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« THE STATE OF BIOLOGICAL MARKERS IN *WISTAR* RATS EXPOSED TO LEAD AND TREATED WITH *Allium triquetrum* L. »

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الحمد لله ...

To *me* 😎

To My Parents ❤️

To My Sister *Fadia* and my brothers

To My Beloved Nephews *Adouma* & *Abduch* ...

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Abstract

Our study aims to evaluate the protective effect of bulbs (B) and leaves (L) of wild garlic aqueous extracts (*Allium triquetrum* L.) on the reproductive, hepatic, and renal functions induced by lead acetate (Pb) in *Wistar* rats. Simultaneously, an *in vitro* study was affected including phytochemical screening, chromatogram (HPLC), and antioxidant activity of *A. triquetrum*. The *in vivo* experiment was realized using male rats that divided into 18 groups; the control (C), the Pb (500mg/Kg BW), the positive controls of B and L at different concentrations (2, 3, 4, and 6 g/Kg BW), in addition to the mixtures of each of Pb-B (Pb-B1, Pb-B2, Pb-B3, Pb-B4) and Pb-L (Pb-L1, Pb-L2, Pb-L3, Pb-L4). After 21 days of treatment, Sperm characteristics were evaluated by CASA system, plasma testosterone, and biochemical parameters, as well as testicular, epididymal, hepatic, and renal oxidative stress markers. The phytochemical screening proved that the extracts of B and L were rich in various compounds (polyphenols, flavonoids, and tannins), while the HPLC profile demonstrated that leaves contain more rutin, and, isoquercetin. Moreover, leaves extracts showed better antioxidant activity (DPPH) than bulbs. The *in vivo* results revealed a significant decrease in the weight of testicles and epididymis, sperm concentration, motility, testosterone, velocity, vitality, round cells, total proteins, albumin, GSH, and GPx level in Pb-treated rats compared to the control, alongside a significant increase in liver and kidney weights, plasma levels of AST, ALT, ALP, urea, creatinine, uric acid, and MDA tissue. However, the co-administration of *A. triquetrum* extracts (Pb-B and Pb-L) displayed a significant difference in the levels of all previous markers compared to the Pb-group, in a dependant dose manner. In conclusion, aqueous extracts of *A. triquetrum* bulbs and leaves have the potential to attenuate the repro-toxicity, hepatotoxicity, and nephrotoxicity of Pb through the modulation of the most studied markers in *Wistar* rats.

Keywords: *Allium triquetrum* L., Pb, Repro-toxicity, Hepatotoxicity, Nephrotoxicity, Protection, Rat.

Résumé

L'intérêt de notre étude est d'évaluer l'effet protecteur des bulbes (B) et des feuilles (L) d'extraits aqueux d'ail sauvage (*Allium triquetrum* L.) sur la fonction reproductive, hépatique et rénale induites par l'acétate de plomb (Pb) chez des rats mâles *Wistar*. Simultanément, une étude *in vitro* a été affectée comprenant le dépistage phytochimique, le chromatogramme (HPLC) et l'activité antioxydante de *A. triquetrum*. L'expérience *in vivo* a été réalisée en utilisant des rats mâles répartis en 18 groupes : le contrôle (C), le Pb (500mg/Kg P.C), les contrôles positifs B et L à différentes concentrations (2, 3, 4 et 6 g/Kg P.C), en plus de groupes combinés de chacun des Pb-B (Pb-B1, Pb-B2, Pb-B3, Pb-B4) et Pb-L (Pb-L1, Pb-L2, Pb-L3, Pb-L4). Après 21 jours de traitement, les caractéristiques des spermatozoïdes ont été évaluées par le système CASA, la testostérone plasmatique et les paramètres biochimiques, ainsi que les marqueurs du stress oxydatif testiculaire, épididymaire, hépatique et rénal. Le criblage phytochimique a prouvé que les extraits de B et L étaient riches en divers composés (polyphénols, flavonoïdes et tanins), tandis que le profil HPLC a démontré que les feuilles contiennent plus de rutine et d'isoquercétine. De plus, les extraits de feuilles ont montré une meilleure activité antioxydante (DPPH) que de Bulbes. Les résultats *in vivo* ont révélé une diminution significative du poids des testicules et de l'épididyme, de la concentration des spermatozoïdes, motilité, la testostérone, vitesse, vitalité, cellules rondes, des protéines totales, de l'albumine, du GSH et du niveau de GPx chez les rats traités au Pb par rapport au contrôle, parallèlement à une augmentation significative du poids du foie et des reins, des taux plasmatiques d'AST, d'ALT, d'ALP, d'urée, de créatinine, d'acide urique et de MDA tissulaire. Cependant, la co-administration d'extraits d'*A. triquetrum* (Pb-B et Pb-L) a montré une différence significative dans les niveaux de tous les marqueurs précédents par rapport au groupe Pb, de manière dépendante de la dose. En conclusion, l'extrait aqueux d'*A. triquetrum* a le potentiel d'atténuer la repro-toxicité, l'hépatotoxicité et la néphrotoxicité du Pb par la modulation de la plupart des marqueurs étudiés chez les rats *Wistar*.

Mots clés : *Allium triquetrum* L., Pb, Repro-toxicité, Hépatotoxicité, Néphrotoxicité, Protection, Rat.

الملخص

تكمن أهمية دراستنا في تقييم التأثير الوقائي المحتمل للبصيلات (B) والأوراق (L) لمستخلصات الثوم المائية (*Allium triquetrum* L.) على الوظيفة التناسلية، الكبدية والكلوية التي يسببها أسيتات الرصاص (Pb) لذكور جرذان ويستار. في نفس الوقت، أجريت دراسة مختبرية تمثلت في فحوصات كيميائية-نباتية، كروماتوجرافية (HPLC) ، بالإضافة إلى نشاط مضادات الأكسدة. أجريت التجربة في الجسم الحي باستخدام ذكور الفئران التي قسمت إلى 18 مجموعة تمثلت في الشاهد (C)، الرصاص (500ملغ /كلغ وزن الجسم)، الشواهد الموجبة (B) و (L) بتركيزات مختلفة (2،3،4، 6 غ/ كلغ وزن الجسم)، بالإضافة إلى المجموعات المدمجة - Pb-B, Pb-B1, Pb-B2, Pb-B3, Pb-B4)و Pb-L (Pb-L1,Pb-L2, Pb-L3, Pb-L4). بعد 21 يومًا من العلاج، قيمت خصائص الحيوانات المنوية عن طريق نظام CASA، التستوستيرون البلازمي، والمؤشرات البيوكيميائية البلازمية، بالإضافة إلى مؤشرات الإجهاد التأكسدي للخصية، البربخ، الكبد والكلى. أثبتت الفحص الكيميائية النباتي أن مستخلصات (B) و (L) كانت غنية بمركبات مختلفة (البوليفينول، الفلافونويد والتانين)، بينما أظهر تحليل HPLC أن الأوراق تحتوي على المزيد من الروتين والأيزوكيرسيتين. علاوة على ذلك، أظهر مستخلص الأوراق نشاطًا مضادًا للأكسدة (DPPH) أفضل من البصيلات. أظهرت النتائج في الجسم الحي انخفاضًا معنويًا في وزن الخصيتين والبربخ، وتركيز الحيوانات المنوية، الحركة، التستوستيرون، السرعة، الحيوية، الخلايا المستديرة، البروتينات الكلية، الألبومين، مستوى الجلوتاتيون و الجلوتاتيون بيروكسيداز في الفئران المعالجة بالرصاص مقارنةً بمجموعة الشاهد، إلى جانب زيادة كبيرة في وزن الكبد والكلى، ومستويات إنزيمات ناقلات الأمين (ALT AST) والفوسفاتاز القلوي (ALP) البلازمية، اليوريا، الكرياتينين، حمض اليوريك، ومستوى MDA . ومع ذلك، أظهرت المستخلصات النباتية المدمجة مع الرصاص (Pb-B وPb-L) فرقًا معنويًا في مستويات جميع المؤشرات السابقة مقارنة بمجموعة الرصاص، مرتبطة بالجرعة المعتمدة. في الختام، أظهرت المستخلصات المائية للثوم ثلاثي الأوراق قدرة على التقليل من السمية التناسلية، الكبدية، والكلوية الناتجة عن الرصاص من خلال تعديل معظم المؤشرات المدروسة في ذكور الجرذان.

الكلمات المفتاحية: ثوم ثلاثي الأوراق (*Allium triquetrum* L.)، رصاص، سمية تناسلية، سمية كبدية، سمية كلوية، حماية، جرذان.

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INTRODUCTION

Detoxification is the process of purifying the body from compounds that have a detrimental effect on cell functions and structures. Modern research has revealed that a wide range of plants extracts can neutralize toxins and protect systems from the toxic effects of drugs and chemicals due to the wide variety of free radical scavenging Phyto-components (**Al-Snafi, 2015**; **Benabderrahim *et al.*, 2019**; **Otmani *et al.*, 2019**; **Aouecheri & Saka, 2020**; **Mansouri *et al.*, 2021a**). Garlic spies is one of the most multipurpose medicinal plants used in traditional herbal medicine to prevent and treat a broad range of ailments, including cardiovascular diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension, and diabetes (**Anwar & Younus, 2017**). In addition, a wide array of therapeutic effects of *Allium* species have attracted particular attention of modern medicine because of their widespread use as antibacterial and antifungal (**Kothari *et al.*, 2020**), as well as to an antioxidant (**El-Sebaey *et al.*, 2019**), for the treatment of various ailments such as antitumor, anti-inflammatory activities (**Vijayakumar *et al.*, 2019**), hypolipidemic, antidiabetic, neuroprotective, antimicrobial activities (**Kothari *et al.*, 2020**), reproductive (**Mansouri & Abdennour, 2011**), hepatoprotective and nephroprotective agent (**El-Sebaey *et al.*, 2019**; **AL-Megrin *et al.*, 2020**; **Moussa *et al.*, 2021**). Likewise, garlic contains several sulfur compounds, enzymes, minerals, vitamins, amino acids, glycolipids, and phospholipids (**Josling, 2005**). The physiological functions of garlic *A. triquetrum* are suitable to its bioactive constituents such as saponin, mucilage, phenols, and especially flavonoids (**Lanzotti *et al.*, 2014**). Furthermore, allicin is a major biologically active component of garlic clove extracts with a potent antioxidant activity (**Butt *et al.*, 2009**). The *A. triquetrum* is a very early blooming species that grows vigorously in cultivations. It grows in water, humid forests, and is consumed raw or steamed in the same way as leeks. *A. triquetrum* possesses several vernacular names (triangular-stalked garlic, three-cornered leek), which refer to different taxa (**Corea *et al.*, 2003**).

Toxicity is the ability of a substance to cause destructive consequences to a cell, organ system, or body. Many chemicals found in the environment such as metals are found in many industries that can affect living organisms (**Drif *et al.*, 2019**). Lead (Pb) is among the metals that need more attention concerning its damaging effects on biota. Previous studies have documented the toxic effects of Pb on biological systems resulting in various pathological and clinical consequences on almost all the organs mainly the brain, kidney, liver, and testis due to their sensitivities (**Kabeer *et al.*, 2019)**. Several pathological lesions were observed in the testis due to Pb-induced toxicity are associated with the risk of

infertility (**Allouche *et al.*, 2009**), through its accumulation in testes, epididymis, vas deferens, seminal vesicle, and seminal ejaculate (**Senapati *et al.*, 2001**), which leads to spermatogenesis impairment, sub-fertility, and decreased testosterone levels (**Berredjem *et al.*, 2014**), sperm count, motility and morphology (**Chowdhury, 2009**). Moreover, when it entered the body, it mainly attacks liver and kidney tissues (**El-Boshy *et al.*, 2019**; **Ezejifor, 2019**; **Almatroodi *et al.*, 2020**), causing several pathologies such as necrosis, vacuolization, and inflammatory cell infiltration for the liver, as well as protein melting, necrosis, glomerulus atrophy, and inflammation for the kidneys (**AL-Mergin *et al.*, 2020**).

Recent studies indicated that lead increased lipid peroxidation levels and inhibited the activities of the antioxidant defense system, including superoxide dismutase, catalase, and glutathione peroxidase, as well as reduced glutathione (**El-Boshy *et al.*, 2019**; **AL-Mergin *et al.*, 2020**; **Sun *et al.*, 2021**). It is also known to provoke damage to membranes, DNA, and proteins (**Elgawish & Abdelrazek, 2014**) and alter metabolic function (**Chowdhury, 2009**). Besides, previous investigations indicated that the administration of herbal plants can alleviate oxidative stress induced by Pb in experimental models (**Corpas *et al.*, 2002**; **Hamadouche *et al.*, 2013**; **EL-Magd *et al.*, 2016**).

So far, no studies have been conducted on the spontaneous species of *Allium triquetrum* L. in alleviating metal toxicity. Considering the valuable phytochemical composition and the antioxidant activity of *A. triquetrum* that could be a promising source of interesting phenolic compounds. Thereby, this investigation provides the first evidence to evaluate the possible protective efficiency of bulbs and leaves aqueous extract of wild garlic *A. triquetrum* against lead acetate-induced physiological alterations in male *Wistar* rats to shed light on its health benefits.

The objectives of this research are to shed light on the possible valuable phytochemical composition and the activity of Allium spp. In view of that, the present study focused to determine the chemical composition of bulbs and leaves aqueous extracts of the wild *A. triquetrum* by high-performance liquid chromatography (HPLC) analysis, in addition to their antioxidant activities.

Since no study has been performed on the detoxifying effect of *A. triquetrum* against lead-induced toxicity in male *Wistar* rats. This work investigates the possible mitigating role of wild garlic against this dangerous metal that is found everywhere in our environment and threatening human health. Therefore, the markers of reproductive, hepatic, and renal functions have been evaluated.

CHAPTER I

BIBLIOGRAPHICAL STUDY

Phytotherapy

Phytotherapy, or herbalism, is defined as the use of plants or herbs as medicines to treat or prevent disease in humans and animals by regulating body functions and to combat the effect of reactive metabolites, such as reactive oxygen species (ROS), and environmental pollutants such as xenobiotics. Several reports have shown the positive effects of plant extracts used in animal feed or as a remedy. The use of plants focuses on the analysis of the body's constituent systems which can reduce the incidence of drug resistance and modulate the immune, neuroendocrine, hormonal, and drainage systems in the prevention of diseases (**Devoyer, 2012**; **Ojo *et al*., 2017**; **Yasmin *et al*., 2020**).

Nowadays, the interest in these medicinal plants is booming due to the integration of modern techniques to evaluate the quality, safety, and efficacy of secondary metabolites on the one hand, and on the other hand; to explore the potential role of drugs developed from these metabolites in health care. Indeed, plants, with their wide variety of phytochemical constituents, have significant potential in the treatment of several human and animal diseases (**Agaie *et al*., 2007**; **Da *et al*., 2015**; **Dongock *et al*., 2018**; **Ouédraogo *et al*., 2019**). Plant constituents vary considerably depending on several intrinsic and extrinsic factors such as genetic, cultural, and environmental factors such as the place of harvesting, the age and part of the plant harvested, the period and time of harvesting, the method of harvesting, drying, storage, transport. Apart from that, phytochemical compounds of therapeutic interest can be derived from many parts of the plant such as bark, leaves, flowers, roots, fruits, seeds, etc. with varying contents. These biologically active compounds can be isolated from the plant by traditional processes such as maceration, decoction, infusion, and extraction (**Ouedraogo *et al*., 2021**).

Vegetable Kingdom

The Plants are divided between the brown line and the green line (*Fig. 1*). The brown lineage includes brown algae and Diatoms (also called Chromophytes). Their chloroplasts result from secondary endosymbiosis. The chloroplasts of the green lineage are from simple endosymbiosis by the incorporation of Cyanobacteria. This lineage includes the red algae (or Rhodophytes), and Chlorobiontes, which themselves are divided into green algae (or Chlorophytes) and Embryophytes.

Embryophytes include all land plants. They are divided between Bryophytes (mosses and sphagnum mosses), and Tracheophytes, which are plants with sap conducting tissues (or plants with vessels). Tracheophytes are divided into two lineages, on the one hand, Pteridophytes including ferns, and on the other hand Spermatophytes or seed plants, characterized by the formation of an ovule. Eventually, the Spermatophytes include the Gymnosperms, which are plants with a naked ovule, and the Angiosperms, which are plants with an ovule, and the Angiosperms or plants with flowers (**Reynaud, 2011**; **Dupont & Guignard, 2012**).

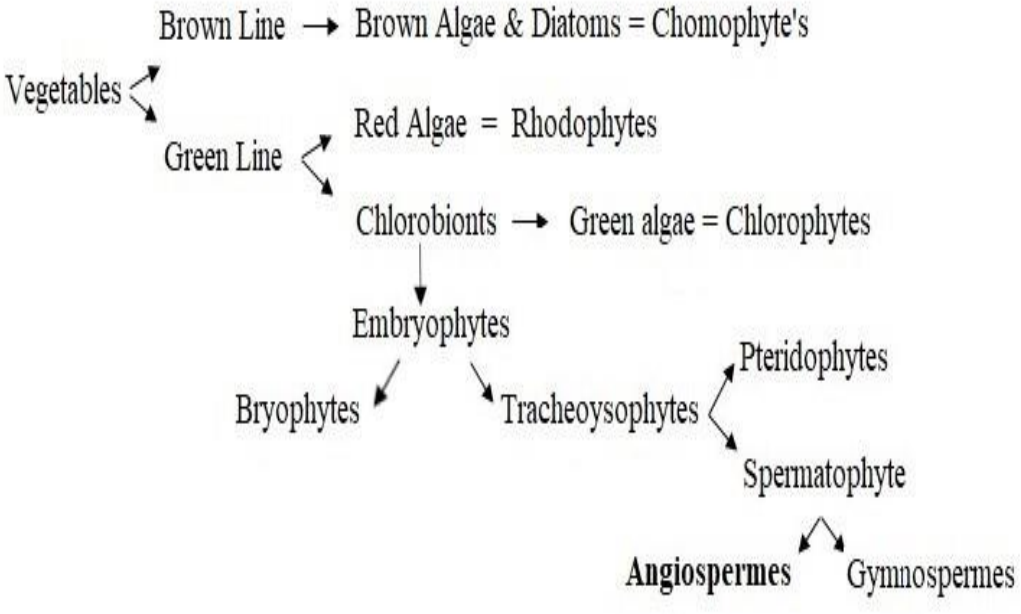

Fig 1. Classification of the Plant Kingdom and Embryophytes.

Part I - The Wild Garlic Allium triquetrum L.

1. General Description of Amaryllidaceae

Amaryllidaceae is a family of more than 1700 species, of which the genus Allium is the majority with about 750 species. Many species are used as ornamental plants, such as Amaryllis, and others are used in food, such as garlic **(Dupont & Guignard, 2012)**.

The Amaryllidaceae are monocotyledonous plants that the seed has only one cotyledon. They are herbaceous, perennial, and bulbous plants. However, in the Allium genus, the bulbs can be perennial such as in the leek, biennial as in onion, or annual as in cultivated garlic. The leaves of Amaryllidaceae are sheathing at the base. The plants of this family present an inflorescence in umbel with regular flowers in general (actinomorphic flowers), bisexual, and which have three parts per whorl (trimeric flowers). The inflorescence is first surrounded and protected before flowering by a membranous spathe, formed by the welding of bracts (generally 2). The perianth is composed of 6 tepals (3 sepals and 3 petals), distributed on 2 whorls. The androecium presents 2 whorls, with 3 stamens on each circle. The gynoecium is made up of 3 fused carpels, thus forming a trilocular ovary. This ovary is super for the species of the Allium genus whereas it is inferior for the other species of Amaryllidaceae. The ovules are anatropous or campylotropous, and their number varies according to the species.

The fertilization gives birth to a dry dehiscent fruit opening in 3 valves. It is a loculicidal capsule with 3 lodges, coming from a syncarpous ovary, i.e., formed from closed carpels that have fused **(Botineau, 2010)**.

The genus *Allium* is one of the largest genera of monocotyledons comprising about 915 species, rich in species for food including culinary herbs as cultivated garlic (*Allium sativum*), rocambole garlic (*Allium scorodoprasum*), onion (*Allium cepa*), shallot (*Allium ascalonicum*), leek (*Allium porrum*), chives (*Allium schoenoprasum*) and green onions like spring onion (*Allium fistulosum*) **(Teshika *et al.*, 2019**). However, several wild species are also known to have medicinal virtues such as *Allium ursinum* and *Allium roseum* L., and others that have been used for food and ornamental purposes as *Allium triquetrum* L (**Corea *et al.*, 2003**; **Muscogiuri *et al.*, 2020**).

***Part I - The Wild Garlic Allium triquetrum* L.**

Table (1). Some common edible *Allium* genus.

Common Name	Scientific Name	References
Red Onion 	*Allium cepa*	**Silmestand *et al*., 2007**
Yellow Onion 		
White onion 		
Italian shallot 	*A. ascalonicum*	**Bonaccorsi *et al*., 2008**
French shallot 		
Ramps 	*A. tricoccum*	**Dabbek *et al*., 2019**
Chinese chives 	*A. odorum* *(A. tuberosum)*	**Miean & Mohamed, 2001**
Welsh onion 	*A. fistulosum*	
Ramson bear's 	*A. tricoccum*	**Oszmianski *et al*., 2013**
Garlic 	*A. sativum*	**Kim *et al*., 2013**
Leek 	*A. porrum*	**Soininien *et al*., 2013**
Three-corner garlic 	*A. triquetrum*	**Kahalerras *et al*., 2021**

Part I - The Wild Garlic Allium triquetrum L.

I- PHYTOCHEMICAL & PHARMACOLOGICAL STUDY

Allium spp. plants are used generally for the treatment of many diseases and in the food industry because of their phytonutrient content.

1- Biological Property

Allium species are useful in reducing the risk of cardiovascular diseases due to their antithrombotic, antihypertensive, hypolipidemic, hypocholesterolemic, and anti-hyperhomocysteine effects. They are also known for other biological properties such as antimicrobial, antiviral, antidiabetic, antiprotozoal, antispasmodic, anticarcinogenic, antimutagenic, antiasthmatic, anti-inflammatory, hepatoprotective, neuroprotective, hypotensive, hypoglycemic, immunosuppressive, prebiotic properties, and strong antioxidant properties. All these activities are mainly due to the high contents of bioactive compounds and other substances such as organosulfur compounds, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, and vitamins (**Elberry *et al*., 2014**; **Lee *et al*., 2014**).

Allium species are revered worldwide as vegetables, condiments, and spices as well as the therapeutic agents in traditional medicine. The bioactive compounds in alliums mainly include organosulfur compounds, polyphenols, dietary fibers, and saponins. Flavonoids, particularly flavonols from alliums, have been demonstrated to have antioxidant, anticancer, hypolipidemic, antidiabetic, cardioprotective, neuroprotective, and antimicrobial activities (**Kothari *et al*., 2020**).

As in the case of *Allium triquetrum*, it has been established that it has therapeutic effects as well as antimicrobial properties that are involved. Along with the antioxidant (DPPH and FRAP), anti-inflammatory and analgesic activity powers in comparison with the molecules used in pharmacology. Consequently, *Allium triquetrum* is a plant of great alimentary interest and with various biological effects, since its chemical composition is very heterogeneous and it does not present any toxicity even at high concentrations. Conversely, the best anti-oxidant, anti-inflammatory, and analgesic activities are noted in the aqueous extract of the leaves (**Menacer *et al*., 2019**).

2- Different Antioxidant Activity Tests Performed on The Allium Genus

Allium genus is a good source of natural antioxidants. Many studies have been carried out to evaluate the antioxidant activities of Allium, and found that onion exhibits strong

antioxidant properties by using a series of *in vitro* assays, including 2,2′- azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1- diphenyl-2-picrilhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), lipid peroxidation, oxygen radical absorbance capacity (ORAC), total antioxidant capacity (TAC), and Trolox equivalent antioxidant capacity (TEAC) assays (**Zhao *et al*., 2021**).

3- The Chemical Characterization of A. triquetrum L.

3.1. Fatty Acids

The chemical composition varies according to the variety grown, the place of cultivation, the time of harvest, and the storage conditions of the plant (**Bruneton, 2009**). The chemical characterization of *A. triquetrum* bulbs, flowers, and leaves allowed the identification of fatty acids as the major family of fractions, mainly represented in flowers and bulbs. A long-chain aliphatic ketone, named hentriacontane-16-one was detected in flowers and leaves. Additionally, flowers and leaves were particularly rich in, respectively alkanes and long-chain aliphatic alcohols. Minor amounts of long-chain aliphatic aldehydes, monoglycerides, sterols, and aromatic compounds were also present in all the studied morphological parts. Furthermore, two other interesting compounds, namely phytol, and α- tocopherol was detected in *A. triquetrum* flowers and leaves (**Rabah *et al*., 2020**). Additionally, it was also demonstrated that the analysis of lipid fractions by GC-MS *A. triquetrum* displayed mostly the presence of saturated, monounsaturated, and polyunsaturated fatty acids, which are palmitic acid and linoleic acid, methyl esters of fatty acids and alkanes, along with sulfur compounds such as Methyl-trans-propenyl-disulfide and Trans propenyl methyl disulfide 1,3-dithiane (**Menacer *et al*., 2019**).

3.2. Mineral Composition

A recent study confirms that *Allium triquetrum* spontaneous species of the Algerian flora is rich in some active secondary metabolites and minerals in potassium and sodium with a Na^+/K^+, calcium, magnesium, phosphorus, sulfur, and chlorine with an absence of nitrates. The analysis of minerals also showed that the plant contains trace elements such as iron, zinc, copper, lead, and manganese (**Menacer *et al*., 2019**).

3.3. Bioactive Compounds

The phytochemical and biological study of *A. triquetrum* wild garlic allowed to determine the chemical composition such as macro and microelements, sulfur compounds,

and phenolic acids, gallic acid, coumarins, and other compounds, mainly phenol, 2,4-bis (1,1-dimethyl ethyl)) (**Menacer *et al*., 2019**).

Some studies have already related the health benefits of *A. triquetrum* to the presence of specific secondary metabolites, and particularly the polar ones. More specifically, *A. triquetrum* bulbs, leaves, and flowers aqueous extracts, containing phenolic components, flavonols, glycosides, and saponins that showed stronger antioxidant activities than methanolic extracts (**Corea *et al*., 2003**; **Menacer *et al*., 2017a**; **2017b**). Likewise, *A. triquetrum* has a chemical diversity by the presence of phenolic compounds, saponosides, cardiotonic heterosides, tannins, iridoids, and terpenes with the absence of alkaloids, anthocyanin, tannins, and quinones. Moreover, the qualitative and semi-quantitative study of some phenolic compounds of *A. triquetrum* by HPLC revealed the presence of gallic acid, ferulic acid, hydroxy-cinnamic acid, chlorogenic acid, naringenin, catechin, rutin, and coumarin with concentrations that differed from one extract to another. However, the highest proportions were observed in the aqueous leaf extract. It should be noted that the determination of polyphenols and total flavonoids also showed the highest concentrations in the leaf extract (**Menacer *et al*., 2019**).

3.4. Antimicrobial Activity

Regarding the biological activities of *A. triquetrum*, it is observed that the fresh plant, in particular the bulbs, are more active on bacterial and fungal microorganisms and more precisely they better inhibit the development of Gram-negative bacteria and yeasts (**Menacer *et al*., 2019**). In addition, **Rabah *et al*.** (**2020**) provided evidence that *A. triquetrum* leaves, bulbs, and particularly flowers' lipophilic fractions can be utilized for pharmaceutical purposes, potentially as antibacterial agents, and as promising sources of bioactive phytochemicals.

Part I - The Wild Garlic Allium triquetrum **L.**

4- Garlic's Action Mechanism

Secondary metabolites can be classified into several major groups, the most important of which are polyphenols, terpene compounds, and nitrogenous compounds including alkaloids. Each of these classes contains a very large diversity of compounds that have a very wide range of activities in human biology (**Tiwari *et al*., 2015**). In parallel, there are other classes of secondary metabolic compounds, which are specific to certain plants, such as sulfur compounds. These represent the key molecules in Allium plants; they confer the characteristic flavors to these species and are responsible for most of their biological effects (**Lanzotti, 2006**; **Rose *et al*., 2006**). Flavonols and anthocyanins are the main subclasses of flavonoids present in the genus Allium (**Gîtin *et al*., 2012**).

The sulfur compounds in garlic are responsible for its activity, notably allicin. In 1991, researchers showed that the activity of garlic is abolished when allicin is removed from the extract (**Sivam, 2001**). Similarly, if allinase, the enzyme responsible for converting alliin to allicin, is inhibited, there is no activity (**Minker, 2012**). Since, Alliin, which is immediately transformed into allicin by the activation of this reaction takes place in the cell vacuole in a few seconds, through to two molecules of 2-propane sulfonic acid, which are chelators that complex several heavy metals that interact with each other and condense to form a molecule of allicin. Thus, allicin is the main active component of garlic, which is the source of garlic's odor. It is volatile depending on the chemical conditions, rapid changes can be observed. This odor is present only when the garlic clove is crushed or cut (**Fischer, 1995**).

The mechanism of action of allicin appears to result from its rapid interaction with the thiol (-SH) groups of enzymes (**Arzanlou *et al*., 2011**; **Guo *et al*., 2012**). Structural differences in bacteria may play a role in their susceptibility to garlic sulfur compounds. The lipids in their cell membranes influence the permeability of allicin and its fat-soluble sulfur derivatives. Therefore, Gram-negative bacteria are generally more sensitive to garlic than Gram-positive bacteria because they contain a higher amount of lipids in their cell wall (**Sivam, 2001**; **Kyung, 2012**).

Part I - The Wild Garlic Allium triquetrum L.

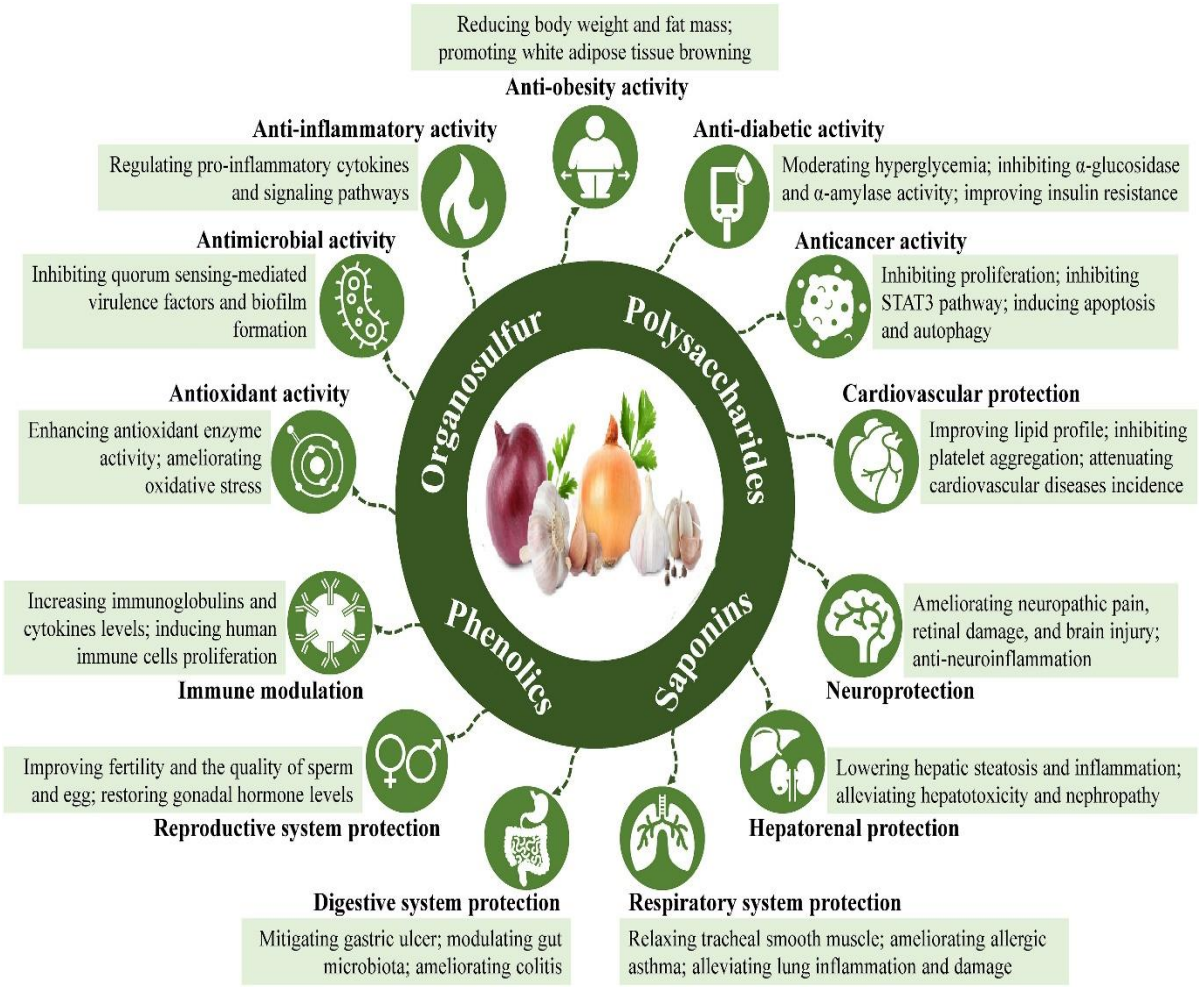

Fig 2. Bioactive compounds and health benefices of garlic and onion (**El-Sebaey *et al.*, 2019**; **Vijayakumar *et al.*, 2019**; **AL-Megrin *et al.*, 2020**; **Batiha *et al.*, 2020**; **Zhao *et al.*, 2021**).

Part II – Lead Acetate (Pb)

The contamination of the environment by heavy metals has been increased by industrial and anthropogenic activities that are affecting human and animal health remarkably. Lead is among the dangerous metal that is characterized by poor conductivity, malleability, high density, and corrosion resistance (**D'Souza *et al*., 2011**). It is a ubiquitous environmental and industrial pollutant that persists and accumulates in ecosystems and biological systems due to its non-degradation (**Andjelkovic *et al*., 2019**; **Mahar *et al*., 2019**).

1. Use of Lead (Pb)

Lead is one of the most abundant elements in nature and is frequently used in various mining activities due to its distinctive physical and chemical properties (**Lu *et al*., 2015**). It is also used in ceramic products (**Berglund *et al*., 2008**), paints, caulking, pipe solder, automotive fuels, battery manufacturing, water piping, roofing, terraces, balconies, noise and vibration insulation, cable protection, production of paints, inks, enamels, plastics, waste combustion, hair dyes, make-up, and jewelry making (**Carocci *et al*., 2016**).

2. Toxicokinetic of Lead

Lead is one of the most toxic heavy metals; its ingestion via the food chain has proven to be a potential health hazard (**Kumar *et al*., 2020**). Lead enters the human body through absorption from food (65%), water (20%), and air (15%) (**Mahmoud & Malik, 2014**). The absorption of inhaled lead is deposited in the ciliated airways at the nasopharynx and tracheobronchial region. Its mucociliary transport to the larynx and then to the gastrointestinal tract represents the main route of particle clearance (**ANSES, 2019**). However, ingested lead is absorbed in the duodenal region of the small intestine (**Bonnard *et al*., 2006**). Lead gases and vapors arrive without difficulty at the level of the pulmonary alveoli and thus pass into the blood. This route depends on particle size and solubility. There are two absorption mechanisms: the first, passive, unlikely for lead, and the second, active, which uses the absorption pathways of calcium, magnesium, and iron (**Haguenoer & Declercq, 2004**). Besides, dermal absorption of inorganic lead compounds is generally considered to be much lower than inhalation or oral absorption (**ATSDR, 2007**).

Upon absorption into the bloodstream, it binds to plasma proteins or enters erythrocytes and binds to hemoglobin, and then moves with the blood to soft tissues mainly liver, kidney, lung, brain, spleen, muscle, and heart (**Schütz *et al*., 2005**; **Kosek-Hoehne *et al*., 2017**), or it is metabolized by oxidation in the hepatic endo-plasmatic reticulum by

cytochrome P450 (**Barbosa *et al.*, 2005**). In plasma, lead is bound to proteins, lipids and only a small fraction of lead exists in the unbound form (**Göen *et al.*, 2012**). After a few weeks, most lead accumulates in bones and teeth where it can persist for many years (**Kosek-Hoehne *et al.*, 2017**). The half-life of lead in soft tissue and blood is about 30 days, and the half-life in bone is very long (**Bouhouch *et al.*, 2016**). Maternal lead can pass through the placenta to the developing fetus (**Rahman *et al.*, 2018**; **Omeljaniuk *et al.*, 2018**).

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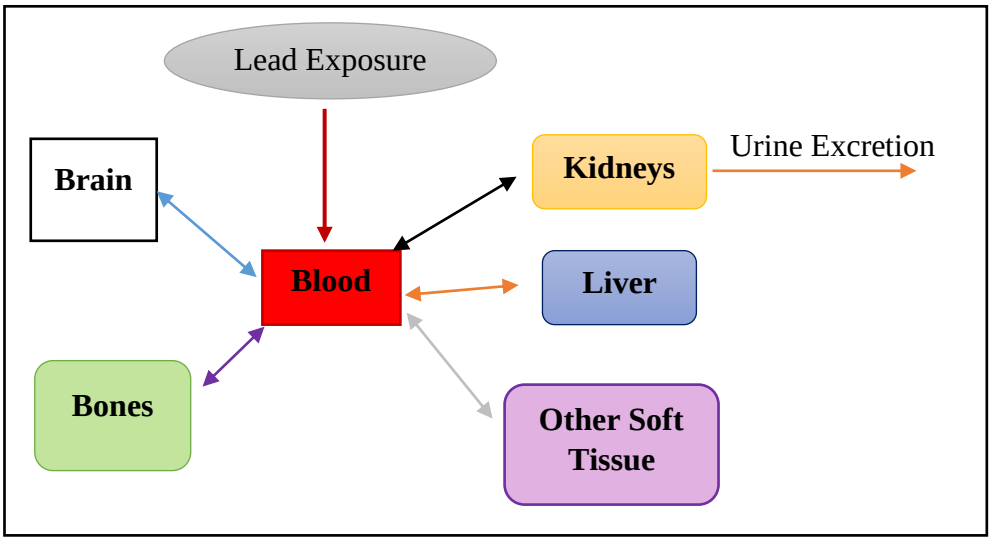


Fig 3. Penetration of lead into the body (**Rădulescu & Lundgren, 2019**).

The main excretion pathway for lead is through the urine, which accounts for 75-80% of total excretion. The secondary route is through the gastrointestinal tract. Other routes, such as sweat, saliva, hair, and nails, account for less than 8% of total excretion. In general, the rate of elimination is very slow, and measurement of renal clearance of ultra filterable lead in plasma indicates that lead undergoes glomerular filtration and net tubular reabsorption. Since different tissues and compartments have different lead storage and exchange characteristics, this may lead to the accumulation of lead in body tissues, particularly bone (**Abadin *et al.*, 2007**). The mechanism for this has not been elucidated and may involve a shift in blood lead distribution to a fraction with a higher glomerular filtration rate (e.g., a lower molecular weight complex), a capacity-limited mechanism in tubular lead reabsorption, or the effects of lead-induced nephrotoxicity on lead reabsorption (**Rădulescu & Lundgren, 2019**).

Part II – Lead Acetate (Pb)

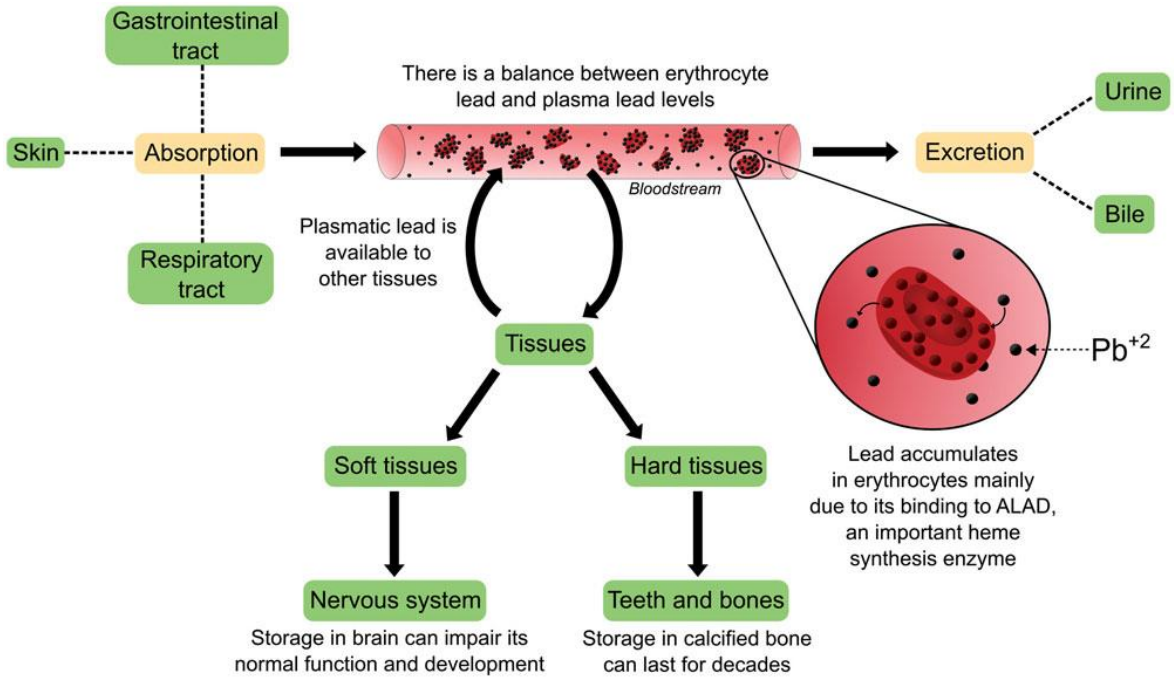


Fig 4. The toxicokinetic of lead poisoning (**De Souza *et al.*, 2018**).

3. Mechanism of Action of Lead

One of the primary mechanisms by which lead exerts its toxic effect is a biochemical process that includes the ability of this metal to inhibit or mimic the actions of calcium and to interact with proteins (**ATSDR, 1999**). It binds to biological molecules and thus interferes with their function by several mechanisms. Lead binds to the sulfhydryl and amide groups of enzymes, altering their configuration and decreasing their activities. Lead can also compete with essential metal cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium (**Flora *et al.*, 2007**), by interfering with processes related to neuronal signaling and intracellular signal transduction. Metal ions have also been found to interact with cellular components such as DNA and nuclear proteins, causing DNA damage and conformational changes that can lead to cell cycle modulation, carcinogenesis, or apoptosis (**Beyersmann & Hartwig, 2008)**.

The mechanism of ionic action of lead is primarily due to its ability to substitute for other divalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations such as Na^{+} (although divalent cations are more easily substituted), thus affecting various fundamental biological processes in the body (**Lidsky & Schneider, 2003**). Significant effects have

been found on various fundamental cellular processes such as intra- and intercellular signaling, cell adhesion, protein folding and maturation, apoptosis, ionic transport, enzyme regulation, neurotransmitter release, etc. (**Garza *et al*., 2006**), and alteration of the capacity to release reserves from organelles, such as the endoplasmic reticulum and mitochondria. The ionic mechanism of replacing calcium ions contributes mainly affecting the bone formation, renal function, and neuronal function (where calcium is essential for processes such as learning and memory), which becomes competent to cross the blood-brain barrier (BBB) at an appreciable speed (**Rădulescu & Lundgren, 2019**). After crossing the BBB, lead accumulates in astroglial cells (containing lead-binding proteins). The toxic effects of lead are most pronounced in the developing nervous system comprising immature astroglial cells lacking lead binding proteins. Lead readily damages immature astroglial cells and impairs myelin sheath formation, both of which are involved in BBB development. Lead, even in picomolar concentrations, can replace calcium, affecting key neurotransmitters such as protein kinase C, which regulates long-term neuronal excitation and memory storage. It also affects the concentration of sodium ions, which are responsible for many vital biological activities such as the generation of action potentials in excitatory tissues for intercellular communication, the uptake of neurotransmitters (choline, dopamine, and GABA), and the regulation of calcium uptake and retention by synaptosomes. This interaction between lead and sodium severely impairs the normal functioning of the above sodium-dependent processes (**Bressler *et al*., 1999**).

Part II – Lead Acetate (Pb)

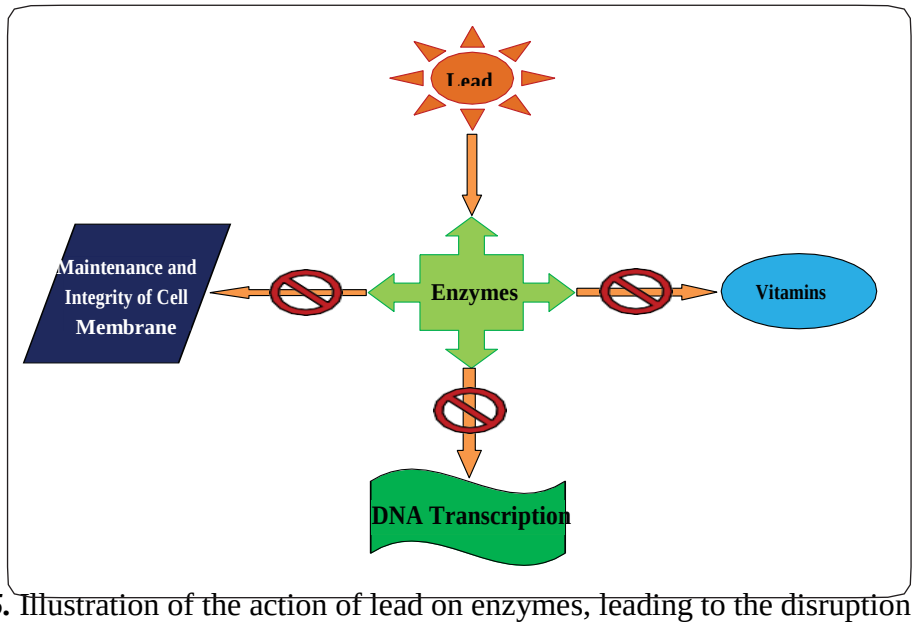


Fig 5. Illustration of the action of lead on enzymes, leading to the disruption of vitamin synthesis, maintenance of cell membrane, and DNA transcription (**Wani *et al.*, 2015**).

4. Mechanism of Lead-Induced Toxicity

Through the various mechanisms postulated about lead-induced toxicity (*Fig. 14*), which is the most important mechanism which involves oxidative stress that causes changes in the composition of membrane fatty acids. Pb can also provoke alterations in gene expression (**Rehman *et al.*, 2018**; **Zhushan & Shuhua, 2020**). The induced generation of ROS by Pb can weaken cells' defense mechanisms (**Flora, 2002**).

The depletion of cells' major sulfhydryl resulting from oxidative stress is an important indirect mechanism of Pb toxicity (**Ercal *et al.*, 2001**). When Glutathione (GSH) is depleted in the body by lead, the body starts making more GSH from cysteine. This antioxidant defense mechanism may be protected by many enzymes. The cofactors like selenium, zinc, copper of many enzymes may be replaced by lead, and thereby, resulting in enzyme inactivation. Studies in lead-exposed animals reported having either elevated lipid peroxidation or decreased intrinsic antioxidant defense in various tissues (**Sangeetha & Umamaheswari, 2020**).

Part II – Lead Acetate (Pb)

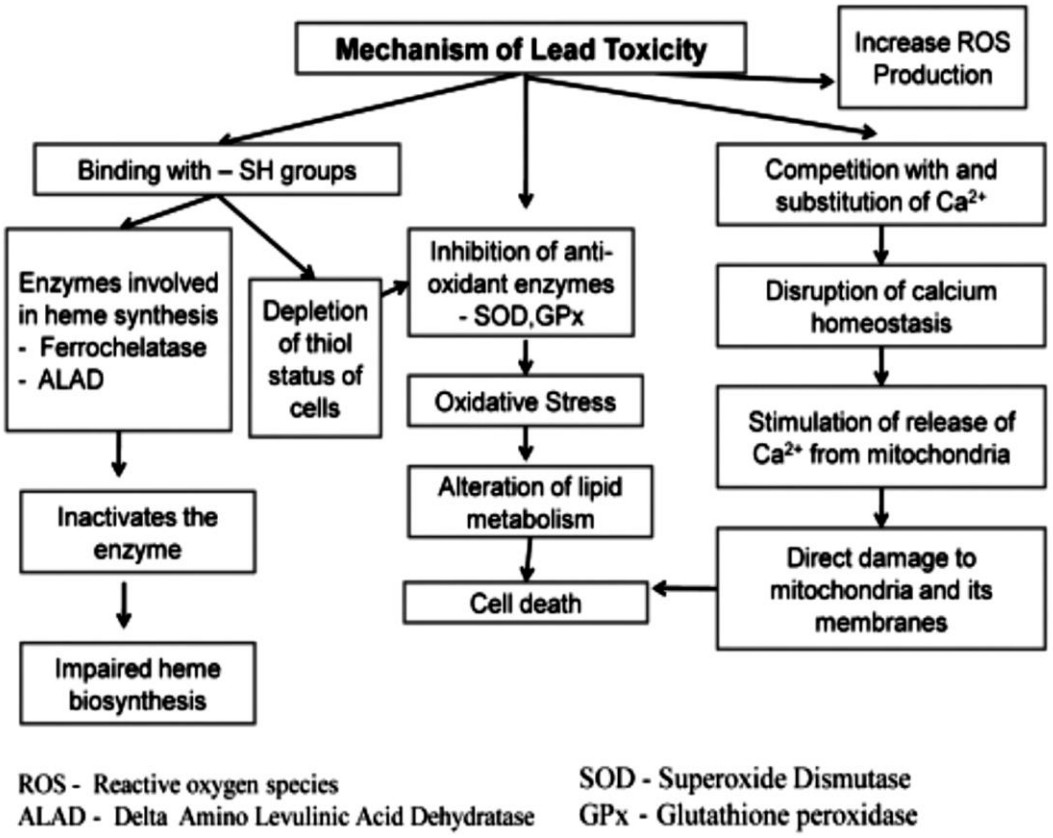


Fig 6. Schematic representation of various mechanisms of lead toxicity (**Sangeetha & Umamaheswari, 2020**).

5. Health Effects of Lead

In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondria, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair. Lead is a cumulative toxicant that virtually affects multiple body systems, including neurological, hematological, gastrointestinal, cardiovascular (**Lamidi & Akefe, 2017**; **Wardani *et al*., 2017**), skeletal, circulatory, endocrinal (**Flora *et al*., 2006)**, histological, biochemical, physiological (**Gargouri *et al*., 2019**; **Mansouri *et al*., 2021a**), genotoxic and carcinogenic (**INRS, 2018**). Lead is easily absorbed into the body and undergoes bioaccumulation in certain target organs as the testis (**El-Magd *et al*., 2016**), brain (**Kabeer *et al*., 2019**), liver, and kidney (**Andjelkovic *et al*., 2019**).

Part II – Lead Acetate (Pb)

a- Effect on the Nervous System

Both the central and the peripheral nervous systems become affected by lead exposure. The effects on the peripheral nervous system are more pronounced in adults, while the central nervous system is more prominently affected in children (**Bellinger, 2004**; **Brent, 2006**). The effect of lead compounds on the hypothalamic-pituitary-adrenal axis suggests that lead acts as a stressor and targets brain meso-corticolimbic dopaminergic systems and affects the sympathetic nervous system directly and indirectly via alterations in neurotransmitter secretion (**Doumouchtsis *et al.*, 2009**). Encephalopathy (progressive degeneration of certain parts of the brain) is a direct consequence of lead exposure where the major symptoms include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory, and hallucinations. More severe manifestations occur at very high exposures and include delirium, lack of coordination, convulsions, paralysis, coma, and ataxia (**Flora *et al.*, 2006**). Repercussions of lead exposure on the peripheral nervous system have also been observed in the form of peripheral neuropathy, involving reduced motor activity due to loss of myelin sheath which insulates the nerves, thus seriously impairing the transduction of nerve impulses, causing muscular weakness, especially of the exterior muscles, fatigue, and lack of muscular coordination (**Sanders *et al.*, 2009**).

b- Effect on the Hematopoietic System

Lead directly affects the hematopoietic system by restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes leads to anemia (**Cornelis, 2005**; **Guidotti *et al.*, 2008**). Anemia caused by lead poisoning can be of two types; hemolytic anemia, which is associated with acute high levels of lead exposure, and frank anemia, which is caused only when the blood lead level is significantly elevated for prolonged periods (**Vij, 2009**). Lead significantly affects the heme synthesis pathway in a dose-dependent manner by downregulating three key enzymes involved in the synthesis of heme (**Piomelli, 2002**). The initial and final steps of heme synthesis take place in the mitochondria, whereas the intermediate steps take place in the cytoplasm.

Part II – Lead Acetate (Pb)

c- <u>Effect on the Reproductive System</u>

Lead causes several adverse effects on the reproductive system in both men and women. Common effects seen in men include reduced libido, abnormal spermatogenesis (reduced motility and number), chromosomal damage, infertility, abnormal prostatic function, and changes in serum testosterone (**Flora *et al*., 2011**). Abnormal sperm formation has been found to arise from oxidative stress alterations on steroidogenic capacity and sperm differentiation (**Budin *et al*., 2017**). In addition, polyunsaturated fatty acids in mammalian sperm membranes were easily vulnerable to peroxidation (**Budin *et al*., 2017**). However, reproductive hormones have an imperative and complex role in the regulation of spermatogenesis and sperm development. A recent study validated the role of increased blood lead and FSH levels in low semen quality with reduced sperm motility, concentration, and altered morphologic changes (**Lu *et al*., 2015**). Lead appears to influence the hypothalamic-pituitary axis leading to reduced TSH and FSH/LH responses to their respective tropic hormones' stimulation (**Doumouchtsis *et al*., 2009**).

The problems with female reproductivity due to lead exposure are more severe. Toxic levels of lead can lead to miscarriages, prematurity, premature membrane rupture, pre-eclampsia, pregnancy hypertension, and premature delivery, low birth weight, and problems with development during childhood (**Park *et al*., 2008**; **Flora *et al*., 2011**). Blood lead levels in mothers and infants are usually similar as the lead present in mother blood passes into the fetus through the placenta and through breast milk (**Dart *et al*., 2004**). Moreover, during the gestation period, a direct influence of lead on the developmental stages of the fetus has also been reported (**Saleh *et al*., 2009**).

d- <u>Effect on the Hepatic and Gastrointestinal Tract System</u>

The gastrointestinal (GI) tract is the main route responsible for the intake and uptake of lead in the general population. Various biological and physicochemical factors in the gastrointestinal tract are the sites of lead absorption and relevant transporters. Interactions of lead with nutrients like calcium and iron affect lead bioavailability in the liver (**Mudipalli, 2007**), which is an important organ that actively participates in many metabolic functions of endogenous molecules such as lipids, carbohydrates, proteins, blood coagulation, immunomodulation, and exogenous molecules such as many xenobiotics where it facilitates their detoxification and removal (**Singh *et al*., 2016**). It plays a major

role in lead metabolism (**Álvarez-Lloret *et al.*, 2014**). It was found that increased lead concentration was associated with increased low-density lipoprotein and decreased high-density lipoprotein (**Yang *et al.*, 2017**) as well as, liver enzymes (ALP, ALT, AST, and GGT) and increased oxidative stress parameters (**Abirami *et al.*, 2007**; **Mazumdar & Goswami, 2014**) such as glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST) and decreased glutathione (GSH) content in hepatocytes and erythrocytes; in addition to malondialdehyde (MDA) and H_2O_2 concentrations (**Omobowale *et al.*, 2014**). Moreover, **Shalan *et al***. (**2005**) demonstrated that the administration of Pb Acetate in rat diet (500 mg/kg/day) for 2, 4, and 6 weeks resulted in hepatic necro-inflammatory lesions which, induced hepatocyte proliferation, portal inflammatory infiltrate, steatosis, apoptosis, and mild fibrosis. Liver injury is associated with a distortion of these metabolic functions and resulted in a disruption of body homeostasis (**Ravichandra *et al.*, 2013**).

e- Effect on the Kidney

The effects of lead poisoning on the renal system have been documented for over a century now, with toxicity ranging from histologic changes to the clinical manifestations of renal dysfunction (**Muntner *et al.*, 2003**). The kidney excretes lead through glomerular filtration and tubular secretion. It has bidirectional transport across the tubular epithelium with a clearance range from 1 to 3 mL/min and is relatively independent of kidney function. An algorithm for the pathogenesis of chronic and acute lead exposure leading to nephropathy proposed in 1997 showed that acute lead poisoning leads to proximal tubular dysfunction and these changes usually disappear with chelation therapy or avoiding lead exposure which, are reported to lead to glomerular hypertrophy and manifests specifically as an increase in the volume of glomerular capillaries. Acute exposure to lead affects renal tubules adversely and leads to generalized defects in solute and amino acid transport in renal tubules, leading to Fanconi syndrome. Chronic exposure to lead may result in progressive tubule-interstitial nephritis, characterized by infiltration of leukocytes, interstitial fibrosis, and tubular atrophy. Since lead tends to induce injury in proximal tubules, kidney injury and alpha glutathione S-transferase (αGST) can prove to be the most appropriate urinary biomarkers for lead-induced renal injury. Besides, one of the primary cellular effects of exposure to lead is the induction of oxidative stress in the renal cells. Exposure of mice to lead acetate enhances the production of ROS and reduces the mRNA

Part II – Lead Acetate (Pb)

expression of enzymes (catalase, superoxide dismutase, glutathione S-transferase, and glutathione peroxidase) necessary to counteract oxidative stress (**Orr & Bridges, 2017**). Furthermore, hemorrhage in the renal tubules, degeneration in epithelial cells, disruption of Bowman's capsule, and necrosis area in kidney tissue were observed (**Abdou & Hassan, 2014**).

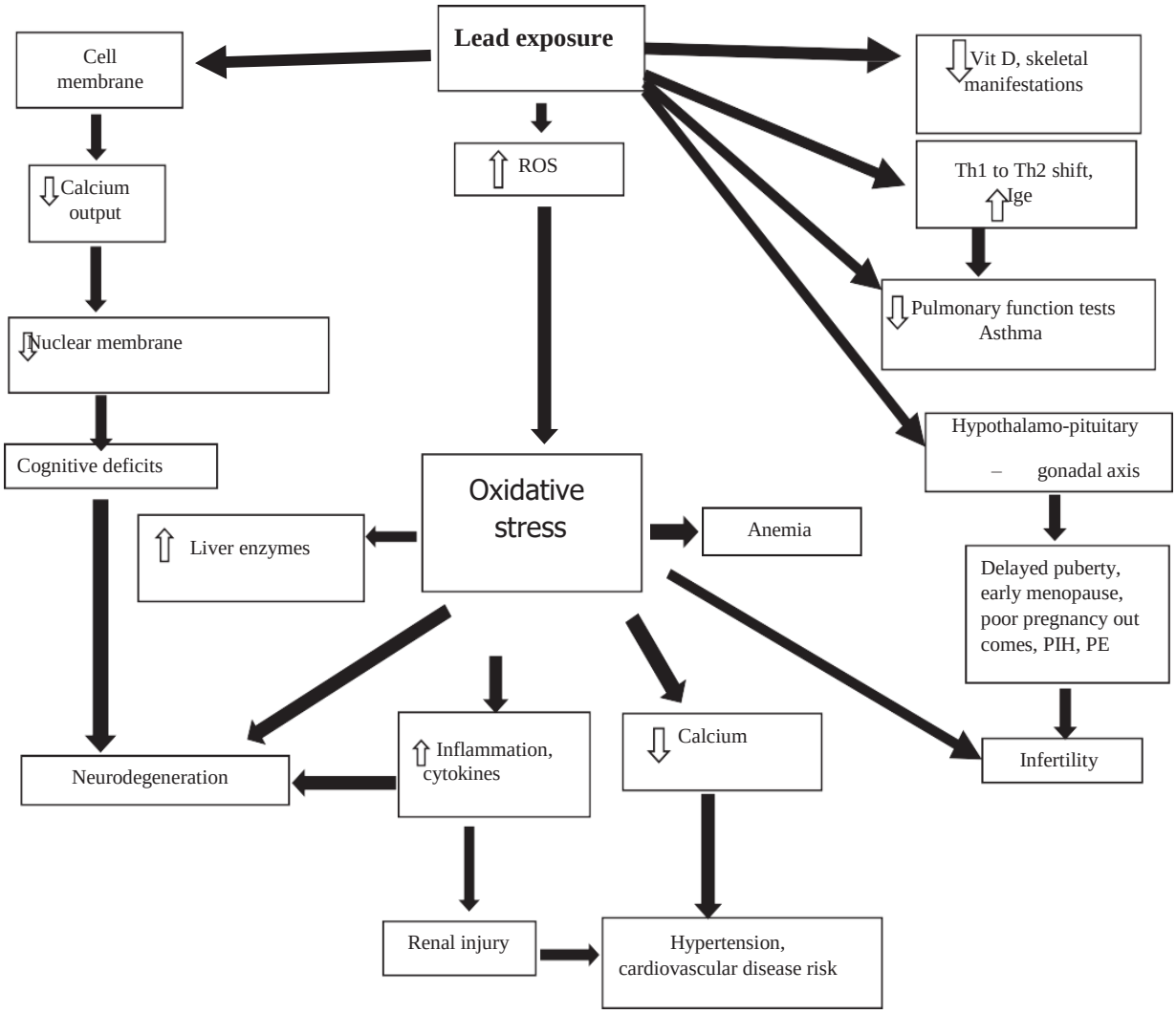


Fig 7. Lead exposure leads to oxidative stress and results in multi-organ manifestations (**Mitra *et al*., 2017**).

Part III – Oxidative Stress

1- Lead and Oxidative Stress

Lead stimulated oxidative stress is a state that involves the generation of free radicals beyond the permissible limits, depleting at the same time the antioxidant reserves and thus hampering the ability of the biological system to reverse the effects. Free radicals' generation starts a chain reaction that results in lipid peroxidation, disruption of the cell membrane, protein oxidation, and oxidation of nucleic acids like DNA and RNA leading to cancer (**Gurer & Ercal, 2000**). Oxidative stress represents an imbalance between the production of free radicals and the biological systems' ability to readily detoxify the reactive intermediates or to repair the resulting damage (**Flora, 2011**). It has been reported as a major mechanism of lead-induced toxicity. Under the influence of lead, the onset of oxidative stress occurs on account of two different pathways operative simultaneously; first comes the generation of ROS, like hydroperoxides (HO_2•), singlet oxygen and hydrogen peroxide (H_2O_2), and second, the antioxidant reserves become depleted (**Flora, 2002**). The antioxidant defenses of the body come into play to nullify the generated ROS. The most important antioxidant found in cells is glutathione (GSH). It is a tripeptide having sulfhydryl groups and is found in mammalian tissues in millimolar concentrations. It is an important antioxidant for quenching free radicals (**Mates, 2000**). Glutathione exists in both reduced (GSH) and oxidized form (GSSG). The reduced state of glutathione donates reducing equivalents (H^+, e^-) from its thiol groups present in cysteine residues to ROS and makes them stable. After donating the electron, it readily combines with another molecule of glutathione and forms glutathione disulfide (GSSG) in the presence of the enzyme glutathione peroxidase (GPX). GSH can be regenerated from GSSG by the enzyme glutathione reductase (GR). Under normal conditions, 90% of the total glutathione content exists in reduced form and around 10% is in the oxidized form (**Flora *et al*., 2012**).

Research findings have suggested that the administration of various antioxidants can prevent or subdue various toxic effects of lead and the generation of oxidative stress. An antioxidant is a substance that, when present at a low concentration as compared to that of the oxidizable substrate, can prevent the oxidation of that substrate. Generally, an antioxidant can prevent lead toxicity in three ways (**Garcia & Gonzalez, 2008**), by inactivating the generated ROS at a molecular level, thereby terminating the radical chain reaction (chain breaking), chelating the lead ion, and preventing further formation of ROS

and by chelating lead and maintaining it in a redox state, which leads to its incompetency to reduce molecular oxygen (**Flora *et al.*, 2012**).

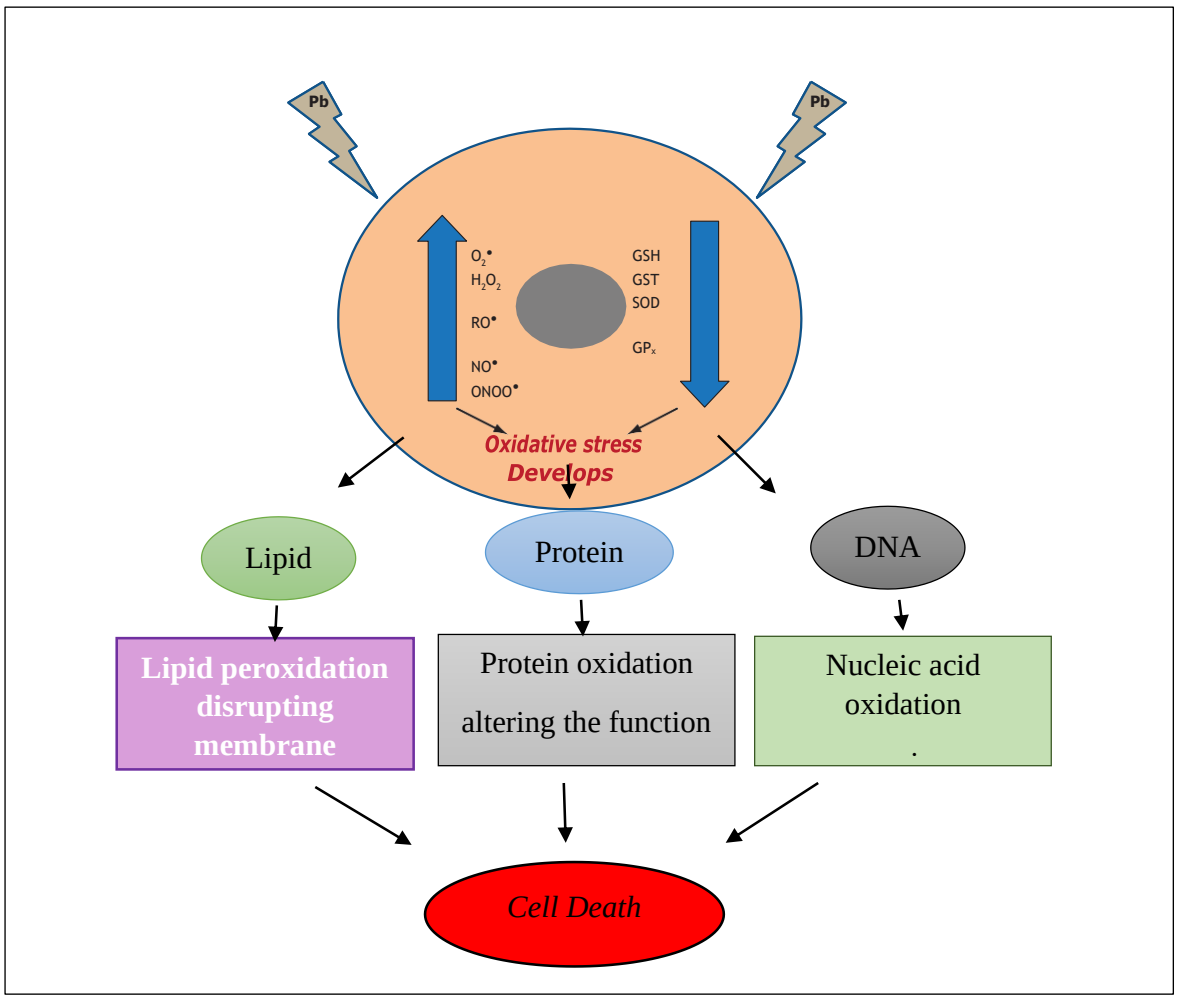


Fig 8. Mechanism and targets for lead-induced oxidative stress in cells (**Flora *et al.*, 2012**).

1-1- Lipid Peroxidation (LPO)

Lipid peroxidation (LPO) is one of the main manifestations of oxidative damage, which plays an important role in the toxicity of many xenobiotics (**Yousef & Salama, 2009**; **Ognjanovic *et al.*, 2010**). Malondialdehyde (MDA) is one of the major products of per oxidized polyunsaturated fatty acids (PUFAs), increased MDA content is an important indicator of OPL (**El-Desoky *et al.*, 2013**). LPO is the oxidative degradation process of polyunsaturated fatty acids (PUFAs) and its occurrence in biological membranes leads to altered membrane function, structural integrity, decreased membrane fluidity, and inactivation of several membrane-bound enzymes (**Valko *et al.*, 2005**).

Part III – Oxidative Stress

1-2- Glutathione (GSH)

GSH, a tripeptide (γ-L-glutamyl-L-cysteinyl glycine), is an endogenous antioxidant and an important cellular defense agent against oxidative damage. Under normal physiological conditions, GSH is mainly reduced. However, under pathological conditions, the GSH/GSSG ratio can decrease significantly. The pentose phosphate pathway regulates the GSH/GSSG ratio by providing NADPH that is required for the reduction of GSSG to GSH-by-GSH reductase (**Aquilano *et al.*, 2014**). GSH can directly scavenge ROS such as H_2O_2 and OH. Or indirectly through the GPx-catalyzed reaction (**Kıvrak *et al.*, 2017**). GSH can regenerate other antioxidants such as vitamin C and vitamin E into their active forms. It has a role in protecting lipids, proteins, and nucleic acids from oxidation (**Lü *et al.*, 2010**).

1-3- Glutathione Peroxidase (GPx)

Glutathione is an abundant non-enzymatic reducing agent that acts as a free radical scavenger and a coenzyme for enzymatic reducing agents, especially GPx (**Cheng & Ko, 2019**). Glutathione peroxidase, a selenium-containing enzyme, catalyzes both the reduction of H_2O_2 and organic hydroperoxides to the corresponding water or alcohol. Generally using glutathione (GSH) as a reductant (**Brigelius-Flohé & Maiorino, 2013**). It is well known that GSH is one of the most important antioxidant molecules and, at physiological concentrations, contributes to maintaining the normal redox state of cells (**Youcef & Salama, 2009**) which spontaneously neutralizes several ROS (**El-Desoky *et al.*, 2013**).

1-4- Superoxide Dismutase (SOD) and Catalase (CAT)

SOD and CAT are primary antioxidant enzymes present in mammalian cells (**Fang *et al.*, 2002**). Catalase is a hemoprotein that catalyzes the oxidation reaction, by H_2O_2 of many metabolites and toxins. Its basic function is to remove H_2O_2 and ROOH peroxide in molecular oxygen to prevent irreversible membrane damage (**Kıvrak *et al.*, 2017**) and converts H_2O_2 to water and molecular oxygen and protect against oxidative damage (**Cheng & Ko, 2019**).

Superoxide dismutase is considered the first line of defense against oxidative breakdown, converting the superoxide radical anion O_2 into H_2O_2 and O_2. SOD offers protection against ROS-induced cellular and histological damage. It reacts very rapidly with NO, reducing the bioactivity of NO and producing ONOO- (**Fukai & Ushio-Fukai,**

Part III – Oxidative Stress

2011). SOD protects CAT from inhibition by superoxide anion (O2-). Conversely, CAT protects SOD against inactivation by H_2O_2. Thus, these enzymes work together to remove ROS from cellular lipids, proteins, and DNA to oxidative damage (**Kalender *et al.*, 2013**).

2- Detoxification of Lead

Dietary-derived antioxidants such as ascorbic acid, vitamin E, carotenoids, polyphenols, and α-lipoic acid have also served as primary lines of defense to overcome free radicals (**Wang & Luo, 2007**; **Uma *et al*., 2012**). Natural antioxidants can delay the progression of many chronic diseases but also the oxidative rancidity of lipids in foods, cosmetics, and pharmaceuticals (**Sridhar *et al.*, 2013**). Antioxidants may play an important role in reducing some health risks from heavy metals (**Ansar & Igbal, 2016**).

2.1. Proteins

Metallothionein's are a group of low molecular weight cysteine-rich proteins that are involved in metal transport, distribution, and detoxification inside the body. These proteins are encoded by the MT gene and are known to have four major isoforms in mammals. MT isoforms are distributed in all body compartments, MT is predominant in the brain, and MT is found mainly in the kidney and GI (Gastrointestinal tract) systems. High numbers of thiol groups enable them to bind to metals effectively and modulate homeostatic functions like absorption, transport, and elimination of metals. Their role in such metal homeostatic regulation has made MT gene variants novel candidates in susceptibility to lead toxicity (**Raudenska *et al*., 2014**).

2.2. Natural Antioxidants

Naturally occurring antioxidants and their role in quenching free radicals generated in the body under various pathologic conditions have been an active area of research. Studies have revealed that antioxidants possess the ability to both prevent and cure the damage caused by the generation of free radicals in the body.

Natural antioxidants can be categorized into enzymatic and non-enzymatic antioxidants like SOD, CAT, GPX which are produced endogenously in the cells, whereas non-enzymatic antioxidants like carotenoids, flavonoids, vitamins, minerals, etc. are constituents of many fruits, vegetables, nuts, grains, and some meats (**Flora, 2009**). The number of antioxidants present under normal physiological conditions is just adequate to

quench the free radicals that are generated at a normal physiological rate. Any further increment in the concentration of free radicals (due to environmental or natural causes) can cause an imbalance between the free radicals and antioxidants, leading to oxidative stress (**Blokhina *et al*., 2003**). This is where the role of exogenous antioxidants becomes important. They are taken through the diet or in the form of supplements to maintain the homeostasis between free radicals and antioxidants and thus prevent various deleterious effects, like heavy metal toxicity, inflammation, cancer, aging, cardiovascular and brain disorders (**Willcox *et al*., 2004**).

a- Polyphenols

Polyphenols are organic compounds found abundantly in plants and have become an emerging area of interest in nutrition over the past few decades. A growing body of research indicates that the consumption of polyphenols may play a vital role in health through the regulation of metabolism, weight, chronic disease, and cell proliferation. More than 8000 polyphenols have been identified so far, although their short- and long-term health effects have not been fully characterized (**Lecour & Lamont, 2011**). Among the phytochemical antioxidants are, flavonoids, tannins, coumarins, phenolics, lignans, and terpenoids that are present in various plant products (such as fruits, leaves, seeds, and oils) (**Jeong *et al*., 2004**).

b- Flavonoids

Flavonoids are the largest group of polyphenols, with over 6000 flavonoids identified (**Harborne & Williams, 2000**). Recent interest in phenolic compounds in general, and flavonoids, has greatly increased due to their antioxidant capacity by the mechanism of scavenging free radicals, including hydroxyl, peroxyl, and superoxide radicals, and can form complexes with catalytic metal ions making them inactive and their possible beneficial implications for human health (**Schroeter *et al*., 2002**). Members of the immediate flavonoid family include: Flavones (Chrysin, Apigenin), isoflavones (genistein, daidzein, and glycitein), flavanols (catechins), flavonols are mainly represented by (quercetin, kaempferol, myricetin, and rutin), flavanones, (Naringin, Naringenin, Taxifoline, Eriodictyol, Hesperidin), anthocyanins (cyanidin-3-glucoside) and anthocyanidins (Epigenidin, Cyanidin, Delphinium, Pelargonidin) (**Losada-Barreiro & Bravo-Díaz, 2017**).

Part III – Oxidative Stress

c- Tannins

Tannins are defined as hydrolyzable or condensed (proanthocyanidins), depending on their chemical structures (**Shahidi & Naczk, 2004**). Condensed tannins are oligomers and polymers of flavonoids, while hydrolyzable tannins are glycosylated gallic acid (**Ferreira & Li, 2000**; **Khanbabaee & Ree, 2001**). They can also inhibit lipid peroxidation and lipoxygenases *in vitro* and can scavenge radicals such as hydroxyl, superoxide, and peroxyl, which are known to be important in the cellular pro-oxidation state (**Gyamfi & Aniya, 2002**). Research has shown that tannins have anti-inflammatory, anti-fungal, anti-tumor, antiviral, and anti-diarrheal properties (**Maïga *et al*., 2005**).

d- Vitamins

Vitamin C (ascorbic acid) is a water-soluble antioxidant that prevents oxidative damage by scavenging ROS. In addition, it has demonstrated anti-apoptotic activities by maintaining mitochondrial membrane potential and protecting mitochondrial DNA from oxidant insults (**Varma *et al*., 2014**; **Chou & Tseng, 2017**). Vitamin C plays a critical role in the brain, including as a cofactor for dopamine beta-hydroxylase. It also protects membrane phospholipids from peroxidative damage and is an effective free radical scavenger in the brain (**May, 2012**).

Vitamin E is a fat-soluble vitamin with antioxidant properties that exist in eight different forms: alpha, beta, gamma, and delta-tocopherol; and alpha, beta, gamma, and delta-tocotrienol, with alpha-tocopherol being the most active form in humans (**Farid *et al*., 2013**). It can react with peroxyl radicals to form a radical (**Mirończuk-Chodakowska *et al*., 2017**). It is a potent chain-breaking antioxidant that inhibits the production of ROS when fats undergo oxidation and during the propagation of free radical reactions. It acts as the first line of defense against lipid peroxidation, protecting cell membranes from free radical attack (**Rizvi *et al*., 2014**).

Vitamin A is a term encompassing a group of unsaturated organic compounds, which include retinol, retinal, and retinoic acid (**WHO, 2009**). It is responsible for the neutralization of $^1O_2$, O_2-, ONOO-, lipid radicals, and its activities are in lipid media (**Fisher-Wellman & Bloomer, 2009**). It is required for normal growth and development, playing a part in reproduction, differentiation of cellular epithelium, regulation of cell

division, genetic regulation, and enhancement of immune responses. Retinol (its basic molecule) is metabolized into many biologically active retinoid compounds, such as retinal (active element of visual pigment) and retinoic acid, an intracellular messenger that modulates cell differentiation (**Edem, 2009**). Furthermore, serum retinol concentrations are depressed during infection and inflammation because retinol-binding protein (RBP) is a negative acute-phase reactant, which makes status assessment challenging (**Tanumihardjo *et al.*, 2016**). Provitamin A carotenoids, which are produced in plants, are also a primary dietary source of the vitamin after enzymatic cleavage. Vitamin A deficiency continues to contribute significantly to the global burden of disease, particularly affecting resource-constrained countries. Vitamin A deficiency disorders include an increased risk of death from infectious diseases (**WHO, 2009**).

2.3. Herbal Extracts and Lead

Numerous studies have been done to find the effect of an herbal product against lead-induced damages. The literature below (*Tab. 2*) shows the protective effect of various plant extracts against lead-induced hepatic damages. The hepatoprotective effects produced by these plants are may be due to the presence of secondary metabolites, polyphenolic compound flavonoids (**Sangeetha & Umamaheswari, 2020**).

Table (2). Herbal uses on lead-induced damage.

Plant name	Inducing agent and dosing	References
Cayratia carnosa	Lead acetate - 20 mg/Kg/BW/ for one day.	**Suganthi *et al*., 2013**
Tinospora cordifolia	Lead nitrate - 5 mg/kg/BW/oral for 30 days.	**Sharma & Pandey, 2010**
Asparagus racemosus	Lead nitrate - 20 mg/Kg BW/oral for 45 days.	**Sharma *et al*., 2012**
Leucas aspera	Lead acetate - 50 mg/kg/BW/oral for 21 days.	**Thenmozhi *et al*., 2013**
Zingiber officinale Roscoe	Lead acetate - 500 ppm by oral 50 for days.	**Attia *et al*., 2013**
Spirulina	Lead acetate - 1.89 mg/kg for 7 days.	**Hemalatha *et al*., 2012**
Coriandrum sativum	Lead nitrate - 40 mg/kg/BW/oral for 7 days.	**Kansal *et al*., 2011**
Ocimum sanctum linn	Lead acetate - 2.10 mg/150 g/BW/oral for 3 days.	**Akilavalli *et al*., 2011**
Vitis vinifera	Lead acetate - 100 mg/kg/BW/ for 7 days.	**Abeer, 2012**
Turmeric and myrrh	Lead acetate - 0.5% oral- 8 weeks.	**El-Ashmawy *et al*., 2006**
Curcuma longa	Lead acetate - 1000 mg/kg/BW/oral for 28 days.	**Baxla *et al*., 2013**
Morocco carob honey	Lead acetate - 2 g/kg.BW/oral for 24 days.	**Fihri *et al*., 2016**
Green tea	Lead acetate - 0.4% oral for 8 weeks.	**Mehana *et al*., 2012**
Murraya koenigii	Lead acetate - 15 mg/kg/BW/ for 7 days.	**Ghosh *et al*., 2013**
Moringa oleifera	Lead acetate - 2000 ppm for 2 weeks after 7 days drug treatment.	**Velaga *et al*., 2014**
Wheat Grass	Lead acetate - 600 mg/Kg/ diet for 6 weeks.	**Mansouri *et al*., 2021a**
Zingiber officinale	Lead acetate - 2 % (20 mg/L) for 3 months.	**Aouacheri & Saka, 2020**
Taraxacum Officinale	Lead acetate - 600 mg/Kg/diet for 6 weeks.	**Mansouri *et al*., 2021b**
Watermelon rind	Lead - 5 mg / kg for 30 days.	**Michael *et al*., 2021**
Eucheuma cottonii	Lead acetate - 20 mg/kg body weight (BW) orally for 21 days.	**Wardani *et al*., 2017**
Thymus vulgaris	Lead acetate - 500 mg/kg/day by oral gavage for 6 weeks.	**El-Boshy *et al*., 2019**

3- Garlic and Lead

Garlic is a medicinal plant; it's used as a condiment; it is credited to have remarkable therapeutic and pharmacological properties. Its active agent is allicin, which imparts its characteristic odor as well as medicinal properties (**Sharma *et al.*, 2010**). Garlic can prevent oxidative stress by chelating lead ions and scavenging free radicals. **Senapati *et al***. (**2001**) reported the prophylactic efficacy of garlic extract in reducing the lead burden from soft tissues. In another study, it was confirmed the ability of garlic to reduce the lead burden from the liver, kidney, blood, and bone (**Pourjafar *et al.*, 2007**). The protective efficacy of aqueous garlic extract was studied against lead-induced hepatic injury in rats and the results indicated the ameliorative ability of garlic towards hepatic injury caused by lead due to generated oxidative stress (**Kilikdar *et al.*, 2011**).

CHAPTER II

MATERIALS & METHODS

Part I- Phytochemical Study of Allium triquetrum L.

I- BOTANICAL DESCRIPTION

1. Classification of Wild Garlic Allium triquetrum L.

According to **Cronquist's, 1981** classification of Angiosperms, garlic belongs to the Liliales, in the Liliaceae family (*Tab. 3*).

Table (3). Classification of the Wild Garlic *Allium triquetrum* according to **Corea *et al.*,** (**2003**); **Tela Botanica** (**2011**); **Deroeck** (**2014**) & **GBIF** (**2021**).

Kingdom: Plantae	
Sub-branch: Angiosperms	
Phylum: Tracheophyta	
Class: Liliopsida	
Subclass: Liliidae	
Order: Asparagales	
Family: Amaryllidaceae	
Subfamily: Allioideae	
Genus: *Allium*	
Species: *Allium triquetrum* L.	

Common Name(s)

The A. triquetrum has different names around the world (*Tab. 4*).

Table (4). Common names of *A. triquetrum* of different languages (paid) (**Philippe, 2020**; **Tela Botanica, 2021**).

English	Three-corner garlic, three corner leek, Triangular-stalked Garlic…
Arabic = Locally	Bibrous, بيبراس, الثوم ثلاثي الأوراق
French	Ail a tige triquètre, Ail a trois angles
Spanish	Lágrimas de la Virgen
Italien	Aglio triquetra
Deutschland	Glöckchen-Launch
Nederland	Driekantige Look

***Part I- Phytochemical Study of Allium triquetrum* L.**

2. Description of A. triquetrum L.

Allium triquetrum L. (Alliaceae), also known as three-cornered leek, triangle onion, or triangular stalked garlic, is a bulbous perennial flowering plant, native to the western and central Mediterranean region, being commonly found in wetland ecosystems. The green striped, white, and pendulous bell-like flowers, as well as the whitish bulbs and ovoid bulbils, distinguish morphologically triangle onions. The vegetative cycle of *A. triquetrum* L. starts in autumn and ends in spring, with the flowering period generally occurring between March and May, depending on the seasonal conditions after bud maturation.

The Three-corner garlic settles in a dry wasteland and prefers a sunny to semi-shaded exposure (*Figs 9, 10*). The soil must be gravelly loam or sandy clay. This species is indicative of humid zones and it supports temperatures until 12 °C (**Kodaira *et al.*, 2000**).

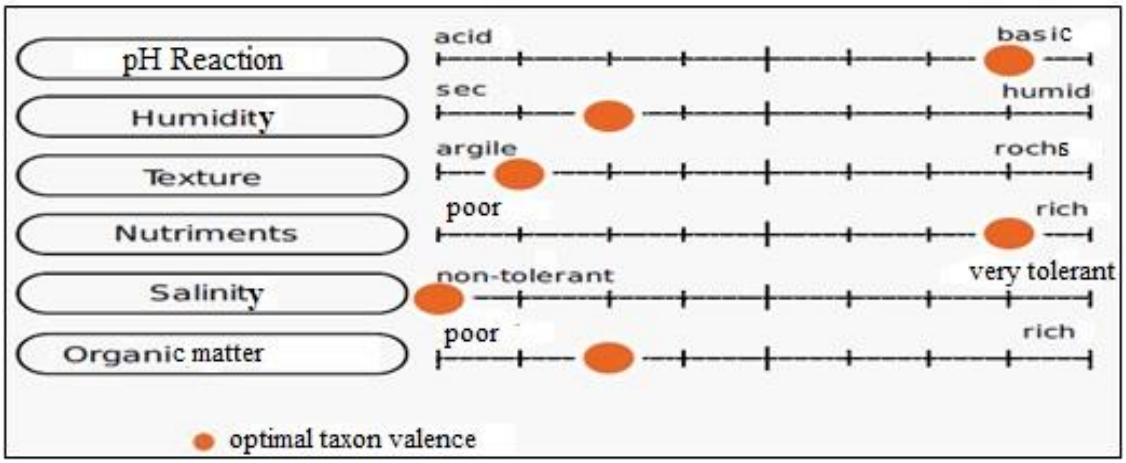


Fig 9. Optimal soil characteristics for the growth of *A. triquetrum* (**Philippe, 2020**; **Tela Botanica, 2021**).

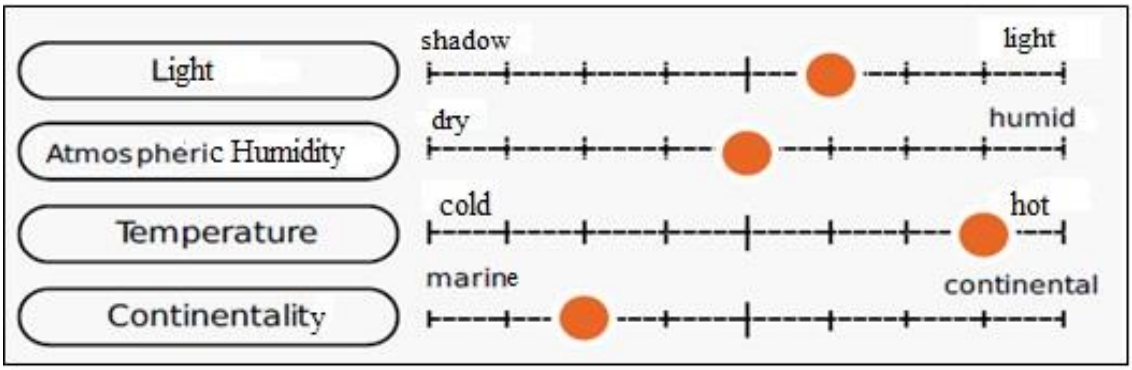


Fig 10. Optimal climatic characteristics for the growth of *A. triquetrum* (**Philippe, 2020**; **Tela Botanica, 2021**).

***Part I- Phytochemical Study of Allium triquetrum* L.**

The description of Three-corner garlic was made from our observation and from that reported in the literature (**Quezel & Santa, 1963**; **Baba Aissa, 1999**; **Blood, 2001**; **Deroeck, 2014**).

2.1. Bulbs

The bulbs and bulblets are generally ovoid, creamy-white with a strong garlic smell (*Fig. 5).* The bulb is an underground storage organ when the foliage dies. They form the young plant for the next growing season. Before its growth, the triqueter garlic is in the form of a small tunneled bulb or a group of bulbils with a cluster of white roots fleshy. The length of the roots varies according to the season and the type of soil.

Fig 11. The bulbs of *Allium triquetrum* L. (*Personal Photo*).

2.2. Leaves and Flowering Stem

Allium triquetrum L. is a perennial, herbaceous plant with a height of 15-50 centimeters that varies depending on the origin and environmental conditions.

The number of leaves differs and each plant usually consists of 2-5 leaves and 1-3 flowering stems per bulb (*Figs 11, 12*). The leaves are 5-15 millimeters wide, green, flat, glabrous, lanceolate, deciduous having a strong garlic smell when cut or crushed.

The peduncle is a little thick, triangular, erect arising directly from the bulb, and most often longer than the leaves. It ends in an umbel containing 6-10 flowers each.

***Part I- Phytochemical Study of Allium triquetrum* L.**

Fig 12. Leaves and flowering stem of *Allium triquetrum* L. (*Personal Photo*).

2.3. Inflorescences and Flowers

The flowers of *Allium triquetrum* are campanulate (bell-shaped) and pendulous. The umbel is subtended by a spathe (united bracts), the flowers are hermaphrodite and are 5-10 per umbel. Their petal is white with a central green stripe. The pedicel is longer than the flower.

The pedicel is longer than the flower, the flowers are actinomorphic and the gynoecium (pistil) includes a three-located superior ovary, a short style, and a three-parted stigma. The ovary has three united carpels, each with 1 to 2 ovules per compartment; the flowers are chasmogamous. **Garcia & Dale** (**2006**) report that the flowers open even when the anthers are still closed. The androecium is composed of six stamens, fused to the base of the tepals, three of which are internal (long) and three are external (small). This difference decreases after flowering from about 1.2 mm to 0.5 mm. The long stamens are almost vertical at the time of the anthesis then bent downwards; the stamens are longer than the style (*Fig. 13*).

Fig 13. Flowers of *Allium triquetrum* L. (*Personal Photo*).

***Part I- Phytochemical Study of Allium triquetrum* L.**

2.4. Fruits and Seeds

After fertilization, one green globular capsule per flower will form. The size of the capsules differs according to the number of seeds; they are generally six per capsule, black and ovoid. Seeds have a short life span of one year and each plant can produce up to 50 seeds per season.

Seeds germinate in autumn, leaves (2 to 4) are formed in winter, and bulbs in late winter and early spring. The plant dies in the fall and survives by the bulbs and bulblets that grow new leaves in the next growing season (Fall). Seeds can be spread by wind, flowering, insects (ants in particular), animals, soil, and water movement.

3. Different Uses or Consummation in Food of Wild Garlic *A. triquetrum*

Triangle onion has been considered for cooking applications, where leaves can be used as the main ingredient in salads, soups, and pies in Italy and used to aromatize pastry or season salads in Algeria (*Fig. 14*), while young leaves and roots' hearts are eaten raw or steamed (**Corea *et al.*, 2003**). The young leaves and the heart of the roots are eaten raw or steamed and they can accompany other vegetables in couscous (**Baba Aissa, 1999**). The juice of triquetra garlic is used as an insect repellent and has antiseptic properties that are common to all species of the Allium genus. Moreover, *A. triquetrum* L. flowers have been traditionally used in folk medicine, especially for wound healing (**Rabah *et al.*, 2020)**.

Fig 14. Some dishes cooked by *Allium triquetrum* L. (*Personal Photo*).

***Part I- Phytochemical Study of Allium triquetrum* L.**

II- COLLECTION OF SPECIMENS

Fresh bulbs and leaves of *A. triquetrum* were collected during the flowering period of March-May from Berrahal, Annaba region, North-East Algeria with geographic coordinates of Latitude 36.83045445, and Longitude 7.488031505; 36° 49' 49. 6" North, 7° 29' 16. 9" East (*Fig. 15*). The identification and authentication of the wild garlic were carried out in the laboratory of plant biology and environment, University of Badji Mokhtar-Annaba.

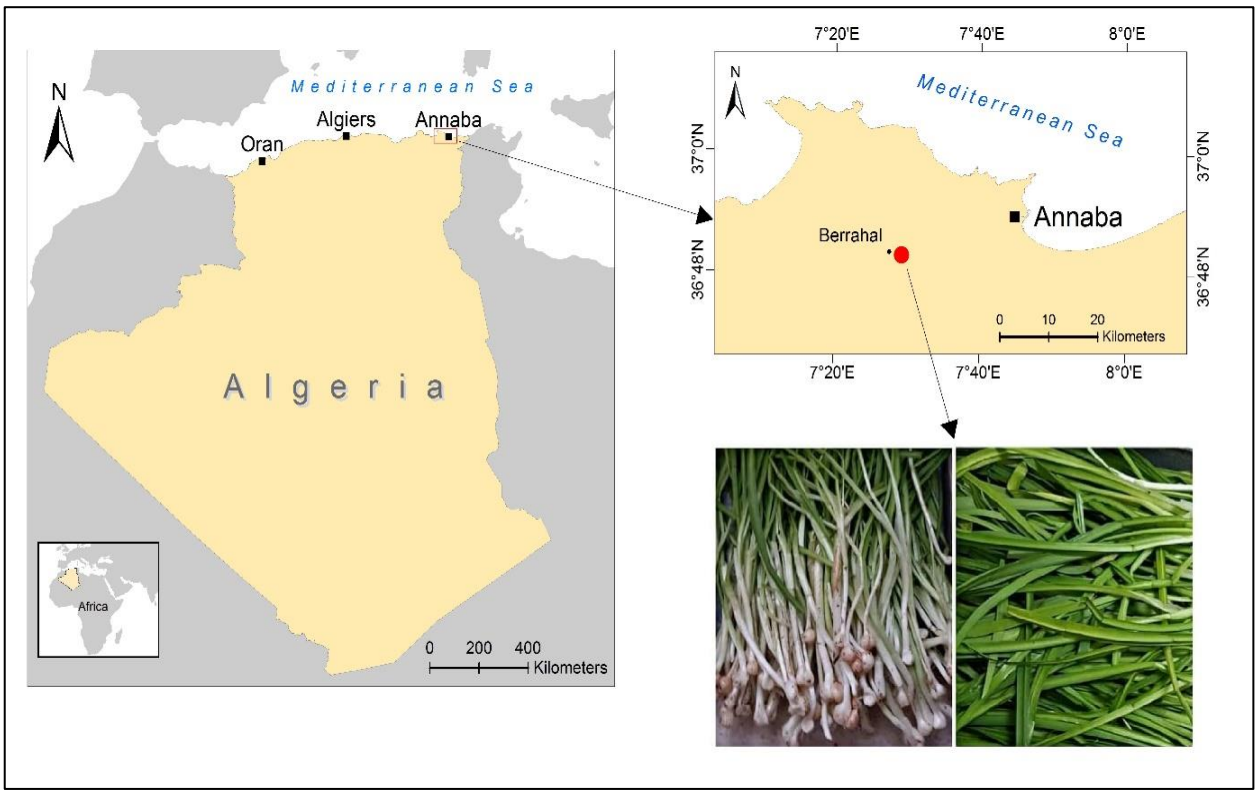


Fig 15. Sampling location of bulbs and leaves of wild garlic *A. triquetrum* collected in spring from Berrahal, Annaba province.

Part I- Phytochemical Study of Allium triquetrum **L.**

III- ANALYSIS METHOD

1. Phytochemical Tests

The phytochemical screening of *A. triquetrum* was realized to know the major constituents of the plant extracts.

Chemical screening is a set of physicochemical reactions to determine the major chemical groups contained in a plant organ. Preliminary tests for the determination of major chemical groups were performed according to **Solfo** (**1973**) **& Harborne** (**1980**).

a- Flavonoid Research

- Weigh 5 g of plant material in 100 mL of boiling distilled water, homogenize, close, and leave to infuse for 15 min.
- Filter into a new Erlenmeyer flask, take 5 mL of filtrate and introduce into a test tube, add 2 mL of 30% NaOH.

Flavonoids give a light-yellow color indicates the presence of flavonoids.

b- Polyphenol's Research

- Weigh 5 g of plant material in an Erlenmeyer, then, add 100 mL of boiling distilled water, homogenize, close, and leave to infuse for 15 min. After that, take 5 mL of filtrate and introduce into a test tube and Add 1 mL of 20% $FeCl_3$.

The formation of a greenish-brown precipitate indicates the presence of hydrolyzable tannins.

The formation of a blackish blue precipitate indicates the presence of condensed tannins (pro-anthocyanidins)

c- Alkaloid's Research

- Weigh 5 g of plant material in 100 mL of H_2SO_4 at 20%, homogenize and let macerate for 24 hours, after filtration in an Erlenmeyer flask, take 15 mL of filtrate and divide it into equal volumes into three test tubes. Then, add a few drops of Dragendorff's reagent in the first one, Mayer's reagent in the second one, and Bouchardat's reagent in the third one.
- Use three control tubes containing atropine sulfate in an aqueous solution.

The formation of an orange-red precipitate after the addition of Dragendorff's reagent indicates the presence of alkaloids.

The appearance of a white precipitate after the addition of Mayer's reagent indicates the presence of alkaloids.

The formation of a reddish-brown precipitate after the addition of Bouchardat's reagent indicates the presence of alkaloids.

d- Total Tannins Research

In a test tube containing 5ml of infusion, add 1ml of 1% ferric chloride ($FeCl_3$). The appearance of a greenish coloration indicates the presence of catechin tannins, a blackish blue coloration indicates the presence of gallic tannins.

e- Anthraquinones Research

- Weigh 1 g of plant material in an Erlenmeyer flask and add 10 mL of dichloromethane, heat carefully in a water bath at 60°C for 3 min, and then, filter hot in a new Erlenmeyer flask under the hood. Later, pour the filtrate into a test tube. After cooling, add 1 mL of diluted NH_4OH and shake.

The formation of red color in the aqueous phase indicates the presence of free anthraquinones.

f- Anthocyanin's Research

- Weigh 5 g of plant material in 100 mL of boiling distilled water, homogenize, close, and leave to infuse for 15 min, filter into a new Erlenmeyer flask, take 5 mL of filtrate, and introduce into a test tube. Afterwards Add 1 mL of HCl, pour in dropwise 1 mL of concentrated NH_4OH solution.

The appearance of a red color after the addition of HCl that turns blue-green after the addition of NH_4OH indicates the presence of anthocyanins.

g- Triterpenes and Sterols Research

- Weigh 1g of plant material in 20 mL of ethyl ether, close, homogenize and let macerate 24 hours, then filter into a new Erlenmeyer flask.

- Pour the filtrate into a capsule and evaporate dry under the host on a hot plate at 30°C. Once cooled, dissolve the residue in 2 mL of acetic anhydride and 2 mL of dichloromethane. Next, pour 4 mL of concentrated sulfuric acid into a second test tube.

The formation of a brownish-red or purple ring at the contact zone of the two liquids and the green or purple coloration of the supernatant layer indicates the presence of poly terpenes and/or sterols.

h- Quinones Research

1g of plant powder is macerated in 30 mL of ethyl ether for 24H, after filtration, a few drops of soda (NaOH) 1N are added. The red color of the aqueous phase indicates the presence of quinones.

i- Coumarins' Research

- Weigh 2 g of plant material in 40 mL of ethyl ether, close, homogenize and let macerate for 24 hours,
- Filter into a new Erlenmeyer flask. Then, pour the filtrate into a capsule and evaporate on a hot plate at 30°C. Once cooled, dissolve the dry residue in 2 mL of hot distilled water and introduce it in a test tube. Finally, add 2 mL of 25% ammonia. After that, observe under UV light at 365 nm.

The appearance of a blue fluorescence indicates the presence of coumarins.

j- Mucilage Research

- Weigh 10 g of plant material in 100 mL of distilled water.
- Heat the mixture under reflux until boiling then let it boil for 10 min, let it cool then filter, take 10 mL of filtrate and introduce it in a test tube. Add 5 mL of ethyl ether and shake.

The formation of a flaky precipitate in the ethereal phase indicates the presence of mucilage.

k- Saponoside Research

- In a series of 10 test tubes 10 mL of the decoctate. Adjust the volume in each tube to 10 mL with distilled water. Shake each tube lengthwise for 15 seconds at a rate of 2

shakes per second. Let stand for 15 min and then measure the height of the foam in each tube.

The foam index (F.I.) is calculated from the numbered tube (N) in which the height of the foam is equal to or close to 1cm.

2. Qualitative Analysis

2.1. Preparation of Aqueous Extracts

Four grams of each part of bulbs and leaves were placed in 200 mL of boiling distilled water (100 °C) for 20 minutes, under magnetic stirring. The obtained two aqueous extracts were then cooled, filtered through Whatman filter paper 4, placed in a petri dish, and left for three days in the oven at 60 °C.

2.2. Preparation of Methanolic Extracts

Allium triquetrum bulbs and leaves were washed, cut into small pieces; left to dry in a laboratory oven (40°C), then powdered using a commercial blender. We deposited 4 grams (gr) of each part studied in 20 milliliters (mL) of methanol in an Erlenmeyer flask for 24 hours (h) at room temperature. After shaking, the extracts are filtered and then evaporated to dryness under reduced pressure using a rotary evaporator at 60°C. The dry residues are taken up in methanol and stored in amber bottles.

2.3. HPLC Protocol

Five milligrams (mg) of each extract were deposited in 100 microliters (µL) of distilled water and 900 µL of Methanol (MeOH).

A C18 column of 5 µm (250×4.6 mm) (USA) was used. The separation was performed on a Shimadzu Prominence-I LC-2030C liquid chromatography (Japan) (*Fig. 18*). Before starting the chromatographic analysis, the mobile phases (phase A: include 19% Acetonitrile, 80% water, 1% formic acid, and phase B: include 59% Acetonitrile, 40% Methanol, 1% formic acid) and the extracts were placed in an ultrasonic tank for degassing. Then, 10 µL of each extract (at concentrations of 5 mg/mL) was injected at 40°C. Standards were prepared in methanol at a concentration of 1 mg / 10 mL. After each injection, the analytical system was rinsed for 1 hour with the mobile phase to ensure that any products that may have remained on the column were dislodged. A peak-free baseline

was the prerequisite for any injection. The solvents used were HPLC quality, with a flow rate of 1 mL/min and a wavelength of 350 nm.

3. Quantitative Analysis

3.1. DPPH Antioxidant Capacity Test

The free radical scavenging activity of the fractions was measured by the 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay (**Burits & Bucar, 2000**). After dissolving the Extract in methanol, the solution of DPPH in methanol (0.04 mg/mL) was prepared and 1250 µL of this solution was added to 50 µL of solution at different concentrations. The mixture was shaken vigorously and then kept in the dark for 30 min at room temperature. Then, the absorbance was measured at 517 nm. BHT was used as standard. Radical scavenging activity was calculated using the following equation:

$$\textbf{Radical scavenging activity (\%)} = \frac{(A\ blank - A\ sample)}{A\ blank} \times 100$$

A blank: Absorbance of the control. A sample: Absorbance of the reagent with the extract.

3.2. Determination of Total Polyphenols in Extracts

The total polyphenols content was determined by the Folin-Ciocalteu method as described by **Li *et al.*** (**2007**). 0.1 mL extracts were mixed with 0.5 mL of Folin-Ciocalteu reagent. After 4 min, 0.4 mL of 7.5 % sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 1h in dark at room temperature. The absorbance of the samples was measured at 760 nm and the results are expressed as microgram of gallic acid equivalents per milligram of extract (µg GAE/mg E).

3.3. Determination of Total Flavonoid in Extracts

Flavonoid determination was determined using the Aluminum trichloride ($AlCl_3$) method cited by **Mouffouk *et al.*** (**2018**). Flavonoids have a free hydroxyl group (OH), which is likely to give in the presence of $AlCl_3$ a yellowish complex by chelation of Al^{3+} ion. The yellow coloration produced is proportional to the number of flavonoids present in the extract (**Basli *et al.*, 2012**). 1 mL of extract was mixed with 1 mL of aluminum chloride ($AlCl_3$) solution (2%) and allowed to stand for 10 min. The absorbance of the mixture was then determined at 430 nm versus a blank equivalent to the prepared extract. The results were expressed as micrograms of quercetin equivalent per milligram dried extract (µg QE /mg E).

***Part I- Phytochemical Study of Allium triquetrum* L.**

3.4. Determination of Total Tannins in Extracts

Total tannins were measured by the Folin-Denis's method (**Polshettiwar *et al*., 2007**). Tannin colorimetry was based on the measurement of the blue color formed by the reduction of phospho-tungsto-molybdic acid by tannins as a compound in an alkaline medium. 1 mL of extract and a standard solution of tannin (100-800 μg/mL) was brought to 7.5 mL with distilled water. Then, 0.5 mL Folin-Denis's reagent and 1 mL of Na_2CO_3 solution were added. The volume was made up to 10 mL with distilled water. The absorbance was read at 700 nm. The total tannin content was expressed as mg of tannic acid equivalent (TAE)/g extract (**Padma *et al*., 2013**).

Part II - Experimental Design

I- MATERIAL USED

1- Animals

A male albino *Wistar* rats were provided from Algiers Pasteur Institute (Algeria) with an average body weight of 200 g ± 10. Rats were kept in a controlled environment under standard conditions of temperature, humidity, and natural light-dark cycle. They were fed *ad libitum* with water and food in the form of 30 g croquettes made of soy, corn, calcium carbonate; phosphate, and cellulose, which was purchased from the agro-food complex (El-Kseur, Bejaia, Algeria).

2- Preparation of Lead Acetate Solution

The solution of Pb-acetate trihydrate (Biochem-Chemopharma, Georgia - USA) was daily dissolved in distilled water respecting the variation in body weight of the rats in the batch where each rat receives 1 mL.

3- Preparation of A. triquetrum Aqueous Extract

Bulbs and leaves were homogenized daily by a commercial blender (Black + Decker BX HBA600E) after adding an appropriate quantity of distilled water to make a final volume of 20 mL after filtration by Aldismed sterilized compresses.

Fig 16. Preparation of the aqueous extract of bulbs and leaves *A. triquetrum.*

Part II - Experimental Design

The prepared solutions of Pb-acetate and garlic extracts were administrated to rats at the same time in the morning by gavage through a gastric tube for three consecutive weeks.

II- THE EXPERIMENT

The animals were divided into 18 groups in which they were treated according to a table (5) for the reproductive function and into 8 groups according to a table (6) for the hepatorenal axis.

Table (5). Experimental design of rats exposed to lead acetate and co-administrated with four doses of bulbs (B1-4) and leaves (L1-4) of *A. triquetrum* aqueous extracts for three weeks.

Groups	Daily treatment
Control (C)	Distilled Water
Lead acetate (Pb)	Pb (500mg/kg BW)
B1	Bulb's extract (2g/kg BW)
B2	Bulb's extract (3g/kg BW)
B3	Bulb's extract (4g/kg BW)
B4	Bulb's extract (6g/kg BW)
L1	Leave's extract (2g/kg BW)
L2	Leave's extract (3g/kg BW)
L3	Leave's extract (4g/kg BW)
L4	Leave's extract (6g/kg BW)
Pb - B1	Pb (500mg/kg BW) + Bulb's extract (2g/kg BW)
Pb - B2	Pb (500mg/kg BW) + Bulb's extract (3g/kg BW)
Pb - B3	Pb (500mg/kg BW) + Bulb's extract (4g/kg BW)
Pb – B4	Pb (500mg/kg BW) + Bulb's extract (6g/kg BW)
Pb - L1	Pb (500mg/kg BW) + Leave's extract (2g/kg BW)
Pb - L2	Pb (500mg/kg BW) + Leave's extract (3g/kg BW)
Pb - L3	Pb (500mg/kg BW) + Leave's extract (4g/kg BW)
Pb - L4	Pb (500mg/kg BW) + Leave's extract (6g/kg BW)

Part II - Experimental Design

Table (6). Experimental design of rats exposed to lead acetate and co-administrated with three doses of leaves (L1-3) of *A. triquetrum* aqueous extracts for three weeks.

Groups	Daily treatment
Control (C)	Distilled Water
Lead acetate (Pb)	Pb (500mg/kg BW)
L1	Leave's extract (2g/kg BW)
L2	Leave's extract (3g/kg BW)
L3	Leave's extract (4g/kg BW)
Pb - L1	Pb (500mg/kg BW) + Leave's extract (2g/kg BW)
Pb - L2	Pb (500mg/kg BW) + Leave's extract (3g/kg BW)
Pb - L3	Pb (500mg/kg BW) + Leave's extract (4g/kg BW)

Part II - Experimental Design

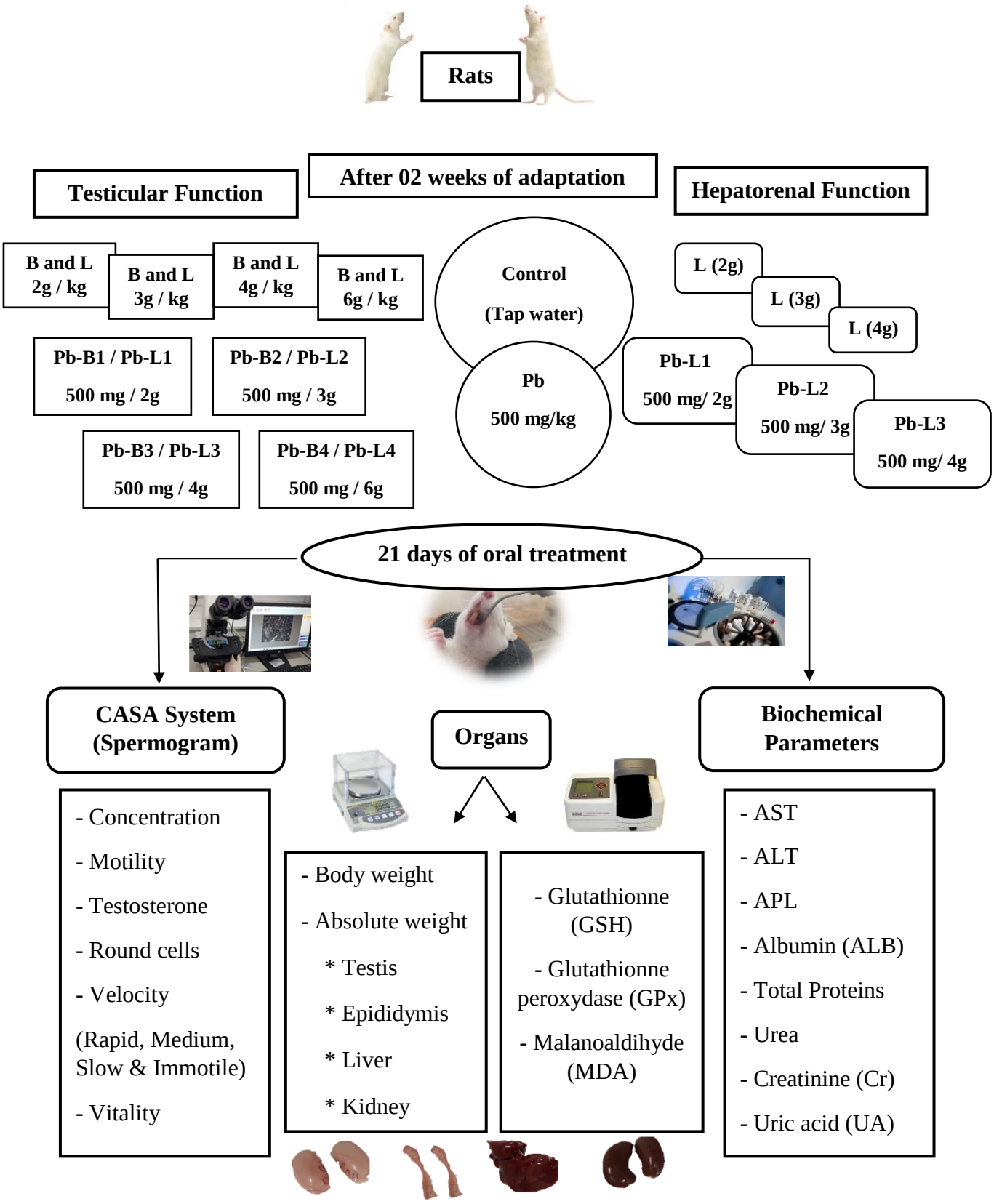


Fig 17. Summary of the experimental protocol.

Part II - Experimental Design

II- 1. Determination Body and Organs Weight

Animal weights were recorded weekly during and at the end of the experimental period. Rats were dissected where testicles, epididymis, liver, and kidneys were removed in a sodium chloride solution (0.9% NaCl) and the absolute weighs were obtained.

II- 2. Sperm Analysis by CASA System

Immediately after decapitation, semen was obtained by making a small incision in the tail of the epididymis using surgical blades, then approximately 1µL of seminal fluid was diluted in physiological solution (NaCl 0.9%) by putting a drop of seminal fluid in a pre-warmed Goldcyto slide (consisting of four counting chambers). The preparation is examined under a microscope Nikon Eclipse E200-LED (×4 objective), integrated with the Sperm Class Analyzer (SCA®, Microptic, Barcelona, Spain), version 6.2.0.0 by applying a computer-assisted semen analysis (CASA System) microcomputer to measure sperm concentration, motility, velocity, and round cells number that have a significant relationship with rat's sperm fertilizing ability. The CASA system has been developed because of its rapid and accurate estimation of sperm characteristics.

Part II - Experimental Design

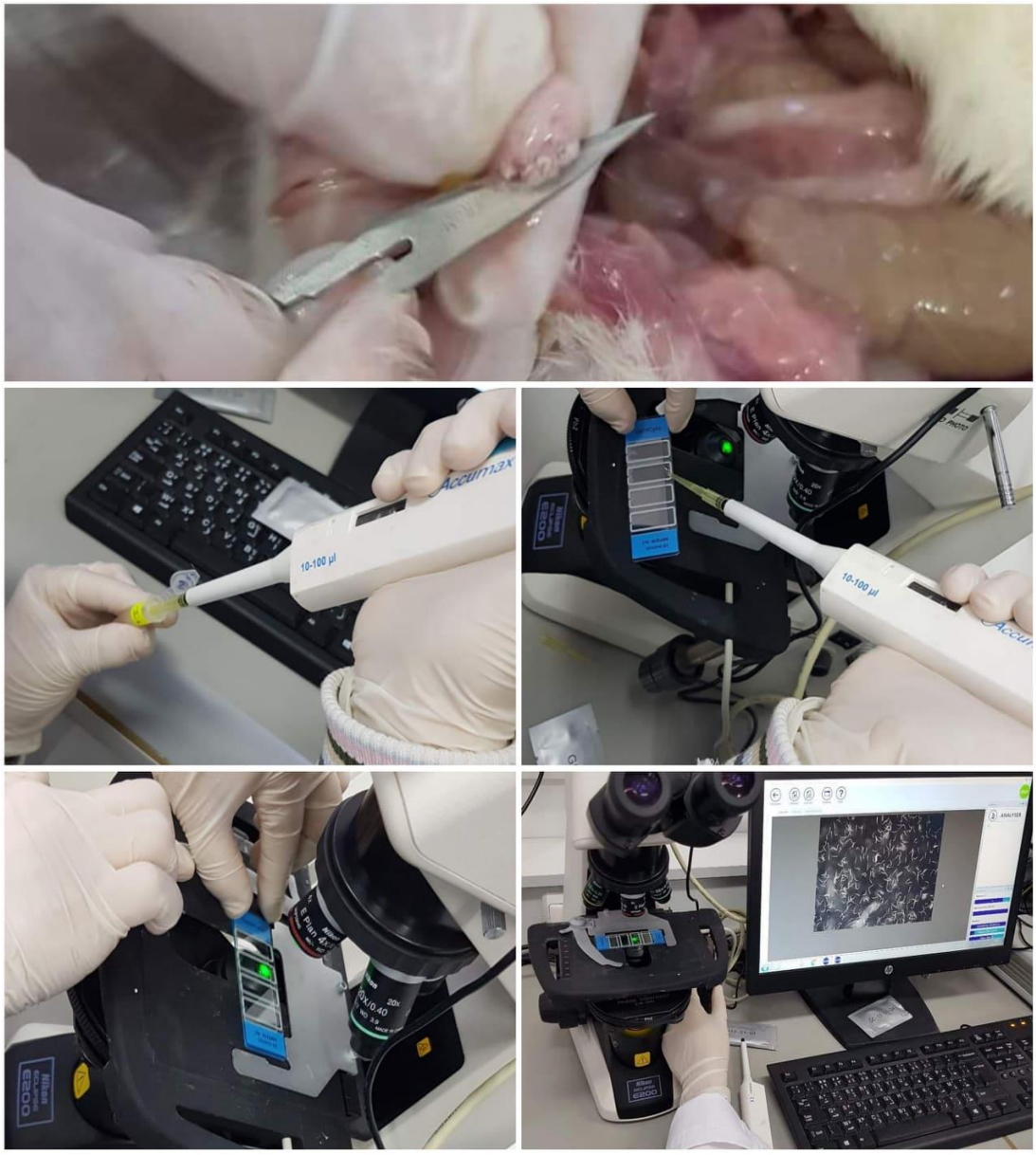


Fig 18. The different steps for sperm characteristics using the CASA system.

2.1. Sperm Vitality Test

Hypo-Osmotic Swelling Test (HOS-test) was used by placing sperm in a hypo-osmotic medium, in which the intact sperm membrane is semi-permeable to water causing its swelling (**Drevius & Eriksson, 1966**). Therefore, HOS-test is used to evaluate the functional and structural integrity of the sperm plasma membrane. It is based on the principle that fluid transport occurs across the intact cell membrane under the hypo-osmotic condition until equilibrium is achieved and results in bulging of sperms, especially, in the tails where the plasma membrane is loosely attached (**Jayendran *et al.*,**

Part II - Experimental Design

1992). This test was issued to determine sperm vitality by using a hypo-osmotic solution that was freshly prepared by dissolving 0.735 g sodium citrate $Na_3\ C_6H_5O_7,\ 2H_2O$, and 1.351 g fructose in 100 mL of distilled water. After pre-warming 1 mL of hypo-osmotic solution in a closed Eppendorf tube to 37 °C for 5 minutes, 0.1 mL of seminal fluid was added, and then mixed gently with a pipette. After half an hour of incubation at 37 °C, the slides of sperm count were estimated under the ×40 objective lens of a light microscope (Leica-Germany). The total number of sperm was expressed as a percentage change in the flagellum out of a total of 100 sperm.

2.2. Determination of Testosterone Levels

Testosterone levels in plasma were estimated by using a chemiluminescence immunoassay-based commercial kit (Access testosterone 33560) and an Access immune assay analyzer (Beckman Coulter Access 2, California, USA).

Part III- Biochemical Parameters Assay

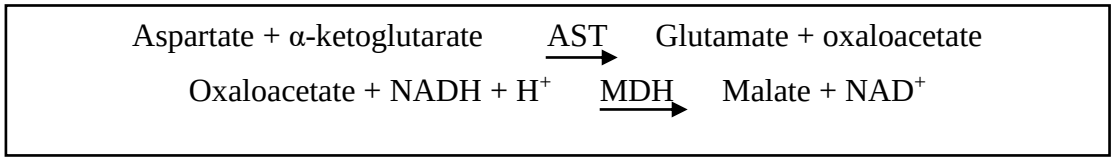
1. Blood Collection

Blood was collected in heparinized tubes, immediately centrifuged at 3000 rpm for 10 minutes, and then the plasma was separated for analysis of the biochemical markers.

The determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total proteins (TP), albumin (ALB), urea, creatinine (Cr), and uric acid (UA) were measured using an auto-analyzer (Mindray BS-240) following the technical instructions of the Spin react Kit (Santa Coloma, Spain).

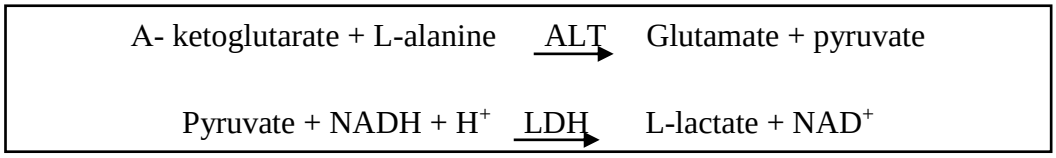
2. Aspartate Aminotransferase (AST / GOT) Measurement

Aspartate aminotransferase (ASAT) also called glutamate oxaloacetate (GOT) catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxaloacetate. Oxaloacetate is reduced to malate-by-malate dehydrogenase (MDH) and NADH, H^+. The rate of decrease in NADH concentration is directly proportional to the activity of aspartate aminotransferase in the sample (serum) (**Murray, 1984a**).



3. Alanine Aminotransferase (ALT/GPT) Measurement

The decrease in NADH concentration is directly proportional to the alanine aminotransferase enzyme activity in the sample (serum) **(Murray, 1984b).**



4. Alkaline Phosphatase (ALP) Measurement

Alkaline phosphatase (ALP) catalyzes the hydrolysis of p-nitrophenyl phosphate at pH 10.4 to give p-nitrophenol and phosphate. The formation of p-Nitrophenol is measured spectrophotometrically, where it is proportional to the catalytic activity of alkaline phosphatase in the sample (serum) (**Wenger *et al*., 1984**).

Part III- Biochemical Parameters Assay

Proteins form an intensive blue-violet-colored complex with copper ions in an alkaline medium. The intensity of the color formed is proportional to the concentration of total protein in the sample (**Koller, 1984**; **Burtis *et al*., 2005**).

5. Albumin (ALB) Measurement

Albumin reacts with bromocresol green (BCG) to form a colored complex. The pH of the medium is maintained at 4.2 by the buffer. After incubation, the absorbance of the mixture is measured at 628 nm (**Doumas, 1971**).

6. Urea (Ur) Measurement

The technique used for the determination of urea is the enzymatic method using urease according to the following reaction:

$$\text{Urea} + H_2O + O_2 \xrightarrow{\text{Urease}} (NH_4)_2 + CO_3^{-2}$$

$$NH_4^+ + \text{Salicylate} + \text{Na ClO} \xrightarrow{\text{Nitroprusside}} \text{Indophenol}$$

Ammonium ions can react with salicylate and sodium hypochlorite to form a green-colored complex, the color intensity is proportional to the concentration of urea present in the sample (serum) (**Fawcett & Scott, 1960**).

7. Creatinine (Cr) Measurement

Creatinine reacts with alkaline picrate to give a colored complex, measured within a defined time interval and proportional to the creatinine concentration of the sample (serum) (**Murray, 1984c**).

$$\text{Creatinine} + \text{picrate alkaline} \xrightarrow{\text{Ph alkaline}} \text{Complexes creatinine-picrate}$$

8. Uric Acid (UA) Measurement

The uric acid present in the sample gives according to the reaction described above a colored complex, quantifiable by spectrophotometry:

$$\text{Uric Acid} + 2\ H_2O_3 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2\ H_2O_2$$

$$2\ H_2O_2 + \text{Amino phenazone} + \text{DCPS} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + 4\ H_2O$$

The intensity of the color formed is proportional to the concentration of uric acid in the sample (serum) (**Schultz, 1984**).

Part IV- Oxidative Stress Analysis

1. Samples' Collection

The organs (testicular, epididymis, liver, and kidneys) were retrieved, weighed, and frozen at -20°C for the determination of the oxidative stress-related markers.

2. Protein Assay

Total proteins content of the tissues was carried out by the method of **Bradford** (**1976**) by using Coomassie Brilliant Blue G-250 (Sigma, St. Louis. USA) as a reagent and bovine serum albumin (BSA) as standard. The BBC reacts with the amine groups ($-NH_2$) of the proteins to form a blue complex. The appearance of this color reflects the degree of ionization of the acidic medium and the intensity establishes the concentration of the proteins. A 5 mL of the CBB Bradford solution was added to 0.1 mL of the homogenate. After 5 min the absorbance was measured at 595 nm.

3. The Glutathione Assay

The non-enzymatic antioxidant activity of reduced glutathione (GSH) is based on the reaction between 5,5'- dithiobis -2-nitrobenzoic- acid (DTNB). The latter results from the reduction of DTNB acid (Ellman's reagent) by the (-SH) groups of glutathione. For this, a deproteinization of the homogenate is essential to keep only the thiol groups specific to glutathione. 200 mg of organs tissues were homogenized in 8 mL of Ethylene Diamine Tetra Acetic Acid (EDTA 0.02 mol) solution. Then 0.8 mL of the homogenate was removed and mixed by adding 0.2 mL of 0.25 % sulfosalicylic acid solution. The mixture was stirred and left for 15 min in an ice bath, then centrifuged at 1000 g for 5 min. 1 mL of Tris (Hydroxymethyl) aminomethane EDTA (pH 9.6) was added to 0.5 mL of the supernatant, mixed then added 0.025 mL of 0.01 mol DTNB, and left to act for 5 min to obtain the yellow color, which then read at 412 nm (**Weckbecker & Cory, 1988**).

- ***Calculation of the concentration***

The concentration of glutathione is obtained by the following formula:

$$\mathrm{GSH} = \frac{\mathrm{OD} \times \mathrm{L} \times 1.525}{13.1 \times 0.8 \times 0.5 \times mg\ proteins}$$

OD: Optical density.

L: Total volume of solutions used in deproteinization (0.8 mL homogenate 0.2 mL of salicylic acid).

Part IV- Oxidative Stress Analysis

1.525: Total volume of solutions used in the determination of GSH in the supernatant (0.5 mL supernatant +1 mL Tris + 0.025 mL DTNB).

13.1: Coefficient of absorbance of the -SH group at 412 nm.

0.8: Volume of the homogenate.

0.5: Volume of supernatant.

4. Glutathione Peroxidase Assay

The determination of glutathione peroxidase (GPx) activity was carried out according to the method of **Flohé & Günzler** (**1984**). This method is based on the reduction of hydrogen peroxide (H2O2) in the presence of reduced glutathione (GSH); the latter is transformed into (GSSG) under the influence of GPx according to the following reaction:

$$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$$

500 mg of tissues were cold-milled using an ultrasonic homogenizer in the presence of 5 mL of a TBS solution (Tris 50 mmol, NaCl 150 mmol, pH 7.4) to obtain a homogenate. The reaction mixture was prepared by adding 0.2 mL of the testicular homogenate to 0.4 mL of GSH (0.1 mmol) and 0.2 mL of the TBS buffer solution. After incubation with a water bath at 25 °C for 5 minutes, 0.2 mL H_2O_2 (1.3 mmol) was added to start the reaction (leave for 10 minutes), then 1 mL Trichloroacetic acid (TCA 1 %) was added to stop the reaction. After the cooling time of 30 min in the ice bath, the tubes were centrifuged at 3000 g for 10 min, and the supernatant was collected. The 2.2 mL TBS buffer solution and 0.32 mL DTNB (1 mmol) were added to 0.48 mL reaction supernatant. After mixing and waiting for 5 min, the absorbance was recorded at 412 nm.

- ***Calculation of the concentration***

The determination of GPx enzymatic activity is done using the following formula:

$$Q = \frac{(\text{OD sample} - \text{DO standard}) \times 0.04}{\text{OD standard}}$$

Q: Quantity of GSH disappeared (oxidized).

OD sample: Optical density of the sample.

OD standard: Optical density of standard

0.04: Substrate concentration (GSH).

$$\text{GPx activity (M GSH / min / mg proteins)} = \frac{\text{Q}}{\text{mg / proteins}}$$

Part IV- Oxidative Stress Analysis

5. Malondialdehyde Assay

Malondialdehyde (MDA) content, which indicates the tissue lipid peroxidation, which is formed during the attack of polyunsaturated lipids by ROS, generated by certain contaminants, was determined by the reaction between TBA and MDA extractable by organic solvents such as butanol according to the method of **Ohkawa *et al*.** (**1979**). 0.5 mL of tissue homogenate was mixed with 0.5 mL of TCA at 20 % (500 mg of the organs in 5 mL of 0.1 mol, pH 7.4 phosphate buffer), then 1 mL of Thiobarbituric Acid (TBA 0.67 %) was added and incubated in a water bath at a temperature of 100 °C for 15 min. After cooling, 4 mL n-butanol was added, centrifuged at 3000 g for 15 min. The supernatant was removed and read at the optical density of 530 nm.

- ***Calculation of the concentration***

The amount of MDA in the sample is expressed in nmol/gram of tissue. It is obtained from a standard curve performed with 1,1`,3,3` tetra-ethoxypropane under the same conditions.

All measurements were realized by a UV/visible spectrophotometer (JENWAY-6300).

Statistical Study

Data were performed using the GraphPad Prism software V. 9 (USA) for windows presented as mean ± standard error of the mean (SEM). The one-way ANOVA was followed by a Tukey post hoc test for multiple comparisons. The criterion for statistical significance was set at $P< 0.05$.

CHAPTER III

RESULTS

Part I - Allium triquetrum L. *Extracts*

1. Phytochemical Screening

The phytochemical screening of bulbs and leaves of *A. triquetrum* aqueous extracts displayed the presence of flavonoids, polyphenols, phenolic derivatives, tannins, sterol terpenes, mucilage, coumarins, and saponosids, with the absence of alkaloids, anthraquinones, anthocyanins, and quinines (*Tab. 7*). This result approved that *A. triquetrum* contains various chemical compounds.

Table (7). Results of phytochemical screening of *Allium triquetrum* L.

Chemical Compounds	Bulbs	Leaves
Flavonoids	+ +	+ + +
Polyphenols	+ +	+ + +
Phenolic derivatives	+	+
Alkaloid	-	-
Tannins	+	+ +
Anthroquinones	-	-
Anthrocyanins	-	-
Sterol terpenes	+ +	+ + +
Quinones	-	-
Coumarins	-	+
Mucilage	+ + +	+ + +
Saponosids	+ + +	+ +
(+): slightly present, (+ +): moderately present, (+ + +): highly present, (-) : absent		

2. Chromatograms

The chromatographic profiles of the aqueous extracts of bulbs and leaves of *Allium triquetrum* analyzed by HPLC appear to contain phenolic compounds such as; flavonoids, hesperidin, coumarins, and chlorogenic acid (*Figs. 20, 21*). Furthermore, leaves' aqueous extract appears to contain rutin and isoquercetin.

Part I - Allium triquetrum L. *Extracts*

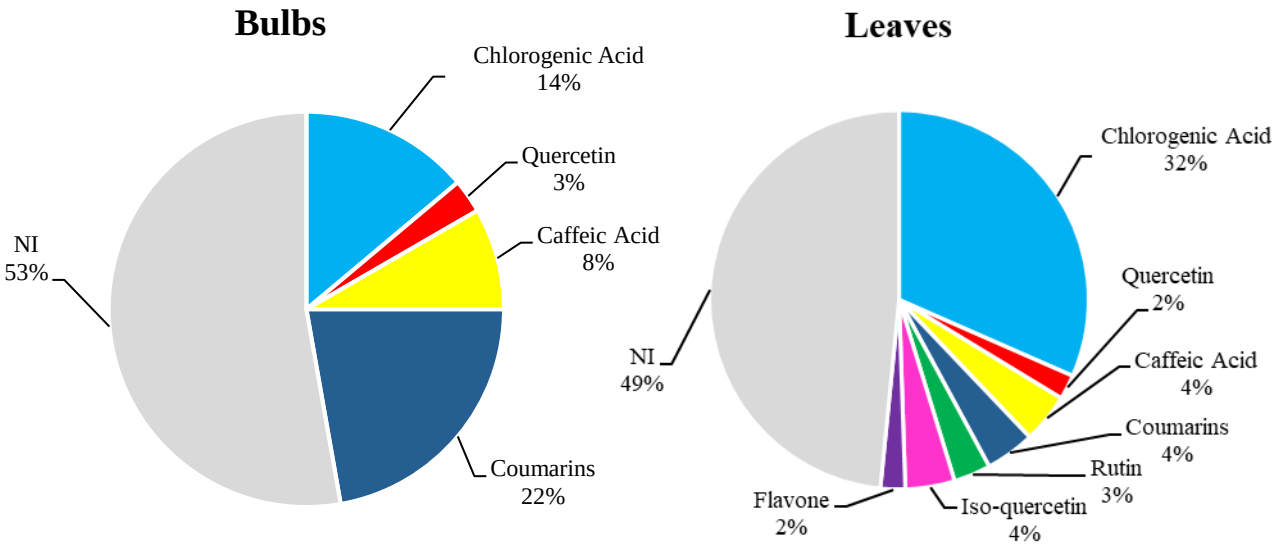


Fig 19. Relativistic circle of bioactive compounds in the aqueous extract of *Allium triquetrum* bulbs and leaves. (NI = Not Identified).

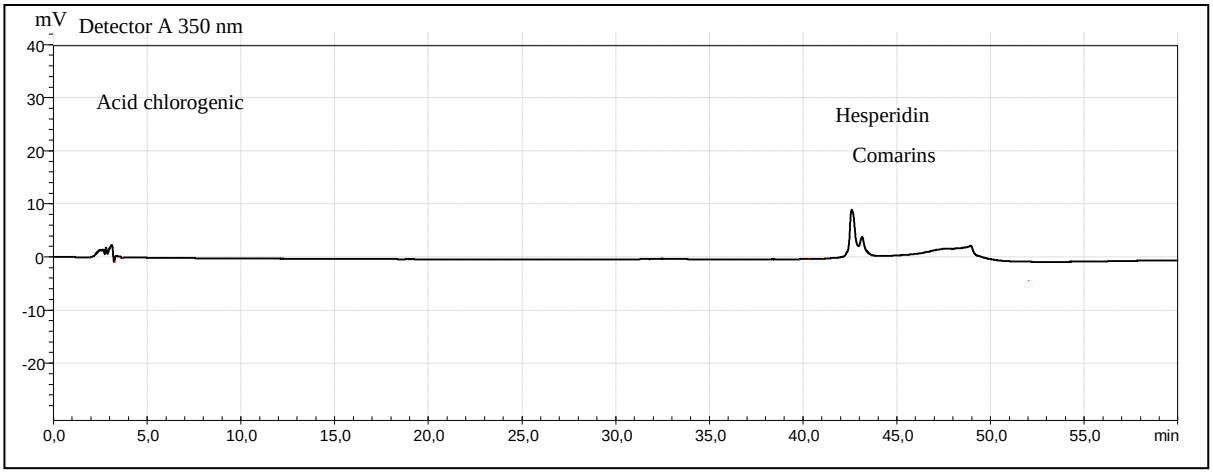


Fig 20. Chromatogram of the aqueous extract of *Allium triquetrum* bulbs.

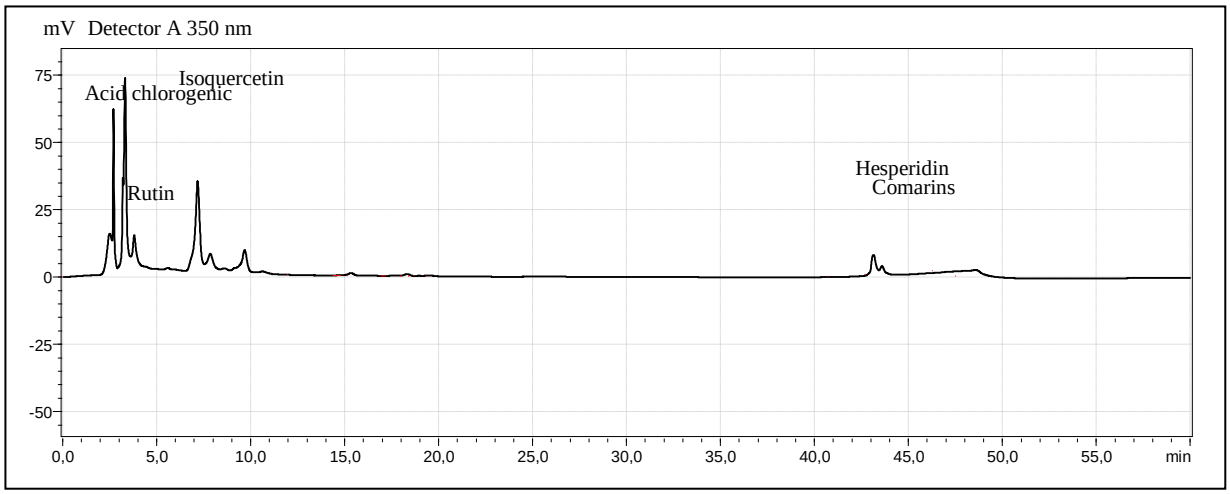


Fig 21. Chromatogram of the aqueous extract of *Allium triquetrum* leaves.

Part I - Allium triquetrum* L. *Extracts

3. Antioxidant Activity

3.1. DPPH Radical Scavenging Capacity

The free radical scavenging activity of fractions has been studied with IC_{50} and inhibition percentage % values and presented in *Fig. 22* which displayed that aqueous extract of bulbs (AEB) and leaves (AEL) had a greater antioxidant activity (DPPH). Moreover, we noticed that leaves contain higher antioxidant activity compared to bulbs.

For the calculation of IC_{50} values, different concentrations of samples were tested against DPPH radicals and it was found that AEL (IC_{50}: 0.962 ± 0.08 mg / mL) is the most potent among all samples and in comparison, with BHT also.

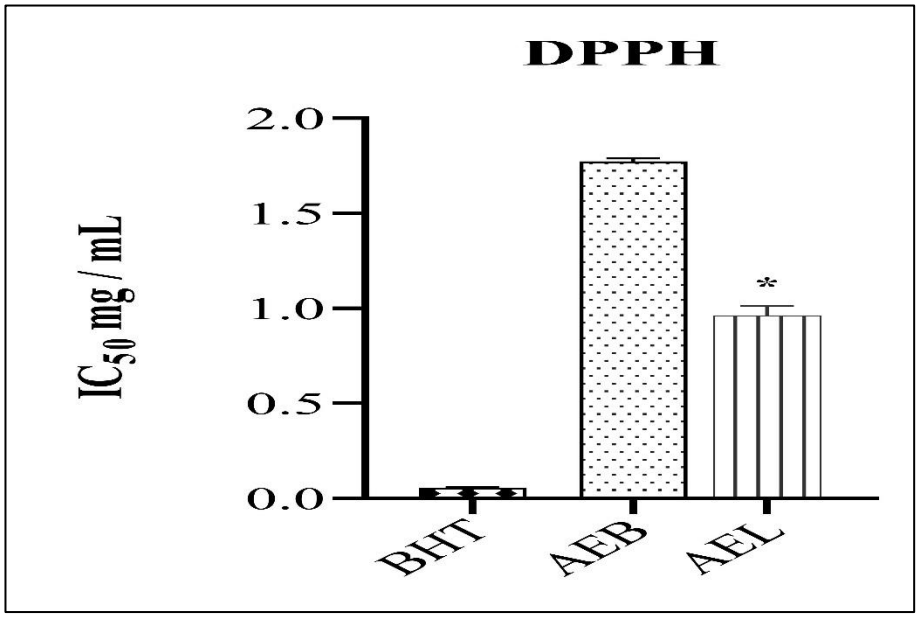


Fig 22. The antioxidant DPPH radical scavenging activity (mg / mL) of bulbs and leaves aqueous extracts of *A. triquetrum*. DPPH = 2,2-Diphenyl-1-picrylhydrazyl, IC_{50} = Inhibitory Concentration.

3.2. Bioactive Compounds

The equations of calibration curves of quercetin and gallic acid were used for the estimation of total polyphenols and flavonoids respectively. The results indicate that the leaves aqueous extract (AEL) had a remarkably higher number of total polyphenols flavonoids, and tannins than the aqueous extract of bulbs (AEB) (*Figs. 23, 24, 25*).

Part I - Allium triquetrum* L. *Extracts

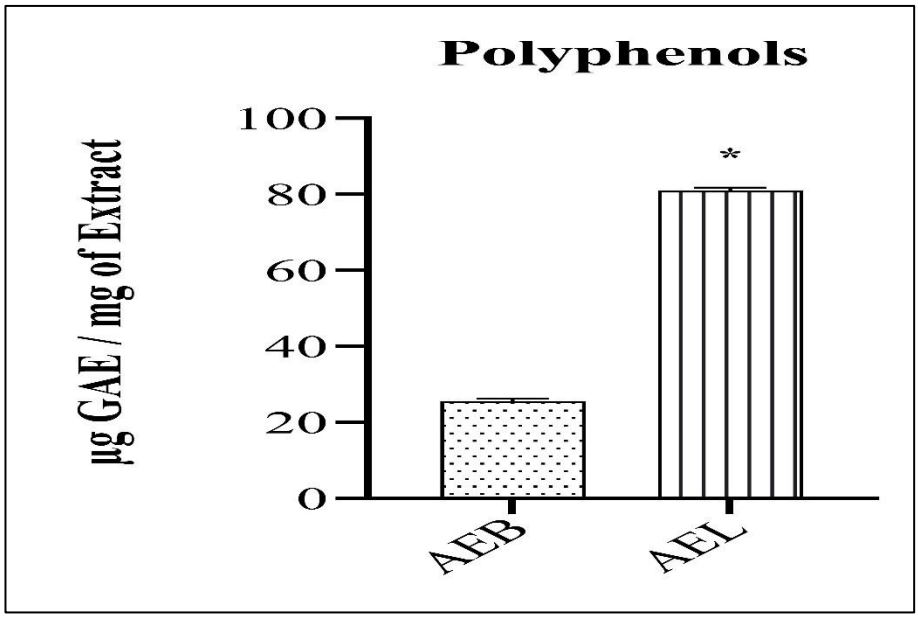


Fig 23. Total polyphenols content (µg GAE / mg E) of bulbs and leaves aqueous extracts of *A. triquetrum* expressed as means ± standard error means (SEM). GAE: Gallic acid equivalent.

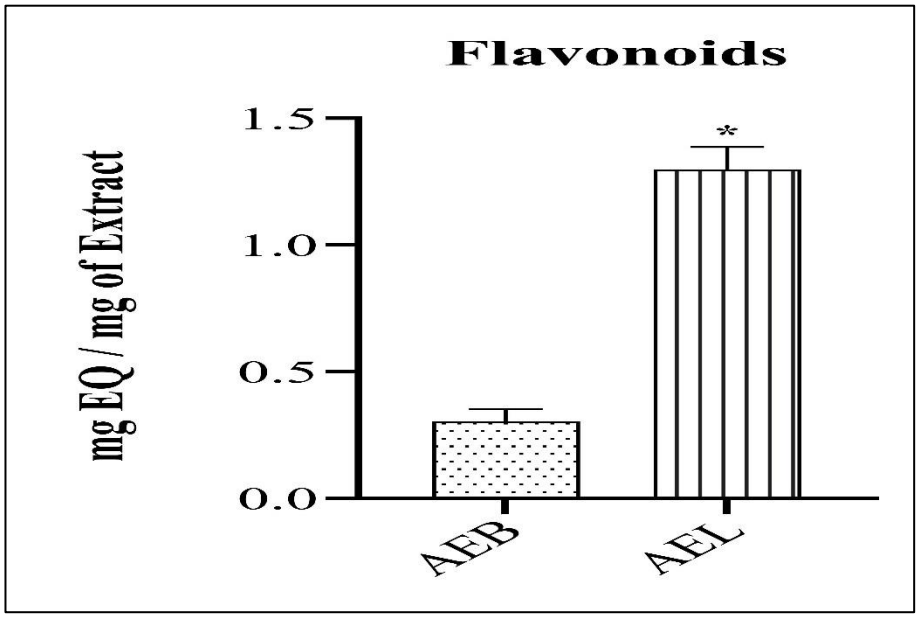


Fig 24. Total flavonoids content (mg QE / mg E) of bulbs and leaves aqueous extracts of *A. triquetrum* expressed as means ± standard error means (SEM). QE: Quercetin equivalent.

Part I - Allium triquetrum* L. *Extracts

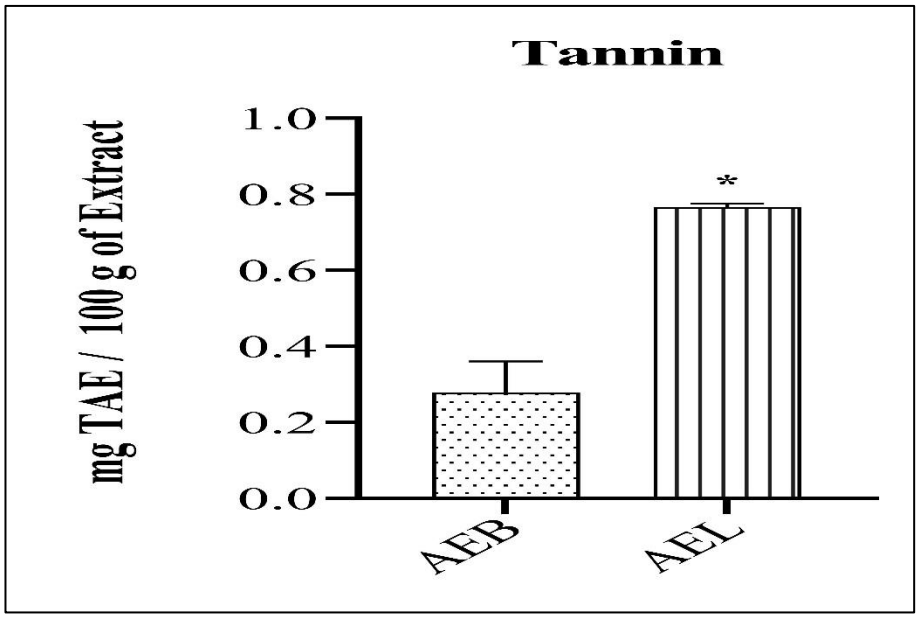


Fig 25. Total tannins content (mg TAE / 100 g E) of bulbs and leaves aqueous extracts of *A. triquetrum* expressed as means ± standard error means (SEM). TAE: Tannic acid equivalent.

Part II - Reproductive Markers

1. General Assessment

During the experimental period, rats of the control group were healthy and showed no signs of toxicity. However, in the rats of the Pb-acetate group, some negative signals such as decreased vitality, muscle weakness, tremor, an abnormal body weight gain, and lack of stability and balance. At the end of the experiment, one rat in the Pb-treated group was nearly paralyzed and three died.

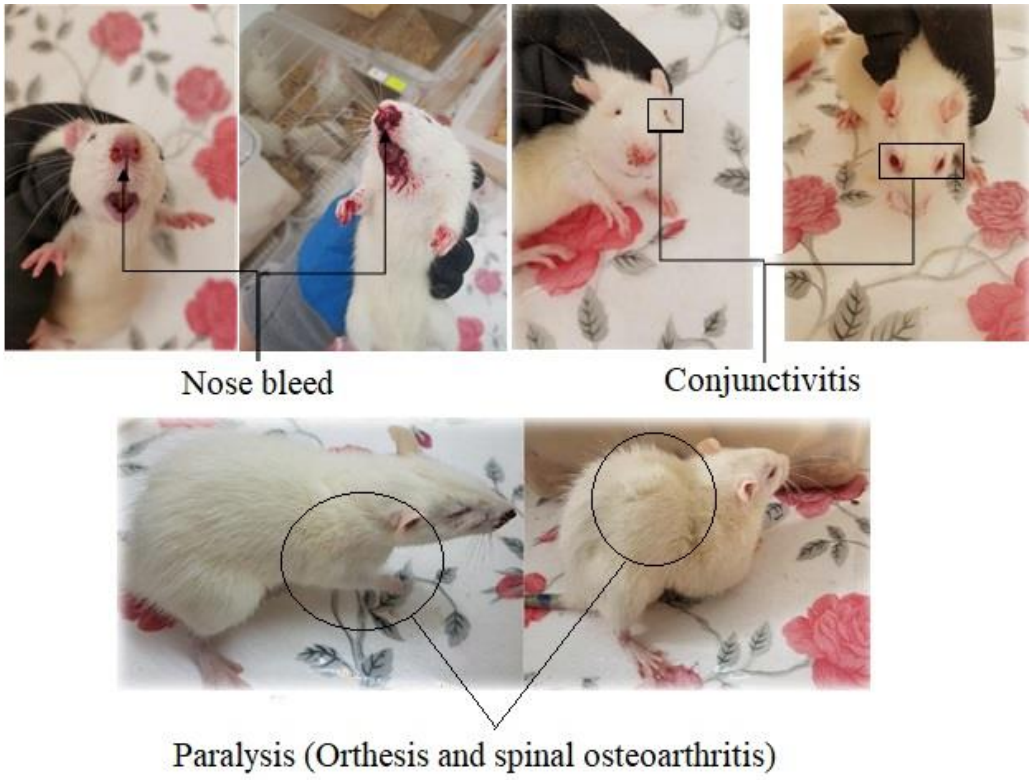


Fig 26. Some signs of toxicity in rats treated with Lead Acetate.

2. Organs and Total Body Weight

The total body weight was significantly decreased in the Pb exposed group compared to the control. In contrast, a significant increase in the rats' weight of the groups treated with the combination of Pb-garlic was observed compared to the Pb group. Treatment of rats with the positive controls of the four doses of garlic extracts (bulbs and leaves) has not made any significant changes in the absolute weight of testis and epididymis as compared to the control. Nevertheless, all Pb-garlic extracts of bulbs and leaves were significantly higher than that of the Pb group (*Figs. 27, 28, 29*).

Part II - Reproductive Markers

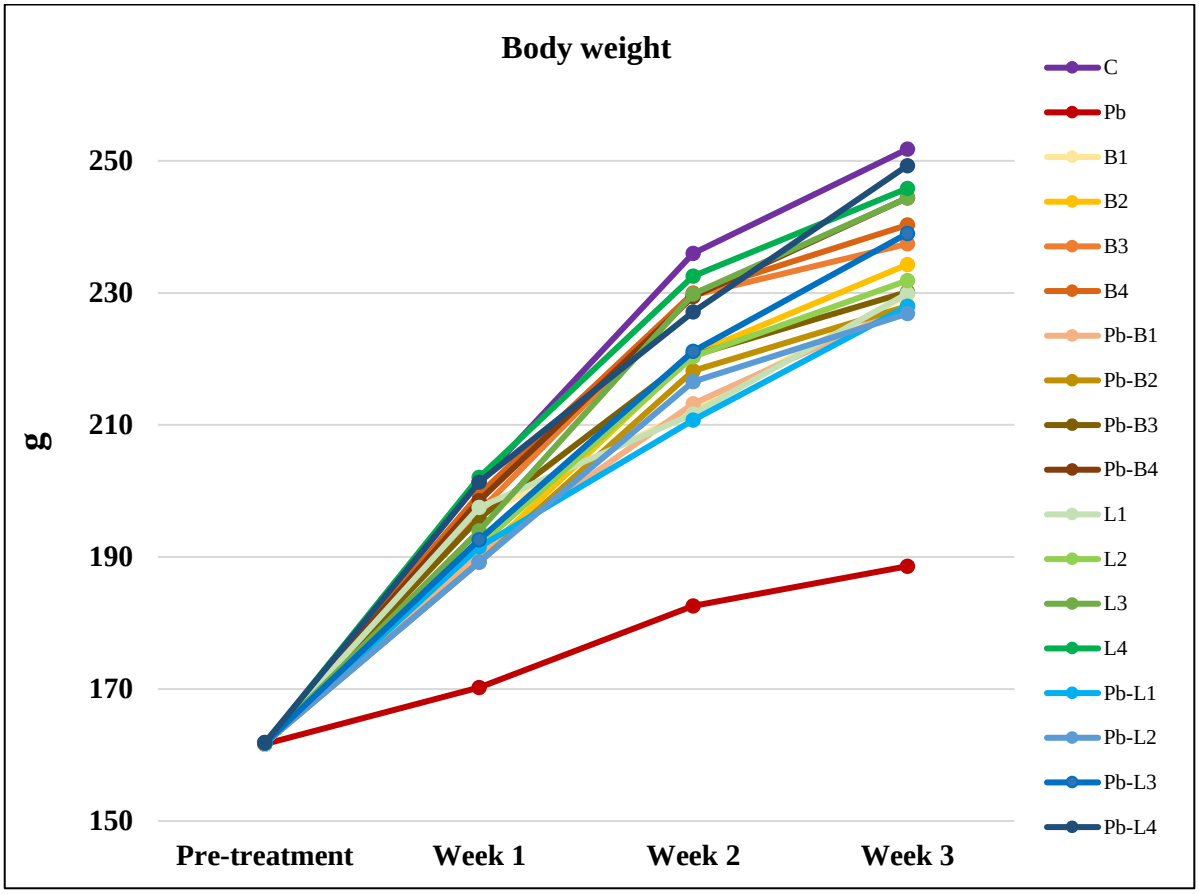


Fig 27. The total body weight (g) (Mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

Part II - Reproductive Markers

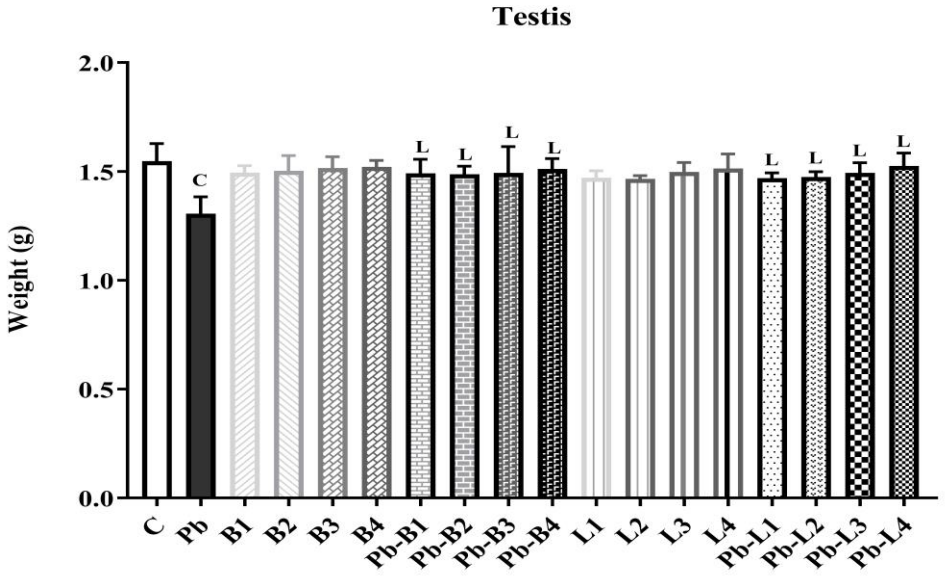


Fig 28. Testis absolute weight (g) (Mean ± SEM) of rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

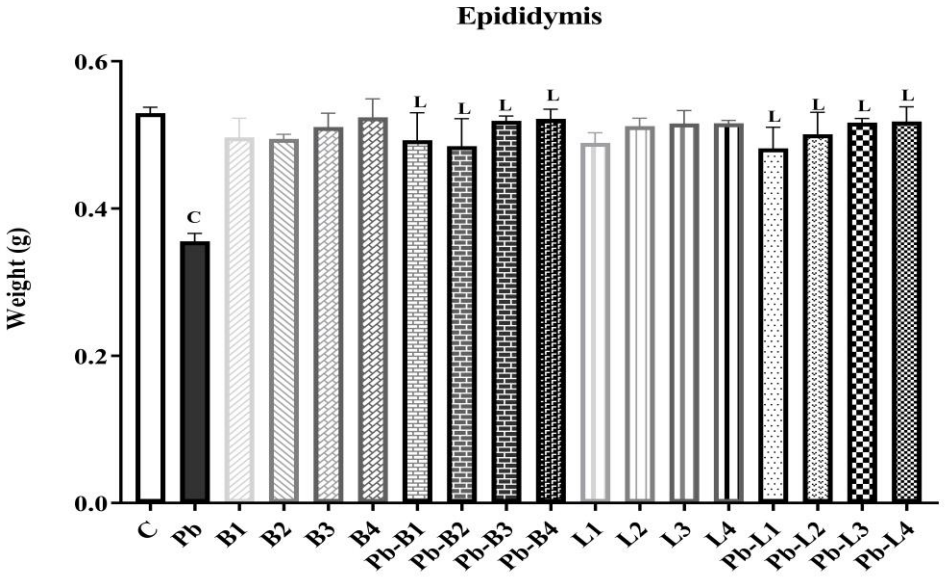


Fig 29. Epididymis absolute weight (g) (Mean ± SEM) of rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

Part II - Reproductive Markers

3. Spermatozoa Concentration and Motility

Rats administered with lead acetate have a significant decrease in sperm concentration and motility, relative to the control group. Contrary, sperm concentration and motility of all combination groups were significantly higher than that of the Pb intoxicated rats.

A significant dose-dependent attenuation was observed in sperm concentration and motility of the Pb-garlic treated rats (*Figs. 30,31*).

4. Testosterone Level

The statistical results obtained in *Fig. 32* illustrate that the Pb group shows a significant decrease in testosterone level compared to the control group. While co-administration of *A. triquetrum* combined with lead acetate at different doses (Pb-B and Pb-L) reported a significant increase compared to the Pb group.

5. Spermatozoa Round Cells

Compared with the control, results revealed a non-significant decrease of round cells counts in the positive controls and the combined treatment groups, with a significant decrease in the Pb group (*Fig. 33*).

6. Spermatozoa Velocity

Results indicate that rapid, medium, and slow sperm velocity of spermatozoa was significantly lower in the Pb group when compared to the control, with a slight increase in the positive controls, and the combined groups. The progressive immotile sperm velocity was significantly higher in the Pb group compared to the control, in contrast to the positive controls (B and L). Spermatozoa velocity of the combined groups (Pb-B, Pb-L) decreased significantly compared to the Pb group (*Fig. 34*).

7. Spermatozoa Vitality

Spermatozoa vitality (%) evaluated by the HSO-test is shown in *Fig. 35*. Rats administered with lead acetate have a significant increase in dead sperm vitality relative to the control group. Contrary, all combination groups were significantly lower than that of the Pb intoxicated rats. However, the percentage of live sperm was significantly lower in the Pb group compared to the control, in contrast to the positive controls (B and L). Nevertheless, Spermatozoa vitality of all Pb-garlic extracts of bulbs and leaves was significantly higher than that of the Pb group.

Part II- Reproductive Markers

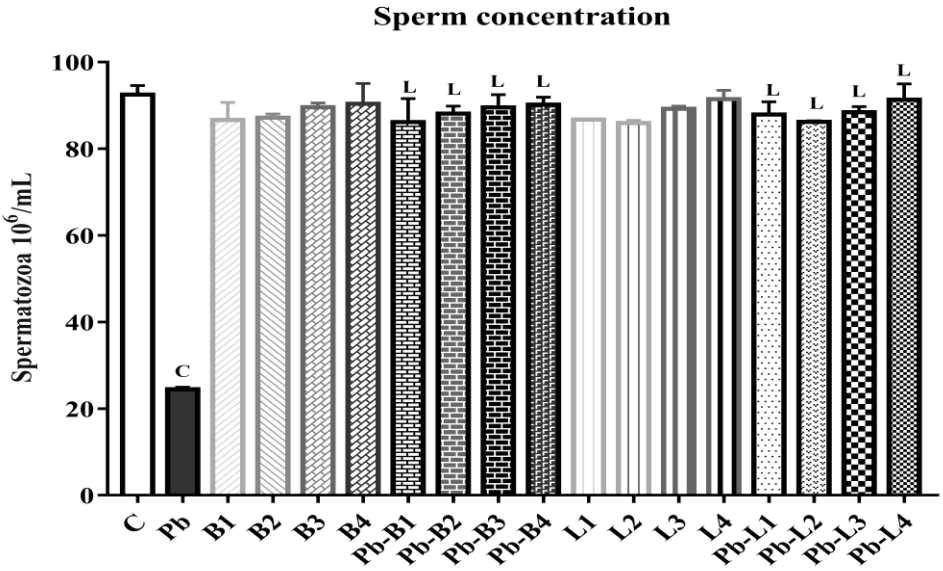


Fig 30. Sperm concentration (Mean ± SEM) of rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

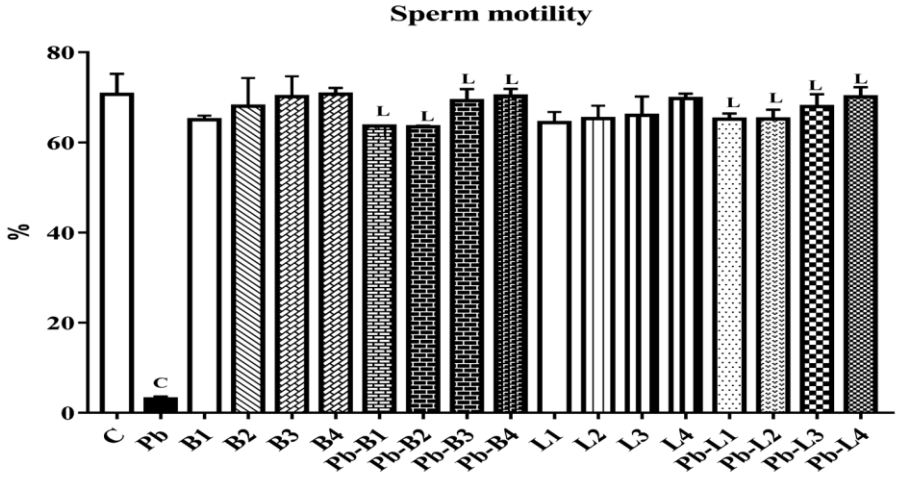


Fig 31. Sperm motility (Mean ± SEM) of rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

Part II- Reproductive Markers

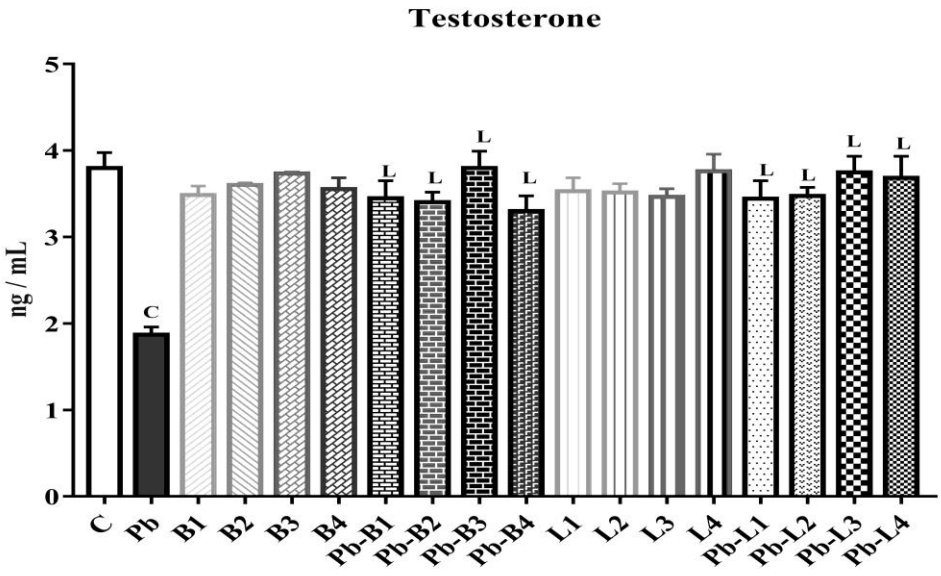


Fig 32. Testosterone levels (Mean ± SEM) of rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

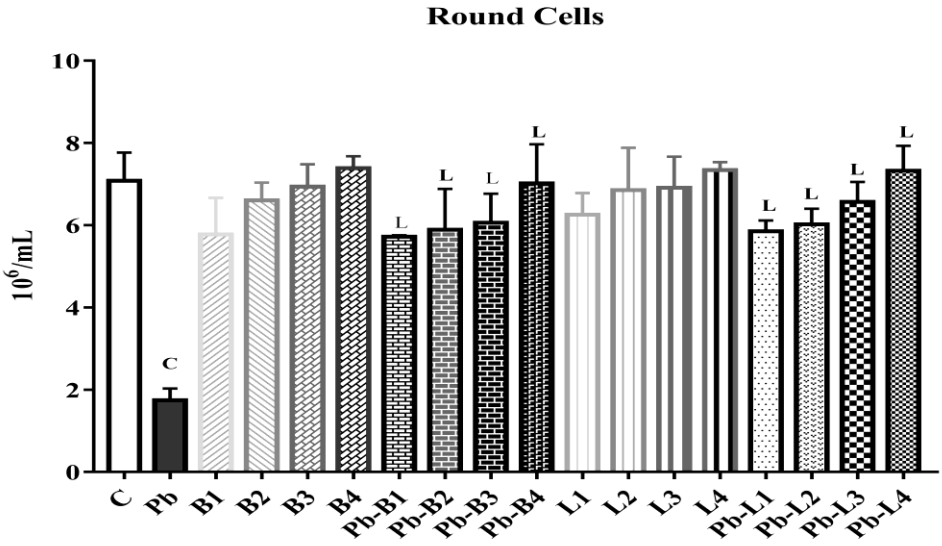


Fig 33. Number of round cells (Mean ± SEM) of rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; L: Leaves; Pb-B: Lead acetate + Bulbs aqueous extract; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

Part II- Reproductive Markers

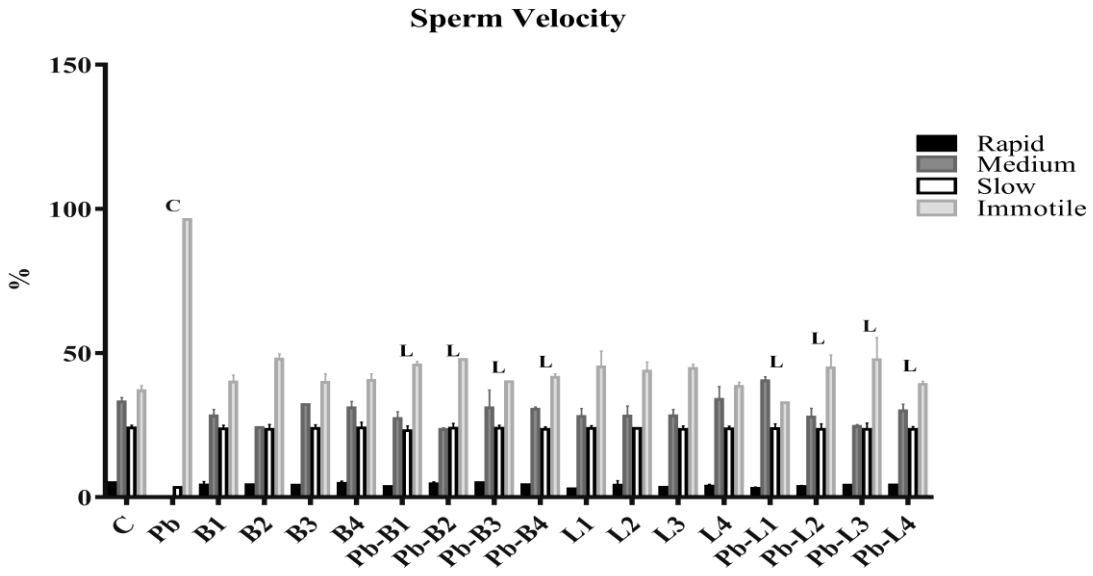


Fig 34. Rapid, medium, and slow spermatozoa velocity (Mean ± SEM) of rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

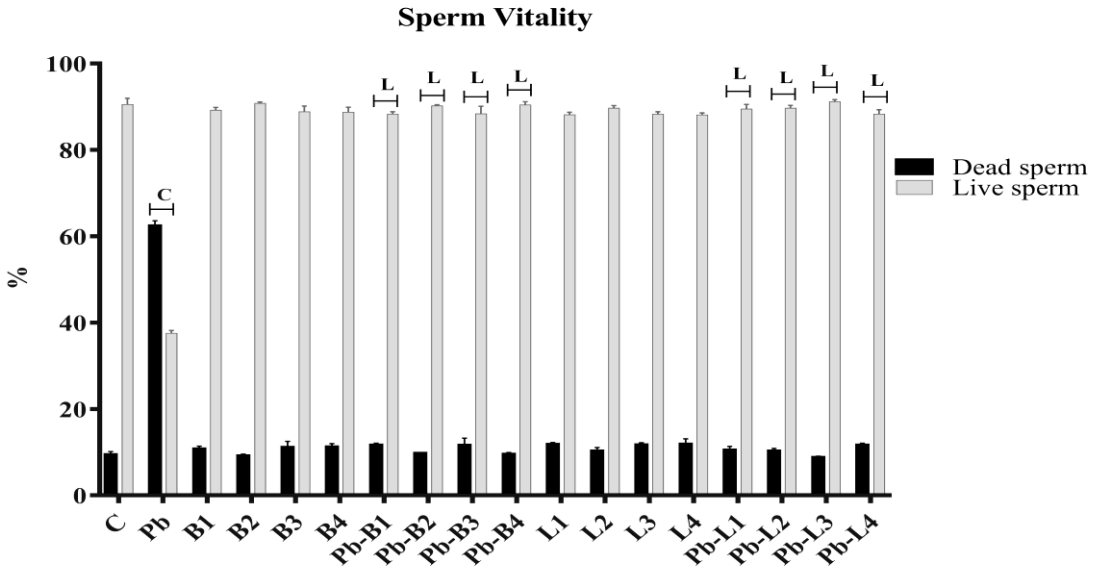


Fig 35. Sperm live/dead count (Mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

Part III - Hepatic and Renal Markers

1. Liver and Kidney Weight

Compared with the control, the results displayed a significant increase in liver and kidney weights in rats treated with lead acetate, with a non-significant in both organ weights in the positive controls (L). While, co-treatments of leaves extracts combined with Pb (Pb-L) have significantly reduced liver and kidney weight compared to the Pb-group (*Figs. 36, 37*).

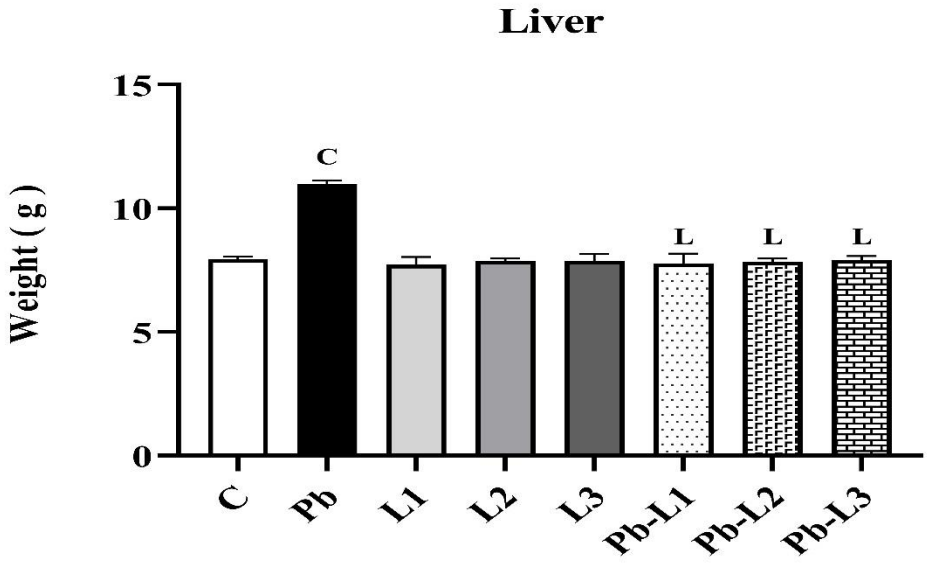


Fig 36. Weight (g) of the rat's liver (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part III - Hepatic and Renal Markers

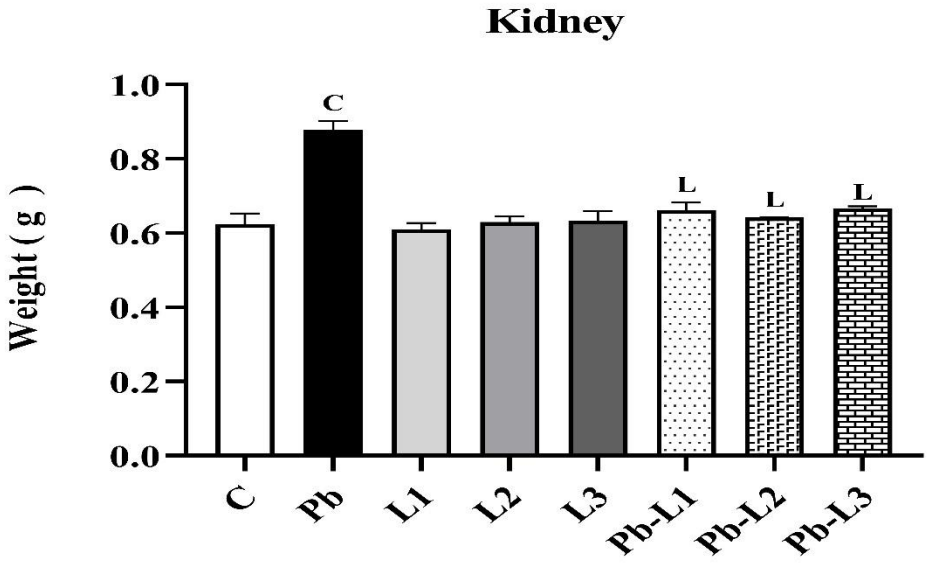


Fig 37. Absolute weight (g) of the rat's kidney (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part III - Hepatic and Renal Markers

2. Blood Chemistry

2.1. Hepatic Markers

Rats exposed to lead acetate exhibited a significant increase in AST, ALT ALP compared with the control (*Figs. 38, 39, 40*). However, supplementation of leaves' aqueous extract as a positive control kept the activities of plasma enzymes constant compared to the control group. Similarly, the co-administration of Pb-L showed a significant decrease in plasma enzyme activities compared to the Pb group.

On the other hand, albumin, and total proteins' concentration (*Figs. 41, 42*) revealed a significant decrease in Pb-intoxicated rats compared to the control, while that of the positive control showed a non-significant augmentation relative to the control. On the other hand, albumin, and total proteins' level of all combination groups (Pb-L) were significantly higher than that of the Pb intoxicated rats.

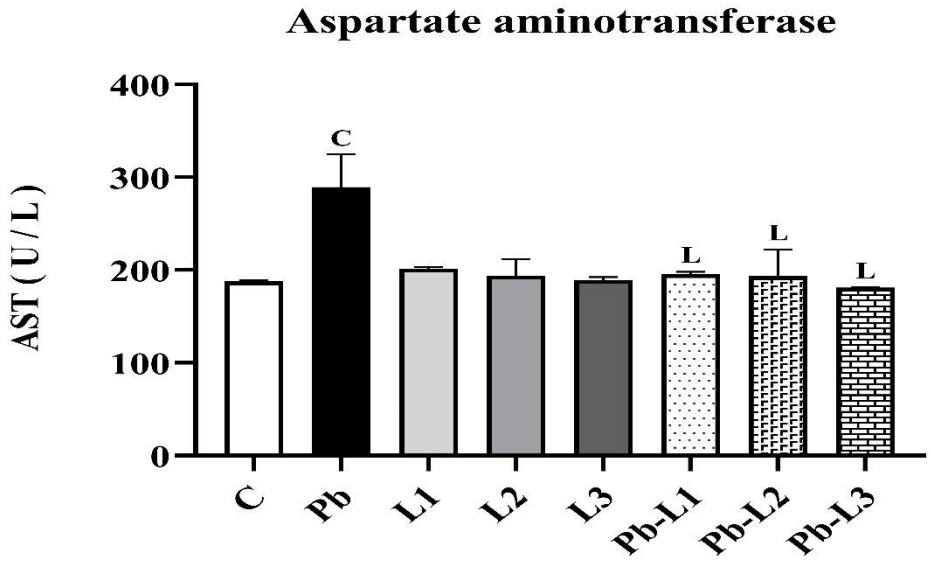


Fig 38. Aspartate aminotransferase (AST) level in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part III - Hepatic and Renal Markers

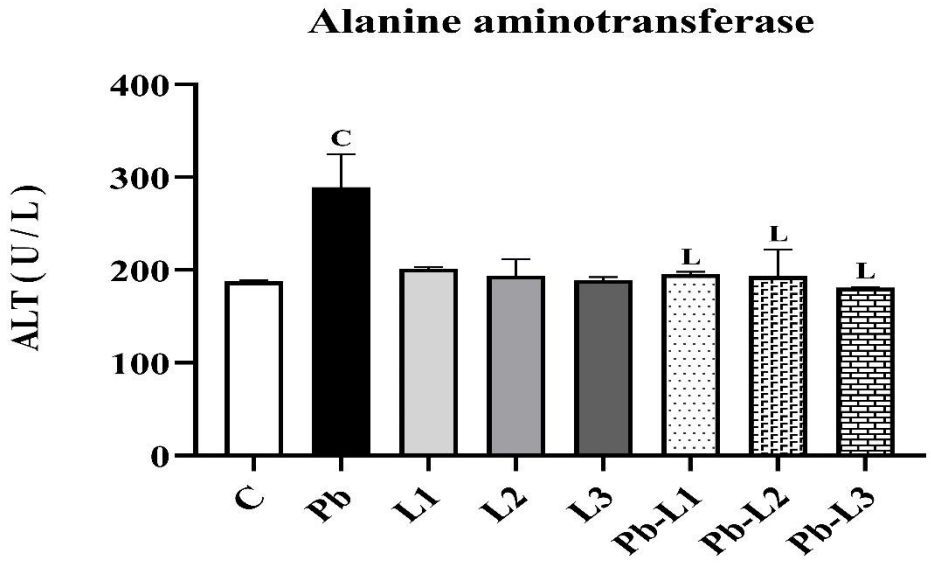


Fig 39. The Alanine aminotransferase (ALT) level in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

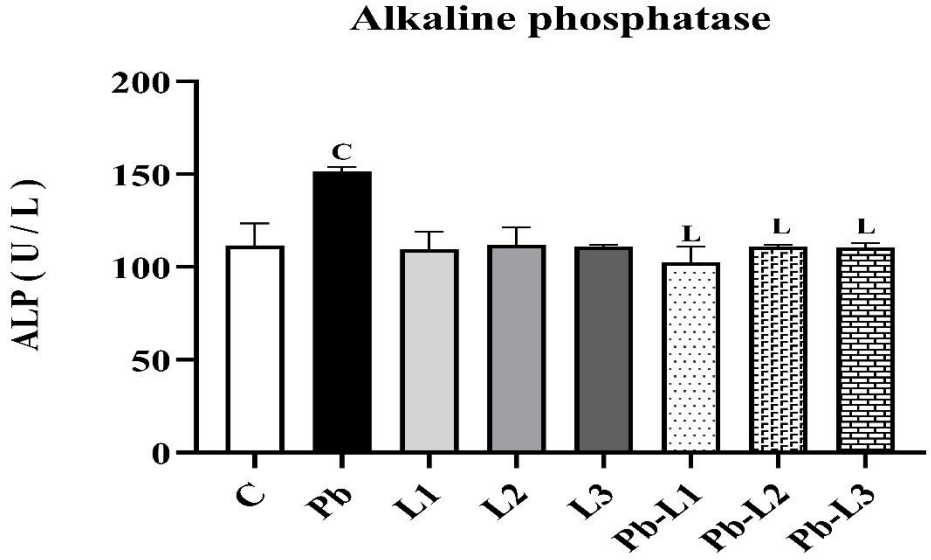


Fig 40. Alkaline phosphatase (ALP) level in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part III - Hepatic and Renal Markers

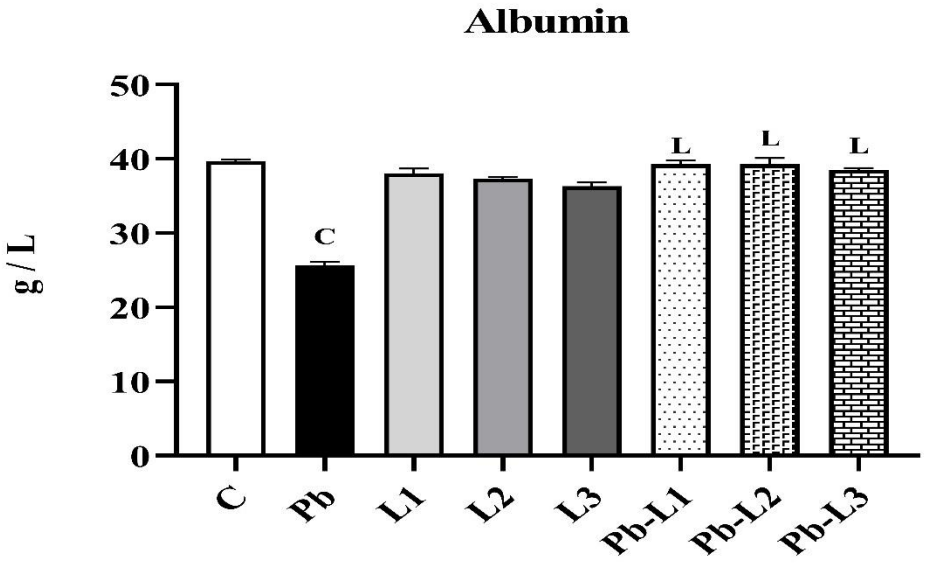


Fig 41. Albumin (ALB) level in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

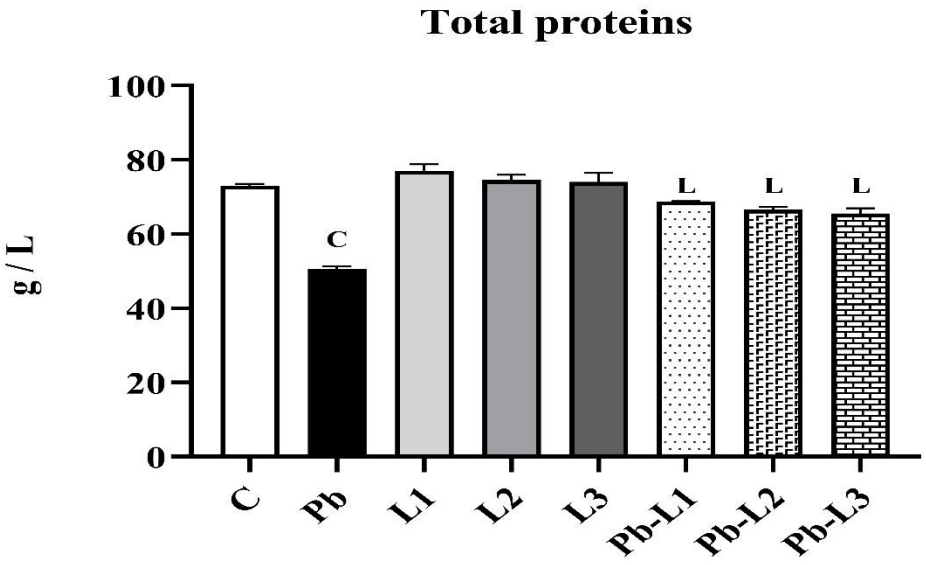


Fig 42. Total protein (TP) level in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part III - Hepatic and Renal Markers

2.2. Renal Markers

The mean value of renal markers in lead acetate-treated rats was significantly augmented compared to the control group. Although, the co-treatments with *A. triquetrum* leaves' extracts had a non-significant decrease in urea, creatinine (Cr), and uric acid (UA) concentration compared to the control. Inversely, results obtained from the combined groups (Pb-L) were significantly lower than those from the Pb-treated rats (*Figs. 43, 44, 45*).

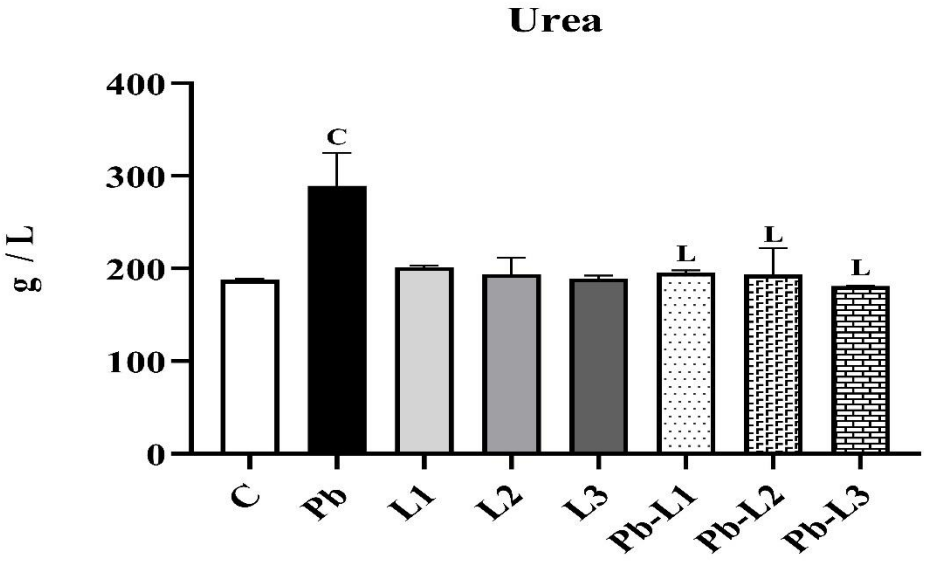


Fig 43. Urea levels in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part III - Hepatic and Renal Markers

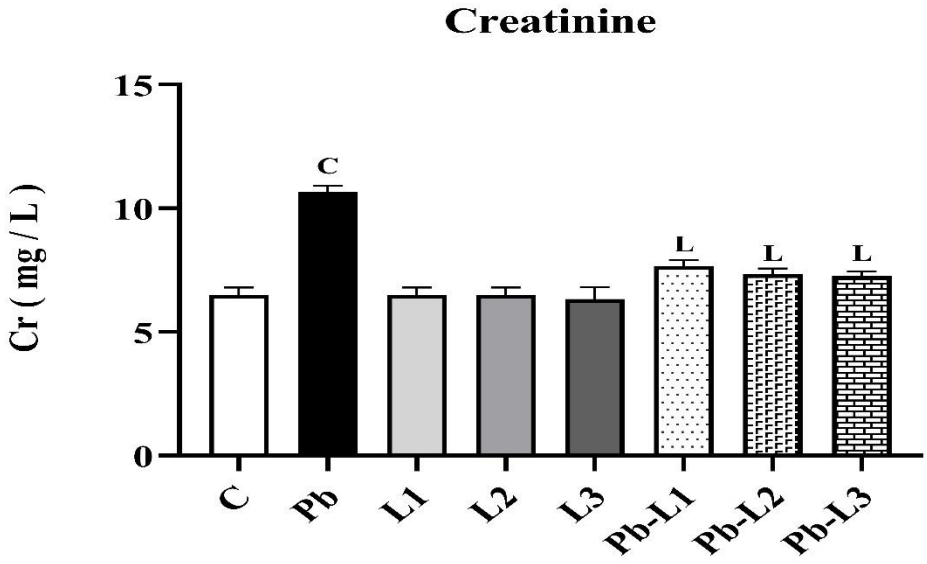


Fig 44. Creatinine (Cr) levels in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

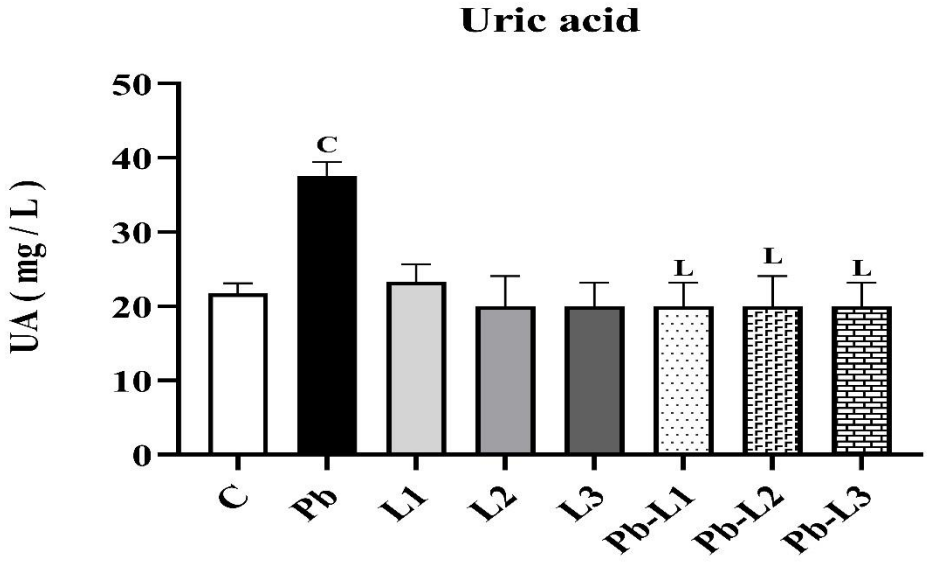


Fig 45. Uric acid (UA) levels in rats (Mean ± SEM) exposed to lead acetate and co-administrated with leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part IV - Oxidative Stress Markers

1- Testicular and Epididymal Markers

The mean value of GSH activity in rats' testis and epididymis was significantly decreased in the Pb group compared to the control. The co-treatments with bulbs and leaves extracts have significantly increased the GSH concentration of testicular and epididymal tissues when compared to the Pb-group (*Fig. 46*).

There was a significant reduction in testicular and epididymal GPx activity of the Pb-treated group compared to the control. The GPx activity in the combined treatments was significantly higher than that of the Pb-group (*Fig. 47*).

Compared to the control, a significant elevation of MDA level in the testis and epididymis was observed in the Pb group. Contrary, the MDA level is remarkably lower in all combined treatments of both organs when compared to the Pb-group (*Fig. 48*).

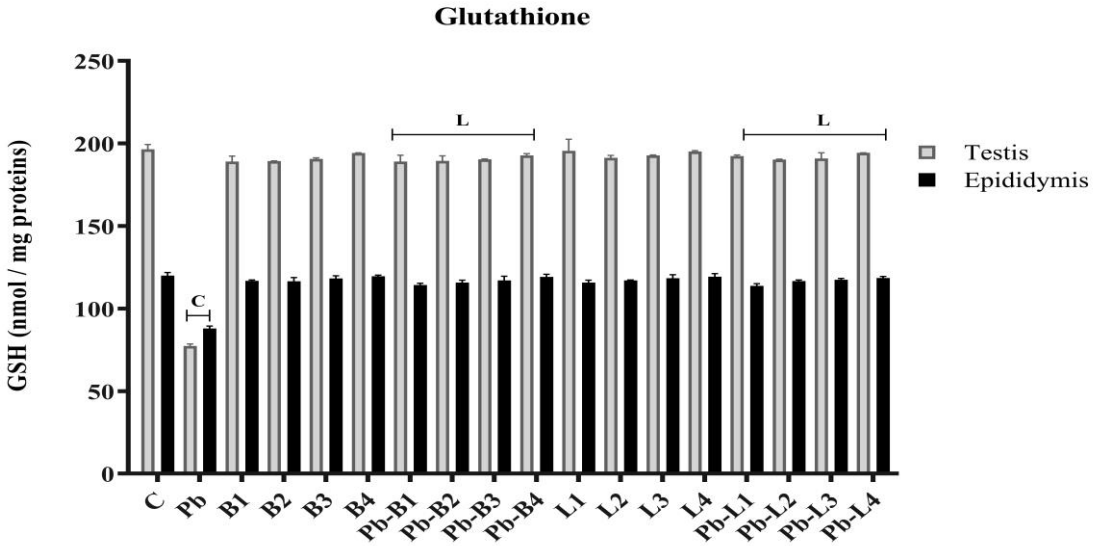


Fig 46. Testicular and epididymal glutathione (GSH) concentration in rats (Mean ± SEM) exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

Part IV - Oxidative Stress Markers

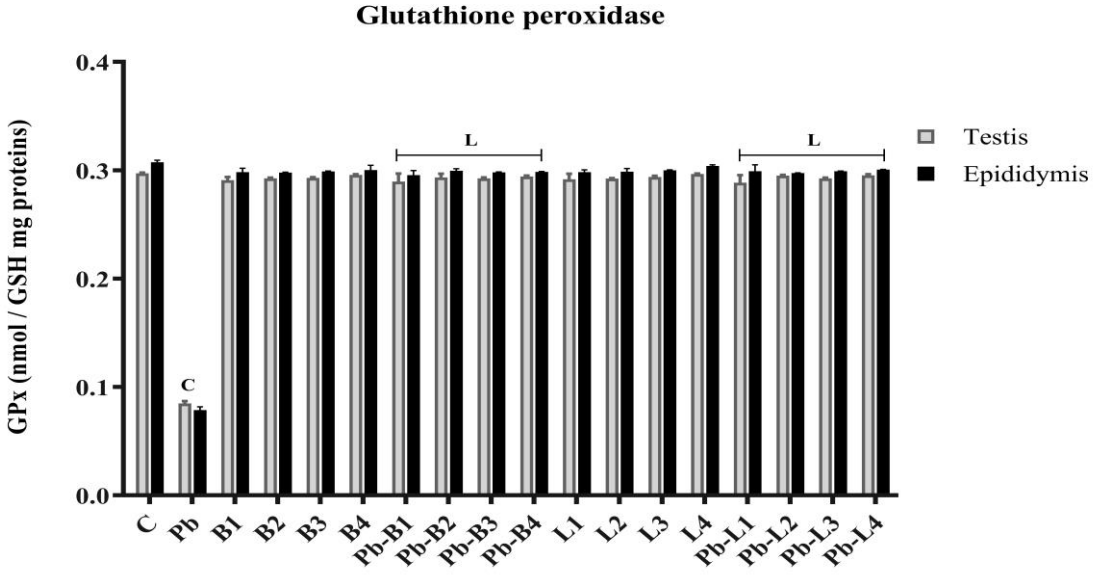


Fig 47. Testicular and epididymal glutathione peroxidase (GPx) activity in rats (Mean ± SEM) exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

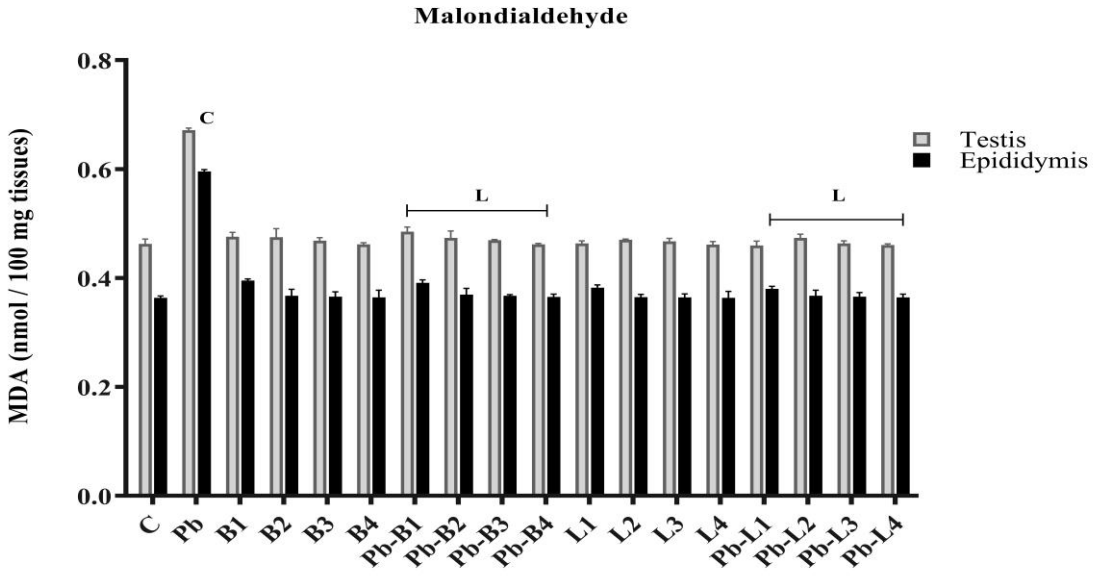


Fig 48. Testicular and epididymal Malondialdehyde (MDA) level in rats (Mean ± SEM) exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control; Pb: Lead acetate; B: Bulbs; Pb-B: Lead acetate + Bulbs aqueous extract; L: Leaves; Pb-L: Lead acetate + Leaves aqueous extract). Statistics: C: versus the control; L: versus Pb group.

Part IV - Oxidative Stress Markers

2- Hepatic and Renal Markers

Compared to the control group the GSH concentration (*Fig. 49*) in the Pb group was significantly reduced, even so, its activity was increased significantly in Pb-L groups when compared to the Pb group.

However, a remarkable diminution in hepatic and renal GPx activity was illustrated in the Pb group. Whereas, the combination of Pb-L significantly increased enzymatic activity compared with the Pb exposed group (*Fig. 50*).

Results of *Fig. 51* designate a significant increase of hepatic and renal MDA level after three weeks of treatment by Pb compared to the control. This level was significantly lower in the combined treatment of Pb-L compared to the Pb-intoxicated rats.

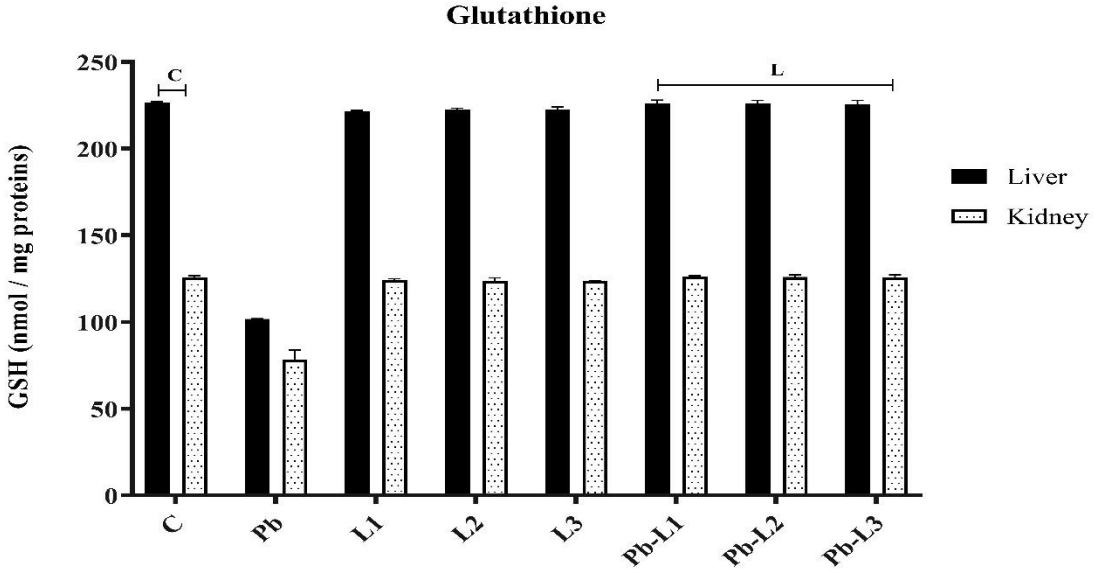


Fig 49. Hepatic and renal glutathione (GSH) concentration in rats (Mean ± SEM) exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

Part IV - Oxidative Stress Markers

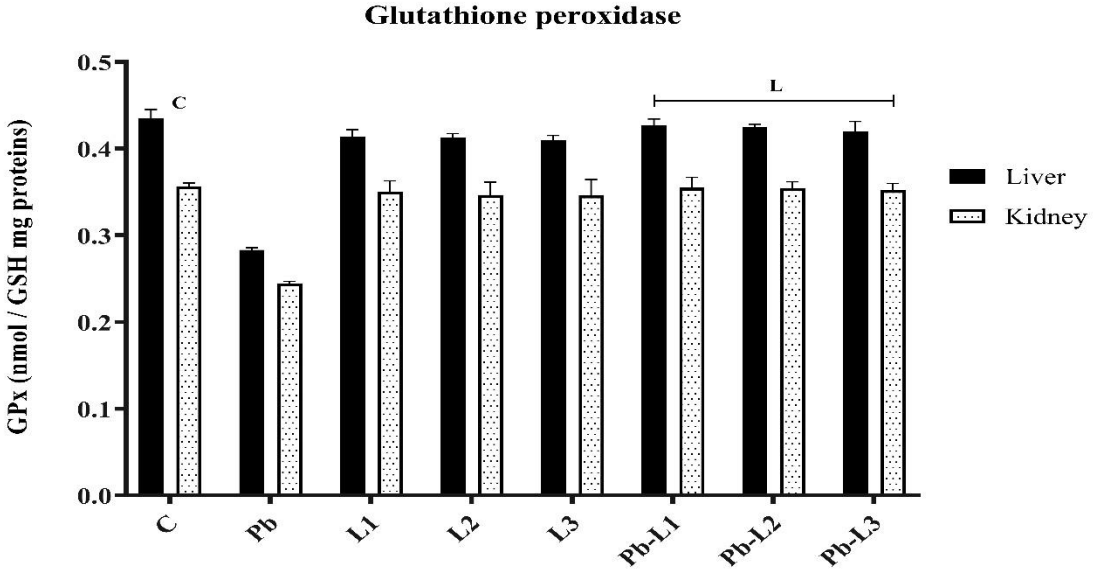


Fig 50. Hepatic and renal glutathione peroxidase (GPx) activity in rats (Mean ± SEM) exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

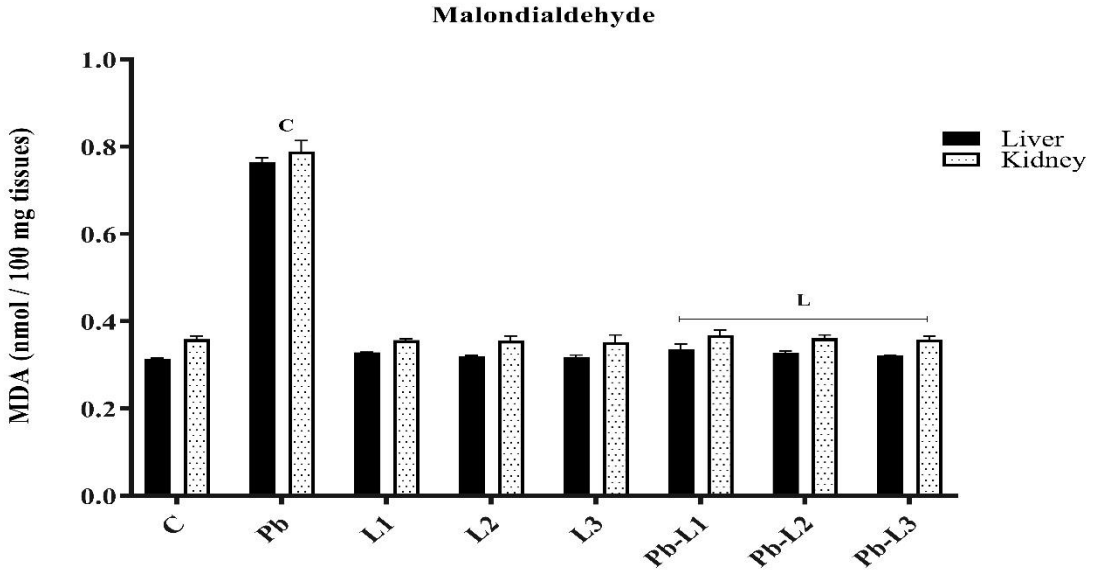


Fig 51. Hepatic and renal Malondialdehyde (MDA) level of rats (Mean ± SEM) exposed to lead acetate and co-administrated with bulbs and leaves' aqueous extracts of *A. triquetrum* for three consecutive weeks. (C: control, Pb: lead acetate, L: leaves, Pb-L: lead acetate + aqueous extract of leaves). Statistics: C: versus control; L: versus Pb group.

CHAPTER IV

DISCUSSION

The phytochemical screening of bulbs and leaves of *A. triquetrum* extracts displayed the presence of flavonoids, polyphenols, phenolic derivatives, sterol terpenes, mucilage, coumarins, chlorogenic acid, and saponosides, with the absence of alkaloids, anthraquinones, anthocyanins, and quinines. Some combined forms of tannins, rutin, and iso-quercetin were present in the leaves. This result approved that *A. triquetrum* contains various chemical compounds. The phytochemicals obtained in *A. triquetrum* from this study were distinguishable from that of **Menacer *et al.*** (**2017a**). As well, an identical trend was observed in the case of and Allium spp., like garlic (*A. sativum*), onion *(A. cepa*), and leek (*A. porrum*) which, contains various chemical compounds as well as polyphenols, flavonoids, saponins, tannins, and glycosides which are the most important secondary metabolites (**Lanzotti, 2006**; **Pârvu *et al.*, 2019**; **Nwonuma *et al.*, 2021**). The study of **Abdulkadir *et al.*** (**2017**) has exhibited the presence of many saponins, tannins, flavonoids, and alkaloids in the Allium cepa. Another interesting study manifested that leaves of *A. roseum* and *A. ampeloprasumthe* contain a high amount of iridoids and coumarins with the absence of anthracenosids and anthraquinons (**Najjaa *et al.*, 2011**). Notably, the richness and diversity of secondary metabolites in the plants of the Allium genus explain their use in the treatment and prevention of many diseases (**Menacer *et al.*, 2017a**). Thus, flavonoids and their derivatives are considered to take part in a crucial role in preventing biological systems from the adverse effects of oxidative processes on macromolecules due to the scavenging reactive oxygen species and inhibition of oxidative stress (**Bhandari *et al.*, 2014**). Besides, several *in-vitro* and *in-vivo* studies reported that most organosulfur compounds protect against oxidative stress due to their high radical scavenging potentials (**Tugbobo *et al.*, 2015**). In addition, phenolic compounds are major plant system constituents that are dispersed as secondary metabolites that can neutralize the free radicals, play a key role as antioxidants, and participate in plant growth and pigment synthesis (**Bhandari *et al.*, 2014**).

Furthermore, the resulting chromatograms demonstrated two major peaks that identified as rutin and iso-quercetin for leaves of *A. triquetrum*. However, the three minor peaks were recognized as coumarins, hesperidin, and chlorogenic acid for bulbs and leaves. These findings are in harmony with the previous work of **Maccelli *et al.*** (**2020**) who illustrated that wild Allium extracts including *A. ampeloprasum, A. roseum, A. ampeloprasum*, and *A. triquetrum* analyzed by ESI FT-ICR mass spectrometric and by HPLC-PDA chromatographic, have the potent antioxidant capacity, medicinal properties owed to the

bioactive compounds, which are polyphenols, organic acids, fatty acids, and lipids. Other compounds have been identified in *A. triquetrum*, like several essential amino acids, choline, 5-oxoproline, hydroxymethylserine, aspartyl-leucine, aspartyl-hydroxyproline, hydrolipoic acid organosulfur compounds, butyric, 4-phenylbutyric acid, anthocyanin, and tetramethoxyflavone (**Maccelli *et al.*, 2020**). Furthermore, ferulic acid, gallic acid, protocatechuic acid, quercetin, and kaempferol were identified by HPLC in four varieties of garlic *A. Cepa* (**Prakash *et al.*, 2007**). Another study indicated that the HPLC analysis of *A. cepa L.* and *A. cornutum* demonstrated the presence of quercetin conjugates, flavonoids, and anthocyanins (**Fredotovi´c *et al.*, 2017**; **Marefati *et al.*, 2021**). The Allium species contains several constituents with different pharmacological properties, which confirm that common commercial garlic and wild Allium species are rich in phenolic metabolites (**Maccelli *et al.*, 2020**).

The antioxidant activities of polyphenolic mixtures are usually evaluated using different *in vitro* spectrophotometric-based assays (**Gu *et al.*, 2019**). Herein, the present results have indicated that *A. triquetrum* leaves aqueous extract is richer in the total phenolic and flavonoids, followed by bulbs suggest of having better antioxidant potential. The actual results agree well with a recent investigation concerning leaves and bulbs of *A. triquetrum* wild garlic (**Menacer *et al.*, 2017a**). Compared with the other Allium species, it was found that *A. cepa* contained high levels of phenolic compounds mainly flavonoids as flavones, flavanones, flavonols, isoflavones, flavanonols, chalcones, and anthocyanins, which have antioxidant properties (**Liguori *et al.*, 2017**). In contrast, the activities of aqueous extracts of *A. sativum*, *A. cepa var. cepa*, and *A. cepa var. viviparum* were lower than that of the present data (**Oboh *et al.*, 2019**). Meanwhile, the different extracts of the aged and the non-aged garlic are characterized with elevated phenol and flavonoid content (**Jang *et al.*, 2018**), while those of *A. commutatum* parts exemplified different content of phenols and flavonoids (**Loizzo *et al.*, 2019**). Moreover, garlic contained more than 20 phenolic compounds such as β-resorcylic acid, followed by pyrogallol, gallic acid, rutin, protocatechuic acid, as well as quercetin (**Nagella *et al.*, 2014**), with higher contents compared to certain common vegetables (**Liu *et al.*, 2018**). Usually, the antioxidant activities of plant extracts are mainly owed to the phenolic-type compounds (**Cai *et al.*, 2004**).

Tannins belong to phenols that are widespread in *A. triquetrum*, which mainly exist in a condensed form and play a key antioxidant activity (**Pardede *et al.*, 2020**). Within, the tannin content recorded the highest level in the leaves extract, which agrees with the dark color observed in the phytochemical screening. These results are consistent with the already reported study realized on garlic *Allium sativum* from aqueous and methanolic extracts **Garba *et al.*** (**2013**). It has been also reported that garlic skin powder ethanolic extraction had a valuable effect on the content of tannin compounds (**Pardede *et al.*, 2020**).

Researchers have shown a close correlation between antioxidant capacity and phenolic content of extracts from various natural sources (**Verzelloni *et al.*, 2007**; **Albishi *et al.*, 2013**; **Sharma *et al.*, 2014**). The results of the DPPH assay are in good accordance with the polyphenolic, flavonoids, and tannins content of *A. triquetrum*. It was revealed a positive correlation between total antioxidant activity and content of polyphenols, particularly quercetin, suggesting that phenolic compounds have a major role in the plant antioxidant properties (**Insani *et al.*, 2016**). Therefore, the aqueous extract of leaves has the highest radical scavenging activity. On the other hand, bulbs aqueous extract exhibited a much lower DPPH scavenging activity than the other studied *A. triquetrum* L. parts. Furthermore, the leaves extracts had higher DPPH free radical scavenging activity than that of bulbs. According to **Stohs & Hartman** (**2015**), leaves are the most nutritive part of the plant, being a significant source of vitamin B and C, provitamin A as beta-carotene, and proteins being among the essential nutrients. Though, the antioxidant activity of Allium spp. has been attributed mainly to a variety of sulfur-containing compounds and their precursors (**Chang *et al.*, 2013**). Besides, scientific evidence displayed that allicin, diallyl disulfide, and diallyl trisulphide emerge to be the main antioxidant compounds. In addition, antioxidant activity is also correlated to other bioactive compounds as dietary fibers, microelements, and polyphenols (**Vlase *et al.*, 2013**; **Kumari & Ranjan, 2014**). Recently, it was postulated a significant positive correlation between phenolic content and antioxidant activity of Allium species (**Beretta *et al.*, 2017**). Moreover, the high antioxidant scavenging activity of Allium species depends on the content of both phenolic and organosulfur compounds (**Zill *et al.*, 2011**).

Lead is a harmful environmental toxicant that induced a wide range of biological alterations and is considered a reproductive toxicant in males, which can provoke deep changes in the sperm quality of rats (**Allouche *et al*., 2009**). Reduced reproductive performance of male rats exposed to lead acetate was linked to decreased steroidogenesis, spermatogenesis, and acrosome function (**Godínez-Solís *et al*., 2019**), as well as to sperm count, motility, and alive sperm (**El-Magd *et al*., 2016**).

In this study, Pb administration for three weeks has reduced total body weight as well as testicular and epididymal absolute weights in rats. Under our study, other authors have reported a shrink in body weight after exposure to lead acetate by gavage (**Fihri *et al*., 2016**), which could be related to the decline in daily food intake. Indeed, results suggest that this metal has adverse effects on rat body growth through the loss of appetite or poor food absorption. Our results were in-line with other studies that reported a decrease in the weight of testis and epididymis of rats supplemented with lead acetate (**Dorostghoal *et al*., 2013**; **Hamadouche *et al*., 2013**). On the contrary, exposure to this metal was not found to alter rat body weight, indicating the absence of obvious toxicity even after exposure to 0.15% lead acetate for 70 days (**Sainath *et al*., 2011**). The testicular weight was largely linked to the mass of the differentiated spermatogenic cells, where testicular weight suppression is an indication of germ cells loss and inhibition of steroid biosynthesis at Leydig cells (**Sainath *et al*., 2011**). Thus, the suppression in testicular weight might be related to the effects of lead on the hypothalamo-pituitary-testicular axis (**Wang *et al*., 2008**), because of its important role in the regulation of male reproductive functions and/or to dropped testosterone production (**Dorostghoal *et al*., 2013**) that could influence spermatogenesis, leading to the deterioration of semen quality and sex organs' functions (**Flora & Agrawal, 2017**). On the other hand, the co-administration of *A. triquetrum* aqueous extract with lead acetate resulted in an elevation in sex organs' mass, which perhaps owed to the fall of free radical generation counteracted by garlic components and confirmed by the increased daily food consumption. According to the phytochemical screening, *A. triquetrum* aqueous extracts were rich in multiple constituents, with a clear difference between the two extracts as leaves contained more tannins than bulbs.

The evaluation of sperm cells is an essential parameter in the examination of sperm quality that could affect male fertility. Computer-assisted sperm analysis (CASA) allows

an objective assessment of different characteristics, as sperm concentration, motility, velocity, morphology, and vitality. The decrease of sperm motility, concentration and testosterone serum levels in our work are consistent with the previous results, which demonstrated that the exposure of rats to lead caused a reduction in the sperm count and motility (**Mansouri & Abdennour, 2011**; **Shubina & Dudenkova, 2016**), as well as the endocrine function of reproductive organs (**Chowdhury, 2009**). The reduction in sperm motility can be explained by the effect of lead ions on the structure and function of the flagellum and the intermediate piece, leading to malformations that make the flagellum more fragile and unable to move sperm. Metal ions can alter spermatozoa head membrane morphology (**Castellini *et al*., 2009**) as well as sperm production (**Wang *et al*., 2008**). Lead acetate was demonstrated to delay spermiation, the release of immature tubular spermatogenic cells (**Corpas *et al*., 2002**), the induction of Leydig cells apoptosis (**He *et al*., 2017**; **Shubina & Dudenkova, 2016**), the decrease of all spermatogenic cells populations, especially the mature forms of spermatids and spermatozoa (**Shubina & Dudenkova, 2016**). Furthermore, observed infertility in lead intoxicated rats was linked to the morphological changes seen in the seminiferous tubules (**Fahim *et al*., 2013**) and to the suppressed gonadotrophins testosterone synthesis that is the key molecule for sperm maturation, which is dependent upon the secretion of LH by the pituitary gland (**Dorostghoal *et al*., 2013**). In contrast, a low Pb dose (0.01%) administrated to *Wistar* rats for 45 days was unable to provoke any changes in sperm concentration and motility (**Godínez-Solís *et al*., 2019**).

The main cellular part of semen is composed of spermatozoa, but it contains also non-sperm elements known as round cells, of spermatogenic or non-spermatogenic origin (**Johanisson *et al*., 2000**). Spermatogenic round cells include immature germ cells and degenerated spermatids, while non-spermatogenic round cells include exfoliating epithelial cells of the prostate, seminal vesicles, and inflammatory cells (**Palermo *et al*., 2016**). Our data illustrate that rat treated with Pb alone has significantly lower round cells, but in animals that received Pb-garlic extracts, round cells were comparable to that of the controls and were dose-dependent. It was suggested that most round cells are originated from immature germ cells that have failed to complete the spermatogenesis. High round cells frequency is a marker of a high turnover of the germinal epithelium within the seminiferous tubules, which is often followed by an increase in sperm production (**Barraud-Lange *et al*., 2011**). Thus, apoptosis of the immature spermatozoa is one of the

mechanisms held within Sertoli cells (**Palermo *et al.*, 2016**). Apparently, very high round cells are an indication of male infertility. Lower round cells in Pb-exposed rats may demonstrate a very low turnover of spermatogenesis within the testicular tubules due to germ cells death during the 30 days exposure since decreased germ cells layer population was observed in rats supplemented with Pb (**Batra *et al.*, 2001**).

The velocity of spermatozoa is one of the determining factors of sperm quality. In this research, the different types of sperm velocity have been affected by Pb exposure, like that of rapid progressive speed, which has decreased along with an increase in low speed and immotile sperm. Such results are similar to those obtained earlier (**Chowdhury, 2009**; **Mansouri & Abdennour, 2011**), in which Pb delayed the activity of live sperm. It can be assumed that Pb has a direct effect on spermatogenesis and their full growth and maturation. Regarding the drop in velocity, it is suggested that this metal can act on the mitochondrial function of the intermediate piece, by inhibiting the energy necessary for sperm movement (**Mansouri & Abdennour, 2011**). Lead can also influence sperm structure, membrane integrity, and functional activity which elucidate the decrease in rat sperm velocity (**Naha & Chowdhury, 2006**). Yet, the supplementation of wild garlic bulbs and leaves together with Pb to rats resulted in a marked increase in rapid and medium sperm velocity, with a decrease in immotile velocity. This finding agrees with the previously reported data on domestic garlic co-administration alongside Pb, which reduced the metal toxicity as garlic contains antioxidants that can play roles as preventive and therapeutic agents (**Mansouri & Abdennour, 2011**).

The treatment of rats with Pb acetate for three weeks caused a remarkable drop in the number of live spermatozoa with a rise in abnormal ones. Therefore, lower HOS reflect abnormalities of the sperm plasma membrane that may be associated with dysfunctional sperm head, mid-piece, and tail proteins, affecting sperm density, motility, morphology, and seminal hyaluronidase activity. These results are consistent with another study, which confirmed the high frequency of chromosomal abnormalities in abnormal sperm of rats treated with 20 mg/kg Pb, indicating the presence of a very large number of dead sperm (**Dorostghoal *et al.*, 2013**; **Hamadouche *et al.*, 2013**). Further, the decrease in the frequency of tail coiled sperms as evidenced by the hypo-osmotic swelling test suggests that membrane integrity of the sperms has deteriorated. Pb exposure also results in severe morphological abnormalities in the head of sperms (**Sainath *et al.*, 2011**). This decrease

may be a consequence of the decrease in the concentration of FSH, which is involved in the activation of Sertoli cells that support the development and maturation of sperm. Therefore, sperm death can be explained by the direct effect of Pb on spermatogenesis, on their growth and maturation (**Mansouri & Abdennour, 2011**).

Furthermore, lead is associated with a decrease in the components of the antioxidant defense system in sperm and can also cause lipid peroxidation, DNA damage, and sperm vitality (**Elgawish & Abdelrazek, 2014**) because Pb may cause premature acrosome reaction (**Godínez-Solís *et al*., 2019**). Distinctly, the oral combined administration of bulbs and leaves extracts with Pb has protected the viability of spermatozoa from such changes, leading to an improvement in the fertility parameters studied in this work. Our findings are in-line with other results (**Mansouri & Abdennour, 2011**; **Berredjem *et al*., 2014**) that demonstrated the protective role of domestic garlic against the observed spermatogenesis damage of rats poisoned with lead for several weeks. Thereby, *A. triquetrum* aqueous extract added to lead acetate may reduce metal toxicity, either directly through the reinforcement of chelating agent synthesis as metallothionein's, or indirectly through several mechanisms such as enzymatic and non-enzymatic antioxidants.

The harmful effects of lead on the liver or kidney either in mice or rats have been affirmed in several studies (**El-Boshy *et al*., 2019**). The *Allium triquetrum* extracts used were confirmed to be safe after oral administration to *Wistar* rats and have potential alleviating activation Pb repro-toxicity. It is obvious via the results of the current study that the injurious effects of lead acetate on the liver and kidney in rats were certain. Consequently, the co-administration of rats with leaves extracts of *A. triquetrum* (2, 3, and 4 g/kg/BW) played a vital role in reducing the alterations of Pb in a dose-dependent manner. The aqueous extract of the leaves is obviously characterized by its antioxidant capacity, which is likely linked to the presence of phenolic compounds and tannins. Herein, the current study was observed that rats subjected to lead exhibited remarkable alterations in the liver and kidney weight. However, the elevation in liver and absolute kidney weight of rats intoxicated with lead acetate was in consistent with **Kaur & Sharma** (**2017**); **Gargouri *et al*.** (**2018**) **& Mohamed *et al*.** (**2020**) who found the same results and attributed that to the expansion of glomerular and mesangial in addition to renal fibrosis. The absorbed Pb is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and affects many

biological activities at the molecular, cellular, and intracellular levels and this explains the cause of significant augmentation of kidney lead level in lead treated rats (**Flora *et al*., 2006**). On the other hand, lead acetate incorporated in *A. triquetrum* extracts attenuated this augmentation in the liver and in the kidney absolute weight. These results concord with **El Kishin *et al.*** (**2015**) who found that treatment of lead intoxicated rats with garlic extract and silymarin resulted a significant decrease in kidney lead level. Although, the present work has scrutinized the adverse effect of Pb toxicity on liver plasma markers as exposure to Pb resulted in Pb accumulation in the hepatic and renal tissues that was associated with the increased levels of AST, ALT, ALP, urea, creatinine, and uric acid with a decrease of albumin and the total proteins, indicating a functional disturbance in these vital organs. Increased levels of renal and hepatic indices in rats exposed to Pb have been recorded previously (**El-Khishin *et al*., 2015**; **El-Boshy *et al*., 2019**; **Almatroodi *et al*., 2020**; **AL-Megrin *et al*., 2020**). Further, cytosolic aminotransferases and ALP activities are usually used as an index for hepatocellular membrane damage, as they leak out into the bloodstream following exposure to chemicals, including drugs and toxic substances (**Al-Brakati *et al*., 2019**). It is apparent that the increase in enzyme activities was associated with necrosis, degeneration, and infiltration of hepatocytes (**Singh *et al*., 2018**) and in the case of cells' integrity alterations (**Nallagangula *et al*., 2017**).

Interestingly, the co-administration of *A. triquetrum* leaves extracts decreased the elevated serological liver markers following administration of lead acetate, which supports the belief that the extract possesses mitigating potential. It has been recently approved that the aqueous extract of *A. triquetrum* leaves is rich in bioactive compounds such as flavonoids, polyphenols, phenolic derivatives, tannins, sterols, terpenes, mucilages, coumarins, and saponosides. Likewise, these findings are confirmed by allicin that neutralized cyclophosphamide toxicity by protecting the hepatocyte membranes' structure (**Sun *et al*., 2021**) and by aged black garlic, which considerably decreased aminotransferase activities (**Shin *et al*., 2014**). In the same context, allicin administration to rats has also been shown to normalize the levels of hepatic markers following p-dimethyl amino azobenzene and phenobarbital exposure (**Pathak *et al*., 2018**). Further, the supplementation of a single garlic clove resulted in a significant decrease of serum ALP, AST, and ALT in carbon tetrachloride-intoxicated rats (**Naji *et al*., 2017**). Similarly, garlic oil has reduced oxidative stress-induced hepatic injuries through normalizing aminotransferase enzyme activities (**Aly *et al*., 2019**).

In this study, plasma total proteins and albumin concentrations were decreased in lead acetate-treated rats, suggesting that Pb may influence hepatic proteins' synthesis or increase their excretion rates owing to kidney damage that was associated with distortions of various functions like albuminuria and reduced glomerular filtration rate (**Navas-Acien *et al.*, 2009**; **Fadrowski *et al.*, 2010**). The observed decrease of total plasma protein concentration suggests an alteration in hepatocytes' functions since the liver is the major organ of blood protein synthesis. This finding agrees with that reported recently after the administration of lead acetate (**Ezejiofor & Orisakwe, 2019**) and lead nitrate to rats (**Lakshmi *et al.*, 2013**). Thus, Pb perhaps affects hepatic genes coding for protein synthesis (**Shalan *et al.*, 2005**; **Dobrakowski *et al.*, 2017**).

The kidneys are the main targets of heavy metal toxicity owed to their ability to accumulate divalent ions (**Lentini *et al.*, 2017**), which result in kidney injury, especially the proximal tubules (**El-Khishin *et al.*, 2015**). In this work, the Pb-induced toxicity has increased the levels of urea, creatinine, and uric acid, which is certainly linked to kidney filtration disturbances that are like previous findings already confirmed tubular damage in rats exposed to Pb (**Zhang *et al.*, 2017**). The increase of uric acid concentrations in this study is possibly due to the degradation of purines or to an increase of uric acid levels by either overproduction or inability of excretion as uric acid is the product of nucleic acids' catabolism (**Navas-Acien *et al.*, 2009**; **Fadrowski *et al.*, 2010**). These alterations were alleviated upon the co-administration of *A. triquetrum* aqueous extracts as it is expected to contain hepatoprotective and nephroprotective substances. This agrees with the previous work of **El-Sebaey *et al.*** (**2019**) who proved that ethanolic extract of *A. Sativum* has immunomodulatory, antioxidant, hepatoprotective, and nephroprotective activities, in addition to its capability in decreasing Pb deposition in renal tissues through chelation and facilitated excretion. The efficiency of garlic in reducing kidney Pb levels was perhaps owed to the presence of sulfur-containing compounds (**El-Khishin *et al.*, 2015**). Thus, all results support the hepato-renal mitigation are in accordance with the finding that mentioned the anti-inflammatory and the antioxidant activity of garlic raw extract against ROS-mediated disorders on liver and kidney functions (**Almatroodi *et al.*, 2020**).

As expected, chronic exposure of rats to lead acetate for three weeks caused a reduction in testicular and epididymal GSH level and GPx activity, thus indicating a high load of free radicals together with a deficiency in the detoxifying efficiency of these vital

reproductive organs. As previously mentioned, there is substantial evidence supporting the role of oxidative stress and ROS as major factors underlying the toxic effects of lead (**Nasr *et al.*, 2017**). Similar observations confirmed a remarkable decrease of GPx activity in Leydig and Sertoli cells (**Yang *et al.*, 2003**), and in testicular tissue following treatment of Pb acetate for 35 days (**Soleimanzadeh *et al.*, 2020**). These findings are possibly related to the influence of Pb on the glutathione protection system, resulting in the generation of peroxides. On the other side, adding different Pb concentrations (0, 3.675, 7.35, 14.7, 29.4, 58.8 mg/L) to crabs did not affect GPx activity after three days of exposure (**Li *et al.*, 2015**). Therefore, the reduced glutathione levels in testis and epididymis tissue as observed in the present study are matched with several studies that reported a decrease of GSH levels in lead acetate-intoxicated rats (**Soleimanzadeh *et al.*, 2020**). Lead has a high affinity to the thiol GSH group, where its decrease could be elucidated by its participation in the detoxification processes of ROS. Lead is excreted in the bile after irreversibly binding to the GSH group, and other sulfhydryl proteins, leading to an increase in lipid peroxidation and DNA damage (**Andjelkovic *et al.*, 2019**). Besides, the increase in the GSH and GPx levels in testis and epididymis of rats exposed to the positive controls of garlic bulbs and leaves may perhaps mediate the induction of antioxidative components. Glutathione is very abundant in the cytosol, nuclei, and mitochondria and its antioxidant capability come from the sulfur atom, which is easily gaining the lost electron generated by Pb toxicity (**Bechara, 2004**). Consequently, cell damage occurs when antioxidant levels become low (**Manna *et al.*, 2009**) that allowing Pb to disrupt cell functions. The co-administration of *A. triquetrum* with Pb to rats resulted in a rise in the levels of GPx and GSH of testicular tissues. Polyphenols have the potential to upregulate the expression of c-glutamyl cysteine synthetase, the enzyme that limits the rate of GSH biosynthesis (**Moskaug *et al.*, 2005**), which may explain the increased level of GSH and GPx in rats exposed to combined treatments of Pb-garlic since *A. triquetrum* aqueous extracts were proved to have the highest antioxidant capacity and considerable levels of polyphenols and flavonoids (**Menacer *et al.*, 2017a**).

The observed high MDA level in the Pb-intoxicated rats after three weeks is an indicator of testicular and epididymal injuries, which coincided with low levels of GPx and GSH. However, in the presence of garlic, MDA concentration has been lowered and was comparable to that of those controls. Similarly, several authors have noticed a remarkable elevation in the testicular MDA levels in Pb exposed rats (**Bechara, 2004**; **Soleimanzadeh**

et al.,* 2020**). In addition, Pb intoxication was responsible for the induction of lipid membrane peroxidation, conducting to the formation of MDA, modifying then the balance between free radicals and the antioxidant system (**Andjelkovic *et al.,* 2019**). On the other hand, the supplementation of *A. triquetrum* to Pb-intoxicated rats led to a reduction in testicular MDA levels, these results are consistent with **Nasr *et al. (**2017**). Recently, it was elucidated that domestic garlic had beneficial action on testis function better than liver and kidney following Pb-intoxicated rats (**Berredjem *et al.,* 2014**). Moreover, Pb organs' level of rats experienced oral co-supplementation of garlic aqueous extract alongside Pb nitrate has been decreased (**Senapati *et al.,* 2001**).

The antioxidant system, involving SOD, CAT, GSH, and GPx, has been shown to protect hepatocytes and renal tissues against lipid peroxidation or inflammation, therefore preventing the occurrence of hepato-renal damage. GSH has a crucial rule in modulating oxidative stress damage (**Zhang *et al.,* 2017**) against Pb toxicity that is responsible for the generation of ROS, the elevation of lipid peroxidation (MDA), and depletion of the antioxidant system (**Flora *et al.,* 2012**; **Zhang *et al.,* 2017**). The obtained results showed the significance of ROS on nephro-hepatotoxicity by increasing MDA concentration and decreasing GSH and GPx levels in Pb-intoxicated rats. Glutathione peroxidase is an antioxidant selenoenzyme that plays a major role in the reduction of hydrogen peroxide to non-toxic products (**Renugadevi & Prabu, 2010**). Therefore, GPx is a potential target of Pb toxicity as it depends on certain essential trace elements for its correct molecular function (**Hsu & Guo, 2002**). Moreover, if the balance between ROS production and antioxidant defense is broken, the enzyme may be exhausted and its concentration could be depleted (**Liu *et al.,* 2010**). In fact, exposure to Pb acetate disturbed the redox homeostasis through increasing MDA level, a lipid peroxidation product. The decrease in GSH is possibly related to the binding of Pb ions with the SH groups, leading to GSH level depletion, thereby interfering with cell antioxidant activity (**Sivaprasad *et al.,* 2004**). Further, many studies have shown that lead has high-affinity tor SH groups in several enzymes such as SOD, CAT, and GPx, thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes (**Liu *et al.,* 2010**). Additionally, Pb is known to enhance ROS generation, lipid peroxidation and to deactivate antioxidant molecules as well as reduce the expression of the corresponding gene (**Liu *et al.,* 2010**; **Gargouri *et al.,* 2019**).

Even though, the alterations observed in the Pb-induced toxicity were remarkably less apparent when *A. triquetrum* extracts were co-administered, irrespective of the dose given, by decreasing MDA concentration and increasing GSH and GPx levels in liver and kidney tissues, demonstrating the mitigating activity of wild garlic. These findings are like the reported protective effect of garlic and cinnamon oils that could reverse the oxidant-antioxidant imbalance during hepatocarcinogenesis induced by lead (**Aly *et al*., 2019**), Such effects are possibly related to the organosulfur contents of garlic like allicin, alliin, and two major organosulfur compounds SAC and S-allylmercapto cysteine, which are potent free radical scavengers (**Asdaq & Inamdar, 2010**). Furthermore, garlic allicin can bind to free radicals, which may reduce lipid peroxidation (**Zhang *et al*., 2017**). It was also shown that onions contain allylsulfides and flavonoids including quercetin, steroid, saponins, and sapogenins which exert antioxidative activities and could reduce hepatocytes apoptosis (**Upadhyay, 2017**). Besides, the ethanolic extract of *A. sativum* extract has reduced the hepatic damage in cadmium chloride-intoxicated rats by enhancing the antioxidant system and decreasing ROS generation (**El-Sebaey *et al*., 2019**).

CONCLUSION

To date, very little research has been recorded on wild garlic *Allium triquetrum* L. The current study was exhibited that the content of bioactive compounds, HPLC analysis, and antioxidant activity of *A. triquetrum* are parts of plant and variety-dependent. The acquired results from this study exemplify that leaves aqueous extract contains a higher content of polyphenols, flavonoids, tannin, and antioxidant activity than bulbs.

On the flip side, it can be concluded that the co-administration of aqueous extract of bulbs and leaves of wild garlic *A. triquetrum* at different doses (2, 3, 4, and 6 g/Kg) with lead acetate (500 mg/Kg/BW) for three weeks to male rats have mitigated lead toxicity by boosting testicular, epididymis, liver and kidneys weight and ameliorating the levels of sperm concentration, motility, serum testosterone, velocity, vitality, round cells. While, preserving certain plasma biochemical markers of the liver (AST, ALT, PAL, TP, ALB) and kidney (Urea, Cr, UA).

Further, garlic extracts have protected testicular, kidneys, and liver damage through increasing organs' GSH and GPx levels and decreasing MDA concentration. Interestingly, *A. triquetrum* aqueous extracts seemingly have a dose-dependent positive effect against Pb toxicity. Hence, wild garlic can be considered a source of many bioactive components that can have promising roles in metal detoxification. It is also important to note that the results of the present study highlight the potential of a new model of garlic production for medicinal purposes.

It would be wise to supplement this research with:

- ✓ *Identify the mitigating molecules of A. triquetrum at the target organs of Pb toxicity as reproductive, nervous, and immune systems;*
- ✓ *Compare the results obtained with the different parts of A. triquetrum extracts such as flowers, roots, seeds by using HPLC;*
- ✓ *Investigate the biological activity of essential oils;*
- ✓ *Test different parts of A. triquetrum on cancerous cells and viruses.*

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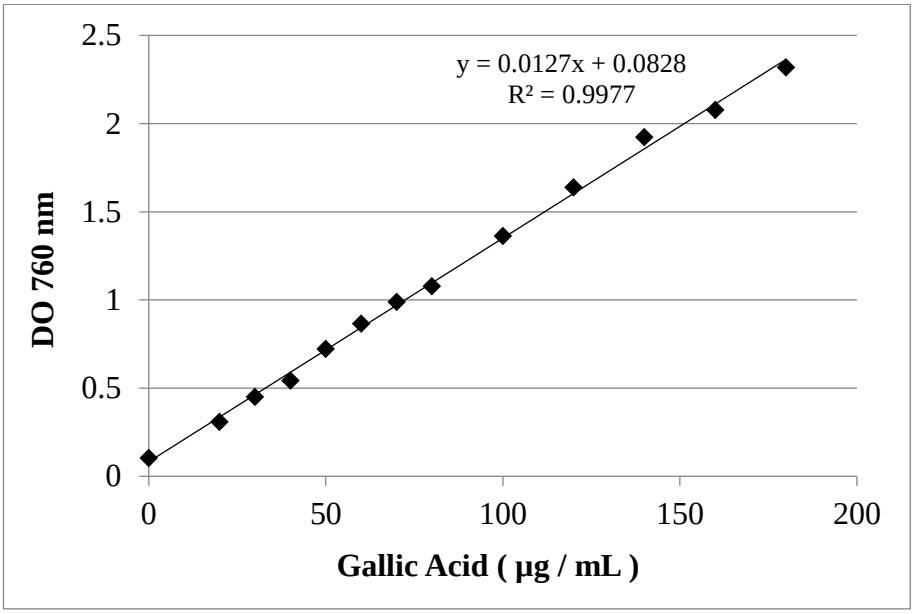
ANNEX

1- ANTIOXIDANT ACTIVITY

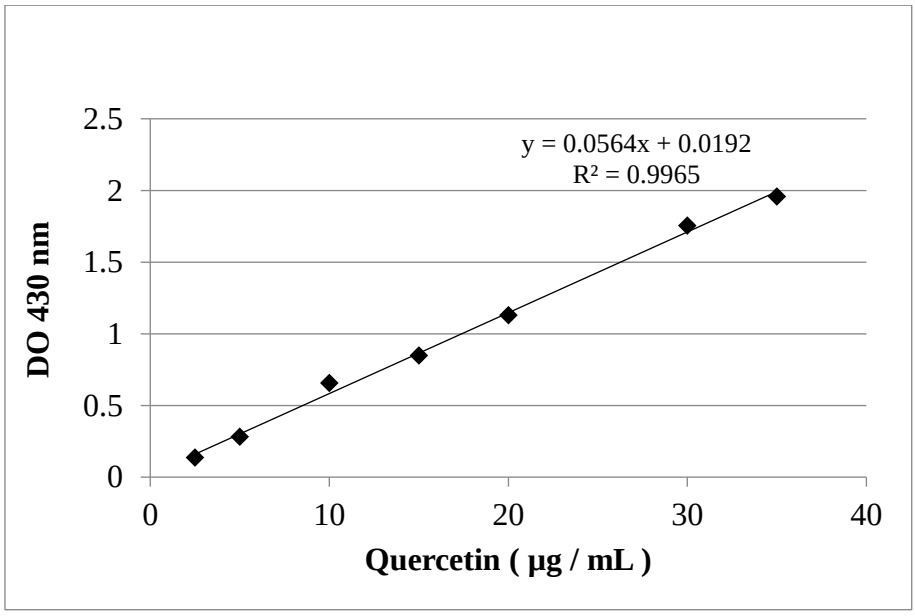
- **Total Polyphenols :**

- Dilution of Folin ciocalteu 1/10: 1 mL of Folin dissolved in 9 mL of distilled water.
- Solution of sodium carbonate Na2Co3 (7.5 %): 7.5 g in 100 mL of distilled water.
- Standard: Gallic acid: 1 mg A. g./1 mL of distilled water.



- **Flavonoïds:**

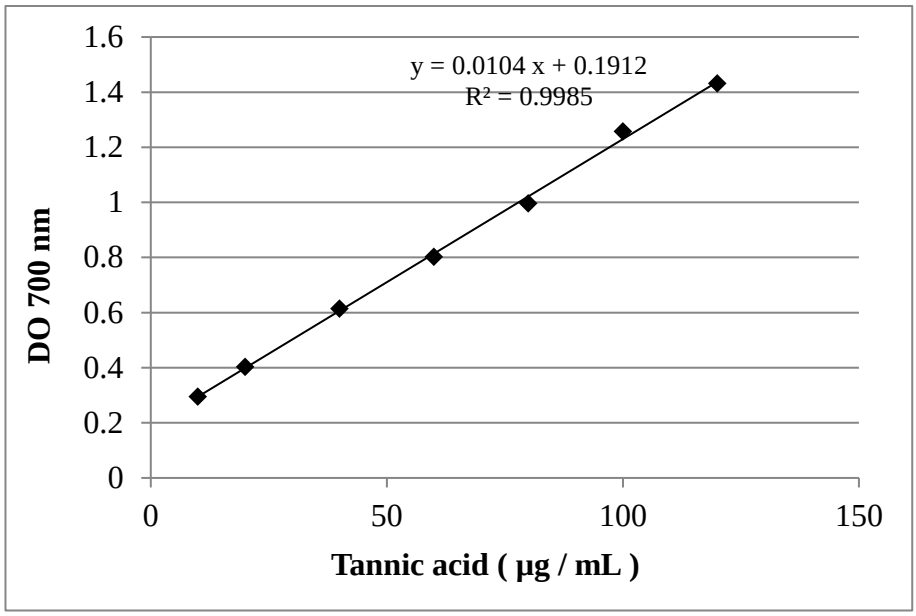
- $NaNO_2$: 0.5g in 10 mL of distilled water.
- NaOH (1N) = 4% solution: 4g in 100 mL of distilled water.
- $AlCl_3$ Solution: 10g dissolved in 100 mL of distilled water.
- Etalon: Quercetin / Catechin: 1mg / 1mL of distilled water.



-

- **Total Tanins :**

- $Na_2 CO_3$ solution (5%): 0.5 g dissolved in 100 mL of distilled water.
- Tannic acid standard: 1mg / 1mL of distilled water.



- **DPPH antioxidant activity test:**

- Methanolic solution: 0.04 g of DPPH/ liter of methanol.

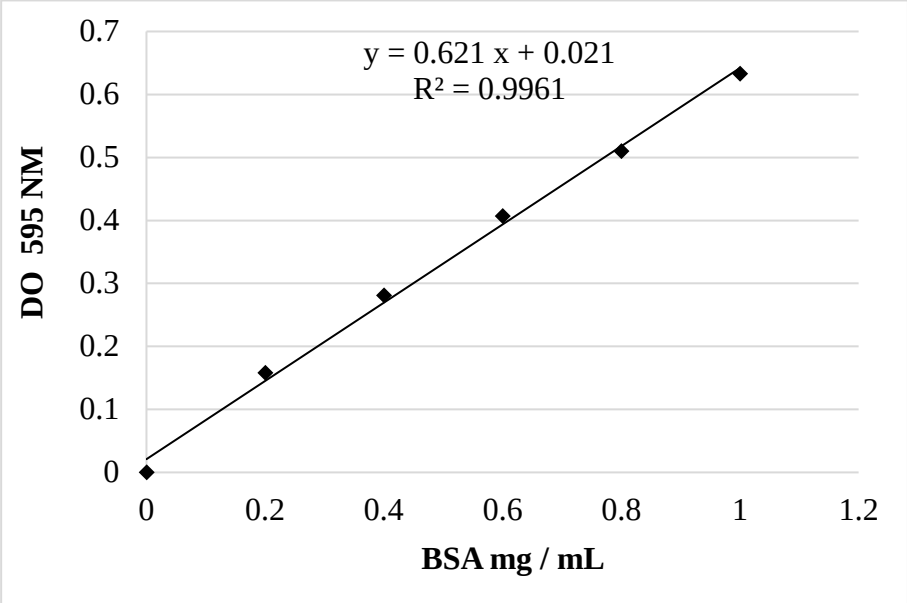
2- OXYDANTIVE STRESS

- **Protein determination by the Bradford method:**

*BSA Solution (1mg / mL): Dissolve 5 mg BSA in 5 ml distilled water.
*Bradford's reagent:

- Dissolve 100 mg of Coomassie blue (G 250) in 50 mL of ethanol (95%). Shake with the stirrer for 2 hours, then add 100 mL orthophosphoric acid (85%) and 850 mL distilled water (to obtain 1 L of solution).

*This reagent should be filtered and stored for a maximum of 1 month at 4°C and protected from light.



- **Glutathione (GSH):**

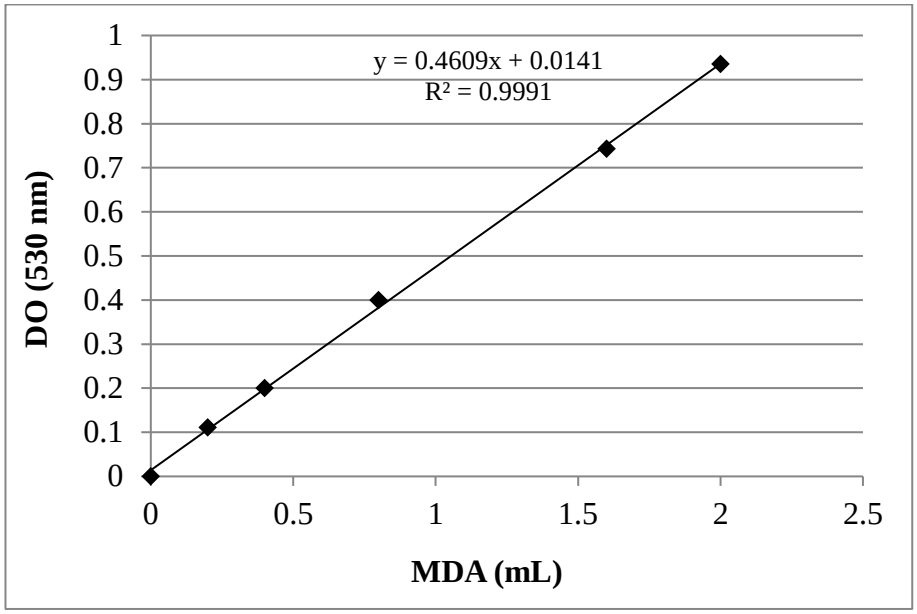
- EDTA solution (0.02 M): Dissolve 5.613 g EDTA in 750 mL distilled water.
- DTNB solution (0.01 M): Dissolve 200 mg DTNB in 50 mL absolute methanol.
- Salicylic acid solution (0.25%): Dissolve 250 mg of salicylic acid in 100 mL of distilled water.

- **Glutathione peroxydase (GPx) :**

- TBS solution: Tris (50 mM, NaCl (150 mM): Dissolve 8.775g NaCl in 1L of distilled water, then add 6.057g Tris and complete the volume to 1L with NaCl solution (150 mM) and adjust the pH to 7.4 by adding HCl or NaOH.
- GSH solution (0.1 mM): Dissolve 3.073 mg GSH in 100 mL distilled water.
- TCA solution (1%): Dissolve 1g TCA in 100 mL distilled water.
- DTNB solution (1.0 mM): Dissolve 100 mg DTNB in 250 mL of absolute methanol.

- **Malondialdehyde (MDA):**

- TCA solution (20%): Dissolve 20 g of TCA in 100 mL of distilled water.
- Tris's solution: Dissolve 0.15 g of tris in 50 mL of distilled water.
- TBA solution (0.67%): Dissolve 0.33 g of TBA in 50 mL of tris solution.



3- CALIBRATION RANGES

- Realization of the calibration range of Gallic Acid:

Gallic Acid (µg/mL)	5	10	25	100	150	200	250	300
DO at 760 nm	0.08	0.14	0.266	0.901	1.262	1.701	1.979	2.339

- Realization of the catechin calibration range:

Quercetin (µg/mL)	25	50	75	100	150	200	250	300
DO at 510 nm	0.018	0.061	0.138	0.206	0.293	0.422	0.566	0.665

- Realization of the calibration range of the Tannic Acid:

Tannic Acid (µg/mL)	10	20	40	60	80	100	120
DO at 700	0.295	0.403	0.615	0.803	0.997	1.258	1.432

- Realization of the protein calibration range:

BSA (mg/mL)	0	0.2	0.4	0.6	0.8	1.0
DO at 595 nm	0	0.158	0.281	0.407	0.510	0.633

- Realization of the MDA calibration range:

1,1,3,3-tetraetoxypropane (mL)	0	0.20	0.40	0.80	1.60	2.00
DO at 530 nm	0	0.111	0.2	0.4	0.743	0.935

SCIENTIFIC PRODUCTIONS

The work presented in this thesis has been published and presented in national and international scientific conferences.

- ***Scientific Publication*** *:*

- Arkoub FZ, Hamdi L, **Kahalerras L**, Hamoudi M, Khelili K (**2022**). Evaluation of *in vitro* and *in vivo* antioxidant potential of *Punica granatum* L. against toluene-induced liver injuries in rats. Vet World *(In progress).*

- Kahalerras L, Otmani I, Abdennour C (**2022**). The *Allium triquetrum* L. Leaves Mitigated Hepatotoxicity and Nephrotoxicity Induced by Lead Acetate in *Wistar* Rats. Biol Trace Elem Res. https://doi.org/10.1007/s12011-021-03052-y

- Kahalerras L, Otmani I, Abdennour C (**2021**). Wild Garlic *Allium triquetrum* L. Alleviates Lead Acetate-Induced Testicular Injuries in Rats. Biol Trace Elem Res 1-8. https://doi: 10.1007/s12011-021-02818-8.

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- ***Scientific Communications :***

  - ***<u>International Communications</u>***

1- Labiba KAHALERRAS and Cherif ABDENNOUR. The Beneficials Effect of Wild Garlic *Allium triquetrum* L. Against Lead Acetate Toxicity. International Online Conference on Environmental Biotechnology and Biodiversity (ICEBB 2021). Lab EcoBiologie Animal (LEBA) 14, 16 December 2021 Algiers (**Oral**). [https://sites.google.com/view/icebb2021/accueil].

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Wild Garlic *Allium triquetrum* L. Alleviates Lead Acetate-Induced Testicular Injuries in Rats

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Abstract
The current study investigates the potential alleviating activity of bulbs (B) and leaves (L) of *Allium triquetrum* aqueous extract (ATE) on repro-toxicity induced by lead acetate (Pb) in male *Wistar* rats administrated orally for 3 consecutive weeks. Eighteen groups of rats were divided into the control, Pb (500 mg/kg body weight/day), positive controls of B and L (2 g, 3 g, 4 g, 6 g/kg body weight/day), in addition to four mixtures of each of Pb-B (Pb-B1, Pb-B2, Pb-B3, Pb-B4) and Pb-L (Pb-L1, Pb-L2, Pb-L3, Pb-L4). The two extracts were subjected to phytochemical screening and HPLC analysis. Sperm characteristics were evaluated by CASA system, as well as the serum testosterone, testicular and epididymal levels of glutathione (GSH), glutathione peroxidase (GPx), and malondialdehyde (MDA). The phytochemical screening proved that bulbs' and leaves' extracts were rich in various compounds and the HPLC showed that leaves contain more tannins. Results revealed a significant decrease in the testicular and in the epididymal weights, sperm concentration, motility, testosterone, velocity, vitality, round cells, GSH, and GPx levels in the Pb-intoxicated rats compared to the control, with the exception of MDA concentration that was significantly increased. However, the co-administration of garlic extracts (Pb-B and Pb-L) exhibited a significant increase in all mentioned markers, except for the MDA level which was reduced. Likewise, Pb caused histological injuries in the testicular seminiferous of rats, while the co-administration of wild garlic has reduced such effect, especially in the higher doses. Both extracts of Pb-B and Pb-L have attenuated Pb toxicity in a dose-dependent manner. In conclusion, aqueous extracts of *A. triquetrum* have the potential to reduce Pb testicular injuries by boosting sperm characteristics and ameliorating oxidative stress markers.

Keywords *Allium triquetrum* · HPLC · Lead · CASA sperm · Reproduction · Rat

Introduction

There is increasingly interest in the use of plants with antioxidant activity for the protection against heavy metals' toxicity, which become a world-wide problem. Recently, the assessment of natural product activities was considered as safe, through the presence of several components [1]. Garlic is considered as one of the best foods known since ancient times that has been used as ingredient of many recipes with important therapeutic potentials [2] such as its ability to act as an antioxidant [3]; its role in the prevention of endothelial dysfunction [4] and for the treatment of various ailments such as hypolipidemia, hypoglycemia, anticoagulant, hepatoprotection [5], antitumor, anti-inflammatory activities [6], chemopreventive activities with immune system improvement [7], antihypertensive, antirheumatic, and antiparalysis; and in the cure of memory loss [8]. Garlic contains several sulfur compounds and several enzymes, minerals, and vitamins. It also contains amino acids, glycolipids, and phospholipids [9], providing a different health benefit [7]. In fact, the physiological functions of garlic are suitable to its bioactive composition such as saponin, alkaloids, flavonoids [10], and especially phenolic compounds compared with other vegetables [11].

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Metals are found in many industries that have the ability to affect living organisms [12]. Lead is easily absorbed into the body and undergoes bioaccumulation in certain target organs as the brain [13], liver, kidney [14], and testis [15]. Pb levels are associated with the risk of infertility [16], by its accumulation in the testes, epididymis, vas deferens, seminal vesicle, and seminal ejaculate [3], which leads to spermatogenesis impairment, sub-fertility, decreased testosterone levels [17] and sperm count, motility, and morphology [18]. Lead can also affect gonad structure and induces testicular oxidative stress leading to poor semen quality and infertility [19]. It is considered as an endocrine disruptor by perturbing steroidogenesis process [20]. Moreover, Pb toxicity can influence aromatase P450, an enzyme that converts androgen into estrogen in testicular cells irreversibly [21] by inducing testicular oxidative stress and altering the male reproductive functions. Oxidative stress is implicated as a major mechanism of lead-induced toxicity, either through increased free radical generation or depletion of the antioxidant enzymes [14] by acting on glutathione system [15]. Pb is known to provoke oxidative stress in testicular tissues, resulting in damage to membranes, DNA, and proteins and inducing dysfunction through lipid peroxidation [22], by irreversible chemical reactions such as malonaldehyde and hydroperoxides that propagate oxidative damage [23] and alter metabolic function and fertility [18]. Moreover, Pb exposure impairs sperm normal function by decreasing the levels of sperm intracellular cyclic adenosine monophosphate and calcium and reducing tyrosine phosphorylation of sperm proteins [24].

Previous investigations indicated that the administration of herbal plants can alleviate oxidative stress induced by Pb in experimental models [13, 19, 25]. The *A. triquetrum* is a very early blooming species which grows vigorously in cultivations. It grows in water and humid forests and is consumed raw or steamed in the same way as leeks. *A. triquetrum* possesses several vernacular names (triangular-stalked garlic, three-cornered leek), which refer to different taxa [26].

So far, no studies have been conducted on the spontaneous species of *Allium triquetrum* L. in alleviating metal toxicity. Thereby, this investigation provides the first evidence to evaluate the possible protective efficiency of wild garlic against lead acetate-induced alterations on reproductive function of *Wistar* rats. Therefore, aqueous extracts of bulbs and leaves were co-administered with lead to study the level of oxidative stress in the testicular and the epididymal tissues, along with sperm characteristics during three consecutive weeks.

Materials and Methods

Allium triquetrum L. Extracts' Preparation

Fresh bulbs and leaves of *A. triquetrum* were collected during the flowering period of March to May from Berrahal, Annaba region, northeast Algeria, with geographic coordinates of latitude 36.83045445 and longitude 7.488031505, 36° 49′ 49.6″ North, 7° 29′ 16.9″ East (Fig. 1). The identification and authentication of the wild garlic were carried out in the laboratory of plant biology and environment, University of Badji Mokhtar-Annaba. Bulbs and leaves (2, 3, 4, and 6 g) were homogenized daily by a commercial blender (Black + Decker BX HBA600E) after adding appropriate quantity of distilled water to make a final volume of 20 mL after filtration by Aldismed sterilized compresses, on the basis that rats take 2, 3, 4, or 6 g/kg bw/day of the prepared extract.

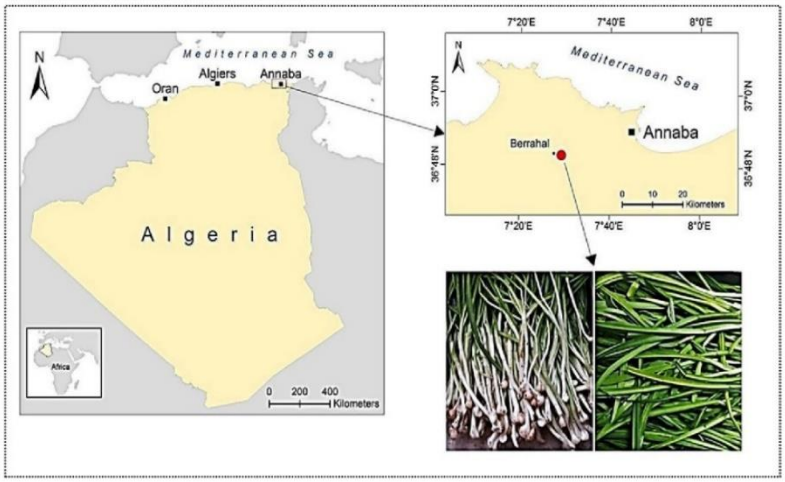


Fig. 1 Sampling location of bulbs and leaves of wild garlic *A. triquetrum* collected in spring from Berrahal, Annaba province

The phytochemical screening of *A. triquetrum* was realized in order to know the major constituents of the plant extracts.

HPLC Protocol

Four grams of each part of bulbs and leaves were placed in 200 mL of boiling distilled water (100 °C) for 20 min, under magnetic stirring. The obtained two aqueous extracts were then cooled, filtered through Whatman filter paper 4, placed in a petri dish and left for 3 days in the oven at 60 °C. Afterwards, 5 mg of each extract were deposited in 100 µL of distilled water and 900 µL of methanol (MeOH).

A C18 column 5 µm (250 × 4.6 mm) (USA) was used. The separation was performed on a Shimadzu *Prominence-I* LC-2030C liquid chromatography (Japan). Before starting the chromatographic analysis, the mobile phases (phase A include 19% acetonitrile, 80% water, and 1% formic acid, and phase B include 59% acetonitrile, 40% methanol, 1% formic acid) and the extracts are placed in an ultrasonic tank for degassing. Then, 10 µL of each extract (at concentrations of 5 mg/mL) was injected at 40 °C. Standards were prepared in methanol at a concentration of 1 mg /10 mL. After each injection, the analytical system was rinsed for 1 h with the mobile phase to ensure that any products that may have remained on the column were dislodged. A peak-free baseline was the prerequisite for any injection. The solvents used were HPLC quality, with a flow rate of 1 mL/min and a wavelength of 350 nm.

Experimental Design

A male albino *Wistar* rats were provided from Algiers Pasteur Institute (Algeria) with an average body weight of 240 g. Rats were kept in a controlled environment under standard conditions of temperature, humidity, and natural light–dark cycle. They were fed ad libitum with water and food in the form of 30 g croquettes made of soy, corn, calcium carbonate, phosphate, and cellulose, which was purchased from the agro-food complex (El-Kseur, Bejaia, Algeria). The solution of Pb-acetate trihydrate (Georgia, USA) was daily dissolved in distilled water. Animals were divided into 18 groups in which they were treated according to Table 1. The prepared solutions of Pb-acetate and garlic extracts were administrated to rats at the same time in the morning by gavage during 3 consecutive weeks (Table 1).

Samples' Collection

After 3 weeks of continuous experimental trial, all animals were fasted overnight and sacrificed by decapitation. The organs (testicular and epididymis) were retrieved, weighed, and frozen at − 20 °C for the determination of the oxidative stress-related markers.

Table 1 Experimental design of rats exposed to lead acetate and co-administrated with four doses of bulbs (B1- 4) and leaves (L1-4) of *A. triquetrum* aqueous extracts for 3 weeks

Groups	Daily treatment
Control (C)	Distilled water
Lead acetate (Pb)	Pb (500 mg/kg BW)
B1	Bulb's extract (2 g/kg BW)
B2	Bulb's extract (3 g/kg BW)
B3	Bulb's extract (4 g/kg BW)
B4	Bulb's extract (6 g/kg BW)
L1	Leaf's extract (2 g/kg BW)
L2	Leaf's extract (3 g/kg BW)
L3	Leaf's extract (4 g/kg BW)
L4	Leaf's extract (6 g/kg BW)
Pb-B1	Pb (500 mg/kg BW) + bulb's extract (2 g/kg BW)
Pb-B2	Pb (500 mg/kg BW) + bulb's extract (3 g/kg BW)
Pb-B3	Pb (500 mg/kg BW) + bulb's extract (4 g/kg BW)
Pb-B4	Pb (500 mg/kg BW) + bulb's extract (6 g/kg BW)
Pb-L1	Pb (500 mg/kg BW) + leaf's extract (2 g/kg BW)
Pb-L2	Pb (500 mg/kg BW) + leaf's extract (3 g/kg BW)
Pb-L3	Pb (500 mg/kg BW) + leaf's extract (4 g/kg BW)
Pb-L4	Pb (500 mg/kg BW) + leaf's extract (6 g/kg BW)

Biometric Markers

Animal weights were recorded weekly during and at the end of the experimental period. Rats were dissected where the testicles and the epididymis (left and right sides) were removed and the absolute weighs were obtained.

Sperm Analysis

Immediately after decapitation, semen was obtained by making a small incision in the tail of the epididymis using surgical blades, and then approximately, 1 µL of seminal fluid was diluted in physiological solution (NaCl 0.9%) by putting a drop of seminal fluid in a pre-warmed Goldcyto slide (consisting of four counting chambers). The preparation is examined under a microscope Nikon Eclipse E200-LED (×4 objective), integrated with the Sperm Class Analyzer (SCA®, Microptic, Barcelona, Spain) version 6.2.0.0, by applying a computer-assisted semen analysis (CASA System) microcomputer to measure sperm concentration, motility, velocity, vitality, and round cell number that have a significant relationship with the rat's sperm fertilizing ability. The CASA system has been developed because of its rapid and accurate estimation of sperm characteristics.

Determination of Testosterone Levels

Testosterone levels in plasma were estimated by using a chemiluminescence immunoassay-based commercial kit

(Access Testosterone 33,560) and an Access Immunoassay analyzer (Beckman Coulter Access 2, California, USA).

Sperm Vitality Test

Hypo-osmotic swelling test (HOS-test) was used by placing sperm in a hypo-osmotic medium, in which the intact sperm membrane is semi-permeable to water causing its swelling [27]. Therefore, HOS-testis used to evaluate functional and structural integrity of sperm plasma membrane. It is based on the principle that fluid transport occurs across the intact cell membrane under the hypo-osmotic condition until equilibrium is achieved and results in bulging of sperms, especially, in the tails where plasma membrane is loosely attached [28]. This test issued to determinate sperm vitality by using hypo-osmotic solution that was freshly prepared by dissolving 0.735 g sodium citrate Na_3, $C_6H_5O_7$, $2H_2O$ (Sigma, St. Louis, USA), and 1.351 g fructose (Sigma, St. Louis. USA) in 100 mL of distilled water. After pre-warming 1 mL of hypo-osmotic solution in a closed Eppendorf tube to 37 °C for 5 min, 0.1 mL of seminal fluid was added and then mixed gently with a pipette. After half an hour of incubation at 37 °C, the slides of sperm count were estimated under the ×40 objective lens of light microscope (Leica-Germany). The total number of sperm was expressed as a percentage changes in the flagellum out of a total of 100 sperms.

Analysis of Oxidative Stress Markers

Specimens from the testis and epididymis from each rat were carefully collected and removed from all rats. Total protein content of the tissues was carried out by the method of Bradford [29] by using Coomassie Blue G-250 (Sigma, St. Louis, USA) as a reagent. A 5 mL of the CBB Bradford solution were added to 0.1 mL of the homogenate. After 5 min, the absorbance was measured at 595 nm.

The non-enzymatic antioxidant activity of reduced glutathione (GSH) is based on the reaction between 5,5'- dithiobis-2-nitrobenzoic-acid (DTNB) and the GSH molecule. A 200 mg of the testes and epidermis were homogenized in 8 mL of ethylene diamine tetra-acetic acid (EDTA 0.02 mol) (VWR International BVBA-EC) solution. Then 0.8 mL of the homogenate was removed and mixed by adding 0.2 mL of 0.25% sulfosalicylic acid (SSA) (BIOCHEM-Chemo-Pharma, France) solution. The mixture was stirred and left for 15 min in an ice bath and then centrifuged at 1000 g for 5 min. A 1 mL of Tris (hydroxymethyl) aminomethane (BIOCHEM-Chemo-Pharma, Montréal, Québec)-EDTA (pH 9.6) was added to 0.5 mL of the supernatant, mixed, then added 0.025 mL of 0.01 mol DTNB (Sigma, St. Louis. USA), and left to act for 5 min to obtain the yellow color, which then read at 412 nm [30].

The determination of glutathione peroxidase (GPx) activity was carried out according to the method of Flohé and Günzler [31]. A 500 mg of testicular and epididymis tissues were cold-milled using an ultrasonic homogenizer in the presence of 5 mL of a TBS solution (Tris 50 mmol, NaCl 150 mmol, pH 7.4) to obtain a homogenate. Reaction mixture was prepared by adding 0.2 mL of the testicular homogenate to 0.4 mL of GSH (0.1 mmol) (Sigma, St. Louis. USA) and 0.2 mL of the TBS buffer solution. After incubation with water bath at 25 °C for 5 min, 0.2 mL H_2O_2 (1.3 mmol) (Sigma, St. Louis. USA) was added to start the reaction (leave for 10 min), and then 1 mL trichloroacetic acid (TCA 1%) (BIOCHEM-Chemo-Pharma, France) was added to stop the reaction. After the cooling time of 30 min in the ice bath, the tubes were centrifuged at 3000 g for 10 min, and the supernatant was collected. The 2.2 mL TBS buffer solution and 0.32 mL DTNB (1 mmol) were added to 0.48 mL reaction supernatant. After mixing and waiting for 5 min, the absorbance was recorded at 412 nm.

Malondialdehyde (MDA) content, which indicates the tissue lipid peroxidation, was determined by the reaction between TBA and MDA according to the method of Ohkawa et al. [32]. A 0.5 mL of tissue homogenate was mixed with 0.5 mL of TCA at 20% (500 mg of the organs in 5 mL of 0.1 mol, pH 7.4 phosphate buffer), and then 1 mL of thiobarbituric acid (TBA 0.67%) (Sigma, St. Louis. USA) was added and incubated in a water bath at a temperature of 100 °C for 15 min. After cooling, 4 mL n-butanol (Sigma, St. Louis, USA) was added and centrifuged at 3000 g for 15 min. The supernatant was removed and read at the optical density of 530 nm.

All measurements were realized by a UV/visible spectrophotometer (JENWAY-6300).

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Histological Examination

Specimens from testicular and epididymal tissues were weighed, rinsed in clean saline, preserved, and then fixed in a 10% formalin solution. The samples were then dehydrated with a graduated series of alcohols at increasing degrees from 70° to 100° by means of a circulating inclusion automaton (Thermo Scientific, Microm STP 120, UK) and incorporated in kerosene (paraffin). After clearing, the tissues were embedded in paraffin, and then they were cut into 2-µm-thick sections using the microtome (Leica Microsystems Nussloch GmbH-Germany). Each section was mounted on a clean glass slide and stained with hematoxylin (Sigma-Aldrich St. Louis, USA) and eosin (BIOCHEM-Chemo-Pharma UK) (H&E). Later, a EuKitt mounting medium

(Emmonya Biotech, LTD-Bulgaria) was dropped on each tissue section to protect and preserve samples of different tissues and a cover slip placed on it and allowed to dry. They were examined with a microscope Nikon Eclipse E200-LED (× 10 objective). Photomicrographs were captured using a digital camera (Pylon, BASLER, Germany) attached to the microscope.

Statistical Study

Data were performed using the GraphPad Prism software V. 7 for graphs, and SPSS software V. 27 for windows presented as mean ± standard error of the mean (SEM). One-way ANOVA was followed by Tukey post hoc test for multiple comparisons. Criterion for statistical significance was set at $P < 0.05$.

Results

General Assessment

During the experimental period, rats of the control group were healthy and showed no signs of toxicity. However, in the rats of the Pb-acetate group, some negative signals such as decreased vitality, muscle weakness, tremor, abnormal body weight gain, and lack of stability and balance were shown. At the end of the experiment, one rat in the Pb-treated group was nearly paralyzed and three died.

Organs and Total Body Weights

The total body weight was significantly decreased in the Pb-exposed group compared to the control. In contrast, a significant increase in the rats' weight of the groups treated with the combination of Pb-garlic was observed compared to the Pb group. Treatment of rats with the positive controls of the four doses of garlic extracts (bulbs and leaves) has not made any significant changes in the absolute weight of the testis and epididymis as compared to the control. Nevertheless, all Pb-garlic extracts of bulbs and leaves were significantly higher than that of the Pb group (Table 2).

Spermatozoa Concentration and Motility

Rats administered with lead acetate have a significant decrease in sperm concentration and motility, relative to the control group. Contrary, sperm concentration and motility of all combination groups were significantly higher than that of the Pb-intoxicated rats.

Table 2 The total body weight (g) and the absolute weight (g) of reproductive organs (mean ± SEM) of Wistar rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

Groups	Body weight	Testis	Epididymis
C	212.7 ± 19.99	1.548 ± 0.03259	0.5296 ± 0.003627
Pb	175.8 ± 6.057 ^{C}	1.306 ± 0.04486 ^{C}	0.3556 ± 0.004789 ^{C}
B1	200.1 ± 14.43	1.495 ± 0.01258	0.503 ± 0.01263
B2	201.7 ± 16.24	1.503 ± 0.02877	0.4945 ± 0.003686
B3	206.4 ± 17.28	1.516 ± 0.01958	0.5107 ± 0.01097
B4	207.9 ± 17.67	1.521 ± 0.01316	0.5239 ± 0.01126
Pb-B1	196.2 ± 13 ^{L}	1.491 ± 0.03235 ^{L}	0.4926 ± 0.02663 ^{L}
Pb-B2	198.2 ± 14.3 ^{L}	1.487 ± 0.01853 ^{L}	0.4848 ± 0.02152 ^{L}
Pb-B3	202.1 ± 15.23 ^{L}	1.494 ± 0.04928 ^{L}	0.519 ± 0.003786 ^{L}
Pb-B4	208.6 ± 18.26 ^{L}	1.512 ± 0.01796 ^{L}	0.5217 ± 0.007623 ^{L}
L1	200.18 ± 14.91	1.471 ± 0.01632	0.4893 ± 0.00975
L2	201.4 ± 15.68	1.466 ± 0.006459	0.512 ± 0.00611
L3	207.5 ± 18.55	1.497 ± 0.02185	0.5156 ± 0.008692
L4	210.6 ± 18.66	1.514 ± 0.02998	0.5159 ± 0.001853
Pb-L1	196.06 ± 13.17 ^{L}	1.469 ± 0.01001 ^{L}	0.4817 ± 0.01663 ^{L}
Pb-L2	198.6 ± 14.62 ^{L}	1.474 ± 0.01098 ^{L}	0.5009 ± 0.01491 ^{L}
Pb-L3	203.6 ± 16.91 ^{L}	1.493 ± 0.01926 ^{L}	0.5167 ± 0.003333 ^{L}
Pb-L4	209.9 ± 18.77 ^{L}	1.525 ± 0.02675 ^{L}	0.5181 ± 0.01005 ^{L}

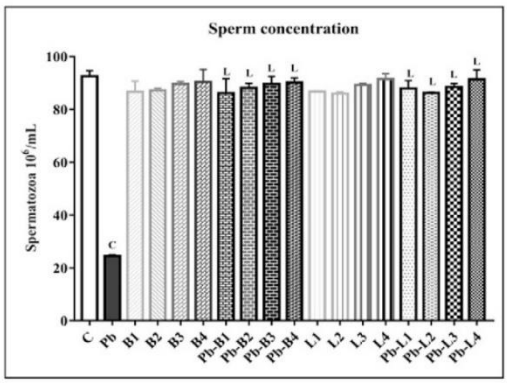


Fig. 2 Sperm concentration (mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

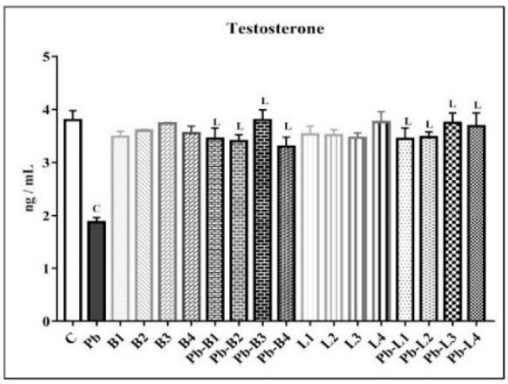


Fig. 4 Testosterone levels (mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

A significant dose-dependent attenuation was observed in sperm concentration and motility of the Pb-garlic treated rats (Figs. 2 and 3).

Testosterone Level

The statistical results obtained in Fig. (4) illustrate that the Pb group shows a significant decrease in testosterone level compared to the control group. The co-administration of *A. triquetrum* combined with lead acetate at different doses (Pb-B and Pb-L) reported a significant increase compared to the Pb group.

Spermatozoa Round Cells

Compared with the control, results revealed a non-significant decrease of round cell counts in the positive controls and in the combined treatment groups, with a significant decrease in the Pb group (Fig. 5).

Spermatozoa Velocity

Results indicate that rapid, medium, and slow sperm velocity of spermatozoa was significantly lower in the

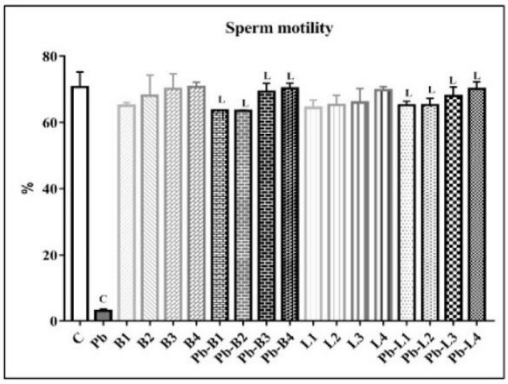


Fig. 3 Sperm motility (mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs, L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

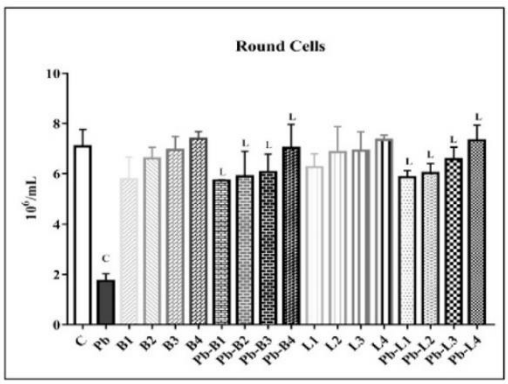


Fig. 5 Number of round cells (mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

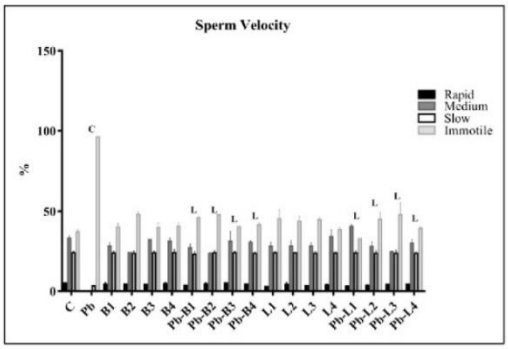


Fig. 6 Rapid, medium, and slow spermatozoa velocity (mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lad acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

Pb group when compared to the control, with a slight increase in the positive controls, and in the combined groups. The progressive immotile sperm velocity was significantly higher in the Pb group compared to the control, in contrast to the positive controls (B and L). Spermatozoa velocity of the combined groups (Pb-B, Pb-L) decreased significantly compared to the Pb group (Fig. 6).

Spermatozoa Vitality

Spermatozoa vitality (%) evaluated by the HSO-test is shown in Fig. 7. Rats administered with lead acetate have a significant increase in dead sperm vitality relative to the control group. Contrary, all combination groups were significantly lower than that of the Pb-intoxicated rats. However, the percentage of live sperm was significantly lower in the Pb group compared to the control, in contrast to the positive controls (B and L). Nevertheless, spermatozoa vitality of all Pb-garlic extracts of bulbs and leaves was significantly higher than that of the Pb group.

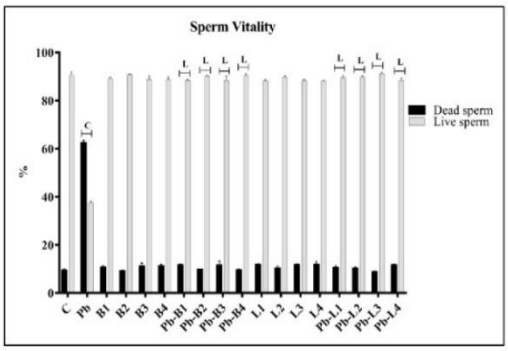


Fig. 7 Sperm live/dead count (mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

Glutathione Concentration

The mean value of GSH activity in the rats' testis and epididymis was significantly decreased in the Pb group compared to the control. The co-treatments with bulbs' and leaves' extracts have significantly increased GSH concentration of testicular and epididymal tissues when compared to the Pb group (Table 3).

Glutathione Peroxidase Activity

There was a significant reduction in testicular and epididymal GPx activity of the Pb-treated group compared to the control. The GPx activity in the combined treatments was significantly higher than that of the Pb group (Table 3).

Malondialdehyde Level

Compared to the control, significant elevation of MDA level in the testis and epididymis was observed in the Pb group. Contrary, the MDA level is remarkably lower in all combined treatments of both organs when compared to the Pb group (Table 3).

Phytochemical Screening

The phytochemical screening of bulbs and leaves of *A. triquetrum extracts* displayed the presence of flavonoids, polyphenols, phenolic derivatives, tannins, sterol terpenes, mucilage, coumarins, and saponosides, with the absence of alkaloids, anthraquinones, anthocyanins, and quinines (Table 4). This result approved that *A. triquetrum* contains various chemical compounds.

Chromatograms

The chromatographic profiles of the aqueous extracts of bulbs and leaves of *Allium triquetrum* analyzed by HPLC appear to contain phenolic compounds such as flavonoids, hesperidin, and acid chlorogenic (Figs. 8 and 9). In addition,

Table 3 Testicular and epididymal GSH concentration, GPx activity, and MDA level (mean ± SEM) of *Wistar* rats exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). Statistics: C, versus the control; L, versus Pb group

Oxidative stress parameters		µmol GSH/mg proteins	nmol/mg proteins	nmol/100 mg tissues
Groups		GPx	GSH	MDA
C	Testis	0.2972 ± 0.000582	120 ± 1.784	0.4626 ± 0.008822
	Epididymis	0.3074 ± 0.001865	196.5 ± 2.917	0.3637 ± 0.003245
Pb	Testis	0.08471 ± 0.002135 [C]	87.98 ± 1.407 [C]	0.6713 ± 0.003911 [C]
	Epididymis	0.07871 ± 0.002986 [C]	77.32 ± 1.2 [C]	0.5957 ± 0.003453 [C]
B1	Testis	0.2908 ± 0.003102	116 ± 0.5536	0.4757 ± 0.007952
	Epididymis	0.2982 ± 0.003737	189 ± 3.323	0.3952 ± 0.00302
B2	Testis	0.2926 ± 0.000551	116.5 ± 2.287	0.4749 ± 0.01569
	Epididymis	0.2978 ± 0.0004143	189.3 ± 0,1824	0.3675 ± 0.01183
B3	Testis	0.293 ± 0.0004999	118.2 ± 1.615	0.4684 ± 0.005616
	Epididymis	0.2989 ± 0.0004435	190.6 ± 0.6804	0.3659 ± 0.00858
B4	Testis	0.2957 ± 0.0005538	119 ± 0.5701	0.4619 ± 0.003068
	Epididymis	0.3002 ± 0.004457	192.8 ± 1,04	0.3643 ± 0.01311
Pb-B1	Testis	0.2896 ± 0.007329 [L]	114.3 ± 1.09 [L]	0.4851 ± 0.008688 [L]
	Epididymis	0.2956 ± 0.004145 [L]	189.1 ± 3.808 [L]	0.391 ± 0.005546 [L]
Pb-B2	Testis	0.2933 ± 0.003416 [L]	115.8 ± 1.39 [L]	0.4739 ± 0.01238 [L]
	Epididymis	0.2996 ± 0.001788 [L]	189.5 ± 3.048 [L]	0.3697 ± 0.01128 [L]
Pb-B3	Testis	0.2925 ± 0.0009546 [L]	117 ± 2.571 [L]	0.4695 ± 0.001085 [L]
	Epididymis	0.298 ± 0.000378 [L]	192.4 ± 0.6557 [L]	0.3672 ± 0.002096 [L]
Pb-B4	Testis	0.2943 ± 0.0006881 [L]	119 ± 1.561 [L]	0.4619 ± 0.001534 [L]
	Epididymis	0.2984 ± 0.000409 [L]	192.8 ± 1.04 [L]	0.3652 ± 0.005205 [L]
L1	Testis	0.2917 ± 0.005018	116 ± 1.38	0.4634 ± 0.00467
	Epididymis	0.2982 ± 0.002158	191,3 ± 1,484	0.3822 ± 0.005013
L2	Testis	0.2924 ± 0.0002418	117 ± 0.3353	0.4701 ± 0.001435
	Epididymis	0.2986 ± 0.003093	192.7 ± 0,2918	0.365 ± 0.004963
L3	Testis	0.2939 ± 0.0008955	118.4 ± 2.162	0.4673 ± 0.00551
	Epididymis	0.3 ± 0.0003183	195.2 ± 0,5142	0.3643 ± 0.006473
L4	Testis	0.2965 ± 0.000511	119.8 ± 1.862	0.4614 ± 0.005375
	Epididymis	0.304 ± 0.001128	195.5 ± 7.116	0.3637 ± 0.01183
Pb-L1	Testis	0.2886 ± 0.006917 [L]	113.7 ± 1.354 [L]	0.4598 ± 0.008089 [L]
	Epididymis	0.299 ± 0.006042 [L]	190.2 ± 0.258 [L]	0.3802 ± 0.004464 [L]
Pb-L2	Testis	0.2951 ± 0.0006735 [L]	116.7 ± 0.5944 [L]	0.4739 ± 0.006643 [L]
	Epididymis	0.2974 ± 0.0002278 [L]	190.4 ± 0.3081 [L]	0.3675 ± 0.01021 [L]
Pb-L3	Testis	0.2925 ± 0.0006495 [L]	117.5 ± 0.7467 [L]	0.4635 ± 0.004519 [L]
	Epididymis	0.299 ± 0.0002413 [L]	190.9 ± 3,443 [L]	0.3659 ± 0.007528 [L]
Pb-L4	Testis	0.2953 ± 0.000942 [L]	119 ± 0.8766 [L]	0.4605 ± 0.002206 [L]
	Epididymis	0.3006 ± 0.0001519 [L]	194.3 ± 0.02282 [L]	0.3643 ± 0.005902 [L]

leaves' aqueous extract appears to contain coumarins and isoquercetin.

Histological Study

The histological assessment of testicular and epididymal sections from different groups of rats after 3 weeks of treatment is laid out in Figs. 10 and 11. The histology of the control displayed that the testis has normal seminiferous tubules with enlarged germinal epithelium lined and germ cells at different stages of spermatogonia, primary and secondary spermatocytes, spermatids, and mature spermatozoa occupying the tubule's lumen. However, testicular parenchyma after lead acetate exposure exhibits atrophic seminiferous tubules as well as dissociated germinal epithelium with reduced spermatogonia numbers. Also, enlarged interstitial spaces were distinguished owed to tubular atrophy, edema, and reduced sperm counts of the lumen, with an absence of Leydig cells.

On the other hand, rats treated with the different doses of bulbs (B) and leaves (L) (2, 3, 4, and 6 g) indicate that the seminiferous tubules are preserved in the size and number and also separated by interstitial tissue. The germinal epithelium is regular in appearance, with

Table 4 Results of phytochemical screening of *Allium triquetrum* L.

Chemical compounds	Bulbs	Leaves
Flavonoids	++	+++
Polyphenols	++	+++
Phenolic derivatives	+	+
Alkaloid	-	-
Tannins	+	++
Anthroquinones	-	-
Anthrocyanins	-	-
Sterol terpenes	++	+++
Quinones	-	-
Coumarins	-	+
Mucilage	+++	+++
Saponosids	+++	++

(+), slightly present; (++), moderately present; (+++), highly present; (-), absent

sperm production observed in the lumen of the tubules defining all stages of spermatogenesis. The testes from rats treated with the highest dose (6 g) showed that the spermatogonia became larger. Besides, a return to normal of the global structure of testicular architecture in animals treated with Pb-B and Pb-L extracts at several doses (2, 3, 4, and 6 g) was observed; this restoration revealed a recovery of spermatogenesis due to the increase in spermatogonia, a respected germinal epithelium with spermatogonia, which demonstrated moderate atrophy of seminiferous tubules and enlarged interstitial space. Nevertheless, as the dose increases, the focal regeneration of spermatogenesis is determined, the seminiferous tubules become more normal, and the lumen is enriched with spermatozoa.

Further, epididymal morphology was altered by exposure to lead acetate, which was demonstrated by the devoid of sperm density in the lumen of the epididymal tubules without germ cells with a fragmented basement membrane compared to the control. Nonetheless, a normal spermatozoa density and intact basement membrane with the presence of sperm cells in the lumen was observed in the groups treated with bulbs' and leaves' aqueous extracts (B and L). Compared to the Pb-treated group, an increase in sperm density of the epididymal ducts with an enormous quantity of spermatozoids and a normal state of the overall epididymal structure was demonstrated in the groups co-administrated with Pb and *A. triquetrum* extracts (2, 3, 4, and 6 g).

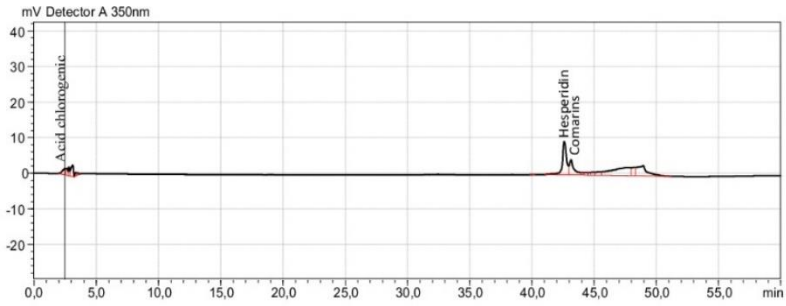


Fig. 8 Chromatogram of the aqueous extract of *Allium triquetrum* bulbs

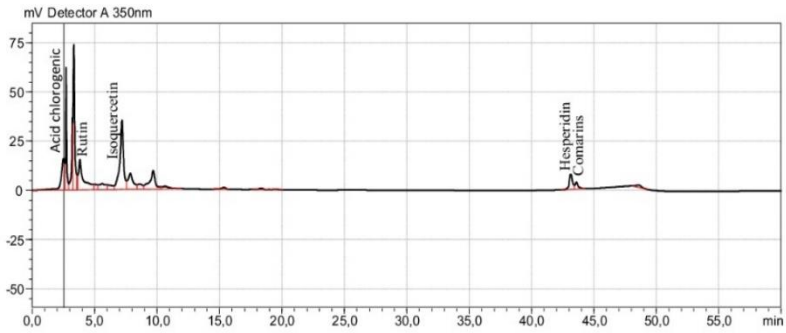


Fig. 9 Chromatogram of the aqueous extract of *Allium triquetrum* leaves

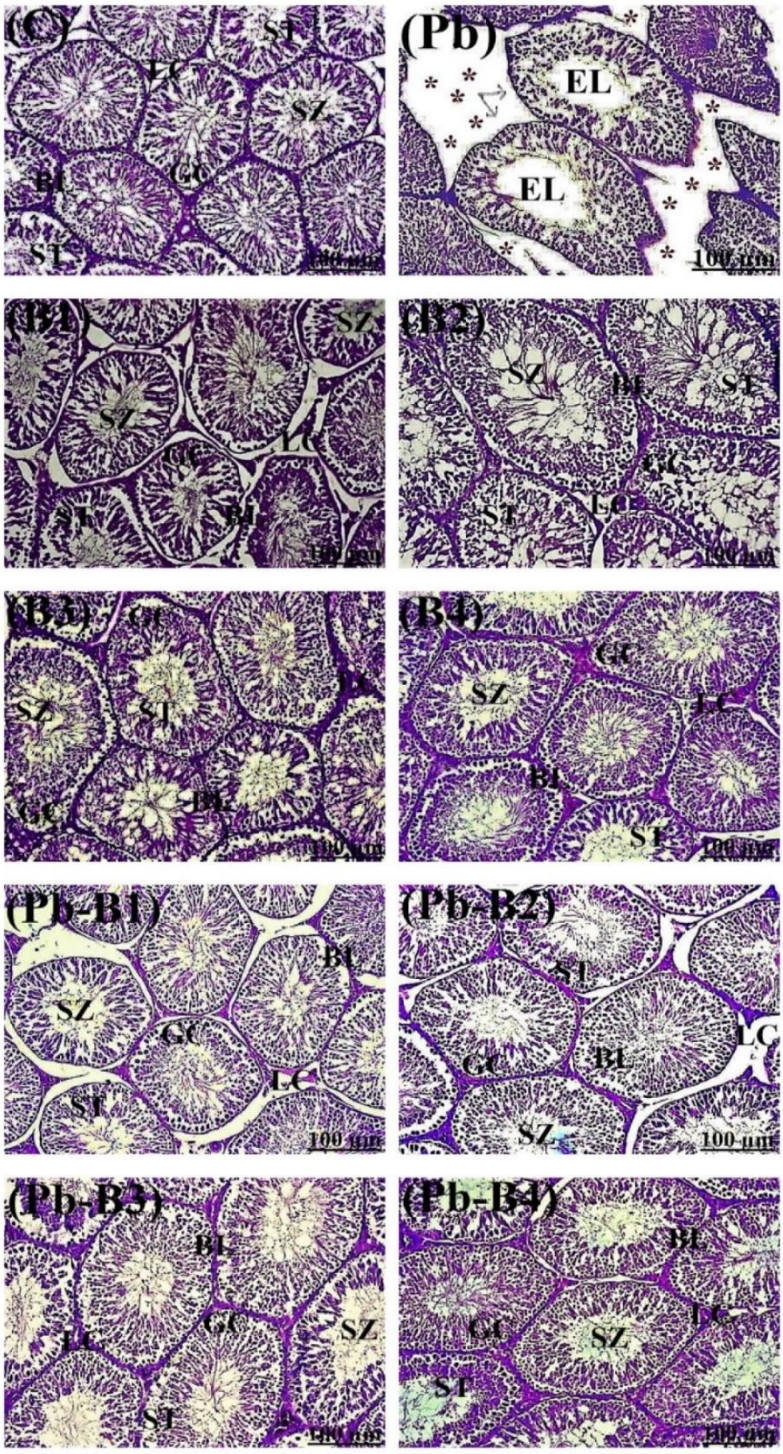
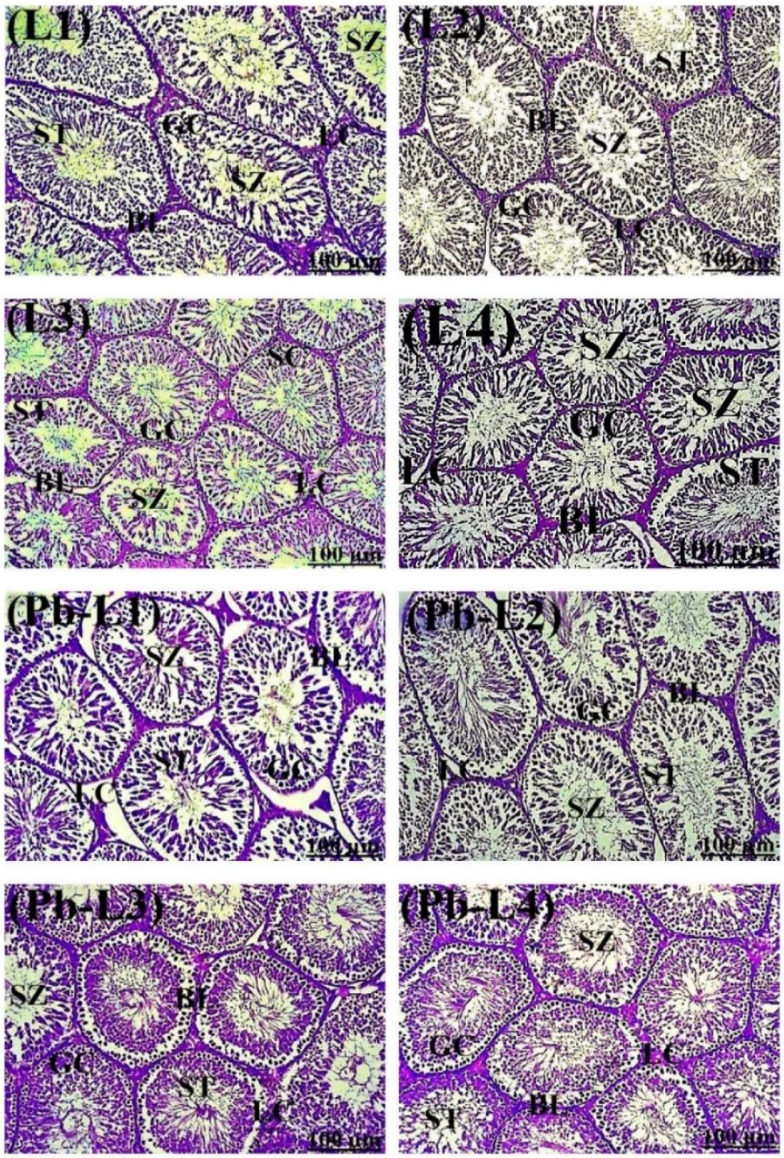


Fig. 10 Light microscopic feature of rat seminiferous tubules exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). H&E staining with magnification of × 100 and the bars represent 100 µm. **LC**, Leydig cells; **GC**, Germinal cells; **ST**, seminiferous tubules; **SZ**, spermatozoa; **BL**, basal lamina of seminiferous epithelium; **EL**, empty lumen; *, interstitial spaces are enlarged due to tubular atrophy and edema. The arrows designate the atrophied tubules

Fig. 10 (continued)



Discussion

Lead is a harmful environmental toxicant that induced a wide range of biological alterations and is considered a reproductive toxicant in males, which can provoke deep changes in sperm quality of rats [16]. Reduced reproductive performance of male rats exposed to lead acetate was linked to decreased steroidogenesis, spermatogenesis, and acrosome function [33], as well as to sperm count, motility, and alive sperm [15].

Lead administration for a period of 3 weeks has reduced total body weight and also testicular and epididymal absolute weights in rats. In accordance with our study, other authors have reported a shrink in body weight after exposure to lead acetate by gavage [34], which could be related to the decline in daily food intake. Indeed, results suggest that this metal has adverse effects on rat body growth through the loss of appetite or poor food absorption. Our results were in line with other studies that reported a decrease in the weight of the testis and epididymis of

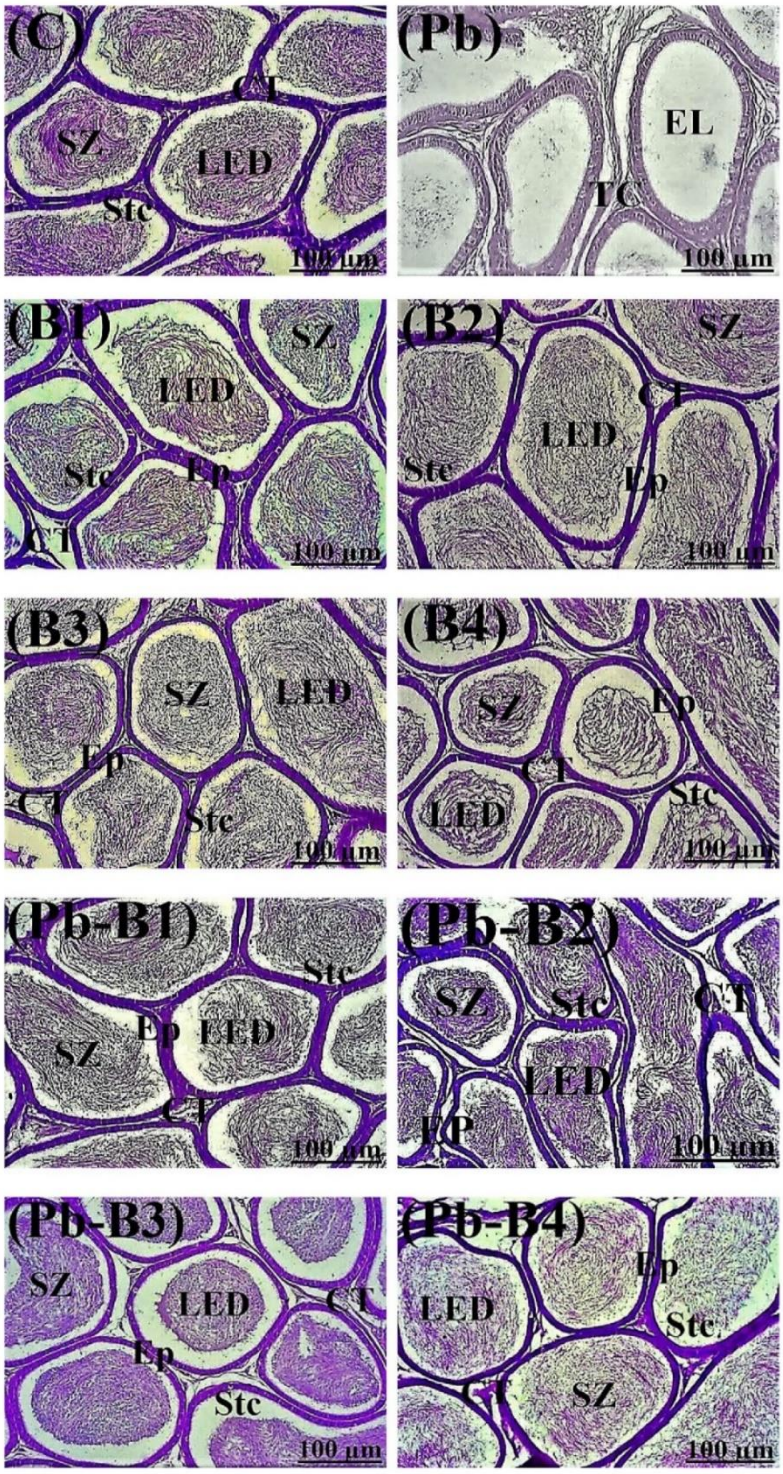
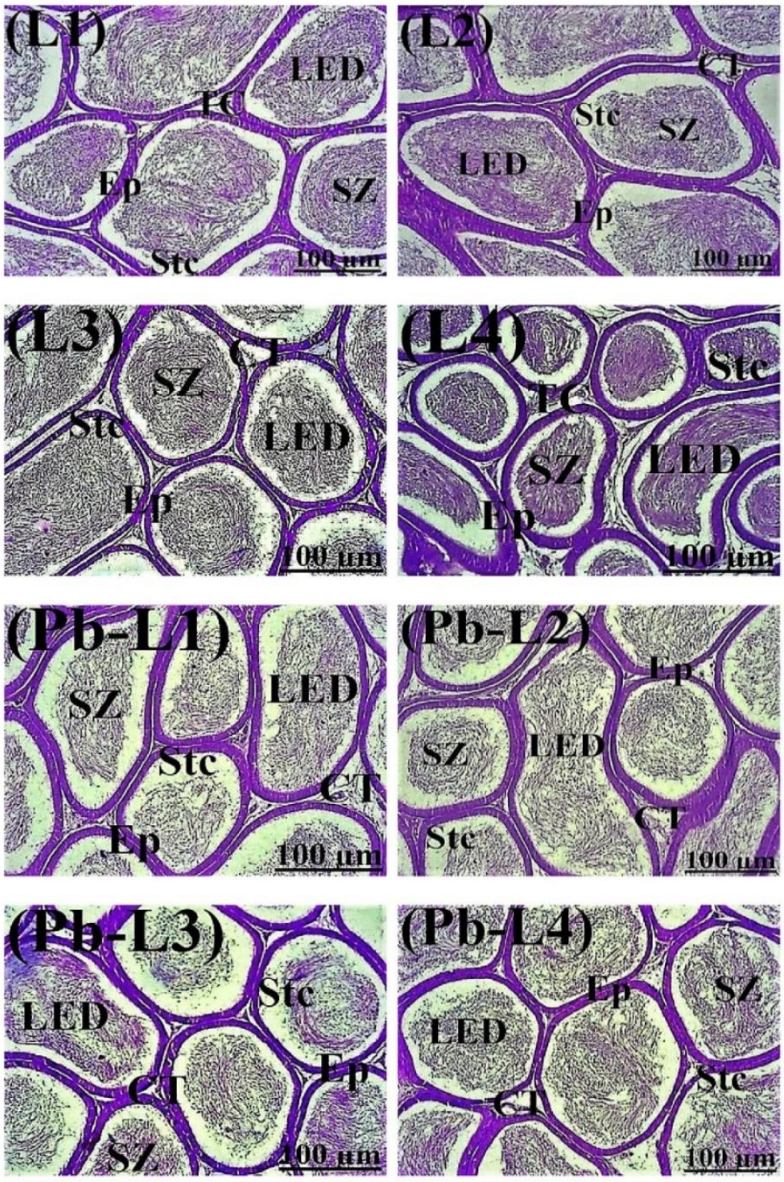


Fig. 11 Light microscopic feature of rat epididymis exposed to lead acetate and co-administrated with bulbs' and leaves' aqueous extracts of *A. triquetrum* for 3 consecutive weeks. (C, control; Pb, lead acetate; B, bulbs; L, leaves; Pb-B, lead acetate + bulbs' aqueous extract; Pb-L, lead acetate + leaves' aqueous extract). H&E staining with magnification of × 100 and the bars represent 100 µm. **CT**, connective tissue, **EL**, empty lumen; **Ep**, epithelium; **LED**, lumen of efferent ducts; **Stc**, stereocilia; **SZ**, spermatozoa

Fig. 11 (continued)



rats supplemented with lead acetate [25, 35]. On the contrary, exposure to this metal was not found to alter rat body weight, indicating the absence of obvious toxicity even after exposure to 0.15% lead acetate for 70 days [19]. The testicular weight was largely linked to the mass of the differentiated spermatogenic cells, where testicular weight suppression is an indication of germ cell loss and an inhibition of steroid biosynthesis at Leydig cells [19]. Thus, the suppression in testicular weight might be related to the effects of lead on the hypothalamo–pituitary–testicular axis [36], because of its important role in the regulation of male reproductive functions and/or to dropped testosterone production [25] that could influence spermatogenesis, leading to the deterioration of semen quality and sex organs' functions [37]. On the other hand, the co-administration of *A. triquetrum* aqueous extract with lead acetate resulted in an elevation in sex organs' mass, which perhaps owed to the fall of free radical generation counteracted

by garlic components and confirmed by the increased daily food consumption. According to the phytochemical screening, *A. triquetrum* aqueous extracts were rich in multiple constituents, with a clear difference between the two extracts as leaves contained more tannins than bulbs.

The evaluation of sperm cells is an essential parameter in the examination of sperm quality that could affect male fertility. Computer-assisted sperm analysis (CASA) allows an objective assessment of different characteristics, as sperm concentration, motility, velocity, morphology, and vitality. The decrease of sperm motility, concentration, and testosterone serum levels in our work is consistent with the previous results, which demonstrated that the exposure of rats to lead caused a reduction in the sperm count and motility [25, 38, 39], as well as endocrine function of reproductive organs [18]. The reduce in sperm motility can be explained by the effect of lead ions on the structure and function of the flagellum and the intermediate piece, leading to malformations that make flagellum more fragile and unable to move sperm. Metal ions can alter spermatozoa head membrane morphology [40] as well as sperm production [36]. Lead acetate was demonstrated to delay spermiation, the release of immature tubular spermatogenic cells [41], the induction of Leydig cells apoptosis [42], and the decrease of all spermatogenic cells populations, especially the mature forms of spermatids and spermatozoa [39]. Furthermore, the observed infertility in lead-intoxicated rats was linked to the morphological changes seen in the seminiferous tubules [43] and to the suppressed gonadotrophin testosterone synthesis that is the key molecule for sperm maturation, which is dependent upon the secretion of LH by the pituitary gland [25]. In contrast, low Pb dose (0.01%) administrated to *WISTAR* rats for 45 days was unable to provoke any changes in sperm concentration and motility [33].

The main cellular part of semen is composed of spermatozoa, but it contains also non-sperm elements known as round cells, of spermatogenic or non-spermatogenic origin [44]. Spermatogenic round cells include immature germ cells and degenerated spermatids, while non-spermatogenic round cells include exfoliating epithelial cells of the prostate, seminal vesicles, and inflammatory cells [45]. Our data illustrate that rat treated with Pb alone has significantly lower round cells, but in animals received Pb-garlic extracts, round cells were comparable to that of the controls and was dose-dependent. It was suggested that most round cells are originated from immature germ cells that having failed to complete the spermatogenesis. High round cells frequency is a marker of a high turnover of the germinal epithelium within the seminiferous tubules, which is often followed by an increase in sperm production [46]. Thus, apoptosis of the immature spermatozoa is one of the mechanisms held within Sertoli cells [45]. Apparently, very high round cells are an indication of male infertility. Lower round cells in Pb-exposed rats may demonstrate a very low turnover of spermatogenesis within the testicular tubules due to germ cells death during the 30-day exposure, since decreased germ cells layer population was observed in rats supplemented with Pb [47].

The velocity of spermatozoa is one of the determining factors of sperm quality. In this research, the different types of sperm velocity have been affected by Pb exposure, as that of rapid progressive speed, which has decreased along with an increase in low speed and immotile sperm. Such results are similar to those obtained earlier [18, 38], in which Pb delayed the activity of live sperm. It can be assumed that Pb has a direct effect on spermatogenesis and on their full growth and maturation. Regarding the drop in velocity, it is suggested that this metal can act on the mitochondrial function of the intermediate piece, by inhibiting the energy necessary for sperm movement [38]. Lead can also influence sperm structure, membrane integrity, and functional activity which elucidate the decrease in rat sperm velocity [48]. Yet, the supplementation of wild garlic bulbs and leaves together with Pb to rats resulted in a marked increase in rapid and medium sperm velocity, with a decrease in immotile velocity. This finding agrees totally with the previously reported data on domestic garlic co-administration alongside Pb, which reduced the metal toxicity as garlic contains antioxidants that can play roles as preventive and therapeutic agents [38].

The treatment of rats with Pb-acetate for 3 weeks caused a remarkable drop in the number of live spermatozoa with a rise in abnormal ones. Therefore, lower HOS reflect abnormalities of sperm plasma membrane that may be associated with dysfunctional sperm head, mid-piece, and tail proteins, affecting sperm density, motility, morphology, and seminal hyaluronidase activity. These results are consistent with another study, which confirmed the high frequency of chromosomal abnormalities in abnormal sperm of rats treated with 20 mg/kg Pb, indicating the presence of a very large number of dead sperm [25, 35]. Further, the decrease in the frequency of tail coiled sperms as evidenced by hypo-osmotic swelling test suggests that membrane integrity of the sperms is deteriorated. Pb exposure also results in severe morphological abnormalities in the head of sperms [19]. This decrease may be a consequence of the decrease in the concentration of FSH, which is involved in the activation of Sertoli cells that support the development and maturation of sperm. Therefore, sperm death can be explained by the direct effect of Pb on spermatogenesis, on their growth, and maturation [38].

Furthermore, lead is associated with a decrease in the components of antioxidant defense system in sperm and can also cause lipid peroxidation, DNA damage, and sperm vitality [22] because Pb may cause premature acrosome reaction [33]. Clearly, the oral combined administration of bulbs'

and leaves' extracts with Pb has protected the viability of spermatozoa from such changes, leading to an improvement in the fertility parameters studied in this work. Our findings are in line with other results [17, 38] that demonstrated the protective role of domestic garlic against the observed spermatogenesis damage of rats poisoned with lead for several weeks. Thereby, *A. triquetrum* aqueous extract added to lead acetate may reduce metal toxicity, either directly through the reinforcement of chelating agent synthesis as metallothioneins or indirectly through several mechanisms such as enzymatic and non-enzymatic antioxidants.

As expected, chronic exposure of rats to lead acetate for 3 weeks caused a reduction in testicular and epididymal GSH level and GPx activity, thus indicating a high load of free radicals together with a deficiency in the detoxifying efficiency of these vital reproductive organs. As previously mentioned, there is substantial evidence supporting the role of oxidative stress and ROS as major factors underlying the toxic effects of lead [49]. Similar observations confirmed a remarkable decrease of GPx activity in Leydig and Sertoli cells [50] and in testicular tissue following treatment of Pb-acetate for 35 days [51]. These findings are possibly related to the influence of Pb on the glutathione protection system, resulting in the generation of peroxides. On the other side, adding different Pb concentrations (0, 3.675, 7.35, 14.7, 29.4, 58.8 mg/L) to crabs did not affect GPx activity after 3-day exposure [52]. Therefore, the reduced glutathione levels in the testis and epididymis tissue as observed in the present study are matched with several studies that reported a decrease of GSH levels in lead acetate-intoxicated rats [51]. Lead has a high affinity to thiol GSH group, where its decrease could be elucidated by its participation in the detoxification processes of ROS. Lead is excreted in the bile after irreversibly binds to GSH group and other sulfhydryl proteins, leading to an increase in lipid peroxidation and DNA damage [14]. Besides, the increase in the GSH and GPx levels in the testis and epididymis of rats exposed to the positive controls of garlic bulbs and leaves may perhaps mediate the induction of antioxidative components. Glutathione is very abundant in the cytosol, nuclei, and mitochondria, and its antioxidant capability comes from sulfur atom, which is easily gaining the lost electron generated by Pb toxicity [53]. Consequently, cell damage occurs when antioxidant levels become low [54] that allows Pb to disrupt cell functions. The co-administration of *A. triquetrum* with Pb to rats resulted in a raise in the levels of GPx and GSH of testicular tissues. Polyphenols have the potential to upregulate the expression of c-glutamyl cysteine synthetase, the enzyme that limits the rate of GSH biosynthesis [55], which may explain the increased level of GSH and GPx in rats exposed to combined treatments of Pb-garlic, since *A. triquetrum* aqueous extracts were proved to have a highest antioxidant capacity and considerable levels of polyphenols and flavonoids [56].

The observed high MDA level in the Pb-intoxicated rats after 3 weeks is an indicator of testicular and epididymal injuries, which coincided with low levels of GPx and GSH. However, in the presence of garlic, MDA concentration has been lowered and was comparable to that of those controls. Similarly, several authors have noticed remarkable elevation in the testicular MDA levels in Pb-exposed rats [53]. In addition, Pb intoxication was responsible on the induction of lipid membrane peroxidation, conducting to the formation of MDA, modifying then the balance between free radicals and the antioxidant system [14]. On the other hand, the supplementation of *A. triquetrum* to Pb-intoxicated rats led to a reduction in testicular MDA levels; these results are consistent with Nasr et al. [49]. Recently, it was elucidated that domestic garlic had beneficial action on testis function better than the liver and kidney following Pb-intoxicated rats [17]. Moreover, Pb organs' level of rats experienced oral co-supplementation of garlic aqueous extract alongside Pb nitrate has been decreased [13].

Histological evaluation of the male reproductive system is a vital way to assess its morphology and function. In view of that, exposure of rats to lead acetate induced histopathological changes in the testes and epididymis, which may be related to increased oxidative stress at the cellular level. Alterations in testicular ultrastructure such as degeneration of the seminiferous tubules, absence of congested spermatogenic series in the testicular blood vessels, and reduced concentration of spermatozoa in the lumen of the tube were observed in the Pb-intoxicated group. Our result is in harmony with that of Nasr et al. [49] and Abdelhamid et al. [57], who reported severe testicular alterations of rats treated with Pb at 50 mg/L during 42 days and 100 mg/kg for 4 weeks, respectively. Lead was also shown to cause degenerative changes in the seminiferous tubules, edema of interstitial tissue, and atrophy in the absence of regular differentiated germ cell steps for sperm maturation, resulting in a decrease in the number of spermatogenic cells [49, 58]. Hence, this toxicity can be attributed to Leydig cells' damage or to the direct toxic action of Pb on gene expression [59]. Furthermore, the testes of rats treated with *A. triquetrum* alone or co-administrated with lead acetate showed a normal histological structure of mature active seminiferous tubules associated with complete spermatogenic series. Notably, both bulbs and leaves of *A. triquetrum* supplements mitigated the deterioration of testicular tissue in lead-poisoned group. Such results agree with the already reported studies, in which garlic aqueous extract have improved testicular structure and sperm count after 4 weeks of testosterone induction that caused oligospermia in *WISTAR* rats [60]. As observed earlier, commercial garlic powder administration (200 mg/

kg bw) had a testicular anti-apoptotic effect, which is provoked by lead acetate in adult male rats for 6 weeks, where seminiferous tubules showed normal diameter and well-differentiated germ cell stages [49]. Likewise, Kasuga et al. [61] noticed that raw garlic juice was effective in the recovery of mice testicular function, after testicular hypogonadism, induced by acetaldehyde intoxication. Likewise, aged garlic extract was efficient in reducing adriamycin-induced testicular damage by improving most of the histopathological changes in germ and Leydig cells of rats [62].

From the current results, it can be concluded that the co-administration of aqueous extract of bulbs and leaves of wild garlic *A. triquetrum* at different doses with lead acetate for 3 weeks to male rats has mitigated lead toxicity by boosting testicular and epididymis weight and ameliorating the levels of sperm concentration, motility, serum testosterone, velocity, vitality, and round cells. Garlic extracts have also protected testicular damage through increasing organs' GSH and GPx levels and decreasing MDA concentration. Seemingly, testicular and epididymal histological architecture is in line with the previous markers in the different treated groups. Interestingly, garlic extracts apparently has a dose-dependent positive effect against Pb toxicity. HPLC analysis of aqueous extracts of *A. triquetrum* showed that leaves are richer in tannin compounds than bulbs. Therefore, wild garlic can be considered as a source of many bioactive components that can have promising roles in metal detoxification.

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Declarations

Ethics Approval Experiments were carried out according to the international animal handling of Helsinki Declaration of 2008 and to the National Ethical Committee of Animal Sciences.

Conflict of Interest The authors declare no competing interests.

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International Communications

People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research

LEBA
مخبر ايكوبيولوجيا الحيوان
LAB ECOBIOLOGIE ANIMALE

ICEBB
1st International Conference on Environmental Biotechnology and Biodiversity (ICEBB)
Online | 14-16 Decembre 2021

Certificate of contribution

This is to certify that

Labiba Kahalerras

Has participated with an oral communication at the 1st International Conference of Environmental Biotechnology and Biodiversity (ICEBB 2021), held online on 14–16 December 2021

Paper : **The Beneficials Effect of Wild Garlic *Allium triquetrum* L. Against Lead Acetate Toxicity**
Author(s): **Labiba Kahalerras & Cherif Abdennour**

Baha mounia
Prof. Dr. BAHA Mounia
General Chair ICEBB

Lebaili nemcha
Prof. Dr. Lebaili Nemcha
Scientific Committee Chair

International Conference on Environmental Biotechnology and Biodiversity

Saad Dahlab University, Blida-1
Faculty of Natural and life sciences

Certificate
OF ATTENDANCE

THE 1ST IWBCE 2021

This certificate is proudly awarded to

Kahalerra Labiba

Co-authors : Ines OTMANI and Cherif ABDENNOUR

Has successfully participated at the

1ST INTERNATIONAL WEBINAR ON BIOLOGICAL AND CHEMICAL ENGINEERING

Held with the support of Faculty of Natural and Life Sciences-Blida1 University-Algeria, on November 10th& 11th, 2021,

By presenting an oral communication entitled:

Evaluation of The Aqueous Extract of Allium triquetrum L. In The Mitigation of Lead-Induced Hepatic Injuries in Rats

Dr. Farida KADRI
Chair of IWBCE 2021

Pr. Smail Megatli
Dean of NLS Faculty

International Communications

الجمهورية الجزائرية الديمقراطية الشعبية وزارة التعليم العالي و البحث العلمي
REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
جامعة سعد دحلب البليدة 1
Université Saad Dahlab BLIDA 1
Faculté des Sciences de la Nature et da la Vie

Attestation de Participation

La présidente du 1er Séminaire International PERTURBATEURS ENDOCRINIENS ENVIRONNEMENT & REPRODUCTION (PEER 2021) tenu du 07 au 08 juillet 2021 a l'université Blida 1 atteste que

Mr / Mme: **KAHALERRAS Labiba**

a participé activement au séminaire et a présenté une **communication E-POSTER** Intitulée:

«Oxidative Testicular Damage Due to Chronic Lead Acetate Exposure in wistar rats Mitigated by Garlic Aqueous Extract »

Co-auteurs : *INES Otmani, CHERIF Abdennour*

Le Doyen de la Faculté des sciences de la Nature et de la Vie
Prof. [illegible]

Présidente du Seminaire
Dr. KHALDOUN Hassina.
PEER – 2021

République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université Chadli Bendjedid- El Tarf
Faculté des sciences de la nature et de la vie
Département de Biologie
Laboratoire de Recherche sur la Biodiversité et la Pollution des Écosystèmes

1er Congrès international de Biodiversité, Risques Environnementaux et Santé Publique
-En Ligne Via ZOOM- CIBRESP, les 07 et 08 Avril 2021

ATTESTATION DE PARTICIPATION

La Présidente du Congrès et le Président du Comité Scientifique attestent par la présente que :

Mme/Mlle/Mr. **Kahalerras Labiba**, Université Badji Mokhtar, Annaba, Algérie
a participé au **1er Congrès international de Biodiversité, Risques Environnementaux et Santé Publique CIBRESP, En ligne - via Zoom- les 07 et 08 Avril 2021** par une communication par affiche intitulée :
« The additional effect of wild garlic on epididymal sperm biology in wistar rat »
En collaboration avec : **Otmani Ines, Abdennour Cherif**

Le Président du Comité Scientifique
Pr. Nasri Hichem

Pr. NASRI Hichem
Professeur et Directeur de Recherche
Laboratoire de Recherche sur la Biodiversité et Pollution des Écosystèmes
Faculté SNV, Université Chadli Bendjedid
E-El-Tarf

La Présidente du Congrès
Dr. Djabali Nacira

Université Chadli Bendjedid - El-Tarf
Dr. DJABALI Nacira
Présidente du 1er Congrès International
"Biodiversité Risques Environnementaux Et Santé Publique CIBRESP" En Ligne Via ZOOM
Les 07 et 08 Avril 2021

International Communications

République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
International Journal of Human Settlements
https://www.aneau.org/ijhs/
ISSN : 2588-1779

Google Meet

ATTESTATION

Cette attestation est délivrée à :

Labiba KAHALERRAS, Laboratory of Animal Ecophysiology. Department of Biology. Faculty of Sciences. University Badji Mokhtar-Annaba, Annaba 23000, Algeria

Ines OTMANI, Laboratory of Animal Ecophysiology. Department of Biology. Faculty of Sciences. University Badji Mokhtar-Annaba, Annaba 23000, Algeria

Cherif ABDENNOUR, Laboratory of Animal Ecophysiology. Department of Biology. Faculty of Sciences. University Badji Mokhtar-Annaba, Annaba 23000, Algeria

Pour leur participation au **SÉMINAIRE INTERNATIONAL SUR LES SCIENCES NATURELLES ET DE LA VIE** en ligne (webinaire), organisé par **International Journal of Human Settlements** le 19 et 20 Février 2021, avec un **poster** intitulé:

THE BENEFICIAL EFFECT OF BULBS EXTRACT EXPOSED TO A CHRONIC DOSE WITH LEAD ACETATE

Oran, Algerie
20/02/2021

L'éditeur
Dr. HAMMA Walid

IJHS Journal

BIODIV 2019

L'Association pour la Conservation de la Biodiversité dans le Golfe de Gabès.

جمعية المحافظة على التنوع البيولوجي بخليج قابس

Le comité d'organisation de la 3ème conférence Méditerranéenne de la Biodiversité «BIODIV2019», Tenue à Hammamet, Tunisie, du 01 au 03 Novembre 2019, atteste que :

LABIBA KAHALERRAS

A participé aux journées par une communication orale N° : 46 intitulée:

PHYTOPROTECTIVE EVALUATION OF GARLIC AGAINST IMMUNOTOXICITY INDUCED BY LEAD ACETATE IN WISTAR RATS

Labiba KAHALERRAS, Cherif ABDENNOUR.

Prof. Lassad Neifar
Président ASCOB-SYRTIS

International Communications

Association Tunisienne des Sciences Biologiques
- Membre de l'IUBMB
- Membre de la FASBMB
- Membre de l'IUSB
- Membre de la FEBS

الجمعية التو نسية للعلوم البيولوجية

Tunisian Association of Biological Sciences
- Member of the IUBMB
- Member of the FASBMB
- Member of the IUSB
- Member of the FEBS

CERTIFICATE OF ATTENDANCE

This is to certify that

Name : KAHALERRAS

Surname : Labiba

Participated and presented the communication:

Entitled : Etude de quelques parametres hematologiques chez le rat wistar

Authors : Kahalerras labiba, Otmani ines, Abdennour cherif

Type : Poster

at the 30th international congress of the Tunisian Society of Biological Sciences (ATSB) held in Sousse, Tunisia on 25-28 march 2019.

ATSB Congress Organization Board
The secretary general
Dr. Manel Ben M'hadheb

Association Tunisienne des Sciences Biologiques

IUBMB (International Union of Biochemistry and Molecular Biology) - FASBMB (Federation of African Societies of Biochemistry and Molecular Biology) - IUSB (International Union of Biological Sciences) FEBS (Federation of European Biochemical Societies)

Association Tunisienne de Biotechnologie et Valorisation des Bio-Ressources

Tunisian Association of Biotechnology and Bio-Resources Valorization

ATTESTATION DE PARTICIPATION

Le Président de l'AT-BVBR, atteste que

LABIBA KAHALERRAS

a présenté au VIIème congrès international de Biotechnologie et Valorisation des Bio-Ressources, organisé par l'AT-BVBR du 20 au 23 Mars 2019 à Tabarka -Tunisie, une communication orale intitulée:

L'EFFET TOXIQUE DU PLOMB SUR QUELQUES PARAMETRES HEMATOLOGIQUES CHEZ LE RAT WISTAR

KAHALERRAS LABIBA, ABDENNOUR CHERIF, OTMANI INES

Président de l'AT-BVBR
Prof. Mohamed Lamjed MARZOUKI

AT-BVBR

International Communications

Université Frères Mentouri Constantine 1

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université Frères Mentouri Constantine 1

Laboratoire de Biologie et Environnement

Université Frères Mentouri Constantine 1

ATTESTATION DE PARTICIPATION

La présidente du **Séminaire International Environnement et Toxicologie** « sinentox'2019 »
qui a eu lieu à Constantine le 18 et 19 février 2019, atteste que :

Mme/Melle/Mr : **KAHALERRAS Labiba**
a participé avec une communication affichée
Intitulée : Effet de l'exposition chronique au plomb sur la reproduction chez le rat Wistar.
Co-auteurs : ABDENNOUR Cherif, OTMANI Ines.

Présidente du comité scientifique

Pr. AFRI-MEHENNAOUI Fatima Zohra

Université Frères Mentouri Constantine
جامعة الإخوة منتوري قسنطينة
Laboratoire de Biologie et Environnement
مخبر البيولوجيا و البيئة

Présidente du séminaire

Pr. AMEDDAH Souad

sinentox'2019, Constantine, 18-19 février 2019

Association Tunisienne de Biotechnologie et Valorisation des Bio-Ressources
Tunisian Association of Biotechnology and Bio-Resources Valorization

ATTESTATION DE PARTICIPATION

Le Président de l'AT-BVBR, atteste que

KAHALERRAS LABIBA

a présenté au VI[ème] congrès international de Biotechnologie et Valorisation des Bio-Ressources, organisé par l'AT-BVBR du 20 au 23 Mars 2018 à Tabarka - Tunisie, une communication par Affiche intitulée

C. AFFICHE N°:66.

L'EFFET PROTECTEUR DU MIEL CONTRE LA REPROTOXICITE INDUITE PAR LE MERCURE CHEZ LE RAT WISTAR

KAHALERRAS LABIBA , OTMANI INES , ABDENNOUR CHERIF

AT-BVBR

Président de l'AT-BVBR
Prof. Mohamed Lamjed MARZOUKI

National Communications

1ère Web conférence nationale sur la gestion des écosystèmes naturels face aux Changements globaux: *A l'occasion de la COP26 à Glasgow (UK) - The 26th session of the Conference of the Parties*
Organisé à Tlemcen le 11 Novembre 2021

ATTESTATION DE PARTICIPATION

Le président du séminaire *CENGEN1 2021* certifie que :

~~Mme~~, Melle, ~~Mr~~ : **Labiba KAHALERRAS**

A présenté une communication affichée intitulée :

"The *Allium triquetrum* Leaves Extenuate Lead Acetate-Induced Hepatotoxicity in Male Rats"

Co-auteurs : Ines OTMANI & CHERIF Abdennour

Président du séminaire — Président du comité Scientifique — Directeur du laboratoire

1ère Web conférence nationale sur la gestion des écosystèmes naturels face aux changements globaux CENGEN' 2021

Pr. HASNAOUI Okkacha

Pr. MERZOUK Abdessamad
ENSEIGNANT CHERCHEUR
UNIVERSITE DE TLEMCEN
abdessamadmerzouk@mail.univ-tlemcen.dz
lecgen2014@gmail.com
Tél. 0771 65 52 85

Ecology and Management Laboratory of Natural Ecosystems
Laboratoire d'Ecologie et Gestion des Ecosystèmes Naturels
مخبر علم البيئة و تسيير النظم البيئية الطبيعية
https://egen.univ-tlemcen.dz
MESRS - DGRSDT - ATRSNV

Site : https://snv.univ-tlemcen.dz/fr, https://egen.univ-tlemcen.dz/, Courriel : lecgen2014@gmail.com

Certificat de Participation

Séminaire National en Ecophysiologie et Environnement

SNEE 10 -11 Novembre 2021, Souk Ahras

Je soussignée, Présidente du SNEE 2021, certifie que **KAHALERRAS Labiba**

a présenté une communication orale intitulée :

" Le Rôle Protecteur Des Bulbes Et Des Feuilles D'ail Sauvage Sur La Peroxydation Lipidique Induite Par L'acétate De Plomb Dans La Fertilité Masculine "

Co-Auteurs : ABDENNOUR C.

Présidente du Séminaire

Président du Premier Séminaire National Ecophysiologie & Environnement
Dr. Amina MERGHAD

Le Doyen

Univ. Med Chérif Messaadia, Souk Ahras. Fac. des Sciences de la Nature et de la Vie
Lab. des Ecosystèmes Aquatique et Terrestre

National Communications

République algérienne démocratique
Ministère de l'enseignement supérieur et de la recherche scientifique
Université M'Hamed Bougara de Boumerdès
Laboratoire de Bioinformatique, Microbiologie
Appliquée et Biomolécules

Certificat

Ce certificat est présenté à:

KAHALERRAS LABIBA

pour avoir participé au *Séminaire National sur le Traitement de la Biocorrosion par les Biotechnologies et la Chimie Verte (SNTBBCV)* qui s'est tenu en ligne le 15 septembre 2021.

A participé avec une communication par affiche intitulée :
EFFET PREVENTIF DE L'AIL CONTRE LES DOMMAGES OXYDATIFS DUS A L'EXPOSITION CHRONIQUE A L'ACETATE DE PLOMB CHEZ LES RATS *WISTAR*.

Co-auteur: ABDENNOUR CHERIF

Présidente du SNTBBCV
BMAB/UMBB
Pr. KEBBOUCHE-GANA S.
Présidente
SNTBTCV

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université Alger 1, Benyoucef Benkhedda
Faculté des Sciences
Laboratoire de Valorisation et Bio-ingénierie des Ressources Naturelles (LBVRN)

LVBRN

Attestation de participation

A

La 1ère Journée scientifique sur
La Biochimie fonctionnelle et la physiopathologie cellulaire (JSBFPC)
Le 06 novembre 2021 à Alger

Je soussigné, Mohamed El Fadel OUSMAAL, président de la JSBFPC atteste que :

Melle/Mme/M. Kahalerras Labiba
A présenté une communication affichée
Intitulée : L'extrait aqueux de bulbe d'ail améliore l'hépatotoxicité induite par le plomb chez le rat
Co-auteurs : CHERIF Abdennour

Fait à Alger, le 06 novembre 2021

Président de la JSBFPC
Dr. Mohamed EL Fadel OUSMAAL

Dr. Mohamed El Fadel OUSMAAL
Maître de conférences classe A
Faculté des sciences - Université Alger 1
Chef d'équipe « Recherche biomédicale et innovation en santé »
Laboratoire LVBRN

Doyen de la Faculté des Sciences
Pr. BENOUADAH Ali

National Communications

الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
University of Laghouat
Faculty of Sciences

Laboratoire de Mécanique Lagh-Univ

DESERTIFICATION & CLIMAT

IEEE GRSS Algeria Chapter

AABRS

UNIVERSITY OF LAGHOUAT

Certificate of Participation

SNPPV_79/2021

This is to certify that

M KAHALERRAS Labiba

Has partipated in the First Online National Seminar on Plant Production and Protection held in the University of Laghouat from 19 to 20, June 2021 with a poster presentation entitled « L'extrait aqueux des feuilles d'ail ajuste le système immunologique des rats WISTAR»

Co-autors : ABDENNOUR Cherif

President of the seminarn

Université Amar Telidji Laghouat
Faculté des Sciences
Président du séminaire (SNPPV1)
Dr . MALLEM Hamida

Head of Department of Agricultural Sciences

REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE
MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE SAAD DAHLAB BLIDA 1
FACULTE DES SCIENCES DE LA NATURE ET E LA VIE
LABORATOIRE DE RECHERCHE EN BIOTECHNOLOGIE, ENVIRONNEMENT ET SANTE (LBES)
DEPARTEMENT DES SCIENCES BIOLOGIQUES

bio

RSDT

Attestation De Participation

Séminaire National : Ressources végétales, Produits Naturels et Santé (RVPNS2021)

Le comité scientifique du séminaire national Virtuel des RVPNS 2021 atteste que :

***Dr KAHALERRAS Labiba ;** Cherif ABDENNOUR*

A participé avec une Communication Affichée intitulée

« Effet Préventif d'Extrait de Feuille d'Ail Sur Les Marqueurs Immunologiques des Rats WISTAR Sous l'Intoxication de l'Acétate de Plomb »

Qui s'est tenu en ligne sous forme de Webinaires « visioconférences », le 9,10,11 juin 2021.

RVPNS-2021

La Présidente du séminaire RVPNS2021

Dr. Cherif Hamida Saida
Présidente
RVPNS-2021

National Communications

République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Centre Universitaire Abedelhafid Boussouf- Mila
Institut de Sciences et Technologie
Département de Sciences de la Nature et de la Vie
Laboratoire : Sciences de la Nature et des Matériaux (LSNM)

Attestation de Participation par Visioconférence

Au 1er Séminaire National sur les Ressources Naturelles face aux Contraintes d'Usage et du Changement Climatique (SENACUC, 2021), le 04 Juillet 2021

La présidente du séminaire atteste que Mme : **Kahalerras Labiba** a présenté une communication orale

Sous le titre : How doses of wild garlic have a potential to attenuate the toxic effect of lead acetate on testicular male infertility.

Co-auteurs : Otmani Ines, Abdennour Cherif.

Directeur de l'Institut — **Présidente du Séminaire (SENACUC, 2021)**

Dr.KHERIEF NACEREDDINE Saliha

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Centre Universitaire Abdelhafid Boussouf-Mila
Institut des Sciences et Technologies, Département des Sciences de la Nature et de la Vie
Laboratoire des Sciences et Matériaux
La Cellule du Qualité d'Enseignement Supérieur et de la Recherche Scientifique

A l'occasion de la Journée Internationale de la Biodiversité
le Centre Universitaire Abdelhafid Boussouf-Mila a l'honneur d'organiser un
Séminaire National sur la Biodiversité Végétale et Animale, Environnement et Santé

ATTESTATION DE PARTICIPATION

La présidente du Séminaire National sur la Biodiversité Végétale et Animale, Environnement et Santé

Atteste que: Melle/Mme/Mr: **KAHALERRAS Labiba**

A présenté une communication orale

Intitulée: **Impact of wild garlic on lead-induced lipid peroxidation in wistar rats.**

Co-auteurs: ABDENNOUR Cherif

Responsable de la Cellule — La présidente du Séminaire

Centre Universitaire Abdelhafid Boussouf-Mila
Institut des Sciences et Technologie
Département des Sciences de la Nature et de la Vie
Présidente du Séminaire
Dr. BOUSMID Ahlem

C.U. Mila, LE 20 Mai 2021

National Communications

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE LARBI TEBESSI – TEBESSA –
FACULTE DES SCIENCES EXACTES ET SCIENCES DE LA NATURE ET DE LA VIE
DEPARTEMENT DE BIOLOGIE APPLIQUEE

Attestation de Participation

Le comité d'organisation de la première journée scientifique sur la biologie des plantes médicinales, tenue à Tébessa le 22 janvier 2020, atteste par la présente que : **LABIBA Kahalerras** a participé à cette manifestation par une communication affichée intitulée :

« Oxidative damage due to chronic lead acetate exposure in wistar rats »

Co-auteurs: INES Otmani, CHERIF Abdennour

Président de la journée
Dr. Samira BOUSSEKINE

RÉPUBLIQUE ALGÉRIENNE DÉMOCRATIQUE ET POPULAIRE
MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITÉ SAAD DAHALB BLIDA 1
FACULTÉ DES SCIENCES DE LA NATURE ET DE LA VIE
LABORATOIRE DE RECHERCHE PLANTES MEDICINLAES ET AROMATIQUES

Attestation de participation

1er Symposium National Biomolécules & Biotechnologies
Blida, 18 - 19 Décembre 2019

Le comité d'organisation atteste que **Kahalerras Labiba** a participé aux travaux du symposium SyNaBio avec une **communication affichée** intitulée :

Le rôle protecteur de l'ail contre l'hémato-toxicité induite par l'acétate de plomb.
LABIBA Kahalerras, INES Otmani., CHERIF Abennour

La Doyenne — Directrice LRPMA — Présidente du Symposium SyNaBio

National Communications

الجمهورية الجزائرية الديمقراطية الشعبية
République Algérienne Démocratique et Populaire
وزارة التعليم العالي والبحث العلمي
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université Mohamed Boudiaf de M'Sila
Faculté des Sciences
Département des Sciences de la Nature et de la Vie

جامعة محمد بوضياف - المسيلة
Université Mohamed Boudiaf - M'sila

1er Séminaire National :

L'Apport des Biotechnologies sur la Protection de l'Environnement

Le 15-16 décembre 2019 à M'sila

ATTESTATION DE PARTICIPATION

Le comité scientifique du Séminaire atteste que :
Melle/Mme/Mr : **LABIBA KAHALERRAS**
A présenté **une communication affichée**
Intitulée: **Effet immunologique de l'extrait aqueux d'ail chez le rat wistar**

Co Auteurs : CHERIF ABDENNOUR

Le Président du Séminaire

Dr. Mouloud GHADBANE

Univ M. B. M'Sila
Faculté des Sciences / SNV
SNABPE 15-16.12.2019

Le Doyen de la Faculté des Sciences

Pr. Ettayib BENSACI

République Algérienne démocratique et Populaire

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

1er Colloque National de Biotoxicologie et Bioactivité

27 Novembre 2019

Université Oran1, Faculté SNV Oran – Algérie

Site Belgaid

جامعة وهران 1 أحمد بن بلة
Université d'Oran 1

Laboratoire BTE-BD-PR

ATTESTATION DE PARTICIPATION

Le Comité d'Organisation Certifie que

Mme/Mr: Labiba Kahalerras

A participé au Premier Colloque national de Biotoxicologie et Bioactivité, par une communication Affichée Intitulée:«Hémato-toxicological evaluation in wistar rats exposed to lead acétate and attenuated by wild garlic »

Co-Auteurs : Chérif ABDENNOUR

Président du Colloque

National Communications

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Université 20 Aout 1955 Skikda

Faculté des Sciences

Département des Sciences de la Nature et de la Vie

SNBBMEE 2019

ATTESTATION DE PARTICIPATION

Séminaire National de Biodiversité, Biologie Médicale et Ecotoxicologie Environnementale
Skikda le 30_31 octobre 2019

Le Président du Séminaire National de Biodiversité, Biologie Médicale et Ecotoxicologie Environnementale, atteste que:

Mme.: **KAHALERRAS LABIBA**

A présentée une communication ***Affichée*** intitulée

LA DETOXICATION DU PLOMB PAR L'AIL LOCAL CHEZ LE RAT WISTAR

Co-auteurs: OTMANI I, ARKOUB F.Z, ABDENNOUR C

Université 20 Aout-1955-Skikda-
Faculté des sciences
Président du SNBBMEE
Séminaire National de Biodiversité, Biologie Médicale
Et Ecotoxicologie Environnementale
SNBBMEE-2019
Dr:LAIB Messaoud

1ER SÉMINAIRE NATIONAL DE TOXICOLOGIE

"PERTURBATEURS ENDOCRINIENS : Risques et Impact sur la Santé"

Annaba les 18 et 19 Septembre 2019

ATTESTATION De Communication AFFICHÉE

Le président du séminaire atteste que :

Mme KAHALERRAS LABIBA
Université BADJI MOKHTAR, Annaba

A affiché le poster **N° 125**, intitulé :

« L'effet Protecteur c'une Plante Algérienne contre L'effet Toxique du Plomb chez Le rat male Wistar »

Co-auteurs : *OTMANI I., ARKOUB F/Z., ABDENNOUR C.*

Le Président

الأستاذ الدكتور
رشيد جعفر

National Communications

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE 20 AOÛT1955 SKIKDA
FACULTE DES SCIENCES
DEPARTEMENT DES SCIENCES DE LA NATURE ET DE LA VIE

SNBES 2018

Université 20 Août 1955 SKIKDA

Séminaire National Biologie Environnement et Santé
SNBES 2018
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ATTESTATION DE PARTICIPATION

Je soussignée Dr. Slimani S., présidente du Séminaire National Biologie Environnement et Santé SNBES 2018, atteste que **KAHALERRAS Labiba** a présenté : une communication affichée

Intitulée : « EFFET DEL'EXPOSITION CHRONIQUE AU PLOMB SUR LA REPRODUCTION ET LES COMPOSANTS SANGUINS CHEZ LE RAT WISTAR ».

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Abstract

Our study aims to evaluate the protective effect of bulbs (B) and leaves (L) of wild garlic aqueous extracts (*Allium triquetrum* L.) on the reproductive, hepatic, and renal functions induced by lead acetate (Pb) in *Wistar* rats. Simultaneously, an *in vitro* study was affected including phytochemical screening, chromatogram (HPLC), and antioxidant activity of *A. triquetrum.* The *in vivo* experiment was realized using male rats that divided into 18 groups; the control (C), the Pb (500mg/Kg BW), the positive controls of B and L at different concentrations (2, 3, 4, and 6 g/Kg BW), in addition to the mixtures of each of Pb-B (Pb-B1, Pb-B2, Pb-B3, Pb-B4) and Pb-L (Pb-L1, Pb-L2, Pb-L3, Pb-L4). After 21 days of treatment, Sperm characteristics were evaluated by CASA system, plasma testosterone, and biochemical parameters, as well as testicular, epididymal, hepatic, and renal oxidative stress markers. The phytochemical screening proved that the extracts of B and L were rich in various compounds (polyphenols, flavonoids, and tannins), while the HPLC profile demonstrated that leaves contain more rutin, and, isoquercetin. Moreover, leaves extracts showed better antioxidant activity (DPPH) than bulbs. The *in vivo* results revealed a significant decrease in the weight of testicles and epididymis, sperm concentration, motility, testosterone, velocity, vitality, round cells, total proteins, albumin, GSH, and GPx level in Pb-treated rats compared to the control, alongside a significant increase in liver and kidney weights, plasma levels of AST, ALT, ALP, urea, creatinine, uric acid, and MDA tissue. However, the co-administration of *A. triquetrum* extracts (Pb-B and Pb-L) displayed a significant difference in the levels of all previous markers compared to the Pb-group, in a dependant dose manner. In conclusion, aqueous extracts of *A. triquetrum* bulbs and leaves have the potential to attenuate the repro-toxicity, hepatotoxicity, and nephrotoxicity of Pb through the modulation of the most studied markers in *Wistar* rats.

Keywords: *Allium triquetrum*, Pb, Repro-toxicity, Hepatotoxicity, Nephrotoxicity, Protection, Rat.