatis, where the hepatic artery and hepatic portal vein enter the liver. The portal vein carries nutrient-laden blood to the digestive system. Below the porta hepatis is the bile duct, which leads to the gallbladder. The liver is supported posteriorly by a system of mesenteries, and is also attached to the diaphragm via the falciform ligament **(Lorente *et al*., 2020)**.

Liver cells consist of hepatocytes and cholangiocytes. Hepatocytes are highly polarized epithelial cells, where their basolateral surface is directly connected to sinusoidal endothelial cells to facilitate the exchange of materials between hepatocytes and blood vessels. Tight junctions between hepatocytes enable the formation of canaliculi. These bile canaliculi collect bile salts and acids, which are transported through the apical surface of the hepatocytes to the bile ducts, where they are finally stored in the gallbladder before release into the duodenum **(Brinkløv and Warrant, 2017)**.

Hepatocytes perform most of the functions generally associated with the liver. They extract and process nutrients and other materials from the blood, and produce both exocrine and endocrine secretions. Liver function is also supported by non-parenchymal cells, comprising numerous cell

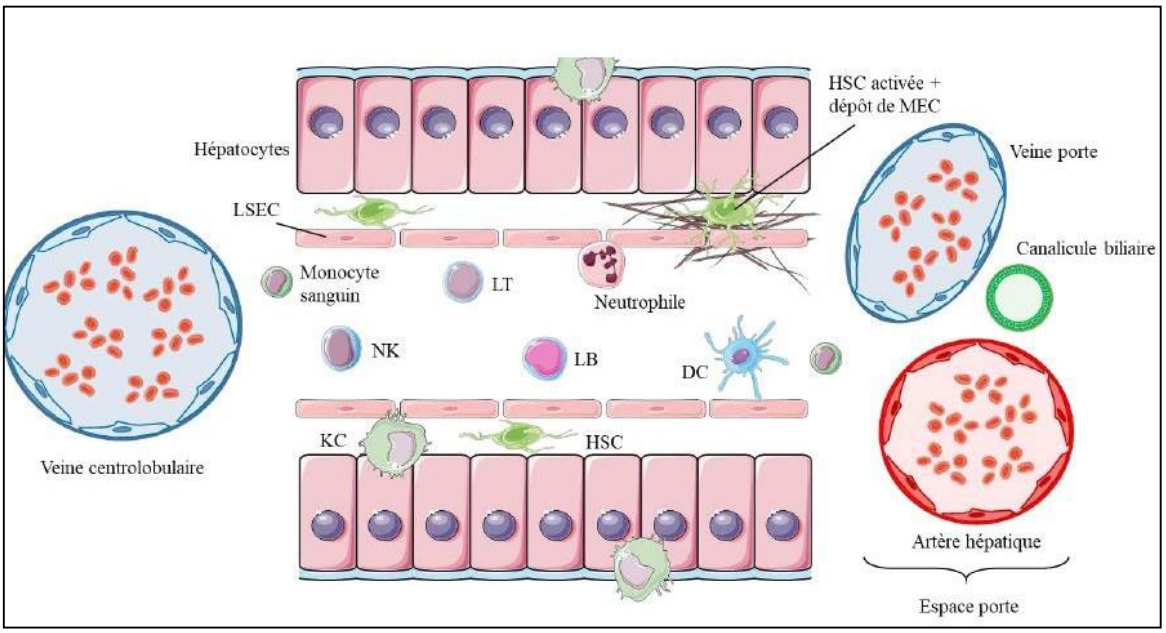


Figure 10: Schematic organisation of liver cell organization (Devisme, 2020)

types such as cholangiocytes, sinusoidal endothelial cells, natural killer cells, Kupffer cells and star cells (figure 10) **(Lorente *et al*., 2020)**.

- ***Liver damage caused by NSAIDs***

Diclofenac is a safe drug in therapeutic doses, but its use can lead to severe liver damage. In fact, DF and its highly reactive metabolites are responsible for excessive ROS generation, involving the phenomenon of "oxidative stress" which can affect mitochondrial membrane permeability, leading to hepatotoxicity. Anti-inflammatory drugs have been shown to increase the expression of heme oxygenase 1 (Hmox1), which is a function of changes in redox status and the imbalance of the pro-oxidant/antioxidant balance. These events lead to the release of pro-apoptotic proteins and the bursting of the mitochondrial outer membrane, resulting in cell lysis **(Delungahawatta *et al*., 2023)**.

II.4.4.2. Nephrotoxicity

- ***Kidney tissue and nephron***

The renal system consists of the kidney, ureters, and the urethra. The overall function of the system filters approximately 200 liters of fluid a day from renal blood flow which allows for toxins, metabolic waste products, and excess ion to be excreted while keeping essential substances in the blood. The kidney regulates plasma osmolarity by modulating the amount of water, solutes, and electrolytes in the blood. It ensures long term acid-base balance and also produces erythropoietin which stimulates the production of red blood cells. It also produces renin for blood pressure regulation and carries out the conversion of vitamin D to its active form **(Ogobuiro and Tuma, 2019)**.

There is a right and a left kidney, both of which are supplied by the main renal artery and may have additional polar arteries. Segmental arteries arise from the main renal arteries, then from the interlobar arteries of the kidney, then from the arches and finally from the interlobar arteries. These arteries branch into glomerular arteries, carrying the blood needed for filtration to the glomerulus. Blood is therefore transported to the glomerulus by the afferent arterioles. Once the urine has been filtered and extracted from the circulating blood, it returns to the circulation via the efferent arterioles. These move towards the medulla to form the vasa recta. This ensures that the tubules exchange urine. And blood. Having ensured this exchange, the vasa recta form a venous

system parallel to the arterial system. From the outside, you can find the renal fascia, perirenal fat, renal capsule, cortex, medulla and urinary tract. Blood is filtered by the glomeruli of the cortex. Glomerular filtered urine passes through the proximal coiled tubule (PCT), the loop of Henle, the distal coiled tubule and the collecting tube (CC) in that order. Urine therefore passes several times from the cortex to the medulla, the loop of Henle is in the medulla and the collecting tube ends there. The urine eventually reaches the nipple and is collected in the calyx and renal pelvis before being sent to the bladder due to ureteral peristalsis (figure 11) **(Ogobuiro and Tuma, 2019; Scholz *et al*., 2021)**.

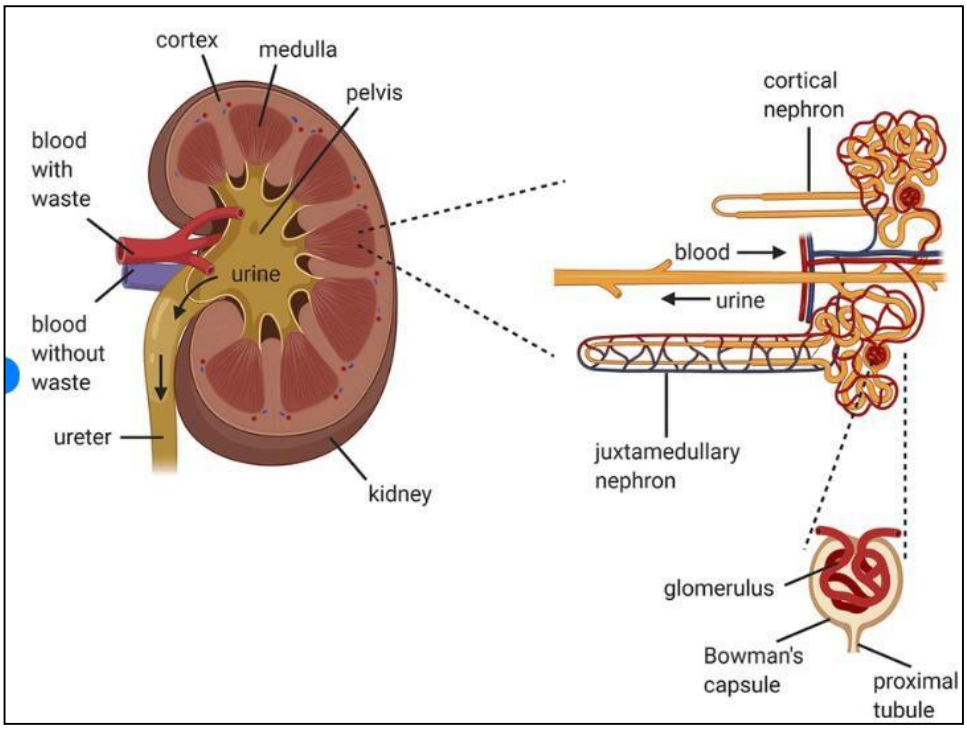


Figure 11: Anatomy of the kidney (Fransen et al., 2021)

- ***Nephrotoxicity of Diclofenac***

The use of DF is often associated with certain toxicities, and increasingly with renal toxicity. Most studies have attributed this phenomenon to the excessive production of ROS, which leads to an accumulation of uric acid in the blood, causing deleterious effects such as gout and increased intracellular ROS. Given the importance of prostaglandins in maintaining renal perfusion, and the reports of fluid retention and edema following DF therapy, regular monitoring of renal function is essential **(Prince, 2018)**.

II.5. Diclofenac and oxydatif stress

Reactive oxygen species (ROS) are produced spontaneously, in small quantities and continuously within the body. The maintenance of a non-cytotoxic level of ROS is ensured by endogenous antioxidant systems such as GSH, GPx and CAT. Diclofenac has been shown to have a cytotoxic effect; induced by excessive production of free radicals via the redox cycle, or by depletion of the hepatic GSH reserve. This depletion is at the origin of a number of cellular damages, of which lipid peroxidation is the most frequent **(Jung *et al*., 2020)**.

Normally when cells use oxygen, the redox process generates free radicals. Free radicals are highly reactive, the high reactivity of them is due to the fact that, unlike common organic molecules, they have an unpaired electron in the outer electron orbital. In this regard, free radicals act as active oxidants, capturing the missing electrons from various compounds and thereby, damaging their structure **(Arman *et al.*, 2019; Neganova *et al*., 2021; Martemucci, 2022)**.

Based on their chemical nature they can be divided into reactive oxygen species (ROS) reactive nitrogen species (RNS) reactive chlorine species (RClS), reactive sulfur species (RSS), and reactive bromine species (RBrS). The most important ones, in both quantitative and qualitative terms, are ROS and RNS. The former includes O_2^-, $HO^\bullet$, and the latter includes $ONOO^-$, NO^-, NO_2^-, etc **(Taurone *et al*., 2022; Geng *et al*., 2023)**.

ROS can have both exogenous and endogenous sources. Many metabolic processes in organisms, mitochondrial electron transport chain (ETC), and oxidative burst of phagocytes can spontaneously produce ROS, and these are referred to as endogenous sources. whereas exogenous sources include specific pharmaceuticals, ionizing radiations, metabolism of environmental chemicals, high partial pressure oxygen, hypoxia, drugs, heavy metals, etc **(Ahmad and Ahsan, 2020; Liu *et al*., 2023; Scarian *et al*., 2024**).

The excess ROS (figure12) directs undesirable changes to the integrity of various biomolecules and results in damage including lipids, proteins, and DNA leading to increased oxidative stress in various human diseases (**Ahmad and Ahsan, 2020; Chaudhary *et al.*, 2023**).

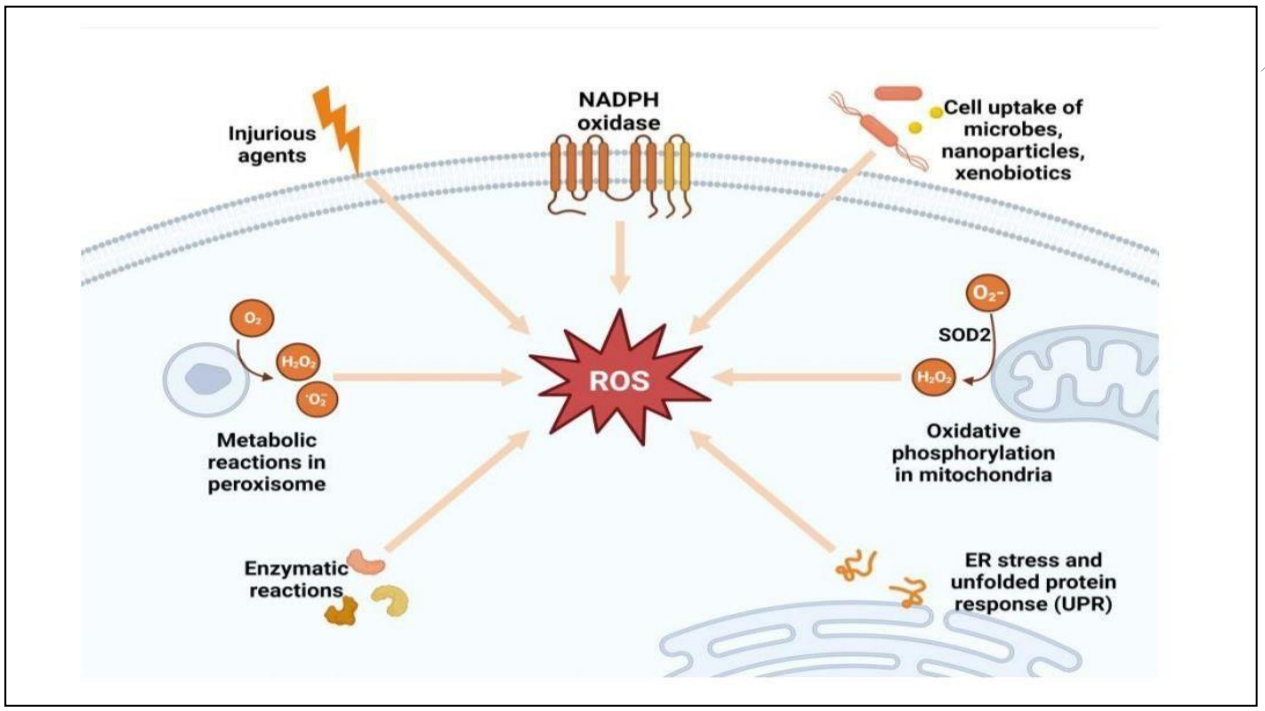


Figure 12: the molecular target of free radicals and oxidative damages (Martemucci et al., 2022)

ROS such as hydroxyl radical and singlet oxygen acting on DNA can produce a wide variety of lesions, and disrupt cellular function through harmful modification with varying mutagenic potentials, which involves base modifications, deletions or translocations, DNA-protein crosslinking, strand breakage, chromosomal rearrangements, hyper- and hypo-methylation and gene expression modulation **(Caliri *et al*., 2021; Martemucci *et al*., 2022; Kıran *et al*., 2023)**.

ROS can induce lipid peroxidation through the degradation of polyunsaturated fatty acids (PUFAs). For instance, the hydroxyl radical is capable of extracting hydrogen from the carbons located between two double bonds of PUFAs. The lipid radical reacts with an oxygen molecule to form a peroxyl radical (ROO^-), which is sufficiently reactive to extract an H^+ from a neighboring PUFA, thus propagating the reaction, resulting in the formation of a variety of products, including malondialdehyde (MDA), 4-hydroxy-nonenal (HNE) and the F2-isoprostane 15(S)-8-iso-prostaglandin F2α (15(S)-8-iso-PGF2α). These products are capable of inactivating many cellular proteins by forming protein cross-linkages. All of these lead to the disruption of the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability which inevitably leads to cell death **(Haleng *et al.*, 2007; Birben *et al.*, 2012 ; Nassar, 2017; Caliri *et al*., 2021)**.

Certain amino acids are more susceptible to oxidation, particularly in proteins containing unsaturated bonds and sulfur. This oxidation can be initiated by radical species like superoxide,

hydroxyl, peroxyl, alkoxyl, and hydroperoxyl, or non-radical species like hydrogen peroxide, ozone, hypochlorous acid, singlet oxygen, and peroxynitrite anion (**Nassar, 2017**). The result of this interaction can inhibit the function and activation of structural proteins and cause the appearance of carbonyl groups, cleavage of peptide chains, and the formation of intra- and inter-chain bi-tyrosine bridges. This damage can also result in the formation of stable and highly reactive products such as protein hydroperoxides, as well as electrophilic molecules that react with proteins possessing nucleophilic side chains (**Martemucci *et al.*, 2022**).

Antioxidant compounds have been extensively researched in biology, medicine and nutrition sciences. An antioxidant is characterized as a chemical or system that can interact with free radicals safely to prevent or delay the oxidation of other molecules, giving rise to more stable derivatives that block the propagation phase. They act either by inhibiting the formation of ROS, by metabolizing them using specific enzymes, or even by acquiring or providing electrons to neutralize free radicals, thereby eliminating the unpaired radical state **(Haleng *et al.*, 2007; Kehili *et al.*, 2017)**.

Antioxidants can be endogenous or exogenous. The former consists of enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase), or non-enzymes (lipoic acid, glutathione, L-arginine, uric acid, bilirubin), proteins (ferritin, transferrin, ceruloplasmin, albumin) and oxidative damage repair systems such as endonucleases. The other source is exogenous and provided by animal or vegetal origin, mainly introduced by diet or nutritional supplementation (vitamins A, C, E, carotenoids, and ubiquinone) and phytochemicals such as isoflavones, polyphenols, melatonin, and flavonoids. In addition, trace elements such as selenium, copper, and zinc are cofactors of antioxidant enzymes **(Pizzino *et al.*, 2017; Martemucci *et al.*, 2022; Ferreira Da Vinha *et al.*, 2023**).

III. Phytotherapy and medicinal plants

Phytotherapy is an integral part of therapeutics. Etymologically, It is considered to be an alternative medicine, according to the WHO (World Health Organisation). Phytotherapy can therefore be defined as an allopathic discipline designed to prevent and treat certain functional disorders and/or certain pathological conditions using medicinal plants and potentially beneficial herbs.

A medicinal plant is a plant of which at least one part contains active chemical compounds which have therapeutic properties **(Wichtl and Anton, 2003; Falch *et al.*, 2013)**.The active

chemical substances extracted from plants are very diverse. They include phenols (anti-inflammatory and antiseptic), flavonoids (antioxidant, anti-inflammatory and antiviral), tannins (anti-infection, anti-haemorrhagic), alkaloids (sedative activity, effects on nervous disorders), vitamins (such as B1, B2, C and E), minerals and many others **(Boudebouz, 2013)**.

Algeria has a great diversity of flora, combined with a centuries-old tradition of traditional plant use. Algeria has more than 3,000 species belonging to several botanical families, 15% of which are endemic. Its geographical location between the Mediterranean Sea and the Sahara has contributed to a varied topography with different elevations, a diversity of soil types, and a variety of climatic zones and microclimates ranging from dry and hot to temperate and humid. All these factors have resulted in a relatively vast geographical area with a wide variety of flora arranged for different uses and traditions. As a result, the older generations of Algerians relied heavily on herbal remedies, and herbs such as sage, thyme, rosemary, lavender, mint and lavender were used to treat their ailments **(Bouabdelli *et al*., 2012)**.

Since the 80s, the field of natural substances has been an area of scientific research in which Algerian teams have focused their studies.

The faculties of Biology and Chemistry at the Frères Mentouri Constantine University have been pioneers in this field. Phyto-pharmacological investigations have also been carried out to find natural sources of new chemical structures with beneficial or protective effects **(Bekhouche, 2019)**.

III.1. Chemical composition of medicinal plants

Plants have a highly complex chemical composition, made up of hundreds or even thousands of substances. Through its roots, it draws elements from the soil (water, minerals, trace elements) and through photosynthesis in its leaves, it produces complex molecules called organic compounds. The substances that the plant produces, known as metabolites, have different levels of interest. A metabolite is an intermediate organic compound derived from metabolism. This term is, generally, limited to small molecules. Metabolites have various functions; including energy, structure, signalling, stimulant, and inhibitory effects on enzymes In plants, there are two main classes of metabolites; primary and secondary **(Cherfia, 2021)**.

III.1.1. Primary metabolites

All living organisms ensure their growth through a complex set of chemical reactions, among them "primary metabolism", which are organic molecules found in all the cells of a plant organism to ensure its survival **(Donatien, 2009)**.

These are constitutive or permanent molecules: directly involved in the main pathways of the cell's basal metabolism, i.e. essential for cell survival (the normal growth, development and reproduction of an organism or cell) **(Dowd and Kelley, 2011; Benslama, 2016)**.

III.1.2. Secondary metabolites

Secondary metabolites are natural products synthesised mainly by bacteria, fungi and plants. They are molecules with a limited distribution in the plant organism and low molecular weight, with diverse chemical structures and biological activities. They play various roles, including defence against external aggression from herbivorous animals and against disease, intercellular communication, regulation of catalytic cycles, etc **(Lattanzio *et al.*, 2008; Akula and Ravishankar, 2011; Wink, 2015)**.

The three main classes of secondary metabolites in plants are alkaloids, terpenoids and phenolics. The class we are most interested in is phenolics.

IV. Polyphenols

The term "phenolic compounds" is used to define chemical substances that have at least one hydroxyl group (OH) substituted on an aromatic ring. Their name comes from the simplest parent compound, phenol (figure 13) **(Dini and Grumetto, 2022)**.

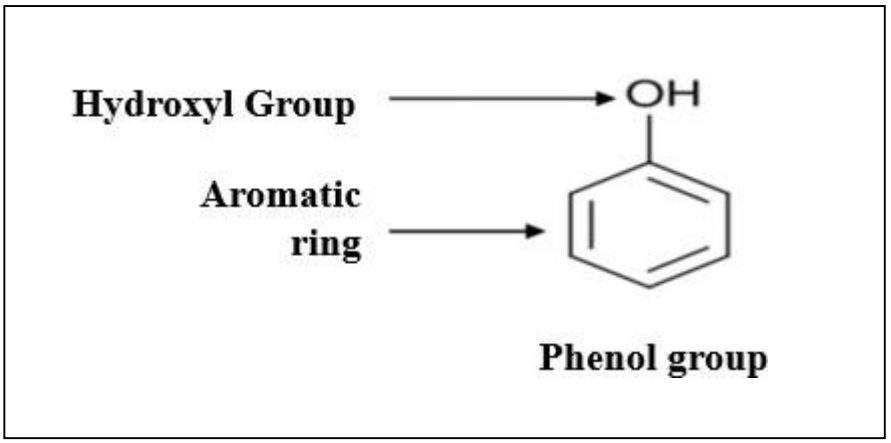


Figure 13: Basic skeleton of polyphenols (Manallah, 2018)

Polyphenols are a large family of organic molecules derived from the secondary metabolism of plants. They are widely present in the plant kingdom, with over 8,000 compounds currently identified, of which more than 4,000 are flavonoids, divided into around ten chemical classes. From a structural point of view, the general term "phenolic compounds" covers mono-, di- and polyphenols whose molecules contain one, two or more 6-carbon benzene rings bearing one or more hydroxyl functions. Their structure varies from simple molecules (simple phenolic acids) to the most highly polymerised molecules (condensed tannins) (figure 14) **(Lassed, 2016)**.

They have attracted attention in recent years because of their anti-inflammatory, anti-atherogenic, anti-thrombotic, analgesic, antibacterial, antiviral, anticancer, anti-allergic, vasodilatory and antioxidant activities **(Zhang *et al*., 2022)**. They are capable of scavenging free radicals and inhibiting lipid peroxidation by reducing hydroxyl, superoxide and peroxyl radicals. They are also capable of trapping metal ions, as they have chelating properties **(Zhang *et al*., 2022; Dini and Grumetto, 2022)**.

These compounds can be classified into different groups according to the number and arrangement of their carbon atoms.

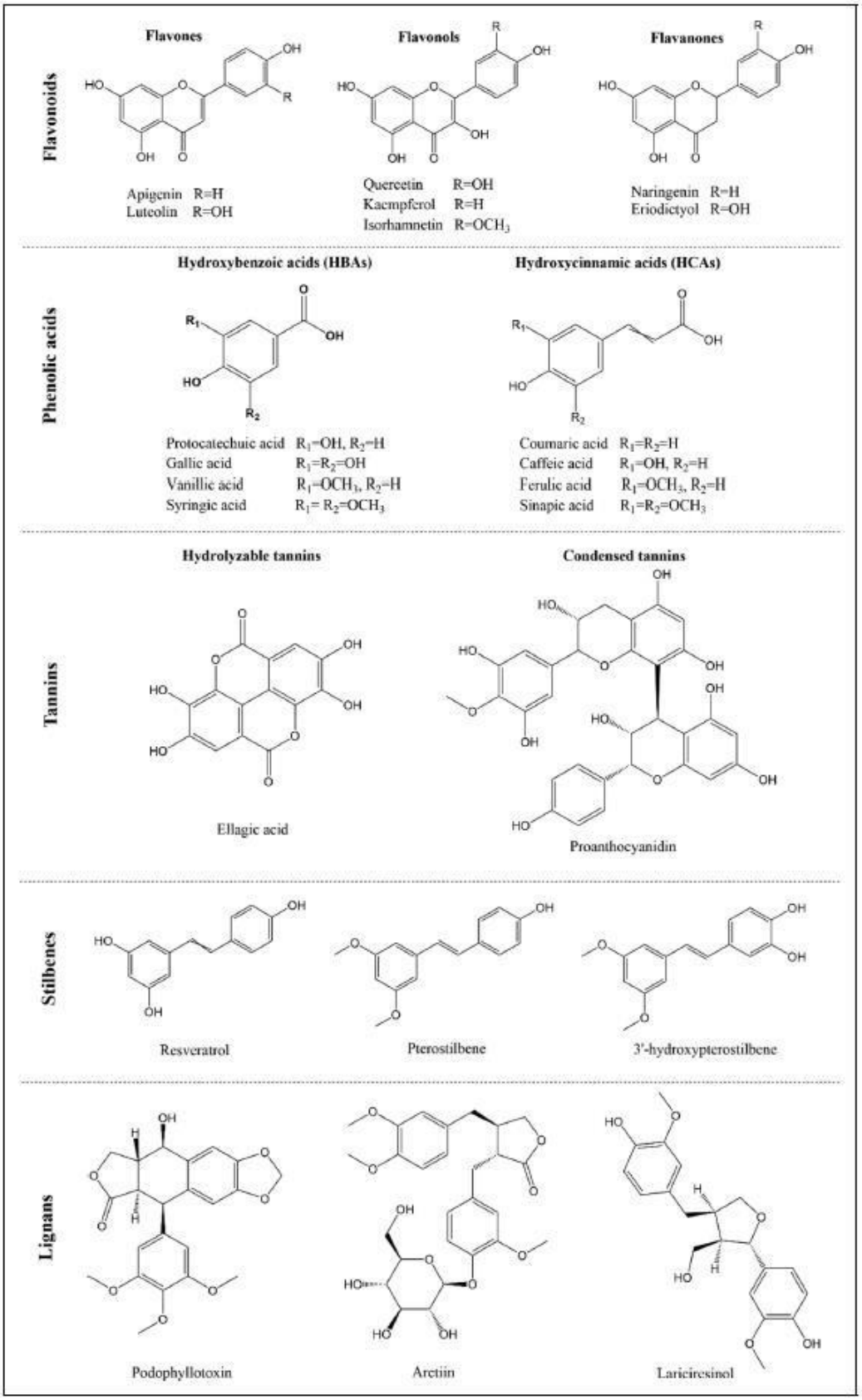


Figure 14: Typical phenolic compounds identified from plants (Zhang et al., 2022)

IV.1.Flavonoids

The term flavonoids refers to a very wide range of natural compounds belonging to the polyphenol family. It is one of the most abundant, and over 9,000 natural structures have been isolated and characterised **(Papastavropoulou *et al*., 2022)**. It is the most representative group of phenolic compounds.

This group has a common basic structure made up of two aromatic rings linked by three carbon atoms to form an oxygenated heterocycle (figure 15).

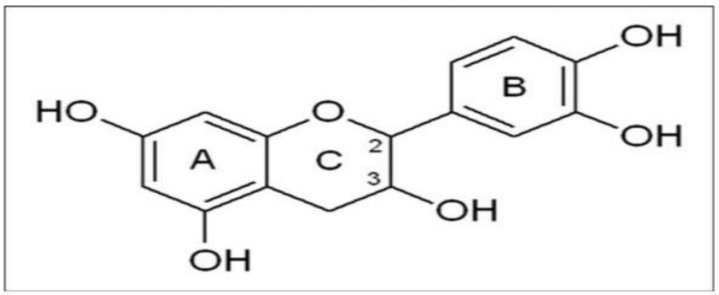


Figure 15: Basic structure of flavonoids (Erlund, 2004)

They are considered to be almost universal plant pigments and can play a part in photosynthetic processes, gene regulation and growth metabolism **(Dini and Grumetto, 2022)**.

Depending on the number and chemical structure of the constituent carbons, as well as on the nature of the substituents and the variations in the type of heterocycle involved, flavonoids are classified into different categories, the most important of which are flavanones, flavonols, flavones, flavanols, isoflavones and anthocyanins (figure 16) **(Tsao, 2010; Kulasari *et al*., 2019)**.

Individual differences within each group arise from variations in the number and arrangement of hydroxyl groups and their degree of alkylation and/or glycosylation.They are generally found in all vascular plants, where they can be found in various organs: roots, stems, wood, leaves, flowers, fruit, and also in honey.

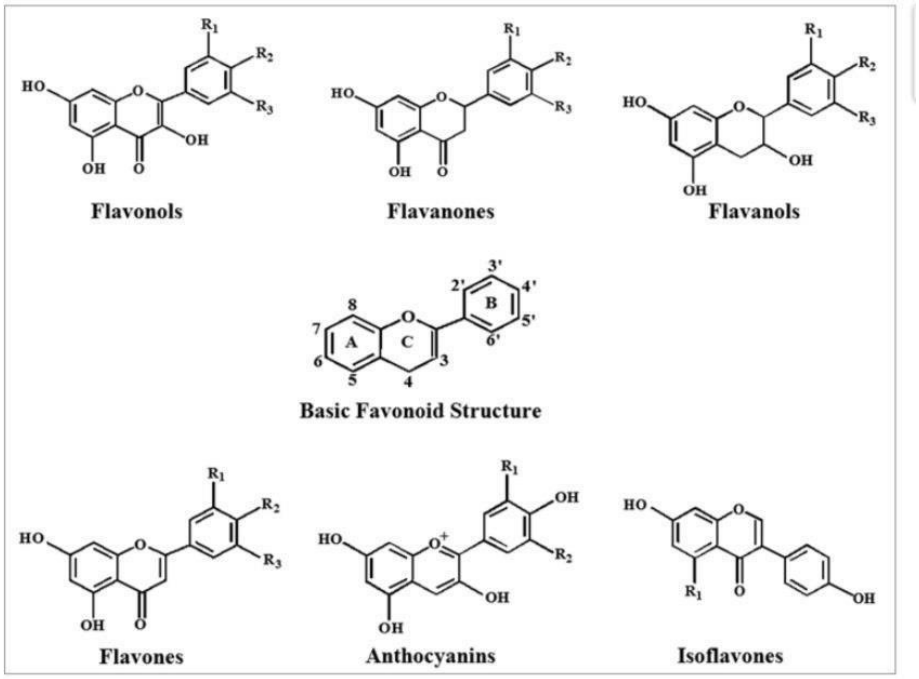


Figure 16: Chemical structure of sub- classes of flavonoids (Pandey and Rizvi, 2009)

IV.1.1. Biological activities of flavonoids

These substances have been studied for their potential health benefits as antioxidants, although numerous studies indicate that flavonoids have anti-inflammatory properties and are capable of modulating the functioning of the immune system. They also have antiviral, antibacterial and anti-carcinogenic properties. They can also interfere with the metabolism of xenobiotics by stimulating detoxification systems **(Dumasa and Yvette, 2014)**.

IV.1.1.1. Antioxidant activity

The production of free radicals in animal cells can be either accidental or deliberate. With the increasing acceptance of free radicals as commonplace and important biochemical intermediates, they have been implicated in a large number of human diseases **(Papastavropoulou *et al.*, 2022)**. The best-known antioxidants are β-carotene, ascorbic acid, tocopherol and phenolic compounds.

Most synthetic or naturally-occurring antioxidants have phenolic hydroxyl groups in their structures and the antioxidant properties are attributed in part to the ability of these natural compounds to trap free radicals such as hydroxyl and superoxide radicals **(Dini and Grumetto, 2022)**. Polyphenols can induce antioxidant enzymes such as glutathione peroxidase, catalase and

superoxide dismutase, which break down hydroperoxides, hydrogen peroxide and superoxide anions, respectively (figur17) **(Tsao, 2010; Kulasari *et al*., 2019)**.

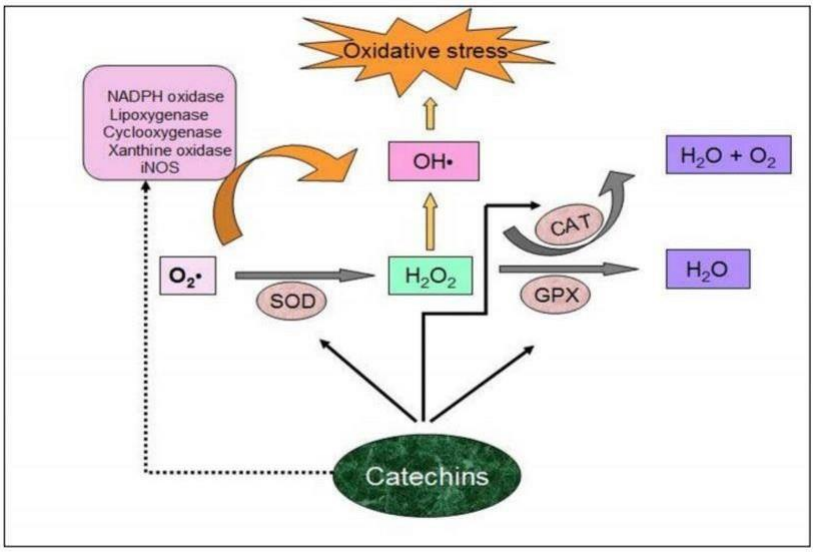


Figure 17: Catechin defence mechanism (Kris-Etherton et al., 2002)

IV.1.1.2. Anti-inflammatory activity

Inflammation-induced oxidative stress is mediated by NF-kB activation. It affects a wide variety of cell signalling processes leading to the production of inflammatory mediators and chromatin remodelling. The latter allows the expression of pro-inflammatory genes such as tumour necrosis factor alpha (TNF-alpha) and iNOS. It can also cause an increase in enzymes such as cyclooxygenase (COX) and lipoxygenase (LPO), which are involved in the release of factors such as interleukins and chemokines. The adverse effects of oxidative stress have been found to be controlled by antioxidants and/or anti-inflammatories, and polyphenols, quercetin in particular, have been shown to inhibit COX and LPO **(Kulasari *et al*., 2019)**. However, catechins manage ROS generation by inhibiting 'prooxidant' enzymes, such as inducible nitric oxide synthase (iNOS) and xanthine oxidase, and by inducing antioxidant enzymes, such as SOD, CAT and GPX **(Li *et al*., 2014)**.

V. Introduction to plant material

Throughout human history, plants have been an essential source of human medicine for millennia to treat various diseases. Almost 80% who live in developed countries are said to be depended on the practice of traditional medicine. Medicinal plants are used to maintain physical, mental, and spiritual health in all cultures and in a variety of capacities and contexts **(Michel *et al*., 2020; Davis and Choisy, 2024)**.

Centaurea is a value-ultimate genus of medicinal plants showing high diversification levels, especially within the Mediterranean basin and is still traditionally recognized as a complicated taxon **(Atia *et al*., 2021)**.

V.1. Centaurea sp.

The *Centaurea* genus (tribe Cynareae) of the Asteraceae family is one of the world's most widespread genera. It comprises some 700 species. This genus is present in Algeria, mainly in the east and south-east, and is represented by 45 species, 7 of which are Saharan species located in the south. It is widespread in southern Europe, the Mediterranean basin, western Asia and the Americas. Centaureas are annual, biennial or perennial herbaceous plants with alternate leaves. As with all composites, the flowers or florets are arranged in capitula and surrounded by an involucre. Their color usually varies from pink to violet, but there are also a few species with yellow flowers. The involucre is made up of unequal bracts in several rows on the artichoke style. These bracts may be ciliated (the most frequent case) or spiny **(Hadjira and Ameddah, 2020)**.

V.1.1. Biological and pharmacological properties of *Centaurea* genus

Several species of this genus are recognized as having medicinal virtues. Species of this genus are used in traditional medicine throughout the world, notably to treat endocrine diseases (diabetes), inflammatory disorders (rheumatic pain, etc.), fever, gastrointestinal symptoms, cardiovascular problems, parasitic and microbial infections and urogenital affections.Several scientific studies have highlighted the biological and pharmacological properties of the *Centaurea* genus, including analgesic, cytotoxic, antibacterial, antifungal and antioxidant activity **(Hadjira and Ameddah, 2020)**.

V.1.2. Plant systematics

Table 3: botanical classification of the Centaurea sp plant (Hilpold et al., 2014)

Kingdom	Plantae
Phylum	**Streptophyta**
Class	**Equisetopsida**
Subclass	**Mangoliidae**
Order	**Asterales**
family	***Asteraceae***
Genus	***Centaurea***

V.1.3. Geographic distribution

The *Centaurea spp* has a center of distribution around the Mediterranean and the Black Sea (figure 18). The highest species numbers can be found in the Balkan Peninsula, Italy, Turkey and the Iberian Peninsula (in order of abundance). Almost all African species are concentrated in the NW of the continent, in the Atlas mountain ranges. A few widespread species reach central Europe and the Baltic Sea and Middle Asia, Afghanistan and Pakistan **(Hilpold *et al*., 2014)**.

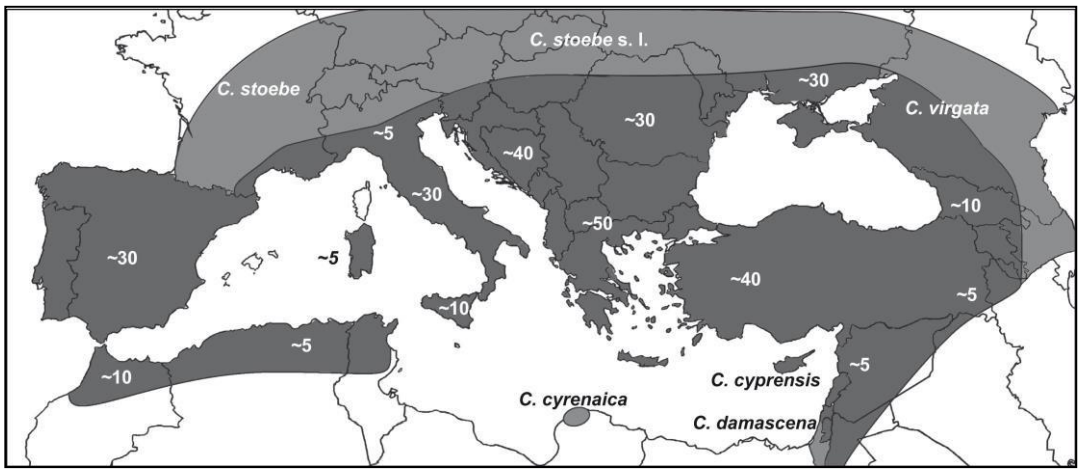


Figure 18: Distribution of sect. Centaurea (Hilpold et al., 2014)

Dark grey: areas with more than one species occurring. Light grey: only one species occurring, speciesname is given. Numbers show the approximate species number in the area. Note that the species numbers reflect, besides real differences in diversity, also the species concepts used in the different areas.

Chapter II:
Material and methods

Material and methods

I. Plant material

Centaurea sp., an endemic species of North Africa was collected during the flowering phase in May 2021 from the area of Biskra. A voucher specimen (GQ/065/04/08) has been deposited in the Herbarium of VARENBIOMOL research unit of university of Constantine1.

I.1. Extraction

Air-dried aerial parts (1130 g) of *Centaurea sp.* were macerated at room temperature with MeOH-H_2O (80:20, v/v) for 24h, three times. After filtration, the filtrate was concentrated and dissolved in H_2O (500 mL) under magnetic stiring. The resulting solution was filtered and successively extracted with petroleum ether, $CHCI_3$, EtOAc, and *n*-butanol. The organic phases were dried with Na_2SO_4, filtered and concentrated in vacuo at room temperature to obtain the following extracts: petroleum ether (0.25 g), chloroform (3 g), EtOAc (4 g) and *n*-butanol (50 g). The extraction was realised by Dr. Boudraa Keltoum.

II. Animals and treatments

II.1. Animals and accommodation

Female *Wistar* rats weighing 180 - 250 g were used in anti-iflammatory test. To evaluate the protective and antioxidant activity *in vivo*, male rats were used, which weigh between 160 and 190 g. They were obtained from the animal facility of the Department of Animal Biology, Faculty of Health Sciences, Nature and Life, Mentouri Constantine 1 University. Animals were housed in cages, minted in an air-conditioned room at 22 to 26 °C with 12-h light and dark cycle and fed on standard rat pellets with free access to food and water ad libitum. Rats were acclimatized to the laboratory environment for 2 weeks, prior to the commencement of the study.

II.2. Formalin-Induced Paw edema

The search for anti-inflammatory properties was conducted in accordance with the acute inflammation model, whereby edema was induced in the paws of rats using formaldehyde (2.5%) as an inflammatory agent and at a rate of 0.1mL per paw. The rats are divided into 4 groups of 6 rats each. The different treatments were administered by intraperitoneal injection:

Group 1: Control rats received 0.9% of NaCl,

Group 2: Receive diclofenac at a dose of 30 mg/kg (**Karrat *et al*., 2022**),

Group 3: Receive the *n*-butanol extract of *Centaurea sp.* at a dose of 100 mg/kg,

Group 4: Receive the *n*-butanol extract of *Centaurea sp.* at a dose of 200 mg/kg.

Half an hour later, paw edema was induced by injecting 0.1 mL of formalin (2.5%) into the plantar aponeurosis of the right hind paw of each rat. Paw volume was measured using water displacement method (**Kebede and Soromessa, 2018**) at 30 min,1, 2, 3, 4 and 5 h after the injection of formalin (**Arzi *et al*., 2015**). The increase in the paw edema volume was considered as the difference from 30 min to 5 h. Before oral administration of drugs, the average volume of the right hind paw of each animal was measured two times (Vo) by a displacement method. The volume of the right hind paw was determined again at 30 min,1, 2, 3, 4 and 5 h after formalin treatment (Vt). The percent of inhibition in increase of edema volume for each group of animals was calculated by the following formula:

***% inhibition of edema= [(Vt - Vo) control - (Vt - Vo) treated]/ (Vt - Vo) control* x100**

Figure 19 : anti-inflammatory test

II.3. Diclofenac induced toxicity

II.3.1. Protective effect of n-butanol extract again acute toxicity induced by Diclofenac (50mg/kg)

To evaluate the protective effect of the *n*-buthanol extract of *Centaurea sp.* on rats for 05 days. The rats were divided into four groups with 7 rats:

Group I (T) : Served as normal and received the vehicle alone (Sterile distilled water, 10 mL/kg) for 5 days.

Group II (EXT): Receive 100 mg/kg of the *n*-butanol extract of *Centaurea sp*. every day by gavage for 5 days.

Group III (DF): Animals received diclofenac (50 mg/kg i.p.) on the 4^{th} and 5^{th} day.

Group VI (EXT+DF): Receive every day (100mg/kg) of the extract by gavage and (50mg/kg) of diclofenac 1 hour later intraperitoneally (on the 4^{th} and 5^{th} day).

The dose of *n*-butanol extract of *Centaurea sp.* was chosen based on *in vivo* observations, and studies performed in our laboratory on the effect of different plant extracts on xenobiotic-induced hepatic and renal toxicity (**Boubekri *et al.*, 2014; Amrani *et al.*, 2017; Amrani *et al.*, 2020 and Mecheri *et al.*, 2024**), while the toxin dose was chosen based on previous studies (**Simon *et al.*, 2019**).

Figure 20: A Rat receives the extract by gavage

Figure 21: A rat receives a dose of diclofenac intraperitoneally

II.3.2. Dissecting rats, collecting blood and organs

After the treatment, the animals were anesthetized with chloroform after 16 h of fasting and were sacrificed, blood was collected for biochemical analysis. The recovered blood is immediately centrifuged at 4000 rpm for 10 minutes, for analysis of biochemical parameters.

Figure 22 : Blood collection

II.3.3. Homogenate preparation

After sacrificing the rats, their organs will be rinsed with a cold solution of NaCl (0.9%) to drain all the blood. Then the organs are rinsed with saline, cut into small pieces, weighed and homogenised in a 1.15% KCl solution. The homogenate was centrifuged at 3000 rpm for 15 minutes to remove cellular debris. Then, the cytosolic fraction was used for tests on MDA, GSH and GPx.

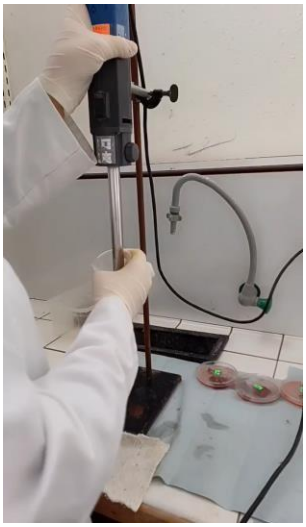


Figure 23: Preparing the homogenate

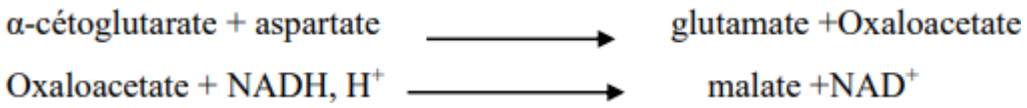
II.3.4. Dosage of biochemical parameters

Enzymatic activities: AST, ALT (liver function biomarkers) are measured. Renal function biomarkers (urea, and creatinine) are estimated. The parameters are determined by colorimetric methods using commercial kits in a biology and medical analysis laboratory.

II.3.4.1. Aspartate aminotransferase (ASAT) assay

Aspartate aminotransferase (AST) is an enzyme found in several parts of your body, including the heart, liver, and muscles. Since AST levels aren't as specific for liver damage as ALT, it's usually measured together with ALT to check for liver problems.When the liver is damaged, AST can be released into the bloodstream. A high result on an AST test might indicate a problem with the liver or muscles.

Aspartate aminotransferase (ASAT) also called oxaloacetate glutamate transaminase (TGO) transfers an amino group from aspartate to alpha-ketoglutarate forming glutamate and oxaloacetate. Oxaloacetate is reduced to malate by malate dehydrogenase (MDH) and NADH, H^+ according to the following reaction:



II.3.4.2. Alanine aminotransferase (ALT) assay

Alanine transaminase (ALT) is used by your body to metabolize protein. If the liver is damaged or not functioning properly, ALT can be released into the blood. This causes ALT levels to increase. A higher than normal result on this test can be a sign of liver damage.

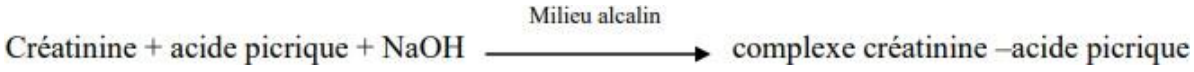
Alanine aminotransferase (ALT) also called pyruvate glutamate transaminase (TGP) transfers an amino group from alanine to alpha-ketoglutarate forming glutamate and pyruvate. Pyruvate is reduced to lactate by lactate dehydrogenase (LDH) and NADH, H^+ according to the the following reaction:

II.3.4.3. Urea dosage

Urease breaks down urea into ammonium and carbon dioxide. The ammonium reacts with salicylate and hypochlorite in the presence of nitroprusside to form a green indophenol according to the reactions below:

2.3.4.4. Creatinine dosage

A creatinine-picrate complex forms when creatinine reacts with picrate in a basic medium. The absorbance at 500 nm increases with the creatinine concentration in the sample.



II.3.5. Oxidative stress test

2.3.5.1. Lipid peroxidation assay (MDA measurement)

MDA is an end product of lipid poxidation that damages cells. It can be detected using a reaction between MDA and thiobarbituric acid (TBA). This forms a pink complex that absorbs light at 532 nm.

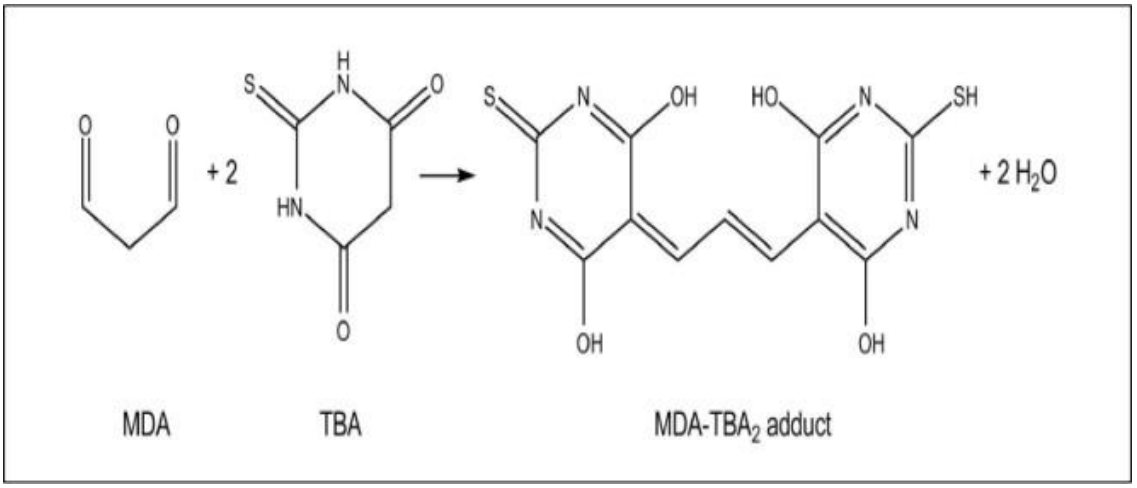


Figure 24: Reaction of malondialdehyde (MDA) with 2 molecules of 2-thiobarbituric acid (TBA) (Weitner et al., 2016)

Lipid peroxidation progression is resolute in the supernatant of all homogenates. It was evaluated by measuring the formation of thiobarbituric acid reactive substances (TBARS) via the colorimetric method of **Uchiyama and Mihara (1978)**. In this experiment, 3 mL of 1% phosphoric acid and 1 mL of 0.67 % thiobarbituric acid (TBA). The aqueous solution was added to 0.5 mL of homogenate (20%) and moved in the centrifuge tube. The mixture was left in a boiling water bath for 45 min, and then it was cooled to room temperature. 5mL of *n*-butanol was added to the mixture and mixed forcefully. Absorbance was read at 532 nm after separation of the *n*-butanol phase by centrifugation. MDA was employed singly as standard. The TBARs content in liver and kidney homogenate were given nmol MDA/mg protein.

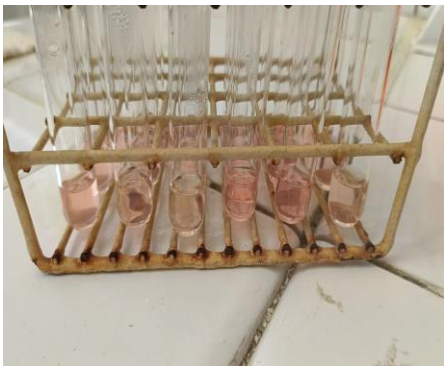


Figure 25: The pink complex forms during MDA dosing

II.3.5.2. Glutathione content measurement

Reduced glutathione (GSH) content in each liver homogenate was tested chemically via Ellman's reagent as reported by **Ellman, 1959**. The basis of this analysis is the reactive cleavage of (DTNB) 5,5'-dithiobis (2-nitrobenzoic acid) by sulfhydryl group and resulting in yellow color with great absorbance at 412 nm against reagent blank with not any homogenate. The GSH content in each liver and kidneys homogenate was given nmol GSH/mg protein (figure 26).

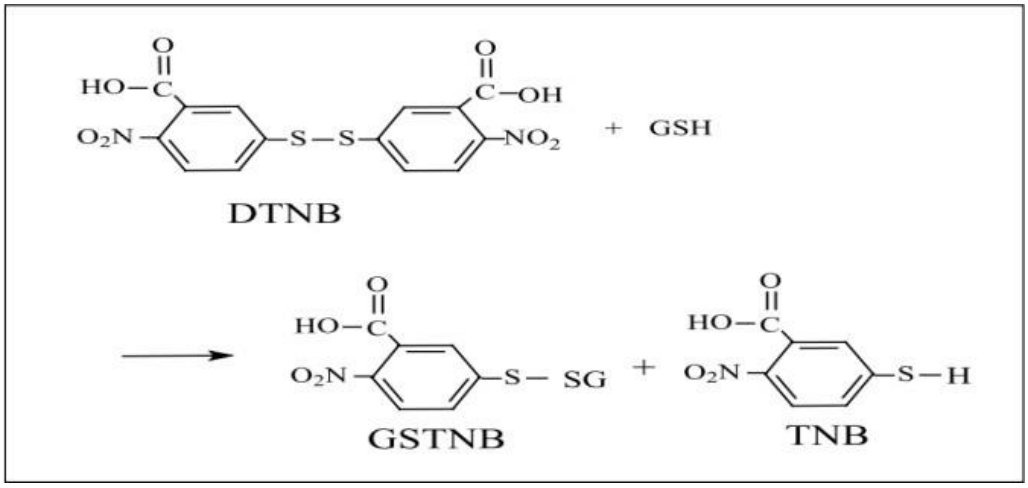


Figure 26: Glutathione dosage principle

II.3.5.3. Evaluation of glutathione peroxidase (GPx) activity

The determination of GPx activity in rat's liver homogenate was performed as designated by **Flohé and Günzler 1984**. In the existence of GSH, GPx causes the hydrogen peroxide (H_2O_2) reduction in the medium. Briefly, 0.2 mL of supernatant disjointed from homogenate was added to 0.4 mL GSH (0.1 mM) and 0.2 mL of Tris-buffered saline (TBS) solution (Tris 50 mM, containing NaCl 150 mM, pH 7.4), and then the tubes were mixed and incubated 5 min at 25°C. 0.2 mL of H_2O_2 (1.3 mM) was added to the mixture. After 10 min, 1 mL trichloroacetic acid (1% TCA) was added in order to end the reaction. Then, the tubes were kept at 0 – 5 °C in an ice bath for 30 min. After centrifugation for 10 min at 3000 rpm, 0.48 mL of supernatant was taken and added to each tube. 2.2 mL TBS solution and 0.32 mL of Ellman's Reagent, 5,5'-Dithiobis-(2-Nitrobenzoic Acid) (DTNB) (1mM) were added 5 min before the measurement of the optical density at 412 nm. The activity was given nmol GSH/mg protein.

$$H_2O_2 + 2\,GSH \xrightarrow{GP_X} GSSG + 2H_2O$$

II.4. Histopathological studies

After the animals have been sacrificed and dissected, they are perfused with a 0.9% NaCl solution to drain any blood remaining in the organs. A piece of the target organ (liver and kidneys) from each rat is immediately removed and preserved in formalin fixative (10%). The histological study was carried out in the pathological anatomy laboratories of the Urology Nephrology and Renal Transplant Clinic (Daksi), Constantine, using a Leica ICC 50 HD photonic microscope. It is based on a semiological analysis comparing normal and pathological tissues, to verify the existence of any changes in the architecture of these organs following administration of the plant (*Centaurea sp.*) and injection of diclofenac.

The various stages in the preparation of paraffin blocks are summarised below:

- After fixation, the tissues are cut into fragments, placed in cassettes and then immersed in a closed container of 10% formalin.

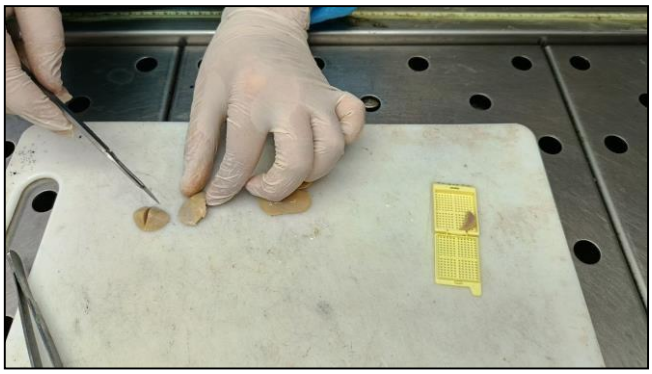


Figure 27: Macroscopic examination

- **Dehydration**

The tissues are immersed in alcohol baths of increasing strength (70°, 80°, 90°, 95°, 99° and finally 100°). Immediately after dehydration, the cassettes are placed in a xylene bath (lightener), so that the degree of penetration can be assessed by the transparency acquired by the piece.

Figure 28: The tissue processor used during the dehydration stage

- **Embedding**

The tissues are embedded in molten paraffin, which is incorporated with the sample and preserves the tissues, enabling very thin histological sections to be made.

Figure 29: Tissue embedding station

- **Cutting**

After the paraffin block has cooled, a microtome is used to cut a ribbon (5 µM). The ribbon obtained from the microtome is spread out on glass slides using water. These slides are placed on a hot plate to ensure that the paraffin containing the histological section is spread and fixed on the

slide. They are then dried in an oven at 40°C for 24 hours to ensure that the section is fixed to the slide.

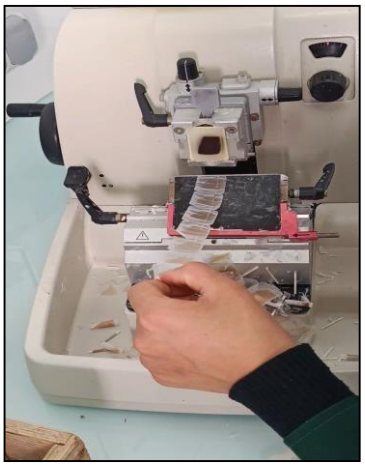


Figure 30: Histological section preparation stage

- **Staining**

To facilitate the penetration of the stains into the section, the slides are first rehydrated. The slides are placed in decreasing degrees of alcohol (100°, 90°, 70°C) for 2 minutes each, then washed with distilled water.

- **Haematoxylin and eosin (HE) staining**

Is the standard two-colour stain used initially, giving us an overall view of tissue morphology and pathological lesions.

- Haematoxylin stains basophilic structures (nuclei) a purplish-blue.
- Eosin colours acidophilic structures (cytoplasm) pink.

Figure 31: Staining stage

- For better microscopic visualisation and long-term preservation against discolouration, a synthetic resin is placed between a slide bearing the histological section and a coverslip.

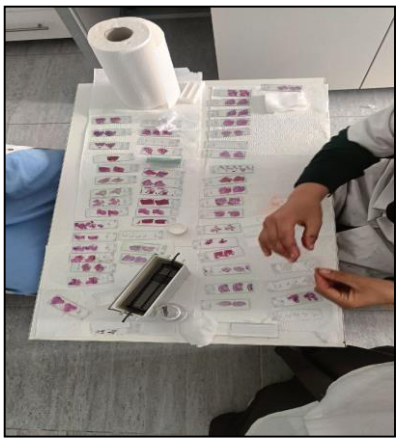


Figure 32: Preparation of histological slides

The slides are ready for microscopic reading, which is carried out using a Leica optical microscope with a camera system.

III. Evaluation statistique

The data was analysed using GraphPad Prism 5 software. The results were expressed as mean and standard deviation. The significance of the results was determined by the Student's t-test. Differences were considered significant when $p \leq 0.05$.

ns: $p > 0.05$ = the difference is not significant

*: $p < 0.01$ = the difference is significant

**: $p < 0.001$ = the difference is highly significant

Chapter III:

Results and discussion

I. Results of the *in vivo* experimental study

I.1. Anti-Inflammatory Activity

The results of the anti-inflammatory effect of *n*-butanol extract of *Centaurea sp*. (100 and 200 mg/kg) on formalin-induced edema in hind paws of the experimental rats are presented in figure 33. The *n*-butanol extract showed a significant reduction ($p < 0.01$) in the edema paw volume in a dose dependent method with a maximum attend at 100 and 200 mg/kg. With all doses 3 h after formalin injection, diclofenac as reference standard (30 mg/kg, ip) produced a significant inhibitory effect comparable to control group ($p < 0.001$).

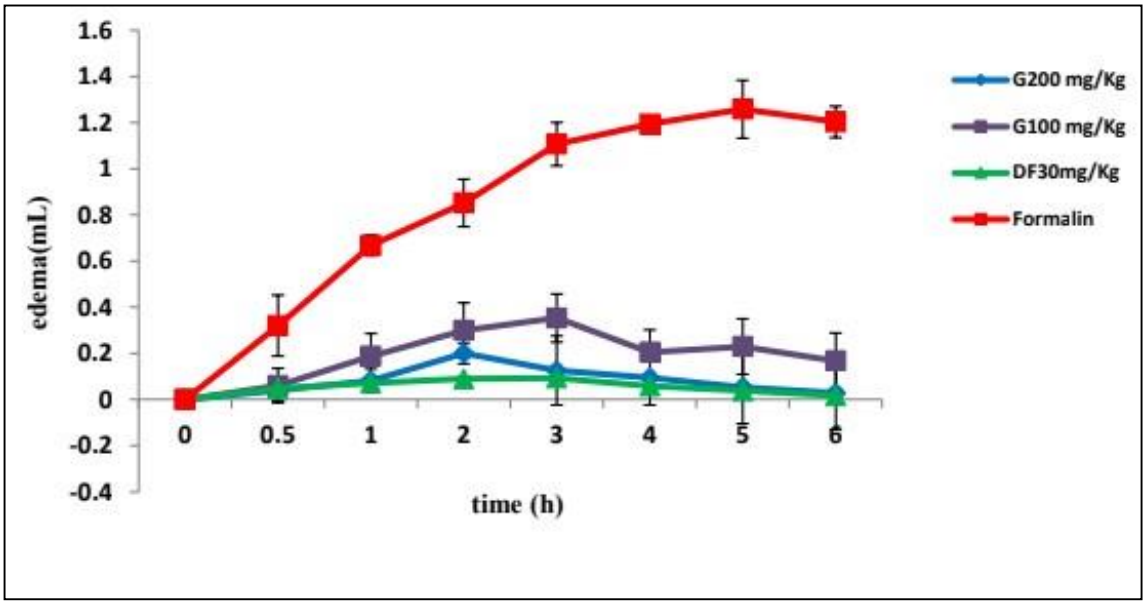


Figure 33: effect of extract on rats paw edema induced by formalin

Administration of the *n*-butanol extract of *Centaurea sp*. plant at doses of 100 and 200 mg/kg significantly prevents plantar edema in rats from the first hour of treatment until the 5th hour with an inhibition percentage of 68.17±2.52 and 91.26±1.36% extracted 100 and 200 mg/kg respectively.

Evaluation of the percentage inhibition of edema shows that *n*-butanol extract has anti-inflammatory activity, which may be due to the richness of this extract in bioactive compounds, mainly polyphenols and flavonoids.

I.2.The protective effect of the plant extract against acute toxicity induced by diclofenac

I.2.1. Serum hepatic and renal injury biomarkers

a. The transaminase enzymes AST and ALT

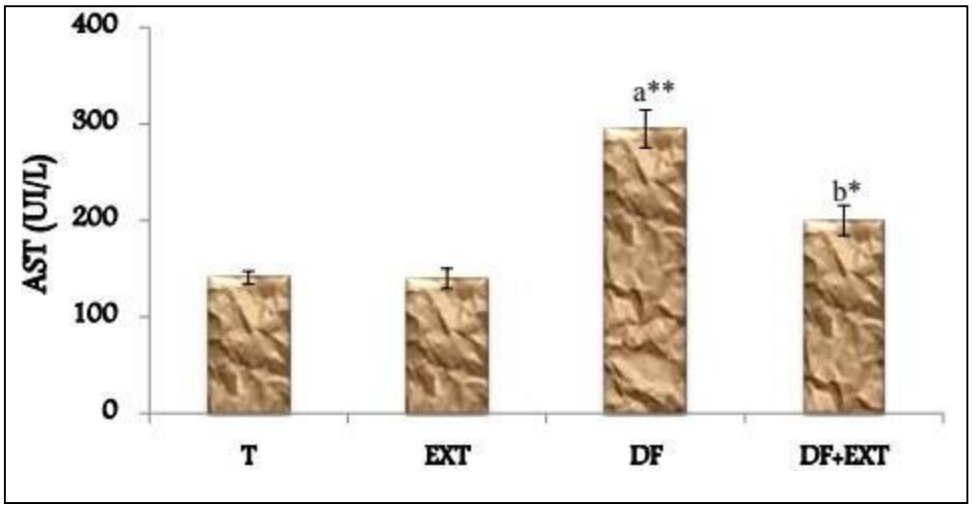


Figure 34: Effect of diclofenac and the extract on the serum concentration of aminotransferases: AST. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

The results presented in Figures 34 and 35 demonstrate that the administration of DF (50mg/kg) induced a significant increase (p<0.01) in plasma levels of AST and ALT compared to the control group. While oral pretreatment with the *n*-butanol extract (100mg/kg) of *Centaurea sp*. significantly reduced the levels of AST and ALT (p< 0.05) in the group co-treated with the extract (EXT + DF) compared to the DF-treated group.

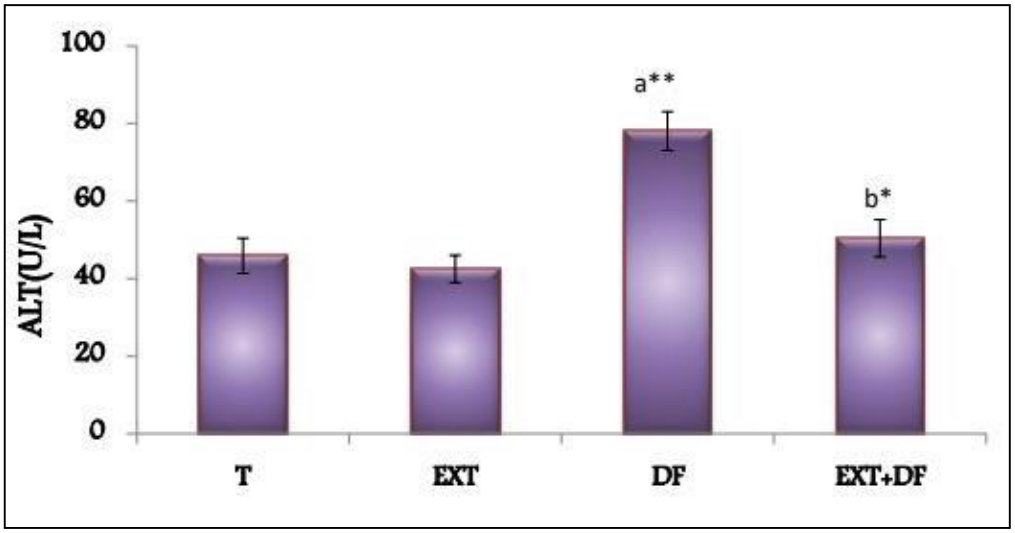


Figure 35: Effect of diclofenac and the extract on the serum concentration of aminotransferases: ALT. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

b. The effect on serum concentration of urea and creatinine

The results obtained in our study (figure 36 and 37) showed that the administration of diclofenac (50mg/Kg) caused a significant increase (p<0.01) in the concentration of creatinine and serum urea compared to the control group (T). A significant decrease (p<0.05) in the serum concentration of the two parameters in the (EXT+DF) group compared to the DF group.

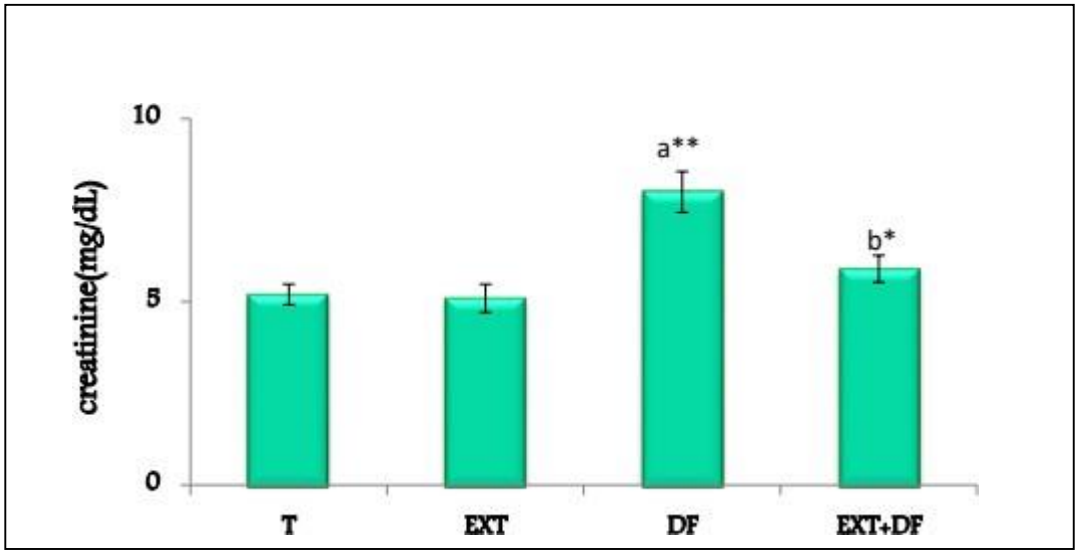


Figure 36: Effect of diclofenac and extract on serum creatinine concentration.
Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01,
a : Groups compared to the control group
b : Groups compared to the diclofenac group

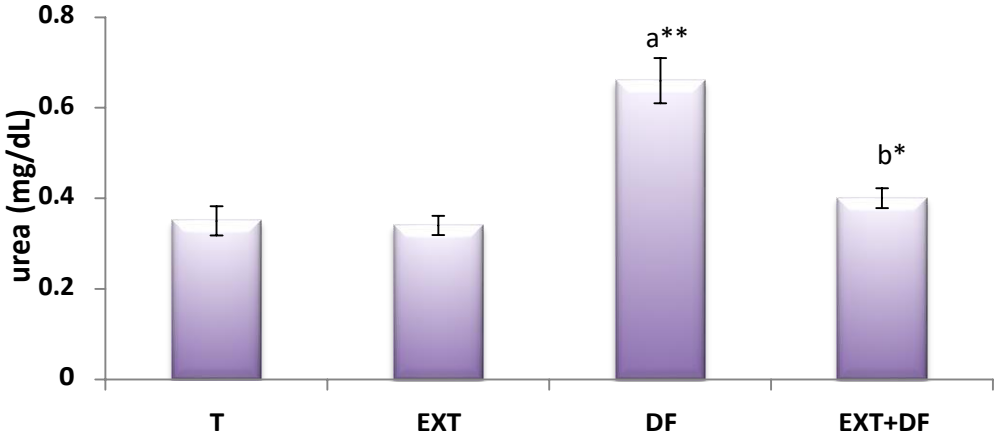


Figure 37: Effect of diclofenac and extract on serum urea concentration
Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01,
a : Groups compared to the control group
b : Groups compared to the diclofenac group

I.2.2. Hepatic and renal oxidative stress and antioxidant markers

a. Effect on lipid peroxidation (MDA levels) in the liver and kidneys

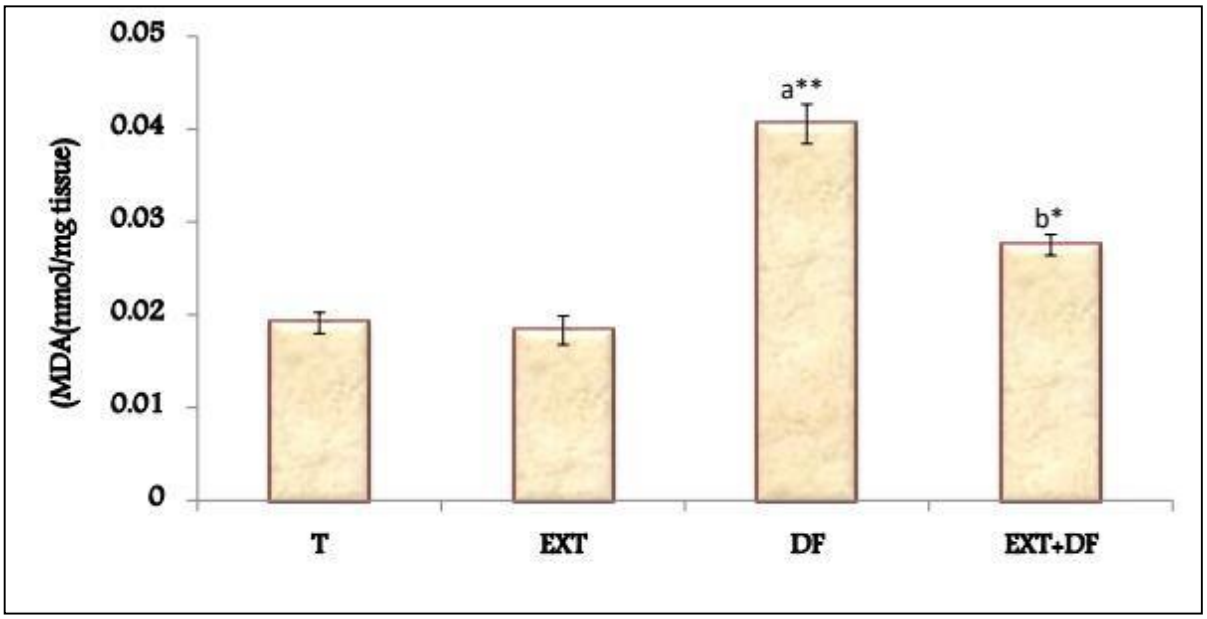


Figure 38: Effect of diclofenac and extract on liver MDA level in different groups. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

Figures 38 and 39 demonstrated the effect of the *n*-butanol extract on the variations of MDA in the liver and kidneys in rats treated with diclofenac. Nephrotoxicity and hepatotoxicity were associated with lipid peroxidation expressed by a significant increase (p<0.01) in MDA in rats receiving diclofenac compared to the control group. Conversely, a five-day treatment with the plant extract (EXT+DF) resulted in a significant reduction (p < 0.05) in the level of renal and hepatic MDA compared to rats treated with diclofenac. Consequently, pretreatment with the extract resulted a reduction in lipid oxidation in rats and a normalization of the MDA value in comparison to the toxic group.

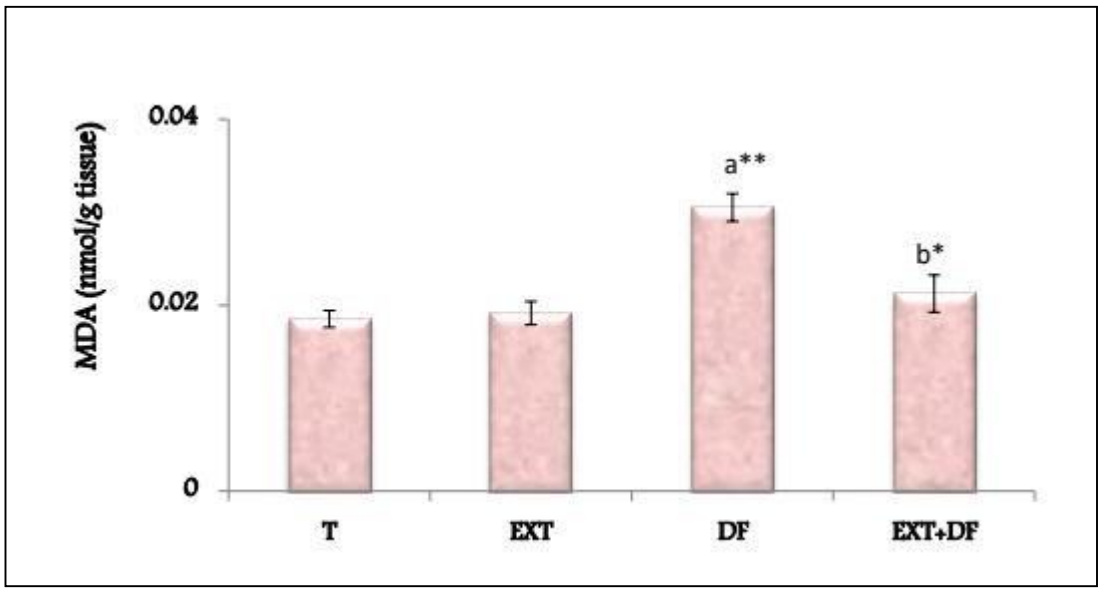


Figure 39: Effect of diclofenac and extract on renal MDA levels in different groups. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

b. The effect on GSH of the liver and kidneys

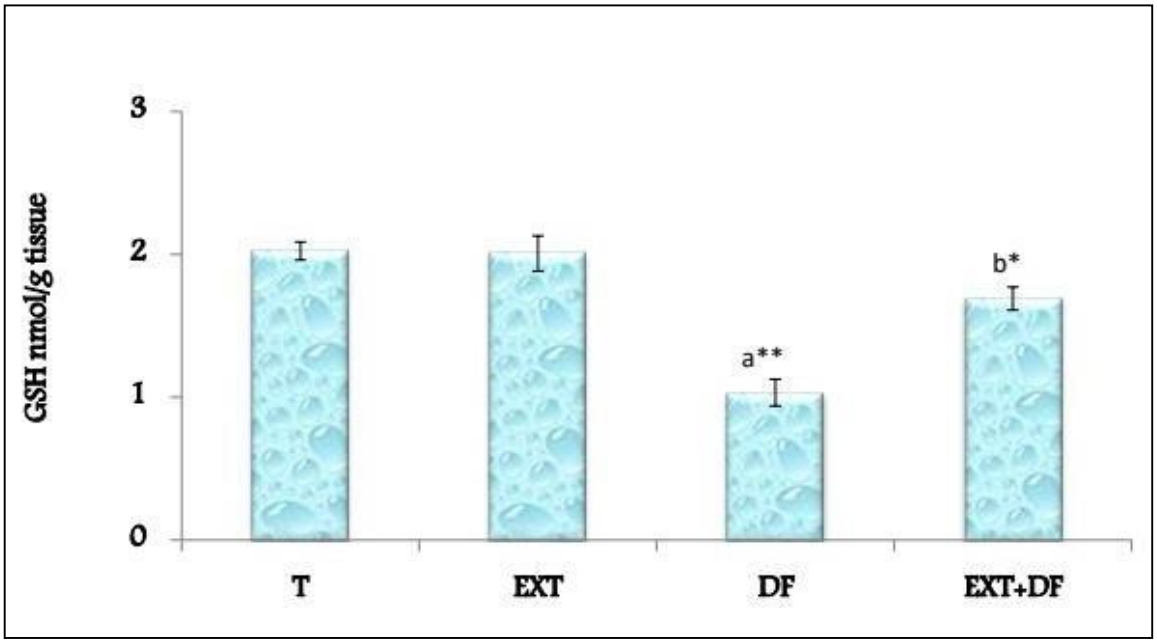


Figure 40: Effect of diclofenac and extract on liver GSH levels in different groups. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

According to the results illustrated in figures 40 and 41, the treatment of rats with DF (50mg/kg) clearly caused a significant depletion (p<0.01) in the level of hepatic and renal GSH compared to the control group. Pretreatment with the plant extract for 5 days at a daily dose of 100mg/kg showed a significant improvement (p< 0.05) in renal and hepatic tissue levels of reduced glutathione (GSH) compared to the DF group.

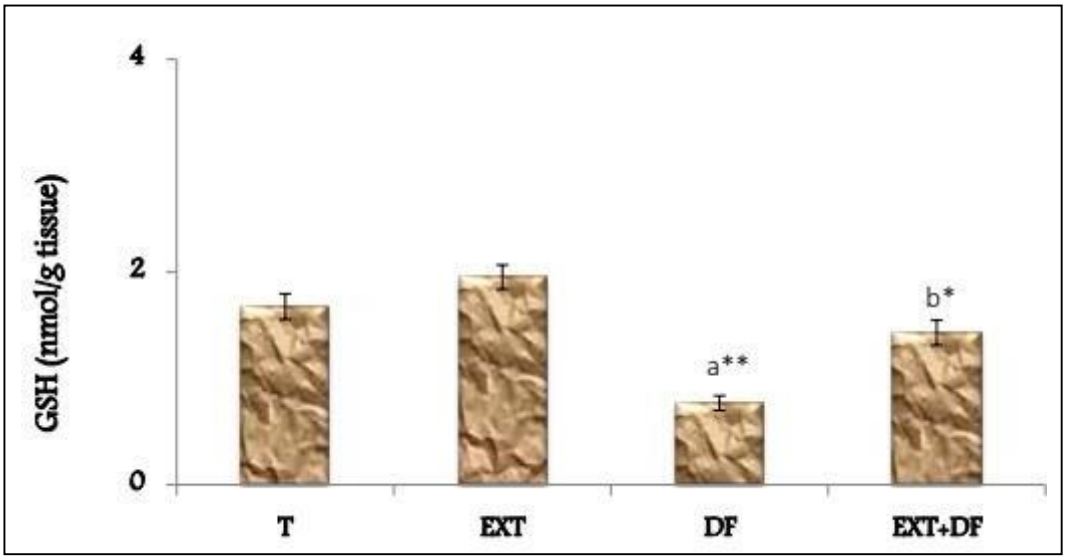


Figure 41: Effect of diclofenac and extract on renal GSH levels in different groups. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

c. Effect on GPx activity in the liver and kidneys

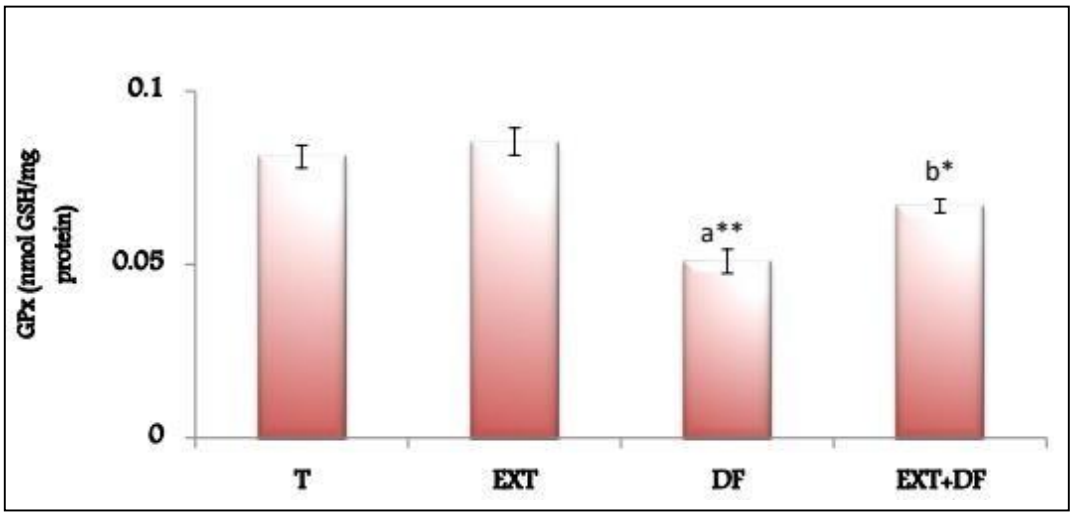


Figure 42: Effect of diclofenac and extract on liver GPx activity in different groups. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

The activity of GPx in the homogenate of the kidneys and liver is significantly reduced (p <0.01) in rats treated with diclofenac alone compared to that measured in control rats. Conversely, concomitant treatment with the *n*-butanol extract (100 mg/kg) caused a significant increase in GPx activity in rats treated with DF (figure 42 and 43).

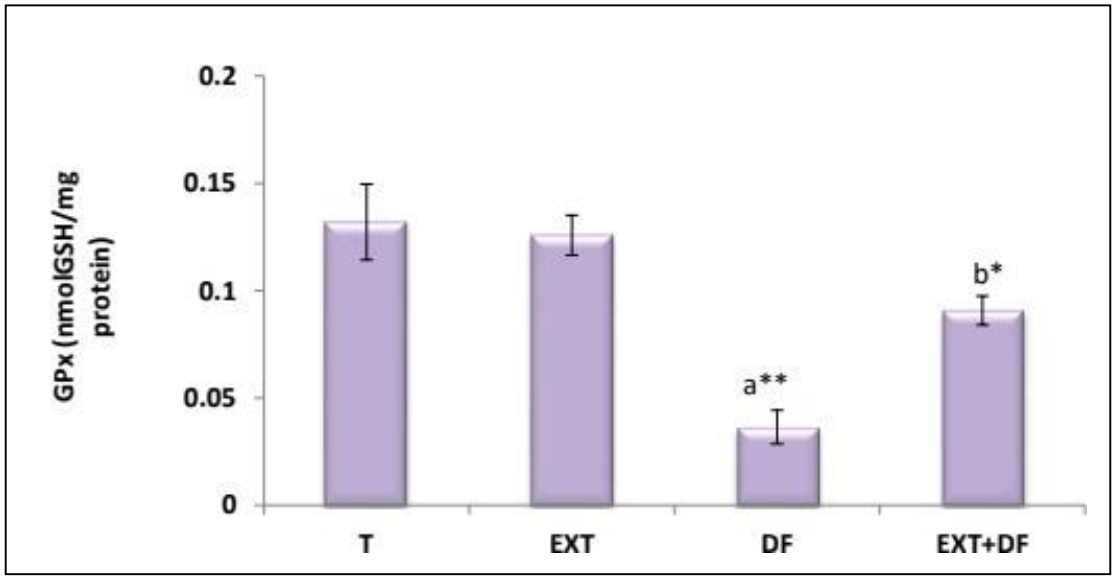


Figure 43: Effect of diclofenac and extract on renal GPx activity in different groups. Data are expressed as mean ± SD. * : P< 0.05, ** : P< 0.01, a : Groups compared to the control group b : Groups compared to the diclofenac group

I.3. Histopathological Assessments

I.3.1. Kidney histopathology

Histological sections of kidney fragments from control group rats showed a normal morphological appearance (figure 44). Furthermore, figure 45 demonstrates that there were no observable morphological changes in the kidneys of the extract-treated group in the microscopic examination. Histopathological alterations were observed in the kidneys of the diclofenac group (50 mg/kg), including moderate glomerular atrophy, proximal tubular necrosis, and an interstitial lymphocytic inflammatory infiltrate (figure 46).

Comparing with the group treated with DF, microscopic examination of histological sections of the kidneys of rats pretreated with the extract at a dose of 100 mg/kg showed almost normal tubules and glomeruli (figure 47).

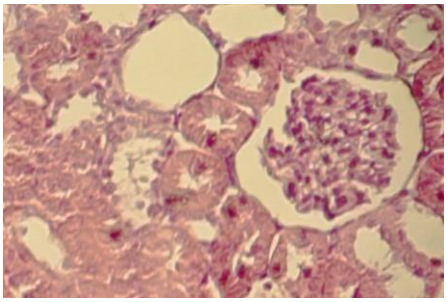


Figure 44: Histology of control rat kidneys (400 x)

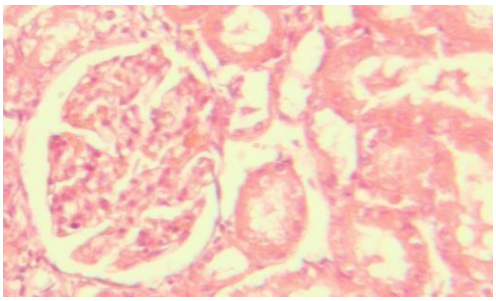


Figure 45: Histology of the kidneys of rats treated with the plant extract (100 mg / kg) H&E (400x)

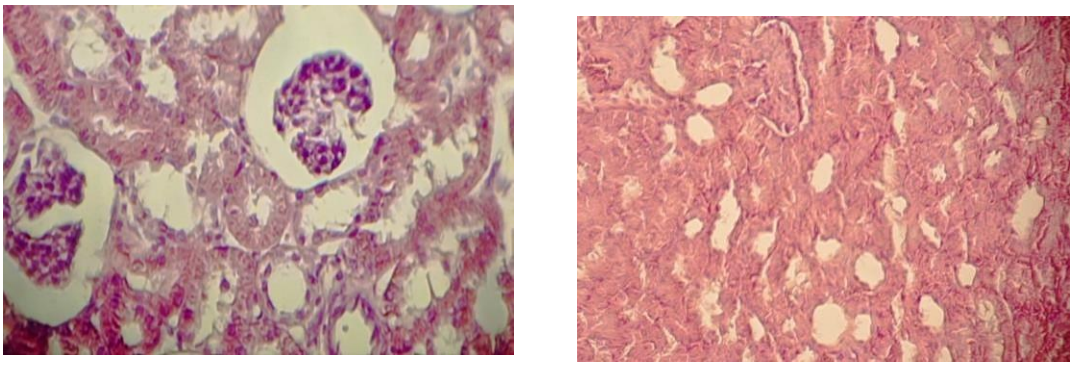


Figure 46: Histology of the kidneys of rats from the toxic group; group treated with diclofenac (50 mg/kg): (1) moderate glomerular atrophy; (2) beginning of necrosis; (3) interstitial lymphocytic inflammatory infiltrate
(100 x et 400x)

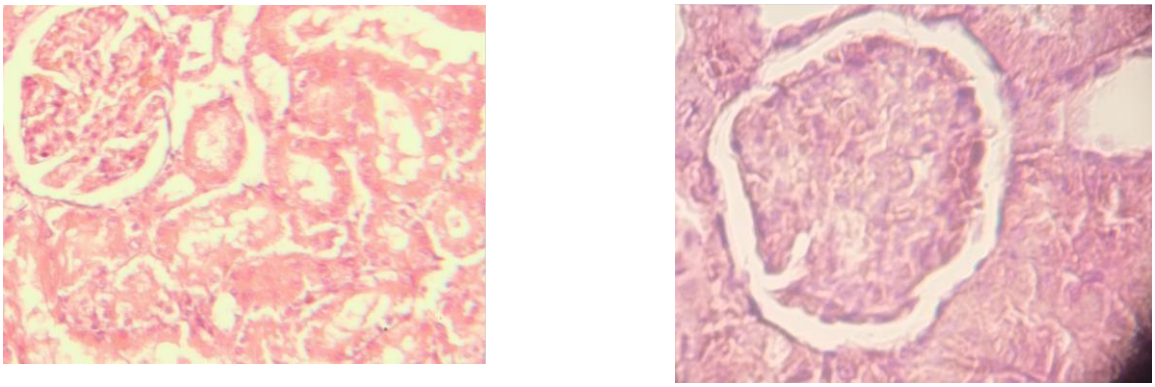


Figure 47: Histology of kidneys of rats treated with DF (50 mg/kg) and n-butanol extract (100 mg/kg) showed a histological picture comparable to that of the control group

I.3.2. Liver histopathology

Histological sections of liver fragments from rats in the control group showed normal cellular architecture, characterized by the arrangement of hepatocytes around the central vein with portal triad and normal sinusoidal spaces (figure 48).

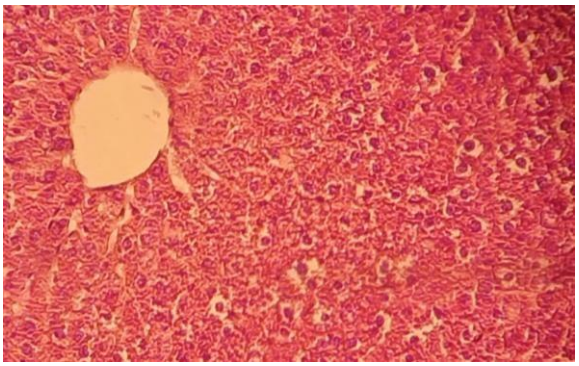


Figure 48: Histology of control rat livers (×100)

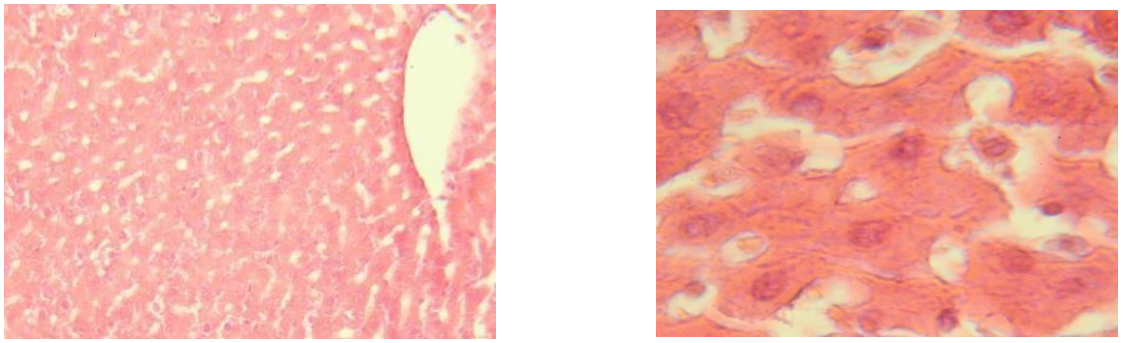
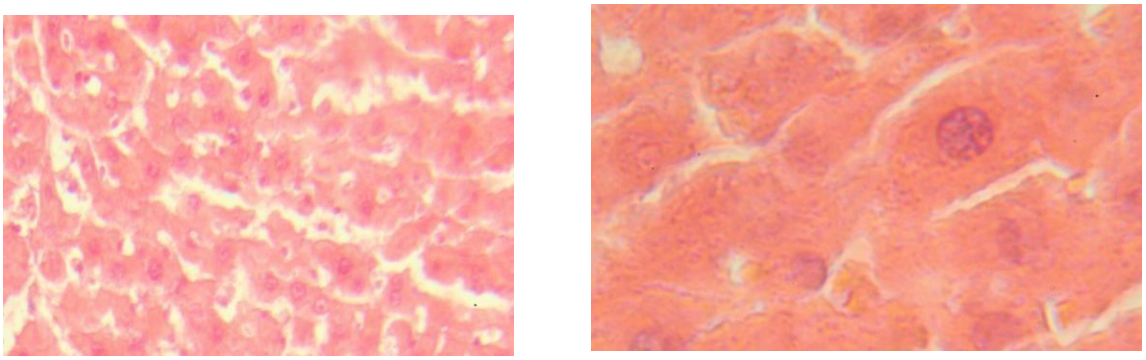


Figure 49: Histology of the livers of rats treated with the plant extract (100 mg / kg) (100x et 400x)

In addition, histological sections of the livers of rats treated with the *n*-butanol extract of *Centaurea sp.* (100 mg/kg) showed normal histology almost similar to that of the control group (figure 49).

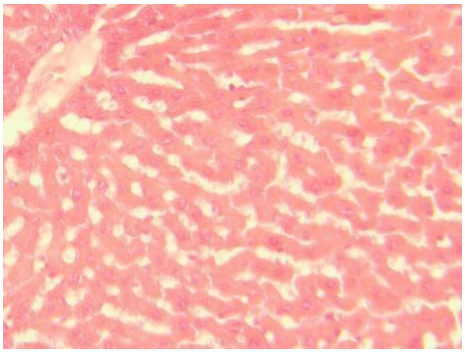


A **B**

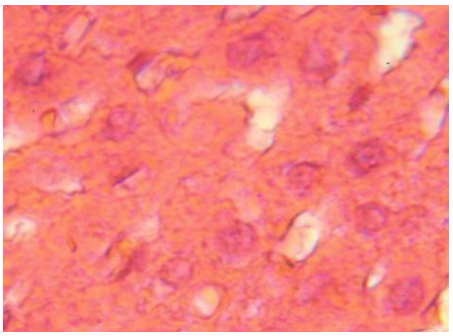
Figure 50: Histology of the liver of rats in the toxic group (group treated with diclofenac (50 mg/kg)): (A) Central perivesicular lymphocytic infiltrate, (B) Periportal and lobular necrosis (100x et 400x)

However, histological sections of the liver of rats treated with diclofenac (50mg/kg) revealed severe histopathological alterations. It is notable that the histological sections of the liver exhibited a perivesicular central lymphocytic infiltrate and focal marked periportal and lobular necrosis (figure 50).

The administration of 100mg/kg of the *n*-butanol extract of *Centaurea sp*. reduced the histopathological changes induced by diclofenac, they showed a histological form comparable to that of the control group with the presence of binucleate cells, hepatic congestion. Histological observation of the liver confirms the results of measurements of biochemical parameters and the antioxidant profile and further confirms the protective effect of the *n*-butanol extract of *Centaurea sp.* plant (figure 51).



C



D

Figure 51: The liver histology of rats treated with DF (50 mg/kg) and Centaurea sp. plant extract (100 mg/kg) showed a histological picture comparable to that of the control group with minimal damage of hepatocytes. (C) Liver congestion, (D) Binucleation of hepatocytes (100 x et 400x)

II. Discussion

Medicinal plants play a very promising role in the treatment of a variety of diseases. The nature has blessed medicinal plants with a wide range of bioactive phytochemicals **(Bibi *et al.*, 2024)**. The present study was designed to evaluate the possible anti-inflammatory and protective role of polyphenols from *Centaurea sp.* against diclofenac-induced toxicity in *Wistar* rats.

Inflammation is the body's defensive reaction to potentially hazardous impulses such as viruses or chemicals that induce cell injury. It triggers inflammatory cells and signaling pathways. The inflammation process is critical in the recovery process because it allows aberrant bodily homeostasis to be restored. Acute inflammation that is not effectively managed can aggravate organ disease and eventually develop into a chronic inflammatory phenotype **(Yeni and Rizky Arcinthya Rachmania, 2022)**. During the inflammatory process, reactive oxygen species (ROS), reactive nitrogen species (RNS), tumor necrosis factor-α (TNF-α), interleukins (ILs), cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX-2) are highly produced in host cells as inflammatory bio-indicators **(Fangkrathok *et al.*, 2013)**. Even though several models are applied to evaluate the anti-inflammatory potential of phytochemicals, protein denaturation and erythrocyte membrane stability were reported as the two mostly used assays for *in vitro* studies **(Otunola and Afolayan, 2018)**. Body inflammation is regulated by numerous signaling pathways forming a complex system. Therefore, drug development has focused on the key targets that antagonized, neutralized, or blocked particular products such as enzyme inhibiting activities by imitating potential phytochemicals used as anti-inflammatory drugs. For the treatment of inflammation, people have been using non-steroidal anti-inflammatory drugs (NSAIDs) as medicines. However, the increasing side effects such as heart attack and strokes due to these drugs are the main need to replace synthetic drugs with minimal risk-causing plant-based medicines **(Gonfa *et al.*, 2023)**.

Extracts of medicinal plants are reported as excellent folk medicine for anti-inflammatory agents worldwide. They are rich sources of phytochemicals applied as herbal medicines and a reasonable basis for producing new anti-inflammatory drugs. Isolated compounds from extracts of medicinal plants are also reported for their effective anti-inflammatory uses **(Verma, 2016; Kiani *et al.*, 2019)**.

Thus, this study was designed to evaluate the curative capacity of *n*-butanol extract of *Centaurea sp.*, against inflammation. The evaluation of anti-inflammatory properties of the extracts was done by formalin-induced inflammation in *Wistar albino* rats.

The search for anti-inflammatory properties was conducted in accordance with the acute inflammation model, whereby edema was induced in the paws of rats using formaldehyde (2.5%) as an inflammatory agent at a rate of 0.1mL per paw. The *n*-butanol extract showed a significant reduction *($p < 0.01$)* in the edema paw volume in a dose-dependent method with a maximum attend at 200 mg/kg, a dose-related inhibition of hind paws edema was observed. With all doses 3 h after formalin injection, diclofenac as a reference standard (30 mg/kg) produced a significant inhibitory effect comparable to control group *($p < 0.001$)*.

Formalin paw edema is a test widely used to determine anti-inflammatory activity as it involves several mediators. Tissue damage and injury are always associated with pain and inflammation. Formalin test is a biphasic response where first phase is the direct effect of formalin, which involves neurogenic pain. The second phase is involved in the inflammatory reactions mediated by prostaglandin, serotonin, histamine, bradikinin and cytokines, such as interleukin-1 beta, interleukin-6 tumor necrosis factor-alpha eicosanoids, and Nitric Oxide **(John and Shobana, 2012; Arzi *et al.*, 2015; Patil *et al.*, 2019)**.

The results demonstrated that anti-inflammatory effects of *n*-butanol extract was dose-dependent and the best anti-inflammatory effect was observed in the doses of 200 mg/kg. This evidence allowed us to suggest that anti-inflammatory actions of this extract are related to the inhibition of one or more intracellular signaling pathways involved in the effects of several inflammatory mediators **(Ananthi *et al.*, 2010; Jayaram and Kuriachan, 2021)**. The obtained results suggest that the extract possibly acts by inhibiting the release and/or action of prostaglandin E2 (PGE2) since the extract showed significant inhibitory activity at the second phase of the edema development. Based on the well-known involvement of free radicals in inflammatory processes it seems that at least a part of the anti-inflammatory effects of *n*-butanol extract may be attributed to its antioxidant constituents **(Hajhashemi *et al.*, 2009; Boubekri *et al.*, 2014)**.

Using female rats, Boubekri *et al.*, (2014) performed anti-inflammatory activity on *Genista quadriflora* munby extract. In the study, the anti-inflammatory activity of the plant extract (100 and 200 mg/kg) was assessed by carrageenan-induced rat paw. The *n*-butanol extract of the *G. quadriflora* demonstrated significant reduction in the edema paw volume in a dose-dependent manner and the aspirin standard (100 mg/kg) produced a significant inhibitory effect comparable to the group that took the plant extract **(Oguntibeju, 2018)**.

The results of our study are consistent with those observed in studies conducted by **(El-Hela *et al*., 2016)** as well as **(Singh *et al*., 2012)**. Pre-treatment with the *n*-butanol extract of the plant had a similar effect to diclofenac. These effects on edema can be explained by the inhibition of the synthesis of pro-inflammatory substances **(Rahmani *et al*., 2016)**, which supports its traditional use for the relief of various inflammatory conditions.

Inflammation disease is one of the most common causes of liver damage or liver dysfunction. With the advancement of research on medicinal plants, it becomes clear that the application of extracts and pure compounds for the treatment of liver diseases is highly effective for the inhibition, blockage, or decreasing of the signal pathways for the induced inflammatory factors in the liver **(Li *et al*., 2019; Gonfa *et al*., 2023)**. Some flavonoids obtained from methanol extract of the aerial part of *B. vulgaris* exhibited significant reduction of liver stress **(Asadi-Samani *et al*., 2015)**. Geniposide compound isolated from the fruit of *G. jasminoides* was reported to inhibit liver fibrosis and suppress expression of CYP2E1 **(Zhang *et al*., 2013)**.

As we know, DF is an anti-inflammatory drug that is used for pain and analgesics. It has been huge concern about the toxicity caused by DF that is hepato, renal and gastro toxicity. Its deposition and its bioactivation to intermediate reactive causes the production of oxidative stress, inflammation, tissue degeneration, and necrosis suggested playing a role in organ damages (**Simona *et al*., 2020**).

Animals treated with diclofenac (50 mg/kg) intraperitoneally on days 4^{th} and 5^{th} developed a liver lesion manifested by a significant increase in the serum levels of the liver enzymes AST, ALT compared to normal untreated animals. The results were found to be consistent with those of previous studies **(Mostafa, *et al*., 2020; Simona *et al*., 2020)**. This increase is attributed to structural liver damage. As hepatic transaminases (AST and ALT) are available in greater quantities in the liver than in other organs, they are biomarkers of choice and remain reference measures in the evaluation of liver damage. ALT is more specific to the liver and is a better parameter to detect liver damage. AST is located in the heart, brain, skeletal muscle tissue and liver. An elevated AST level is indicative of hepatic impairment, although it may also be indicative of myocardial infarction or muscle injury **(Metushi *et al*., 2012)**.

In contrast, serum levels of urea and creatinine have been employed as prognostic factors in renal disease. The serum creatinine level has been employed to predict the glomerular filtration

rate. The renal function test demonstrated a significant increase in the levels of urea and creatinine in the groups that received diclofenac compared to the control group. This indicated an increase in the conversion of ammonia into urea, an elevated production of free radicals, and a low glomerular filtration rate due to kidney dystrophy. The results obtained are in agreement with those previously reported by **Simon *et al*., 2019; Moradi *et al*., 2020.** Non-steroidal anti-inflammatory drugs have been demonstrated to have a specific affinity for kidney tissue. Furthermore, they are well-known to be nephrotoxic, with their nephrotoxic effects being attributed to the induction of mitochondrial permeability transition pore (MMPT), a phenomenon of mitochondrial degeneration initiated by the flow of calcium ions into the mitochondria due to the inducing peroxidants inorganic phosphate or reactive oxygen species **(Jung *et al*., 2020)**.

Inhibition of renal prostaglandin biosynthesis has been demonstrated to be an unlikely primary cause of DF-induced nephrotoxicity (NT), although induction of oxidative stress and lipid peroxidation by DF may be a proposed mechanism for acute NT. Interestingly, DF-induced NT develops through a strong release of pro-inflammatory substances. Blood urea nitrogen and serum creatinine are considered traditional biomarkers of NT and renal failure **(Islas-Flores et *al*., 2013; Al-Kuraishy *et al*., 2019)**.

Medicinal plants have strong therapeutic purposes and have been used since centuries for the preparation of functional drugs. Plants are in use as medicine for human beings at least 60,000 years back to the middle Paleolithic period. Medicinal plants have very important role in treating various fatal diseases. All parts of the plants contain various active compounds which can be used as a remedy for different diseases. Phytochemicals also known as bioactive nutrients or compounds which naturally found in plants which are released or produced by the plants for health benefits **(Shinwari *et al*., 2017; Bibi *et al*., 2024)**.

In the last 15 years, there has been a growing interest in the polyphenolic components found in plants of the genus *Centaurea* (Asteraceae). It is a large genus, including several hundred representatives, originating from the Mediterranean basin, but it is widespread on practically most of the continents, especially since the plants are quite expensive and do not require special soil conditions **(Kubik *et al*., 2022)**. Many plants belonging to this genus have been tested for therapeutic properties. Studies have revealed that extracts from plants belonging to the genus *Centaurea sp*. act as digestive enhancers, stimulants of the production and secretion of bile and lowering agents for blood pressure. Due to the high availability and common nature of these plants, they are often used in folk medicine as antidiarrheal, astringent, antipyretic, anti-

inflammatory, and anti-rheumatic agents, as well as antifungal, antibacterial, and antidiabetic agents. They also have valuable compounds with strong antioxidant and even anti-cancer potential **(Alper and Güne, 2019)**.

The antioxidant and anti-inflammatory activities are related to each other. Free radicals are produced in cells/tissues during normal physiology and play important roles that are necessary for normal function. However, excessive production is also formidable because it can cause oxidative stress and damage cells, lipids, and proteins. Simultaneously, oxidative stress induces the expression of cyclooxygenase and lipooxygenase, which triggers the secretion of inflammatory mediators **(Wang *et al*., 2020)**.

Pre-treatment with the dose (100 mg/kg) of the *n*-butanol extract of *Centaurea sp*. plant demonstrated a protective effect on the liver and kidneys against diclofenac-induced injury, as indicated by a significant restoration of serum levels of ALT, AST, urea, and creatinine. These findings are consistent with those of **Simon *et al*., 2019; Abed Al-Kareem *et al*., 2022; Mendoza-Fernández *et al*., 2023**.

The work of Mendoza-Fernández and colleagues demonstrates that pretreatment with the methanolic extract of *T. integrifolia* (100, 200, and 400 mg/kg/day orally) significantly attenuated the elevation in serum levels of ALT, AST, urea, and creatinine compared to the diclofenac group. In the study by Allwell and colleagues (2024), the ethanol extracts of plantain root plant demonstrated nephroprotective and hepatoprotective effects against diclofenac-induced toxicity (50mg/kg).

In addition, treatment of rats with DF (50 mg/kg) significantly increased MDA levels in the liver and kidneys compared to untreated rats. This elevation in MDA levels suggests that extreme free radical production and lipid peroxidation are involved in the pathogenesis of numerous diseases **(Messarah *et al*., 2013)**. The results of our study indicate that the administration of DF to rats resulted a significant reduction in glutathione (GSH) levels and glutathione peroxidase (GPx) activity in kidneys, and liver. These results are consistent with those of **Tandoh *et al*., 2021; Eman, 2022 and Villa-Jaimes *et al*., 2023**.

The accumulation of MDA is an indicator of lipid peroxidation which was observed in the kidneys and liver of rats treated with diclofenac, while the level of GSH was significantly reduced, these results demonstrated the occurrence of oxidative stress and lipid peroxidation in the kidneys and liver, probably due to ROS produced during the metabolism of diclofenac, which

is metabolized by CYP3A4 to 5-0H-diclofenac, which is then conjugated urinary to mercapturic acid in humans which explains the decrease in the GSH reserve **(Simon *et al*., 2019)**.

The GSH/GPx enzyme system removes reactive oxygen species (ROS) and reactive nitrogen species (RNS). GSH depletion can lead to increased ROS and RNS generation, increased mitochondrial complex activity and nicotinamide adenine dinucleotide phosphate (NADPH) oxidation, decreased cell viability, and impaired adenosine triphosphate (ATP) generation. GPx catalyses the reduction of many oxidising species, including hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), using GSH as a substrate. Depletion of GPx increases oxidative stress and leads to endothelial dysfunction and apoptosis **(Panday *et al*., 2020)**.

Co-treatment with the *n*-butanol extract reduced MDA levels and preserved GSH and GPx levels, reflecting the ability of our extract to reduce the renal and hepatic damage caused by diclofenac. These results are consistent with the work of **Oyinloye *et al.* (2023)**, who show that the ethyl acetate fraction of *Mucuna pruriens* leaves at a dose of 400 mg/kg protects the liver against diclofenac-induced toxicity.

Possible antioxidant mechanisms of the *n*-butanol extract include its ability to scavenge ROS or increase the levels of endogenous antioxidant enzymes. This radical scavenging activity of the extract may be related to the nature of the phenolic compounds, thus contributing to their electron transfer/hydrogen donating capacity **(Li et Kong, 2009)**.

Phenolic compounds are a group of secondary metabolites with highly effective free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory action. Flavonoids are a group of phenolic compounds with beneficial abilities ranging from their ability to scavenge a wide range of oxygen, chlorine, and nitrogen species such as hydroxyl ions, peroxynitrous acid, superoxide, reactive oxygen, peroxyl radicals, and hypochlorious acid to their ability to chelate ions by decreasing the metal ions pro-oxidant capacity **(Nwozo *et al*., 2023)**.

The results of our histological study show that treatment of male rats with diclofenac (50 mg/kg) causes morphological changes in the liver and kidneys. DF administration caused a perivesicular central lymphocytic infiltrate, focal marked periportal and lobular necrosis. Furthermore, diclofenac-induced nephrotoxicity was confirmed by the histological alterations recorded, including moderate glomerular atrophy, proximal tubular necrosis, and interstitial lymphocytic inflammatory infiltrate **(Prathima *et al*., 2018; Oyinloye *et al*., 2023; Allwell *et al*., 2024)**.

The histopathology change becomes predominant with DF treated rats has more tissue damage and inflammation with a dose of 50mg/kg. Since the hepatocytes receive blood flow, which has a reduced level of essential nutrients and oxygen that causes hepatic degeneration and periportal inflammation causes liver damage **(Simon *et al.*, 2019)**.

In contrast, co-treatment with plant extract (100 mg/kg) reduced the morphological changes induced by diclofenac (50 mg/kg) in the liver and kidneys compared with the control group in which showed almost normal tubules and glomeruli for the renal tissue and either an almost normal one with the presence of binucleate cells and hepatic congestion for the hepatic tissue. These results are consistent with those of **(Salman *et al.*, 2020; Sagástegui-Guarniz *et al.*, 2020)**.

All the above results confirm the protective effect of the *n*-butanol extract of *Centaurea sp.* plant and its antioxidant and anti-inflammatory properties.

Conclusion and prospects

Today, medicinal plant therapy is a genuine human heritage in the field of public health, where the diversity of biological properties is certainly linked to the therapeutic virtues attributed to an extraordinary range of bioactive molecules synthesised by the plant. This is what we have been able to demonstrate through our work.

The experimental part enabled us to respond to the various hypotheses raised in the introduction and to formulate a conclusion stipulating that the *n*-BuOH extract of the *Centaurea sp.* Plant (100mg/kg) acts via several mechanisms in the protection against toxicity induced by diclofenac as a model for NSAIDs in rats.

The first part of this study, which evaluated the anti-inflammatory activity elucidated by the formalin-induced oedema test, concluded that the anti-inflammatory effect of the *n*-BuOH extract is very promising, even better than the standard drug itself. This means that the extract exhibits anti-inflammatory action against the release of the mediators of acute inflammation; histamine, bradykinin and prostaglandins. This plant has pharmacological power, which supports its traditional use for the relief of various inflammatory conditions.

The results obtained in the second part of this experiment, which involved evaluating the antioxidant, nephroprotective and hepatoprotective activity of the extract studied, showed a correlation between the polyphenols and flavonoids present in the extract and their significant antioxidant activity against the toxicity caused by diclofenac. The toxicity of this agent was demonstrated by hepatic and renal dysfunction (release into the blood of hepatic transaminases, creatinine and urea) and oxidative stress in intoxicated rats. In contrast, co-treatment of the rats with *n*-BuOH extract appears to be able to balance endogenous antioxidant activity by improving GSH levels, restoring GPx activity and maintaining MDA levels.

Taking these data together, we can summarise that *n*-BuOH extract comprises polyphenolic compounds that are endowed with numerous beneficial properties.

However, this research is still in its early stages and requires further exploration, including the isolation, identification, and characterization of the active compound, reliable methods. It also requires investigations into the mechanisms of action of the plant extract to establish correlations between their pharmacological activities and chemical constituents. Additionally, further research into the active ingredients and testing for other biological activities will enhance our understanding of these species.

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Evaluation of the possible anti-inflammatory and protective role of polyphenols from *Centaurea sp.* against diclofenac-induced toxicity in *Wistar* rats

Mémoire pour l'obtention du diplôme de Master en Toxicologie

Abstract

Inflammation is an essential response provided by the immune system that ensures survival during infection and tissue injury. Diclofenac (DF) is administrated to treat pain, inflammatory disorders, and dysmenorrhea but kidney and liver problems are the main worries of the agent. *Centaurea sp.* is widely used in traditional medicine for its therapeutic properties essentially attributable to natural bioactive compounds. In the present study, we evaluated the possible anti-inflammatory activity using formalin-induced paw edema, and the protective role of polyphenols from *Centaurea sp.* against diclofenac-induced toxicity in *Wistar* rats.

Rats were orally administered *n*-butanol extract (100 mg/kg b.w.) for 5 days and diclofenac was administered on the 4^{th} and 5^{th} day (50mg/kg, ip). Serum transaminases, creatinine, urea, lipid peroxidation (LPO), reduced glutathione (GSH), and glutathione peroxidase (GP_X) were estimated to access liver and kidney damage. A histological study was determined.

The results showed that *n*-butanol extract (100 and 200mg/kg) exhibited a significant reduction of edema in formalin-induced rat paw edema. Significant changes in biochemical indicators (ALT, ASAT, urea, creatinine) and oxidative stress (malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPx)) in the group treated with diclofenac (50 mg/kg). This dysfunction was accompanied by alterations and changes in hepatic and renal architecture. While, these levels were restored to control value in animals treated with plant extract. The regularized levels of LPO, GSH, transaminases, creatinine, urea and GPx activities revealed the antioxidant properties of the extract plant. The histological study showed the hepatoprotective and nephroprotective effect of our extracts against diclofenac-induced toxicity.

These results reveal the promising potential of extract of *Centaurea sp.* as antioxidant, anti-inflammatory agent, and protector against the toxicity of anti-inflammatory drugs (DF). Thus, opening new perspectives in the field of medical research and pharmacology for the development of complementary therapies.

Mots-clefs : Diclofenac, *Centaurea sp*., Hepatotoxicity, Nephrotoxicity, Polyphenol, Antioxidant activity, Anti-inflammatory activity

Laboratoires de recherche : laboratoire de VARENBIOMOL (U Constantine 1 Frères Mentouri)

Présidente du jury : Pr. Amrani Amel (PROF - U Constantine 1 Frères Mentouri)
Encadrant : Dr. Boubekri Nassima (MC(A) - U Constantine 1 Frères Mentouri)
Examinateur : Dr. Kabouche Sami (MC(A) - U Constantine 1 Frères Mentouri)