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كلية العلوم الطبيعية والحياة
FACULTY OF NATURAL LIFE AND SCIENCES
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# Master's Thesis

In order to obtain a diploma of an Academic Master In biological sciences

Specialty: Applied Biochemistry

## Theme

**Statistical study on the prevalence of the use of the *Ephedra alata* in the El-Oued region and Study of its protective effect on Bleomycin induced toxicity and oxidative stress in Albinos rats**



*Presented by*

**Meriem Chadou**

**Ouafa Zouari Ahmed**

**Wissal Ben Ghalia**

*Jury members*

| | | |
|---|---|---|
| **President : Dr . TLILI Mohammed Laid** | **MCA** | **El-Oued University** |
| **Examiner : Dr . RAMDANE Farah** | **MCA** | **El-Oued University** |
| **Supervisor : Pr . DEROUICHE Samir** | **Prof** | **El-Oued University** |

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# إهداء

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ ﴿وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ﴾ (التوبة:105)

الحمد لله الذي ما تم جُهد ولا خُتم سعي الا بِفضله، بفضلٍ من الله وتوفيقه، نجحت اليوم اللهم ليس بجدي واجتهادي إنما بكرمك وفضلك ومنتك علي، نجحت و تخرجت بفضل الله ثم دعاء الوالدين، شكرا لكل من كان عوناً لي بعد الله.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ ﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ﴾ (المجادلة: 11)

أبتدئ بشكر **المولى عز وجل** الذي رزقني العقل وحسن التوكل عليه

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أهديكم هذا العمل المتواضع

**وفاء**

# إهداء

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ ﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ﴾ (المجادلة: 11)

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## Abstract

The objective of this work was to do a statistical study on the prevalence of the use of the *Ephedra alata* in the El-Oued region and study the effect of aqueous extract of *Ephedra alata* L. for protection of toxicity induced by Bleomycin in rats. For statistical study, 52 persons interviewed were chosen; some of the informations related to the interrogators and others about the plant were adopted in the survry. For *in-vitro* study, qualitative and quantitative phytochemical compounds and biological activities were assess on the *Ephedra alata* L aqueous extract. For *in-vivo* study, Twenty female albino Wistar rats. were divided into 4 groups (n = 5); Control group, *E. alata* group (AEEA), Bleomycin group (BLM) and rats co-treated with BLM and AEEA group ( BLM+ AEEA). BLM induced toxicity using intraperitoneally single dose 10 U/kg. *E. alata* treated orally at a dose 200 mg/kg/day for 30 days. Various hematological, biochemical, and oxidative stress markers were estimated. Histopathology of liver, lungs and kidneys tissues was observed. The results obtained show that in traditional medicine, men and people between the ages of 31 and 60 years are more interested in using *Ephedra alata*. The aerial part of the plant is mostly used by decoction method to treat cancerous diseases and diabetes. Results of *in-vitro* phytochemical results revealed that *E. alata* contains most of active compounds, especially flavonoids of various kinds with high antioxidant and anti-inflammatory activity. Results of *in-vivo* rats study show that BLM treated rats induced alteration in Hematological and biochemical parameters compared to control group. Also BLM treated rats induced oxidative stress and histological alteration in lungs cells, liver and kidney compared to control rats. Co-treatment of BLM with AEEA were partially reversed all of previous parameters, Without any negative effect for the use of the plant in healthy rats. This study indicated that the anti-inflammatory and antioxidant property of leaves aqueous extract of *Ephedra alata* allowed to use them to protect organs from the side effects of drugs or from the destructive effects of various diseases. Other studies are necessary to delve into these findings and to determine the factors responsible for the beneficial effects of the plant.


**Kywords**: *Ephedra alata* L, Bloemycin, side effect , lungs, polyphenols, oxidative stress, Wistar rats.

**ملخص**

الهدف من هذا العمل هو إجراء دراسة إحصائية حول الاستعمالات الشعبية لنبتة العلندة في منطقة الوادي ودراسة تأثير المستخلص المائي لهذه النبتة في حماية سمية دواء البليوميسين عند الجرذان. بالنسبة للدراسة الإحصائية تم توزيع استبيان على 52 شخصا موزعين على مختلف مناطق الوادي. في هذا الاستبيان تم الاعتماد على بعض المعلومات المتعلقة بالأشخاص وأخرى متعلقة بالنبتة واستعمالاتها. اما بالنسبة للدراسة البيولوجية فقد تم تحديد بعض المركبات الكيميائية من اجل التحاليل النوعية والكمية ودراسة بعض الانشطة للنبتة المدروسة في المختبر، من جهة اخرى قمنا بدراسة التاثير العلاجي لنبات العلندة حيث تم استعمال عشرون أنثى من جرذان ويستار تم تقسيمها إلى 4 مجموعات (5 جرذان في كل مجموعة): المجموعة الشاهدة و مجموعة النبتة و مجموعة دواء البليوميسين واخيرا مجموعة الدواء المعالجة بالنبتة. بالنسبة للدواء فقد تم حقنه للجرذان بجرعة واحدة (10 وحدة/كغ) داخل الصفاق. اما نبتة العلندة فقط اعطيت للجرذان عن طريق الفم بجرعات يومية (200 ملغ / كغ ) لمدة 30 يومًا .تم اختيار بعض المعايير البيوكيميائية ومعايير الدم وكذلك معايير الاجهاد التأكسدي وايضا اعتماد بعض المقاطع النسيجية لأنسجة من الكبد والرئتين والكلى. النتائج التي تم الحصول عليها من خلال الدراسة الاحصائية وجدنا ان الرجال اكثر استعمالا للنبتة من النساء وايضا الفئة الاكثر استخداما للنبتة في العلاج هم الأشخاص الذين تتراوح أعمارهم بين 31 و 60 عامًا. كما ان الجزء الهوائي من النبات هو المستخدم في العلاج وان اغلب الاجابات في الاستبيان بينت ان النبتة تحضر بطريقة الغلي وان اغلب الامراض المعالجة بالنبتة في المنطقة هي لعلاج السرطان وامراض السكري وامراض الجهاز التنفسي. من جهة اخرى، أظهرت النتائج في المختبر ان النبتة تحتوي على معظم المركبات النشطة، وخاصة مركبات الفينولات و الفلافونويدات كما تميزت بأنشطة بيولوجية عالية كمضادات الأكسدة ومضادات الالتهابات. النتائج على الجرذان اظهرت ان الجرعة المحقونة من دواء البليوميسين تسبب في احداث تغييرات مهمة في معايير الدم و وبعض المعايير البيوكيميائية مقارنة بالمجموعة الشاهدة. كما ان البليوميسين احدث اجهاد تاكسدي و خلل في انسجة كل من الرئتين والكبد والكلى مقارنة بأنسجة الجرذان الشاهدة. النتائج المتحصل عليها اظهرت ان العلاج بالمستخلص المائي لنبات العلندة حسن بشكل معتبر اغلب الاضطرابات السابقة مع عدم وجود أي مؤشرات سلبية لاستعمال النبتة عند الجرذان السليمة. في الختام من خلال هذه الدراسة تبين ان لنبتة العلندة خاصية مضادة للالتهابات ومضادة للأكسدة وايضا يمكن استعمالها لحماية الجهاز التنفسي من الاثار الجانبية للأدوية .كما ان هناك حاجة لدراسات أخرى للتعمق في هذه النتائج وتحديد العوامل المسؤولة عن الآثار المفيدة للنبات.

**الكلمات المفتاحية:** نبات العلندة، البليوميسين، الاثار الجانبية، البوليفينول، الرئتين، الاجهاد التأكسدي، فئران ويستار.

# ABBREVIATIONS LIST

**ABC:** ATP-binding cassette (ABC) family
**Abs:** Absorbance.
**AEEA:** Aqueous extraction *Ephedra alata*.
**AGPIOO - :** Lipid peroxidation by repairing the pyroxyl radical.
**AGPTOOH:** Hydro peroxide.
**AKt:** Proteine kinase B.
**ALP:** Alkaline phosphatase.
**ALT:** Alanine aminotransferase.
**AST:** Aspartate aminotransferase.
**ARF:** Acute renal failure.
**ATP:** Adenosine triphosphate.
**$AlCl_3$:** Aluminum chloride.
**A1:** Absorbance 1.
**A2 :** Absorbance 2.
**Ax10:** control group x10.
**A x 40:** control group x40.
**BHA:** Butylated hydroxyl anisole.
**BHT:** Butylated hydroxyrotoluene.
**BLM:** Bleomycin.
**Bleomycin-Cu (I):** complex bleomycin-Copper(I).
**BSA:** Bovine serum albumin.
**Bx10:** Ephedra group x10.
**Bx40:** Ephedra group x40.
**CBC:** Complete Blood Count.
**CAT:** Catalase.
**$CH_3COONa$:** Sodium acetate.
**$Cl^-$:** Chlorine.
**CNS:** Center nervous system.
**Co(III):** carbon monoxide III.
**Cu(I):** Copper.
**Cu-sod:** Copper- Superoxide dismutase.
**P450:** cytochrome p450.
**C4′:** Carbon atom.
**Cx10:** Belomycin group x10.
**Cx40:** Belomycin group x40.
**DCs:** dendritic cells.
**DNA:** Deoxyribonucleic Acid.
**DPPH:** 2,2-diphenyl-1-picrylhydrazyl.
**DTNB:** 5,5'-dithiobis-2 nitro benzoic acid.
**Duox :** 1–5, dual oxidases
**D x10:** (Belomycin+*Ephedra*) group x10.
**Dx40:** (Belomycin+*Ephedra*) group x40
**E :** *Ephedra*
**$e^-$:** Electron
**EDTA:** Ethylenediamine tetraacetic acid.
**EOR:** Reactive oxygene Species.
**Etc:** Etcetera
**ETC:** Electron transport chain
**ER:** Endoplasmic reticulum
**FC:** Ferric chloride.
**Fe(II):** Fer(II).
**Fe(III):** Fer(III).
**Fe(IV):** Fer(IV).
**$Fe^{+3}$:** Ferric ion.
**$Fecl_3$:** Ferric chloride.
**FOXO1:** Forkhead box protein O1.
**FRAP:** Ferric-tripyridyltriazine.
**GSH:** Glutathione.
**GSH-Px :** Glutathione peroxidases.
**GSK-3β:** Glycogen synthase kinase-3 beta.
**GST:** Glutathione S-transferase.
**GSSG:** Glutathione disulfide.
**GR:** glutathione reductase.
**GPx:** Glutathione peroxidase.
**HCL:** Hydrogen chloride.
**HCT:** Hematocrit.
**HGB:** Hemoglobin.
**$H_2N$-OC:** Molecule exhibits tautomerism.
**$H_2O_2$ :** Hydrogen peroxide.
**$HO^{2\cdot}$:** Hydroperoxyl.
**$HOCl^-$ :** Hypochlorous Acid.
**HOONO:** Superoxide anion peroxynitrite.
**$H_2SO_4$:** Sulfuric acid.
**$H_3PO_4$:** Phosphoric acid.
**IC50:** Half-maximal inhibitory concentration 50 %.
**IL-1B:** Interleukin-1b.
**IL-8:** Interleukin-8.
**IL 10:** Interleukin-10.
**IP:** Inhibition percentage.
**LDH:** Lactate dehydrogenase.
**LOOH:** Lipid hydroperoxides.
**MCH:** Mean corpuscular Hemoglobin.
**MCHC:** Mean Corpuscular Hemoglobin Concentration.
**MCV:** Mean corpuscular volume.
**MDA:** Methylene dioxyamphetamine.
**Mn(II):** Manganese(II)
**MnSod:** Manganese superoxide dismutase.
**mTOR:** mechanistic target of rapamycin.
**M ± SD:** Mean ± standard deviation.
**Nacl:** sodium cloride.
**NAD:** Nicotinamide adénine dinucléotide.
**$NH_2$:** Nitrenium ion.

| | |
|---|---|
| **NADP:** Nicotinamide adénine dinucléotide phosphate. | **RBC:** Red blood cell count . |
| **Ni(III)**: Nickel (III) oxide. | **RH:** Rhesus. |
| **NO:** nitric oxide. | **RNA:** Ribonucleic acid. |
| **$NO^{2-}$ :** Nitrogen dioxide. | **$RNH_2$:** primary amine. |
| **$N_2O_3$:** Dinitrogen trioxide. | **RNHCL:** Hypochlorous acid and simple chloramines. |
| **$NO_2CO_3$:** Sodium Carbonate. | **RNS:** Reactive Nitrogen Species. |
| **NOS1-3**: Nitric oxide synthases isoforms 1–3. | **ROS:** Reactive Oxygen Species . |
| **$O^{\cdot-}$:** Superoxide radical. | **Se - OH:** Selenoles- peroxide. |
| **$O^{2-}$:** Oxygen radical. | **$SQ^{\circ-}$ :** Radicals (Square Roots). |
| **OH·:** Hydroxyl radical. | **SOD:** Superoxide dismutase. |
| **8-OH-Dg:** 8-hydroxy-2'-deoxyguanosine. | **TBA:** Thiobarbituric acid . |
| **OXL:** Pathogen facteur. | **TBS:** Saline buffer. |
| **P38:** Mitogen-activated protein kinase. | **TBARS:** Thiobarbituric acid reactive substances . |
| **1xpbs:** Phosphate Buffered Saline. | **TCA:** Trichloroacetic acid. |
| **Pgp:** P-glycoprotein. | **TNFα:** Tumor necrosis factor-α . |
| **Ph:** Potential hydrogen. | **UI:** Unité internationale. |
| **PI3K:** Phosphoinositide 3-kinase. | **USA:** United States of America. |
| **PGN:** Peptidoglycan. | **UV:** Ultra-violet. |
| **PKA:** protein kinase A. | **WBC:** White blood cell. |
| **PNS:** peripheral nervous system | **Zn(II):** Zinc(II). |
| **PUFAs:** polyunsaturated fatty acids. | **Zn-Sod:** Zinc- Superoxide dismutase. |
| **$R^2$:** R squared | |

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# SUMMARY

# Introduction

## Introduction

The general term "Cancer" applies to a large group of diseases that can affect any part of the body we also speak of abnormal multiplication of cells. These cells have lost their normal control mechanisms and are therefore in able to multiply continuously, invade nearby tissues, migrate to distant parts of the body and promote the growth of new blood vessels from which they draw the nutrients (Hamour et Ferrah, 2021) Cancer is an abnormal growth of cells caused by multiple changes in gene expression leading to dysregulated balance of cell proliferation and cell death and ultimately evolving into a population of cells that can invade tissues and metastasize to distant sites, causing significant morbidity and, if untreated, death of the host (Ruddon, 2007). Cancer chemotherapy emerged as a means for treating systemic disease in the 1960s. Prior to this time, the primary treatment for cancer involved surgery and radiation. Neither therapeutic modality was designed to treat the problem of systemic diseases due to metastasis. This limitation ultimately became the rationale for a new therapeutic approach to deal with the systemic nature of this disease، eventually involving the use of cytotoxic drugs (Crawford, 2013). Bleomycin is An antibiotic agent with antitumor activity, Bleomycin exerts its antitumor effect by inducing tumor cell death, while inhibition of tumor angiogenesis may also be important (Sleijfer, 2001). Subacute and chronic interstitial pneumonitis is a serious, life-threatening side effect of bleomycin, which has been described in up to 10% of patients receiving the drug (Lyman *et al*., 2007; Reinert *et al*., 2013; Viala, 2021). The toxicity with bleomycin is initiated by direct oxidative damage, which then leads to subsequent inflammation and fibrosis mediated by generation of both extracellular ROS and intracellular ROS (Allawzi et al., 2019). Oxidative stress is an important factor causing metabolic and physiological alterations and various diseases in the body (Derouiche *et al*., 2022).

Medicinal plants are receiving much attention nowadays to meet the public concern of replacing synthetic medicine with those from natural origins, The growing problem of the appearance of new diseases rises up the necessity for finding replacements from natural origins (Ghanem et El-Magly, 2008). Medicinal plants have always been important in many fields worldwide and certain contain various types of bioactive compounds with multiple therapeutics effects (Saidi *et al*., 2022). One such herb is *Ephedra* is a genus of non-flowering seed plants belonging to the Ephedraceae family (Hadjadj *et al*., 2020), which includes approximately 69 species, mainly in the desert areas of Asia, America, Europe and North Africa. Among these species, 15 ones and four varieties can be found in China (González-Juárez *et al*., 2020). *E. alata* is used in traditional medicine as a stimulant, antifungal and to treat allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, and headaches (Soumaya *et al*., 2020),to treat kidney, as well as used for treatment of cancer also the plant stems are chewed for treatment of bacterial and fungal infections (Jaradat *et al*., 2015). Moreover, *Ephedra* can also be used to treat COVID-19 infections to improve the symptoms (Tang *et al*., 2023). The medicinal value of plants lies in some chemical active substances that produce define physiological action on the human body (Yadav *et al*., 2017). The objective of the present work is the *in-vitro* evaluation of the phytochemical and biological activities of *Ephedra alata* aqueous extract and *in-vivo* protective action against Bleomycin chemotherapy side effect in rats.

# first part

## Bibliographic synthesis

# Chapter I

## Ephedra alata

## and

## Oxidative stress

# 1. *Ephedra alata* alenda

## 1.1. Gender *Ephedra*

The origin of *Ephedra* has sometimes been considered to be ancient, possibly as early as or prior to the breakup of Pangaea (ca. 230 Myr ago in the Middle Triassic) on the basis of the early occurrence of "ephedroid" pollen fossils (Huang et Price, 2003).

*Ephedra* is a medicinal plant belonging to the Ephedraceae family (Al-Rimawi *et al*., 2017), It is a species of *Ephedra* that grows mostly in the desert. genus *Ephedra* contains more than 60 species of non flowering seed plants distributed throughout Asia, America, Europe, and North Africa (Chouitah, 2019; Poli *et al*., 2018), including *Ephedra alata*, *Ephedra Lristanica*, *Ephedra Sarcocarpa*, *Ephedra strobiliacea*, *Ephedra procera*, and *Ephedra pachyclada* (Rustaiyan *et al*., 2011). is low broom-like shrubs or small trees. The leaves are very reduced, usually limited to opposite-decussate scales. Most are dioecious, with the male (stamens) and female (ovules) organs borne in small cones (Philippe, 2001).

This species has a reputation for high tolerance to water deficiency in the Saharan regions, It also presents an extremely powerful system of lateral roots which is able to extend over several dozens of meters (Derbel *et al*., 2010).

*Ephedra* species have a long history in traditional Chinese medicine (approximately 5000 years), with uses in the treatment of allergies, nasal congestion, bronchial asthma, coughs, and flu (Danciu *et al*., 2018).



### 1.1.1. Subspecies *Ephedra alata* alenda

#### 1.1.1.1. Botanical description

It is one of the rare shrubs in the saharan zones (Figure 01), This species, which is renowned for its high tolerance to water deficiency in the Saharan regions (Djenidi *et al*., 2022; Kebili, 2016). *Ephedra alata* is light green densely branched dioecious small and perennial stiff shrub, about 50-100 cm tall, the twigs appear leafless and the leaves reduced to small scales, cones sessile shaped, clustered in the axils or at branch tips (Abdel-Kader *et al*., 2003).

At the ecological level, the plant is an excellent species for fixing aeolian sand (Abdallah et Chaieb, 2007), as this is an excellent wind sandal. However, the quality of its charcoal, prized by the people of the Sahara, suggests that it is in danger of deforestation (Djenidi *et al*., 2022).

**Figure 01:** *Ephedra alata* L. (original photo)

### 1.1.1.2. Taxonomical Classification

According to Bell and Bachman. 2011, and the catalog of Dobignard and Chatelain 2010, the systematic position of *Ephedra alata* is as follows (table 01):

**Table 01:** Classification of *Ephedra alata*

| Taxonomical Rank | Taxon |
|---|---|
| Kingdom: | Plantae |
| Phylum: | Spermaphytes |
| Subphylum: | Gymnosperms |
| Class: | Gnetopsida |
| Order: | Ephedrales |
| Family: | Ephedraceae |
| Kind: | *Ephedra* |
| Species: | *E. alata* |
| Subspecies(Common name): | alenda |



### 1.1.1.3. Origin and geographical distribution

*Ephedra* (Ephedraceae) is a genus of gymnosperms of about 69 species widely distributed in temperate and subtropical regions of the world, except southern Africa and Australia. Thus, its species are widely distributed on the Eurasian continent, in North Africa and in North and South America. They grow in open and arid habitats, such as deserts and rocky slopes. Although similar suitable habitats are widespread in southern Africa and Australia, no extant species occur in these areas and no well-authenticated fossils are known (Huang *et al*., 2003; González-Juárez *et al*., 2020).

- Africa: Algeria; Egypt, Libya, Morocco, Tunisia, Mauritania, Chad and Mali;

- Asia: Saudi Arabia, Iraq, Iran, Palestine, Lebanon, Jordan and Syria. It grows mainly in northern and western China, northern India;
- America: in the United States. Ephedra grows along the Rocky Mountains (Rashed, 2021; Al-Snafi, 2017).

In Algeria, *E. alata* is found in the northern and western Sahara at the level of sandy soils, regs and the sandy beds of wadis. It is even encountered in the sand of the tropical level and the Hamada of Tinghert. This plant is an excellent sand-fixing species. However, the quality of its charcoal, appreciated by the Saharan populations, exposes it to the phenomenon of deforestation (Figure. 2) (Ozenda, 1991; Hedhoud et Madoui, 2022).

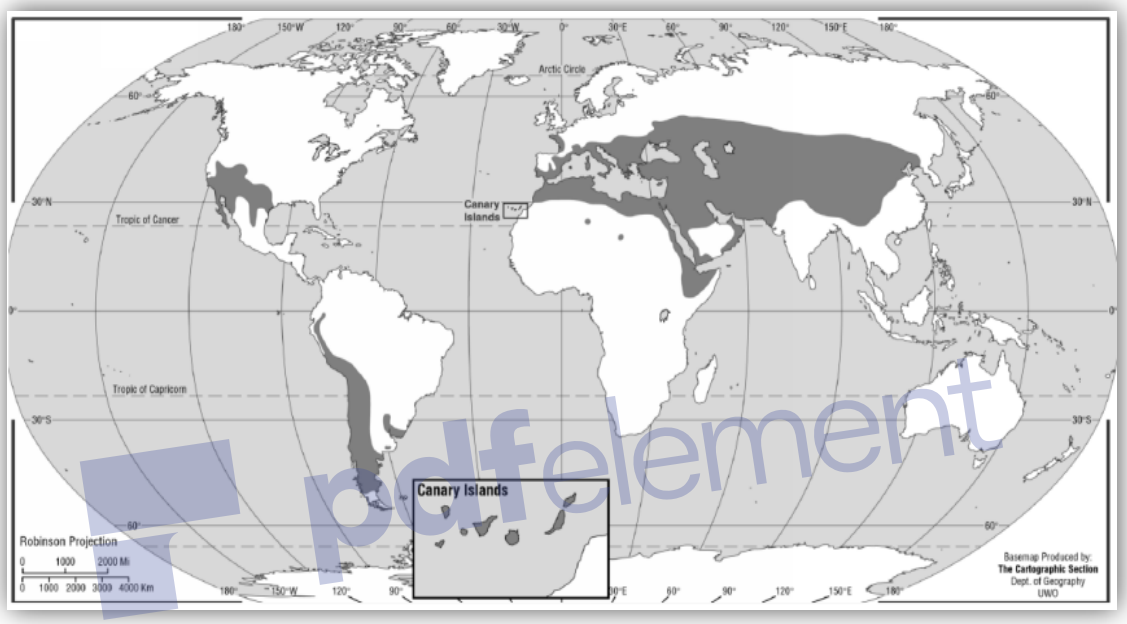



**Figure 02 :** Geographical distribution of *Ephedra* in the world (kabili, 2016)

#### 1.1.1.4. Chemical constituents

The phytochemical screening showed that the *Ephedra alata* plant extract contain a mixture of phytochemicals as cardiac glycosides, reducing sugars, flavonoids, phenolic compounds and alkaloids (Jaradat *et al*., 2015). *Ephedra* species contain alkaloids ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, and methyl pseudoephedrine. Beside the E-type alkaloids, ephedroxane, and macrocyclic spermidines called ephedradine A-D, which isolated from some Eurasian *Ephedra* species (Al-Snafi, 2017).

#### 1.1.1.5. Pharmacological effects

*Ephedra alata* alenda stem contains ephedrine which is the main alkaloid of this plant (40 to 90% of the total alkaloids) and whose content varies according to the species (0.5 to 2%). Ephedrine is accompanied by pseudoephedrine and the corresponding nor and N,N-dimethyl derivatives (Boubekri *et al*., 2020), which are used for the treatment of certain serious illnesses: cancer; inflammatory diseases, asthma, antifungal, and antimicrobial…. etc (Ghanem et El-Magly, 2008), also The pharmacological effect of the different *Ephedra* species depends on the

phytoconstituents of each one. As shown in general studies, the *Ephedra* species were characterized by the alkaloids and phenolic compounds content, such as trans-cinnamic acid, catechin, epicatechin, symplocoside, and flavonol-3-O-glycosides, and proanthocyanidins (Jaradat *et al*., 2021). The stem and leaves (which also contain of pseudoephedrine) are used for medical purposes. The synthesis of the pseudoephedrine will follow. Due to their molecular structure, these sympathomimetic amines act by stimulating the system of adrenergic receptors at the junction between sympathetic fiber and smooth muscle of the vascular walls. They thus simulate the vasoconstrictor action of norepinephrine physiologically produced by nerve fiber (Laccourreye *et al*., 2015). This plant is widely used for the treatment of cardiovascular diseases, hypotension, colds, diabetes mellitus, and cancer. In addition, it is also used for the treatment other respiratory diseases (Ben guedouad et Taleb, 2021).

This is due to bioactivities of *E. alata*, which is represented in:

- **Antioxidant effect**: *E. alata* pulp could be a source of natural antioxidant against free radicals. In fact, the differences between *E. alata* and other species might be attributed to their biomolecule content (Mufti *et al*., 2023).
- **Anti-cancer effect:** Some species of ephedra have been attributed anticancer potential against various cell lines (Danciu *et al*., 2018). In an ethnopharmacological study in Palestine, *E. alata* was themost used phytomedicine for the treatment of cancer (Jaradat et al., 2016).
- **Hypoglycemic effect:** Alcoholic extract of *E. alata* exerted hypoglycemia, one hour after administration to fasting rats. The same extract failed to reduce blood glucose levels in alloxanized rats compared to the positive control, glibenclamide (Rashed, 2021).
- **Antimicrobial effects:** *E. alata* has been shown to have high antiviral activity against HSV (Soltan et Zaki, 2009). Work done by Egyptians have shown that the aqueous extract of this plant which collected from Egypt has significant inhibition potential *in vitro* and *in vivo* against the growth and production of aflatoxins by *Aspergillus flavus* (Al-Qarawi *et al*., 2011; Chouikh, 2020).

The activity of different extracts of *E. alata* stem was investigated against yeast and fungi. Four fungi, *Aspergillus fumigatus, Penicillium italicum Syncephalastrum racemosum,* and *Candida albicans* were used as test microorganisms. Acetonitrile extracts exhibited the most potent antimicrobial effect with a broad spectral range. Thin layer chromatographic separation of active constituents in acetonitrile extracts revealed the presence of seven fractions. All fractions showed antimicrobial activities with four fractions having a potent inhibitory effect (Al-Snai, 2019).

- **Anti-inflammatory effect :** The percentages of inhibition of edema in the mice of the batches treated with *E. alata* at the dose of (300 mg/kg) show that both treatments possess anti-inflammatory activity important. After 4 hours, *E. alata* show a percentage inhibition of no significant and significant respectively when comparing with the effect of the drug (Samir et Benasam, 2018).
- **Diuretic effect:** It was reported that alkaloids from *Ephedra* stem have the function of clearing and regulating the distribution and excretion of water *in vivo* to exert the diuretic and antioncotic effects, and D-pseudoephedrine shows the strongest pharmacological

activity among all the alkaloids. Experiment results showed that urine volume can be extended to two to five times that of pre-dose when anesthetized dogs are intravenously injected with D-pseudoephedrine (0.5–1.0 $mg \cdot kg^{-1}$), and the pharmacological time of single administration canreach 30–60 min (Rashed, 2021).

- **Analgesic and Hypolipidaemic Effects:** The results of the protective effect of hyperlipidaemia caused by Triton X-100 showed that there are noticeable changes in the biochemical and oxidative stress parameters. The treatment with the methanol extract of *E. alata* normalized the biochemical parameters and the stress state. The histological study confirmed the biochemical results. The values obtained for the methanolic extract of *E. alata* regarding the analgesic activity are similar to those obtained for indomethacin (Chouikh, 2020).
- **Effect on body mass:** Mixture of *Ephedra* and guarana effectively promotes short-term (8 weeks) weight loss in overweight subjects. Such an effect was mainly attributed to an increase in the sympathomimetic tone resulting in increased lipolysis and glycogenolysis, with sympathetic stimulation of the central satiety center leading to appetite suppression (Boozer, 2001).
- **Effect on blood pressure:** Chinese beliefs claim that the aerial and underground part of *Ephedra* have opposite effects. This has been confirmed, for the action on blood pressure, by tests on animals. A polyphenol called Ephedrannine a isolated from the roots of the plant as well as a minor type of alkaloids in the plant isolated from its roots, named Ephedradine, exhibited a hypotensive effect. On the other hand, ephedrine has a hypertensive action (Kebili, 2016).

#### 1.1.1.6. Toxicological effects

Tachycardia, hypertension, hyperperspiration, bronchodilation, agitation, and mydriasis can all be symptoms of this. *Ephedra* use has also been linked to gastrointestinal and psychological side effects (Anteur et Rezkallah, 2022). Results add further weight to the concerns that the chronic ingestion of ephedra can potentially increase the risk of cardiovascular events, including myocardial ischaemia, stroke, cardiomyopathy and dysrhythmias Studies have reported that acute treatment with ephedra in healthy volunteers could lead to an increase in HR without the induction of cardiac arrhythmias . also found there were no cardiac arrhythmias induced after taking ephedra among them our subjects during the examination. Despite the induction of cardiac arrhythmias not being noted, there are significant effects on it autonomic nervous modulation after ingestion of ephedra in this study. found that the autonomic nervous activity was chronically changed after ingestion of multiple-dose of ephedra, although the baseline HR was not affected. Since the status of the autonomic nervous system, although often ignored by clinicians, is a major one determinant of cardiovascular health and prognosis, the present results should remind physicians of the potential acute and chronic effects of *Ephedra* on autonomic nervous modulation (Chen *et al*., 2010). many events adverse cardiovascular and cerebrovascular events have been associated with the use of dietary supplements containing ephedra alkaloids (Herem et Saadaoui, 2022).

## 2. Oxidative stress

### 2.1. Definition

Oxidative stress is defined as a severe imbalance in the balance between production of radioactive free and anti-oxidant defenses, leading to graves damage to cellular structure and metabolism degrade many targets such as : proteins, lipids and nucleic acids (McCord, 2000).

Reactive Oxygen Species (ROS) can play, and in fact they do it, several physiological roles (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism; despite this, environmental stressors (i.e., UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics (i.e., antiblastic drugs) contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage (oxidative stress) (Pizzino *et al*., 2017).

Oxidative stress is reported to associate with the development of several metabolic, chronic disorders or cancers (Finkel et Holbrook, 2000; Reuter *et al*., 2010; Aminjan *et al*., 2019).

Cells deploy an antioxidant defensive system to protect themselves (Reuter *et al*.,2010).

### 2.2. Free Radicals

#### 2.2.1. Definition of Free Radicals

A free radical can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence. Thus the attacked molecule loses its electron and becomes a free radical itself, beginning a chain reaction cascade which finally damages the living cell (Phaniendra *et al*., 2015).

Reactive Oxygen Species (ROS) are highly reactive molecules that include free radicals, such as $O2^{\cdot-}$ and hydroxyl ($^{\cdot}OH$), which have one or more unpaired electrons, as well as compounds such as $H_2O_2$ that are not free radicals. $O2^{\cdot-}$ is produced by single-electron reduction of molecular oxygen, and it undergoes dismutation by superoxide dismutase (SOD) to generate $H_2O_2$ which is usually broken down by catalase or glutathione peroxidase to $H_2O$. The single-electron reduction of $H_2O_2$ results in the formation of highly reactive and potent hydroxyl radical ($^{\cdot}OH$), via the Fenton or the metal catalysed Haber-weiss reactions. Superoxide may react with local $NO^{\cdot}$ to generate peroxy nitrite ($ONOO^{\cdot}$) which is itself a potent ROS. In contrast, $H_2O_2$ can diffuse across cell membranes and therefore has the potential to act at more distant sites (Mishra et Samanta, 2012).

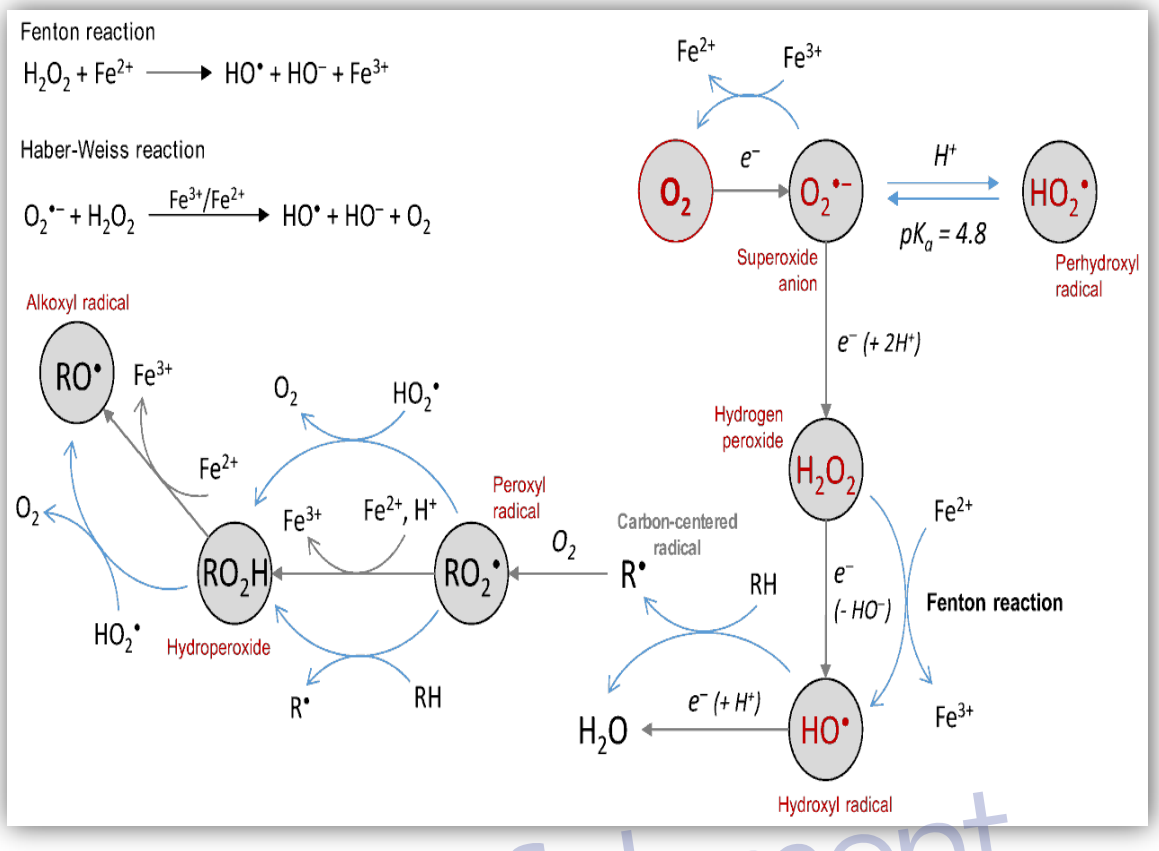



**Figure 03**: Mechanism of reactive oxygen species (ROS) production (Collin, 2019)

### 2.2.2. Roles of free radicals

The role is very complex because they can have a physiological role or a toxic effect depending on their concentration. Reactive oxygen species physiologically, ROS exist in cells and tissues at low but measurable concentrations. When they are produced in a specific cellular compartment, they can participate in the functioning of certain enzymes, intervene in the immune defense, act as a second cellular messenger, intervene in signal transduction pathways and regulate cellular functions (Dikalov *et al*., 2007) and the phenomenon of apoptosis or activate transcription factors (Haleng *et al*., 2007).

Free radical nitric oxide or $NO^{\bullet}$ is an important compound; it is notably synthesized by endothelial cells via the action of NO synthases on L-arginine. It is a very diffusible labile molecule, whose regulatory effects are exerted on most of the physiological functions of the organism (maintenance of vascular tone, neurotransmission, renal functioning) (Hare, 2004). However, $NO^{\bullet}$ can form with the superoxide anion peroxynitrite (HOONO), a powerful and diffusible oxidant, capable of damaging many organic molecules.

Most importantly, the excess ROS can damage the integrity of various biomolecules including lipids, proteins and DNA leading to increased oxidative stress in various human diseases (Phaniendra *et al*., 2015).

### 2.2.3. Sources of free radicals

**A. Endogenous sources of ROS**

The main endogenous sites of cellular redox-reactive species generation-including ROS and reactive nitrogen species (RNS) comprise mitochondrial electron transport chain (ETC), endoplasmic reticulum (ER), peroxisomes, membrane-bound NADPH oxidase (NOX) isoforms 1–5, dual oxidases (Duox) 1 and Xanthine oxidase and 2 complexes, and nitric oxide synthases isoforms 1–5 (NOS1–3). The complexes I and III of mitochondrial ETC produces superoxide anion (Sharifi-Rad *et al*., 2020).

- **Mitochondria**

The mitochondrial ETC is considered to be the primary endogenous source of ROS but other internal sources are also present. Other sources of ROS, primarily $H_2O_2$, are microsomes and peroxisomes (Sharifi-Rad *et al*., 2020). The consequences of this mitochondrial activity are double and paradoxical. On the one hand, the mitochondria provide the cell with an important source of energy since 36 high-potential ATP molecules energy are generated during the reduction oxygen. On the other hand, under the conditions physiological, about 0.4 to 4% of electrons escape, react directly with oxygen (Haleng *et al*., 2007) to form superoxide. The superoxide dismutase family of enzymes is comprised of MnSOD located in the mitochondrial matrix, and Cu, ZnSOD located in the mitochondrial intermembrane space, cytosol and extracellular space. These key enzymes catalyze the dismutation (disproportionation) of superoxide anion radical to hydrogen peroxide and molecular oxygen. In doing so, they protect cells against oxidative damage and regulate the cellular concentration of $O_2^{\cdot-}$ and its reactive progeny under both physiological and pathological conditions. Increased understanding of the role of mitochondria and its enzyme, MnSOD, has motivated researchers to explore existing and new compounds for their ability to enter mitochondria and mimic MnSOD. This compounds that reportedly possess fair SOD-like properties (are catalysts of $O_2^{\cdot-}$ dismutation), or are stoichiometric scavengers of $O_2^{\cdot-}$, and for which evidence exists that they are efficacious in attenuating mitochondrial dysfunction (Miriyala *et al*., 2012).

**B. Exogenous sources**

Environmental triggers, such as exposure to cigarette smoke, UV radiation, heavy metal ions, ozone, allergens, drugs or toxins, pollutants, pesticides, or insecticides, may all contribute to the increase of ROS production in cells (Mehdi Sharifi, 2020).

### 2.3. Biological targets of oxidative stress

✓ **Nucleic acids**

Deoxyribonucleic acid (DNA) is a prime target for AOEs. Guanine, for example, can react with •OH to form 8-hydroxy-2'-deoxyguanosine (8-OH-dG) which, instead of pairing with cytosine, will associate with adenine, resulting in mutations within the DNA and leading to alterations of the genetic message involved in the onset of cancer and aging (Haleng *et al*., 2007).

✓ **Proteins and amino acids**

Oxidative damage to proteins EORs are indeed able to react with different acids amino chains of proteins, also altering their function. The most sensitive to their action are the amino acids aromatics such as tryptophan, tyrosine, histidine, on which the $OH^{\bullet}$ radical is added, modifying the conformation of the protein. On amino acids containing a sulfur atom such as cysteine and methionine, oxidation by free radicals leads to the formation of bridges disulphides, therefore to the aggregation of several protein molecules (Koechlin-Ramonatxo, 2006).

✓ **Lipids**

The hydroxyl radical is capable of tearing a hydrogen on the carbons located between two double bonds of polyunsaturated fatty acids (PUFA): this is the initiation phase. The radical lipid reacts with an oxygen molecule to form a peroxyl radical ($ROO^{\bullet}$), reactive enough to snatch an $H^{+}$ to an PUFA neighbor, thus propagating the reaction. This results in an alteration of membrane fluidity which inevitably leads to death cellular. Generated peroxides will be neutralized by glutathione peroxidase or continue to oxidize and fragment into aldehydes (malondialdehyde, 4hydroxynonenal) whose pro-atherogenic activities are well known (Haleng *et al*., 2007).

✓ **Lipoproteins**

The radical attack of circulating lipoproteins leads to the formation of oxidized LDL, which will be captured by specific receptors macrophages the activity of these receptors not being regulated by the intracellular concentration of cholesterol, macrophages are gradually transformed into foam cells (role Important in the early stages of atherosclerosis) (Haleng *et al*., 2007).



## 2.4. Antioxidant systems

### 2.4.1. Definition of antioxidant systems

Antioxidants have been defined as substances that, when present at low concentrations compared to an oxidizable compound (e.g., DNA, proteins, lipids or carbohydrates), delay or prevent oxidative damage caused by the presence of ROS (Bouayed et Bohn, 2010).

Thereby blocking the propagation chain reaction. produced by this oxidant (Tang et Halliwell, 2010). They are present in many forms and can intervene in the prevention of the formation of free radicals, as well as to participate in their elimination. To protect themselves from oxidative stress, organisms have developed an arsenal of antioxidants (Fetoni *et al*., 2019). Several molecules, including some enzymes, are part of this natural body defense (Gbadegesin *et al*, 2014).

### 2.4.2. The different types of antioxidants

**A. The enzymatic antioxidant system**

Enzymes exist endogenously and help protect cells against free radicals produced physiologically during normal cellular metabolism. The main enzyme systems include super

oxide dismutases (SOD), catalase (CAT) and several forms of glutathione peroxidases (GSHPX) (Jacob *et al*., 2006; Garrel *et al*., 2007; Menon et Goswami, 2007) (Figure 04).

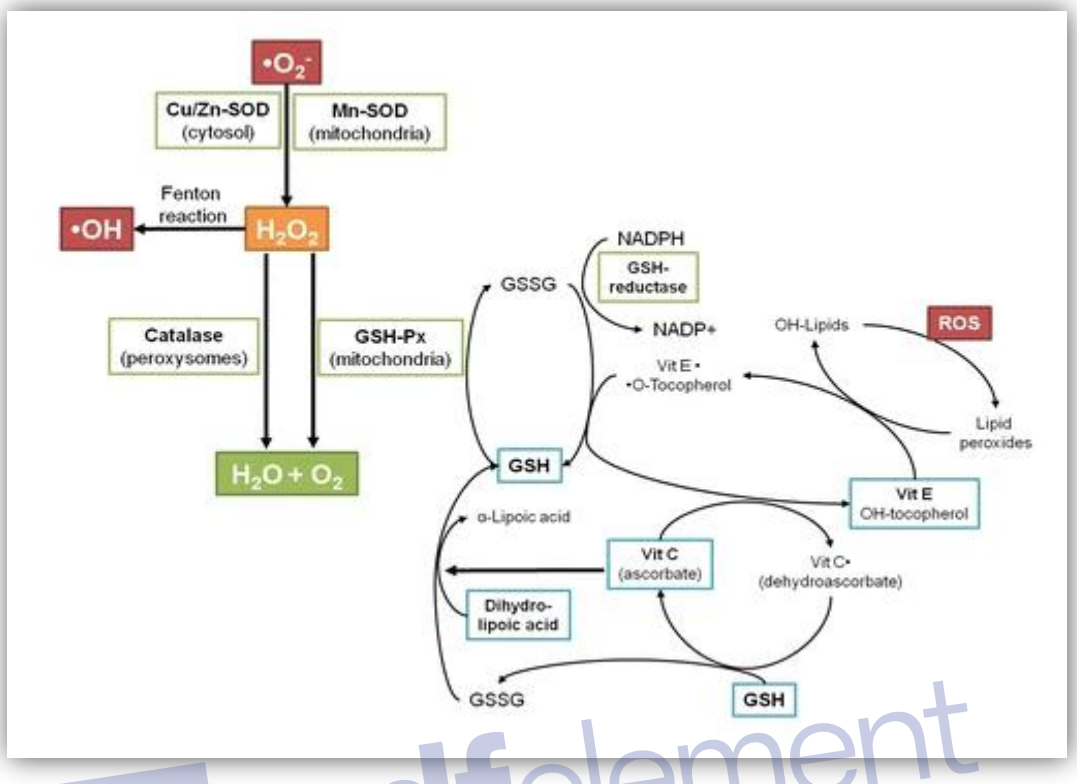



**Figure 04:** Antioxidant defenses in the organism (Kurutas, 2015)

### Superoxide dismutases (SOD)

Superoxide dismutase is a metalloprotein that catalyzes the disproportionation of $O_2^{\bullet-}$ into $H_2O_2$ and $O_2$, thus representing an important defense system against free radicals (Haleng *et al*., 2007). The reaction mechanism is catalyzed by a metal located at the heart of the enzyme, the nature of which will distinguish a specific type (Chandel et Budinger, 2007). SOD converts superoxide into hydrogen peroxide and molecular oxygen according to the following reaction (Mates, 2000): $2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$.

In mammals, three enzymatic isomers of SOD are found. They differ by their location in the cell and by the nature of the metal that exists in their structure: Cu/Zn-SOD is found in the cytosol and nucleus, Mn-SOD exists in mitochondria and Ec-SOD is found outside the cell (Servais, 2004), localized in extracellular fluids (Zelko *et al*., 2002). They accelerate the rate of dismutation of the superoxide anion into hydrogen peroxide (Rahman *et al*., 2006; Garrel *et al*., 2007).

### Catalase (CAT)

Catalase was the first antioxidant enzyme to be characterized and catalyses conversion of hydrogen peroxide to water and oxygen (Stocker et Keaney, 2004): $H_2O_2 \rightarrow 2H_2O + O_2$.

Catalase consists of four subunits, each containing a haem group and a molecule of NADPH (Ergul, 2016). It is present in peroxisomes (Valko *et al*., 2006). In humans, this enzyme occurs in abundance in the body, with the greatest activity in the liver, followed by erythrocytes, and then the lungs (Ratnam *et al*., 2006). Catalase is an iron-dependent enzyme, which competes with GSH-Px for $H_2O_2$, its use becoming important when the amounts of $H_2O_2$ are high (Sayre *et al*., 2005). according to the following reaction (Gutteridge et Halliwell, 1992):

$$\text{Catalase} + Fe^{+3} + H_2O_2 \rightarrow \text{Compose 1} + H_2O$$

$$\text{Compose 1} + H_2O \rightarrow \text{Catalase-}Fe^{+3} + H_2O + O_2$$

- **Glutathione peroxidase (GPX)**

Glutathione peroxidase is a selenium dependent enzyme (Akbas *et al*., 2005). It is a selenoprotein made up of four subunits each containing a selenium atom, it eliminates free radicals that can oxidize the DNA molecule (Belkheiri, 2010). There are two forms of GPX; Selenium-independent (Glutathione-S-transferase GST) and selenium-dependent (GPx) the latter known by the addition of two electrons to reduce the peroxide have forming the Selenoles (Se-OH). It is found in the mitochondria and in the cytosol. It is expressed in most cells (Comhair et Erzurum, 2002). This enzyme can react with $H_2O_2$ and with the hydroperoxides that result from the oxidation of cholesterol and fatty acids (Herbette *et al*.,2007). GPX catalyzes the following reactions (Ganther, 1999):

$$H_2O + GSH \rightarrow 2H_2O + GSSG$$

$$ROOH + 2GSH \rightarrow ROH + H2O + GSSG$$



**B. Non-enzymatic antioxidant systems**

Most of the natural antioxidants are derived from plant materials, such as fruits, vegetables, herbs and spices. These are particularly rich in phenolic compounds, vitamins and carotenoids (Lourenço *et al*., 2019).

- **Polyphenols**

Are secondary metabolites (Sharifi-Rad *et al*., 2020). It show a large diversity of structures, from simple molecules to polyphenols like tannins and flavonoids. Regarding vitamins, the most important include Vitamins E and C (Lourenço *et al*., 2019). The key enzyme [phenylalanine ammonia-lyase] is only present in plants (Rasouli *et al*., 2016), several biological functions have been ascribed to polyphenols, including anti-inflammatory, antioxidant, antimicrobial and antimelanogenesis effects (Sharifi-Rad *et al*., 2020), It in fact, potent activity as a scavenger for superoxide anions, $H_2O_2$, lipid peroxides, •OH, and several RNS (Imam *et al*., 2017).

- **Glutathione and protein thiols**

Glutathione is a tripeptide (glutamic acid-cysteine-glycine). It is present in essentially reduced form (GSH). The GSH/GSSG ratio is considered an excellent marker of lipid peroxidation and makes it possible to objectify the importance of stress. During aging and during intense exercise,

this ratio tends to decrease. The other antioxidant properties of GSH are numerous: cofactor of GPx, chelator of transition metals, final regenerator of vitamins E and C (Haleng *et al*., 2007).

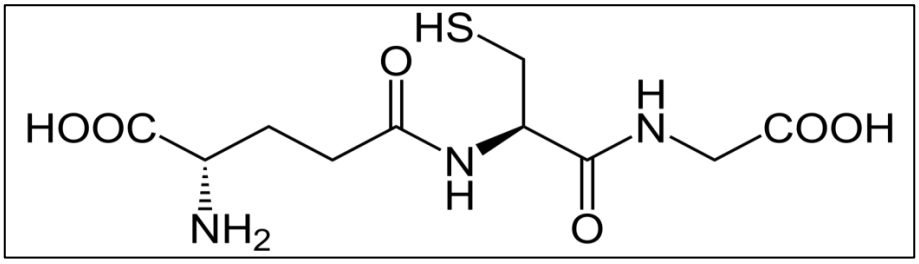



**Figure 05:** Structure of glutathione (Belkheiri, 2010)

- **Vitamin C**

Vitamin C is one of the main water-soluble antioxidants present in intra- and extracellular fluids. The spatial organization of vitamin C allows it to bind to the aqueous phase of oxidized vitamin E in the cell membrane, and quickly give up its electron. After giving up its electron, vitamin C forms a very unreactive radical, which will then be converted back into vitamin C by a reductase enzyme, which uses GSH (Morris et Carson, 2003). Vitamin C is mainly antioxidant, but in too high doses and in the process of immune defense, it can exert a prooxidant action through its ability to reduce ferric ion ($Fe3^{+}$) to ferrous ion ($Fe2^{+}$) which is a powerful catalyst for several redox reactions such as the Fenton/Haber-Weiss reaction (Sayre *et al*., 2005). By increasing the availability of ferrous iron, vitamin C paradoxically stimulate the repair of oxidized DNA (Duarte *et al*., 2009).

- **Vitamin E**

Vitamin E is fat soluble and has been shown to be the primary antioxidant in cell membranes, particularly those of mitochondria (Traber et Atkinson, 2007). Its low concentration would not allow it to be a preponderant antioxidant and its use for this purpose could compromise cell function (Azzi, 2007). It can stop the spread of lipid peroxidation by repairing the pyroxyl radical ($AGPIOO^{\bullet}$) through the formation of hydro peroxide (AGPIOOH). In this scavenging reaction, vitamin E in turn becomes radical and vitamin C regenerates it (Zhou *et al*., 2015). Its general antioxidant role is protection against lipid peroxidation as donating hydrogen.

- **Flavonoids**

These are natural polyphenols contained in the form of glucosidic derivatives in many fruits and vegetables. That means that they have no direct functions in the fundamental activities of the plant organism, such as growth or production. (Yilmaz, 2006). Flavonoids are made up of two aromatic rings, phenol and pyridine, joined by three carbon atoms that often come from an oxygenated heterocycle (Galleano *et al*., 2010). They exert their antioxidant activity by inhibiting enzymes or by capturing remnants of free radical-producing metals and/or stimulating antioxidant enzymes (Montoro *et al*., 2005). They have a chelating activity of metals such as copper and iron, which, in the free state, can be at the origin of the production of free radicals by the reactions of Fenton and Haber Weiss (Puppo, 1992).

# Chapter II

# Bleomycin Chemotherapy

# 1. Chemotherapy

## 1.1. Definition

Chemotherapy is a cancer treatment that involves treating cancer drugs that kill dividing cancer cells quickly. These chemotherapy drugs destroy the genetic material of rapidly dividing cells, which impairs cell division and therefore tumor growth (Heniche *et al*., 2022). This is the most common type of systemic treatment and improves the chances of recovery. It is one of the main methods of treating cancer (Martin, 2017; Descôteaux, 2013). It uses chemicals, antitumor or anticancer drugs, aims to eliminate cancerous cells even those that are not detected during examinations. It is completely adapted to each patient according to the characteristics of the tumor to be treated (Bouchard et Ayoub, 2005).

## 1.2. Different types of chemotherapy

Each treatment is individual, there are national and international recommendations, but individual adaptations are possible. Chemotherapy may be the only treatment used against cancer, but it is often combined with one or more other therapies (Ray-Coquard, 2021).

According to the time of administration (before or after the surgical act) and the objective, there are:

### a) Curative chemotherapy

Curative chemotherapy can cure completely and definitely certain cancers. It can be used alone or with surgery, radiotherapy and / or hormone therapy. If the cancer can not be completely cured it can also induce remission, cancer is no longer detected by medical tests, it is to-on the remission can be long-term and a normal life can be lived. It is generally believed that a patient's cancer is cured after 3 to 5 years of (Bouchard, 2005).



### b) Adjuvant chemotherapy

Adjuvant chemotherapy given after the operation or radiotherapy in the aim of eliminating the remaining cancer cells (Bouchard, 2005; Joseph, 1996). That is reduce the risk of recurrence at the origin of the original tumor or elsewhere in the body. It extends over 5 to 6 months on average but can last up to two years (Christophe *et al*., 2003).

### c) Néoadjuvant chemotherapy

Neoadjuvant chemotherapy is practiced before surgery, and aims to reduce the size of the tumor and thus facilitate the operation, as well as reduce the risk of recurrence of the disease (Christophe *et al*., 2003; Pr Ferdi, 2018).

### d) Chemo-radiotherapy

Chemotherapy that is administered at the same time as radiotherapy for a synergistic (fortifying) effect (Vander., 2017).

**e) Palliative chemotherapy**

The aim is to extend survival and improve quality of life by reducing symptoms by reducing tumor volume (Ray-Coquard, 2021). And are expected to prolong the lives of patients and improve their comfort (Samaké, 2012). it can slow the evolution of the disease: decreased the size of the tumor, destruction or decrease in the number of metastases, etc. This has the effect of prolonging your life expectancy, sometimes several years (Arnold et Nakamura, 2008). Chemotherapy is also given to patients with leukemia to destroy cancer cells prior to bone marrow or stem cell transplantation. This is called myeloablation or myelosuppression. (Heron, 2013).

### 1.3. Action of chemotherapy

Cancer chemotherapy uses drugs that destroy cancer cells as they divide or inhibit growth. They kill preferentially cancer cells because they multiply more frequently than other cells in the body. However, it is not possible to prevent their action on normal cells which are also dividing: this explains adverse effects of treatment. But unlike tumor cells, cells healthy have, in most cases, the ability to regenerate. So the side effects subside after the end of treatment. It involves drugs loaded interfere with cell function. They thus make it possible to obtain

- The arrest of cell proliferation, these are cytostatic drugs;
- Cell death is cytotoxic drugs (Charline,2018).

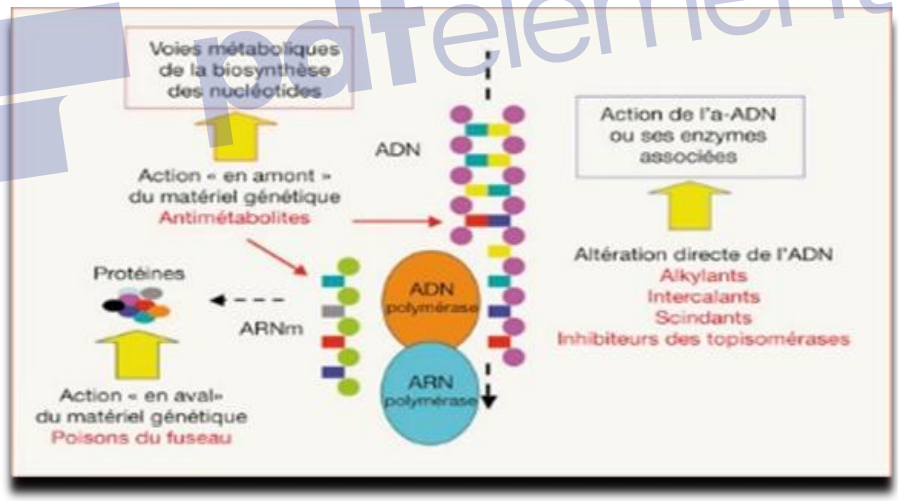



**Figure 06**: Cytotoxic mechanisms of action chemotherapy (Carol, 2017)

### 1.4. Place of administration

Chemotherapy consists of the intravenous or oral administration of molecules cytotoxic chemicals that destroy cancer cells. Its purpose is to eradicate micro metastases and any undetectable tumor cells that have escaped from the primary tumour (Chemsa, 2020).

### 1.5. Mechanisms of resistance

Unfortunately, cancer cells develop resistance mechanisms against most agents commonly used in chemotherapy. Among these we find the inactivation of the agents by glutathione, but

also an increase in the rate of DNA repair or a greater tolerance to the damage caused to the DNA by the agents, the loss or the mutation of the therapeutic target as well as the pump mechanisms. The most studied of these pumps is P-glycoprotein (Pgp), an ABC family transport protein. Pgp recognizes molecules that are inside the cell, binds them and releases them outside the cell (Ricard, 2006).

### 1.6. Kinetics of action of chemotherapy

Tumor regression depends on the dose of the drug administered (dose effect), the duration between each cycle of chemotherapy (time effect) and its mode of administration. The kinetics of action of many chemotherapies is cyclical: 4 to 5 days of treatment followed by a 21-day interval allowing reconstitution of hematopoietic precursors faster than the growth of cancer cells, repeated 4 to 6 times in order to obtain a maximum tumor regression and avoid the risk of recurrence. These kinetics cannot be generalized to all the drugs used in chemotherapy and depends on the optimal dose/intensity (i.e. a high ratio between the dosage of the treatment and its total duration) (Fofana, 2022).

### 1.7. Adverse effects of chemotherapy

#### a) Hematological toxicity

Myelotoxicity Hematological toxicity is the oldest and most common acute toxicity of cytotoxic drugs. It results from the destruction of hematopoietic stem cells being differentiated. It may involve red blood cells, white blood cells and/or platelets. These are called anemia, leukopenia and thrombocytopenia respectively (Heniche *et al*., 2022). It usually starts from one to two after weeks of chemotherapy of varying duration, but usually somewhere around the corner one week. This decrease is generally without clinical translation and is not it is often not noticed by the patient (Akoua Tchikahane, 2014).

#### b) Cardiac toxicity

Cardiac consequences of chemotherapy. This can happen even years after the initial treatment. The anthracyclines , a group of powerful chemotherapies agents widely used in the treatment of many tumors are known to cause acute and chronic cardio toxicity. Progressive, irreversible and destructive cardiomyopathy .The pathophysiology is thought to consist of association Anthracycline metabolites of cellular iron, toxic constituents . Oxygen free radicals that cause myocardial fibrosis, hypotension, tachycardiaa cardiac arrhythmias during treatment chemotherapy. Chronic cardio toxicity presents as congestive Heart failure, Most often (>80%) within 1 year treatment (Dietz et Van der Hem, 2003).

#### c) Neurotoxicity

Chronic neurotoxicity with repeated courses of chemotherapy, 50 to 70% of patients develop a persistent and severely disabling peripheral neuropathy, similar to that seen with cisplatin and carboplatin. It is mainly manifested by a sensory deficiency، symmetrical and distal, characterized by persistent paresthesia between cycles of chemotherapy, Numbness of the extremities and spontaneous pain. In severe cases, patients may exhibit sensory ataxia as well as loss of superficial and deep sensitivity, leading to functional deficits in the subtle gestures of

everyday life (typing, buttons, jacket, engine, etc.) (Tofthagen *et al*., 2011). Damage caused by neurochemotherapy may involve the5 organ. Center nervous system peripheral nervous (CNS) or peripheral nervous system (PNS), or both simultaneously (Psimaras *et al*., 2009).

**d) Digestive disorders**

✓ **Vomiting and nausea**: They can occur at the start of treatment and lead to complications Physical and psychological supplement. Taking cyclophosphamide or cisplatin causes vomiting Frequent and for several hours after the injection. Its intensity and frequency are very difficult to tolerate and can lead to this refuse treatment. Repeated vomiting can cause serious side effects such as: Peptic ulcers and/or bleeding, associated metabolic disorders Dehydration and malnutrition. Every patient is different, so tolerance varies from person to person identical treatment, therefore all treatments must be perfect fit for every patient (Sidibé *et al*., 2009).

✓ **Diarrhea**: Diarrhea is the second most common gastrointestinal disorder along with oral chemotherapies. They occur with erlotinib, gefitinib, imatinib, lapatinib, pazopanib, sorafenib and sunitinib (Ifrah *et al*., 2022).

**e) Pulmonary toxicity**

✓ **Busulfan**: concerns less than 5% of patients during long-term oral treatment (cumulative dose of 3000mg). It is an interstitial pneumopathy leading to an irreversible fibrosis of evolution unfavorable.

✓ **Carmustine**: 20 to 70% for cumulative doses >1200 mg/m2, dyspnoea and pulmonary fibrosis more or less severe, Early or delayed (up to 12 years after treatment).

✓ **Gemcitabine**: dyspnoea, hypoxia, reversible bronchospasm, risk of interstitial pneumonitis.

✓ **Bleomycin**: due to the strong pulmonary distribution and the absence of hydrolase responsible for the lung damage (Faure, 2010).

**f) Renal toxicity**

The problems of tolerance and toxicity of drugs in oncological chemotherapy have become a major problem. Poisoning renal function in these therapies may be due to hemodynamic changes, parenchymal damage and/or obstruction of the excretory tract. In addition, minor renal disorders such as tubular functional changes (acid-base and electrolyte abnormalities), urinary sediment abnormalities haematuria), generally associated with cancer treatments, are often underestimated. Therefore, the actual occurrence of nephrotoxicity Cancer drugs are difficult to define. There most episodes of acute renal failure are reversible with a return to basal renal function upon discontinuation of treatment. However, chronic kidney damage its presence leads to permanent or renal failure prolonged proteinuria.

Kidney damage was the most common event during treatment an anticancer drug is acute renal failure (ARF) characterized by a rapid rise in serum creatinine (Launay-Vacher et al., 2008).

**g) Gonadal toxicity**

Gonadal functions may be affected by chemotherapy, the most criminalized drugs are alkylating agents. According to the sex of the patient, Oligospermia, amenorrhea, or even

amenorrhea can be observed Chemotherapy induced according to the patient's age. Fertility in the se young patients it can be stored by storing sperm and eggs (Akoua Tchikahane, 2014).

**h) Hepatotoxicity**

Pathologic features of OXL-induced liver injury they include hepatic sinus hyperplasia and platelet aggregation in the sinusoids Intrahepatic‹ Hepatic steatosis, clinically important adverse reactions it is characterized by a bluish discoloration of the liver, an enlarged spleen and thrombocytopenia:78%,they have varying degrees of sinusoidal liver damage. Changes liver histopathology occurs in approximately 59% of cases Liver , and 10-60% are present hepatic impairment, as well as an increased incidence of irregular events during chemotherapy, studies have shown that oxidative stress is a major factor mechanisms involved in OXL-induced liver injury (Heniche *et al*., 2022).

**i) Dermatological toxicity**

The main dermatological manifestation of chemotherapy anti-cancer is alopecia. This side effect is transient (the hair usually grow back as soon as the treatment is finished) and is not serious. But its psychological burden is difficult to bear. We should try to avoid it. However, in some cases, this hair loss is unavoidable, either because of the type of medication used, i.e. the doses of medication administered. The rest of the cutaneous manifestations of cancer chemotherapy can go from a simple hyperpigmentation, to the weakening of the nails or ulcerations cutaneous (Akoua Tchikahane, 2014).

## 2. Bleomycin

### 2.1. Definition

Bleomycin (BLM) is an antibiotic chemotherapeutic agent with antitumor activity, bleomycin was discovered by Umezewa in 1966 and was originally isolated from the fungus Streptomyces verticillus (Dulohery *et al*., 2016; Elgendy *et al*., 2021; Reinert *et al*., 2013). BLM, a family of glycopeptide antibiotics, are currently used clinically in combination with a number of other agents for the treatment of malignant tumors (Yang *et al*., 2018; Huang *et al*., 2012) , against a range of lymphomas, head and neck cancers and germ-cell tumours and Hodgkin disease (Chen et Stubbe., 2006).

### 2.2. Chemical formula and structure

Bleomycin exhibits potent cytotoxic, DNA-damaging activity, which is a result of its unique structure. its chemical formula (C55 H84 N17 O21 S3), where he owns four distinct regions: a metal-binding domain, a DNA-binding domain, a linker region, and a disaccharide (Figure 07). The metal-binding region places metal cofactors in close vicinity to DNA bound by the DNA-binding region. These two regions are joined by a linker, and the disaccharide is involved in cellular recognition and uptake (Sankaranarayanan *et al*., 2014; Steele *et al*., 2019; Madkour, 2019).

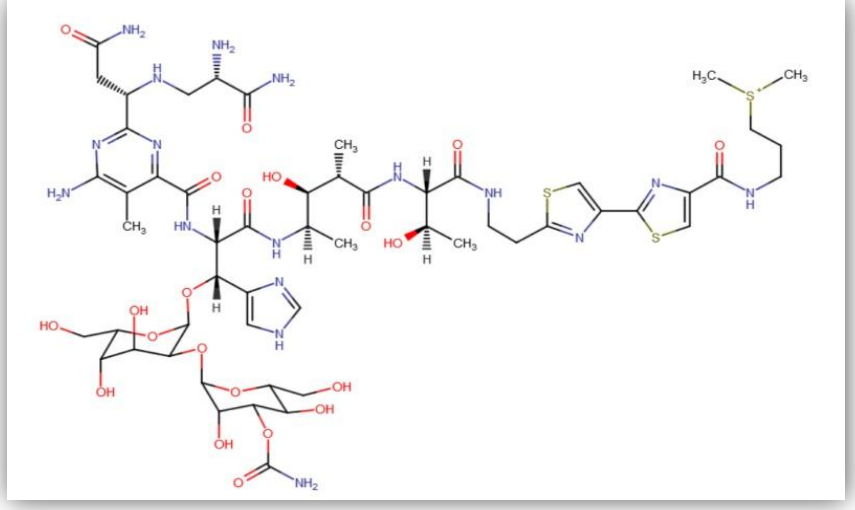


**Figure 07:** Structure of bleomycin (US, 2012)

### 2.3. Pharmacokinetics

Since bleomycin is not absorbed following oral administration, it is most commonly injected intravenously, intramuscularly, and subcutaneously. It is also instilled in the bladder for local treatment of bladder cancer. Following systemic administration, bleomycin is found in low concentrations in most body tissues, except for the skin and lung where it accumulates. Because of this, these two organs are particularly susceptible to bleomycin toxicity. Bleomycin is inactivated by an enzyme that is not found in the lungs and skin. This enzyme, which acts like an aminopeptidase, converts the beta-aminoalanine moiety in bleomycin to the corresponding desamidobleomycin. Most of an administered dose is excreted in the urine by glomerular filtration within 24 hours (Scholar, 2007; Sprangers *et al*., 2020).

### 2.4. Mechanism of Action

The bleomycin require a reduced transition metal, Fe(II) or perhaps Cu(I), oxygen, and a one-electron reductant to generate an “activated” bleomycin (Avendaño et Menéndez, 2015). When bleomycin, in its metal-free form, is administrated intravenously, it rapidly binds to Cu(II) in the blood serum to create a stable complex, bleomycin-Cu(II). When this complex is transferred inside a cell, bleomycin is reduced to bleomycin-Cu(I). The new complex can enter the nucleus and exchange with Fe(II) to form bleomycin-Fe(II) (Murray *et al*., 2018; Wu *et al*., 1994).

Both Fe(II) and $O_2$ function as co-factors in DNA cleavage, when bleomycin-Fe(II) binds to $O_2$, forming bleomycin-Fe(II)-OO•, which then accepts an electron and H+ to generate the "activated bleomycin" complex, bleomycin-Fe(III)-OOH. immediately afterwards, DNA damage and Fe(II) oxidation occur. In the presence of a reducing agent, Fe(III) is substituted by Fe(II). The role of the reducing agents is probably the production of Fe(II) from Fe(III), an effect that the superoxide is also able to perform through the Haber-Weiss reaction (Murray *et al*.,2018; Claussen et Long, 1999; Della Latta *et al*., 2015; Simpson et Scott., 2017). In addition to oxidative cleavage in the presence of Fe(II) and $O_2$, metal-free bleomycin can also mediate

sequence-selective RNA cleavage by a mechanism involving phosphoryl transfer, i.e. a "hydrolytic" mechanism (Hecht, 2002).

The complex of activated BLM binds to DNA in an intercalative manner. The interaction of the positively charged bithiazole moiety with the negatively charged polynucleotide chain of DNA probably delivers the "active" iron-oxygen components to appropriate DNA, leading to subsequent DNA damage that includes deoxyribose modification and release of free bases without scission (Della Latta *et al*., 2015), where The primary mechanism of action of the bleomycin is the generation of single- and double-strand DNA breaks and is initiated by the abstraction of a desoxyribose 4′-hydrogen (Avendaño et Menéndez, 2015; Westre, 1995). Activated bleomycin participates in the abstraction of the C4′ hydrogen atom from the deoxyribose moiety of a pyrimidine nucleotide 3′- to a guanine (Figure 08) (McLean *et al*., 1989; Abraham *et al*., 2001; Wu *et al*., 1985).

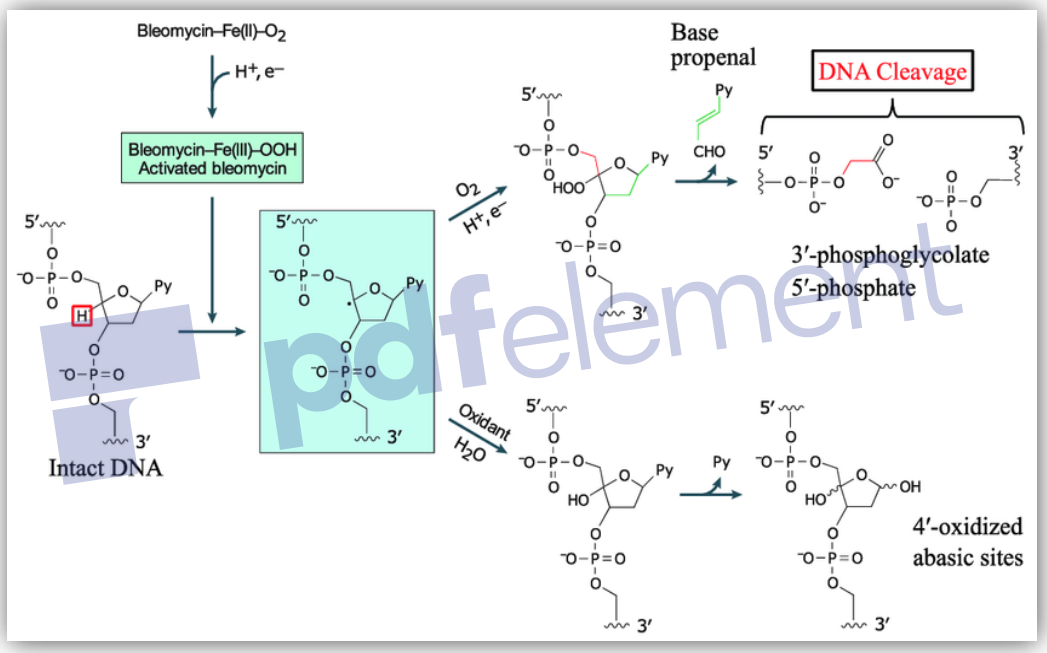



**Figure 08:** Mechanism of bleomycin-mediated DNA cleavage (Murray *et al*., 2018)

The Bleomycin-Fe(III)-OOH activated form is generated in the presence of a one-electron reductant, Fe2+ and oxygen. The activated bleomycin then abstracts the hydrogen atom (red square) from C4' of the deoxyribose moiety of DNA to form the intermediate 4' radical. This intermediate can partition into two pathways. In the abundance of oxygen, the 4' radical initiates a series of chemical transformations, leading to a direct strand break and producing 3'-phosphoglycolate and 5'-phosphate ends, and release of a base propenal. However, in the absence of oxygen, the intermediate reacts with an oxidant in the presence of water, generating 4'-oxidized abasic sites (Chen et Stubbe, 2005; Rabow *et al*., 1990; Murray *et al*.,2018). As a consequence, the free radicals produced by this process cause DNA breaks that ultimately lead to cell death (Della Latta *et al*., 2015; Claussen et Long, 1999; Galm *et al*., 2005).

One possible mechanism explaining the formation of the activated bleomycin "bleomycin–Fe(V)¼O", which is analogous to the one postulated for the case of heme-dependent enzymes such as cytochrome P450, involves the heterolytic cleavage of the O–O bond, initiated by a protonation step. This reaction gives a bleomycin–Fe(V)¼O species or its alternative Fe(IV) resonating form, which can abstract a hydrogen atom from DNA, initiating the series of events that culminate in strand cleavage. Alternatively, the O–O bond in Fe(III) hydroperoxo complex could be homolytically cleaved, giving the bleomycin– Fe(IV)¼O and a hydroxyl radical, any of which can abstract the DNA 4'-hydrogen. A concerted reaction of Fe(III) hydroperoxo complex with DNA with concomitant O–O bond homolysis to give bleomycin– Fe(IV)¼O is also possible. The bleomycin molecule can be viewed as finely tuned for its function, and its various structural portions act synergistically to effect efficient DNA cleavage, with the roles summarized in Figure 09 (Avendaño et Menéndez, 2015; Claussen et Long, 1999; Solomon *et al*., 2000; Boger et Cai, 1999).

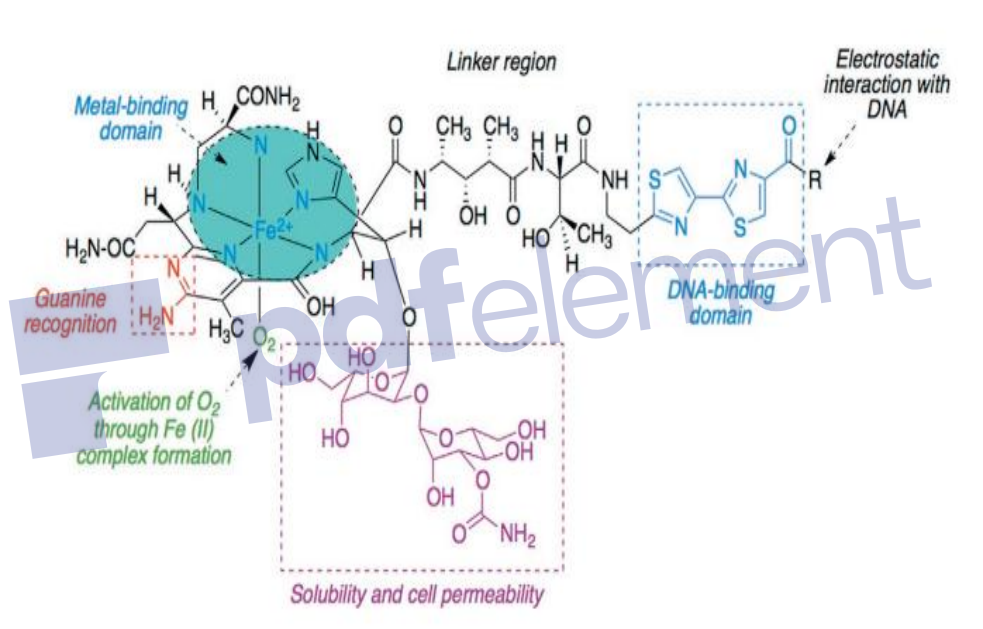



**Figure 09:** Roles of bleomoycin structural fragments (Avendaño et Menéndez, 2015)

Other transition metals such as Cu(I), Co(III), Mn(II), Ni(III), Ru(II), VO(IV) and Zn(II) are also able to recognize and bind BLM, promoting DNA strand scission. In fact these ions may form coordination complexes with several amine groups of the pseudopeptidic moiety of BLM (Della Latta *et al*., 2015).

Activated bleomycin has also been reported to catalyse the degradation of other cellular components including RNA, lipids, and proteins. Cleavage of RNA by bleomycin was shown to be selective, depending on the secondary and tertiary structure of the substrate. The significance of these bleomycin cellular targets is not fully understood since the cytotoxic nature of bleomycin is thought to mainly stem from its ability to mediate double-strand DNA cleavage (Murray *et al*., 2018; Tounekti *et al*., 2001; Morgan et Hecht, 1994).

### 2.5. Bleomycin transport

The cytotoxicity of bleomycin to certain tumour types might be related to the efficiency of drug uptake. Bleomycins are large molecules (ca. 1.5 kDa), hydrophilic and therefore they are probably unable to diffuse through cell membranes. The mechanism of cellular uptake has been the focus of several investigations. At present, there is no general consensus on either the efficiency of uptake into the cell or into the nucleus of the cell. After administration, it has been proposed that they bind rapidly and irreversibly to Cu (II) in plasma. It is believed that both the free bleomycin and the bleomycin-Cu (II) complex are transported into the cells. as that bleomycin binds to a receptor protein on the plasma membrane that is then transferred into intracellular endocytotic vesicles by receptormediated endocytosis. The Cu (II) complex is intracellularly reduced to bleomycin-Cu (I), which can react with oxygen to initiate a series of reactions leading to DNA strand scission. On the contrary, this complex is less stable than the one from Cu (II) and it can dissociate, allowing the formation of the bleomycin-Fe (II) complex and its transformation into the activated bleomycin species (Figure 10). Bleomycin transport is probably critical to the success of chemotherapy, and the use of internalizing antibodies for this purpose is currently being studied (Avendaño et Menéndez, 2008; Chen et Stubbe, 2005; Roy et Horwitz, 1984; Kénani *et al*., 1994).

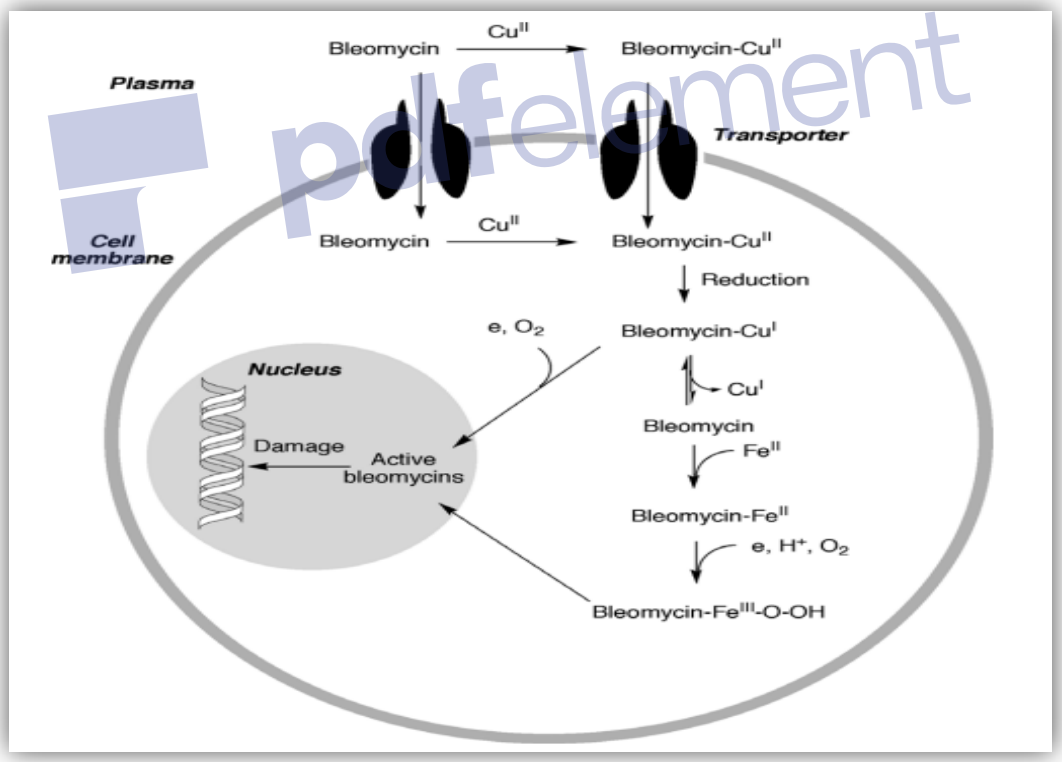



**Figure 10:** Bleomycin transport and bioactivation (Avendaño et Menéndez, 2008)

### 2.6. Adverse effect

The most important toxic effects of bleomycin are to the lungs and skin. Subacute and chronic interstitial pneumonitis is a serious, life-threatening side effect of bleomycin, which has been described in up to 10% of patients receiving the drug (Lyman *et al*., 2007; Reinert *et al*.,

2013; Viala, 2021). Signs of this begin with a dry cough, fine rales, and diffuse basilar infiltrate on x-ray. It may progress to life-threatening pulmonary fibrosis. Other adverse responses include hyperthermia, headache, nausea, and vomiting as well as an acute fulminant reaction in patients with lymphomas (Scholar, 2007). One of the potential determinants of bleomycin toxicity is bleomycin hydrolase, the enzyme that is primarily responsible for metabolizing bleomycin to nontoxic molecules. Interestingly, the two organs that are the most common sites of bleomycin toxicity (the lungs and the skin) have the lowest levels of the enzyme (Reinert *et al*., 2013; Ferrando *et al*., 1997). Bleomycin is more slowly degraded in the lungs than in other tissues, such as the bone marrow or liver, because of lower bleomycin hydrolase activity (Reinert, 2013). The mechanisms of lung damage include the formation of highly reactive oxygen species (Boukhenouna *et al*., 2018). There is usually little myelosuppression except in those with severally compromised bone marrow function (Scholar, 2007). Risk factors for bleomycin pulmonary toxicity include age greater than 70 years, preexisting COPD, higher doses of bleomycin, bolus infusion, and prior chest irradiation, and high supplemental oxygen exposure (Kwok *et al*., 2017; Clarke-Pearson, 2012). Skin reactions are the most common side effects and include erythema, hyperpigmentation of the skin, striae, and vesiculation. Skin peeling, thickening of the skin and nail beds, hyperkeratosis, and ulceration may also occur. These manifestations usually occur in the second and third weeks after treatment, when the cumulative dose has reached 150–200U. Directly after its administration, fever chills and sometimes hypotension can occur. Skin changes often leave a residual hyperpigmentation and may recur when patients are treated with other anticancer drugs (Reinert *et al*., 2013; Scholar, 2007; Sleijfer, 2001; ChuandV et DeVita Jr, 2011). Other common side effects are alopecia, stomatitis, and fatigue. Vascular events, including myocardial infarction, stroke, and Raynaud's phenomenon, are occasionally reported (Reinert *et al*., 2013).

### 2.7. Bleomycin and oxidative stress

Bleomycin is a commonly used in cancer therapeutic that is associated with oxidative stress leading to pulmonary toxicity. The toxicity with bleomycin is initiated by direct oxidative damage, which then leads to subsequent inflammation and fibrosis mediated by generation of both extracellular ROS and intracellular ROS (Allawzi *et al*., 2019). Several studies have indicated that reactive oxygen species (ROS) are involved in BLM-induced lung injury (Teixeira *et al*., 2008), there is convincing evidence that its antitumour action is linked with free radical formation. It has been suggested that the activity of BLM results from its reaction with DNA to cleave the backbone. The mechanism of this cleavage apparently involves the initial formation of Fe(II) BLM and then a redox reaction between the iron center and oxygen to produce Fe(III)-BLM and a ROS as the hydroxyl radical, thus forming an activated complex capable of releasing damaging oxidants in close proximity to DNA. In lung, ROS may inactivate these enzymes provoking genetic injury and death of cells sensitive to oxygen, resulting in a typical alveolar cells injury (Teixeira *et al*., 2008; Kaiserova *et al*., 2006).

# Second part

## Experimental part

# Chapter I

## Materials and methods

# 1. Materiels

## 1.1. Statistical study

### 1.1.1. Study area

The city of El-Oued situated to the southeast of Algeria are 33.3676° N latitude and 6.8516° E longitude (Hadjadj *et al*., 2020; Attia *et al*., 2021). El-Oued is part of the northern Sahara Northeast, is limited by the state of Tebessa in the North-East, the state of Khenchela in the North, the state of Biskra in the North-West, by the state of Djelfa in the West, the state of Ouargla in the West and the South, and east by Tunisian-Algerian border (Figure 11) (Remini et Kechad, 2011; Hacini *et al*., 2022). It is characterized by an arid climate. The average annual temperature is of the order 22 ,66°C, the warmest month is July with 34.43° C, the coldest month is January with 10.76°C. The warm period lasts from May to October, with an average of 29.98° C (Zeghdi *et al*., 2016), average rainfall varies between 80 and 100 mm/year (period from October to February). The flora in El-Oued region is characterized by the speed of change, adaptation to soil and climate, the small number of species, the discontinuous nature of the plant material (Mayouf *et al*., 2014).

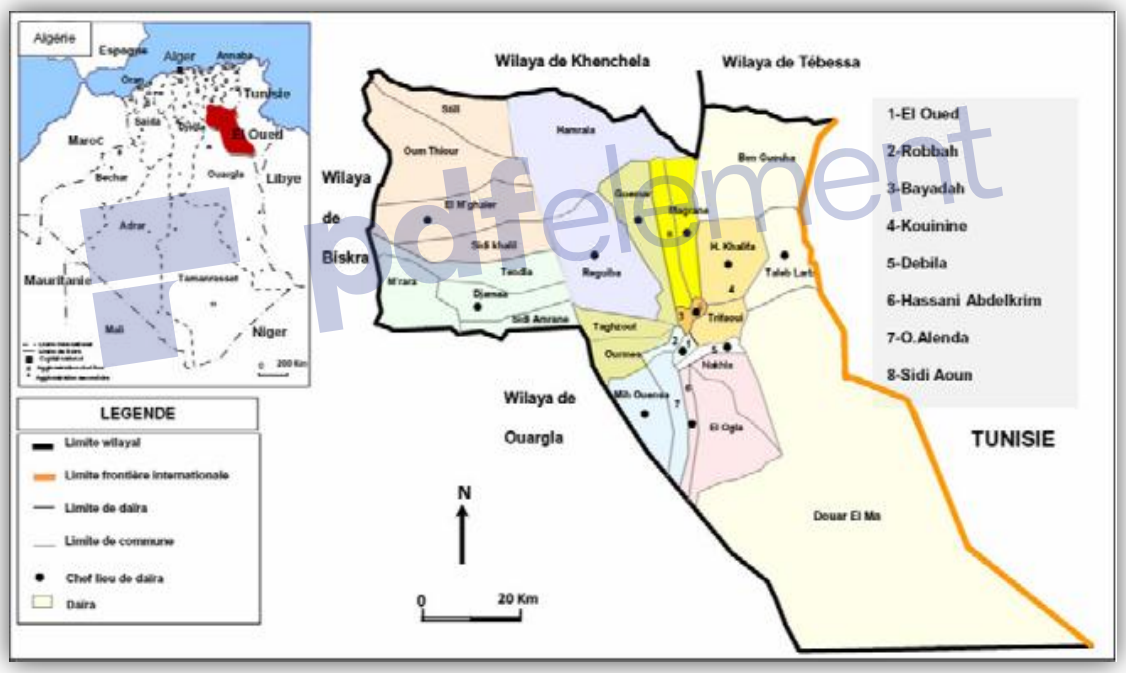



**Figure 11**: The geographical location of the study area (El-Oued) (DPAT, 2007)

## 1.2. *In vitro* study

### 1.2.1. Chemicals

Bleomycin, BLM (BLOICIN-S ®).Other utilized chemicals and reagents were of analytical grade and commercially available. Sodium Chloride (Nacl), Ethanol, Chloroform, Comassie Blue, Phosphoric Acid ($H_3PO_4$), Bovine Serum Albumin (BSA), Gallic Acid, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), Butylated Hydroxytoluene (BHT), hydrochloric acid (HCl), Tris, Salicylicacid, DTNB (5-5'-dithiobis2-nitrobenzoic acid), hydrogenperoxide ($H_2O_2$),

Ferric chloride ($FeCl_3$), Fehling liquor, sulfuricacid ($H_2SO_4$), Aluminum chloride ($AlCl_3$), Folin-Ciocalteu (FC), Sodium Carbonate ($Na_2CO_3$), Sodium acetate ($CH_3COONa$).

### 1.2.2. Plant materials

The aerial part of *Ephedra alata* was collected in 14 November 2022 from khouinza District, El-Oued Province, Algeria. The plant material was washed using water, dried at room temperature, were powdered by mechanical grinder until a fine powder was obtained, The powders of *E. alata* are stored at room temperature in airtight containers protected from bright light until the beginning of the experiment.

**Figure 12**: *Ephedra alata* L (original photos)

### 1.2.3. Aqueous extract preparation

To extract it About 50 g of the aerial parts powder of *E. alata* was soaked in 500 ml of distilled water and kept at room temperature in the dark for 24 h. Then, it was filtered through filter paper. After extraction, the water was removed first using a rotary evaporator and then incubated at 60°C to dry completely. The extract was weighed and stored in a refrigerator at 4°C (Murugan et Parimelazhagan, 2014).

## 1.3. *In vivo* study

### 1.3.1. Animal materials

#### A. Animals and care

A total of 20 female albino rats, weighing 80-150 g, were obtained from the animal house of Pasteur Institute, Algeria. They were placed and kept in the animal house of the Molecular and Cellular Biology Department, University of El-Oued, Algeria. Animals were adapted for 10 days under the same laboratory conditions of photoperiod (12 h light/12 h dark) with relative humidity 64±2% and room temperature of 19±1°C. Standard rat food (Southon *et al*., 1984) and tap water were available ad libitum for the duration of the experiments.

### B. Treatment animals

After a period of adaptation, the animals were divided into four groups, each containing 5 rats as follows:

- **Group I (Control group):** was serving as a control and received normal water;
- **Group II (AEEA treated group):** was treated orally 200 mg/kg, b.w / day of AEEA daily for 4 weeks;
- **Group III (BLM treated group):** was treated by injection 10 U/kg, b.w of BLM (BLOICIN-S ®) On the first day of the experiment;
- **Group IV (BLM+AEEA treated group):** treated the first day of the experiment by BLM (BLOICIN-S ®) 10 U/kg, b.w then treated orally 200 mg/kg, b.w / day of AEEA daily for 4 weeks.

Body weight was recorded periodically during the experiment days.

### C. Samples preparation and Blood collection

Mice were slaughtered after fasting for 16h hours, under light anesthesia with chloroform (94%) by inhalation, blood samples were collected during the slaughter of animals into EDTA tube to carried CBC and dry tubes. The serum was obtained by centrifugation for 10 min at 3000 tour/min and used for biochemical analysis assays; blood glucose level measured during rat's slaughter using glucometer.



## 2. Methods

### 2.1. Statistical study

#### 2.1.1. Ethnobotanical survey

The survey was carried out between September 2022 and February 2023. During this period different places El-Oued city were visited, with 52 persons interviewed, their age ranged from 14 to 70, all people interviewed had been informed about the objective of this study. Each interview followed a questionnaire aimed at obtaining the following information: record socio-demographic data (gender, age, marital status, educational level) and the use of medicinal species (illnesses treated, part used, method of preparation).

### 2.2. In vitro study

#### 2.2.1. Phytochemical analysis

The methods of qualitative phytochemical analysis as described in study of Balamurugan et al (2019), Banu et Cathrine (2015) and Morsy (2014) were used to identify the phytochemicals provides in the extract.

- **Phenolic**

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

- **Saponins**

0.5 mg of extract was vigorously shaken with few ml of distilled water. The formation of frothing is positive for saponins.

- **Flavonoids**

To 5ml of dilute ammonia the plant extract is added and shaken well. The aqueous portion is separated and concentrated $H_2SO_4$ is added. The yellow colour indicates the presence of flavonoids.

- **Steroids**

2ml of extract with 2ml of chloroform and 2ml of concentrated $H_2SO_4$ are added, the appearance of red colour and yellowish green fluorescence indicates the presence of steroids.

- **Tannins**

To 5ml of extract few drops of neutral 5% ferric chloride solution was added, the production of dark green colour indicates the presence of tannins.

- **Terpenoids**

3ml of the extract was taken and 1ml of chloroform and 1.5 ml of concentrated $H_2SO_4$ are added along the sides of the tube. The reddish brown colour in the interface is considered positive for the presence of terpenoids.



- **Reducing Compond**

1 mL of filtrate solution is hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of reddish precipitate indicates the presence of reducing sugars.

- **Alkaloids**

Wagner's test (iodine – potassium iodine reagent): To about an ml of extract few drops of Wagner's reagent were added. Reddish –brown precipitate indicates presence of alkaloids.

### 2.2.2. Total phenolic and flavonoids compounds

#### A. Total phenolic

The polyphenols are determined by the Folin-Ciocalteu method. This method, initially described by Slinkard and Singleton (Slinkard et Singleton, 1977), makes it possible to know the total polyphenolic content of a given sample. The sample of the aqueous extract of the *E. alata* (0.5 ml) and 2 ml of sodium carbonate (75 g / l) were added to 2.5 ml of 10% (v / v) Folin-Ciocalteau with gallic acid as standard. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm. The tests were carried out three times in order to ensure the reproducibility of the results. The total phenolic content was expressed in mg Equivalent of Gallic Acid per gram of sample.

**B. Total Flavonoids**

Determination of the total flavonoid content of the aqueous extract of the *E. alata* is carried out by the method described by Derouiche et al (2022). 0.5 ml of a 2% $AlCl_3$-ethanol solution was added to 0.5 ml of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as a standard for plotting the calibration curve. The tests were carried out three times in order to ensure the reproducibility of the results. The results were expressed in milligram equivalent Quercetin per gram of sample.

### 2.2.3. Antioxidant activity DPPH test

The in vitro antioxidant activity to *E. alata* was evaluated by measuring the scavenging power of the DPPH (1,1-diphenyl-2-picryhydrazyl) radical according to the method described by Burits and Bucar (Burits et Bucar, 2000), where 3ml of various concentrations (25,50,100,200,400,800,1000,1200,1400,1600,1800 et 2000 µg/ml) of *E. alata* samples were added to75µL of methanolic solution of DPPH (1.3mg/ml) . Absorbance measurements were read at 517 nm after 30 min of incubation time at room temperature (A1). Absorbance of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control (A0).

The percentage inhibition [(A0-A1/A0) × 100] was plotted against the phenol content and IC50 was determined.



### 2.2.4. Antioxidant activity FRAP test

The Antioxidants are determined to *E. alata* by colorimetry. The ferric-tripyridyltriazine complex is reduced to the ferrous-tripyridyltriazine in presence of the antioxidants; the complex loses its yellow color to a dark blue. This coloration measured at 595 nm is proportional to the concentration of antioxidants present in the samples. The method is standardized by Trolox (Oyaizu, 1986; Lim et al., 2007). were Taken 500µl of sample, then we Add to it 1.25ml of the buffer solution (0.2 M, PH = 6.6), then The mixture were incubated 20 min in a water bath at 50 ° C, after that we Add 1.25ml of the aqueous TCA solution (10%) to stop the reaction and put it in Centrifugation at 3000 rpm for 5 minutes. 1.25 ml of supernatant are then mixed with 1.25 ml distilled water and 250 µl $FeCl_3$ (0.1%).Finally was measured at 700 nm against a blank. The results expired by IC50, after calculating of the ferric reducing antioxidant power values according to (Yazdani., *et al* 2019) as follows: FRAP (%) = 100– (OD control /OD sample) × 100

### 2.2.5. Anti-inflammatory assay

**A. Protein denaturation inhibition**

The anti-inflammatory activity is measured of protein denaturation inhibition in presence of the anti-inflammatory compound, which is studied through in vitro assay. The measured turbidity at 660 nm is proportional to the concentration of anti-inflammatory compound present in the sample (Djouadi et Derouiche, 2021), has been added different concentrations (10–100 µg ml -1 ) of the sample to bovine serum albumin (BSA) solution (1%), then The mixture were incubated 30 min at room temperature. The pH of the solution was adjusted to 2 using dropwise

addition of concentrated HCl. After incubation, the mixture is heated at 72 °C for 30 min, Finally all tubes were cooled for 10min and the turbidity was measured at a wavelength of 660nm. Diclofenac was used as standard. The results expired by IC50, after calculating of inhibition percentage (IP) as follows:

IP (%) = [(Acontrol –Asample)/ Acontrol] × 100, where Acontrol is the absorbance of the control,and Asample is the absorbance of the sample extract/standard (Vamanu et Nita, 2014).

**B. Hemolysis assay**

The Hemolysis assay to *E. alata* is done as described by (Vinjamuri., *et al* 2015) that determined the protective effect of the antioxidant compound presented in the sample against the membrane erythrocyte lysis which induced by 1X PBS. The detection of membrane RBCs lysis by measuring the concentration of hemoglobin in blood plasma at 540 nm by spectrophotometer. 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation, then done The blood centrifuged at 1000 rpm for 10 min at 40C, Plasma is removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min, after that The Washed erythrocytes were stored at 4 o C and used within 6 h for the hemolysis assay, and we Add 50 µL of 10 dilutions (100 µL Erythrocytes suspension and 900 µL 1XPBS) of erythrocytes suspension was mixed with 100 µL of test samples (20-80ng/mL), 100 µL of 1XPBS was used as a control. Finally, Reaction mixture is incubated at 370 C water bath for 60 min. where The volume of reaction mixture is made up to 1 mL by adding 850 µL of 1XPB, The reaction mixture is centrifuged at 300rpm for 3min, then The resulting hemoglobin in supernatant is measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. The percentage hemolysis is calculated as follows:

Hemolysis inhibition percentage (%) = 100– (OD sample /OD control) × 100

**2.3. In vivo study**

**2.3.1. Biochemical parameters analysis**

Some biochemical parameters urea, creatinine, uric acid, Cholesterol, Triglycerides, AST and ALT activity were measured using commercial kits obtained from Spinreact (Barcelona, Spain ).

**2.3.2. Hematological parameters analysis**

Hematological analysis was performed using an automatic hematological analyzer Medtronic (Coulter Beck-man, USA). The parameters included: white blood cell (WBC) count, lymphocytes and granulocyte counts.

**2.3.3. Histopathological study**

After rats were sacrificed, the part of liver, kidneys and lung tissues was removed and immersed in fixative (solution 10% formaldehyde) until the time of slices preparation. It was dehydrated in ascending graded series of ethanol, cleaned with toluene, immersed in paraffin, and colored with hematoxylin and eosin. Histopathological evaluation was performed with a light microscope.

### 2.3.4. Oxidative stress parameters

**A. Preparation of the homogenate**

One gram of the liver, kidneys and lungs of each rat from the different batches studied was ground and homogenized in TBS (50 mM Tris, 150 mM NaCl, pH 7.4). The homogenate is centrifuged at 5000 rpm for (10 minutes, 4°C). The supernatant obtained is recovered and then stored at -80°C. while waiting to perform the assays of the oxidative stress parameters.

**B. Measurement of malondialdehyde (MDA)**

✓ **Principle**

Malondialdehyde is one of the end products of the decomposition of polyunsaturated fatty acids (PUFAs) as a result of lipid peroxidation and reacts with thiobarbituric acid (TBA) at 100°C temperature and in acidic environment (pH 2-3).

The principle of this assay is based on the condensation of MDA in an acidic and hot medium with thiobarbituric acid. A molecule of MDA is condensed with two molecules of thiobarbituric (TBA) to form a colored complex in pink (reading at 532 nm) (Yagi, 1976).

✓ **Reagent**

Trichloroacetic acid (TCA) 20% W/V; Thiobarbituric acid (TBA) 0.375% W/V; Butylhydroxytoluene (BHT) 0.01% W/V; Hydrogen chloride (HCl) 1 N.

375mg of TBA, 20 g of TCA, 0.01 g of BHT, 25 ml of 1 N HCl and 50 ml of distilled water were introduced into a beaker. The solution obtained was heated to 40° C. in a water bath until the TBA had completely dissolved, then transferred to a 100 ml flask and the volume made up with distilled water to the mark.



✓ **Procedure**

Pipette 100µl of sample, 400 µl of TBA reagent into glass and screw-top test tubes and close tightly. Heat the mixture in a water bath at 100°C for 15 minutes. Then cool in a cold water bath for 30 minutes, leaving the tubes open to allow the gases formed during the reaction to escape. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer.

✓ **Expression of results**

The concentration of TBARS was determined using the molecular extinction coefficient of MDA (å = 1.53 105 $M^{-1}$ $cm^{-1}$). The results were expressed in mol/l (Djiala et Maffo., 2018).

$$MDA(mole/l) = \frac{\text{OD sample}}{1.53 \times 10^5}$$

- **C:** Concentration in mole/l;
- **OD:** Optical density read at 532 nm.

**C. The enzymatic activity of catalase (CAT)**

✓ **Principle**

The catalase activity consists in measuring the catalase-induced $H_2O_2$disappearance contained in the sample by measuring the absorbance of $H_2O_2$ at 560 nm using a UV / visible spectrophotometer.

✓ **Procedure**

Briefly in test tubes, mix 1 ml of phosphate buffer (0.1M, pH7.2), 0.975 ml of freshly prepared $H_2O_2$ (0.091M) and 0.025 ml of the enzyme source (serum). Absorption read at 560 nm every minute for 2 minutes (Aebi, 1984).

✓ **Calculation**

Calculate the enzymatic activity of CAT: by the following formula:

$$CAT\ activity(\text{UI /g}) = (2{,}3033\ /T) \times (\ log\ A1\ /\ log\ A2\ )$$

- **A1:** Absorbance at the first minute;
- **A2:** Absorbance at the second minute;
- **T:** Time interval in minutes.

**2.4. Statistical analysis**

Data were expressed as the mean ± standard deviation (M ± SD). Statistical analysis was carried out by using the student T-test to compare means between two groups. Results were evaluated using the Minitab and EXCEL software. Differences were considered significant at $p < 0.05$.

# Chapter II

# Results and discussion

# 1. Results

## 1.1. Statistical study

### 1.1.1. Characterization of study population

The studied population contains 52 people, including 29 men and 23 women. A large number of individuals surveyed are uneducated (53.84%) and educated (46.15%). The number of married people is large (88%), while single people (12%).

**Table 02:** Socio-demographic characteristics of the people surveyed

| Characteristic | Number of respondents | Percentage (%) |
|---|---|---|
| **Sex** | | |
| Men | 29 | 65 |
| Women | 23 | 44 |
| **Ages (years)** | | |
| <20 | 4 | 7.69 |
| 20-30 | 5 | 9.16 |
| 31-40 | 10 | 19.23 |
| 41-50 | 10 | 19.23 |
| 51-60 | 10 | 19.23 |
| 61-70 | 9 | 17.30 |
| >71 | 4 | 7.69 |
| **Level of education** | | |
| Education | 24 | 46.15 |
| Uneducated | 28 | 53.84 |
| **Marital status** | | |
| Single | 6 | 12 |
| Married | 46 | 88 |



### 1.1.2. Use of the plant according to sex

Among the 52 people interviewed, of 29 men use *Ephedra alata* subsp. alenda in the treatment of different diseases, however 23 of women resort to *E. alata* (Figure 13). The figure recorded among men is explained, on the one hand, by their conviction and confidence in the alternative or natural medicine. And it recorded among women is explained, by that women assume that the woman does not go to hospital except in cases emergencies and prefers to take care of themselves at home.

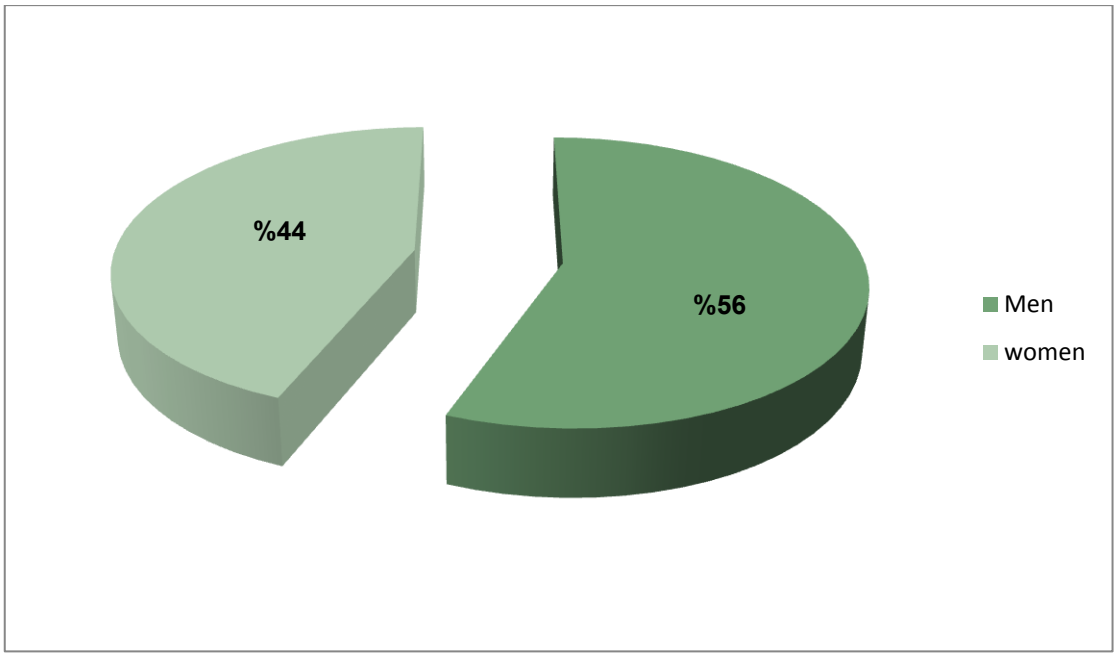
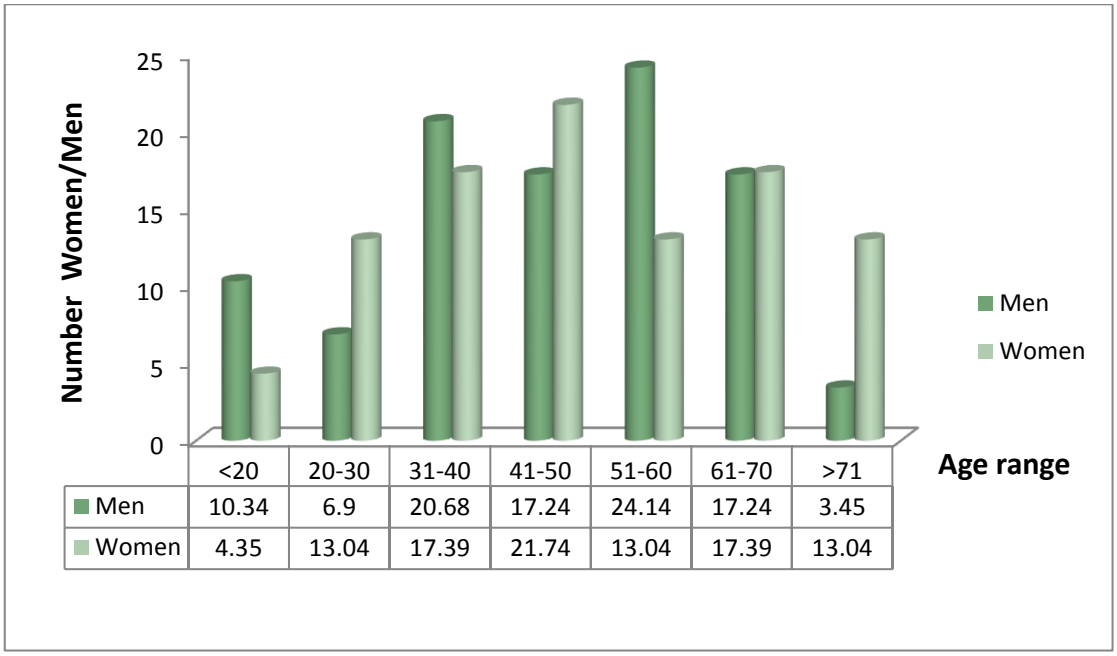



**Figure 13:** Use of *Ephedra alata* according to sex

### 1.1.3. Use of the plant according to age group

It appears from the analysis of Figure 14 that the therapeutic use of the plant for women and for men is much more dominant among people between the ages of 51 and 60. People ages 41 to 50 come in second, followed by people in the 31 to 40 age group. People between the ages of 61 and 70 and those between the ages of 20 and 30 rank fourth and fifth, respectively, in terms of use of the plant in traditional medicine. follows them over the age of 70 rank sixth and those under 20 rank seventh.






| | <20 | 20-30 | 31-40 | 41-50 | 51-60 | 61-70 | >71 |
|---|---|---|---|---|---|---|---|
| Men | 10.34 | 6.9 | 20.68 | 17.24 | 24.14 | 17.24 | 3.45 |
| Women | 4.35 | 13.04 | 17.39 | 21.74 | 13.04 | 17.39 | 13.04 |

**Figure 14:** Ratio of use the plant according to the age group of the respondents

### 1.1.4. Used parts of the plant

Various organs of *Ephedra alata* are used in traditional medicine. We have noted that the commonly used organs are: the aerial part or green stems. The green stems are the most used organs with 100%.

### 1.1.5. Methods of preparation and modes of administration

Results of our survey showed that the decoction was the primary mode of preparation for plant remedies (51%) followed by infusion (44%), powder (3%) and inhalation (2%). The oral route was used in 98% of applications (Figure 15).

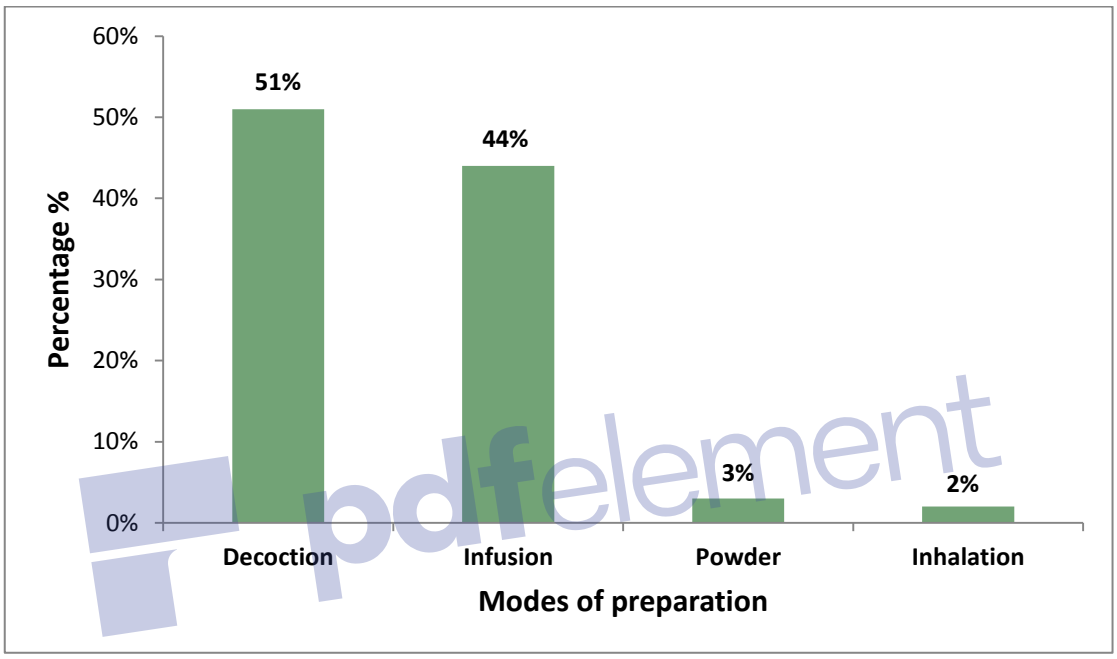



**Figure 15:** Percentage of preparation modes used by the respondents

### 1.1.6. Therapeutic uses of the plant

The survey revealed that those interviewed in the El-Oued region use *Ephedra alata* subsp. alenda for mainly treat cancer, diabetes, Ovarian disease, Diseases of immune origin (weakened immunity, blood purification...), Diseases of inflammatory origin (cystitis, arthritis and dislodgment of kidney stones) and other diseases (pain in the stomach, treatment of the spine and weight reduction, skin allergies, flu... ) (Figure 16).

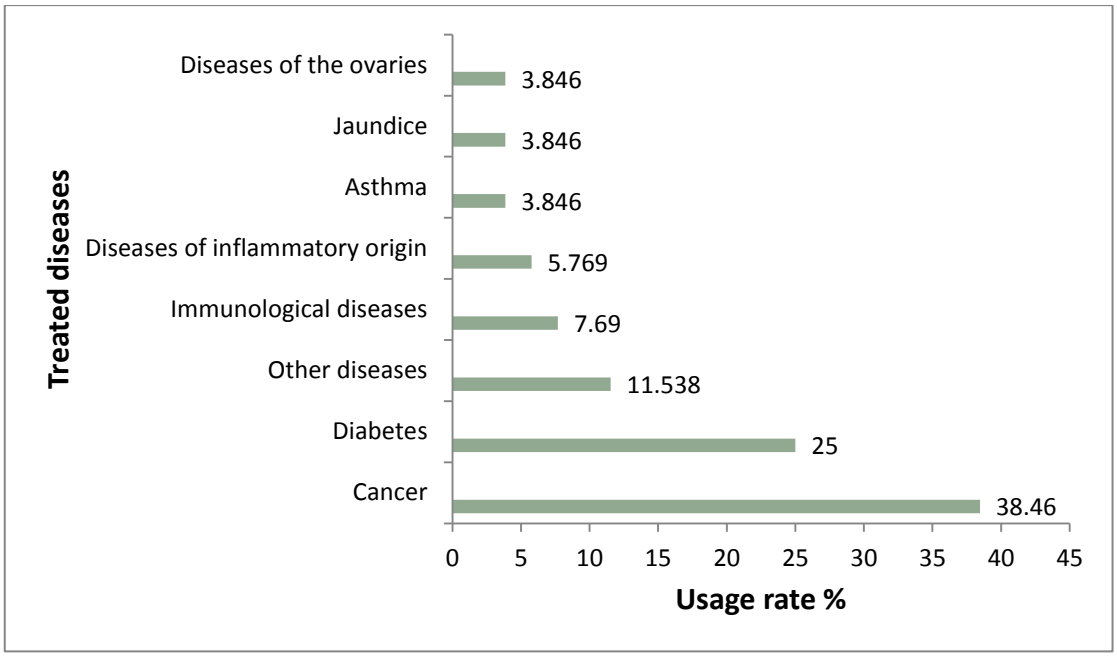



**Figure 16:** Diseases treated by *Ephedra alata* subsp. alenda according to the population studied

### 1.2. *In vitro* study

#### 1.2.1. Phytochemical Screening

##### 1.2.1.1. Qualitative phytochemical analysis of *E. alata*

Results of preliminary phytochemical analysis (Table 03) showed the presence of a wide range of bioactive secondary metabolites including, phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compound and alkaloids.



**Table 03:** Phytochemical compounds of *Ephedra alata* aqueous extract

| Phytochemical | Aqueous extract of *E. alata* |
|---|---|
| Phenol | +++ |
| Saponins | +++ |
| Flavonoids | +++ |
| Steroids | +++ |
| Tannins | +++ |
| Terpenoids | +++ |
| Reduced compounds | +++ |
| Alkaloids | +++ |

(+++ presence)

### 1.2.2. Total phenols and flavonoids concentration

Results of quantitative phytochemical analysis show that the studied plant has a large amount of total phenols as well as significant amounts of flavonoids, as the percentage more than 5% of the total phenols.

**Table 04:** Total phenols and flavonoids concentration in *E. alata* aqueous extract

| **Compounds** | **Polyphenols** (mg of GAE/g of extract) | **Flavonoids** (mg QE/g of extract) |
|---|---|---|
| **Aqueous Extract of *E. alata*** | 36.324±0.172 | 1.845±0.0376 |

### 1.2.3. Antioxidant activity and IC50 value

Results appeared the antioxidant activity of AEEA, based on DPPH and FRAP assays, results show that the curve of inhibition percentages (IP) of free radicals were increased proportionally with extract concentration (annex 04) and through the standard equation (y=0.0443x+16.795) for DPPH and (y=0.056x+44.273) for FRAP, we calculated the inhibition concentration (IC50) values, which expressed the important antioxidant activity, as shown in the table 05.

**Table 05**: Inhibition concentration 50 of *Ephedra alata* aqueous extract

| | **DPPH antioxidant activity** | | **FRAP antioxidant activity** | |
|---|---|---|---|---|
| **IC50 mg/ ml** | AEEA | Vit C | AEEA | Vit C |
| | 0.749 | 0.046 | 0.102 | 0.454 |



### 1.2.4. Anti-inflammatory activity

The *in vitro* anti-inflammatory activity evaluated by the ability of AEEA to inhibit heat-induced albumin denaturation and the hemolysis tests. Results show that the curve of inhibition percentages were increased proportionally with extract concentration (annex 04) and through the standard equations (y=0.516x+30) for protein denaturation and (y=0.049x+48.96) for hemolys (annex 04). The IC50 values against protein denaturation of AEEA, diclofenac and the hemolysis test were presented in the table 06.

**Table 06**: Inhibition concentration 50 of *Ephedra alata* aqueous extract

| | **Inhibit protein denaturation** | | **Hemolysis** |
|---|---|---|---|
| **IC50 µg/ ml** | AEEA | Diclofenac | AEEA |
| | 38.708 | 37.929 | 20.947 |

## 1.3. *In vivo* study

### 1.3.1. Growth parameters

The analysis of body weight and relative organ weight is summarized in Table 07. As shown in the table, exposure of rats to BLM caused a highly significant reduction in weight gain,

compared to the control group, a significant reduction in gain weight was also observed in rats treated with AEEA and BLM+ AEEA. The results analysis of the relative weight of the liver shows a decrease in AEEA and BLM+ AEEA group , and a non-significant difference in BLM group, compared to the control. Analysis of the results of the relative weight of the kidneys shows a non-significant difference in the different groups. Results of the relative weight of the Lungs shows a non-significant difference in AEEA and BLM group, except for BLM+ AEEA group , where a significant decrease is observed compared to control group.

**Table 07:** Initial weight**,** Weight gain and Relative Organ Weights in Control and Experimental Groups

| | **Control** | **AEEA** | **BLM** | **BLM+AEEA** |
|---|---|---|---|---|
| **Initial weight** (g) | 104±9.18 | 103.20±2.73 | 103.40±3.36 | 106.20±2.87 |
| **Weight gain** (g/d/Rat) | 2.666±0.0122 | 2.308±0.0273*** | 2.366±0.0558** | 2.155±0.0670**a |
| | **Relative weight** | | | |
| **Liver (%)** | 2.886±0.0056 | 2.686±0.0201*** | 2.923±0.033 | 2.420±0.0567***c |
| **Kidneys (%)** | 0.642±0.0094 | 0.612±0.0134 | 0.627±0.0088 | 0.612±0.0182 |
| **Lungs (%)** | 1.010±0.0981 | 0.983±0.0907 | 1.220±0.127 | 0.760±0.0506**c |

Values represent mean ± mean standard deviation (n=5). *p<0.05; **p<0.01; *** p < 0.001 comparison with the control group. a p < 0.05; b p < 0.01; c p < 0.001 comparison with BLM group.

### 1.3.2. Hematological parameters

Results of hematological parameters illustrated in Table 08 showed that in BLM group a highly significant increase (P<0.001) of MCH, (P<0.01), MCV, MCHC (P<0.05), RBC and HCT compared to control. In addition, results showed a highly significant decrease (P<0.001) of WBC and no significant change of HGB in BLM group compared to control group. In AEEA group, results showed a significant decrease (P< 0.01) of HGB, HCT, WBC and MCV and a significant increase (P< 0.05) in MCHC but no significant change in RBC and MCH compared to control group. Concerning the treated group (BLM+ AEEA) results showed a significant increase (P< 0.01) in MCHC, (P< 0.05), RBC and a significant decrease (P< 0.01) in HCT and a no significant difference in WBC, HGB, MCV and MCH compared to control group. In addition, results showed a highly significant decrease (P< 0.001) of HGB, HCT, MCV and MCH. And a significant increase (P< 0.05) in WBC in AEEA treated group compared BLM group.

**Table 08:** Hematological parameters of control and experimental groups

| | Groups of rats | | | |
|---|---|---|---|---|
| | **Control** | **AEEA** | **BLM** | **BLM+ AEEA** |
| **WBC count** ($\times 10^9$ /L) | 5.233±0.306 | 5.200±0.161** | 3.733±0.072*** | 5.733±0.627[a] |
| **RBC count** ($\times 10^{12}$ /L) | 7.408±0.138 | 7.383±0.050 | 8.397±0.326* | 7.542±0.072* |
| **HGB** (g/dL) | 14.340±0.374 | 13.540±540*** | 15.520±0.541 | 14.12±0.183 |
| **HCT** (%) | 39.644±0.826 | 37.080±0.213*** | 42.10±1.09* | 38.400±0.286**c |
| **MCV**(fl) | 51.657±0.116 | 50.673±0.276** | 52.985±0.223** | 51.311±0.146[c] |
| **MCH** (pg) | 18.701±0.084 | 18.437±0.139 | 19.489±0.106*** | 18.718±0.098[c] |
| **MCHC** (%) | 36.194±0.174 | 36.515±0.118* | 36.948±0.183** | 36.760±0.148** |

Values represent mean ± mean standard deviation (n=5). *p<0.05; **p<0.01; *** p < 0.001 comparison with the control group. a p < 0.05; b p < 0.01; c p < 0.001 comparison with BLM group.

### 1.3.3. Biochemical parameters

According to the results obtained in figure 17, there was a significant increase of Blood glucose (P< 0.01) in BLM group and non-significant change in AEEA group compared to the control group. In addition, results showed a highly significant decrease (P< 0.001) of blood glucose in BLM+ AEEA group compared to BLM group.

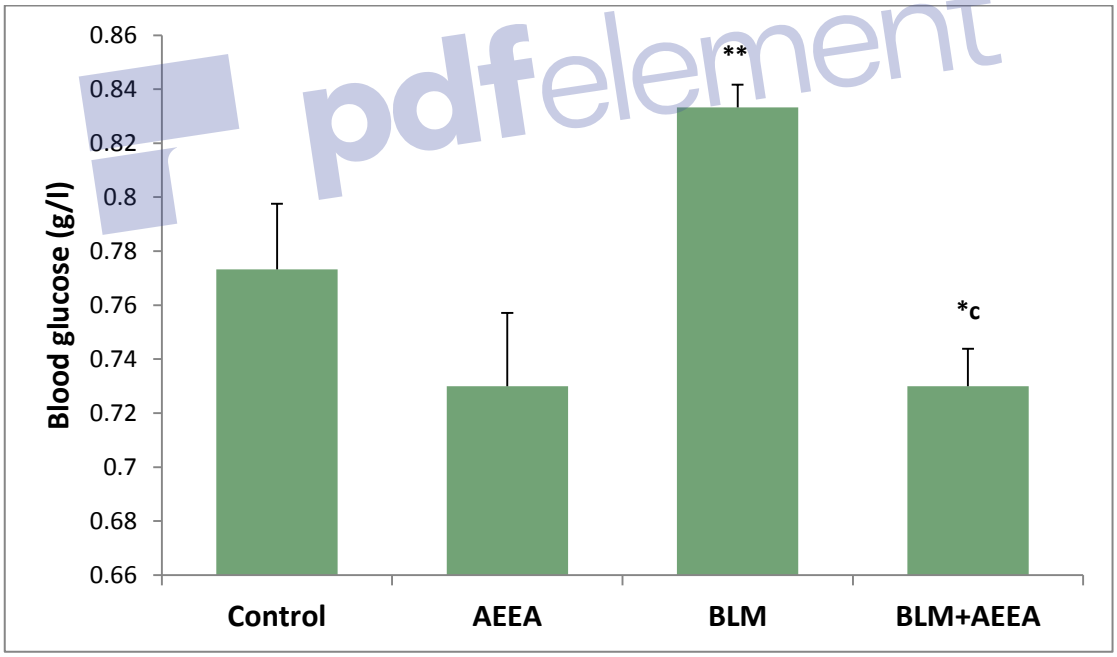



**Figure 17:** Blood glucose level in the control and experimental groups

In the other side, The effects of BLM and AEEA on the renal parameters were presented in figure 06. results observed a highly significant decrease ( P< 0.01) of urea, creatinine and uric acid in BLM group compared to the control group. In addition, in AEEA group, results showed a significant decrease (P< 0.01) in urea and a no significant change of creatinine and Uric acid compared to the control group. In the other hand, in the BLM+ AEEA group a significant decrease (P< 0.001) of urea and (P< 0.05) creatinine and no significant change of uric acid compared to the control group. But in plant (AEEA) treated group, results showed a significant

increase (P≤ 0.05) in urea and no significant change (P≥ 0.05) in creatinine and uric acid compared to the BLM group.

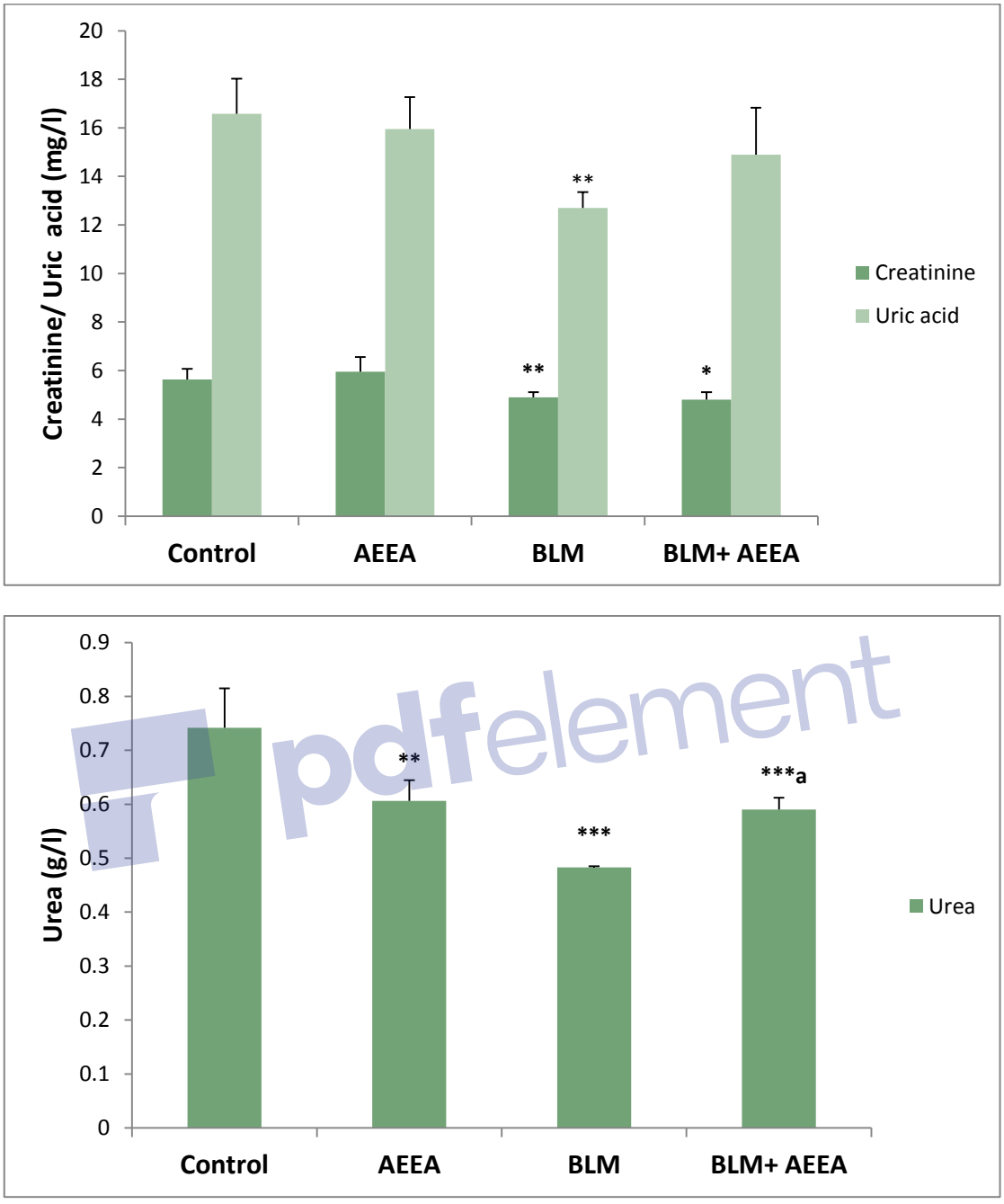



**Figure 18:** Creatinine, Uric acid and urea levels of control and experimental groups

Results of transaminases activities in the different groups studied are represented in figure 19. The results showed a significant increase (P< 0.05) of AST activity and no significant change of ALT in BLM group compared to the control group. The same results showed a significant increase (P< 0.01) in transaminases activities in AEEA group compared to control group. In addition, in BLM+ AEEA group, results showed a significant decrease (P< 0.05) in AST activity and no significant change of ALT activity compared to BLM group.

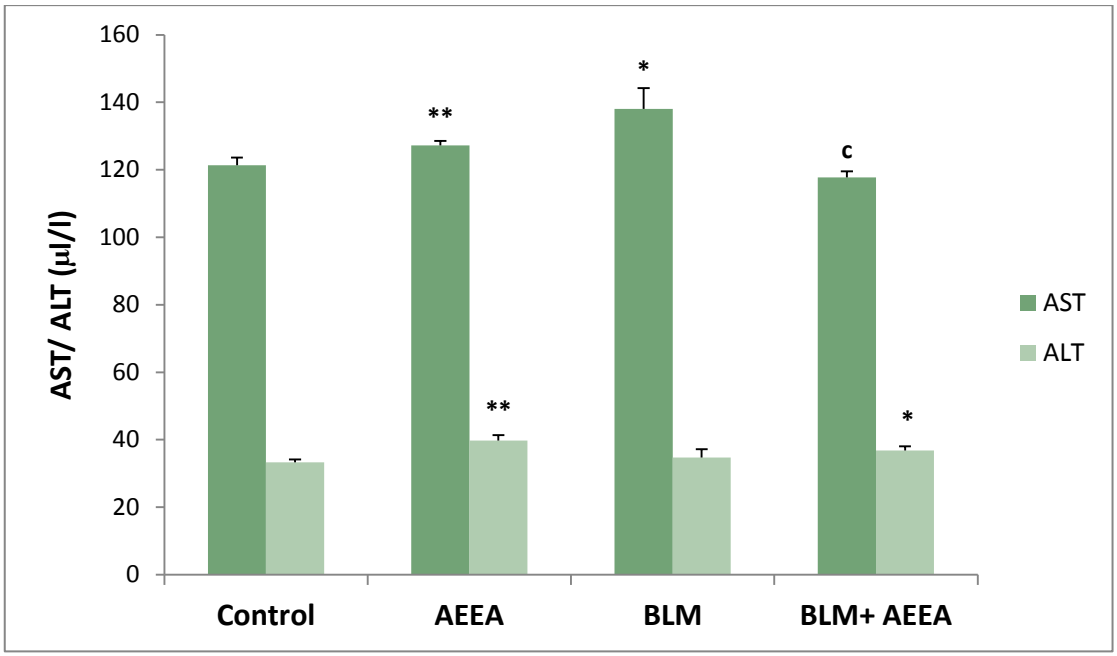



**Figure 19:** Transaminases (AST, ALT) activities in the control and experimental groups

The effects of BLM and AEEA on lipid profile were represented in figure 20. The results show a highly significant increase (P< 0.05) in cholesterol and triglyceride in AEEA and BLM groups compared to the control group. In addition, in BLM+ AEEA group, showed a significant amelioration (p< 0.01) in cholesterol and triglyceride levels compared to BLM group.

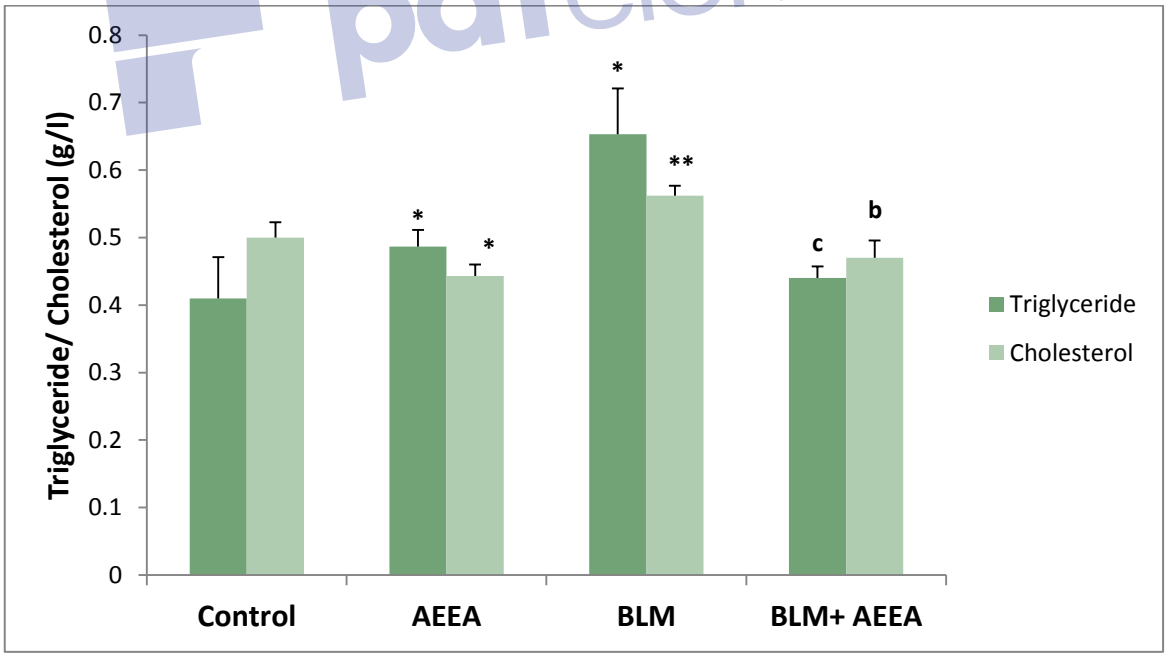



**Figure 20:** Lipid profile of control and experimental groups

### 1.3.4. Oxidative stress parameters

#### a) Malondialdehyde (MDA) levels

Regarding the tissues MDA levels (figure 21), our results showed a significant increase ($P<0.01$) of lungs and liver MDA and no significant change of kidney MDA level in the BLM group and no change of tissues MDA levels in the AEEA group compared to the control group. In addition, the treatment groups of BLM by AEEA cause an amelioration of this parameter in all tissues compared to the BLM group.

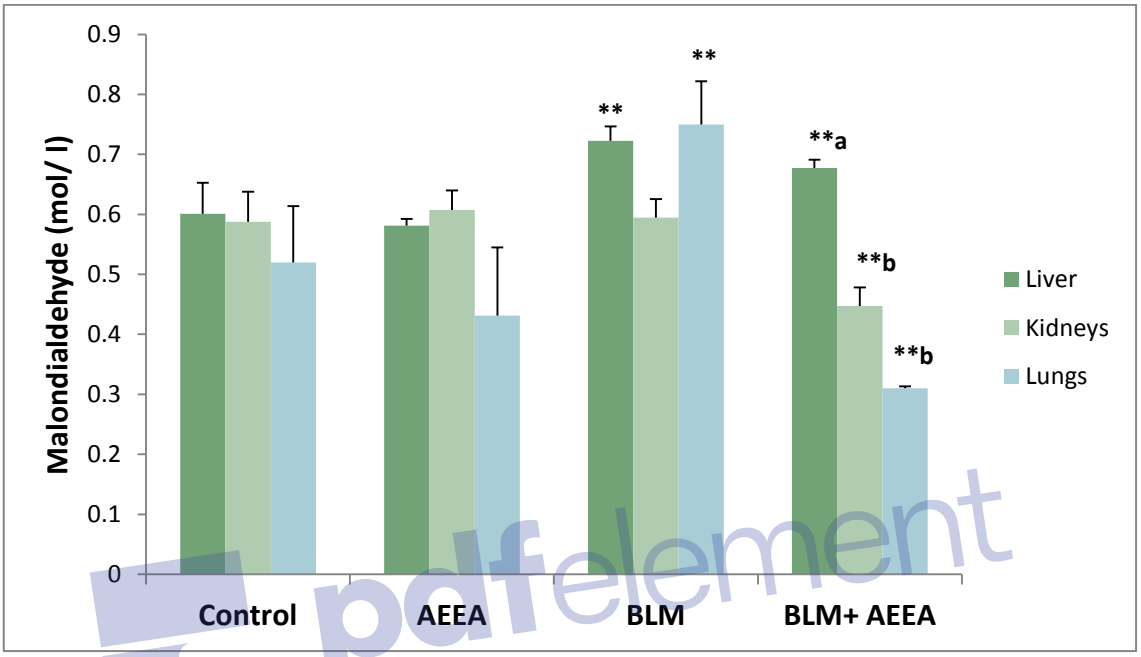



**Figure 21:** Malondialdehyde levels in liver, kidneys and lungs in control and experimental groups

#### b) Catalase activity (CAT)

Concerning the catalase activity (figure 22), our results show a significant decrease in catalase activity of all studied tissues in the BLM and AEEA group compared to the control group. In addition, results showed a significant amelioration ($P<0.001$) of catalase activity in kidney of the group (BLM+ AEEA) and no significant change in the other tissues compared to the BLM group.

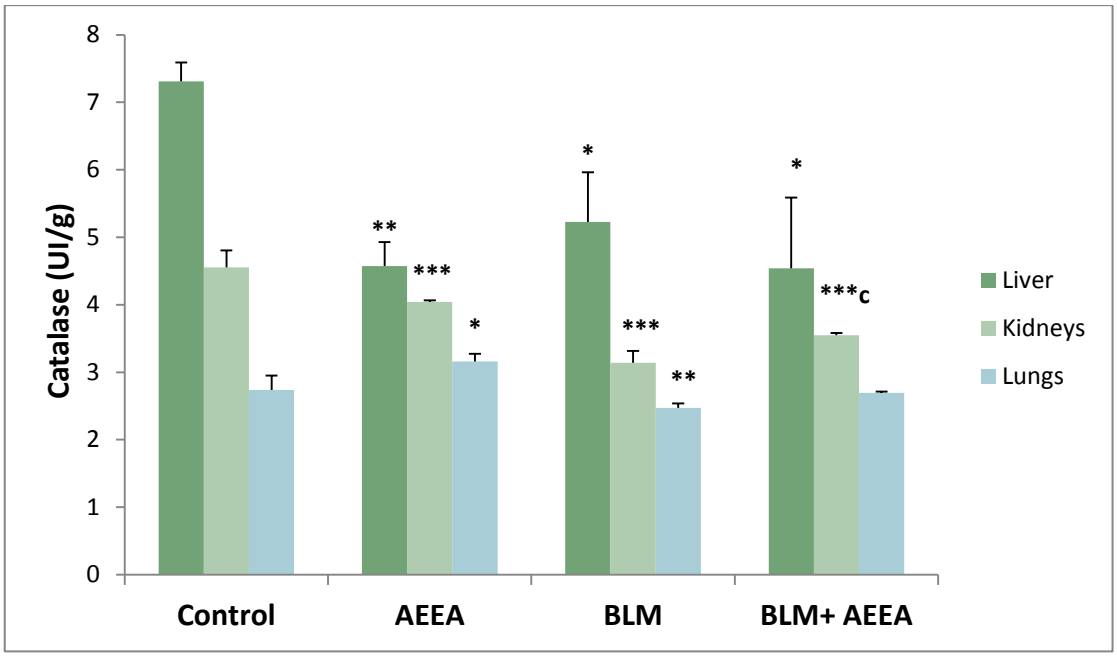



**Figure 22:** Catalase activity in liver, kidneys and lungs of control and experimental groups

### c) FRAP activity in liver, kidneys and lungs

Results of FRAP activity were presented in figure 23, our results showed a significant decrease ($P<0.05$) of FRAP activity in kidney and lungs and a significant increase ($P<0.05$) of FRAP activity in liver and no change in the other groups compared to the control group. In addition, in the BLM group treated by AEEA, a significant ($P<0.01$) amelioration of FRAP activity in all tissues compared to the BLM group.



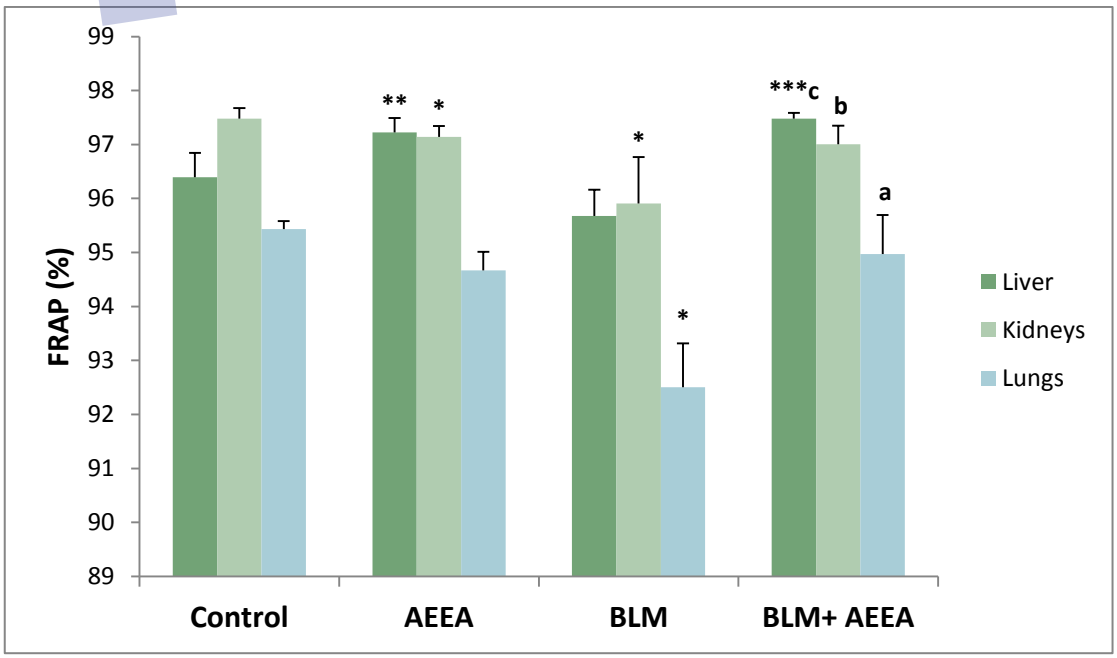
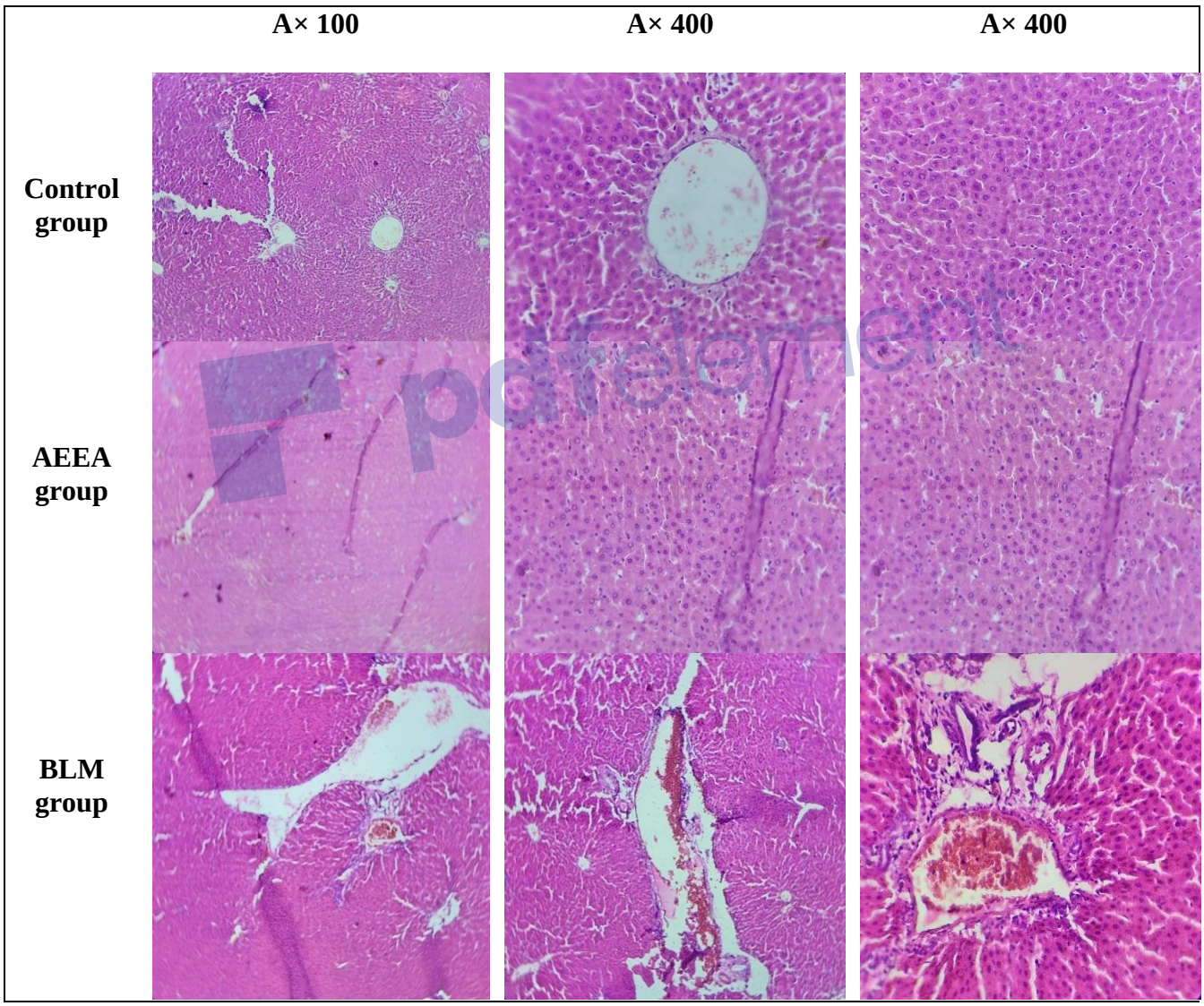



**Figure 23:** Tissues FRAP activity in control and experimental groups

### 1.3.5. Histological Results

### A. Liver histological results

The liver histological sections of rats under different experimental conditions reveals significant differences between the rats treated with BLM compared with the control rat and the rat treated with AEEA (figure 24). Photomicrograph of the liver tissues of control showing the normal tissue structure with striations and branched appearance and normal nucleus. In liver cells, treatment with single dose of BLM causes a marked cell lysis (damage to the level of tissue) and causes fairly marked cell necrosis. Necrosis predominantly peripetous and usually accompanied by inflammation. Scattered vacuolations are also observed as well as macrocytic steatosis. For rats treated with AEEA for 30 days of them noticed a partially improvement in liver tissue level.



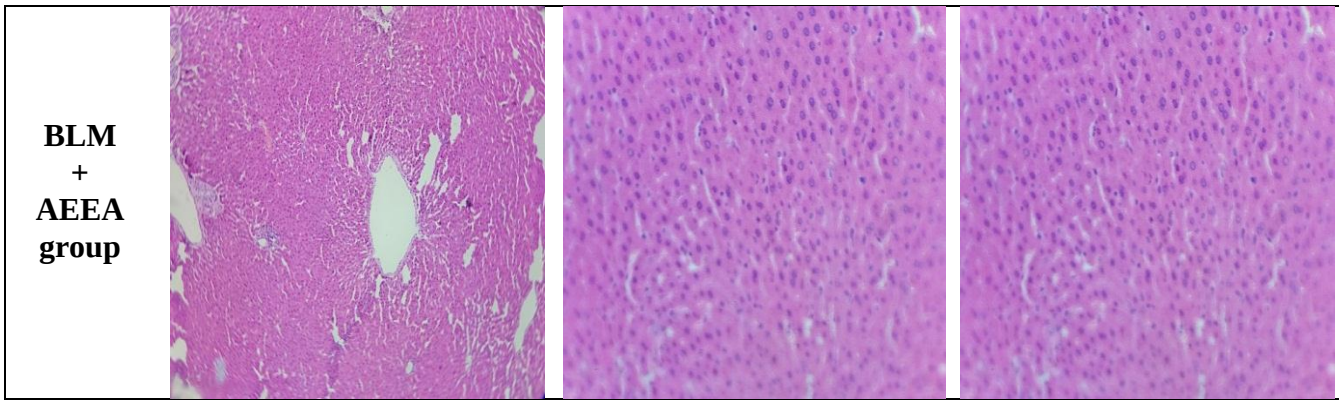
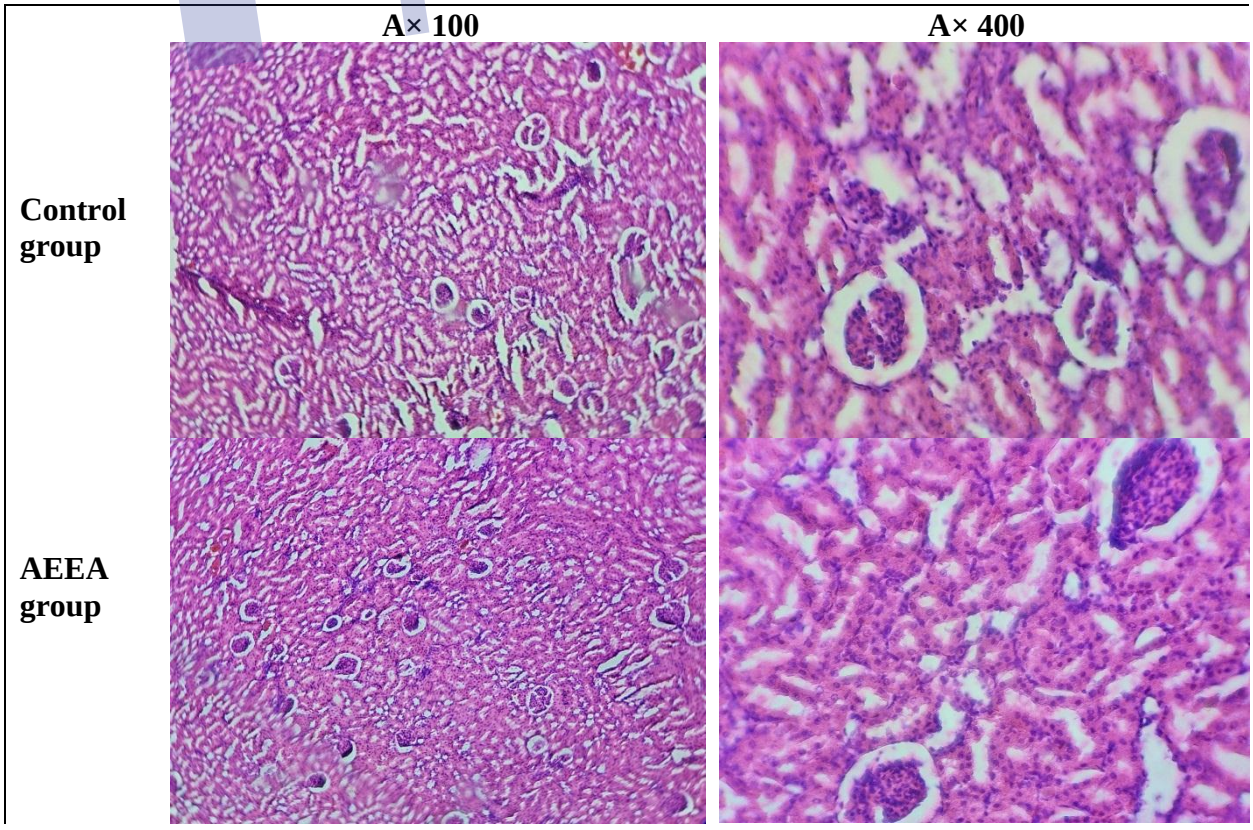



**Figure 24:** Photomicrograph of histopathological examination of liver sections of control, AEEA group, BLM group and BLM+AEEA treated group, coloration with hematoxylin and eosin

### B. Kidneys histological results

For kidneys histopathological study as show in (figure 25) results indicated normal cells layer structure glomerulus similar sizes and narrow bowmen's space in control and AEEA group, conversely in BLM group the histological results show a bowmen's space expansion with hemorrhagic necrosis and inflammation at the level of tissue cells. Histological observations of the kidneys morphology of the BLM rats treated with AEEA show slight correction in morphological with the survival of necrosis and the expansion of a bowmen's space at the level of some cells.





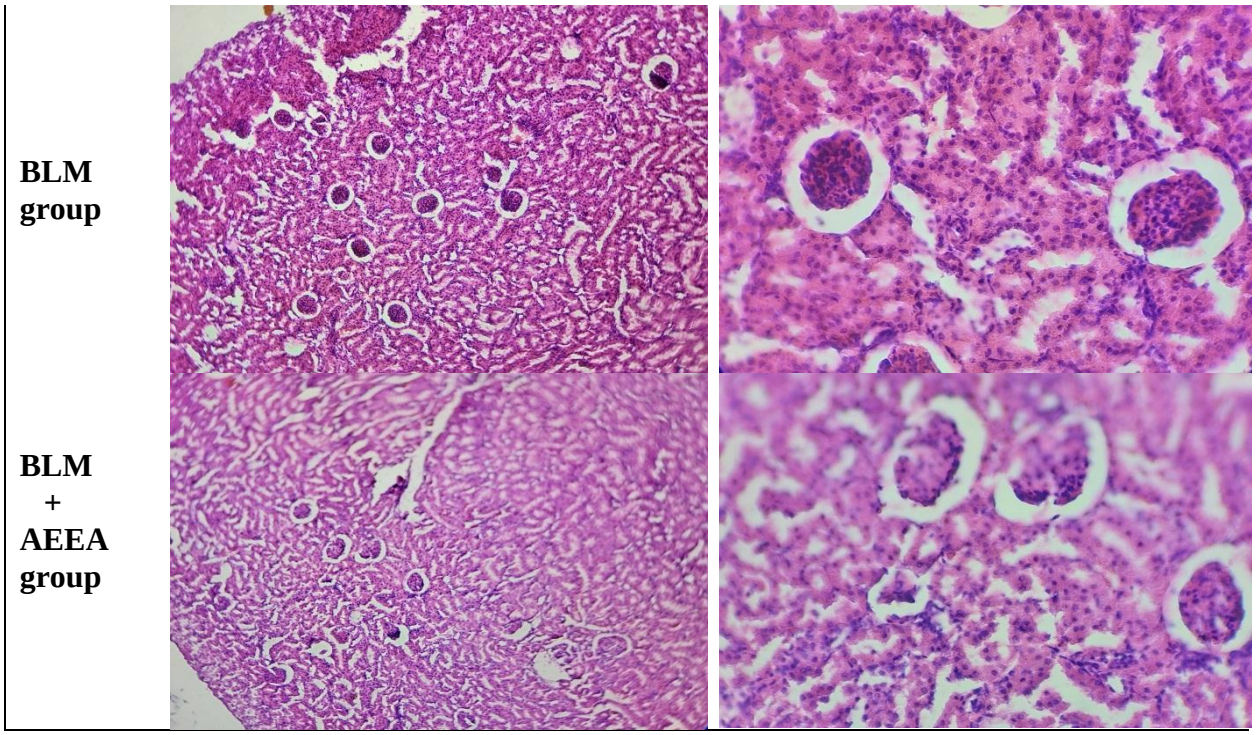
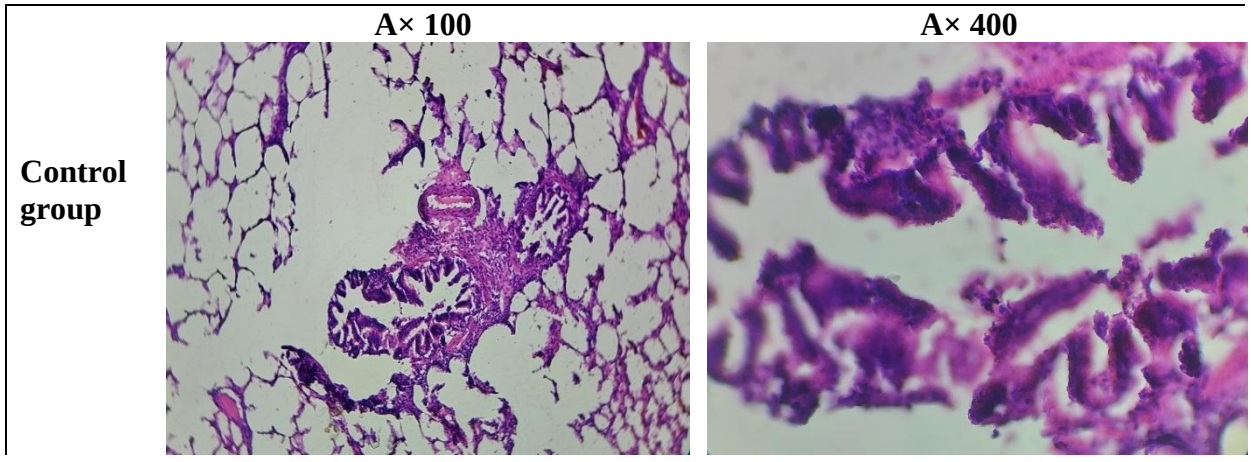



**Figure 25**: Photomicrograph of histopathological examination of kidney of control, AEEA group, BLM group and BLM+AEEA treated group, coloration with hematoxylin and eosin



### C. Lungs histological results

For lung histopathological study as show in (figure 26) results indicated normal cells in control and AEEA group, on the other hand, in BLM group the histological results show an alveolar cell damage, hemorrhagic, necrosis and inflammation at the level of tissue cells. Histological observations of the lung morphology of the BLM rats treated with AEEA show slight correction in morphological with the survival of necrosis and inflammation at the level of some cells.



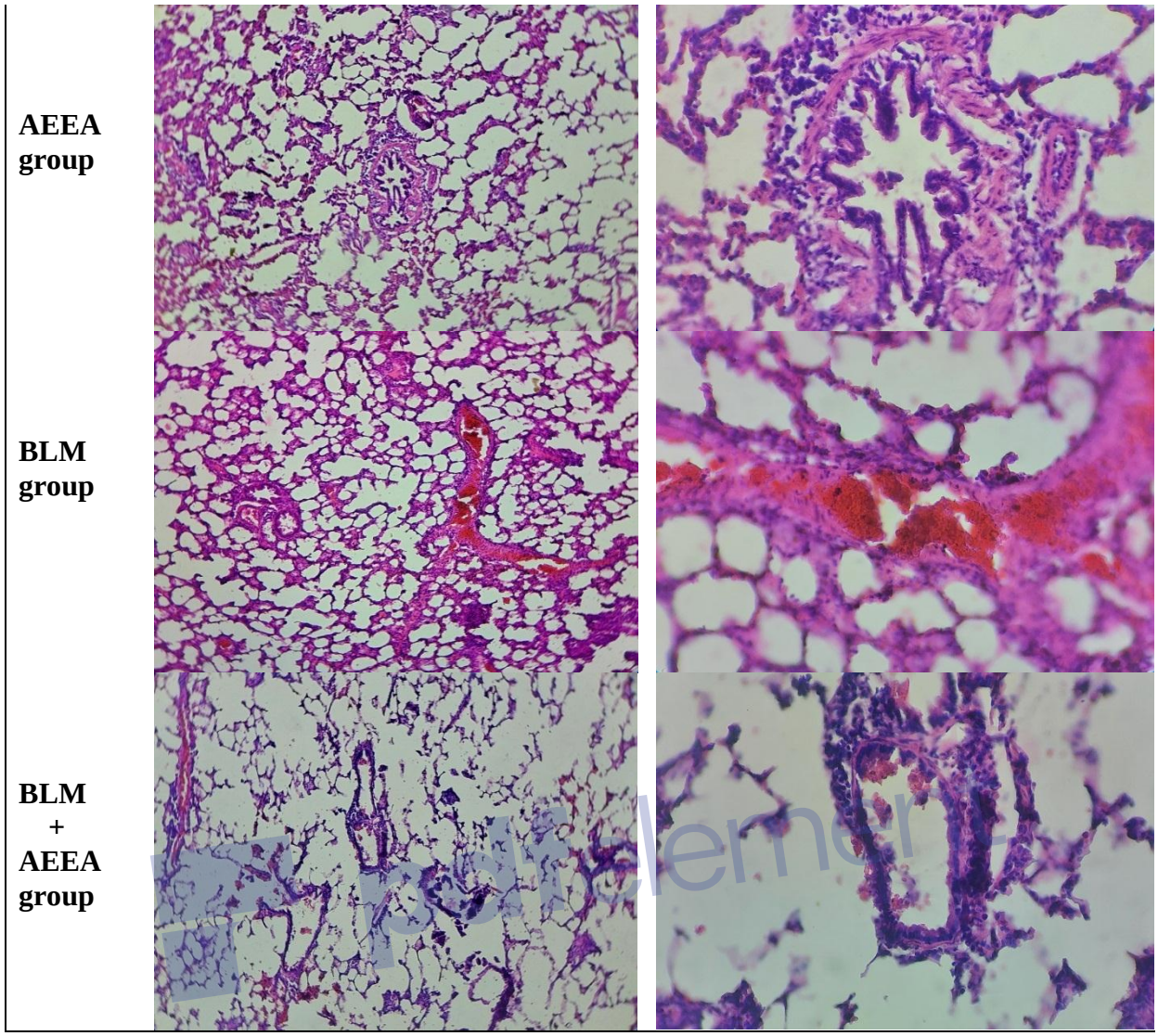



**Figure 26:** Photomicrograph of histopathological examination of lungs of control, AEEA group, BLM group and BLM+AEEA treated group, coloration with hematoxylin and eosin

## 2. Discussion

### 2.1. Statistical study

Previous studies have reported that *Ephedra alata* exert identical biological activities as antibacterial, liver function protection, prevention of cardiovascular diseases, and prevention and amelioration of cancer (Hamden et al., 2009). When comparing with other peoples using *Ephedra alata* collected from the southern Palestine as study of Jaradat et al (2016) which indicated that *Ephedra* is the most commonly used plant for breast cancer treatment. In Morocco, *Ephedra alata* subsp. alenda is used against diabetes (Ghourri et al., 2013). Research conducted by Fuad Al-Rimawi, et al., (2017) indicated that this plant grows widely in Palestine and very used in traditional medicine to treat allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, headaches. This plant also shows antimicrobial and anticancer activities. wilaya of Naâma using this plant as an anticancer, antidiabetic and hypotensive (Hadjadj et al., 2019).

Most of the respondents agree with the use of *Ephedra*, and especially in the case of cancer and problems of the respiratory system. Previous study show that *E. alata* is used against influenza, cough and rheumatism in many region as Bechar, Tunisia and in Tuareg from the Illizi region (Hibi et al., 2022; Sioud et al., 2020; Miara et al., 2019).

Chinese herbal formulae incorporating *Ephedra* have been investigated primarily for the treatment of lung cancer and other tumors, but in-depth research on the molecular mechanisms through which *Ephedra* functions in the treatment of various cancers has not yet been conducted, which may affect the subsequent clinical application of *Ephedra* (Tang et al., 2023).

## 2.2. *In vitro* study

### 2.2.1. Qualitative and quantitative phytochemical analysis

Preliminary phytochemical results revealed the presence of a wide range of bioactive secondary metabolites including, phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compounds and alkaloids with high concentrations of phenolic and flavonoids compounds. Other studies on *E. alata* Decne in both Bechar and southern Algeria showed bioactive components in the aqueous extract and compared to our study on *E. alata* in El-Oued state; On the abundance of steroids, phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compound and alkaloids for *E. alata* of El-Oued state, while the results showed that *E. alata* Decne for the state of Bechar did not contain terpenoids and *Ephedra alata* Decne for southern Algeria lacked the presence of both alkaloids and glycosides and was *E. alata* of El-Oued is very rich in the rest of the biologically active components compared to both types of *E. alata* Decne from Bechar and southern Algeria because it contains a lower percentage. (Benarba et al., 2021; Hibi et al., 2022).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities, several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Yadav et Agarwala, 2011). The high scavenging property of AEEA may be due to hydroxyl groups existing in the phenolic compounds that can provide the necessary component as a radical scavenger. Free radicals are often generated biological reactions or from exogenous factors. An increase of phenols in plant tissue may enhance plant resistance to stress conditions. Furthermore, they can be a source of important antioxidants for human health: for example, caffeic and gallic acids inhibit carcinogenesis (Ertani et al., 2016). In terms of exploiting the diverse saponin structures that display anti-cancer properties, presumably by targeting a range of different metabolic pathways (Osbourn et al., 2011). Saponins has the property of precipitating and coagulating red blood cells (Okwu et Josiah, 2006). Flavonoids, the main group of polyphenol compounds, are the most effective antioxidants and were associated with a wide gamut of pharmacological effects including antimicrobial, anti-inflammatory, and inhibition of platelet aggregation (Peng et al., 2018). Aromatic steroids exhibit a wide range of biological activities, but mostly they likely possess anticancer, anti-inflammatory action of steroids might be effective to relive symptoms caused by inflammation (Dembitsky et al., 2018; Mardani, 2012). Tannins play a major role in various antibiotics used intreating common

pathogenic strains (Mehta et Shahista, 2021), Tannins are defined as phenolic compounds and plants secondary metabolites which have beneficial effects on protein metabolism in ruminants, decreasing rumen degradation of dietary protein and increasing absorption of amino acids in the small intestine (Hassanpour et al., 2011), whereas the inhibition potency on condensed tannin that can be considered as preservative agent for antibacterial, antiyeast and antioxidant activities (Sulaiman et al., 2011). Terpenoids are reported to have anti-inflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and anti-bacterial activity (Indumathi et al., 2014), and play diverse functional roles in plants as hormones (McGarvey et Croteau, 1995), also a wide range of terpenoids have demonstrated pharmaceutical activity against human ailments such as cancer (Roberts, 2007). Glycosides are known to lower the blood pressure (Gilani et al., 2000), cardiac glycosides induce inhibition of cell proliferation and / or cell death in several cancer cell lines (Trenti et al., 2014; Winnicka et al., 2006). Alkaloids are one of the important classes of secondary metabolites which are found to possess important biological properties like analgesic, muscle relaxant, antioxidant (Roy, 2017). These are used to help humans and have been shown to be beneficial for some life-threatening diseases, as these compounds possess bactericidal, anti-histaminic, anticancer, central nervous system stimulant and depressant, herbicidal, insecticidal and fungicidal properties (Kaur et Arora, 2015), it is effective in anti-lipid peroxide production and act as antioxidant, antiradical (Pérez et al., 2003). Thus, the results obtained in this study suggest that these plants are proving to be an increasingly valuable reservoir of bioactive compounds with considerable medicinal value.

### 2.2.2. Antioxidant activity

In our work we assess the antioxidant activity by using DPPH assay. DPPH, a purple-colored, stable free radical is reduced to the yellow-colored diphenylpicrylhydrazine when antioxidants are added. The antioxidant capacity of the extracts were estimated and compared with ascorbic acid (positive control) using the stable DPPH radical (Kaouachi et Derouiche., 2018). The effect of antioxidants on DPPH radical scavenging was presumed to be due to their hydrogen donating ability. The reduction capability of DPPH radicals was determined by the decrease in its absorbance induced by antioxidants. It is visually noticeable as a discolouration from purple to yellow. There is a direct correlation between antioxidant activities and the reducing power of the components of some plants. The results of antioxidant activity are shown ascorbic acid had a very potent anti-radical activity compared to our extracts. Also, Our results indicate that the aqueous extract for *Ephedra alata* has a high activity against scavenging assay of free radical DPPH. The effective antioxidant activity shown by the results may be due to the abundance of bioactive components contained in this plant extract. The extract reduces and discolours the DPPH radical due to their ability to yield hydrogen to the free radicals produced during peroxidation. Phenolic compounds are well known as antioxidants and directed against free radicals associated with oxidative damage. Tannin and flavonoids act on the complications of diabetes by their antioxidant and anti-enzymatic properties, neutralizing the effect of free radicals and limiting the inflammatory reaction in different tissues (Zebidi et al., 2018), also Polyphenolic compounds prevent degenerative diseases such as cancer and cardiovascular disorders (Mahmoudi et al., 2023). Flavonoid shows antioxidant activity due to the presence of free -OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo (Sunil, 2014).

In the other hand, in our study we assess the FRAP assay in plant study. The reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. whereby the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the sample. In this assay system, the presence of antioxidants causes the reduction of the $Fe^{3+}$/ferricyanide complex to the ferrous form ($Fe^{2+}$), which is monitored by measuring the formation of Perl's Prussian blue at 700 nm (Qingming et al., 2010; Do et al., 2014), all extracts show some degrees of electron-donating capacity in a concentration-dependent manner. The results showed a high and close reducing power between ascorbic acid (positive control) and AEEA at their different concentrations. The data presented here indicate that the marked reducing power of AEEA seem to be attributed to their antioxidant activity. Flavonoids are a group of natural compounds with different phenolic structures, present in plants, are powerful antioxidants against free radicals, because they act as "radical-scavengers". This activity is attributed to their hydrogen-donating ability. Indeed, the phenolic groups of flavonoids serve as a source of a readily available "H" atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure, as The antioxidant activity of flavonoids depends on the arrangement of functional groups around the core structure. The configuration, substitution and total number of hydroxyl groups significantly affect several mechanisms of antioxidant activity, such as free radical scavenging and metal ion chelation ability (Tripoliet al., 2007; Heim et al., 2002).

### 2.2.3. Anti-inflammatory activity

The in-vitro anti-inflammatory activity or the ability of AEEA to inhibit protein denaturation was studied through inhibiting heat-induced albumin denaturation of tissue proteins results in its loss of function and causes inflammation. Compared to the standard, diclofenac, the tested compounds have shown high anti-inflammatory activity. The anti-inflammatory activity of *E. alata* back to presence of flavonoids in the plant (Jaradat, 2015), Also this is considered the last are powerful inhibitors of the production of prostaglandins, very active pro-inflammatory molecules. Flavonoids inhibit the migration of leukocytes by blocking their adhesion to the vascular wall (Lakache et al., 2021). As this beneficial effect may be due to the presence of polyphenols in *E. alata* extracts as it has been shown, in various in vivo and in vitro studies, that polyphenols decrease markers of inflammation and acted on many molecular targets at the center of inflammation signaling pathways (Lekouaghet et al., 2021; González-Gallego et al., 2010; Santangelo et al., 2007). Many studies have shown that many flavonoids and related polyphenols contribute significantly to the anti-inflammatory activities exerted by many plants (Marrassini et al., 2018), Terpenoids are reported to have anti–inflammatory (Indumathi et al., 2014).

In the hemolysis test. We found that extract was in High protected for the erythrocytes. The results revealed that *Ephedra alata* contains biologically active compounds that counter the decomposition of red cells (this is mainly due to the properties of its compounds such as phenols, flavonoids, and antioxidant tannins in eliminating reactive oxygen species). That have the ability to inhibit oxidative stress (Khattabi et al., 2022). Because the rate of haemolysis is much higher when red blood cells are treated with hydrogen peroxide .This could be attributed to the oxidative nature of hydrogen peroxide and its ability to destroy the cell membrane and

consequently the release of haemoglobin from cells. According to $H_2O_2$ cause degradation of hemoglobin in erythrocytes thus formed Fe ions generated by the reaction of the OH hydroxyl radical. The antihemolytic activity of plant extracts may be due to inhibition of the radical by the bioactive compounds in the extract which releases electrons to $H_2O_2$ thus neutralizing a water molecule (Hegaziet al., 2011; Lippi et al., 2006; Nagababu et al., 2013; Mohamed et al., 2012).

### 2.3. *In vivo* study

#### 2.3.1. Growth parameters

The influence of BLM and AEEA on body weight was evaluated in this study. Results showed that AEEA and BLM decrease the body weight compared to the control group. As Significant decreased diet consumption in AEEA group and BLM group were observed during the experimental days, This explains the lack of increase in body weight. The results of our study were consistent with other studies that demonstrated the effectiveness of *E. alata* in decrease weight (Tiss et al., 2020; Tiss et al., 2022), And also the lack of increase in body weight caused by BLM (Matsumoto et al., 1989).

Furthmore, the results showed that there was no significant increase in the relative organ weight (Liver and Lungs) of the BLM group compared with the control group. This might be explained by the fact that the significant decrease of body weight due to BLM occurred through loss of fat and muscle, and not a reduction of internal organ weight (Matsumoto et al., 1989). Also result indicate that AEEA treatment in BLM group cause a Significant decreases in the Relative liver and lungs weight compared to BLM group. This might be explained by the fact that the significant decrease of body weight due to AEEA occurred through loss of fat and muscle, and a reduction of internal organ weight.

#### 2.3.2. Hematological parameters

The results of hematological parameters, showed a decrease in WBCs level in the BLM group compared to the control group. This explains that BLM suppresses the immune system and decrease the number of WBCs (Thor et al., 2019; Zhu et al., 1996). On the contrary, a significant improvement of WBCs levels in the AEEA + BLM group compared to the BLM group. Through these results, we link this effect to the ability of the *E. alata* to strengthen immunity, The phytochemical analysis of AEEA showed the presence of phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compond, alkaloids; compounds that were reported to have medicinal activity against various diseases. Flavonoids and phenolic compounds of plants play important preventive role in cancer development. In several studies, flavonoids showed a wide range of antibacterial, anticancer, antiviral, and anti-inflammatory activities (Al-Awaidaet al., 2018).

In the other side, results show an increase in HCT, MCV, MCH and MCHC and a decrease in HGB level in BLM group compared to control. The histological results of the lung are shown the pulmonary changes induced by BLM. As confirmed by many studies of BLM-induced pulmonary fibrosis in rats, as you suggest that the total RBC count will be high during the progression of pulmonary fibrosis (Gouda et al., 2020). Pulmonary fibrosis results in lung dysfunction, including low arterial oxygen saturation, and this explains the rise in RBCs, as the

body tries to adapt to the situation by increasing the production of RBCs in a larger size, and thus will be followed by an increase in both HCT, MCV, MCH and MCHC. Decreased oxygen binding to RBCs may cause toxicity, Oxygen toxicity caused by a higher concentration of oxygen may be explained by increase of active oxygen species such as superoxide anion, hydroxyl radical and peroxides. The active form of BLM appeared to be the ternary complex: BLM-Fe(III)-$O_2$ . This complex containing active oxygen should give the pulmonary damage as the active oxygen species do (Ekimoto et al., 1984).

Results showed also that there was an amelioration of hematological parameters when treated by AEEA in BLM group. AEEA administration showed their regulatory effects on hematology parameters such as WBC, RBC, HCT, etc. The present study confirmed that *E. alata* intervention alleviated the levels of RBC, HCT, MCV, MCH and MCHC. This showed that AEEA could effectively modulate the levels of hematology parameters during the early progression of BLM-induced pulmonary fibrosis in rats.

### 2.3.3. Biochemical parameters

The present investigation revealed an increase of blood glucose level in the BLM group compared to controls group, these data indicate that hepatic dysfunction might occur due to BLM that target the PI3K/AKT/mTOR inhibition of this pathway may also lead to hyperglycemia by interrupting the intracellular response to insulin, bleomycin is able to cause cell damage independent from its effect on DNA by inducing lipid peroxidation (Hay et al., 1991).

Belomycin increase the risk of hyperglycaemia by decreases in pancreatic insulin secretion, increases in insulin clearance, decreases in glucose utilization in the periphery, causing decreased glucose transport, decreased glycogen synthesis, and increased glycolysis, and increases in gluconeogenesis all appear to contribute to the hyperglycemic effect of these drugs. Activation of this target inhibits nuclear localization of the transcription factor FoxO1, preventing transcription of genes involved in gluconeogenesis, and this traget is also involved in activation of glucose transport into the cells and glycogen synthesis, which plays a key role in nutrient sensing of the cell. Glucose metabolism is mediated through activation of hypoxia-inducible factor 1α, a transcription factor that upregulates expression of glucose transporters and glycolytic genes. Chronic inhibition of mTOR has been linked to decreased proliferation and destruction of insulin-producing pancreatic β cells (Goldmanet al., 2016), this suggestion is supported by Matsumoto et al (1989). In the opposite side AEEA treatment improve the blood glucose induced by BLM. In the present study, we found that the AEEA a crucial role in lowering serum glucose concentration, either by stimulation of insulin secretion or by extra-pancreatic action and therefore by the influence of glucose uptake and its utilization by different tissues. It has been reported that several bioactive molecules isolated from plants such as terpenes and our dishes are rich in flavonoids and have antioxidant influence pancreatic β cells and stimulate insulin secretion through their antioxidant activities (Sarkhail et al., 2007).

In this study we mentioned a decrease in urea, creatinine and Uric acid in BLM rats compared to normal rats. Creatinine, urea and uric acid are waste products produced by protein metabolism. They are eliminated by the kidneys, and generally used as an indicator of proper kidney function. When renal failure sets in, the levels of these parameters increase (Gowda et al.,

2010). These results may be explained by the degradation of proteins into amino acids then into urea and creatinine and the degradation of nucleic acids into uric acid, which will induce a decrease in protein compounds and the degradation of nucleic acids into acid urics, which will induce a decrease in protein compounds in the body (Hosten, 1990). This suggestion is opposite by the study of Kawai et al (1998).

Concerning the transaminases activities results show that AST activity was increased in BLM group compared to control. The release of transaminase from hepatocytes is largely due to hepatocellular damage or death. These enzymes are normally released at a constant rate with their usual levels in health being the equilibrium between the normal turnover of hepatocytes due to programmed cell death and the clearance of these enzymes from plasma. ALT is present prevalently in the hepatocyte cytoplasm, whereas AST is present in both hepatocyte cytoplasm and mitochondria (Zoppini et al., 2016).

The increase in serum enzymes may be due to tissue damage in the liver, kidney, and heart as a result of changes in cell membrane permeability, or also due to increased synthesis or decreased destruction transaminases induced by belomycin. This suggestion is opposite by Matsumoto et al (1989). We found that oral and daily administration of the crude extract of *Ephedra alata* on the BLM group at a daily dose of 30 days significantly increased plasma activity of AST compared to BLM group. These results suggest that the plant extract may have protected the liver from damage caused by belomycin. These data are in concurrence with those reported by Gong et al (2015), who obtained results showed an increase in the serum level of AST and ALT, these effects could be a consequence of the disturbing of several antioxidant system functioning.



### 2.3.4. Lipid profile

Our study showed that plasma cholesterol and serum triglyceride levels were significantly increased in rats treated with BLM. This increase can be explained on the one hand by insulin deficiency which inhibits 3-hydroxy-3 methyl glutamyl coenzyme A reductase (HMG- coA reductase) a key enzyme for cholesterol biosynthesis and, by the intense degradation of lipid compounds in adipose tissue. Hyperglycemia can cause a metabolic disorder resulting in changes in the normal glucose pathway, where part of the glucose is transformed into fatty acids and glycerol and then the synthesis of triglycerides and cholesterol (Jain et Surana, 2016) and due to BLM cytotoxicity has been demonstrated to be a result of reactive oxygen species generated during its metabolism, where BLM interacts with molecular oxygen and iron, producing superoxide anion and other oxygen metabolites (Desai et al., 2000).

Belomycin leading to an elevated level of lipid hydroperoxides (LOOH), a low paraoxonase activity, thus, cadmium toxicity causes disruption of lipid metabolism in addition to disruption of pro-inflammatory cytokine levels (Balci et al., 2012), increased serum cholesterol levels can be a sign of liver damage.

A decrease in cholesterol is recorded in the co-treatment with *E. alata* and BLM, this explained by the role of ephedrine and the molecule bioactive in *Ephedra* like Tanin, flavonoid and saponins, which are active substances widely found in plants and mushrooms. Among them,

saponins are abundant and mostly have good anticancer, anti-inflammatory, immunomodulatory and other medicinal values. The saponins are used as lipid membrane modulating substances. which increases the rate of the reducing metabolism of adipose tissue, these results agree with those of Murray et al (1984) and Williams et al., (1986).

### 2.3.5. Oxidative stress statue

Our results showed clearly the oxidative disturbance induced by BLM, which can be detected by catalase and FRAP activities depletion and the increase in MDA level in lungs, kidneys and liver of rats compared to control. It's demonstrated that the cytotoxic effect of BLM involves the formation of reactive oxygen species (ROS) that implicate the oxidation of Fe (II) to Fe (III), the FRAP method is based on $Fe^{3+}$ being reduced to $Fe^{2+}$ by the examined substance. Bleomycin forms a complex with Fe (II), which is subsequently oxidized to Fe (III) therefore decreased in the FRAP activity, inducing a reduction of oxygen to free radicals (Bahri et al., 2020; Reinert et al., 2013). Intracellular ROS formation lead to cell membrane attack via polyunsaturated fatty acids disintegration causing MDA formation, which explain in consequence MDA increase following BLM treatment. ROS generation can also induce several damages in cell organelles, so that intracellular antioxidant enzymes can no longer act properly against oxidative stress (Sleijfer, 2001). Our results showed clearly catalase activities depletion after acute BLM treatment (Bahri et al., 2020). Similar results are found by Teixeira et al In terms of increased MDA and decreased catalase (Teixeira et al., 2008).

Our results showed clearly the enhancement of oxidative stress parameters by AEEA treatment after using bleomycin. AEEA contains flavonoids and phenolic compounds which are possess very good antioxidant properties. Phenolic compounds are recognized as antioxidants due to their ability to donate hydrogen atoms. Furthermore، flavonoids are a large group of naturally occurring plant phenol compounds that inhibit lipid oxidation by scavenging radicals this is what led to a decrease in MDA in the kidneys and liver (Abdulazeez et al., 2019; Rice-Evans, 2001). Intracellular ROS formation lead to cell membrane attack via polyunsaturated fatty acids disintegration causing MDA formation, which explain in consequence MDA increase following BLM treatment in the liver, it appears that AEEA reduced lipid oxidation compared to BLM group (Bahri et al., 2020). Increased level of FRAP in the liver and is due to decreased Fe(III) and increased Fe(II), Polyphenols may react with radicals according hydrogen transfer from the antioxidant to the radical, electron transfer proton transfer, sequential proton transfer, sequential proton loss-electron, transfer and interaction of BLM with di-iron and its transformation into tri-iron corresponds to the transformation of tri-iron into di-iron by the action of the phenolic substances present in the plant (Kriston et al., 2009) and level normal level of FRAP in kidneys and lungs, because of the stability in the conversion of trivalent iron to binary iron. Decreased level of catalse enzymes in the liver, kidneys and lungs is due to In the presence of some flavonoids of AEEA the formation of unreactive catalase compound II has been detected (Krych et Gebicka, 2013). This is what led to a decrease in catalase in the liver, kidneys and lungs, and this is due to the inhibition of the catalase enzyme.

### 2.3.6. Histopathological section

In this present study, BLM causes a destruction and deep injury in lungs and liver cells compared to control. We can explain this effect as follows: BLM cytotoxicity has been demonstrated to be a result of reactive oxygen species generated during its metabolism, where BLM interacts with molecular oxygen and iron, producing superoxide anion and other oxygen metabolites (Desai et al., 2000). This may be particularly important in the lung and in part account for its ability to cause alveolar cell damage and subsequent pulmonary inflammation. The lung injury seen following BLM comprises an interstitial oedema with an influx of inflammatory and immune cells. This may lead to the development of pulmonary fibrosis, characterized by enhanced production and deposition of collagen and other matrix components. In this article we review our current understanding of the mechanism for BLM toxicity with special reference to the damage it causes to the lung (Hay et al., 1991), to induce the formation of toxic DNA lesions and to cause cell death (Brahim et al., 2008).

The BLM-induced ROS production is based on intracellular iron-binding. That is –iron complex reduces molecular oxygen to superoxide ($O_2^-$) and hydroxy radical ($OH^\bullet$). Free radicals causing strand cleavage of DNA and lipid peroxidation , it can also utilize $H_2O_2$ to cleave DNA in the presence of ferric ions (Kasper et Barth, 2014), Similar observations were reported by Su et al (2017).

Pulmonary fibrosis led to a decrease in lung function and eventually re-respiratory failure (Shi et al., 2016), that the liver of rats treated with belomycin showed focal coagulative necrosis and severe hydropic degeneration, by production ROS, liver oxidation was associated with the inflammation process, since both proteins have been linked to this process. It was reported that IL-1β and IL-8, as well as TNF-α, can promote inflammatory response. Similar study were reported by Su et al (2017).

In histological results of AEEA shows a high protection against tissues lysis induced by BLM, our in-vitro result appeared that AEEA containing different second metabolic compounds which they have anti-inflammatory property such as flavonoids inhibit leukocyte migration by blocking their adhesion to the vascular wall, ephedrine, pseudoephedrine, and ephedrine analogy were found to have strong anti-inflammatory activities in vivo. Ephedrine administration can produce an anti-inflammatory effect by stimulating the expression of IL-10 in dendritic cells (DCs) and inhibiting the production of induced inflammatory factors (such as TNF-α) through the PI3K/Akt and PGN pathways. Moreover, ephedrine can act on PI3K, Akt, and the downstream GSK-3β and p38 pathways، leading to the increased expression (Tang et al., 2023). Treatment with AEEA backs the cellular arrangement around the central vein and reduced necrosis. Crude extract of *E. alata* also protects the liver from BLM-induced damage, which may be due to its antioxidant property.

# Conclussion and Prespectives

## Conclusion

Phytotherapy is the use of medicinal plants that make it possible to treat everyday problems in a natural way, and it is being used increasingly as the results of the current study provide sufficient evidence that medicinal plants still play an important role in the primary health care system of the people of the El-Oued state.

- The results of the survey show that the *Ephedra alata* is widely used and emphasizes the importance of its effectiveness in treating many diseases in the region like diabetes, cancer, asthma…
- The phytochemical screening showed that the *Ephedra alata* plant extract contain a mixture of phytochemicals as phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compond and alkaloids with important quantitative value of total phenols and flavonoids which may contribute to the plant being a source of treatment for many diseases.
- The in vitro study of *Ephedra alata* aqueous extract appeared an essential antioxidant and anti-inflammatory activities which may suggest these products to be an effective treatment for many diseases associated with oxidative stress and inflammatory factors.
- The hematological analysis concluded that the rats treated by AEEA may have a positive impact in the hematopoietic and immune systems which protect against anemia and immune diseases.
- The positive influence of *Ephedra alata* aqueous extract, on restoring some biochemical markers that demonstrate the beneficial effect of treatment against the physiologic and metabolic diseases
- The action of AEEA was observed in the decrease of MDA and increases of catalase and FRAPS levels which may give it the property of antioxidant activity which can protect against several diseases.
- The high protection capacity of our treatment was extended to microscopic level which shows their high protection in cells stabilization against lungs, kidneys and liver injury induced by BLM. We shed the powerful effect of AEEA in our histological sections. This allows us to consider that this treatment has potential effect to limiting the histological alteration which associated with disease complications.

**Perspective**

For future studies, we hope that studies will focus more on:

- Detect all compounds of *Ephedra alata* aqueous extract;
- Testing them in anti-diabetic, inflammation and anticancer action by cell culture and in-vivo methods;
- Try to make extraction and the separation these compound;
- Try to make a specific drug form *Ephedra alata*.

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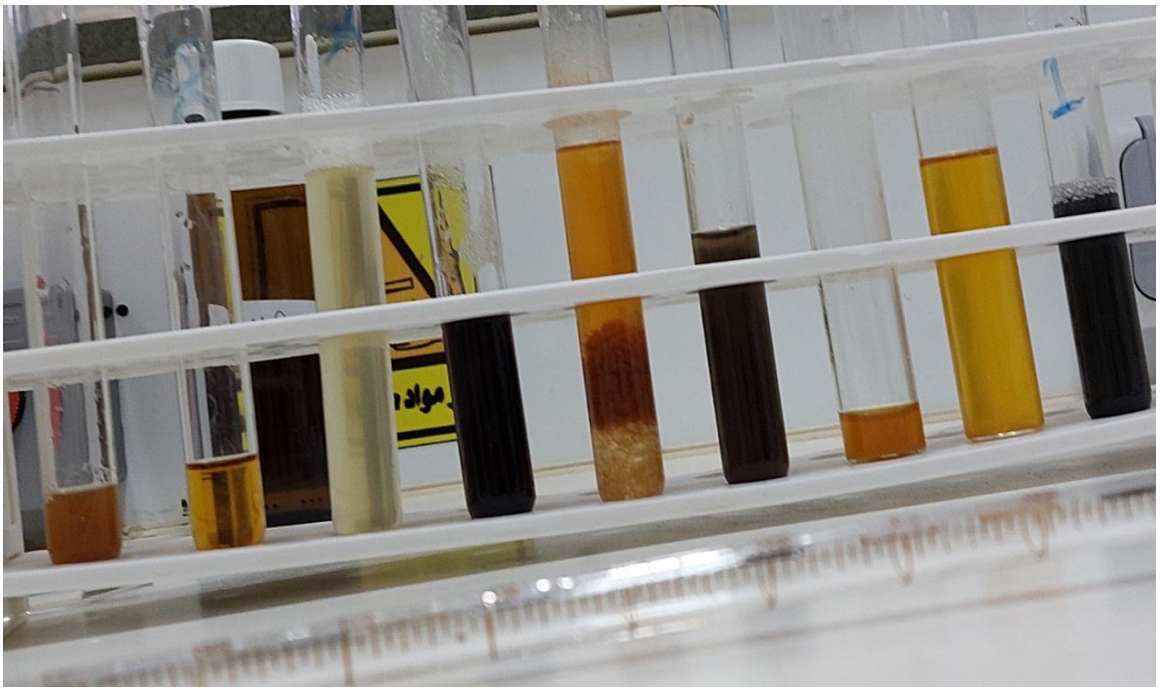
# Annex

**Annex 01:**

| N° | Question | Yes | No | Notes |
|---|---|---|---|---|
| 01 | Sex | | | |
| 02 | Age | | | |
| 03 | Weight | | | |
| 04 | Height | | | |
| 05 | Have you used alenda before? | | | |
| 06 | Do you know someone who has used alenda? | | | |
| 07 | Why did you use it? | | | |
| 08 | Did you use it in one way or in multiple ways? | | | |
| 09 | How you use it? | | | |
| 10 | How long do you use it? | | | |
| 11 | Did you use it continuously or intermittently? | | | |
| 12 | What is the time between one dose and the other? | | | |
| 13 | The time you use it? | | | |
| 14 | Did you achieve your purpose of using it? | | | |
| 15 | Were you using other medicines or plants while you were using them? | | | |
| 16 | Do you suffer from other diseases? | | | |
| 17 | What is the type of food you eat? | | | |
| 18 | Do you eat sweets and how much do you eat them? | | | |
| 19 | Do you have negative symptoms? Mention it if any | | | |
| 20 | The concentration of the dose you used? | | | |
| 21 | Did it reduce the symptoms of the disease or get rid of it completely? | | | |



**Annex 02:**



**Annex 03:**

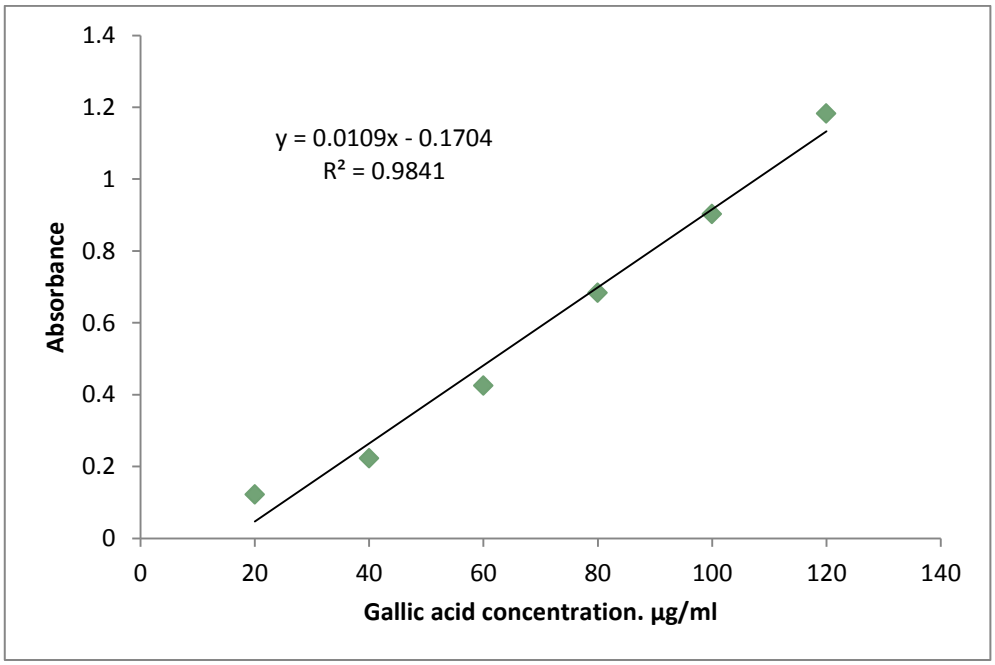



**Figure:** Gallic acid calibration curve for the determination of polyphenols

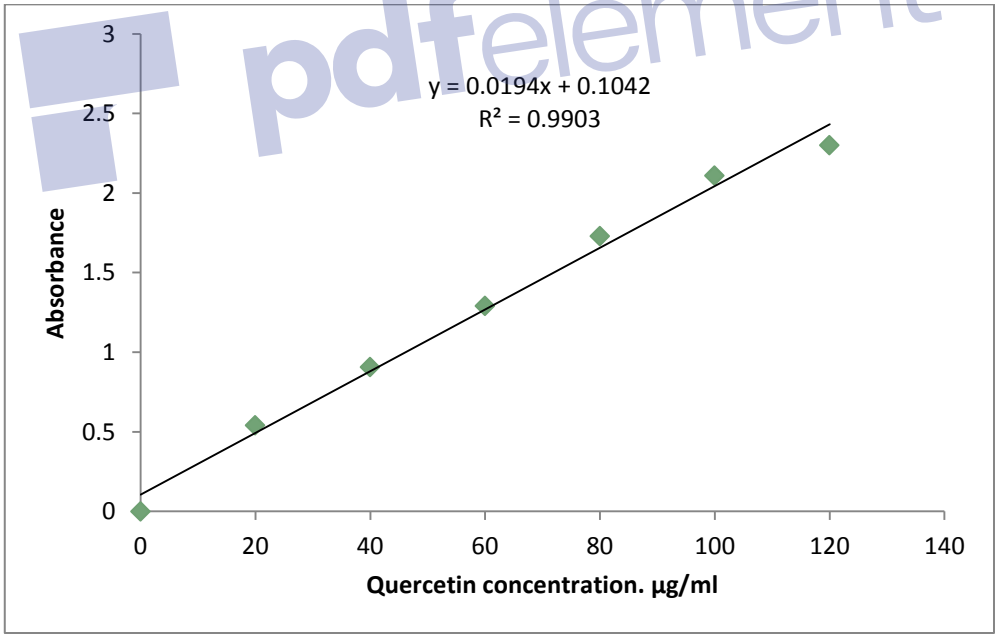



**Figure:** Quercetin calibration curve for flavonoid assays

**Annex 04:**

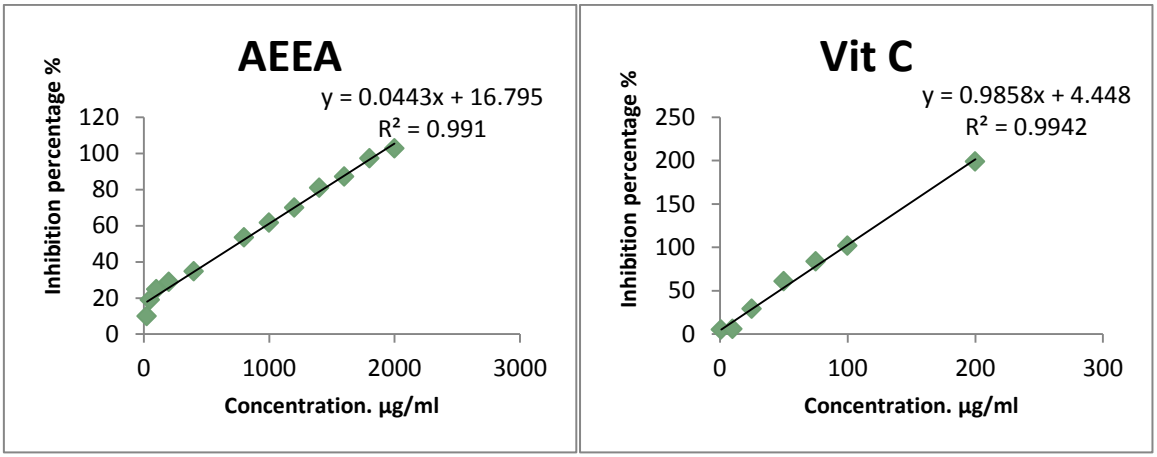



**Figure:** Inhibition percentage versus ascorbic acid and AEEA concentrations for the determination of IC50 for DPPH activity.

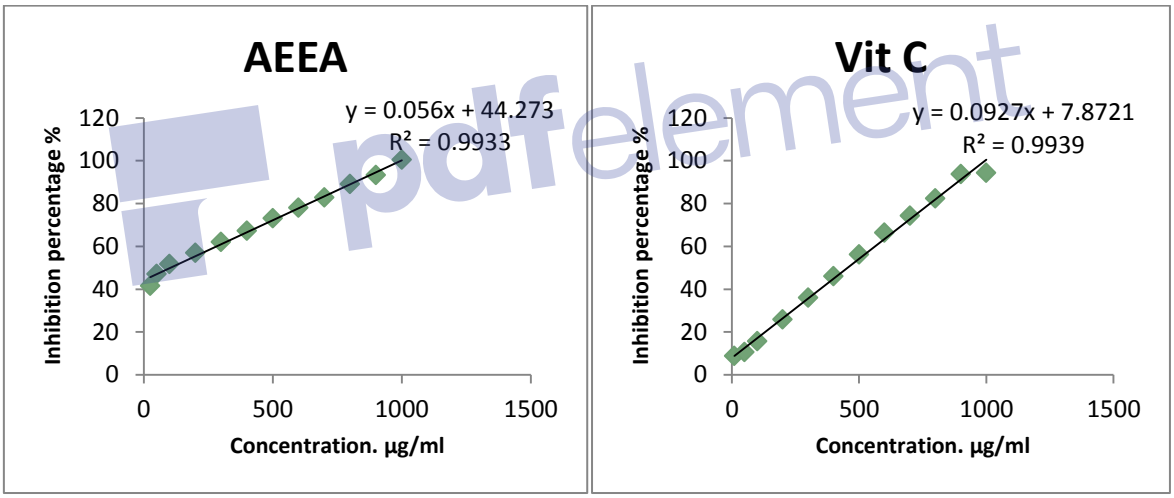



**Figure:** Inhibition percentage versus ascorbic acid and AEEA concentrations for the determination of IC50 for FRAP activity.

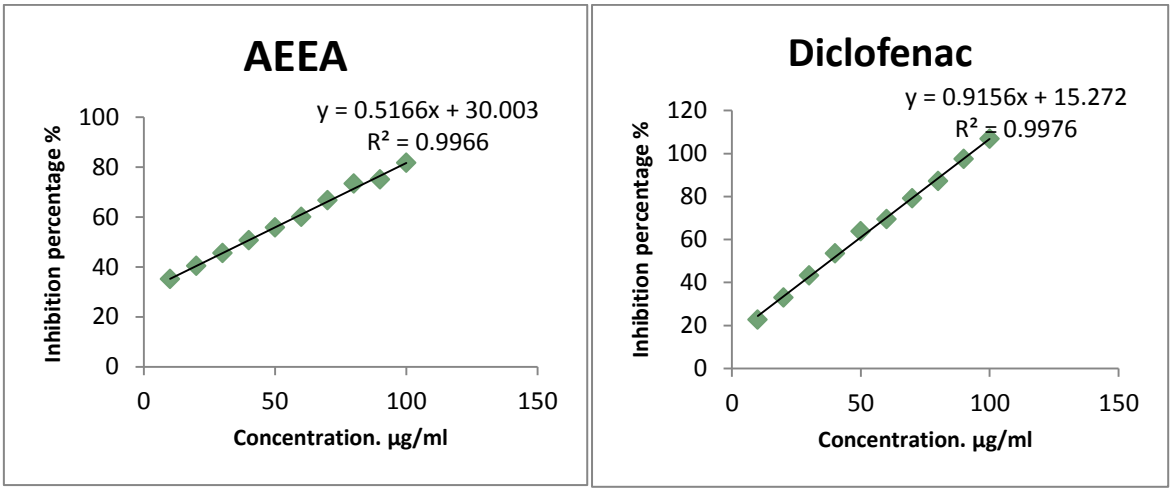



**Figure:** Anti-inflammatory activity of AEEA

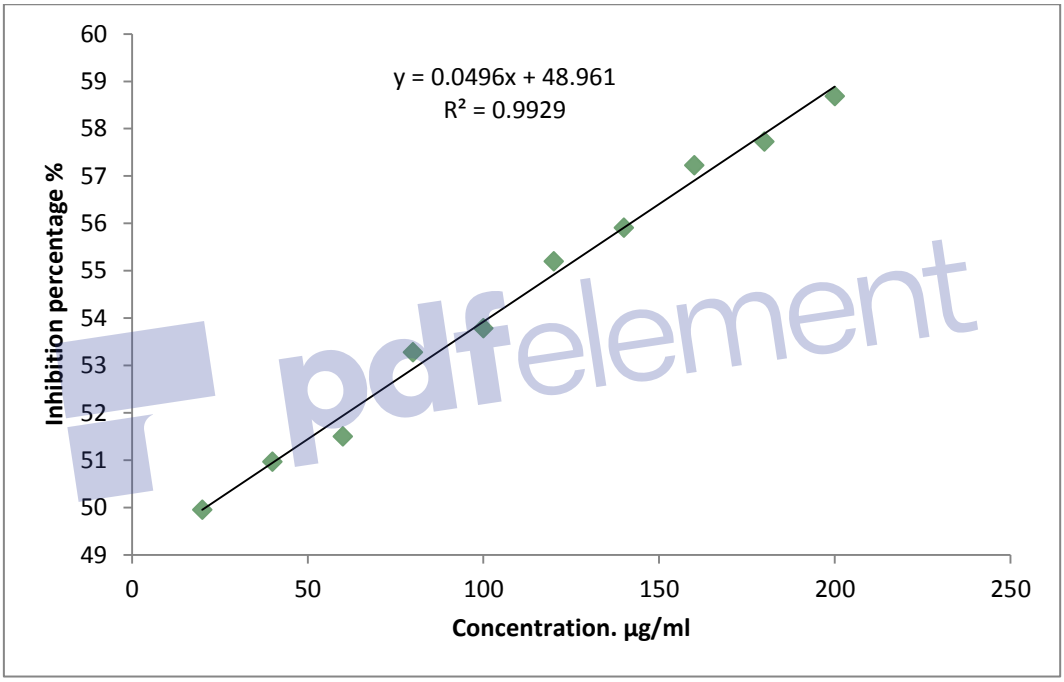



**Figure:** Hemolysis activity of AEEA

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INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

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# Nature de la demande de protection *

Brevet d'invention ☑ | Extension de la demande internationale selon le PCT ☐ | Certificat d'addition ☐

[71] - DEPOSANT[S] : *Nom, Prénom, [dénomination], et Adresse complète*

Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

*Nationalité du ou des déposants* Algérienne

[72] - INVENTEUR[S] : *Nom, Prénom, Adresse*

ZOUARI, Ahmed Wafa, Cité Ali Derbal, Robbah, El Oued, 39000, Algérie
CHADOU, Meriem, Elnezla, El Oued, 39000, Algérie
BEN GHALIA, Wissal, Cité Ali Derbal, Robbah, El Oued, 39000, Algérie
CHETEHOUNA, Sara, Cité belle vie, El Oued, 39000, Algérie
BOULAARES, Islam, cité sidi Abdallah, El Oued, 39000, Algérie
DEROUICHE, Samir, Pôle Universitaire, Cité Echott, El Oued, 39000, Algérie



[54] - TITRE DE L'INVENTION :

Gouttes naturelles contre les infections pulmonaires

[30] – REVENDICATION DE PRIORITE (S)

| [31] - N°[s] de dépôt | [32] - date[s] : | [33] - pays d'origine | Nature de la demande |
|---|---|---|---|
| | | | |

| Numéro de dépôt | Date de dépôt | Heure |
|---|---|---|
| 22 1059 | 21 DEC. 2022 | 13:23 |

N° de la demande internationale et date internationale de dépôt

Visa

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EL BOUANI EL Hafsi
Service Dépôt
D Z P
Chef de Service