MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE AKLI MOHAND OULHADJ – BOUIRA
FACULTE DES SCIENCES DE LA NATURE ET DE LA VIE ET DES SCIENCES DE LA TERRE
DEPARTEMENT DE BIOLOGIE

Réf : ……./UAMOB/F.SNV.ST/DEP.AGR/2017

MEMOIRE DE FIN D'ETUDES

EN VUE DE L'OBTENTION DU DIPLOME MASTER

Domaine: SNV **Filière**: Sciences Biologiques
Spécialité: Analyses Biologiques et Biochimiques

Présenté par:

LAOUFI Thiziri et LADJ Mouna

Thème

Artemisia herba-alba anti-inflammatory activity and gastro-protective effects in mice

Soutenu le: 03 /07 / 2017 **Devant le jury composé de:**

Nom et Prénom	**Grade**		
Mme BOUTELDJA Razika	MAA	Univ. de Bouira	Présidente
Mr. HAMZAOUI Soufiane	MCB	Univ. de Bouira	Promoteur
Mr. LAMINE Salim	MAA	Univ. de Bouira	Examinateur

Année Universitaire: 2016/2017

Abstract

Artemesiaherba-alba commonly named "chih" is a medicinal plant, very wide spreade in North Africa to treat several diseases.

The objective of this study was to evaluate both anti-inflammatory effect and protective effect of the stomach of the aqueous extract of the white wormwood (1 ml / kg during 21 days) in white mice.

The obtained results, have shown, firstly that the inflammation of the mice induced by the stress during their grouping in a single cage has been diseapeased after feeding the mice by the aqueous extract of the white wormwood and the usean ointment formed by the powder of this plant mixed with water and applies against lesions of the skin.

In the second place, our study showed that the gavage of the aqueous extract of white wormwood 60 minutes before the administration of the ethanol to the mice was able to provide partial protection of the gastric mucus.

Key words: White wormwood, aqueous extract, inflammation, stomach ulcer, ointment.

Résumé

Artemesia herba alba communément Appelée “Chih”, est une plante médicinal très répandue dans l’Afrique du nord largement utilise pour traiter plusieurs maladies.
L’objectif de cette étude était d’évaluer l’effet anti inflammatoire et l’effet protecteur de l’estomac de l’extrait aqueux de l’armoise herbe blanche (1ml/kg pendant 21 jours) chez les souris blanches.

Les résultats obtenu montrent en premier lieu que l’inflammation des souris induite par le stress lors de leur regroupement dans une seule cage a disparue après avoir nourri les souris par de l’extrait aqueux de l’armoise herbe blanche et l’application d’une pommade formée a partir de poudre de cette plante, mélangée avec de l’eau et appliquée sur les lésions de la peau.

En deuxième, notre étude a montrée aussi que le gavage de l’extrait aqueux d’armoise blanche 60 minutes avant l’administration de l’éthanol a 50% aux souris a pu assurer une protection partial dela mucus gastrique.

Mots clés : armoise herbe blanche, extrait aqueux, inflammation, ulcère d’estomac, pommade

ملخص

يعتبرالشيح*Artemisia Herba-Alba*من بين أهم الاعشاب الطبية التي تستخدم في الطب التقليدي في شمال افريقيا لمعالجة العديد من الأمراض . الهدف من هذه الدراسة هو معرفة التأثير المحتمل للشيح على التهاب الجلد و القرحة المعدية (1 ملل /كغ الفئران البيضاء).

و قد أظهرت النتائج المتحصل عليها بان الفئران التي وضعناها في قفص واحد لمدة 15 يوما قد أصيبت بالتهابات على مستوى الجلد بسبب الشجارات التي حدثت بينها (يعود ذلك الى التوتر) قد شفيت بعد21يوما. بعدما قمنا بمعالجتها بالمحلول المائي للشيح و مرهم محضر من بودرة الشيح م الماء المقطر .

ما أظهرت دراستنا أيضا أن للمحلول المائي المحضر من الشيح تأثير إيجابي على المعدة حيث قام بحمايتها من الضرر الذي سببه الايثان

الكلمات المفتاحية :المستخلص المائي للشيح, الالتهاب ,القرحة المعدية,المرهم

Acknowledgment

Above all, I thank ALLAH for giving us the will in order to finish this humble work.

Wewould also like to thank my supervisor *Mr.HAMZAOUI Soufiane,* lecture at the Faculty of Sciences of Nature and Life at the University of Bouira, who followed and guided us throughout this work and for his confidence and encouragement.

We would also like to thank *Ms BOUTELDJA*, a assistant professor in the Faculty of Nature and Life Sciences at the University of Bouira, for accepting to chair the jury.

We, thank *Mr. LAMINE Assistant* professor at the Faculty of Nature and Life Sciences at the University of Bouira, for having accepted to judge this work

We would like to thank Ms. *HAMICHE Thiziri* for her assistance, especially to complete the practical part of this thesis. We also want to thank *Ms. Nadjma* for helping us and doing her best to provide us what we needed.

We also want to thank assistant Professor *CHIRIFI,* who has never hesitated to help us. And also Professors *HAMDIS* and *CHIBANE* about their assistance.

We want to thank the student *DERRICHE M'hamed* and *MERZOUK Oussama* for the unlimited assistance they gave us to accomplish this work. Finally, we thank the security officials especially *Mustafa, Farhat* and maintenance workers, especially *BOURAS*.

Dedication

I dedicate this modest work to my dear parents, the source of my joys and the secret of my strength, you will always be the model: my father *Hacene*, in your determination, your strength and your honesty, my mother *Louiza*, And your dedication to us. Thank you for your sacrifices. I owe you this success.
To the spirit of my grandfather *Mahfoud*.
To my brothers "*Houssam*" "*Gillas*" and my sister "*Safia*" ,to my grandmother also to my uncle and his wife, children and to my aunts.
To my friends *siham,Mssou,oussama,M'hamed*.
To all who love me.

(LAOUFI Thiziri)

I dedicate this modest work to my dear parents, the sources of my joys and the secret of my strength, you will always be the model: my fatherAbdenour, in your determination, your strength and your honesty, my mother *Dalila*, And your dedication to us. Thank you for your sacrifices. I owe you this success.
To my uncle *Madjid* and his wife *Nedjma*
To my brothers *Hocine* and *Lamine*
To my friends *Siham, Karim, Misssou, Souhila, Amel*

(Ladj Mouna)

List of abbreviations

ADP: Adenosine diphosphate.

AHA*: *Artemisia herba alba.

ATP: Adenosine triphosphate.

CBC: Complete Blood count.

CCL2: Chemokine Ligand 2.

CCR2: C-C Chemokine receptor type 2.

C5a: Complement Component 5a

DAP: Dihydroxyacetone phosphate.

EDTA: Ethyldiamine

G-CSF: Granulocyte Colony-Stimulating Factor.

GK: Glycerol kinase.

GLDH: 2-L-Glutamat dehydrogenase.

GM-CSF: Granulocyte –Macrophage Colony-Stimulating Factor Human.

GPO: Glycerol Phosphate oxidase.

G-6-P: Glucose-6-phosphate.

G-6-PDH: Glucose-6-phosphate dishydrogenase.

HBA: Hydroxybenzoic acid.

H.E: Hematoxylin-Eosin.

HES: Hematéine-Eosine-Saffron.

HK: Hexokinase.

HMG-coA: 3-Hydroxy-3-Methylglutaryl-Coenzyme A.

H_2O_2: Hydrogen peroxide.

IGg: Immunoglobulin G.

IL6: Interleukin-6.

LDL: Low Density Lipoprotein.

LTB4: Leukotriene B4. .

MAPK: Mitogen activated protein kinase.

MMG: May -Grunwald / Giemsa.

MO: Optic microscope.

NSAID: Non-steroidal anti inflammatory drugs.

NSAIDs: Nonsteroidalanti-inflammatory drugs.

NAD: Nicotinamide adenine dinucleotide.

NADH: Nicotinamide adenine dinucleotide reduced.

NH_3: Ammoniac

NH_4: Ammonium

PGE: Prostaglandins of the E type.

PUD: Peptic ulcer disease.

TG: Triglycerides.

TNFα: Tumornecrosis factor

UV: Ultra violet.

4-AAP: 4-aminoantipyrine.

4-CP: 4-chlorophenol.

Tables list

Table 01: Different cells involved in the inflammatory reaction……13

List of figures

Figure 01: The plant Artemisia herba alba……3

Figure 02: Distribution map of *Artemisia herba alba In the world* ……..……... 4

Figure 03: Anatomy of the stomach**.....**.....9

Figure 04: histological structure of stomach**... ..**.....9

Figure 05: Location map of sampling……..22

Figure 06: The mice used during the experiment…….**.....**23

Figure 07: The infused aqueous extract…….**.....**24

Figure 08: Ointment of *AHA*……24

Figure 09: A fixed mouse on a polystyrene support……**....**31

Figure 10: The organs of the mouse after dissection…….**.....**32

Figure 11: The stomach of the mouse after dissection……**....**32

Figure 12: The stomachs of mice to study in cassettes……34

Figure 13: Automaton device of inclusion…….**.....**34

Figure 14: The inclusion process that lasted 22 hours…….35

Figure 15: Paraffin coating apparatus……..36

Figure 16: The steps of coating a tissue sample…….**.....**36

Figure 17: Realization of histological sections using a microtome…….37

Figure 18: Label the blades with a diamond pencil…….38

Figure 19: Histological sections in a bath Marie…….38

Figure 20: The mounting of the blades……37

Figure 21: Blood smear of a mouse in dicates the preasence of leukocyte cells after 72 h of inflammation observed in OM with magnification (X40)…….……38

Figure 22: Blood smear of a mouse observed under MO with magnification (X40)……..39

Figure 23: White blood cells of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days ……..40

Figure 24: Neutrophil levels of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days ……41

Figure 25: Platelet levels of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days ……..…42

Figure 26: Glucos levels of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days …….……43

Figure 27: Cholesterol levels of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days ……44

Figure 28: Triglyceride levels of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days ……45

Figure 29: Urea levels of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days ……46

Figure 30: Creatinin levels of different group of mice (treated or not treated by aqueous extract of *AHA*) for 21 days ……46

Figure 31: Variation of body weight of the different groups of mice…….....47

Figure 32: Histological section of mouse stomach observed under light microscope with magnification (X4, X40) (Fundique region)……48

Figure 33: Histological section of mouse stomach observed under light microscope with magnification (X40) (Fundique region)……..…….48

Figure 34: The villi of stomach of witness mouse ……..…….49

Figure 35: Histological section of mouse stomach treated with ethanol for 15 min observed under OM with magnification (X10, X40)……...…49

Figure 36: The villi of not treted mouse by aqueous extract of AHA observed under OM with magnification (X10, X40)..……50

Figure 37: Histological section of mouse stomach observed under OM with magnification (X10) ……..…….…..50

Figure 38: Magnification (X40) of the area A of the figure 37……...……51

Figure 39: Magnification of zone B of the histological section of the stomach of a mouse observed with the M (X40)……51

List of tables

Table 01: Different cells involved in the inflammatory reaction…...……..13

Table of contents

List of figures and tables

List of abbreviations

Introduction…….....1

Literature review

Chapter I: *Artemisia herba-alba*

I-Botanical aspects of *Artemisia herba alba* (*AHA)*…….....3

I-1-Denominations…….....3

I-3-Morphology……..3

I-4-Taxonomy…….3

I-5-Distribution……...4

I-6-Phytochemestry..…….....5

I-6-1-Sesquiterpene……..5

I-6-2-Flavonoids……...……..……5

I-6-3-Phenolic compounds and waxes……6

I-6-4-Essential oils……...6

Chapter II: Peptic Ulcer Disease (stomach ulcers)

II-1-Historical Perspective……..7

II-2-Definition……7

II-3-Signs and symptoms……...……7

II-4-Anatomy……...…....8

II-5-Pathophysiology……...……10

II-6-Etiology…….....10

II-6-1-Ethanol:……..10

II-7-Epidemiology……...….11

Chapter III : Skin inflammation

III-1-Definition…….....12

III-2-Etiologies …….....12

III-3-Cells of inflammation……...13

III-4-Actors and course of inflammatory reaction……13

III-4-1-Notions of acute inflammation and chronic inflammation……...….13

III-5-General procedure of the different stages of the inflammatory reaction…….....14

III-5-1-Vasculo-exudative reaction……...14

III-5-1-1-Active congestion……...14

III-5-1-2-Inflammatory Edema…….....15

III-5-1-3-Leukocyte Diapedesis……...15

III-6-Cell reaction……....16

III-6-1-Blood……....16

III-6-2-Local connective tissue……....16

III-7-Debridement……16

III-7-1-Internal Debridement……17

III-7-2-External Debridement……...17

III-8-Repair and healing……17

Experimental part

Chapter I: Materials and methods

I-Inflammatory assays……...21

I-1-Plant material……...21

I-1-1-Conservation…….....21

I-2-Biological material……..21

I-3-Experimental design……...22

I-3-1-Groups of mice…….22

I-3-2-Preparation of the infused aqueous extract……..22

I-4-Preparation of the ointment……23

I-5-Blood sampling and weight measurement…….....23

I-6-The biological parameters realized during the experimentation…….24

I-7-Methodes of determination of biochemical blood parameters……24

I-7-1-Dosing of glucose…….24

I-7-2-Determination of total cholesterol……25

I-7-3-Determination of triglyceride……...25

I-7-4-Complete blood count (CBC) or Hemogram……...26

I-7-5-The creatinine dosage……...26

I-7-6-The urea dosage……27

I-8-Realizations of blood smear……27

II- Peptic ulcer disease (PUD)……..28

II-1- Test solution preparation…….....28

II-2- Experimental design…….28

II-3-Dissection of mice……29
II-3-1-Material…… ……29
II-3-2-The method of dissection……...**Erreur ! Signet non défini.**
II-4- Realization of histological sections……..31
II-4-1- Fixation……...……31
II-4-2- Inclusion……..32
II-4-3- Sections……..……...32
II-4-4- The colorations……..…….....32
II-4-5- Mounting……...……32
II-5-Preparation technique in the laboratory of anatomopathology (stomach of mice)……...33
II-5-1- Fixation……33
II-5-2- Preparation before inclusion: Dehydration……...33
II-5-3-Inclusion / coating…….....34
II-5-4- Histological section…….....36
II-5-5-Attaching the cut to the blade……36
II-5-6-Dewaxing……...37
II-5-7-Dewaxing / coloring HE……38
II-5-8- Mounting……...38

Chapter II: Results and discussion

II-1-Results of blood smear study after 72 h of inflammation……..42
II-2- Results of the blood smear study after 21 days of treatment with the aqueous extract of *Artemisia herba-alba*……42
II-3-Effect of the aqueous extract of *Artemisia herba-alba* on biochemical parameters…….43
II-3-1-White blood cells……43
II-3-2- Neutrophile……44
II-3-3- Platelet……...45
II-3-4-Blood glucose…….....47
II-3-5-Cholesterol…….....48
II-3-6-Triglycerides……..48
II-4-The tests performed to identify the presence of toxicity……...49
II-4-1-Urea……49
II-4-2-Creatinine ……..50

II-5- The influence of *AHA* administration on body weight change…….51

II- 6-Peptic ulcer disease……..52

II-6-1- Histological sections…….....52

Conclusion……53

References

Appendices

Introduction

Introduction

Medicinal plants have significant contribution for the drugs development and discovery, still provide abundant and promising source for lead structures as drug candidates. Many plants have been used for decades to treat various human diseases including inflammatory diseases.

Recently, focus on plants research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems.

Within the *asteraceae* family there are a large number of different plants that include white wormwood or the *Artemisia* genus, which includes more than 400 species distributed throughout the world **[1].**

On a small scale, the Romans in the first century used dry heads obtained from several species of the genus *Artemisia,* for the treatment of the disease caused by ascaris, enterobius, solitary worm infections and it became an important element of the pharmacopoeia in the early 20^{th} century. *Artemisia* species were the source of the remedies, such as *Artemisia* (*absinthium, Artemisia Annua, Artemisia vulgaris* or *Artemisia herba alba).*

Which are incorporated into the pharmacopoeias of several countries. The genus *Artemisia* contains artemisinin, a drug against malaria isolated from the Chinese plant *Artemisia annua*, but artemisinin, which is a sesquiterpene lactone, is not the only drug component in this genus, there are other sesquiterpene lactones and flavonoids that are used with a low risk of toxicity to mammals **[2].**

Inflammation is a body response to injuries induced by a various stimuli, such as infectious agents from microorganisms, noxious substances, physical damages, and changes induced by malignant cells. Various pathological conditions, including atherosclerosis, sepsis, cancer, arthritis and metabolic syndromes are related with inflammatory condition. Currently, no satisfying drug is available for the treatment of these inflammatory-related diseases. There are two anti inflammatory drugs available in the clinic: corticosteroids and non-steroidal anti inflammatory drugs (NSAID) **[3].** Despite the fact that corticosteroids and NSAID remain the common choice for the treatment of inflammatory diseases, the usage of these drugs are restricted by their undesirable side effects and the limited potency to reduce the symptoms of inflammation. Moreover, chronic use of corticosteroids anti inflammatory drugs has been limited as they exhibited a weight gain, osteoporosis and immunosuppressive effects. Whereas high dose NSAID medication leads to gastrointestinal tract-related toxicities**.** *Artemisia herba-*

alba leaves are widely used as medicinal herbs in Algeria. The infusion of these plants is usually prescribed against gastric discomfort and diarrhea.

Polyphenols present in these plants are regarded as the active compounds. Numerous data are now available showing a wide array of biochemical and pharmacological actions of flavonoids **[4].** The Crude tannin rich aqueous extract of *Artemisia herna alba* prevented gastric lesion formation induced in mice by ethanol, indomethacin, reserpine and serotonin.

Peptic ulcer is a common disorder of the alimentary tract and in various countries its prevalence is estimated at 5-10% of the adult population. Several polyphenols including tannic acid, ellagic acid, flavone, flavanone and quercetin have been reported to inhibit gastric acid secretion and to protect the stomach against necrotizing agents **[5].**

- The aim of this work was to investigate the cytoprotective properties of *Artemisia herb-alba* (AHA) aqueous extracts against Ethanol-Induced Damage of the mice stomach and to figure out the anti-inflammatory activity of AHA in mice.

Literature review

Chapter I: Artemisia herba-alba

I-Botanical aspects of *Artemisia herba alba (AHA)*

I-1-Denominations

Name in Arabic: Chih [6].

Name tamazight: Ifsi [7].

Names in French: Armoise herbe blanche [7].

Names in English: Desert wormwood or white wormwood [6].

I-3-Morphology

Artemisia herba-alba is a greenish-silver perennial herb **[8].** Grows 30-60 cm in hight characterized by a thymol odor **[9].** The leaves are hairy, silvery, small and deeply, bi-pennated with linear stripsit is chamaeophyta (the buds giving rise to new growth each year are borne close to the ground) **[10].**

The stems are rigid and erect, the flowering heads are sessile, oblong and tapering at base. The plants flowering from September to December. Plants are found on the steps of the middle East and North Africa where they are common and sometimes stand-forming. It has a seasonal dimorphism losing its wide winter leaves at the dry season and replacing them with smaller summer leaves whose anatomical structure is different **[10].**

I-4-Taxonomy

Artemisia herba alba ***fig 01*** is classified into:

Kingdom: *Plantae*

Subkingdom: *Tracheobionata*

Superdivision*: spermophyta*

Division: *Magnoliophyta*

Classe: *Magnoliopsida*

Subclass: *Asteridae*

Order: *Asterales*

Kingdom: *Plantae*

Subkingdom: *Tracheobionata*

Superdivision*: spermophyta*

Division: *Magnoliophyta*

Classe: *Magnoliopsida*

Subclass: *Asteridae*

Figure 01: The plant *Artemisia herba alba*

Order: *Asterales*

Family: *Asteraceae*

Subfamily: *Asteroideae*

Order: *Asteraceae*

Tribe: *Anthemideae*

Subtribe: *Artemisiinae*

Genus: *Artemisia*

Subgenus: *Seriphidium* and species: *Artemisia herba alba Asso* **[8].**

I-5-Distribution

In the world

Artemisia herba alba is a medicinal and aromatic shrub that grows wild in arid areas of the Mediterranean area **[11].**

The white wornwood develops in bioclimatic stages whitchrange from the upper semi-arrid to the saharian prevailing on salt soils and poorly drained areas **[12].**

This plant grows commonly on the steppes of north Africa (Morocco, Algeria and Tunisia), Egypt, Sinai desert, Middle East, Weastern Asia, the Canaries and South-eastern Spain, extending into northwestern Himalayas **[11] (Figure 02).**

In Algeria

We can find *Artrmisia herba-alba* in the following areas:

Méchria, Boussaada, Biskra, M'sila, Dejelfa, Benefouda, Bougaa, Boutaleb, Bordj Bouarreridj , Biskra , Laghouat ,Ghardaia , Southern Algeria **[3].**

Figure 02: Distribution map of *Artemisia herba-alba* **[8].**

I-6-Phytochemistry

I-6-1-Sesquiterpene

Terpenes are polymers consisting of C5 units (isopentyl pyrophosphate). Monoterpenes (C10) are slightly volatile substances that form essential oils **[13].** They protect plants against parasites, inhibit bacterial growth and attract pollinating animals. The main monoterpenes identified in the White grass mugwort are thujone (monoterpene lactone), 1,8-cineol and thymol **[14].** Alcoholic monoterpenes (yomogi alcohol, santoline alcohol) have been demonstrated. Sesquiterpenes (3-C5 units) and sesquiterpenes lactones have also been identified in several chemotypes in the Middle East **[15].**

Thujone is probably one of the most bioactive terpenic constituents of *Artemisia*. Its name comes from Thuya (*Thuja occidentalis*) plant from which it was extracted for the first time. It has also been identified in other species, such as Absinth (*Artemisia absinthium*) and Roman *Artemisia* (*Artemisia pontica*). Structurally bound to menthol, it consists of a C6 (cyclohexane) ring, in addition, an isocyanate exocyclic group and a lactone group **[16].**

Thuyone is a naturally occurring chiral compound in the form of two stereo isomers: alpha thuyone and beta-thuyone **[17].**

Many scientists have studied the chemistry of *AHA* growing in Egypt. Most studies have been concerned with the sesquiterpene lactones. All the lactones isolated differ from those found previously. Few works studied the chemical constituent of Moroccan *AHA* species, and the Algerian one, which proven that this genus is rich in sesquiterpenes **[18].**

I-6-2-Flavonoids

There are phenolic compounds that contribute to the pigmentation of the plants. Some of them play the role of phytoalexins, metabolites synthesized by plant to control various parasitoses. Flavonoids are encountered in the free state (soluble) or linked to a sugar (glycosides) in the vacuolar liquid. The coloring of derivatives depends on the different substitutions of the hydrogen atom on various rings, the formation of complexes with metal ions ($Fe3^{+}$, $Al3^{+}$) and pH **[19].** The main isolated flavonoids from *AHA* are hispidulin, cirsimaritine **[20].** Flavones glycosides such as 3-rutinoside-quercetin and isovitexin have been demonstrated in chemotypes of Sinai **[21].**

I-6-3-Phenolic compounds and waxes

Chlorogenic acid was observed in *AHA* when a chemical survey of 49 Moroccan medicinal plant species was performed by ESR (Electron Spin Resonance) spectroscopy. During a survey for antiulcerogenic principles of *AHA*, eight polyphenolics and related constituents were isolated. These included chlorogenic acid-4-5-*O*-dicaffeoylquinic acid, isofraxidin 7-*O*-D-glucopyranoside, 4-*O*-D-glucopyranosylcaffeic acid,rutin, schaftoside, isoschaftoside, and vicenin-2 **[8].** In a study of the components of *AHA* wax, obtained in 0.23 % yield by extraction of the dry plant with ether, contained 32.1 % saturated C16-32 acids (35.2 % C28 and 26.5 % C30), 23.2 % saturated. C21-31 hydrocarbons (67.7 % C29 and 24.2 % C31), 27.1 % esters (mainly of saturated C18, C19, and C20 acids and saturated C22 and C24 alcohols) and 16.96 % saturated C16-26 alcohols. (C16, 24.71 %, C20 10.34 %, C22, 32.88 % and C24 22.96 %)

I-6-4-Essential oils

In Spain, essential oil from *Artemisia herba alba* showed that monoterpene hydrocarbons and oxygenated monoterpenes are the most abundant skeletons. But large amounts of sesquiterpenes also were found in some populations. Camphor, 1,8-cineole, p-cymene and davanone were the major chemical compounds found. Two oils types were found for plants grown in Sinai those of cineole-thujane bornane type and the pinane type with monoterpene skeletons **[11].**

In Jordan, regular monoterpenes were predominant and the principal components were α- and β-thujones, classifying the *Artemisia herba alba* as being a thujone chemotype .

In Morocco, sixteen chemotypes were found, and twelve of them have monoterpenes as major components of essential oils. The remaining four chemotypes, have sesquiterpene skeletons as the major fraction. Investigations reported no correlation between chemotypes and geographic distribution **[22].**

In Algeria, essential oils, monoterpenes were the major components, essentially camphor α and β-thujones, 1-8-cineole and chrysanthenyl derivatives .

In Tunisian oil, oxygenated monoterpenes were found to be the major components of *AHA* oil extracted from aerial parts **[23].**

Chapter II : Peptic Ulcer Disease

(Stomach Ulcers)

II-1- Historical Perspective

The first description of a perforated peptic ulcer was in 1670 by Princess Henrietta of England **[24].** John Lykoudis, a general practitioner in Greece, treated patients for peptic ulcer disease with antibiotics, beginning in 1958, long before it was recognized that bacteria were a dominant cause for the disease**.**

In their original paper, *Warren* and *Marshal* contended that most gastric ulcers and gastritis were caused by colonization with this bacterium (*Helicobacter pylori*) not by stress or spicy food as had been assumed before **[25].**

II-2- Definition

Peptic ulcer disease (PUD) is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus **[26].** An ulcer in the stomach is known as a gastric ulcer while that in the first part of the intestines is known as a duodenal ulcer.

II-3- Signs and symptoms

Abdominal pain, naturally epigastric with severity relating to meal times is usually after 1 hour of taking a meal. Duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it, bloating and abdominal fullness; water brash (rush of saliva after an episode of regurgitation to dilute the acid in oesophagus); nausea, copious vomiting; loss of apetite and weight loss **[28].** Hematemesis (vomiting of blood); melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin); heart burn, gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion of peptic ulcer; and sudden increase in the abdominal pain or sharpness in the quality of the pain; vomiting blood or material that looks like coffee grounds; blood in stool or black, tarry stools **[29].**

A gastric ulcer will give epigastric pain during the meal as gastric acid is secreted, or after the meal, as the alkaline duodenal contents reflux into the stomach **[30].**

Symptoms of duodenal ulcers will manifest mostly before the meal-when acid production stimulated by hunger is passed into the duodenum. This is not considered as reliable sign in clinical practice **[30].**

II-4- Anatomy

The stomach is located in the upper part of the abdomen just beneath the diaphragm The stomach is distensible and on a free mesentery, therefore, thesize, shape, and position may vary with posture and content **[31].** An empty stomach is roughly the size of an open hand and when distended with food, can fill much of the upper abdomen and may descend into the lower abdomen or pelvis on standing. The duodenum extends from the pylorus to the ligament of Treitz in a sharpcurve that almost completes a circle. It is so named because it is about equal in length to the breadth of 12 fingers, or about 25 cm. It is largely retroperitoneal and its position is relatively fixed. The stomach and duodenum are closely related in function, and in the pathogenesis and manifestation of disease. The stomach may be divided into seven major sections **[32].** The cardia is a 1–2 cm segment distal to the esophago gastric junction. The fundus refers to the superior portion of the stomach that lies above an imaginary horizontal plane that passes through the esophagogastric junction. The antrum is the smaller distal one-fourth to one-third of the stomach. The narrow 1–2 cm channel that connects the stomach and duodenum is the pylorus. The lesser curve refers to the medial shorter border ofthe stomach, whereas the opposite surface is the greater curve. The angularis is along the lesser curve of the stomach where the body and antrum meet, and is accentuated during peristalsis **(Figure 03).**

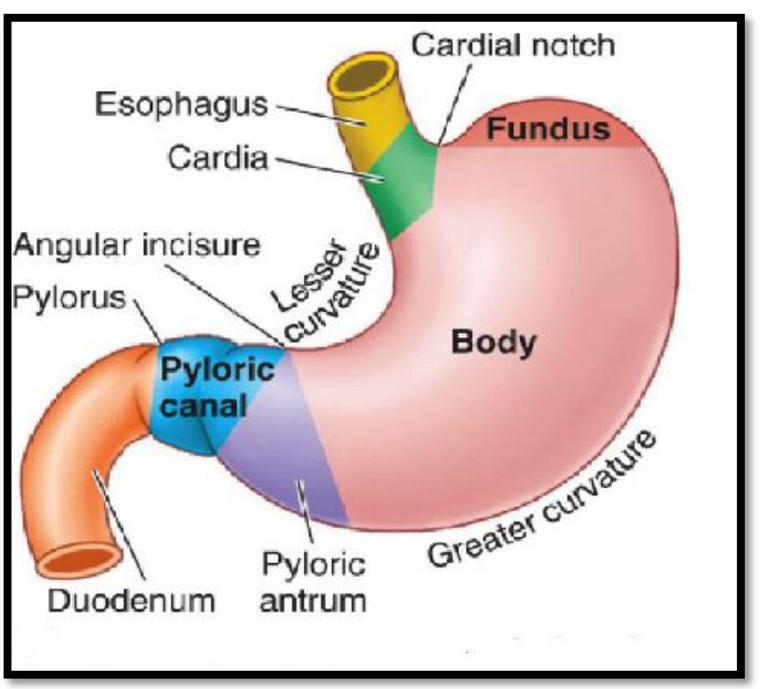

Figure 03: Anatomy of the stomach

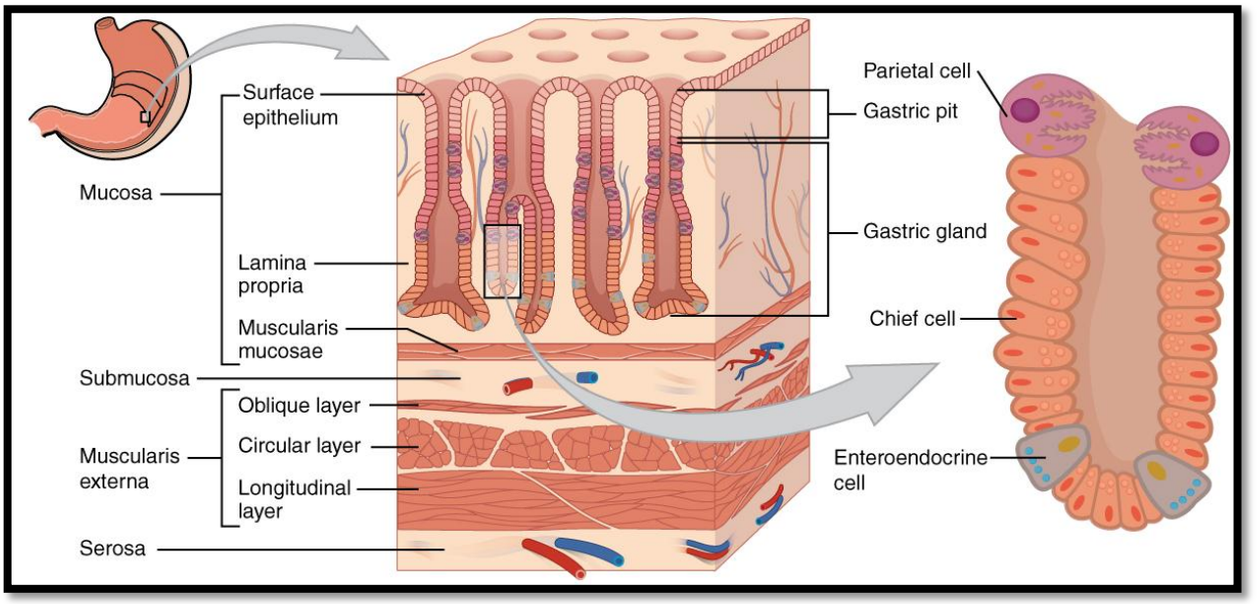

Figure 04: histological structure of stomach.

II-5- Pathophysiology

Peptic ulcers are defects in the gastric or duodenal mucosa that extend through the muscularis mucosa. The epithelial cells of the stomach and duodenum secrete mucus in response to irritation of the epithelial lining and as a result of cholinergic stimulation. The superficial portion of the gastric and duodenal mucosa exists in the form of a gel layer, which is impermeable to acid and pepsin **[33].** Other gastric and duodenal cells secrete bicarbonate, which aids in buffering acid that lies near the mucosa. Prostaglandins (PG) have an important protective role, because PG increases the production of both bicarbonate and the mucous layer.

In the event of acid and pepsin entering the epithelial cells, additional mechanisms are in place to reduce injury **[34].** Within the epithelial cells, ion pumps in the basolateral cell membrane help to regulate intracellular Ph by removing excess hydrogen ions. Through the process of restitution, healthy cells migrate to the site of injury. Mucosal blood flow removes acid that diffuses through the injured mucosa and provides bicarbonate to the surface epithelial cells **[35].** Under normal conditions, a physiologic balance exists between gastric acid secretion and gastro duodenal mucosal defense. Mucosal injury and, thus, peptic ulcer occur when the balance between the aggressive factors and the defensive mechanisms is disrupted **[36].** Aggressive factors, such as NSAIDs, *Helicobacter pylori* infection, alcohol, bile salts, acid, and pepsin can alter the mucosal defense by allowing back diffusion of hydrogen ions and subsequent epithelial cell injury [**33].** The defensive mechanisms include tight intercellular junctions, mucus, mucosal blood flow, cellular restitution, and epithelial renewal.

II-6- Causes

According to **Mohammed Hosein & al, (2014)** Peptic ulcer disease may be due to any of the following**:**

- *Helicobacter pylori* infection
- Drugs
- Life style factors
- Severe physiologic stress
- Hyper secretory states (uncommon)
- Genetic factors [**37].**

II-6-1- Ethanol

The mechanism of ethanol induced gastric lesion is varied, including the deplesion of gastric mucus content, damaged mucosal blood flow and mucosal cell injury **[34].** It has been reviewed that ethanol causes severe damage to the gastrointestinal mucosa starts with micro vascular injury results in increase vascular permeability; edema formation and epithelial lifting. **SZABO & al, 2013**, suggested that after intra gastric administration of ethanol a rapid time dependent release of endothelin-1 into the systemic circulation preceded the development of the hemorrhagic mucosal erosions by vasoconstriction **[38].** Moreover, by decreasing the secretion of biocarbonate (HCO_3^-) and mucus production .Ethanol produces the necrotic lesions in gastric mucosa. Further ethanol also has been reported to activate TNFα and Mitogen Activated Protein Kinases (MAPK). Also, ethanol has also initiate apoptosis which lead to cell death. Further, ethanol after metabolism has been reported to releases superoxide anion and hydroperoxy free

radical. Which lead to an increased lipid peroxidation **[39].** Increase in lipid peroxide content and oxygen derived free radicals results in marked changes in cellular levels and causes membranes damage, cell death, exfoliation and epithelial erosion.

II-7- Epidemiology

In the United States for example, PUD affects approximately 4.5 million people annually. Approximately 10% of the US population has evidence of a duodenal ulcer at some time. Of those infected with *Helicobacter pylori* **[40].** The life time prevalence is approximately 20 %. Only about 10 % of young persons have *Helicobacter pylori* infection; the proportion of people with the infection increases steadily with age. Overall, the incidence of duodenal ulcers has been decreasing over the past 3-4 decades. Although the rate of simple gastric ulcer is in decline, the incidence of complicated gastric ulcer and hospitalization has remained stable, partly due to the concomitant use of aspirin in an aging population. The hospitalization rate for PUD is approximately 30 patients per 100,000 cases **[28].**

The prevalence of PUD has shifted from predominance in males to similar occurrences in males and females. Lifetime prevalence is approximately 11-14% in men and 8-11% in women. Age trends for ulcer occurrence reveal declining rates in younger men, particularly for duodenal ulcer, and increasing rates in older women **[40].** Trends reflect complex changes in risk factors for PUD, including age-cohort phenomena with the prevalence of *Helicobacter pylori* infection and the use of NSAIDs in older populations.

The frequency of PUD in other countries is variable and is determined primarily by association with the major causes of PUD is *Helicobacter pylori* and NSAIDs **[41].**

Chapter III: Skin inflammation

III-1- Definition

The inflammation is the first biological response of the immune system to infection or irritation, the word “inflammation” comes from the Latin“ inflammo” meaning “ I set alight”, “Iignite”. A verity of stimuli such as physical damage, ultra violet irradiation, microbial invasion and immune reactions are responsible for inflammation **[42].**

The inflammation is characterized by redness, heat, swelling, and pain **[43].** As a result of many associated changes such as vasodilatation increased vascular permeability, and plasma extravasations **[44].** On the bases of pathological features, there are two major forms of inflammation: acute and chronic. Chronic inflammation is characterized by persistent inflammation. On other hand, acute inflammation occurs over seconds, minutes, hours and days **[43].**

III-2- Etiology

The causes of the inflammatory reaction are multiple and represent pathogens. These causes cause cellular and tissue damage that will trigger inflammation:

• Infection: contamination by micro-organisms (bacteria, viruses, parasites, fungi).

• Physical agents: trauma, heat, cold, radiation.

• Chemical agents: caustics, toxins, venoms.

• Coreign bodies: exogenous or endogenous.

• Lack of vascularization: inflammatory reaction secondary to necrosis by ischemia.

• Dysimmune agression (abnormal immune response, allergies, autoimmunity).

It should be emphasized that:

• The pathogen can be endogenous or exogenous **[43].**

• Infectious micro-organisms are only a part of the causes of inflammation. An inflammatory reaction is therefore not synonymous with infection.

•The same pathogen can cause different inflammatory reactions depending on the host, particularly depending on the state of immune defenses.

• Several causes can be associated in triggering an inflammatory reaction.

III-3- Cells of inflammation

This table shows a different cells involved in the inflammatory reaction

Table 01: The different cells involved in the inflammatory reaction **[45].**

Circulating blood cells	**Residual Tissue Cells**
Polynuclear neutrophil	Macrophages
Monocytes	Histiocytes
Polynuclear eosinophils	Mast cells
Basophils	Endothelial Cells
Platelets	Fibroblasts
Lymphocytes	
Plasma cells	

III-4- Actors and course of inflammatory reaction

Inflammation involves cells, vessels, changes in the extracellular matrix and many chemical mediators that can be pro or anti-inflammatory and that can alter or maintain the inflammatory response. Whatever its location, and the nature of the pathogen, the course of an inflammatory reaction presents general morphological features and common mechanisms. Nevertheless, the different stages present variations related to the nature of the pathogen, the organ where the inflammatory reaction takes place, to the physiological ground of the host. All these factors determine the intensity, the duration of the inflammatory reaction and the lesional aspect **[46].**

III-4-1- Notions of acute inflammation and chronic inflammation

III-4-1-1- Acute inflammation

Acute inflammation is the immediate responses to an aggressive agent, of short duration (a few days or weeks), often brutal and characterize by intense vasculo-exudative phenomena. Acute inflammation heals spontaneously or with treatment, but may leave squeal if tissue destruction is important **[47].**

- The acute inflammation goes through three phases: vascular phase with vasodilatation, plasma transudation, edema and fibrinoformation.
- Cell phase made up of an interstitial extravascular influx of leucocytes, mainly neutrophilic polynuclear cells, followed by mononuclear cells in a second phase.
- Phase of resolution with termination of inflammation in which apoptosis would play an important role **[48].**

III-4-1-2- Chronic inflammation

Inflammations having not endency to spontaneous healing and which evolve in persistent or worsening for several months or years. There are two types of the occurrencces of chronic inflammation:

- Acute inflammation develop into prolonged sub acute inflammation and chronic pathogens persists in the tissues (cleansing incomplete). Or when acute inflammation recurred repeatedly in the same at each episode, less and less tissue destruction well repaired
- Inflammation can sometimes manifest themselves in a form apparently chronic. The acute vasculo-exudative phase passed unnoticed because short or asymptomatic. This is often the case with autoimmune disease or of disease where the dysimmunitary mechanisms are predominant for exemple chronic active hepatitis secondary to infection with hepatisis B or C viruses **[47].**

III-5- General procedure of the different stages of the inflammatory reaction

The inflammatory reaction is a dynamic process involving several successive stages the vasculo-exudative reaction, the cellular reaction, the debridement, the terminal phase of repair and healing.

III-5-1- Vasculo-exudative reaction

It is clinically introduced by:

• Four classic cardinal signs of acute inflammation: redness, heat, swelling, pain.
• It has three phenomena: active congestion, inflammatory edema, leukocyte diapedesis **[49].**

III-5-1-1- Active congestion

It is an arteriolar vasodilation then capillary in the locally affected area, resulting in an increase in blood supply and a slowing of the circulatory current **[49].**

Congestion is triggered rapidly by a nervous mechanism (vasomotor nerves) and the action of chemical mediators **[49].**

III-5-1-2- Inflammatory Edema

Inflammatory edema results from the passage into the interstitial connective tissue or the serous cavities of a liquid called exudate consisting of water and plasma proteins.

Its clinical translation is a swelling of the tissues which, by compressing nerve endings, is responsible for the pain (also caused by some chemical mediators). Its microscopic translation is a pale, uncoloured and distended aspect of the connective tissue.

The inflammatory edema results from an increase in hydrostatic pressure due to vasodilation and especially an increase in the permeability of the wall of the small vessels under the effect of chemical mediators **[50].**

• Local supply of chemical mediators and defenses (immunoglobulins, clotting factors, complement factors).

• Dilution of accumulated toxins in the lesion.

• Restriction of the inflammatory focus by a fibrin barrier (derived from plasma fibrinogen), which prevents the spread of infectious microorganisms.

•Slowing of the circulatory current by hemoconcentration, which promotes the following phenomenon: leukocyte diapedesis **[51].**

III-5-1-3- Leukocyte Diapedesis

Leukocyte diapedesis corresponds to the migration of the leucocytes outside the microcirculation and their accumulation in the lesional focus.

Leukocyte diapedesis: passage of leukocytes through the wall of an expanded capillary

It involves first the polymorphonuclear cells (during the first 6 to 24 hours) and then later (in 24 to 48 hours) monocytes and lymphocytes. It is an active crossing of the vascular walls which comprises several steps:

1- Margination of leukocytes in the vicinity of endothelial cells, favored by the slowing of the circulatory current.
2- Adherence of leukocytes to endothelial cells, by the use of adhesion molecules present on the leucocyte membrane and on the endothelium **[52].**
3- Trans-endothelial passage of leukocytes. The leukocytes emit pseudopoda that penetrate between the intercellular junctions of the endothelial cells and then cross the basal membrane through transient depolymerization caused by their enzymes.

III-6- Cell reaction

The cellular reaction is characterized by the formation of inflammatory granuloma or inflammatory granulation tissue.

Cellular Composition The inflammatory focus grows rapidly in cells derived from the blood or local connective tissue **[53].**

III-6-1- Blood

Polymorphonuclear cells, monocytes and lymphocytes. After diapedesis, these leucocytes leave the perivascular territory and migrate to the lesional focus by chemotactism. Chemotactic agents, produced by altered tissues, by bacteria and by leukocytes already present in the inflammatory focus, bind to leukocyte membrane receptors, which leads to the activation of their cytoskeleton and to their mobilization.

III-6-2- Local connective tissue

Locally some cells will multiply and this is the case of fibroblasts, lymphocytes, endothelial cells, and to a lesser extent macrophages) and / or will transform or differentiate **[54].**

• Accumulation of polymorphonuclear cells with a short life (3-4 days). Their enzymes are released into the inflammatory focus. The intake of new neutrophils must be sustained in the initial phases of inflammation by increased haematopoietic production.

• Monocytes become activated macrophages capable of phagocytosis, secretion of many mediators and cooperation with lymphocytes for the development of the immune reaction (presentation of antigenic molecules to lymphocytes). Their life span is longer than that of the polymorphonuclear cells.

• Transformation of B lymphocytes into plasma cells secreting immunoglobulins.

Activation of T lymphocytes: secretion of numerous mediators, acquisition of cytotoxic properties and cooperation with B lymphocytes.

• Modification of fibroblasts in myofibroblasts: acquisition of contractile properties and synthesis of constituents of the extracellular matrix **[54].**

III-7- Debridement

It gradually succeeds the vasculo-exudative phase, and is contemporaneous with the cell phase. The debridement can be compared to a cleansing of the lesional focus: it is the

elimination of necrotic tissues (resulting from the initial aggression or the inflammatory process itself), pathogens and exudate. The debridement necessarily prepares the terminal phase of repair-healing. If the debridement is incomplete, the acute inflammation will evolve into chronic inflammation. Detersion is carried out according to two mechanisms: internal and external deburring **[48].**

III-7-1- Internal Debridement

This involves the elimination of necrotic tissues and certain pathogens (infectious microorganisms, foreign bodies) by phagocytosis, while the fluid of edema is drained into the lymphatic circulation and resorbed by the macrophages by pinocytosis. Phagocytosis is defined by the inclusion in the cytoplasm of the phagocyte of a living or inert foreign particle, usually followed by digestion of this particle by lysosomal enzymes. Digestion is complete or incomplete with residues discharged from the cell or accumulated in the macrophage **[55].** Phagocytes are represented by polynuclear cells, capable of phagocyticizing bacteria and small particles and by macrophages capable of phagocytating macro-particles.

III-7-2- External Debridement

• Spontaneous: the debridement takes place by liquefaction of the necrotic material (pus, caseium) and elimination by fistulisation to the skin or in a natural bronchial, urinary or intestinal duct.

• Surgical: the debridement is performed by surgical trimming often indispensable when the lesions are too extensive or soiled.

III-8- Repair and healing

The tissue repair follows a complete debridement. It leads to a scar if the injured tissue cannot regenerate (neurons or myocardial muscle cells) or when tissue destruction has been very prolonged and / or prolonged. The repair can lead to a complete restitution of the tissue: there is no longer any trace of the initial aggression and the subsequent inflammation. This very favorable evolution is observed during limited, brief, little destructive aggressions in tissue capable of cellular regeneration **[48].** The repair process involves many growth factors and complex interactions between cells and the extracellular matrix to regulate cell proliferations and biosyntheses.

Fleshy bud

The repair involves the constitution of a new connective tissue called a fleshy bud which gradually takes the place of the inflammatory granuloma and will replace the tissues destroyed during the inflammation **[51].**

Creation of a scar

The scar is the definitive mark sometimes left by the inflammatory focus after the fleshy bud phase. It is formed of a fibrous connective tissue (predominance of collagen) taking the place of tissues definitively destroyed. The structure of a scar changes gradually over several months **[44].**

Cutaneous scar: under the thinned epidermis, the dermis is dense in collagen and the appendages pilosebaceous and sweat have disappeared

Epithelial regeneration

It appears parallel to the conjunctive repair. The destroyed epithelial cells are replaced by the proliferation of healthy epithelial cells around the inflammatory focus.

- At the level of a coating (skin, mucous membranes), the epithelium regenerates, from the periphery to the center of the tissue loss, when this is filled by the fleshy bud **[46].** This regeneration can be done in a metaplastic mode (regeneration of the bronchial cylindrical epithelium in the form of a squamous epithelium) or an atrophic mode with disappearance of certain specialized functions (disappearance of vibrating eyelashes).
- At the level of a parenchyma (liver, exocrine glands, kidney) the quality of the epithelial regeneration depends, on the one hand, on the importance of the initial destruction of the tissue and in particular on the intensity of the destruction of the other hand, the mitotic power of the epithelial cells [44].

Experimental part

Chapter I: Materials and Methods

I-Inflammatory assays

I-1-Plant material

The space of *Artemisia herba-alba* was collected in April 2017 in the south of Algeria from Djelfa province (fig 05)**.**

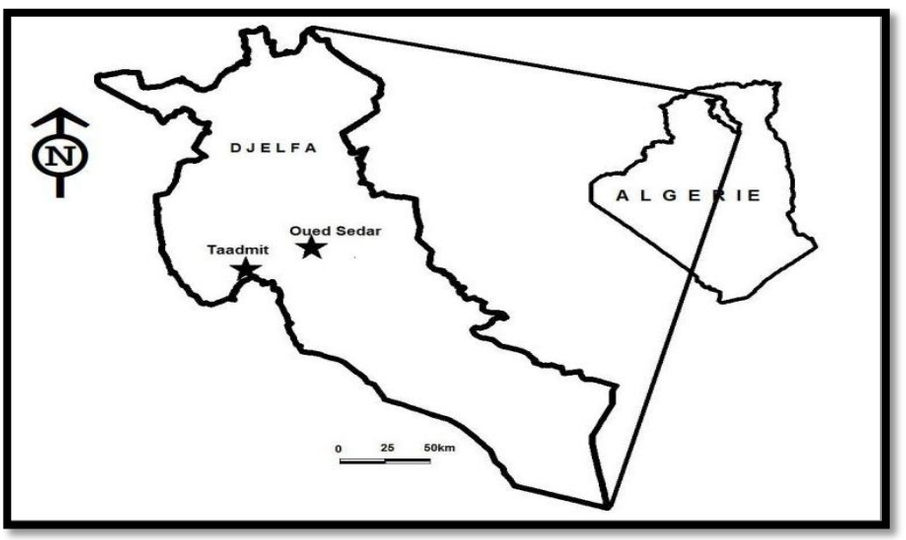

Figure 05: Localisation of Djelfa province in Algeria map.

I-1-1-Conservation

The freshly harvested plant is allowed to dry after washing with a sterile water in the oven at a temperature of 40 ° C for 48 hours, Thereafter the plant is crushed with a mixer in order to obtain a very fine powder.

I-2-Biological material

In our work we used the mice as an animal model which was obtained from Pasteur institute Algiers. The animals placed in plastic cages standardized laboratory conditions of ventilation and lighting (15:9 hours light: dark) and the temperature was between (24-26 °C). Males' mice were used in the experiment and the weight was (20 +2 g) at the beginning of the experiment and old (6-8) weeks, they had a free access for food and water, (figure 06).

Figure 06: Experimental mice used in this work

I-3-Experimental design

We put 15 mice in a cage (50cm x 30cm) for 15 days (figure 06), after which we observed that there were rat bites due to stress. The blasts caused wounds to the skin of the mice, which led to inflammation. Then we separated them into three groups, 5 mice for each.

I-3-1-Groups of mice

Group I: 5 healthy control mice who received daily water and food for 21 days.

Group II (5 mice) with inflammation: these mice received each day water and food for 21 days.

Group III (5 mice) inflammation + Aqueous extract of *AHA*: mice that received daily twice doses of the aqueous extract of *AHA* by gavage for 21 days.

I-3-2-Preparation of the infused aqueous extract

The extraction of the bioactive substances contained in the aerial part of *AHA* is made by infusion. In 100 ml heat distilled water we put 2g of powder aerial part of AHA. We left the infusion for 30 minutes with agitation using magnetic agitator **(Figure 07)**. The aqueous extract obtained was filtered with filter paper to get rid of the plant debris.

Figure 07: The infused aqueous extract.

I-4-Preparation of the ointment:

After a few days of the inflammation, we prepared an ointment using waxy powder of *AHA* to cover the wounds **(figure 08).** We mixed 2 g of powder with a quantity of distilled water. We applied the mixture twice a day.

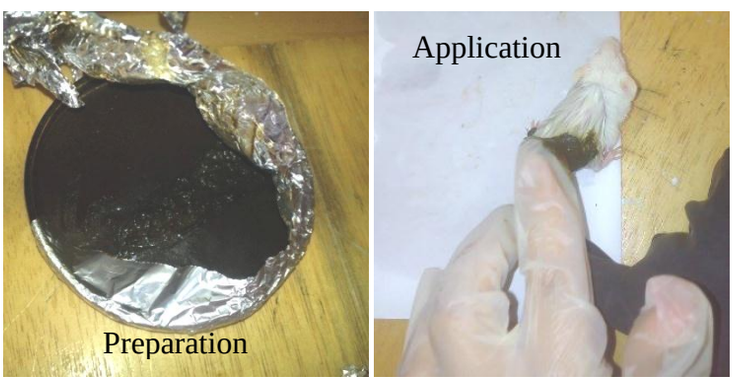

Figure 08: Preparation and application of ointment of *AHA*.

I-5-Blood sampling and weight measurement

The blood was taked from the neck by decapitation and put in tubes containing heparin and EDTA to prevent coagulation. (1 day before the start of the experiment, 72 hours after inflammation and after 21 days).

After each blood sample, the blood was placed in heparinized tubes, and EDTA, centrifuged at 6000 rpm for 15 minutes and then the serum was recovered and used for biochemical assays of blood glucose, urea, creatinin, total cholesterol, TG and CBC. The weight measurement is performed daily on fasting mice.

I-6-The biological parameters realized during the experimentation

In order to obtain control results we determined the following parameters: blood glucose, urea, creatinin, CBC 72 hours after the inflammation. CBC and blood smear were used as confirmation parameters to ensure the presence of inflammation. After 21 days, we have measured the parameters of toxicity: urea, creatinine.

I-7-Methodes of determination of biochemical blood parameters

I-7-1-Dosage of the glucose

Blood glucose can be dosed by many methods, including the oldest are colorimetric method. Whereas, those currently practiced are enzymatic. In our study, the blood glucose was determined according to an enzymatic method (Hexokinase / G-6-PDH) using the REF 3L82-20 glucose reagent kit by a auto-analyzer of type (ARCHITECT c Systems).

I-7-1-1-Principle of the method

Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ion, thereby producing glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dishydrogenase (G-6-PDH) oxide in particular 6-phosphogluconate G-6-P with simultaneous reduction of material and methods nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide reduced (NADH).

β-glucose + ATP $\xrightarrow{\text{hexokinase}}$ **Glucose-6-phosphate + ADP.**

Glucose-6-phosphate + NAD $\xrightarrow{\text{G-6-PDH}}$ **6-phosphogluconate + NADH.**

A micromole of NADH is produced for each micromole of glucose consumed. The NADH product absorbs light at 340 nm and this increase in the absorbance can be detected by spectrophotometry.

1000 µl of reagent is placed in the test tube and 10 µl of serum are added. After a reaction of 10 min. Using a UV spectrophotometer, measures the absorbance A of the colored substance in solution for the wavelength ($\lambda = 470$ nm) **[57].**

I-7-2-Determination of total cholesterol

Cholesterol can be dosed by many methods, including the oldest are colorimetric whereas those currently practiced are enzymatic.

In our study, cholesterol was determined using an enzymatic method (reaction of Trinder) by an auto-analyzer of the type (ARCHITECT Systems) using the cholesterol reagent (REF 7D62).

I-7-2-1-Principle of the method

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase which decompose them into cholesterol and free fatty acids. Free cholesterol, including initially present, is then oxidized by cholesterol oxidase to form cholesten-4-one-3 and hydrogen

peroxide. Hydrogen peroxide combines withHydroxybenzoic acid (HBA) and 4-aminoantipyrine to form a chromophore(Quinoneimine) quantified at 500 nm.

Esterified Cholesterol + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestene-4-one-3+H_2O_2

H_2O_2 + Hydroxybenzoic acid + 4-aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinone imine

The intensity of the staining of the quinone imine measured at 500 nm is directly proportional to the amount of cholesterol present in the serum sample.

1000 µl of reagent is placed in the test tube and 10 µl of serum are added. We wait 10 min for the reaction. Using a UV spectrophotometer, we measures the absorbance A of the colored substance in solution for the wavelength λ (= 505 nm) **[56].**

I-7-3-Determination of triglycerides

The triglycerides were assayed by the same autoanalyzer (ARCHITECT cSystems) using an enzymatic colorimetric method of triglycerides (glycerol Phosphate oxidase) using the glyceride reagent kit REF 7D74.

I-7-3-1-Principle of the method

Rests on the enzymatic assay of free glycerol after lipase action. The triglycerides are enzymatically hydrolysed by lipase to release fatty acids and glycerol. Glycerol is phosphorylated by adenosine triphosphate (ATP) and glycerol kinase (GK) to produce Glycerol-3-phosphate and adenosine diphosphate (ADP). The glycerol-3-phosphate is oxidized to dihydroxy acetone phosphate (DAP) by glycerol Phosphate oxidase (GPO) to produce hydrogen peroxide (H_2O_2). During the colored reaction catalysed by peroxidase, H2O2 reacts with 4-aminoantipyrine (4-AAP) and 4-chlorophenol (4-CP) to produce a red dye.

Triglycerides $\xrightarrow{\text{Lipase}}$ Glycerol + fatty acids.

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3-phosphate + ADP.

Glycérol-3-phosphate $\xrightarrow{\text{Glycerol-3-phosphate oxidase}}$ Dihydroxyacetone phosphate + H_2O_2.

$2H_2O_2$ + 4-chlorophenol + 4-aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinone imine + $4H_2O$.

The intensity of the staining of quinone imine measured at 505 nm is directly proportional to the amount of triglycerides in the serum sample

1000 µl of reagent is placed in the test tube and 10 µl of serum are added. We wait 10 min for the reaction. Using a UV spectrophotometer to measure the absorbance of the colored substance in solution **[57].**

I-7-4-Complete Blood Count (CBC) or Hemogram.

The blood count or hemogram is an essential examination that provides information about blood cells, immune defense processes, hemostasis and reveals evocative changes in a large number of diseases. It includes the numeration (calculation of the absolute number of these different cells in a certain volume of blood) of the different figurative elements of the blood: red blood cells, white blood cells or leukocytes and platelets.

The hemogram is performed by *CBC automata* (Switzerland) equipped with counters optical or electronic devices able to assess the number of erythrocytes and leukocytes, dose hemoglobin, and calculate hematocrit and erythrocyte levels.

I-7-5-The creatinin dosage

Creatinin forms in an alkalin medium a colored complex with picric acid measured at 520 nm. The rate of formation of this complex is proportional to the concentration of creatinin. In a test tube 500 µl of first creatinin reagent are added and 500 µl of 2nd reagent and then 100 µl of serum are added. The value of the absorbance A is read directly by the UV spectrophotometer **[58].**

I-7-6-The urea dosage

The enzymatic determination of urea by kinetic method is carried out according to the following reactions:

$$\mathbf{Urea + H_2O \xrightarrow{ureas} 2NH_3 + CO_2.}$$

$$\mathbf{2NH_4 + 2alphocetoglutarate + 2NADH \xrightarrow{GLDH} 2\ Glutamat + 2\ NAD + 2H_2O.}$$

500 µl of the first reagent of the blood urea are placed in a test tube and 5 µl of serum, 500 µl of second reagent is added after 5 min. The value of absorbance A bound after10 min by the UV spectrophotometer and with a wavelength λ (= 340 nm) **[58].**

I-8-Realizations of blood smear

Smear or spreading of thin-layer cells on a glass slide represents the standard method of cytological blood sampling. The smear allows the observation of the blood-figure elements

The realization of a blood smear involves spreading a drop of blood over the entire surface of the glass slide to obtain a very thin layer of cell. The smear is subsequently fixed and colored by the double-staining method May -Grunwald / Giemsa (MMG).

Material necessary to realize this test is as following: Clean and dry glass blades, cotton, alcohol, steril needles, two colors: May-Grunwald and Giemsa, distilled water, immersion oil, optical microscope.

Using a needle sterilized with alcohol, prick the end of the tail of the mouse cleanly washed with alcohol. Recover the drop of blood and place it directly on one end of the blade as shown in the figure 09. Place on the drop another blade inclined at 45° in front of the drop of blood so that the blood is spread under the blade by capillarity Spread immediately with the second blade held at 45°. The smear should cover about half the blade**.** Dry the smear by stirring in the open air.

Coloring: Turn on the dry smear 8 to 15 drops of May Grunwald so as to completely cover the ftottis. Let it act for 3 min, add 15 drops of distilled water and leave to act 2 min.

Rinse under a weak stream of water to remove the dye add 6 to 10 drops of Giemsa and leave to act for 15 minutes, rinse with a small jet of distilled water to remove excess dye.

Leave the blade in the air in an inclined position after having lowered the lower side with paper towel. (University protocol).

After staining, all the blood cells can be observed under an optical microscope, the nuclei of the leukocytes are colored blue or violet, and the cytoplasm is light blue. The erythrocytes combine with pale pink discs in the center. Purple more intense than red blood cells.

II- Peptic Ulcer Disease (PUD)

II-1- Test solution preparation

Aqueous extracts were prepared by boiling 2 g of the powdered material *AHA* in 100 mL of distilled water for 30 min. The resulting solutions were filtered and then diluted three times with water (test solutions) **(Figure 07).**

II-2- Experimental design

Male mice, weighing 25–35 g, were deprived of food for 48 h but allowed free access to water until 1/h before the experiment. During the fasting period, the animals were kept individually in cages.

Mice were randomly divided into 3 groups of 5 mice. Control groups were gavaged with water 60 min before oral administration of test solution of ethanol (concentration of 50 %).

Treated groups were given the solution of ethanol 60 min after administration of the test solution.

All administered solutions were given at a dose of 5 ml / kg. 15 min after administration of the necrotizing agent (Formaldehyd), the animals were killed by cervical dislocation and their stomach rapidly removed.

II-3-Dissection of mice

II-3-1-Material of dissection

This dissection of mice involves simple material. The usual dissection material may be more complete and generally includes.

Scissors: -large scissors: to cut the skin, the bones, the muscles.
- Fine scissors: for delicate sections (vessels, nerves.)
- Grooved probe: allows scissors to be guided during large incisions.
-Scalpel: allows to dissolve the tissues surrounding the organs.
- Pincers: - strong pincers: to hold the thick structures (bones).
- Fine straight lines: for delicate dissections.
Curves: for the vessels.
- Arteries: to stop blood flow.
-wire needle: allows to manipulate ligatures

II-3-2-The method of dissection

The mouse is placed in a polystyrene tank as it shows in the figure 09.

Often the dissecting tanks contain at the bottom a cork plate which allows most the pins to be firmly anchored. Dissection tanks are made of plastic or stainless steel **(Figure 09),** but must in any case be resistant to the usual decontamination techniques (heat, chemical disinfectants.).

Figure 09: A fixed mouse on a polystyrene support

A-In order to access the organs, the first part of the dissection work consists of opening the skin. An incision is made with a chisel on the lower abdomen: it is called a buttonhole.

B-A grooved probe is used to cut the skin without damaging the organs with the scissors. This is inserted from the buttonhole to the chin. The grooved probe will then serve as a guide for the scissors**.**

C-The cutting of the skin is practiced following a certain number of axes. There is no point in trying to cut the whole skin "freehand". If the incisions are made correctly, all the organs will be easily accessed by simply stretching the skin.

D-Once incised, the skin is detached carefully from the abdomen and different tissues. It is then folded outwards and held in place with pins.

E-The skin constitutes an external tunic for an organism, but it is not in direct contact with the central organs. There is another inner tunic that protects the abdominal wall: it must be cut by creating a buttonhole in order to access the organs.

F-The organs of the thoracic cavity are protected by the thoracic cage (or thoracic chest plate). It is cut to access the heart and lungs.

J-In order to access all the organs constituting the digestive system, it is necessary to unwind the entanglement of organs**.(figure10, figure 11).**

Figure 10: The organs of the mouse after dissection.

- To recover the stomach we used a chisel

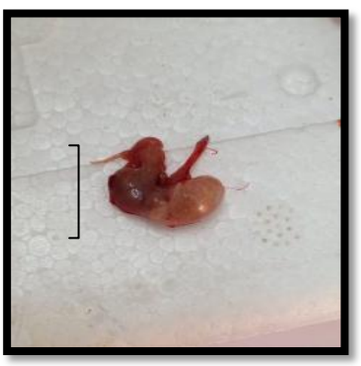

Figure 11: The stomach of the mouse after dissection.

II-4- Realization of histological sections

To make visible what we wants to observe, it is necessary to implement techniques (Sample preparation) that is applied to the material. For observation in MO, the cuts examined are the result of technical procedures requiring several successive steps: fixation, inclusion, cutting, coloring, assembly.

II-4-1- Fixation

The purpose of the fixation is to preserve the structures and harden the parts. It shall be made immediately after sampling by immersion of the material in a large volume of fixing liquid. The most commonly used fixing fluids are formalin orbouin's liquid (mixture of formaldehyde and picric acid). The duration of fixation varies depending on the volume of sampling (a few hours for a small biopsy fragment to several weeks for an entire human brain).

II-4-2- Inclusion

The purpose of inclusion is to allow for fine and regular cuts. The environment most commonly used is paraffin. Since paraffin is hydrophobic, the must first undergo dehydration (by immersion in alcohol baths) of increasing degree and then in toluene baths) before being poured into a mold containing molten paraffin by heating and become liquid, which then infiltrates the room. After cooling, a paraffin block, hard, the inside of which the sampled part is included. In some cases, inclusion media (celloidin, plastic resins...).

II-4-3- Sections

The cuts of the paraffin block are made with a microtome making it possible to produce section slices (sections) of 2 to 5 µm thickness. Cuts are collected on blades of glass.

II-4-4- The coloration

The colorings made on blades, accentuate the contrasts to better recognize the different elements of the preparation. Since the dyes are in aqueous solution, the cuts must first undergo rehydration. This is carried out after dewaxing (by heat and toluene baths) by immersing the slides in alcohol baths of decreasing degree then in distilled water. The most frequently used combine two or three different dyes: Hematoxylin-Eosin (H.E.) combines hematein which colors the nuclei into violet and eosin cytoplasm’s in pink; The usual trichromatic staining’s are Hematéine-Eosine-Saffron (H.E.S.) by adding saffron coloring the collagen fibers in yellow, and massontrichrome which associates a nuclear dye (hematoxylin), a dye cytoplasmic and a blue or green colorant dyeing the collagen fibers. Many special colors (so-called signatures) make it possible to visualize different structures or components of the tissues (reticulin fibers by colorations silver or elastic fibers by orcein).

II-4-5- Mounting

After having been dehydrated (by alcohol baths of increasing degree then toluene baths), the colored sections are mounted between the blade and the slide with a synthetic resin whose refractive index is close to that of the glass. Then, of a "microscopic preparation" (simply called "blade" in the language current) ready to be observed in MO

II-5-Preparation technique in the laboratory of anatomopathology (stomach of mice)

II-5-1- Fixation

Put the anatomical piece (mice stomach) in a 10 % formalin bath between 24h and 48h. (It is preferable to leave 48 hours), care must be taken to prepare a bath which is 10 times the mass of the part to be fixed. Take a fragment of the organ to be studied. Place it in a cassette that is closed and which will be dipped in successive baths. (Be careful to cut the fragment of the organ). **(figure 12)**

Figure 12: The stomachs of mice to study in cassettes.

II-5-2- Preparation before inclusion: Dehydration

Put in successive baths of alcohols of increasing concentration to dehydrate the organ knowing that the paraffin is very hydrophobic.

Figure 13: Automaton device of inclusion of the hospital of Bouira.

This device allows dehydration and infiltration of biological tissues automatically **(figure 13)**. The apparatus, of the carousel type, consists of 12 metal tanks filled with solutions of alcohol with increasing content (70 to 100 %), xylene and then paraffin. The vats for paraffin are heated to 65 C° to keep the paraffin in a liquid state. The system is semi-closed to reduce exposure to solvent vapors. The samples are placed in a basket

(capacity: 80 cassettes) which is successively transferred from one bath to another according to user-defined programming. In this automated processor, the water of the previously fixed tissues is substituted by the alcohol, which is in turn substituted by toluene, a paraffin solvent, and finally gradually substituted with paraffin, which will be solidified at the outlet, after coating **(figure 14).**

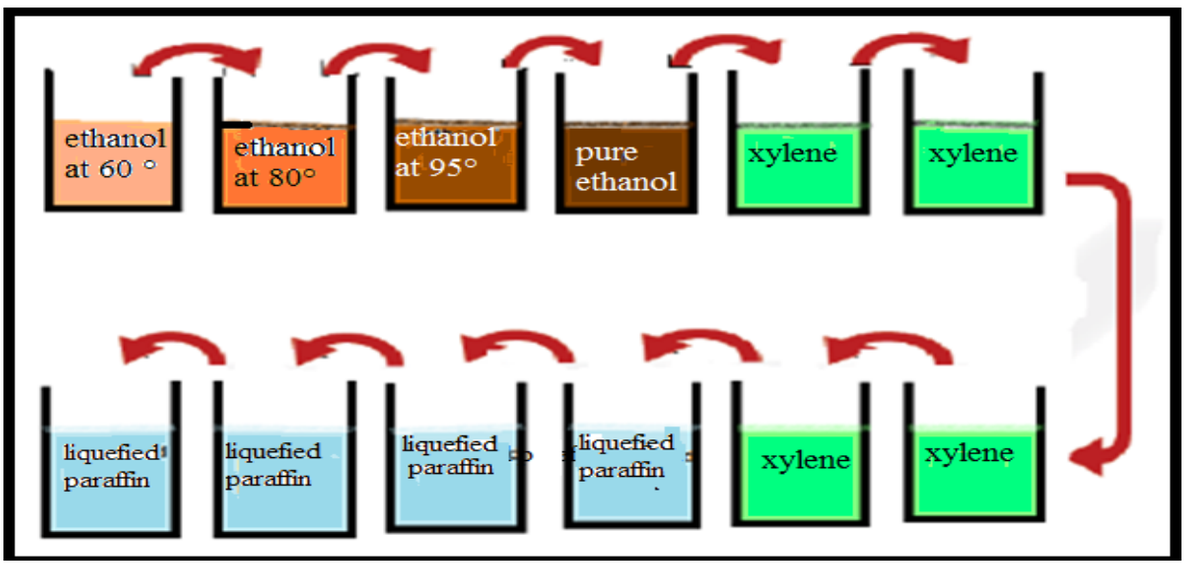

Figure 14: The inclusion process that lasted 22 hours

II-5-3-Inclusion / coating:

This device as it shows in the figure (15) is a compact and programmable station for the coating of biological tissues in paraffin. It is designed to allow the final assembly and hardening of biological tissues impregnated in paraffin to be subsequently cut with the help of a microtome. The apparatus has a metal bath heated to 60 °C to preserve the samples before assembly. The working platform is separated into two surfaces, a tempered surface that maintains the liquid paraffin to allow handling and orientation of the sample, and a cooled surface for solidification of the paraffin. The paraffin reservoir, the mold tray and the paraffin dispenser are also tempered to facilitate coating.

Figure 15: Paraffin coating apparatus

The following pictures in the figure 16 show the steps of coating a tissue sample using this apparatus.

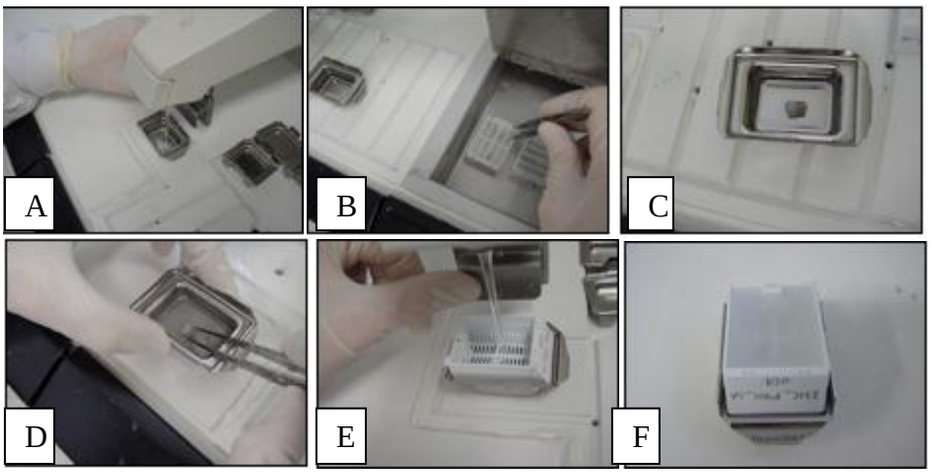

Figure 16: The steps of coating a tissue sample

A-The bottom of the preheated metal molds is filled with the hot paraffin.

B-held on the temperate surface. The cassette containing the sample is taken out of the hot paraffin bath .

C-the fabric rapidly transferred to the mold.

D-The mold is placed gently on the cooled surface to fix the sample to the center (cold paraffin curing).

E-The upper part of the cassette, with tissue identification, is placed on the mold .

F-The mold is then passed over the cold surface for 30 minutes to cure the paraffin .

II-5-4- Histological section

The blocks are cut into very thin strips using a microtome (**figure 17)** according to the following steps:

- Move the object holder to the maximum
- Place the block in the object holder without fixing it
- Place the razor face engraved on the outside: fix (facingcut in a vertical plane, parallel to the wire of the razor, the twohorizontally
- Longest block edges by hand
- Adjust the thickness of the sections (5 um)
- Put the ratchet
- Cut

Figure 17: Realization of histological sections using a microtome

II-5-5-Attaching the cut to the blade

On a heating plate maintaining a temperature of 50°C, a slide is placed on which is deposited a solution of distilled water with 1% albumin, taking care to make a dome of water on the blade to avoid air bubbles. The histological section is then placed on the slide.

Figure 18: Label the blades with a diamond pencil

The blade is drained by simply reversing the water on a sopalin, place the right blade to dry well. The slide is then placed in an oven at 56 °C for 1 hour.

This will make it easier to remove the remaining paraffin and to allow the fixation of the cut on the blade.

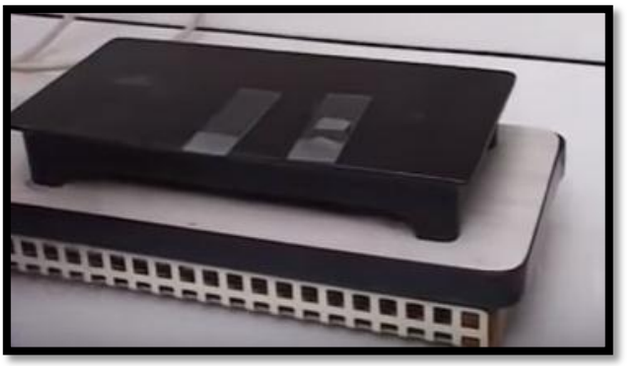

Figure 19: Histological sections in a bath Marie.

II-5-6-Dewaxing

Then it is necessary to remove the paraffin. (Taking care not to remove the cut from the blade) by passing the slide in 2 toluene baths. (Taking care of the cleanliness of the last bath), passage into alcohol to rehydrate the sample.

The slide is placed in a tap water bath for a few seconds. The slide is placed in a distilled water bath for a few seconds.

II-5-7-Dewaxing / coloring Hematoxylin-Eosin (HE):

The slides obtained are colored in order to facilitate the observation of the cellular structures under the optical microscope.

Steps:

Xylene: 20min
Xylene: 20min } **Dewaxing**

Alcohol: 100°
Alcohol: 90°
Alcohol: 70° } **Rehydratation**

Bath with hematoxylin, allows to color the nuclei: 5 min
Rinse tap water: 3 min
Rinsing distilled water
Eosin bath, allows to color the cytoplasm in pink: 3 min
Rinsing distilled water
Bath in 95% alcohol: 3 min
Bath in 100% alcohol: 3 min
Xylene: 3 min } **Hematoxylin/ Xylene coloring**

II-5-8- Mounting

In order to preserve the cut an object-covered slide or a transparent film is fixed on the sample carried by the slide with the aid of a synthetic resin. Due to their good optical properties, the most commonly used resins are polymethacrylamites of methyl **(figure 20).**

After assembly, the slides are observed under an optical microscope.

Figure 20: The mounting of the blades.

Chapter II: Results and discussion

II-1- Results of blood smear study after 72 h of inflammation

The following figure 21 shows the blood smear results of the two groups of healthy mouse and mouse with inflammation after 72h of inflammation.

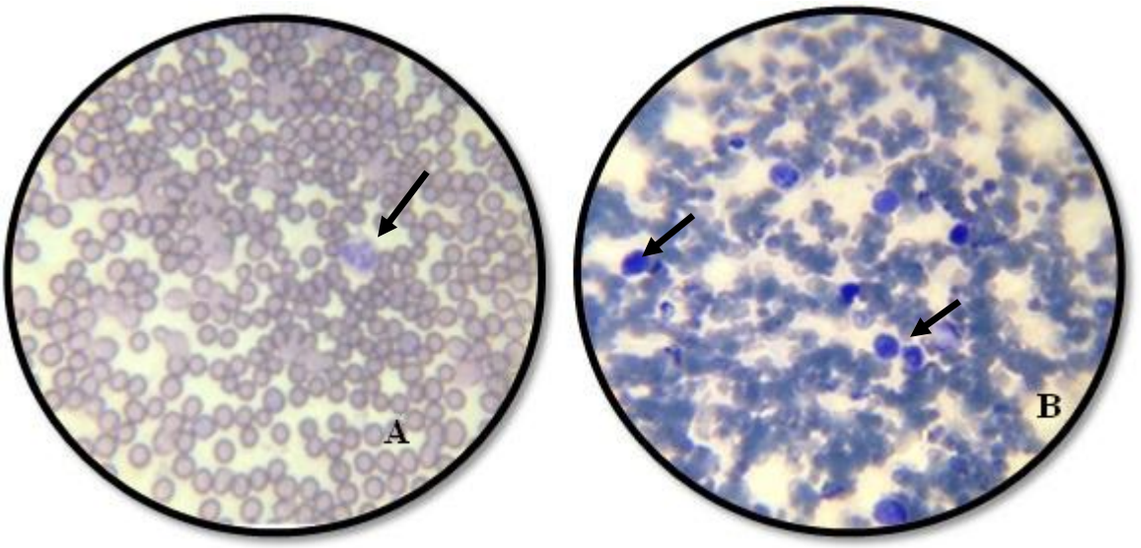

Figure 21: Blood smear of a mouse, indicates the presence of leukocyte cells after 72 h of inflammation observed in OM with magnification (X 40).

(A): Normal smear of a healthy control mouse. **(B):** Smears with the presence of leukocyte cells of a mouse after 72 h of inflammation (leukocyte cells indicated by arrows).

comparing the blood smear of a mouse with inflammation with a healthy control mouse, the presence of excess leukocyte cells is noticed, this confirm the presence of inflammation

II-2- Results of the blood smear study after 21 days of treatment with the aqueous extract of *AHA*.

The figure 22 shows the blood smear results of the two groups of healthy control mice and mice with inflammation after 21 days of treatment with *AHA.*

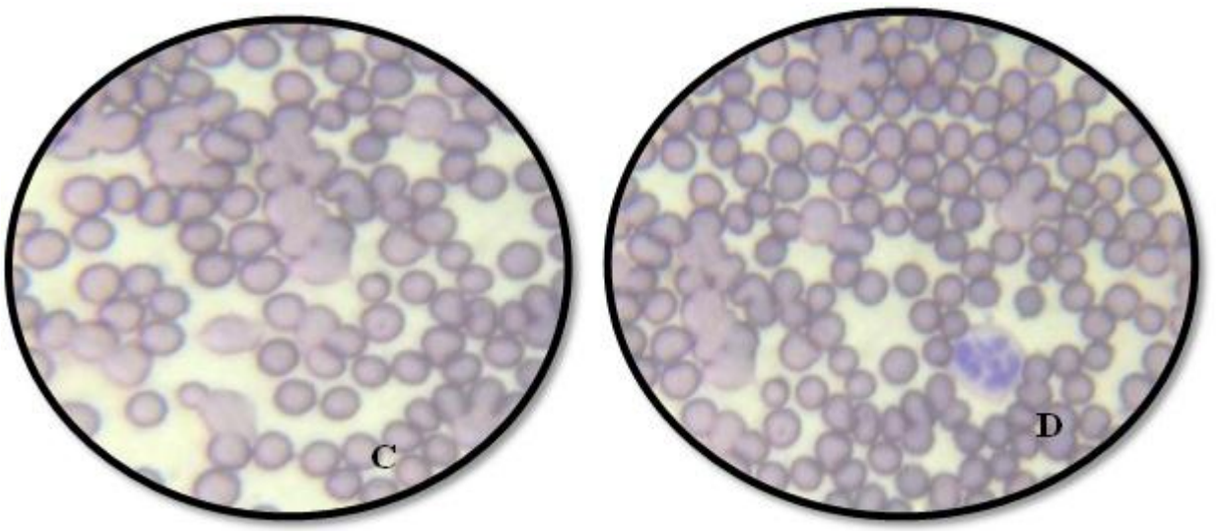

Figure 22: Blood smear of a mouse observed under MO with magnification (X40), indicates the presence or (absence) one leukocyte cell after 21 days of inflammation magnification X 40. **(C):** Normal smear of a healthy control mouse. **(D):** Smear with the presence of one leukocyte cell of a mouse after 21 days of treatment with the aqueous extract of *artemisia herba alba* (leukocyte cell showed by arrow).

In comparison with the control mouse, the treated mouse smear shows the presence of a single leukocyte cell after 21 days of treatment with the aqueous extract of *AHA*, this means that inflammation is disappeared, thus our plant has an anti-inflammatory effect.

II-3- Effect of the aqueous extract of *Artemisia herba alba* on biochemical parameters

II-3-1- White blood cells

The figure 23, shows the variation in the concentrations of white blood cells in different groups of mice (witness, treeated, not treated) before and after treatment.

In the group of untreated mice the rate of GB reached its maximum after 72 hours of inflammation 17,1 $.10^3/mm^3$. 21 days after treatment with water the rate decreased by 5.88 %. On the other hand in the group of mice treated the rate of the GB reached $17.10^3/mm^3$ after 72h of the inflammation.

This concentration decreased by 29.47% towards the third week of treatment with the aqueous extract of *Artemisia herba alba*.

The leucocytosis is due to the cytokines and chemokines synthesized during the inflammatory reaction by the active cells.

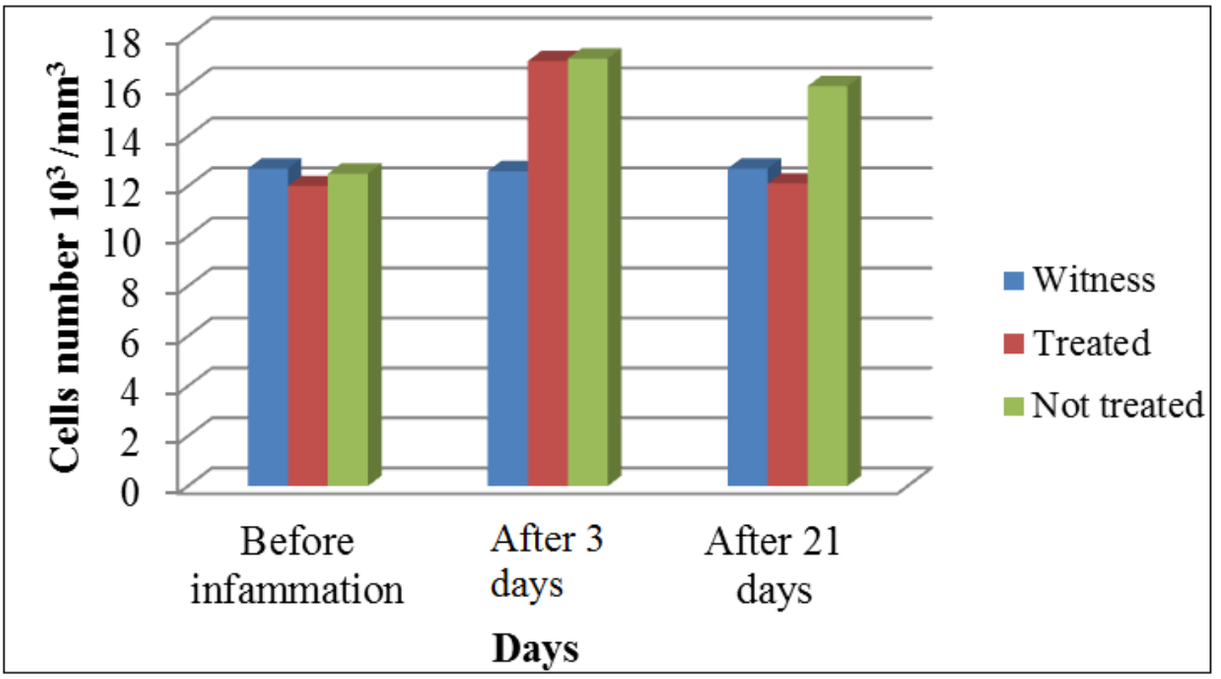

Figure 23: White blood cells of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days.

II-3-2- Neutrophile

The figure 24 shows the variation in the concentrations of neutrophile level in different groups of mice before and after treatment.

In the group of untreated mice the rate of neutrophile reached its maximum after 72 hours of inflammation $10.10^2/mm^3$. 21 days after treatment with water the rate of decreased by 9.80 %. On the other hand in the group of mice treated the rate of the neutrophile arrived at a value of $9.10^2/ mm^3$ after 72h of the inflammation.

This concentration decreased by 30.50 % towards the third week of treatment with the aqueous extract of *Artemisia herba alba.*

Polynuclear production is medullary from pluripotent stem cells. Their maturation and their proliferation is controlled mainly by 2 cytokines: GM-CSF and G-CSF. There is a baseline production of PNN, which increases when needed. The maturation of the PNN requires about 5 days and the lifetime of a PNN is 2 days. Their action in inflammation is exerted via surface receptors: different chemotactic receptors (for LTB4, C5a). The activation of these receptors generates the migration of the PNN to the site of inflammation but also the production of oxygenated free radicals and the expression of adhesion molecules.

Receptors for opsonins: Fc receptors for the Fc fragment of Ig G, receptors for fragments of the activated complement. Receptors for endothelial cell adhesion molecules.

Once activated, PNNs synthesize products first stored in primary (lyzosome) or secondary

granules and then released either inside the cell and acting on the phagocytosed substances or in the extracellular medium.

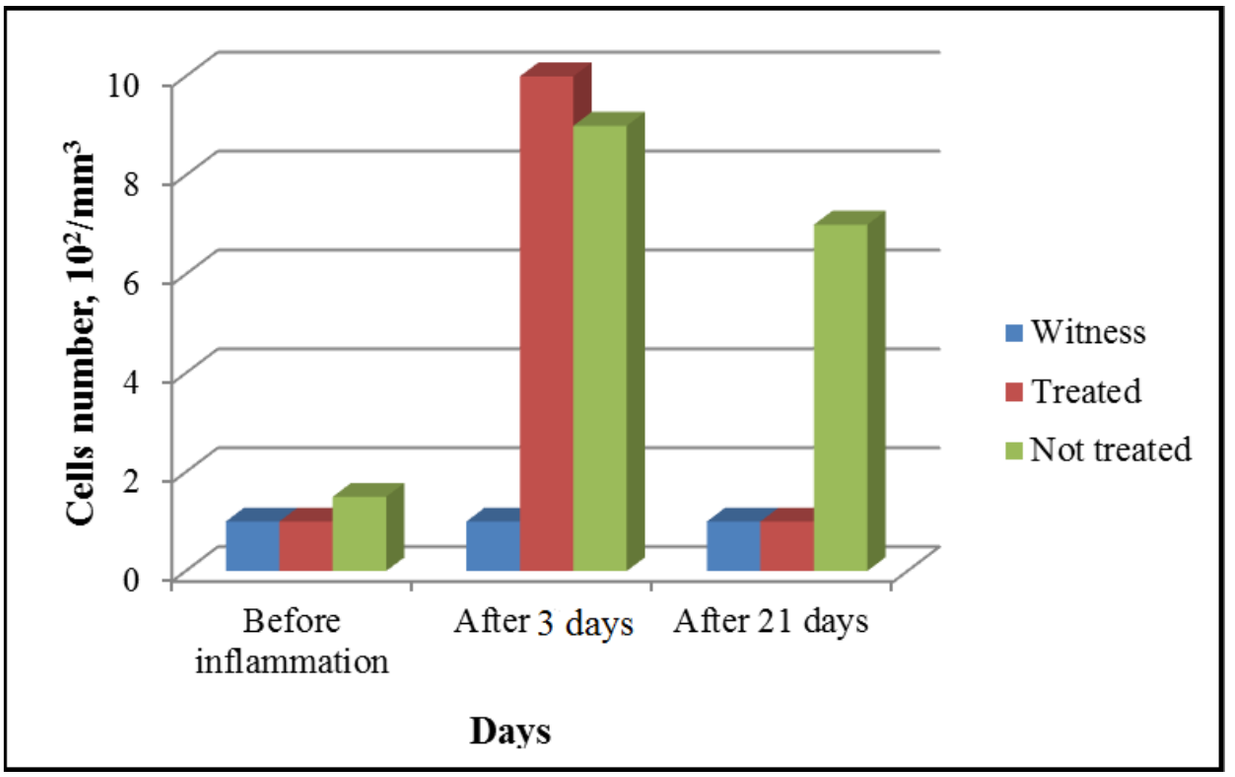

Figure 24: Neutrophil levels of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days.

II-3-3- Platelet

The figure 25 shows the variation of the platelet level in the different groups of mice (healthy witness, milking, untreated) before and after treatment.

In the group of untreated mice the platelet count reached its maximum after 72 hours of inflammation 1190 / mm^3 .21 days after treatment with water platelet counts decreased by 7.39 %. On the other hand in the group of mice treats the platelet rate is arrived at a value of 1200 / mm3 after 72 hours of inflammation. This concentration decreased by 15.25 % towards the third week of treatment with the aqueous extract of *AHA.*

There is a parallelism between the importance of hyperplaquettosis and that of the inflammatory state. The secretion of pro-inflammatory cytokines (including IL-6) is responsible for the stimulation of megakaryopoiesis.

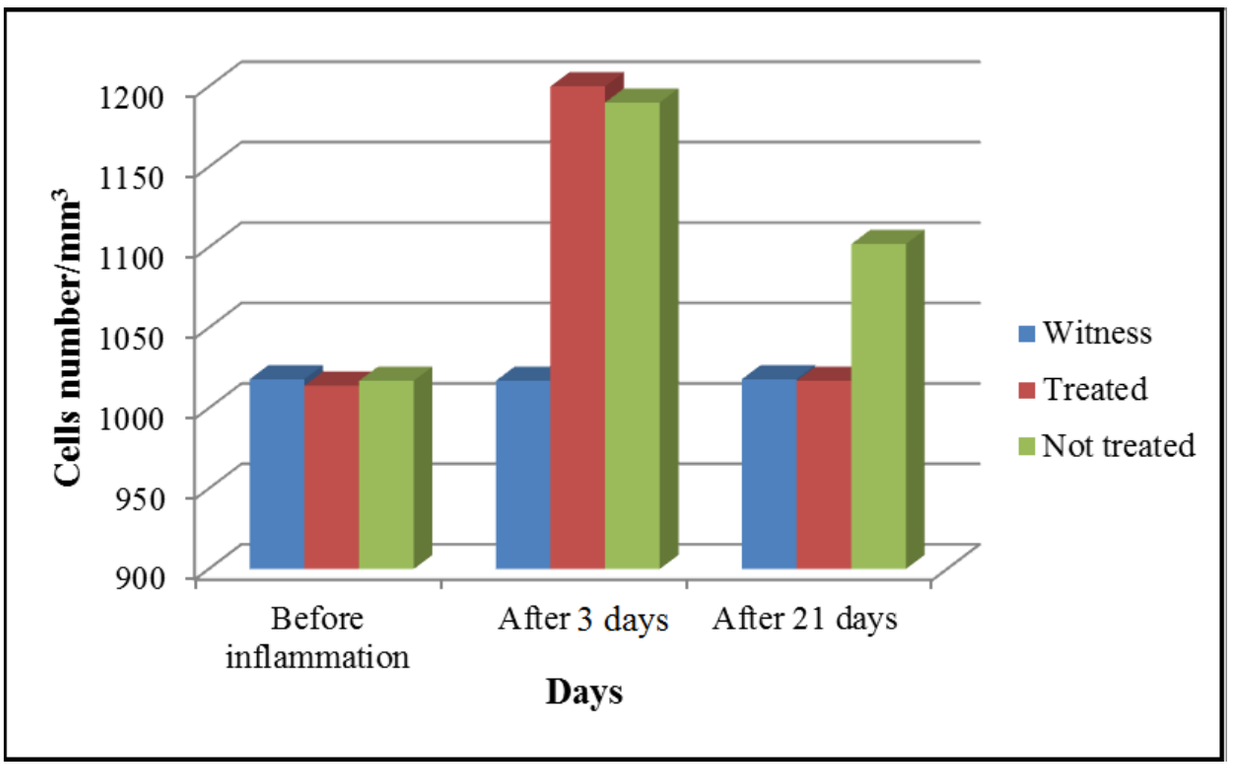

Figure 25: Platelet levels of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days.

II-1-Blood glucose

The figure 26 shows the variation in blood glucose concentration in the treated (untreated) mouse groups compared to the control healthy mice before the treatment: 1,1 g / l 0,95 g / l 1,02 g / l and After treatment.

In the group of untreated mice the concentration of blood glucose continued to increase and reached its maximum after 21 days (1.48 g / l). On the other hand, in the other group of mice with treated inflammation, the oral administration of the aqueous extract of Artemisiaherba alba caused a 7.27% decrease in blood glucose after 72 hours of inflammation and a significant decrease of 41.60% after 21 days of treatment compared to the healthy control. According to our results, we have found that the aqueous extract of *AHA* has been able to play a crucial role in the decrease of the glucose concentration, either by the stimulation of insulin secretion or by extra-pancreatic action and thus by the influence of the glucose absorption by the various tissues. Briefly, *Artemisia herba alba* has a hypoglycemic effect.

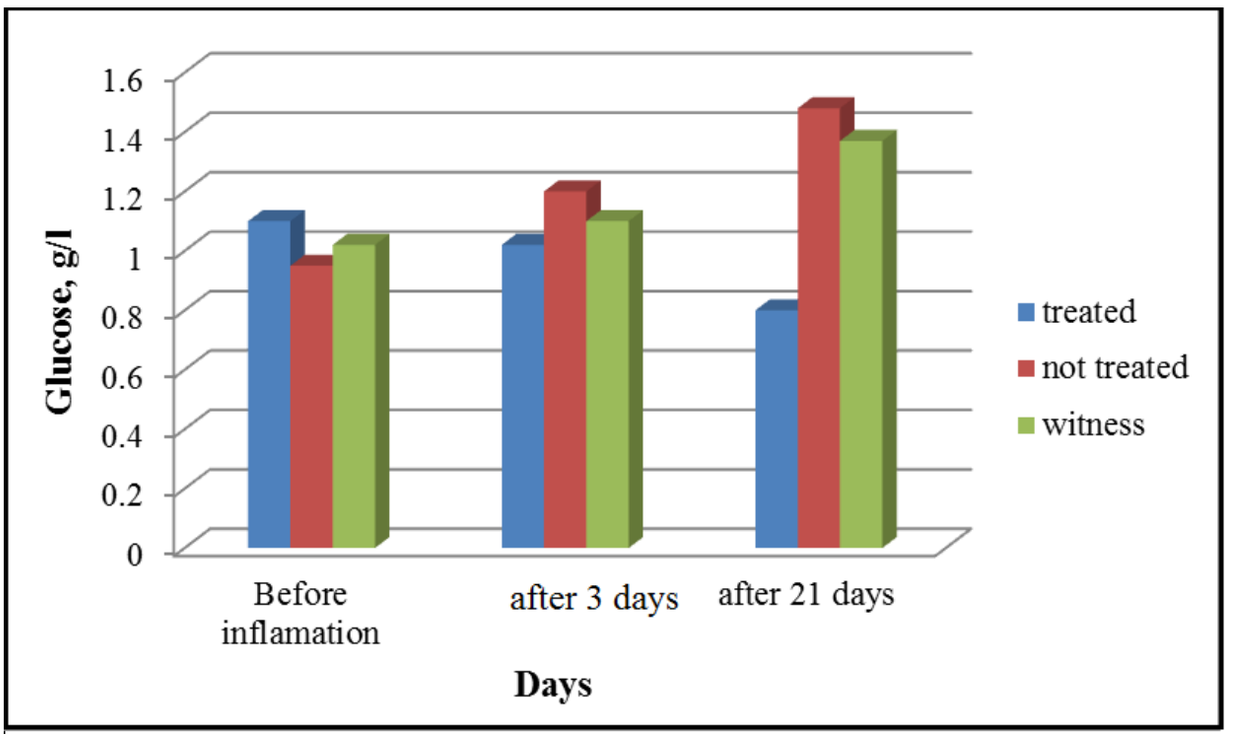

Figure 26: Glucose levels of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days

III-2-Cholesterol

The figure shows the concentration of plasma cholesterol in two groups of mice (treated, untreated) relative to the healthy mouse group after 21 days of treatment. In the group of untreated mice the concentration of cholesterol increased by 14.69% until reaching a value of 2.11 g / l.

In the group of treated mice the cholesterol level had a significant decrease of 35.55%, which is represented by a value of 1.16 g / l.

According to our results we have observed that the administration of the aqueous extract of *AHA* may have caused a reduction in the cholesterolic concentration of the underlying mechanism by which the freeze-dried aqueous extract of *Artemisia herba alba Asso.* The effect of cholesterol on in the intestinal tract and the increase in biliary excretion appears to be a reduction in intestinal absorption of cholesterol. The aqueous extract Of *AHA* could also act by decreasing the biosynthesis of cholesterol specifically by decreasing the activity of HMG-CoA reductase

In addition, the aqueous extract of *Artemisia herba alba* decreased serum cholesterol by altering lipoprotein metabolism: enhancing LDL uptake by increasing LDL receptors **[56]** and / Or by increasing activity of Lecithin-Cholesterol Acyl Transferase (LCAT).

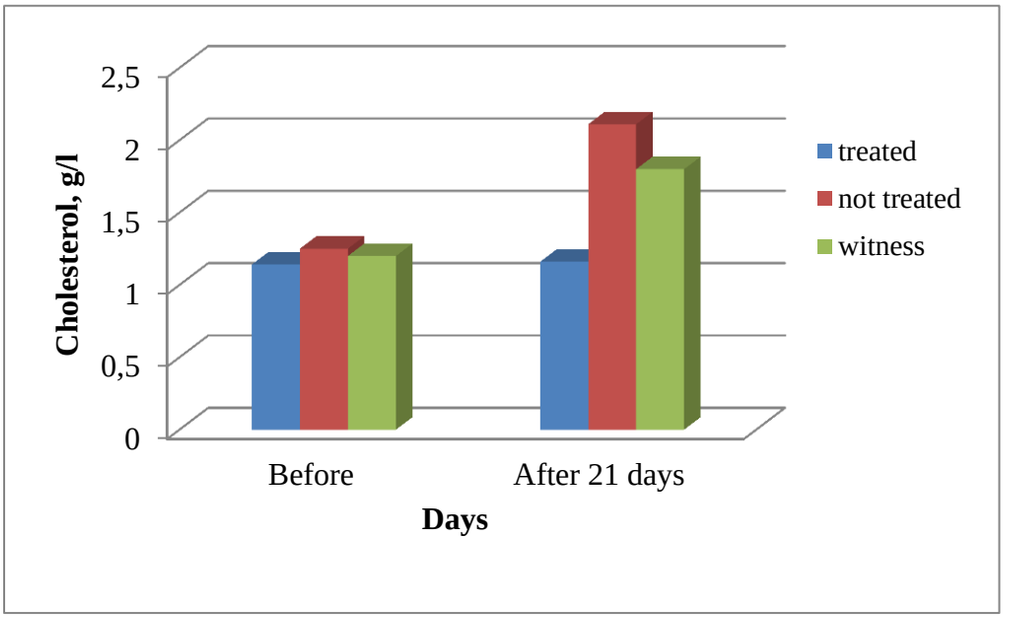

Figure 27: Cholesterol levels of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days

III-3-Triglycerides

The figure 28 shows the concentration of plasma triglycerides in two groups of mice (treated, not treated) relative to the healthy mouse group after 21 days of treatment. In the group of untreated mice the concentration of TG increased by 9.09% until reaching a value of 1.65 g/l.

In the group of treated mice, the rate of TG decreased significantly by 17.33%, which is represented by a value of 1.24 g / l. It was also found that the daily administration of the aqueous extract d '*Artemisia herba alba* pendant three weeks an increased LDL catabolism, LCAT activation and tissue lipases and the reduction of serum triglyceride levels by decreasing fatty acid synthesis and / or inhibition of acetyl-COA carboxylase.

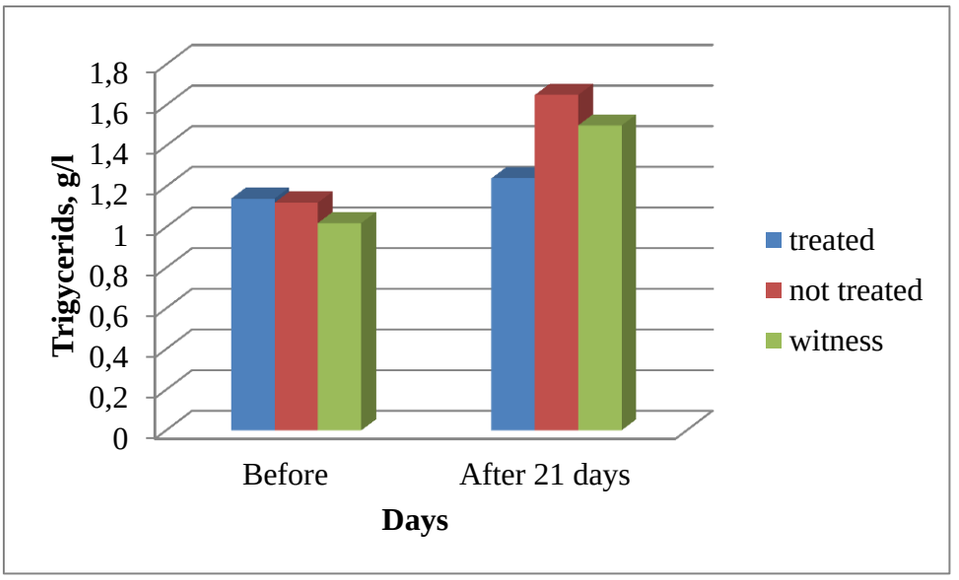

Figure 28: Triglyceride levels of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days

IV-The tests performed to identify the presence of toxicity

To evaluate the presence of toxicity, two tests were carried out: urea and creatinine.

IV-1-Urea

The results obtained in our study "Figure 29" showed that the aqueous extract of *artemisia herba alba* caused a slight decrease in the concentration of urea in the mice treated after 21 days of treatment compared to the healthy mice control and Mice not treated 2 g / l 2.9 g / l 3.11 g / l this means that the dose used during the experiment is not toxic.

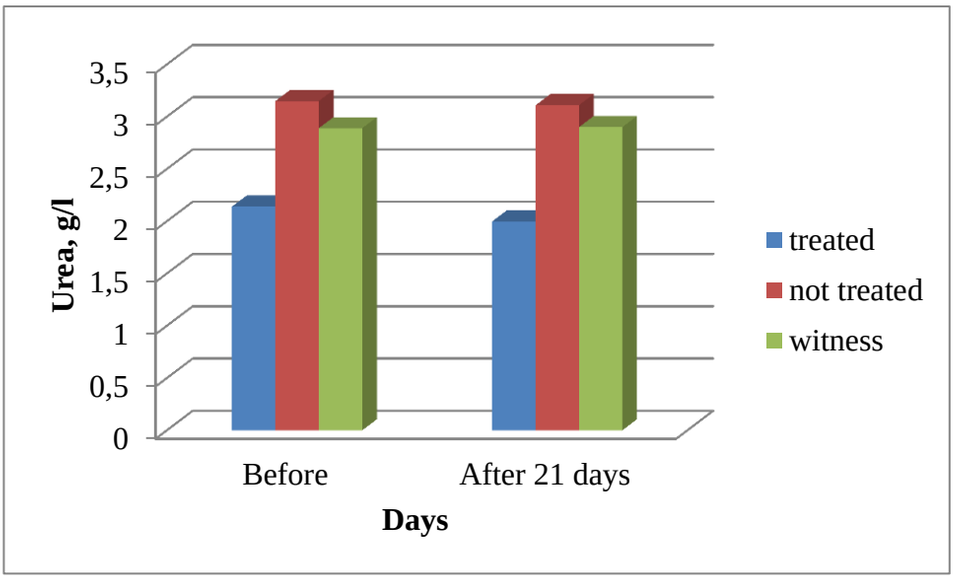

Figure 29: Urea levels of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days

IV-2-Creatinin

The results obtained in our study "Figure 30" showed that the aqueous extract of *AHA* caused a slight decrease in the concentration of urea in the mice treated after 21 days of treatment compared to the control mouse. Mice not treated 3.8 g / l 3.79 g / l 3.91 g / l this means that the dose used during the experiment is not toxic

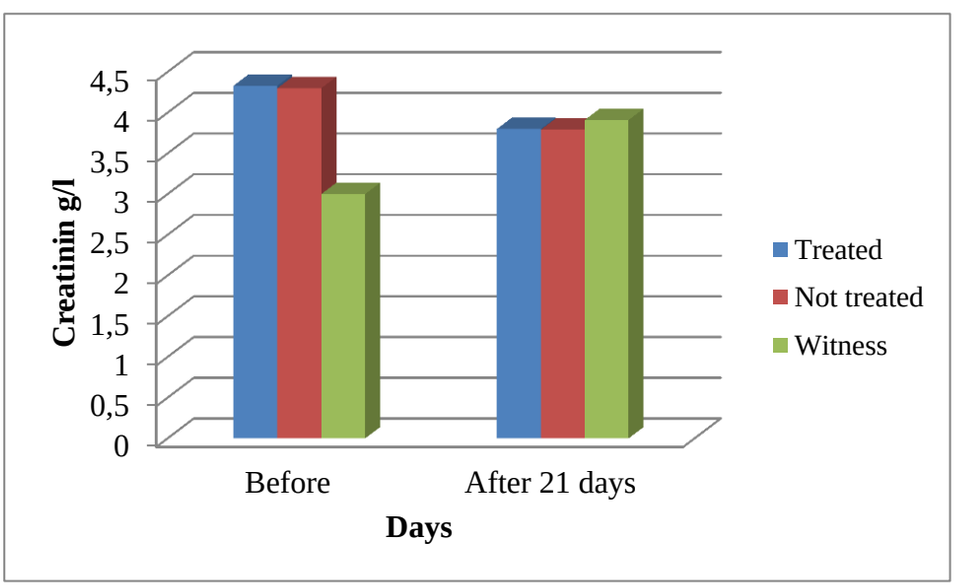

Figure 30: Creatinin levels of different group of mice (treated or not treated by aqueous extract of *AHA)* for 21 days

V-The influence of *AHA* administration on body weight change

Figure (31) shows the results obtained from the variation in body weight of the

AHA different groups of mice (normal, mice with inflammation, mice with inflammation treated) after a daily treatment of 21 days or with an aqueous extract of the aerial part of Has a dose of 1 ml / kg.

The results obtained in our study show that the inflammation caused a remarkable weight loss from the first week until reaching a stable value towards the third week in the group of untreated. This decrease is in order Of 9.09%, 4.34%, up to the third week, relative to body weight, after each week of treatment with distilled water. In addition, the group of mice treated suffered during the same periods an increase of 23.07%, 10.34% and 19.44%.

According to our results we found in the group of untreated mice a loss of weight and this is due to a loss of appetite under the action of an inflammatory protein named CCL2 which can act directly on the neurons. Binding to its CCR2 receptor and decreasing their activity and thus their ability to secrete the MCH peptide. Thus the action of CCL2 on certain neurons of the hypothalamus could explain the loss of appetite, the increase in energy expenditure and the weight loss associated with a state of inflammation.

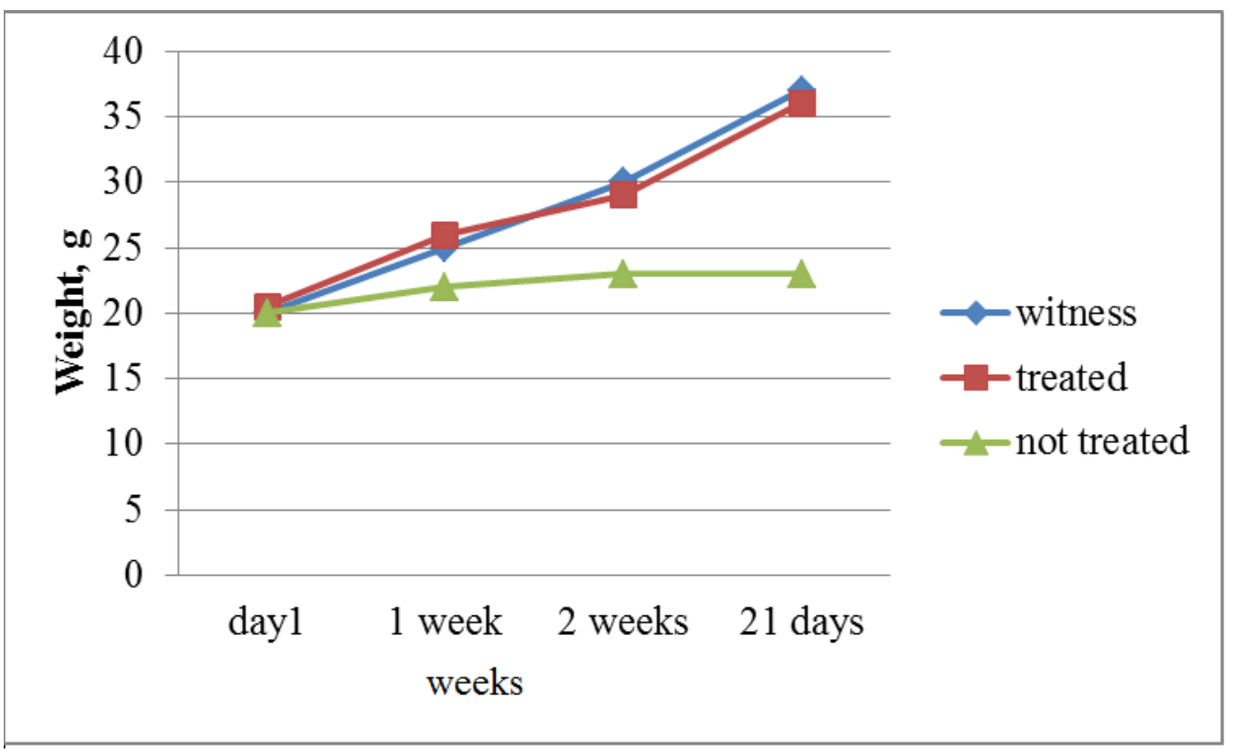

Figure 31: Variation of body weight of the different groups of mice treated and not treated by the aqueous extract of *AHA*

VI- Peptic Ulcer Disease

VI-1- The results of the histological sections

VI-1-1 Stomach control (witness)

The figure 32 shows the normal organization of the different layers of the mouse (fundic region) observed under an OM (magnification X4, X10).

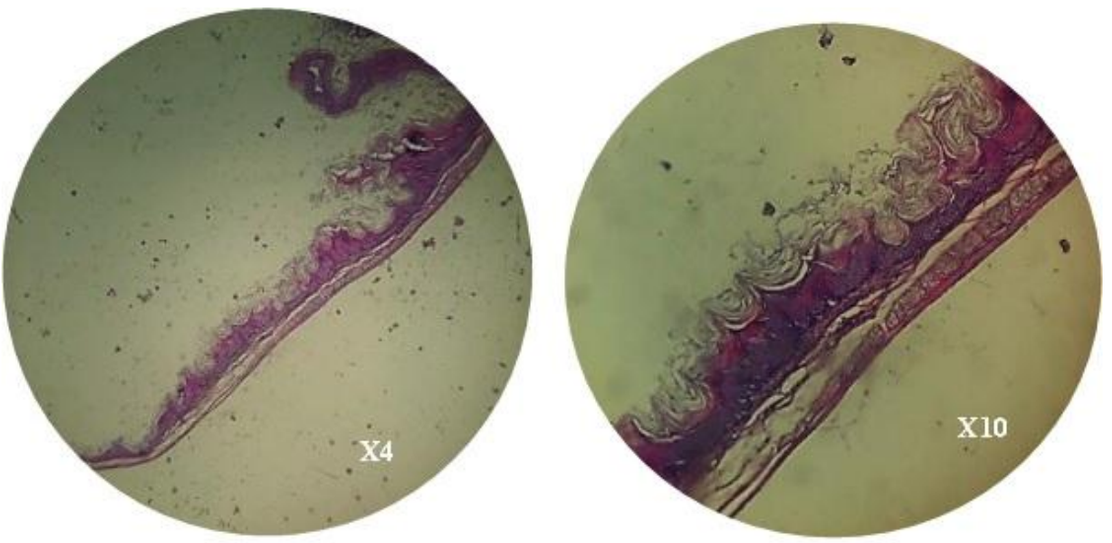

Figure 32: Histological section of mouse stomach observed under OM with magnification X 4, X 10 (Fundic region).

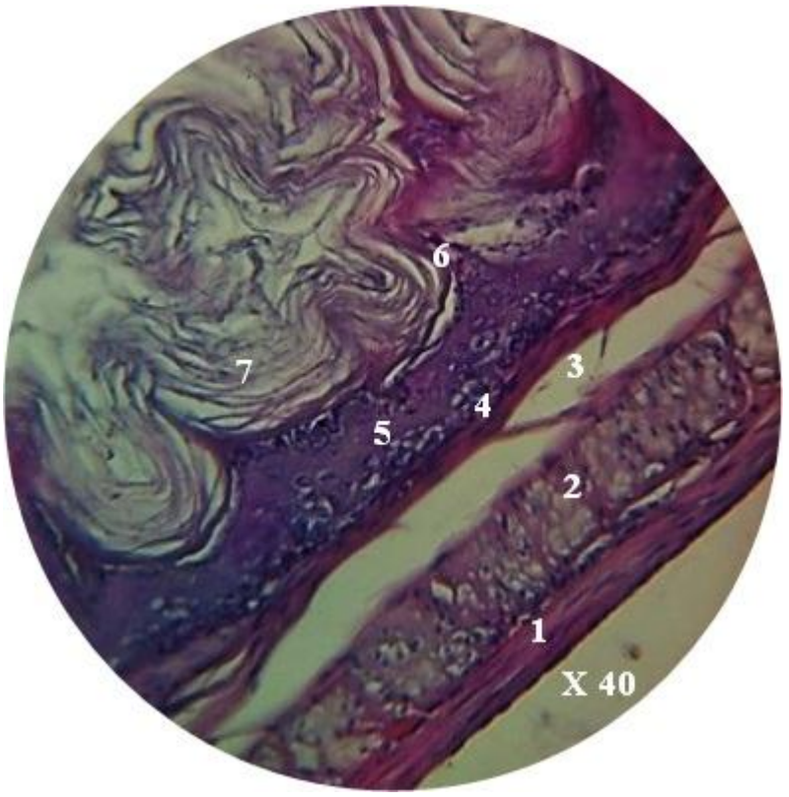

Figure 33: Histological section of mouse (witness) stomach observed under OM with magnification X 40 (Fundic region).

1-Outer longitudinal muscle layer

2-Inner circular muscle layer

3-Chorion of the submucosal

4-Muscularis mucosae

5-Lamina propria

6-Fundial glands

7-Cryptic epithelium

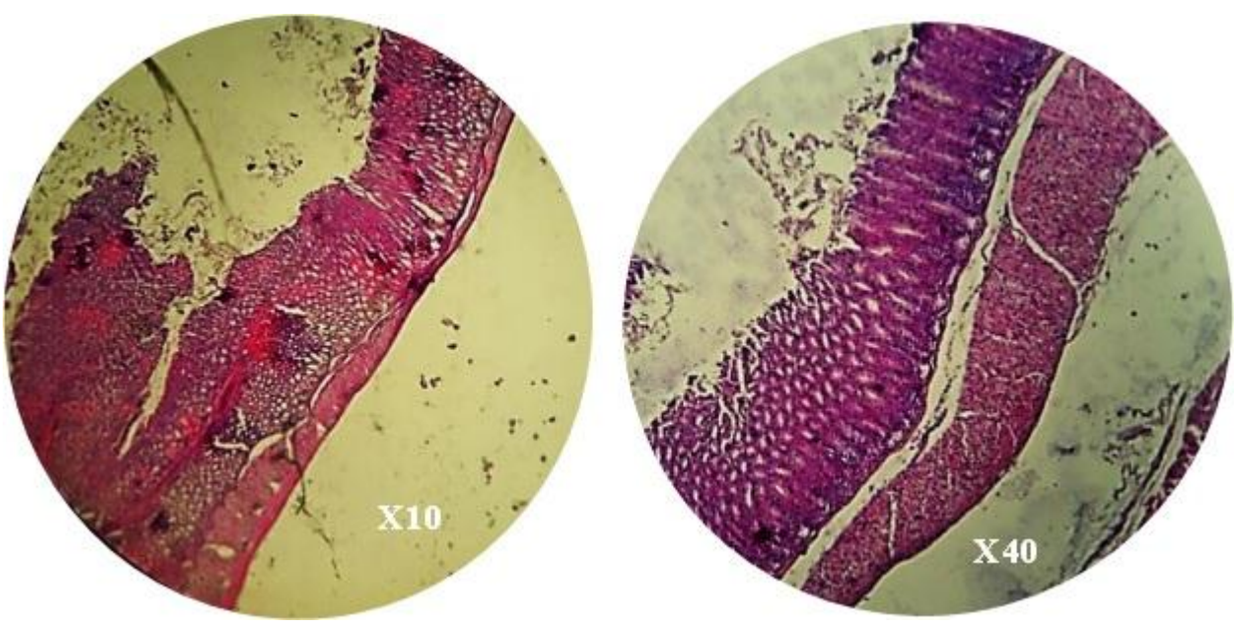

Figure 34: The villi of stomach of witness mouse.

The stomach of untreated mice

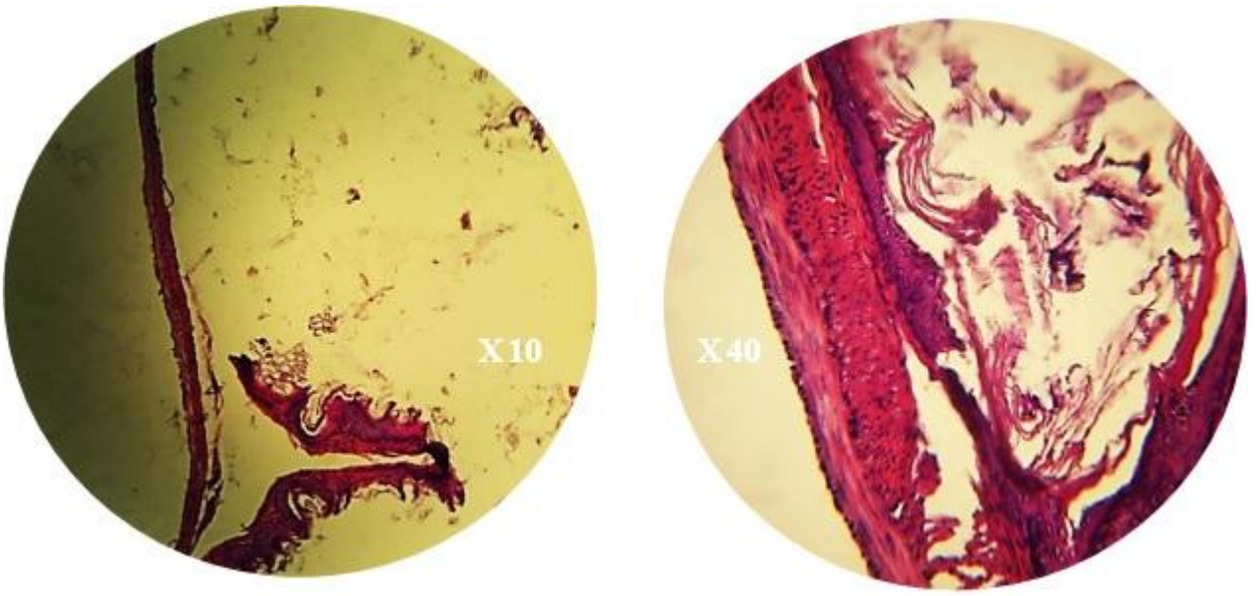

Figure 35: Histological section of mouse stomach treated with ethanol (50%) for 15 min observed under OM with magnification X 10, X 40 (Fundic region).

The figure 35 shows the results obtained after realization of the histological sections on mice treated with ethanol for 15 min.

We notice that in magnification X4 there is a detachment of the internal mucosa, and in magnification X40 we noted that the epithelial crypt is completely disappeared and the appearance of the necritic cells.

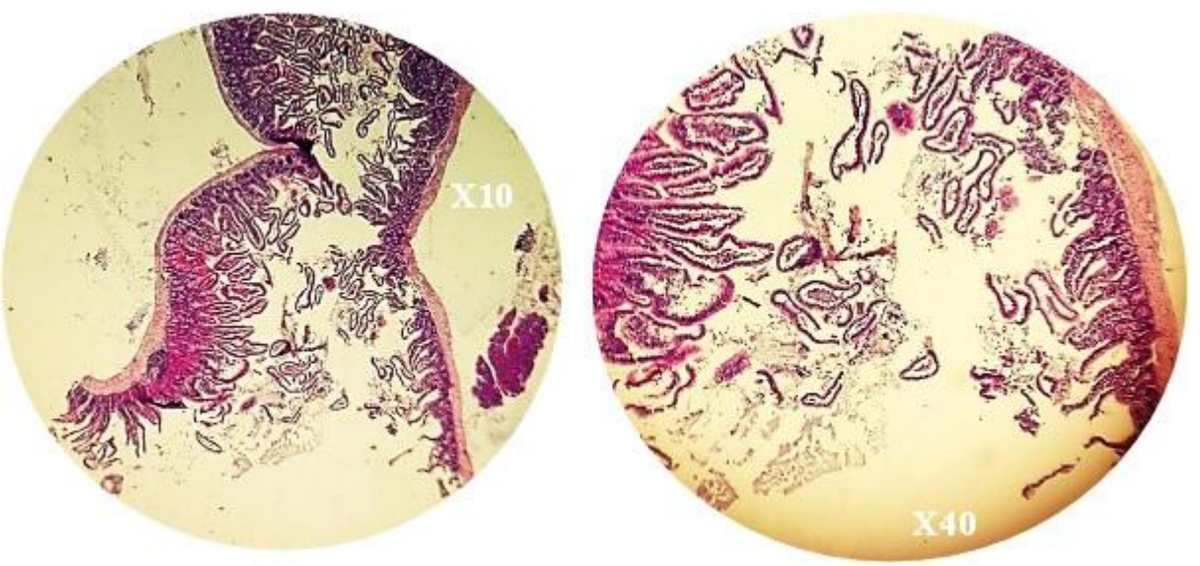

Figure 36: The villi of not treated mouse by aqueous extract of *AHA* observed under OM with magnification X10, X40

It is noted that there is a total disruption of the later by the effect of ethanol.

The stomach of treated mice

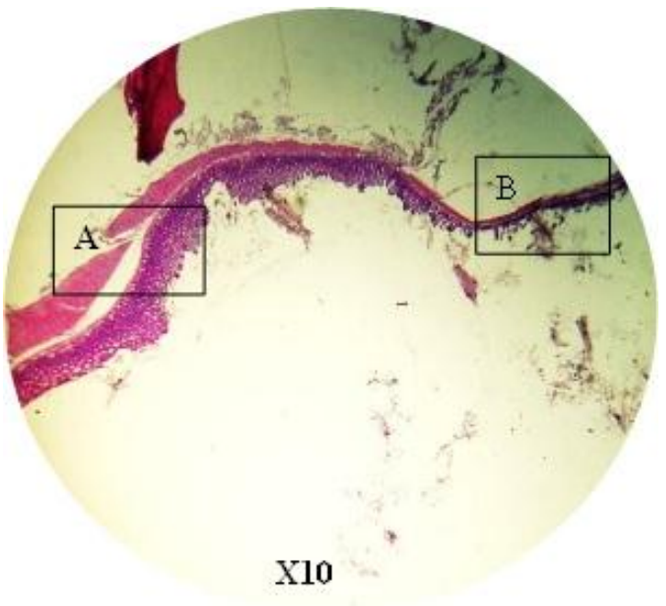

Figure 37: Histological section of mouse stomach observed under OM with magnification X10 (Fundic region after treatment with aqueous extract of *AHA). Zone A* affected, zone B not affected

It is noted that ethanol caused destruction of the mucosa on the one hand (B), and on the other hand the aqueous extract of *AHA* provided protection of the mucosa cells (A).

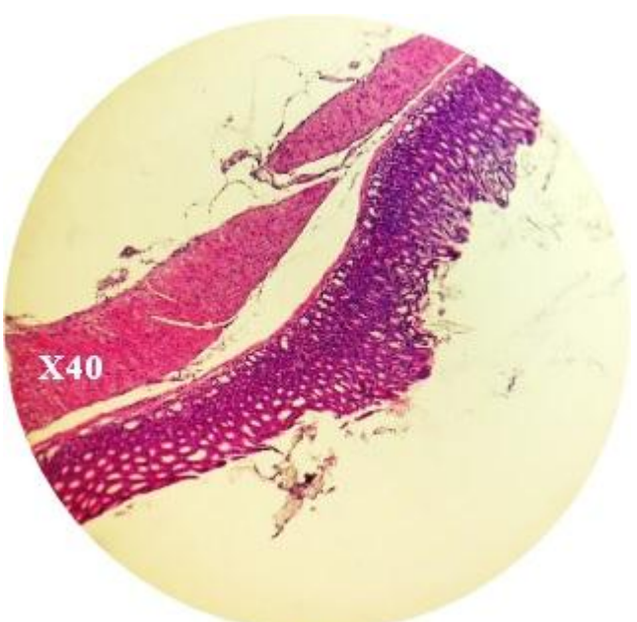

Figure 38: Magnification (X40) of the area A of the figure 37.

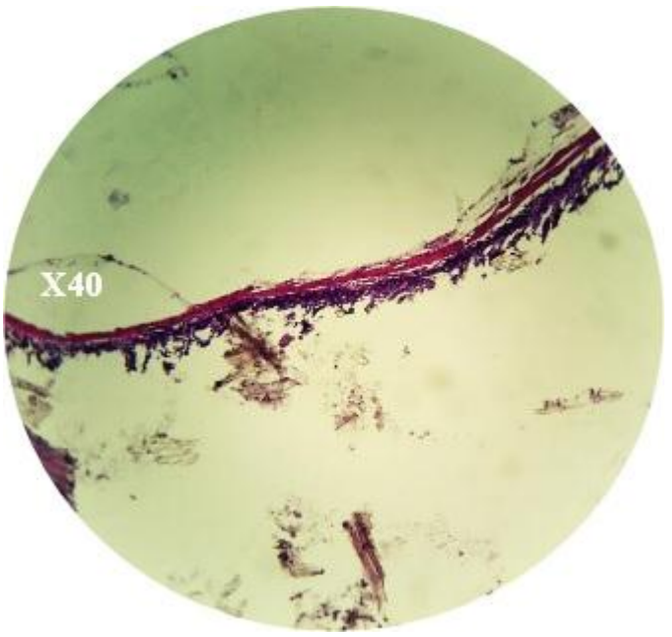

Figure 39: Magnification of zone B of the histological section of the stomach of a mouse observed with the MO (X40) (figure 37)

According to this figure 38 it is observed that the mucus is disappeared under the effect of ethanol, whereas the mucosa remains intact (homogeneous staining to the extrimites) which means that the cells are alive and were not affected by ethanol.

In figure 40 There is a total disappearance of mucus and the beginning of the dissection of the mucosa by the effect of ethanol.

Discussion

SZABO & al, 2013 suggested that after intra gastric administration of ethanol a rapid time dependent release of endothelin-1 into the systemic circulation preceded the development of the hemorrhagic mucosal erosions by vasoconstriction. Moreover, by decreasing the secretion of biocarbonate (HCO_3^-) and mucus production. Ethanol produces the necrotic lesions in gastric mucosa and also initiate apoptosis which lead to cell death **(figure 36) [58].**

According to **Kamel Gharzouli and al, 1999** the inability of *AHA* extract to precipitate haemoglobin suggests that the Prussian blue-reactive substances are mainly monomeric flavonoids. The ability of these plant extracts to protect the stomach against ethanol injury suggests that both monomeric and oligomeric polyphenols participate in enhancing the mucosal barrier. Besides inhibition of the proton pump, polyphenols present free-radical-scavenging properties, a stimulatory effect on prostaglandin E2 and therefore of mucus secretion the three main components of the gastric mucosal barrier **(figure 38) [59].**

Conclusion

Conclusion

In Algeria, traditional medicine is widespread and holds a major place in the treatment of inflammation and stomach ulcers, the number of studies on the search for new molecules capable of preventing or even delaying appearance of complications related to inflammation, remains very limited.

All of our work has highlighted the beneficial effects of the administration of the *AHA* aqueous extract either in the treatment of inflammation and ulcer or in the positive influence of the latter on the rate of certain biochemical parameters.

The results obtained in the present study clearly show that during the inflammation these parameters have increased.

In the first part, we confirmed the anti-inflammatory effectof the aqueous extract of *AHA*. Our results indicate that this extract exhibits a high anti-inflammatory activity in inflamed mice.

On the other hand, the aqueous extract of *AHA* also shows a hypolipidemic activity (cholesterol, triglycerides) and hypoglycemiante.

In the second part of this work, we evaluated the cytoprotective activity of the *AHA* aqueous extract. Our results show that the extract of the plant favors a partial protection of the gastric mucosa. These results leave great hope for the future with the protective role that can confer in the prevention and limitation of the side effects of inflammation and ulcer.

Indeed, it appears from the present work that the *AHA* is a very interesting product and rich in therapeutic possibilities. Our results have a panamount importance because they open up in the future experimental perspectives which should allow us to clearly identify the molecules involved in the anti-inflammatory, cytoprotective, hypoglycemic and hypolipidemic effect of AHA and to advance towards a better knowledge of the molecular mechanism(s) involved in the observed pharmacological effects.

References

References

[1] BENCHEQROUN, H, GHANMI, K, SATRANI, B and al. *Activité antimicrobienne des huiles essentielles d'Artemisia mesatlantica, plante endémique du Maroc*. Bulletin de la Société Royale des Sciences de Liège, 2012, 81, 4-21p.

[2] SQUIRES, J-M, FERREIRA, J-F, LINDSAY, D-S and al. *Effects of artemisinin and Artemisia extracts on Haemonchuscontortusin gerbils (Merionesunguiculatus).* Veterinary Parasitology, 2012, 15, 1152-1159p.

[3] REKKA, S, ABAZA, I, CHEBANI, S and al. *Chemical composition of the essential oil of aerial part of AHAA from Oum El bouaghi (Algeria) and chemotaxonomy survey*. DMES, 2016, 7, 12, 4383-4390p.

[4] MIDDELTON, S, HUDAIB, M, ABURJAI, T-A. *Composition of the essential oil from Artemisia herba alba grown in Jordan*. J- Essent Oil Resn, 1993, 18, 301–304p.

[5] ATTAGUI, A, MIGHRII, H, AKROUT, A and al. *Influence of drying time and process on Artemisia herba-alba Asso essential oil yield and composition*. Journal of Essential Oil Bearing Plants, 1995, 12, 8, 358–364p.

[6] SEDDIEK, S-A, ALI, M-M, KHATER, H-F and al. *Anthelmintic activity of the white wormwood Artemisia herba alba against Heterakis gallinarum infecting turkey poults*. Journal of Medicinal Plants Research, 2011, 5, 16, 3946-3957p.

[7] EL-GHAFFARI, L. *Catalogue des plantes potentielles pour la conception de tisanes*. L'organisation non gouvernementale italienne, MOVIMONDO, 2008, 11p.

[8] ABOU El-HAMD H-M, El-SAYED, M, MAGDI, A and al. *Chemical constituent and Biological Activities of AHA*. Raccords of natural products , 2010, 4, 1, 1-25p.

[9] CHAABNA, Naila. *Activité anticoccidienne des extries d'Artemisia herba alba*, Mémoire de magister : Biologie et physiologie vegetale, Setif, Valorisation des ressources vegetales, 2014, 61p.

[10] MOUFID, Abderrahmane and EDDOUKS, Mohamed. *Artemisia herbaalba : A Popular Plant with Potential Medicinal Properties*. Pakistan Journal of Biological Sciences, 2012, 15, 1152-1159p.

[11] SALIDO, Safia, VALENZUELA, Luis-R, ALTAREJOS, Joaquin and al. *Composition and intraspecific variability of AHA from southern Spain*, ELSEVIER, 2004, 32, 265-277p.

[12] FENARDJI, F, KLUR, M, FOURLON, C and al. *White Artemisia (Artemisia herba-alba).*Rev. Elev. Med. Vet. Pays Trop, 1974, 27, 2, 203-216p.

[13] DUKE, XL, Shen, M, Nielsen, M-R and al. *Advanced Glycation End-Products and the Progress of Diabetic Vascular Complications,* 1992, 15, 5, 385-389p.

[14] SEGAL, R, BREUER, A, FEUERSTEIN, I. *Irregular monoterpene alccols from Artemisia herba alba*. Phytochemistry, 1990, 19, 12, 2761-2762p.

[15] MESSAI, Laid. *Etude phytochimique d'une plante medicinale de l'Est de l'Algerie*, Mémoire de master: Chimie organique, phytochimie, 2011, 60p.

[16] BOURAOUI, J. *Pharmacognosie, phytochimie, plantes médicinales, Technique & documentation*, Lavoisier, Paris, 2003.

[17] U, Lüttge, M, Kluge, G, Bauer. *Botanique: traité fondamental* (traduction française), Ed.Tec. & doc. Lavoisier, Paris, 1992, 205-218p.

[18] N, Saleh, S, El-Nougoumy, S-M, Abd-Allah and al. *Phytochemistry*, 1994, 24, 1, 201-203p.

[19] S, KHEBRI. *Etude chimique et biologique des huiles essentielles de trois Artemisia.* Mémoire de master: chimie organique, Boumerdes, 2011, 70p.

[20] Z, Fleisher, A, Fleisher, R-B, Nachbar. *Chemovariation of Artemisia herba alba Asso*. Aromatic Plants of the Holy Land and the Sinai, Part XVI. J Essent Oil Res, 2002, 14, 156–160p.

[21] T, Dob, and T, Benabdelkader. *Chemical composition of the essential oil of Artemisia herba-alba Asso grown in Algeria,* J Essent Oil Res, 2006, 18, 685–695p.

[22] Najm, WI. *Peptic ulcer disease. Primary care*. Phytochemistry, 2011, 38, 33, 83–94p.

[23] SNAWDDEN, A, TAHRAOUI, A, El-HILALY, J and al. *Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province)*. Journal of Ethnopharmacol, *2011*, 175, 103-108p.

[24] Sandhya, S, Venkata-Ramana, K, Vinod,R-R and al. *Scope of medicinal flora as effective anti-ulcer agents* ,2013, 7, 11, 504-512p.

[25] BERROTERAN, A, PERRONE, M, *CORRENTI, M and al*. *Detection of Helicobacter pylori DNA in the oral cavity and gastroduodenal system of a Venezuelan population*. J. Med. Microbiol, 2002, 51, 764-770p.

[26] RIGAS, B. *Peptic Ulcer Disease*. ELSAVIER, 2002, 30, 27, 74-88p.

[27] Marshall, BJ and Warren, J-R. *Unidentified Curved Bacilli In The Stomach Patients With Gastritis And Peptic Ulceration*, Lancet I. 1984, 8390, 1311-3115p.

[28] MEDINA, M-L, MEDINA, M-G, MARTIN, GT. *Molecular detection of Helicobacter pylori in oral samples from patients suffering digestive pathologies*. Med. Oral Patol. Oral. Cir. Bucal, 2010, 15, 38-42p.

[29] ARES, J-J, OUTT, P. E and RANDALL, J-L. *Synthesis and biological evaluation of substituted avones asgastro protective agents* .Med. Chem. 1995, 38, 4937-4943p.

[30] MOTILVA, M, Al-YAHYA, M, TARIQ, I-A and al. *Artemisia herba-alba*, Saudi *plants*: *A phytochemical and biological Approach. College of pharmacy*, King Saud University, Riyadh, 1992, 50, 44-49p.

[31] SHAIMA, A, Majeed, Noor-A, AL-Taai, Ibrahim and al. *The effect of aqueous alcohol Artimisiaherba alba and Thymus vulagaris extract on the cryptosporidium parvum in the white mouse (BALB/C),* International journal of advaneed research, 2015, 3, 2, 31-35p.

[32] KAUR, Amandeep, SINGH, Robin, SHARMA, Ramika and al. *Peptic ulcer: A review on etiology and pathogenesis*, International research journal of pharmacy, 2012, 3, 6, 34-38p.

[33] RUPESH, Gautam and SHARMA, S. *Anti-ulcer activity of Punica granatumlinn diabetic rats*. International journal of pharmacy and pharmaceutical sciences, 2012, 4, 3, 459-461p.

[34] ODE, OJ, SANNI, Saka and G-M, Oladele. *The global relevance of traditional medicine and herbal plants, The Nigerian perspective*. International journal of applied Biology and pharmaceutical technology, 2011, 2, 4, 280-289p.

[35] Francesca, Boreeli and Angelo A, Izzo. *The plant Kingdom as a source of anti-ulcer Remedies*. Phytotherapyresearch. 2000, 14, 7, 581-591p.

[36] Mohammed, H, Farzaei, R, Rahimi, Z and al. *An evidence based review on Medicinal plants used for the treatment pf peptic ulcer in traditional Iranian Medicine*. International journal of pharmacology, 2013, 10, 13, 1-17p.

[37] M-H, Farzaei, Mohammed, Meza-Shams, Zahra, Abbasabadi and al. *Scientific evaluation of edible fruits and spices used for the treatment of peptic ulcer and traditional Iranian Medicine*. Hindawi, 2014, 12, 7, 1-12p.

[38] Baheti, J-R, Navale, S-R, Awati, S-T and al. *Anti-ulcer herbal drugs –A Review*. Deccan pharma journal series, 2010, 1, 4, 76-92p.

[39] SANDIA, A, El-ABED, D and KAMBOUCHE, N. *Les huiles essentielles.* Edition Dar el Gharb, Maroc, 2012, 200p.

[40] Lennard, J-F and Babavis, N. *Effect of different foods on the acidity of the gastric contonents in patient with duodenal ulcer*. 2000, 6, 113, 113-117p.

[41] DEBNATH, Treshna, KIM, Dahye, OU-LIM, Beong. *Natural products as a source of anti-inflammatory agentsassociated with inflammatory bowel disease. Open acces molecules* **[en ligne]**. 2013, 18, 10, 7253-7270p.

[41] DINESHKUMAR, K, Dangar, CHINTANKUMAR, J-Tank, RISHARD and al. *Natural Products in treatment of inflammatory bowel disease.* International journal for pharmaceutical research scholars, **[en ligne].** 2016, 5, 1, 208-213p.

[42] ELSAYED, A, Elsayed, EL-ENSHASY, Hesham ,WADAAN, Mohammad A-M and al. Mushooms: *A Potential natural source of anti-inflammatory compounds for medical application*. Hindawi Publishing coporation, 2014, 10, 1-15p.

[43] KOHLER, Chantal. *Les cellules sanguines*. Collége universitaire et hospitalier des histologistes embyryologistes. 2010-2011.

[44] **ITALIANI, Paola and BORASCHI, Diana.** *From monocytes to M1/M2 macrophage :phenotypical vs. Functional differenciation.* Frontiers in immunology. **[en ligne].** 2014, 5, 514p.

[45] MC-Rousselet, J-M, Vignaud, P, Hofman, f-P, Chatelet. *Inflammation et pathologie inflammatoire*, 2005, 75p.

[46] BLETRY, Olivier, KAHN, Jeane-emmanuel, SOMOGYI, Alexandre. *Immunopathologie réaction inflammatoire*. 2^{e}édition. FRANCE : Masson, 2006, 370p.

[47] BONNEVILLE, Marlène. *Physiopathologie de l'inflammation cutanée : Rôle de l'activation de l'immunitée innée cutanée dans le développement de l'eczéma allergique de contact*. **[en ligne]** Université Claude Bernard-Lyon, 2006, 1, 206p .

[48] HORN, Florian, LINDENMEIE, Gerd, MOC, Isabelle and al. *Biochimie humaine.* Paris : Flammarion, 2005, 596p.

[49] MAYOL, Katia, CAVALI, François, DAVOUST-NATAF, Nathalie. *Les médiateurs de l'inflammation*, 2013, 3p.

[50] HENROTIN, Y, DEBY-DUPONT, G, REGINSTER, J-Y. *Les médiateurs biochimiques de l'inflammation*. **[en ligne]**. Rev. Med. Liège, 2012, 56, 6, 433-442p.

[51] HELAL, Malik. *Phtalazinones et 2,3-benzodiazépinonesn dérivées de l'azélastine :synthéses et activités anti-cytokine*. chimie organique. Université louis pasteur (Strasbourg I) : 2007, 324p.

[52] ABBAL, M, ALRIC, L, CANTAGREL, A and al. *Réaction inflammatoire: aspects biologiques et cliniques*. Conduite à tenir, module 8-Item 112, 2004, 25p.

[53] ABADJIAN-A, Gérard. *Inflammation et cicatrisation*. Pathologie général, hôtel-Dieu de France faculté de médecine, 2012.

[54] GABRIELA, C, VASIL, E, DUMITRU, C and al. *L'étude de l'activité des quelques enzymes du stress oxydatif dans le tissu musculaire a des différents cyprinidés de culture*. Annale de SAUAC, 2005, 7, 1, 1-4p.

[55] ALIS, S. *Characterization of Biophysical and Metabolic Properties of Cells labeled with Super paramagnetic Iron Oxide Nanoparticles and Transfection Agent fo rCellular MR Imaging.* Annale de RSNA, 2003, 229, 3, 1-9p.

[56] BABADI, Y. *Evaluation of Iron Oxide nanoparticles effects on tissue and enzymes of Thyroid in Rats* .Annale de l'ISSN, 2013, 2, 7, 1.

[57] SHARMA, SB,NASIR, A, PRABHU, RM and al .*Hypoglycemaemic and hypolipidic effect of ethanolic extract of seeds of Eugenia Jambolana in alloxan induced diabetic rabbits, journal of ethnopharmacologie*, 2003, 85, 201-206p.

[58] SZABO, S, SANDOR, Z and al. *Vascular approach to gastro duodenal ulceration, new studies with endothelins and VEGF*.1998, 43,40-45p.

[59] GHAZOULI, K, KHENNOUF, S, AMIRA, S and al. *Effects of aqueous extract from Quercus ilex, L .Root Bark, Punica granatum L. Fruit feel and Artemisia herba alba asso leaves on ethanol-induced gastric damages in rats.* Phytotherapy research, 1999, 13, 42-45p**.**

Appendices

Appendices

Appendix 1

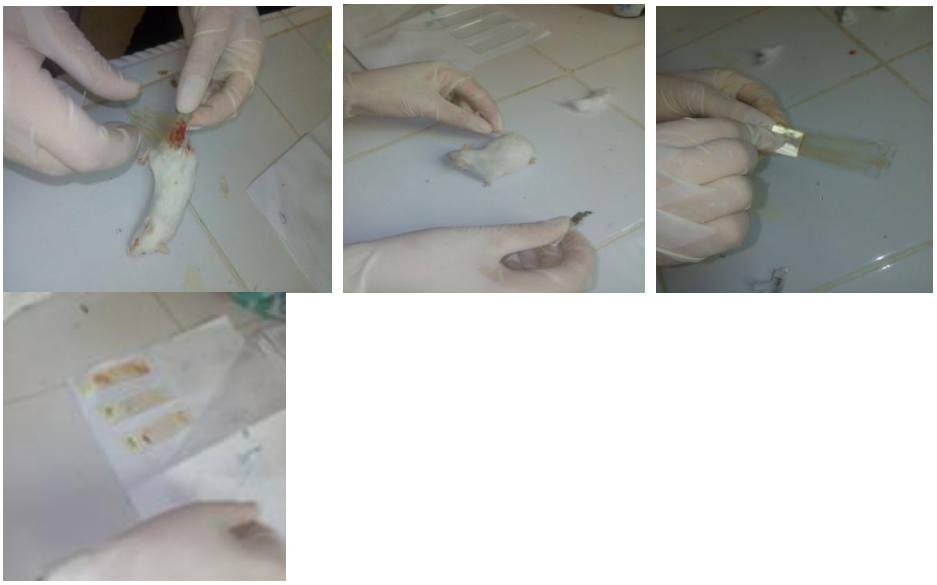

Figure: Main steps for the realization of a blood smear.

Figure: A mouse after being euthanized with chloroforme

Figure: The use of grooved probe.

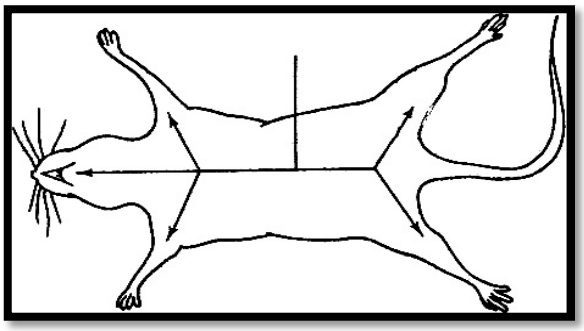

Figure: Diagram of incisions made

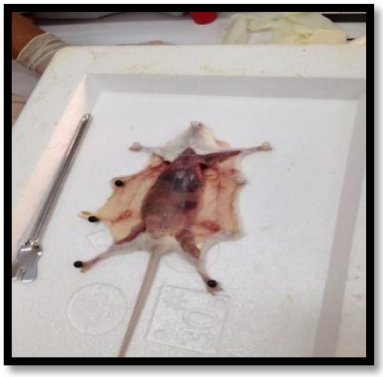

Figure: The skin is carefully removed from the abdomen.

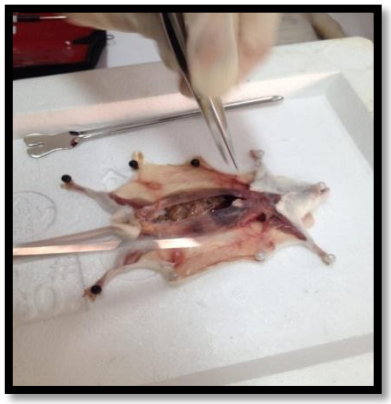

Figure: Incising the layer of abdominal muscles (thin and translucent) which covers the viscera.

Figure: Cutting the thoracic cavity

Appendix 02

TABLE 01 : Species of *Artemisia* used in Traditional Medicine(shahba& al,1994).

Plant	**Geographical area**	**Traditional use**
Artemisiaabysinica	Middle East	
Artemisiaabsynthicum	Europe,North Africa,West Asia	Anthalmintic
Arcemisiaafra	Africa	
Artemisiadracunculus	NorthAmerica	
Artemisia herba-alba	Middle East	Antidiabetic
Artemisiajudaica	NorthAfrica	Antidiabetic
Artemisiavulgaris	Europe	Appetite, stimulant, Anthelmintic Emmenagogue.

Table 02:Chemical composition of *Artemisia herba-alba* Asso. essential oil according to Rekkab and al, 2016.

N°	**Compound**	**RIa**	**%**
01	(2,3,3-Trimethyl-2-oxiranyl)methanol	927	1.0
02	Pinene	932	0.4
03	Camphene	946	2.8
04	Thuja-2,4(10)-diene	953	0.3
05	β-Pinene	974	0.1
06	Mesitylene	994	0.8
07	δ-2-Carene	996	0.4
08	1,2,3-Trimethylbenzene	1020	0.2
09	β-Phellandrene	1025	0.2
10	1,8-Cineole	1026	8.9
11	γ-Terpinene	1054	0.1
12	Terpinolene	1086	0.1
13	Filifolone	1087	2.0
14	α-thujone	1101	9.1
15	β-thujone	1112	14.2
16	Chrysanthenone	1124	16.2

17	*trans*-Pinocarveol	1135	1.3
18	Camphor	1141	15.6
19	Eucarvone	1146	0.3
20	Pinocarvone	1160	2.0
21	Borneol	1165	1.6
22	Terpinen-4-ol	1174	0.6
23	Myrtenol	1194	0.4
24	Safranal	1196	0.1
25	Shisofuran	1198	0.2
26	Verbenone	1204	1.6
27	*cis*-Ascaridol	1234	0.1
28	(E)-Ocimenone	1235	0.5
29	*trans*-Chrysanthenylacetate	1235	1.8
30	Isopiperitenone	1240	0.6
31	Bornylacetate	1284	0.4
32	Piperitenone	1340	7.6
33	Piperitenoneoxide	1366	1.3
34	Germacrene D	1484	0.1
35	Spathulenol	1577	0.1
36	Globulol	1585	0.1
37	β-Copaen-4α-ol	1590	0.1
38	Viridiflorol	1592	0.2
39	α-Cedrol	1600	0.1
40	(E)-γ-Atlantone	1706	0.1

Identified Compounds 93.6%

aRI: (retention index) measured relative to *n*-alkanes (C6-C24) on the non-polar DB5-MS column.