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The detoxification of mercury by Algerian medicinal plants (*Urtica dioica* and *Raphanus sativus)* in Wistar rats

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ABSTRACT

Objective: The aim of this study is to evaluate the protective role of two Algerian plants (stinging nettle leaves *Urtica dioica* and red radish roots *Raphanus sativus*) against the oxidative stress induced by chronic mercury exposure. **Materials and methods:** A total of 42 males (6 groups) and 42 females *Albinos wistar* rats (6 groups) were distributed as follows: The group (1) has served as a control (0+0); group (2) has received an experimental diet containing 0.8 g $HgCl_2$/kg food (Hg+0); group (3) has received the infusion of nettle 1.5 ml/rat *per os* (UD+0); group (4) has received fresh radish juice 1ml/rat *per os* (RS+0); group (5) has received 0.8 g $HgCl_2$/kg food +1.5ml nettle infusion/rat *per os* (Hg+UD) and group (6) has received 0.8 g $HgCl_2$/kg food +1ml radish juice/rat *per os* (Hg+RS), for 30 consecutive days. Biometric, biochemical and fertility markers, in addition to reduced glutathione level (liver, kidney and testis) and the histological profiles of liver, kidney, testis and epididymis were evaluated.

Results of *Urtica dioica*:

Mercury induced negative effect on growth and organs absolute weights (liver, kidney, testis and epididymis) in the two sexes compared to controls.

Compared to the control, in both males and females, the levels of glucose, triglycerides, urea, creatinine, ALT, AST and ALP were significantly raised in the Hg group. In the latter group, the concentrations of minerals; Mg, Fe and Ca were significantly decreased. Besides, Hg+UD group has only showed raised AST activity and reduced Mg level.

Concerning the fertility markers, Hg has provoked significant decrease in the spermatozoa's concentration and motility and in plasma testosterone level as well.

Furthermore, hepatic, renal and testicular GSH concentrations have declined significantly in the Hg treated rats compared to the control. A remarkable enhancement of the fertility markers and also in the GSH level of the UD group, accompanied with normal levels of these markers in the Hg+UD group.

Histological profiles showed some hepatic and renal impairment in rats exposed to Hg. It revealed marked degeneration of most seminiferous tubules, with few sperms in the epididymis ducts. However, the Hg+UD rats have demonstrated an improved histological structure with the presence of important numbers of sperms. In addition, an increased sperms' numbers were noted in the UD supplemented rats.

Abstract

Results of *Raphanus sativus*:

Mercury disrupted rats' growth and the absolute organs weights of both males and females rats (liver, kidney, testis and epididymis).

In males and females exposed Hg, data indicated a significant increase of glucose, triglycerides, urea and creatinine levels, in addition to the activities of AST, ALT and ALP. The levels of minerals Mg, Fe and Ca were affected in the Hg group. Fortunately, fresh juice of radish supplementation has kept the levels of glucose, triglycerides, urea, creatinine, ALT, ALP and Fe within their normal biochemical ranges.

On other side, the exposure of rats to Hg caused significant decrease in the fertility markers (spermatozoa's concentration, motility and testosterone level) and in the hepatic and renal GSH content. Though, no significant difference was seen between the Hg+RS group and the control concerning the fertility markers and GSH levels. A clear enhancement of reproductive markers and testicular GSH was noted in the RS group compared to the control.

The histological profiles showed injuries of the hepatic tissues and vascular impairment accompanied by degeneration of renal glomerulus with marked testicular degeneration of the most seminiferous tubules, characterized with a few sperms in the lumen of epididymis ducts in male rats treated by the Hg. However, the supplementation of *R. sativus* has ameliorated the histological architecture of liver and kidney, especially the reproductive organs by increasing the number of sperms.

In conclusion, nettle and red radish have moderated some damaging effects of mercury on both sexes against the hepatic, the renal and the reproductive functions, in addition to boosting testicular GSH level. Their supplementations would be a natural, easy and cheap method to protect exposed individuals from adverse effects of mercury.

Key words: Mercury, toxicity, nettle, radish, fertility, oxidative stress, rat.

RESUME

Objectif: L'objectif de cette étude est d'évaluer le rôle protecteur de deux plantes algériennes (l'ortie *Urtica dioica* et le radis rouge *Raphanus sativus*) contre le stress oxydatif induit par l'exposition chronique au mercure. **Matériels et méthodes:** 42 rats mâles (6 groupes) et 42 femelles (6 groupes) de genre *Albinos wistar* de 7 rats chacun : le groupe (1) a servi comme un témoin (0+0); le groupe (2) a reçu une diète expérimentale contenant 0.8 g de $HgCl_2$/kg de nourriture (Hg+0); le groupe (3) a reçu l'infusion d'ortie 1.5 ml /rat *per os* (UD+0); le groupe (4) a reçu le jus frais de radis rouge 1 ml/rat/ *per os* (RS+0); le groupe (5) a reçu 0.8g $HgCl_2$/kg de nourriture+1.5 ml d'infusion d'ortie /rat *per os* (Hg+UD); le groupe (6) a reçu 0.8g $HgCl_2$/kg de nourriture+ 1 ml jus de radis rouge/rat per os (Hg+RS), pendant 30 jours consécutifs. Les paramètres biométriques, biochimiques et les marqueurs de fertilité, en plus, la concentration de GSH (le foie, les reins et les testicules) et les profils histologiques (foie, reins, testicules et l'épididyme) ont été évalués.

Résultats d'*Urtica dioica*:

Le Mercure a induit un effet négatif sur la croissance des rats et le poids absolu de quelques organes (foie, reins, testicules et l'épididyme) chez les deux sexes comparativement aux témoins.

En comparaison avec le témoin, chez les mâles et les femelles, la concentration du glucose, triglycérides, urée, créatinine, ALT, AST et ALP ont élevés d'une façon significative dans le groupe d'Hg. Dans ce dernier, les concentrations de minéraux; Mg, Fe et Ca ont diminué d'une manière significative. En outre, le groupe Hg+UD a seulement montré une augmentation de l'activité de l'AST et une réduction de la concentration de Mg. Concernant les marqueurs de fertilité, l'Hg a provoqué une diminution significative de la concentration et la mobilité des spermatozoïdes et la concentration de testostérone plasmatique. De plus, une diminution de GSH (foie, reins et testicules) d'une façon significative dans les rats traités par l'Hg par rapport au témoin. Une remarquable amélioration des marqueurs de fertilité et le taux de GSH ont été observés dans le groupe d'UD avec des niveaux normaux dans le groupe Hg+UD. Les profils histologiques ont montré une certaine déficience glomérule rénal et hépatique chez les rats exposés au mercure. Il a révélé une dégénérescence marquée dans la plupart des tubules séminifères, avec un peu de spermatozoïdes dans les conduits épididymaires. Toutefois, le groupe d'Hg+UD a démontré une amélioration de la structure

histologique avec la présence d'un nombre important des cellules spermatiques. En plus, une augmentation de nombre des spermatozoïdes a été noté dans le groupe traite par UD.

Résultats de *Raphanus sativus* :

Le Mercure a perturbé la croissance des rats et le poids absolu des organes (foie, reins, testicules et épididymes) chez les mâles et les femelles. Dans le groupe d'Hg, chez les deux sexes, les données ont montré une augmentation significative du glucose, triglycérides, urée et créatinine, et les activités d'AST, ALT et ALP. Les concentrations des minéraux Mg, Fe et Ca ont été affectés dans le groupe traité par Hg. Heureusement, le jus de radis rouge a maintenu le taux du glucose, triglycérides, urée, créatinine, ALT, ALP et Fe dans leurs valeurs biochimiques normaux. D'une autre côté, l'exposition des rats à Hg a provoqué une diminution significative des marqueurs de fertilité (la concentration et la mobilité des spermatozoïdes, ainsi que le taux de testostérone) et le taux de GSH hépatique et rénale. Cependant, aucune différence significative n'a été observée entre Hg+RS et le témoin concernant les marqueurs de fertilité et le taux de GSH. Une amélioration remarquable des marqueurs de reproduction et GSH testiculaire a été notée dans le groupe RS par rapport au témoin. Les profils histologiques ont montré une dégénérescence des tissus hépatiques et une altération vasculaire accompagnée d'une dégénérescence du glomérule rénal avec un déclin testiculaire des tubules séminifères, complété avec un peu de spermatozoïdes dans les conduits épididymaires chez les rats mâles traités par l'Hg. Toutefois, la supplémentation de *R. sativus* a amélioré l'architecture histologique du foie et des reins, notamment les organes reproducteurs en augmentant le nombre de spermatozoïdes.

En conclusion : L'ortie et le radis rouge ont modéré certains effets nocifs du mercure chez les deux sexes contre l'insuffisance dans les fonctions hépatique, rénale et les marqueurs de reproduction, en plus, de stimuler le taux de GSH testiculaire. Leurs dispositions complémentaires seraient un moyen facile, naturel et utile pour défendre quiconque exposé au mercure de ses effets toxiques.

Mots clés:

Mercure, toxicité, ortie, radis, antioxydant, stress oxydatif, rat.

الملخص

يهدف هذا العمل إلى تقييم الدور الوقائي لاثنتين من النباتات الجزائرية (القراص *Urtica dioica* و الفجل الأحمر *Raphanus sativus*) ضد الجهد التأكسدي الناجم عن التعرض المزمن للزئبق. **المواد والطرق**: وزع 42 ذكرا (6 أفواج) و 42 أنثى (6 أفواج) جرذ من نوع ويستار كما يلي: فوج (1) كان بمثابة شاهد (0+0)؛ تلقى فوج (2) نظاما غذائيا يحتوي على 0.8 غ كلوريد الزئبق/كلغ غذاء (0+Hg)؛ و تلقى فوج (3) نقيع نبات القراص بمعدل 1.5 مل/جرذ عن طريق التزقيم (0+UD)؛ في حين أعطي فوج (4) عصير الفجل الأحمر الطازج بمعدل 1مل/جرذ عن طريق التزقيم (0+RS)؛ كما أعطي فوج (5) 0.8 غ كلوريد الزئبق/كلغ غذاء + 1.5 مل من نقيع القراص/جرذ (UD+Hg)؛ وقد تلقى فوج (6) 0.8 غ كلوريد الزئبق/كلغ غذاء + 1 مل من عصير الفجل/جرذ (Hg+RS)، لفترة دامت 30 يوما متتالية. قيست المعايير البيو مترية، البيوكيماوية ومؤشرات الخصوبة، بالإضافة إلى مستوى الجلوتاثيون (الكبد، الكلى والخصية) والدارسات التشريحية (الكبد ،الكلى ،الخصية والبربخ).

نتائج القراص:

أحدث الزئبق أثارا سلبية على نمو الجرذان وعلى الوزن المطلق للأعضاء (الكبد، الكلى، الخصية والبربخ) مقارنة بالشاهد في كلتا الجنسين.

مقارنة بالشاهد، ارتفع مستوى الجلوكوز، الدهون الثلاثية، اليوريا، الكريتيانين، ناقلات الأمين (ALT, AST)، والفوسفتاز القاعدي (ALP) بشكل كبير عند ذكور وإناث الجرذان المعرضة للزئبق. عند هذا الأخير، سجل انخفاض في تركيز المغنيسيوم، الحديد والكالسيوم بشكل ملحوظ. بالإضافة إلى ذلك، فقد اظهر فوج الزئبق+قراص ارتفاعا في نشاط الانزيم ناقل الأمين (AST) وانخفاضا في تركيز المغنيسيوم فقط.

فيما يتعلق بمؤشرات الخصوبة، فقد أحدث الزئبق انخفاضا كبيرا في تركيز و حركة الحيوانات المنوية، وكذلك في تركيز هرمون تستوستيرون. بالإضافة إلى ذلك، فقد انخفض مستوى الجلوتاثيون (الكلى، الكبد والخصية) بشكل كبير في الجرذان المعالجة بالزئبق مقارنة بالشاهد. كما لوحظ وجود تحسن في مؤشرات الخصوبة وكذلك في مستوى جلوتاثيون كل الأعضاء لدى الجرذان المعالجة بالقراص فقط، مصحوبة بمعدلات طبيعية لهذه المؤشرات عند تلك التي أعطيت معدن الزئبق وأوراق القراص معا.

أظهرت الدراسات التشريحية تلف في نسيج الكبد والكلى مع تخريب للأوعية الدموية مصحوبا بتدمير للكبيبات الكلوية لدى الجرذان المعرضة للزئبق. كما لوحظ تدهورا ملحوظا في معظم الأنابيب المنوية، مع عدد قليل من الحيوانات المنوية في قنوات البربخ. ومع ذلك، أظهرت الجرذان المعرضة للزئبق + قراص تحسنا في تركيب الأنسجة مع وجود عدد كبير من الخلايا المنوية. بالإضافة إلى ذلك، لوحظت زيادة في عدد الحيوانات المنوية في المجموعة التي تلقت المعالجة بالقراص لوحده.

نتائج الفجل:

سبب الزئبق اضطرابا على نمو الجرذان والوزن المطلق للأعضاء (الكبد، الكلى، الخصية والبربخ) عند كلتا الجنسين.

الملخص

أظهر الفوج المعالج بالزئبق عند الذكور والإناث ارتفاعا كبيرا في معدل الجلوكوز، الدهون الثلاثية، اليوريا والكريتيانين، إلى جانب زيادة في نشاط الانزيمات (AST، ALT و ALP). في حين سجل انخفاضا ملحوظا في تركيز معدن المغنيسيوم، الحديد والكالسيوم لدى الجرذان المعاملة بالزئبق. لحسن الحظ، حافظ عصير الفجل الأحمر على مستويات الجلوكوز، الدهون الثلاثية، اليوريا، الكريتيانين، ALT، ALP والحديد عند المعدلات الحيوية الطبيعية.
من ناحية أخرى، سبب تعرض الجرذان للزئبق انخفاضا كبيرا في مؤشرات الخصوبة (تركيز وحركة الحيوانات المنوية ، وكذلك هرمون تستوستيرون) وفي معدل الجلوتاثيون الكلوي والكبدي. ومع ذلك، لم يلاحظ أي فرق بين المجموعة المعالجة بالزئبق + الفجل الاحمر والشاهد فيما يخص مؤشرات الخصوبة و الجلوتاثيون. وقد لوحظ تحسن كبير في مؤشرات الخصوبة و جلوتاثيون الخصية في الفوج الذي تلقى الفجل الأحمر لوحده مقارنة بالشاهد.
أظهرت الدراسة النسيجية تلفا لأنسجة الكبد مع تخريب للأوعية الدموية مصحوبا بتدميرالكبيبات الكلوية وتلف لمعظم الأنابيب المنوية للخصية، مع عدد قليل من الحيوانات المنوية في تجويف قناة البربخ للجرذان المعرضين للزئبق. ومع ذلك، فان إضافة عصير الفجل ادى الى تحسن البنية النسيجية للكبد والكلى، خاصة الأعضاء التناسلية عن طريق زيادة عدد الحيوانات المنوية.
الاستنتاج: أمكن لنباتي القراص والفجل الأحمر أن يقللا من بعض الآثار الضارة للزئبق عند كلتا الجنسين على الوظائف الكبدية والكلوية ومؤشرات الخصوبة، بالإضافة إلى تحفيز جلوتاثيون الخصية. إن إضافة هاتين النبتتين يمكن أن تكون وسيلة طبيعية، سهلة و مفيدة لحماية الأشخاص ضد التأثيرات السامة للزئبق.

الكلمات الدالة: زئبق، سمية، قراص، فجل، خصوبة، جهد تأكسدي، جرذ.

LISTS OF FIGURES

LIST of TABLES

LIST OF CONTENTS

Chapter II : Materials & Methods

Chapter III : **Biochemical markers**

Chapter IV: **Reproductive profile**

LIST OF ABREVIATIONS

ALP	:	**Alkaline phosphatase**
ALT	:	**Alanine aminotransferase**
ANOVA	:	**Analysis of variance**
AST	:	**Aspartate aminotransferase**
BSA	:	**Bovine serum albumin**
Ca	:	**Calcium**
DTNB	:	**5,5′-Dithiobis (2-nitrobenzoic acid)**
EDTA	:	**Ethylene diamine tetraacetic acid**
Fe	:	**Iron**
Fe^{+2}	:	**Ferrous ions**
Fe^{+3}	:	**Ferric ions**
GSH	:	**Reduced glutathion**
H_2O_2	:	**Hydrogen peroxide**
Hg	:	**Mercury**
$HgCl_2$	:	**Mercury chloride**
LDH	:	**Lactate dehydrogenase**
Mg	:	**Magnesium**

NaCl : **Sodium chloride**

NADH : **Nicotinamide adenine dinucleotide**

OH : **Hydroxyl radical**

RS : ***Raphanus sativus***

TBS : **Tris-buffered saline**

UD : ***Urtica dioica***

INTRODUCTION

INTRODUCTION

In latest years, there is a growing health concern about several effects of chronic exposure to various environmental contaminants such as trace metals, which arise from different sources, especially the anthropogenic activities. Trace metals are considered to be the most hazardous substance which could enter into the body of both animals and humans by many ways.

In Algeria, Hg used to be refined in "Mercurial complex of Azzaba", which produces mercury from rocks found in the form of HgS. The production of Hg was up to 900t/year during the eighties. The operations of rocks' crushing and grinding resulted in contamination of the environment, and exposing workers to the risk of poisonings (**Abdennour *et al.*, 2002**). The levels of mercury found in the groundwater of the region are very high and exceed the standard range 1µg/L, thereby demonstrating a contamination of water bodies in the surrounding of the factory (**Benhamza, 2007**) where it getting up to 170µg/l (**Radji, 2006**).

Additionally, many medicinal uses of Hg including various medications, creams, teething powders, cosmetics and paints (**Guzzi *et al.*, 2008**). Besides, mercuric chloride acted as a corrosive sublimate for treatment of syphilis, from its first appearance in the West during the 15^{th} century up to World War **II** (**O'Shea *et al.*, 1990**). Moreover, in agriculture Hg is used as a pesticide, fungicide, dental amalgams and vaccines and in chemistry as a mediator in production of other mercury compounds (**Ratcliffe *et al.*, 1996).**

Mercury has been regarded as a priority pollutant by many international agencies **(Wang *et al.*, 2004**), because it is widely used in different fields of human life. It has been known that mercury toxicity could provoke neurological, digestive, heamatological, renal, respiratory, immune, and reproductive disorders, which are dependent upon the dose, the chemical form and the exposure route (**Bridges and Zalups, 2010).** In fact, mercury has a high affinity and a stable complex to sulfhydryl (SH) groups and other biomolecules which might disturb some structures as enzymes **(Omotayo *et al.*, 2011)** and metabolic processes **(Wiggers *et al.*, 2008)**. Consequently, oxidative stress was proposed as one of the most mechanisms of Hg pathological exposure **(Clarkson, 1997)**.

Mediterranean region for centuries is very rich in traditional methods by the use of medicinal plants for treating various common illnesses (**Saad *et al.*, 2006**). In some developing countries 70–95% of the populations rely on these traditions for the first care (**Robinson and Zhang, 2011**).

In Algeria as in all countries of the Maghreb, plants are used mainly by the elderly who are still using traditional folk medicine which remains the major source to cure minor and serious ailments (**Reguieg, 2011).** The rapid growth of medicinal plants utilisation is due to several factors, including their availability, cheap or free and also herbal products are safe and effective.

Many therapeutic properties such as neuroprotective, cardioprotective, chemoprotective, anti-carcinogenic, hepatoprotective and anti-inflammatory (**Campanella *et al*., 2003; Visioli *et al*., 2000**), antistress, growth promotion, appetite stimulation and immunostimulation activities (**Citarasu *et al*., 2010**) have been qualified to herbal preparations. However, healing plants are rich in antioxidants that may be used to improve human body, in particular by reducing or suppressing active oxygen species and free radicals (**Gülçin *et al*., 2002**).

The objectives

The objectives of this study is to evaluate the therapeutic efficiency of nettle ***Urtica dioica*** and red radish ***Raphanus sativus***, local natural plants, to test their protective roles against chronic mercury intoxication. Nettle is generally unusable by the majority of the population due to the ignorance of the nutritional and healing properties. Although it is widely distributed across northern Algeria, nettle is used only by some people, especially the elderly, who know its medicinal benefits for long time.

Red radish is characterised by an exceptional tastes and aromas which has an important nutritious and health benefits. It is also available in the market, especially during the cold season, but its consumption is very limited.

In this study, male and female *Wistar* rats were chronically intoxicated by inorganic mercury ($HgCl_2$) and supplemented with ***U. dioica*** and ***R. Sativus*** in an attempt to detoxify this metal. For this reason, the following markers have been evaluated:

- Biochemical markers (glucose, triglycerides, urea, creatinine, AST, ALT, ALP, Mg, Fe, Ca and glutathione);
- Reproductive markers (testosterone and sperm concentration and motility);
- Histological profile of liver, kidney, testis and epididymis.

Such biomarkers are generally assayed in order to know the health status of the organism and in particular the functions of vital organs and tissues as that of blood, liver, kidneys, testes and epididymis.

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CHAPTER I:
LITTERATURE REVIEW

Mercury pollution

Industrial revolution has increased global atmospheric levels of mercury up to 300%. Mercury is distributed into the atmosphere from natural sources, one third from degassing of the earth's crust, and from industrialized sources, two thirds primarily from the burning of coal (**WHO, 2003**). Due to atmospheric transport Hg is deposited locally and globally, in soil and water. Mercury undergoes a series of complex chemical and physical transformations as it cycles between air, sol, and water. Humans, plants, and animals are daily exposed to Hg and accumulated during this cycle; named 'the Hg cycle' (**Mostafalou and Abdollahi, 2013**). Upon deposition, terrestrial and aquatic microbes transform elemental and inorganic Hg into a methylated, organic form that is extremely absorbable through ingestion. The organic form, methyl Hg (CH_3Hg), is 100 times more poisonous than inorganic Hg **(NRC, 2000).** Mercury from the environment entering human body comes from the food chain, principally from the ingestion of fish. Other major sources include natural gas, crude oils, the refining of petroleum products, sewage treatment facilities, batteries, light bulbs and thermometers **(Ngim *et al*., 1989; Ratcliffe *et al*., 1996).**

Global anthropogenic emissions of Hg are estimated to range between 2000 and 6000 metric tons per year. China alone is believed to emit about 1000 tons of Hg annually. The only long-term sink for removal of Hg from the biosphere is thought to be deep-sea sediments (**Clarkson, 1997; Goldman and Shannon, 2001**).

Mercury has a unique physical (density, liquid states at environmental temperatures and volatility) and chemical (ease of reduction) properties, which make it a useful industrial reagent. It is true, that Hg may cause many problems when it exceeds the safe limit. Mercury has been traditionally used by Chinese since 1000 years before the birth of Christ as the red dye pigment vermilion, moreover, in the Greco-Roman world. Since then its toxicity has become well famous in metal workers, dyers and paint manufacturers.

For thousands of years, Hg has been playing a positive role in the field of medicine and dentistry in a variety of therapeutics as pharmaceuticals containing mercuric chloride such as antiseptics and disinfectants (**Merck Index, 1983).**

On the basis of toxicological characteristics, there are three forms of mercury; elemental mercury (liquid and vapor), inorganic mercury (as $HgCl_2$) and organic compounds (CH_3Hg methyl mercury and ethylmercury found in vaccine preservative).

The metallic element (Hg^0) is a liquid metal at normal ambient temperatures and pressures. Most of the mercury encountered in the atmosphere is elemental mercury gas (**Clarkson, 2002; Mousavi *et al.*, 2011**). Though, dimethyl mercury was revealed to be the final product in the aquatic bacterial methylation of mercury and is the most toxic form of mercury **(Wood *et al.*, 1968).**

CH_3CH_3Hg	CH_3Hg	Hg^{++}	Hg
Dimethyl Mercury	Methyl Mercury	Mercuric Ions	Elemental (atomic)
Lipid soluble	Lipid Soluble	Water Soluble	Liquid metal/ Lipid Soluble

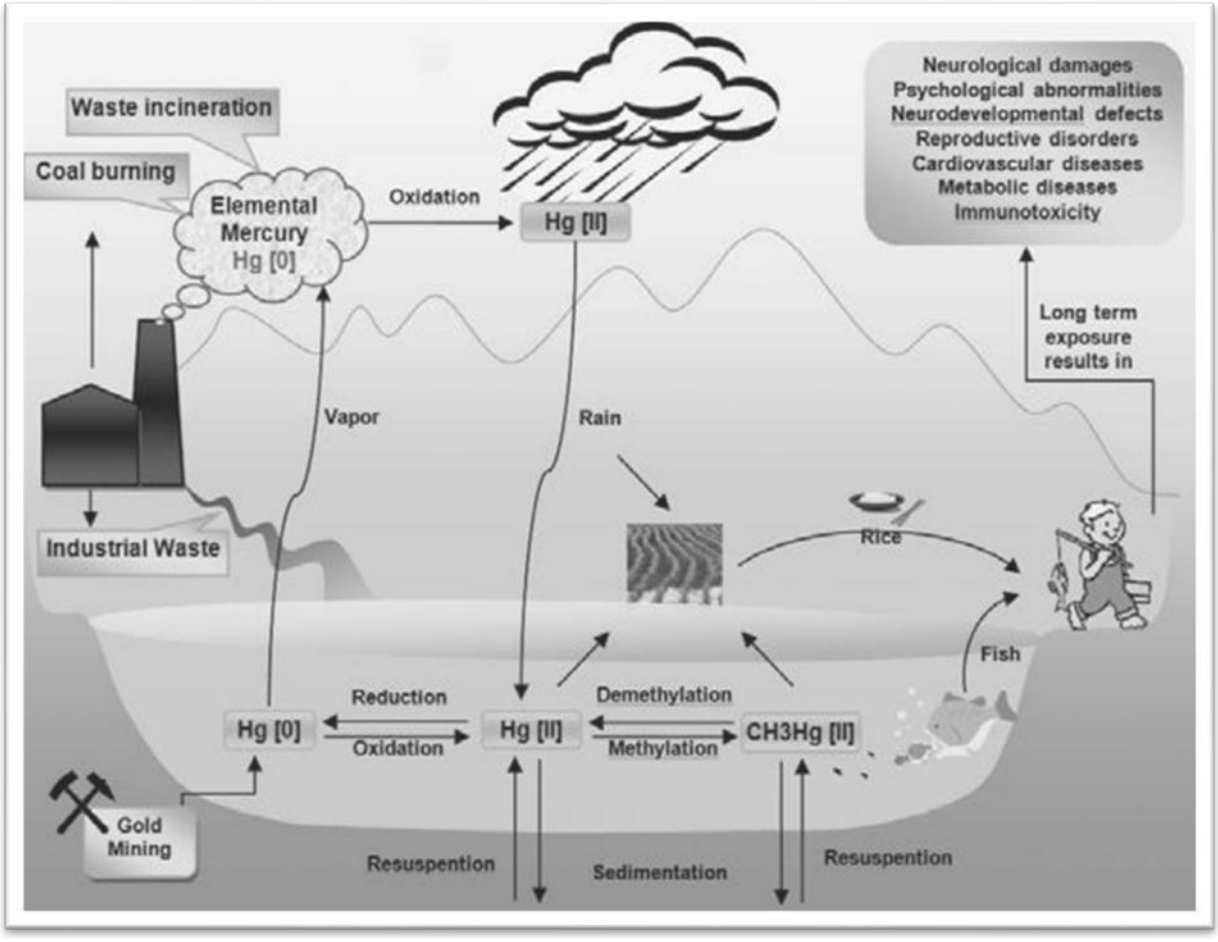

Figure N°01. Schematic view of Hg environmental recycling from the atmospheric emission, deposition, exposure and bioaccumulation **(Mostafalou and Abdollahi, 2013).**

Mercury entry to the body is mainly by digestion of mercury salts from herbal remedies, or, through the skin as mercury salts which used for their antiseptic, fungicidal and bactericidal properties (**WHO, 2003**). In addition, the utilisation of skin lighteners would result in a significant exposure due to its dermal absorption (**IPCS, 1991**).

Following inhalation, the level of absorption of inorganic mercury aerosols depend on particle size but on average, approximately 10 % is absorbed, as most particles will be found in the respiratory system (**IPCS, 1991; WHO, 2003)**.

Mercury poisoning could happen via inhalation, ingestion, or absorption through the skin. The main route of inorganic mercury exposure is through ingestion (**WHO, 2003**). In humans, approximately 5–10 % of inorganic mercury in food is absorbed after ingestion (**DEFRA, 2002**).

Mercury intoxication causes some serious illnesses and may influence different organs (**Sener *et al*., 2003; Holmes *et al*., 2009**), depending upon the dose, the chemical form, the exposure route (**Bridges and Zalups, 2010; Hong *et al*., 2012**), and the period of exposure. A recent study showed that mercurial compounds would readily cross the placental barrier and the blood–brain barrier, damaging the developing brain of the foetus (**Christinal and Sumathi, 2013**).

The accumulation of Hg reaches the highest concentration (about 85-90%) in the kidneys followed by the liver. Thus, the kidney is the critical target organ for chronic mercuric chloride poisoning (**Emanuelli *et al*., 1996; Clarkson, 1997**), which can be taken up by the proximal tubules (**Verma *et al*., 2010; Parket *et al*., 2012**), bound to metallothionein (**Piotrowski *et al*., 1974; Cherian *et al*., 1978**). The second largest depot of mercury occurs in the liver, resulting in severe hepatic damages (**Wadaan, 2009**). Inorganic mercury is complexed with glutathione in the liver and secreted in the bile as a glutathione-mercury complex or cysteine mercury, and lesser amounts is found in epithelial tissues, and testes. Furthermore, $HgCl_2$ may combine with plasma proteins or enter into red blood cells, then it distributes to all tissues. Hg content of hair, blood and nail are correlated well with the Hg recent exposure (**NRC, 2000**). Hair, nail and have high sulfhydryl groups and mercury has high tendency to bind sulfur.

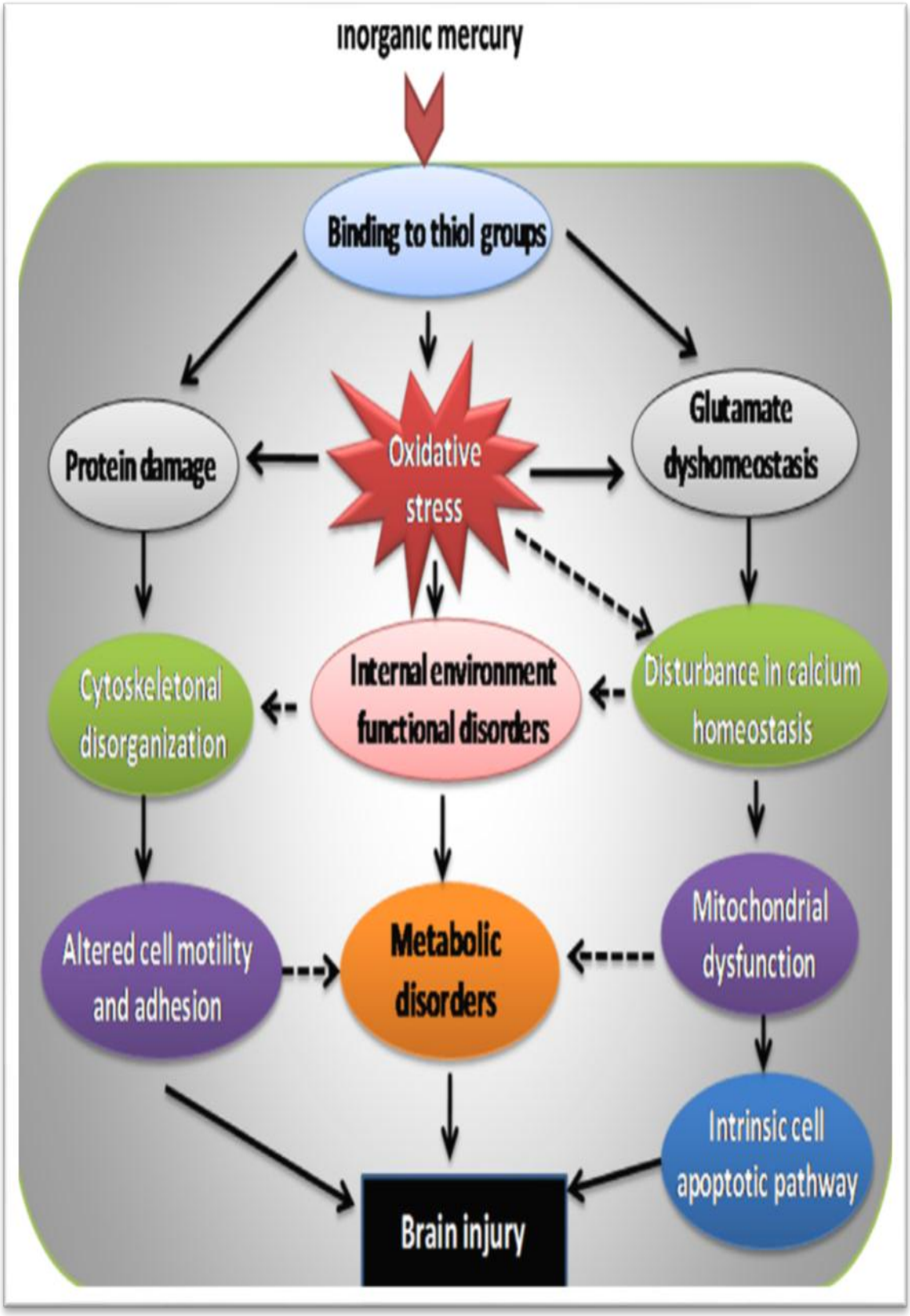

Figure N° 02. The proposed scheme illustrating cellular events resulting from inorganic mercury toxicity **(Yuyu *et al*., 2015).**

Reactive oxygen species (ROS) such as superoxide is well known to increase in the intracellular following exposure to Hg (**Bando *et al*., 2005**). Mercury has a great affinity for SH groups of endogenous biomolecules such as a various enzymes, amino acids, and glutathione (GSH), as a result, Hg has bound to any free thiol available and the thiol in the highest concentration will be the most frequently bound (**Divine *et al*., 1999**), which comprises about 10% to 50% antioxidant capacity of both membrane and plasma proteins (**Salonen *et al*., 2000**). The reaction rate is almost instantaneous (**Clarkson, 2002**). In other words, higher concentrations of thiols appear to protect against mercury accumulation, both *in vivo* and *in vitro* (**Divine *et al*., 1999**). Glutathione is the most common low molecular weight sulfhydryl-containing compound in mammalian cells, present in millimolar amounts in most cells (**Sen, 2003**).

Glutathione (GSH) is known to interact with Hg causing the depletion of the former (**Zalups and Lash, 1997; Gatti *et al*., 2004**), and can bind to the cysteine residues of certain enzymes, inhibiting their activity (**Frasco *et al*., 2007**), which induce biochemical damage to tissues that provoke an oxidative stress (**Perottoni *et al*., 2004**) and causes severe oxidative changes **(Hansen *et al*., 2006)**, furthermore, Hg played toxic health effects to tissues and genes through diverse mechanisms such as interruption of microtubule formation, changing intracellular calcium balance, disrupting membrane potential, troubling or inhibition of enzymes, inhibition of protein and DNA synthesis and disturbing immune functions (**Yee *et al*., 1996**), mitochondrial damage, lipid peroxidation (**NRC, 2000**), oxidation of membrane lipids can lead to the loss of cellular or organelle membrane integrity which can eventually result in cell death and pathological injury (**Girotti, 1998**). As consequence, the reactive oxygen species (ROS) generated by exposure to the mercurial forms may be one of the causes of the toxic effects (**Hussain *et al*., 1997; Shanker *et al*., 2005**).

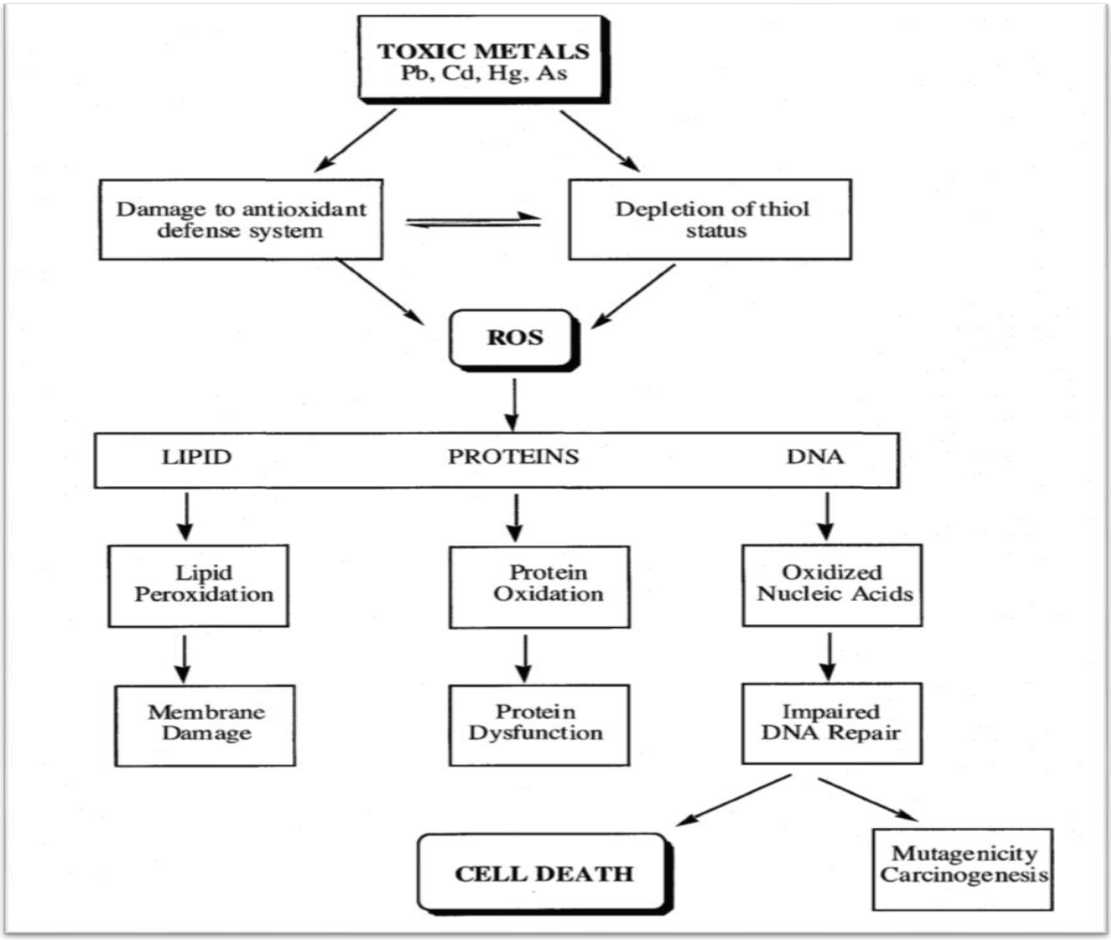

Figure N° 03. Possible mechanisms for metal-induced oxidative stress (**Ercal *et al.*, 2001**).

The elimination of mercury may be achieved mainly through urine, faeces, or expired air although a number of other, minor routes exist (**ATSDR, 1999**) with a body burden half-life of approximately 1-2 months (**Clarkson, 1989; WHO, 2003**), although significant amounts are shed through sweat, tears, breast milk, and saliva (**Berlin *et al.*, 2007**). Following an acute exposure to mercuric chloride, the half-life elimination from urine was estimated to be 25 days (**Suzuki *et al.*, 1992**). Approximately 60-75% of absorbed Hg was excreted as sulfhydryl mercury compounds, primarily with cysteine and little if any metallic mercury was in the urine (**Winship, 1985**). Urinary excretion involves active tubular transport and glomerular filtration, which is probably passive (**Berlin, 1986**).

The form of Hg found in the faeces is predominantly inorganic form. Intestinal flora can convert organic mercury to inorganic, which then promotes its faecal excretion (**Rowland *et al.*, 1980**). Half-lives appear to be multiphasic, as with metallic mercury, with human studies suggesting an effective half-life of 42 days for 80% of an oral dose; the other 20% did not appear to have a measurable rate of excretion (**Rahola *et al.*, 1971)**.

Elimination of inorganic Hg from the blood and brain is a biphasic process such as an initial rapid elimination phase followed by as lower phase. Inorganic mercury may also be reduced to form elemental mercury, which is exhaled as elemental mercury vapour or excreted in the breast milk (**WHO, 2003**). Mercury vapor absorbed via the lungs converted to divalent mercury (Hg^{2+}) in tissues and excreted in bile as glutathione conjugates, which are after eliminated in faeces (**Custodio *et al*., 2005**).

The half-lives of elimination of the various forms are influenced by species, strain, dose and sex. Age is also an important factor on body burden, with neonates and children showing greater rates of absorption and retention. Body half-life estimates have been made of 70–80 days for MeHg, 58 days for elemental mercury and 1–2 months for inorganic forms (**NRC, 2000**).

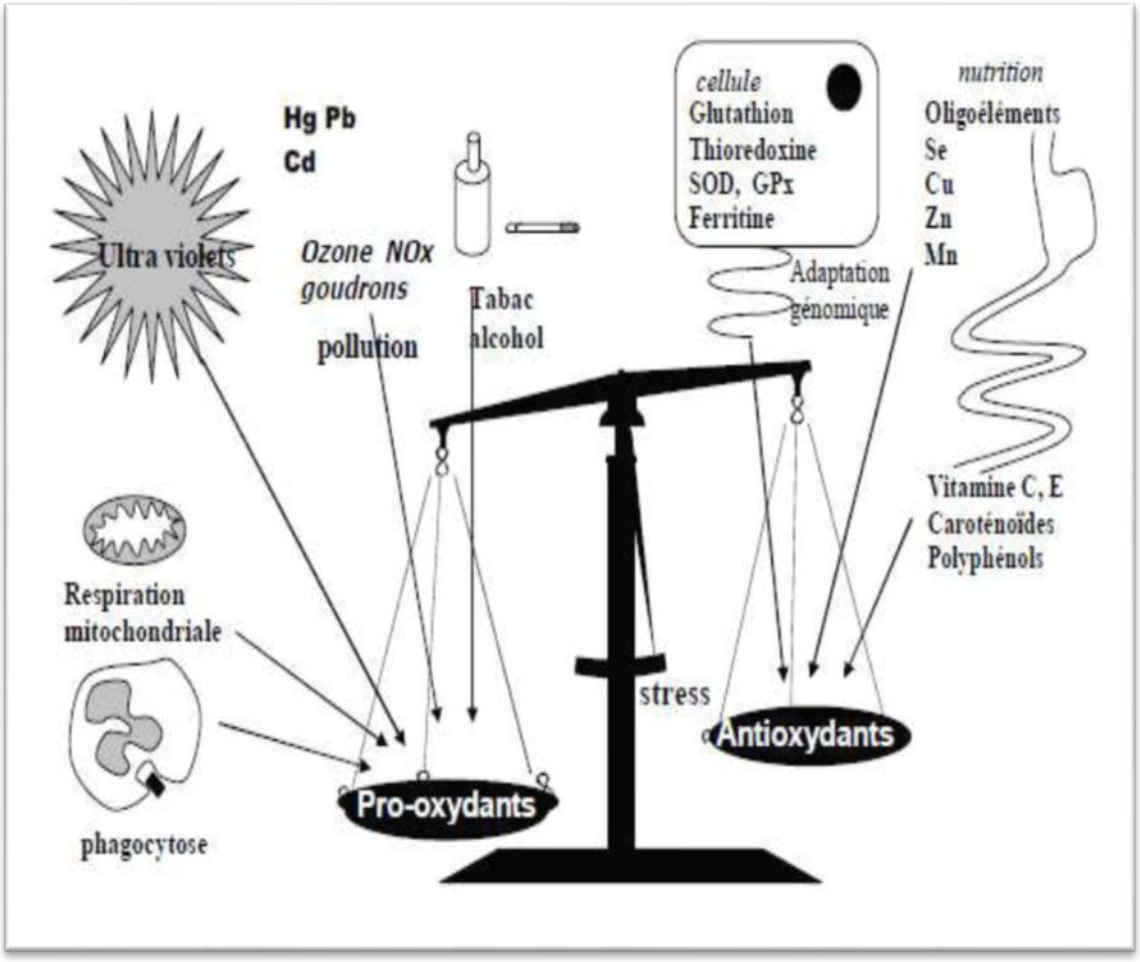

Figure N°04: The balance between antioxidant and pro-oxidant (**Favier, 2006**).

Immuno-dysregulation by inorganic mercury might provoke either auto immunity or immuno-suppression depending on the genetic of rat strains tested **(Robert *et al*., 1995)**. Immune dysfunctions include hypersensitivity reactions to mercury exposure, as asthma, dermatitis, various types of autoimmunity, suppression of natural killer cells (**Ilback *et al*., 1991**) and disruption of various other lymphocyte subpopulations (**Berlin *et al*., 2007).** A direct interaction between the immune system and Hg exposure leads to the reduction of white blood cells' activation (**Gallagher *et al*., 1995**). Even at subacute, chronic exposure, immune-modulatory effects of mercury were seen (**Hemdan *et al*., 2007**).

Acute toxicity of Hg could affect the different systems including the kidneys, gastro-intestinal tract, lungs, nervous and immune systems (**EU, 2007**). Clinical symptoms of acute intoxication include swelling of the salivary glands, stomatitis, loosening of the teeth, nephritis, and hepatitis occur (**Stockinger, 1981**).

Ingestion of mercuric chloride can cause jaundice, an increase in liver enzymes (**WHO, 2003**), histopathological and ultrastructural lesions of liver evidenced by degeneration and cell necrosis (**El-Shenawy and Hassan, 2008**).

Coronary heart disease, myocardial infarction, hypertension (**Houston, 2011**) and tachycardia, following the ingestion of inorganic mercury were reported (**ATSDR, 1999**).

Inhalation of mercury vapours and contact with mercurous chloride in teething powders and ointments can be the reason of acrodynia, a pink discolouration of hands and feet frequently accompanied by insomnia, irritability, and light sensitivity (**WHO, 2008**).

Acute oral poisoning causes gastroenteritis and colitis, and then damages the kidney (**Asano *et al*., 2000**), this may produce death, and surviving patients commonly develop renal tubular necrosis with anuria (**Barnes *et al*., 1980)**. Kidneys are particularly susceptible to mercury salts resulting in different alterations such as acute renal tubular necrosis and autoimmune glomerulonephritis (**Tchounwou *et al*., 2003**).

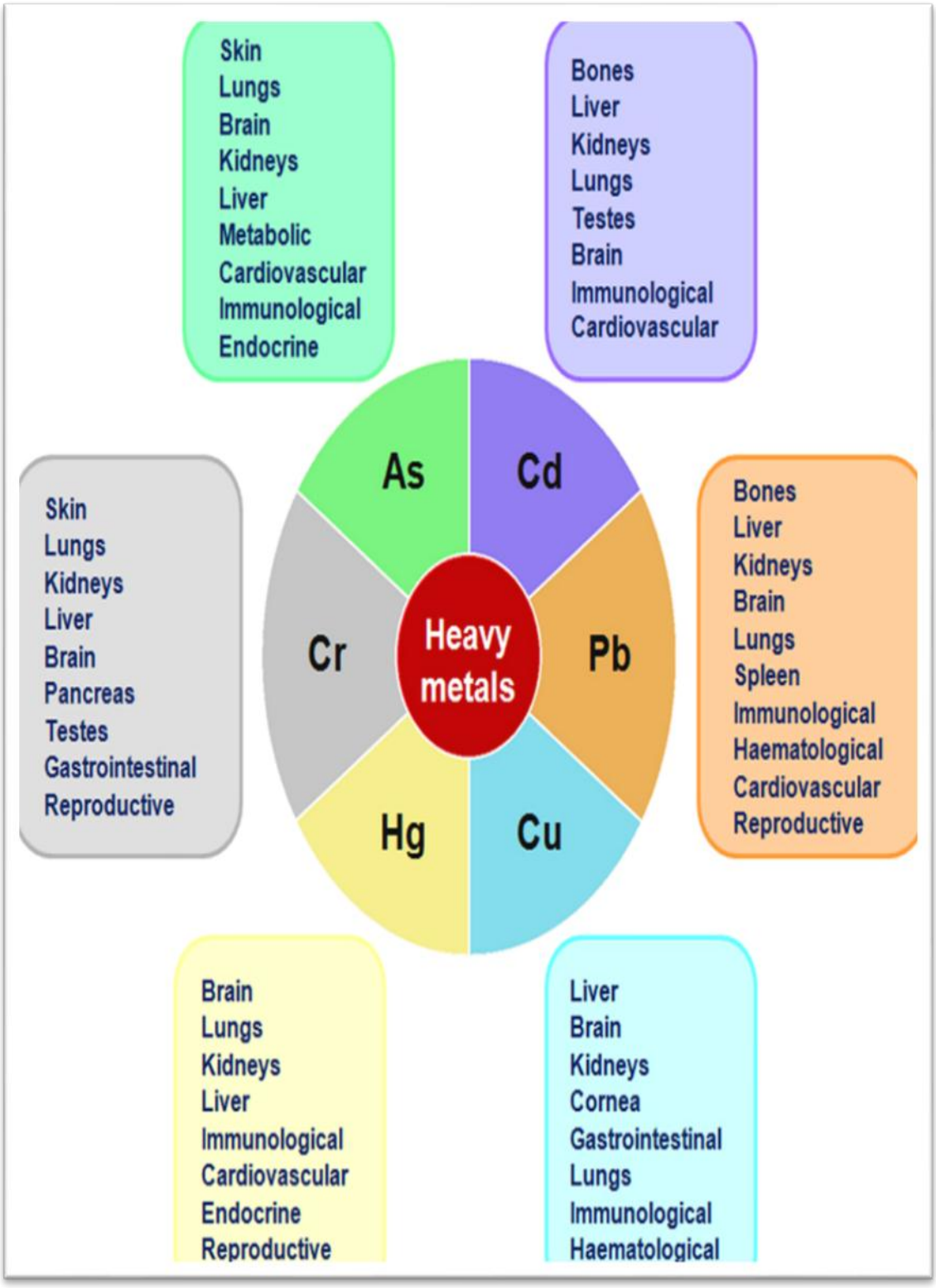

Figure N° 05. Main organs and systems affected by environmental and occupational exposure to heavy metals (**Garcia-Nino and Pedraza-Chaverri, 2014**).

Chronic exposure of humans to elemental or inorganic mercury might be experienced in some occupational situations which include damage to the central nervous system, progressive anaemia, gastric disturbance, excessive salivation and metallic taste in the mouth (**Boadi *et al*., 1992**). Chronic exposure of chloralkali workers and mercury miners has also been suggestive of cardiovascular toxicity (**Kobal *et al*., 2004**).

Nervous system is known to be the most sensitive target organ, as results, it would produce neurological problems including tremors, abnormal irritability or responsiveness to stimulation, emotional ability, insomnia, memory loss, neuromuscular changes, headaches, polyneuropathy, and deficits in cognitive and motor function tests (**WHO, 2008**). Furthermore, chronic inorganic Hg salts intoxication may lead to development of lip tremor, tongue, severe salivation, losing teeth, anorexia, and weight loss (**Nelson *et al*., 2011**).

Reproduction of animals was affected when treated chronically with mercuric chloride over 3 months, where it showed an alteration in testicular tissue and a decrease in spermatogenic cell counts (**IPCS, 1991; Rao *et al*., 2001; Abdennour *et al*., 2011**). In the same way, dentists exposed to metallic mercury have spontaneous abortions, stillbirths, congenital malformations and irregular, painful or haemorrhagic menstrual disorders (**Sikorski *et al*., 1987**). Furthermore, prolongation of the oestrous cycle as seen in animals exposed to mercury (**Baranski and Szymczk, 1973).** Also, exposure of mice to $HgCl_2$ throughout pre-mating; mating and gestation periods caused marked effects on fertility and pup survival indices (**Khan *et al*., 2004**). Mercury concentrations in cord-blood correlate also well with that of foetal-brain during the third trimester (**NRC, 2000**).

Herbal medicine

Medicinal plants are an essential resource of healthiness to humanity since the oldest time (**Ladeji *et al.*, 2003**). According to World Health Organization, traditional medicines, including herbal medicines have been, and continue to be, used in every country around the world. Complementary and Alternative Medicine (CAM), including herbal remedies are used throughout the world and almost civilizations which represented the original sources of the greatest treatments and drugs (**Cooper, 2005; Tsao, 2005**).

Arabic medicine has contributed greatly to the advancement of modern medicine in Europe and remains one of the principle sources of western medicine (**Azaizeh *et al.*, 2006**). Traditional Arab-Islamic medicine is still being plasticised by the Mediterranean as well as most Arab and Islamic countries. However, the recent form of Arab herbal medicines has historical origins in Greco-Arab and Islamic medicine, which was the leader in the golden age (seventh to fifteenth century) of the Islamic civilization. In general, Medicine and pharmacology are considered to be one of the most illustrious and best known faces which Arabs most shined (**Oumeish, 1998; Saad and Said, 2010; Saad, 2011**).

The plant kingdom has provided an endless source of medicinal plants first used in their simple forms as herbal teas, syrups, infusions, ointments, liniments and powders.
In human body, the chemical constituents of medicinal plants interact directly or indirectly with the body chemistry. Once the active constituents are absorbed into the blood stream, these constituents circulate and affect the blood system to spring the essential benefits and hold off the waste as well as free radicals bounded to the fibres of the plants and, as consequence, purify valuable contents into body cells.
Depending on the type of plants, one part including (flowers, leaves, branch or roots) or whole sectors (aerial parts or roots) with their unending advantages with healing properties could be expected to be used widely for the treatment of acute or chronic diseases, food products, synthesis of beneficial drugs and nutritional improvement, moreover, ensure the psychological health.

Health benefits of herbal teas of many plants are rich in phenolic compounds, which are confirmed to apply an important antioxidant activity and are suggested to be involved in human diet as health promoters (**Seeram *et al.*, 2001; Kim *et al.*, 2005; Piccolella *et al.*, 2008; Khoo *et al.*, 2011**).

Stinging nettle

Stinging Nettle known as *Urtica dioica* (Urticaceae) is a wild-growing, annual and perennial herb belongs to the family of *Urticaceae* and is originating in Eurasia; the nettle has spread in all temperate regions of the world. It is found more in Northern Europe than in southern Europe, North Africa, Asia and widely distributed in North and South America (**Borchers *et al*., 2000**). It grows in nitrogen-rich soils between 0 and 1800 m (**Pignatti *et al*., 1982**) and temperate zones. Raw material of the nettle such as herbs (*Urticae herba*), leaves (*Urticae folium*), and roots (*Urticae radix*) are recommended as helper supportive treatment for many diseases and is one of the valuable plants used in phytotherapy as both monotherapy and in combination therapy.

The nettle is one of the few plants that we can identify with closed eyes. Regarded as a "bad grass", it is in reality a plant rich in vitamins and minerals and is equipped with many virtues. Its use is multiple sections; it is used in agriculture, food, cosmetics, dyeing the textile industry and for medicinal purposes (**Bertrand and Jeanne, 2008**).

U.dioica has been frequently consumed by humans for medicinal purposes. Its stinging effect is widely known by many who have been surprised by its bite. Its genus name *Urtica* is derived from *uro*, to burn, or *urere*, meaning to sting (**Grieve, 1931**).The stinging nettles species name *dioica* is Latin for "two houses", from the Greek word *oikia*, meaning house, and refers to the plant's dioecious nature, bearing male and female flowers on separate plants (**Woodville, 1810**).

The benefit of *U. dioica* is known for long time, where people have taken benefits of this sting by flailing arthritic or paralytic limbs with fresh nettle to stimulate circulation and bring warmth to joints and extremities in a treatment known as "urtication" (**Green, 1824**).

Ancient Egyptians also reportedly used the infusion for the relief of arthritis and lumbago (**Harrison, 1966**). The Roman troops were flailed themselves with stinging nettle to keep warm. This practice of urtication became more popular in folk medicine as a remedy for arthritis, rheumatism, and muscular paralysis and perhaps, it was the most ancient medicinal use of this herb.

Nettle is a traditional remedy used for years against the anaemia and the lack of energy: it is said that it is an excellent herb to its high content of iron and other minerals. It is also said that it stimulates the digestive functions (**Wichtl and Anton, 2003**).

The herbal tea of nettles is always proposed by herbalists as traditional remedy for gout and rheumatism. In Germany, the herbal tea of nettle is used as diuretic, but it is not powerful enough to be associated with a treatment of hypertension or heart problems. While in Russia, the nettle is also used for the biliary disorders and liver (**Valnet, 1983**).

Nettle chemical constituents contain a fairly wide variety of chemicals although only a few compounds belonging to various classes of natural products have been identified. *U.dioica* has been used for hundreds of years in folk and officinal medicine as haemostatic and vitamin substances (**Dar *et al.*, 2013**). It is rich in vitamins such as ascorbic acid (20–60 mg/100 g of dry material), vitamins B, K (0.16–0.64 mg) and E, and pro-vitamin A (**Guil-Guerrero *et al.*, 1999**), and minerals such as calcium (853–1050 mg/100g), iron (2–200 mg/100g dry material), magnesium (175 mg/100g), phosphorus (50–265 mg/100g), potassium (532–613 mg/100g), and sodium (16–58 mg/100g) (**Frank *et al.*, 1998**). Other important substances present are all of the essential amino acids and a very high content of chlorophyll (0.08–0.3% in fresh leaves and 0.6–1% in dry leaves) (**Frank *et al.*, 1998**), tannins, phytoncides, glycoside urticin, organic acids, sterols, chlorophyll (up to 5%) and alkaloids (**Reprintseva *et al.*, 2011**) and rarely carbohydrates (**Martinez-Para *et al.*, 1980).** Furthermore, **Mavi *et al (2004)*** reported that *U. dioica* contains phenolic compounds, especially flavonoids, which have antioxidant potential. The compounds responsible for the stinging/burning action of the hairs of leaves are acetylcholine, histamine, and serotonin (**Czarnetzki *et al.*, 1990**).

Nettle is recommended as an important medicinal herb in human health, it is nutritionally high in minerals (iron, manganese, potassium, and calcium), chlorophyll, amino acids, lecithin, carotenoids, and vitamins A, C and D as well as flavonoids, tanins, sterols, fatty acids, polysaccharides, and lectins (**Asgarpanah *et al.*, 2012**).

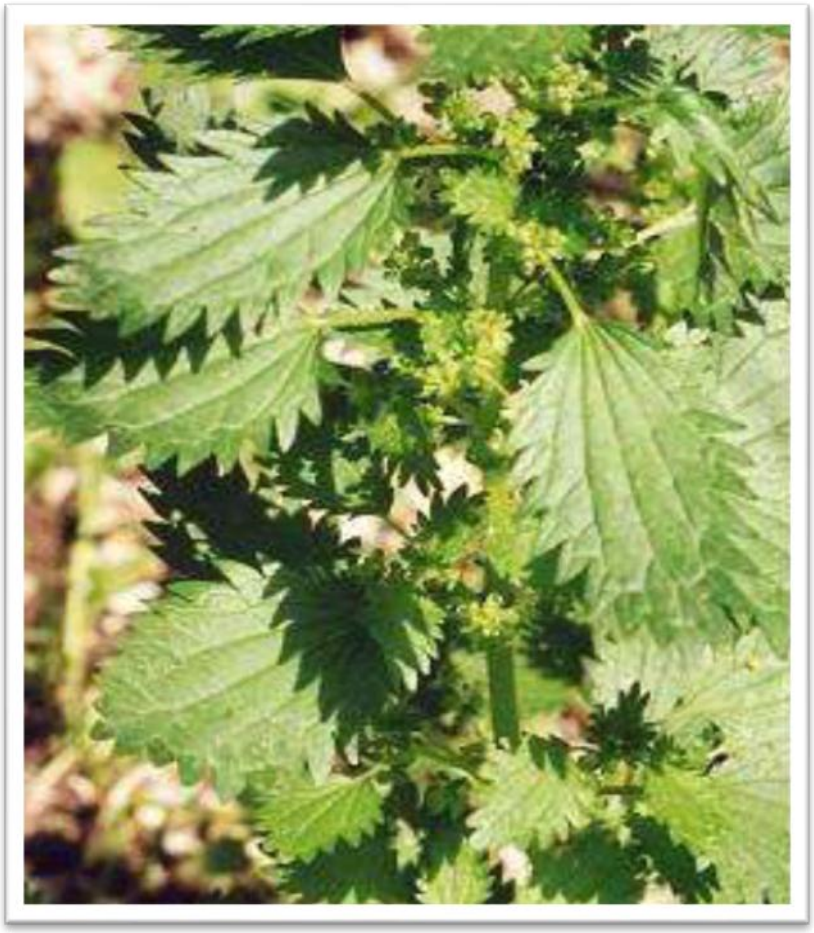

Figure N° 06: Picture of **s**tinging nettle *Urtica dioica* taken from North east Algeria in spring.

Antioxidants of nettle are found in both leaves and roots and are used therapeutically. So, nettle is a natural powerful antioxidant and as a possible food supplements (**Kanter *et al*., 2005**).

The various antioxidants seven in low doses may be effective as strong hydrogen giving ability (or their reducing power), free radical scavenging and metal chelating activities. The phenolic components appear as mostly responsible for the antioxidant activity of aqueous extracts (**Khalil *et al*., 1999; Gülcin *et al*., 2004**). In addition, antioxidants are associated with decreased DNA damage, lipid peroxidation, boost the immune performance and reduce cells' metamorphosis (**Torbeyns, 2012–2013**).

It has been demonstrated that *U.dioica* leaf extracts administered to rats as pre-treatment has decreased the oxidative stress in muscles, suggesting that nettle is giving a protection to cells against oxidative stress (**Cetinus *et al*., 2005**).

Cardiovascular diseases' treatment has been reinforced by the traditional use of *U.dioica* (**El Haouari *et al*., 2006; Daher *et al*., 2006**). Moreover; the herb was indicated in the treatment of oedema as a result of cardiac or renal insufficiency (**Wichtl and Anton, 2003**). The study in animals indicates that the extracts of *U.dioica* significantly inhibit platelet aggregations and improve lipid profile (**Daher *et al.*, 2006**).

Hypoglycaemic effect of nettle has been stated in ancient medical texts. The hypoglycaemic component has been termed "Hypoglycaemic principle= 'urticin' and nettle has been used for the treatment of high blood sugar (**Said *et al*., 2008**).This effect may be caused in part by the reduction of intestinal glucose absorption (**Bnouham *et al.*, 2003**). Hypoglycemic activity of *U.dioica* was detected in a large pharmacological screen of European species with known potential of anti-diabetic effects (**Kavalali *et al*., 2003**).
Most importantly, nettle has been applied as a diuretic in the treatment of urinary, bladder and kidney problems (**Kavalali, 2003**). It was demonstrated the diuretic, the natriuretic and the hypotensive effects of stinging nettle in animals (**Tahri *et al*., 2000**), accompanied with increased excretion of chlorides and urea. Thus, flavonoids and high potassium content may contribute to the diuretic action (**Bradley, 1992**).

The anti-inflammatory of *U. dioica* extracts is attracting attention for their actions (**Gülçin *et al*., 2004**) and it has been reported to possess inhibitory effects on prostatic hyperplasia (**Zhang *et al*., 2008**). The leaves and seeds are suggested to be useful for patients suffering from neutrophil function deficiency (**Basaran *et al*., 1997**) that could stimulate the proliferation of human lymphocytes (**Wagner *et al*., 1989**).
U.dioica has been used as a remedy for rheumatism, as it provides temporary relief from pain (**Alford, 2007**), and allergic rhinitis (**Sezik *et al*., 1997**). Besides, nettle extract in synthetic condition can halt the viral propagation such as those causing aids and hepatitis (**Chrubasik *et al*., 2007**), and it was used against liver insufficiency (**Ye¸silada *et al*., 1993**) and to treat stomachache (**Ye¸silada *et al*., 2001**). Moreover, *U. dioica* can be used in the prevention of liver damage of rats (**Lebedev *et al*., 2001; Kanter *et al*., 2005**).

The side effects of *U.dioica* are seen as rare phenomena of allergy, skin infections and oedema (**Wichtl and Anton, 2003**). The use of fresh nettles can be the origin of pitting and rarely lead to severe allergic reactions in sensitive individuals (**Alternative Medicine**

Review, 2007). However, the internal use of nettle is not associated with significant adverse effects.

The therapeutic applications of nettle are summarised in table N° 01.

Table N° 01: Summary of the important therapeutic properties of ***Urtica dioica***.

Therapeutic Properties	Actions	References
-Treatment of prostate cancer and benign hypertrophy of the prostate.	The effects of nettle roots in the treatment of HBP.	**-Konrad *et al*, 2000;** **-Schneider and Rubben, 2004;** **-Durak *et al*., 2004.** **-Safarinejad, 2005;** **-Hoffman, 2006;**
-Hypotensive.	The roots of nettle can produce a hypotensive through vasodilator effects.	**-Newail *et al*., 1996;** **-Blumenthal, 2000;** **-Tahri *et al*., 2000;** **-Testai *et al*., 2002;** **-Legssyer *et al*., 2002.**
-Diuretic.	-Increases the urinary excretion	**-Blumenthal, 2000;** **-Tahri *et al*., 2000.**
-Hepatic-protective, -Depurative.	-Elimination of accumulated toxins in the body. -The leaves help to regulate the inflammatory factors.	**-Turkdogan *et al*., 2003;** **-Yener *et al*., 2008.**
-Anti-platelet aggregation.	High iron content in the leaves.	**-El Houari *et al*., 2006.**
-Anti-allergic.	-Useful in the treatment of allergies to pollen, long-term treatment. - Effects on the key receivers and the enzymes associated with allergic	**-Gulcin *et al*., 2004;** **-llhami *et al*., 2004;** **-Roschek *et al*., 2009.**

	rhinitis(leaves)	
-Anti-inflammatory, -Immuno- stimulator.	- Inhibitory activity on rat legs oedema(the roots) -An immuno-stimulant neutrophils (flavonoids glycosides from leaves).	**-Glusker and Rossi, 1986;** **- Wagner, 1994.**
-Effect on brain function and memory.	The leaves are capable to reduce the transcription of inflammation factors, and stimulate the cerebral performance.	**-Wichtl and Anton, 2003.**
-Antihyperglycemic, -Anti-diarrheic, -Antioxidants.	- Inhibits significantly glucose absorption of rat small intestine. -Due to the presence of tannins. -In relation to different oxidative systems (phenols, flavonoids).	**-Guillaume, 1991;** **-Bnouham *et al*., 2003;** **-Cetinus *et al*., 2005.** **-El Haouari *et al*., 2006;** **-Morel, 2008;**
-Anti-asthenic (against fatigue), -Anti-anemic.	-Vitamins A, B2, B5, C, E, K, its minerals magnesium, phosphorus, sulfur, silicic acid, potassium, calcium , its eight essential amino acids and its principles iron, folic acid, chlorophyll.	**-Dahout and Wuyts, 1991.**

Red radish

Brassicaceae family is the eldest cultivated plants known to humans. Some confirmation has been proved that a brassica (cruciferous) vegetable was widely consumed as early as 10,000 years ago (**Snowdon *et al*., 2007**) with varied kind of uses; biofuel, edible oil, human food and animal feed.

Cruciferae vegetables including broccoli, cabbage, cauliflower, radish, garden cress, horse radishes, and wasabi, belong to family of Cruciferaceae which have an

exceptional tastes and aromas but also come with both important nutritious and health valuable benefits.

Cruciferae vegetables are rich in carotenoids, vitamin C, fibre, flavonoids, and in particular, a group of health-promoting metabolites known as glucosinolates which linked to cancer prevention as well as having antioxidant properties (**Fahey *et al.*, 1997; Barillari *et al*., 2005**). This is maintained by strong epidemiological evidence for the association of Cruciferae vegetables consumption with a highly significant reduction of inflammatory response (**Holst and Williamson, 2004; Moon and Kim, 2012**). Moreover, Cruciferae was reported to reduce the risk of many sorts of cancer (**Verhoeven *et al*., 1996; Higdon *et al*., 2007**).

Consequently, numerous studies have made attention on finding the bioactive compounds from this family as a source of potential chemopreventive agents. The family has a unique sulphur-containing compounds responsible for their strong aroma and spicy (or bitter) taste (**Drewnowski and Gomez- carneros, 2000**).

Red radish *R .sativus* L. is originated from Europe and Asia. It can be cultivated in moderate climates at altitudes between 190 and1240 m. It is 30–90 cm high and its roots are thick and of various sizes, forms, and colours. They are comestible with a spicy taste.

R. sativus is a root crop spicy or sweet in taste with a lot of juice. Roots have many variable shape and skin color, but the round, red skinned variety is the best one; is generally cultivated all over the world for its edible roots and leaves and its great source of medicinal compounds (**Herman-Lara *et al*., 2012**).

Health and nutritional benefits of Radish is multiple. It is rich in folic acid, vitamin C and plenty of anthocyanins in red radish extract (**Patil *et al*., 2009**). About 12 to 34 kinds of anthocyanins have been identified in the red radish (**Fuleki, 1969; Wu and Prior, 2005**).

Different parts of radish; including, roots and leaves have been reported to possess a wide range of pharmacological activities (**Nadkarni, 1976; Gilani and Ghayur, 2004**).

Radishes have been applied as laxative, stimulant, digestive, appetizer, and in other disorders of stomach digestion, and as bile flow stimulants (**Chevallier, 1996**). Furthermore, the juices of the fresh leaves have been used as a diuretic and laxative agent (**Chopra *et al*., 1986**). The leaves, seeds and roots have also been used to treat asthma and

other chest diseases (**Duke and Ayensu, 1985**). In particular, radishes have been used in traditional medicine as carminative and stomachic agents, especially as anti-cancer and/or anti-inflammatory agents (**Duke and Ayensu, 1985**). Some isolated glucosinolates of *R. sativus*, seeds are responsible on cancer-chemoprotective property (**Barillari *et al*., 2005; Duan *et al*., 2006**).

Aqueous extract of radish was reported to cause an increase in the contractions of the duodenum, jejunum, and ileum (**Yong *et al*., 2000**).

Flavonoids and vitamin C of radish may inhibit lipid peroxidation, promote liver and red blood cell catalase, and inhibit XOD activities in animal's tissues. Radish can be suggested for the treatment and prevention of cardiovascular disease and cancer (**Jin *et al*., 2001**).The leaves and roots of *R. sativus* are used as hepatoprotective (**Zaman and Ahmad, 2004**), cardioprotective (**Zaman, 2004**), antioxidant (**Barillari *et al*., 2006**) and anti-urolithiatic (**Vargas *et al*., 1999**) effects. Furthermore, the freshly squeezed root juice of *R. sativus* has been recommended to stimulate antiulcer activity (**Alqasoumi *et al*., 2008**).

Figure N° 07. Picture of Red radish *Raphanus sativus* grown in Algeria taken from the east of Algeria **(Al-Hadjer)**.

The therapeutic uses of radish are summarised in table N° 02.

Table N° 02: Summary of the most therapeutics proprieties of ***Cruciferae***.

Therapeutics proprieties	Actions	References
-Beneficial in reducing the risk of incidence and progression of type II diabetes by improving insulin sensitivity; -Beneficial in Coronary heart disease (CHD) and inflammation.	-Total fat levels tend to be relatively low and contain negligible amounts of detrimental saturated fats. -There are appreciable levels of polyunsaturated fatty acids (PUFAs).	**-(Harris *et al*., 2008);** **-(Risérus *et al*., 2009);** **-(Lopez, 2010);** **-(Ortega *et al*., 2012).**
-Good drugs for anaemia, rickets, influenza, mortality, poor immune function, and cognitive decline.	The cruciferous vegetables are good source of many vitamins (A and K, C) and minerals (Fe, Ca, Se, Zn).	**-(Lee and Kader, 2000);** **-(Beck *et al*., 2001);** **-(Rayman, 2012);** **-(Chapapis *et al*., 2012).**
-Reduced incidence of some cancers; -Supposed dietary preventive routes for several cancers as colon, lung, and potentially breast and prostate cancers; -Stimulates expression of body's own protective antioxidant and detoxification proteins, and the damaging effects as cell cycle arrest and apoptosis.	Glucosinolates and their catabolites (metabolism-derived breakdown products).	**-(London *et al*., 2000) ;** **-(Plate *et al*., 2003);** **-(Hayes *et al*., 2008);** **-(Steinbrecher *et al*., 2009).**
-Reduce risks of age-related chronic diseases, like cancer and cardiovascular disease, and advantageous for gut micro-biota.	-Flavonoids & phenols -Glutathione reductase (roots and leaves).	**-(Harborne *et al*., 1993);** **-(Lugasi *et al*., 2000);** **-(Vitoria *et al*., 2001).**

-Anti-oestrogenic properties; -Anti-inflammatory, diuretic, and anti-HIV activities; -Hypo-lipidic activity.		**–(Clifford, 2004);** **-(Graf *et al.*, 2005);** **-(Lee *et al.*, 2006);** **-(Terao *et al.*, 2008).**
-Antidiabetic; -Antiherpes; -Analgesic; -Activation of macrophage.	Lipopolysaccharides (roots).	-(**Genichiro *et al.*, 1993**).

Radish Glucosinolates are a class of metabolites found in the seeds of *R. sativus*; their hydrolysis products possess health-promoting including anticancer properties (**Holst and Williamson, 2004**). Glucosinolates from Brassica genus might exert neuro-protective effect through modulation of inflammatory responses in the central nervous system (**Noyan-Ashraf *et al.*, 2005; Cuzzola *et al.*, 2013**).

Anthocyanins of red radish is a red pigments found in the outer skin. Anthocyanins are water-soluble natural pigments belong to the flavonoids class, which act as an antioxidants helping to defend plant tissue from UV irradiation **(Holton and Cornish, 1995)**. Anthocyanins were found to have the strongest antioxidant power out of 150 flavonoids **(Elliott *et al.*, 1992).** In addition, anthocyanins are very effective antioxidants, and are the most characteristic bioactive nutrients in berries and other ‘‘red–purple healthy food’’. Anthocyanins are the largest group of water-soluble pigments in the plant kingdom responsible for the attractive red, orange, blue and purple colours of most fruits (**Mazza and Miniati, 1993**) and vegetables and health benefits (**Konczak and Zhang, 2004**).

Moreover, anthocyanin can play an antioxidants, or antibacterial agents (**Kong *et al.*, 2003**), which can be clarified by several antioxidant mechanisms, including hydrogen donation, metal chelating and protein binding (**Kong *et al.*, 2003**). The functions of anthocyanins result from the chemical structure of these compounds, particularly from the presence of hydroxyl groups in the ring moiety. They can give electrons or transfer hydrogen atoms from hydroxyl moieties to free oxidative radicals (**Castnˇeda–Ovando *et al.*, 2009**). Additionally, anthocyanins also chelate metal ions, such as Fe^{3+} and Cu^{2+}

(**Seeram and Nair, 2002**). Besides, Anthocyanins have shown to exert anticarcinogenic activities against multiple cancer cells in vitro (**Renis *et al.*, 2007; Shih *et al.*, 2007**) and in animal tumour models in vivo (**Cooke *et al.*, 2006; Lala *et al.*, 2006**). Potential cancer chemo-preventive mechanisms of anthocyanins include scavenging of reactive oxygen species, inhibition of cell proliferation, and promotion of cell apoptosis and differentiation (**Stoner, 2008**). Epidemiological and experimental studies indicate that anthocyanin intake protects against the risk of cardiovascular diseases and other chronic degenerative conditions (**Seeram, 2008; Wang and Stoner, 2008**).

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Alternative Medicine Review Volume 12, Number 3 September 2007.

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Chapter II:
Materials & Methods

MATERIALS & METHODS

1. Materials:

1.1. Biological Material

The experimental study was carried out on 50 males and 50 females of albinos Wistar rats weighing between 170–230 g which were obtained from Pasteur Institute (**Algiers**). The rearing of rats was achieved at the "Pet Center" of the University Badji Mokhtar-Annaba. Animals were housed in polyethylene cages lined with litter of wood chips and cleaned daily. The group number and the dates were written on each cage. Rats were adapted for approximately one month under standard husbandry conditions, room temperature 23 ± 1 ^{0}C and natural photoperiod. They were given standard diet obtained from ONAB (**Bejaia**) and potable water. The quantity of food and water were evaluated daily.

1.2. Chemicals

Pure inorganic mercury has been used (pharmaceutical company **PROLABO**, made in **EC**). It is easily soluble in distilled water. A concentration of 0.8 g/kg bw was chosen according to literature.

1.3. Preparation of Plant Material

- **Stinging nettle**

The nettle (***Urtica dioica***) was collected from clean area of **Souk Ahras** region (north-east Algeria) at the beginning of spring, cleaned with distilled water and prepared by infusing fresh leaves in boiling water (16 gr in 25 ml of distilled water) during 5 minutes to get a green solution**.**

Table N° 03. Scientific classification of ***Urtica dioica*** **(Quezel and Santa ,1963).**

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants

Class	Magnoliopsida – Dicotyledons
Subclass	Hamamelididae
Order	Urticales
Family	Urticaceae – Nettle family
Genus	*Urtica* L. – nettle
Species	*Urtica dioica* L. – stinging nettle

❖ **Red radish**

The Freshly harvested radish roots were collected from clean area of **Annaba** region (east Algeria) and washed to remove soil and other surface contaminants. They were crushed in a juicer for few minutes to obtain a relatively thick fresh juice.

Table N° 04. Scientific classification of ***Raphanus sativus***:

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae
Order	Capparales
Family	Brassicaceae/Cruciferae – Mustard family
Genus	*Raphanus* L. – radish
Species	*Raphanus sativus* L. – cultivated radish

1.4. Experimental procedure:

After one month of adaptation under the same laboratory conditions, rats were distributed into six groups of seven each, which they were treated for 30 days.

Group 1: The control group received potable water and standard diet *ad libitum* **(0 + 0).**

Group 2:	Rats received potable water and standard diet contaminated with mercury chloride **(0.8 g Hg/kg diet)** *ad libitum* **(0 + Hg).**
Group 3:	In addition to potable water and standard diet *ad libitum*, rats received infusion of nettle **(1.5 ml)** by gavage **(0 + UD).**
Group 4:	In addition to potable water and standard diet *ad libitum*, rats received the fresh juice of red radish **(1 ml)** by gavage **(0 + RS).**
Group 5:	Rats received potable water and standard diet contaminated with mercury chloride **(0.8 g Hg/kg diet)** *ad libitum* **+** infusion of nettle **(1.5 ml)** by gavage (**Hg+UD**).
Group 6:	Rats received potable water and standard diet contaminated with mercury chloride **(0.8 g Hg/kg diet)** *ad libitum* **+** fresh juice of red radish **(1 ml)** by gavage (**Hg+RS**).

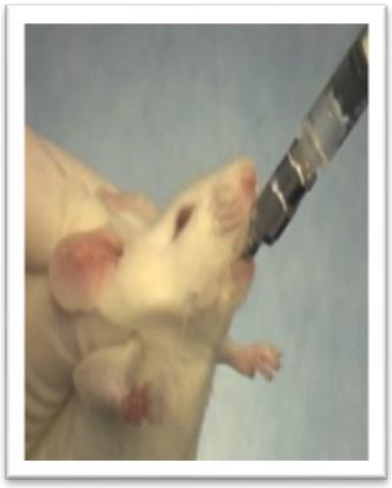
1.5. Biological Samples:

Animals were sacrificed by decapitation after one month of treatment and blood was immediately collected in tubes containing the anticoagulant:

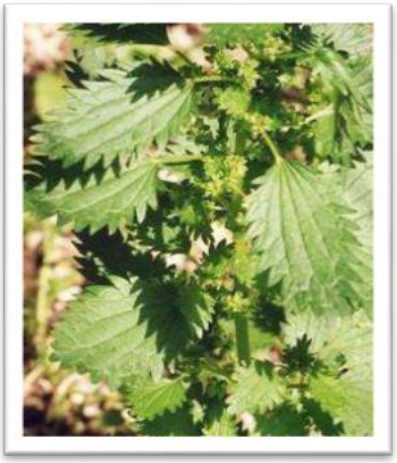
- ✓ Labelled polypropylene test tubes containing heparin. Blood was centrifuged at 4000 rpm/15 minute, and then the plasma biochemical markers were evaluated (glucose, triglycerides, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), magnesium (Mg), iron (Fe), calcium (Ca) and testosterone.
- ✓ After sacrifice, liver, kidney, testis and epididymis were immediately weighed and then stored in the freezer (-20)°C for the determination of the parameter of the oxidant stress (GSH) and a part of these organs were collected and preserved in 10% neutral buffered formalin to achieve histological profile.

Pet center

Wistar rats inside the cages



Gavage of rats



Fresh stinging nettle leaves

Nettle leaves infusion

Raw red radish roots

Fresh radish juice

Figure N° 08. Rat housing, gavage, and the preparation of nettle and radish extracts.

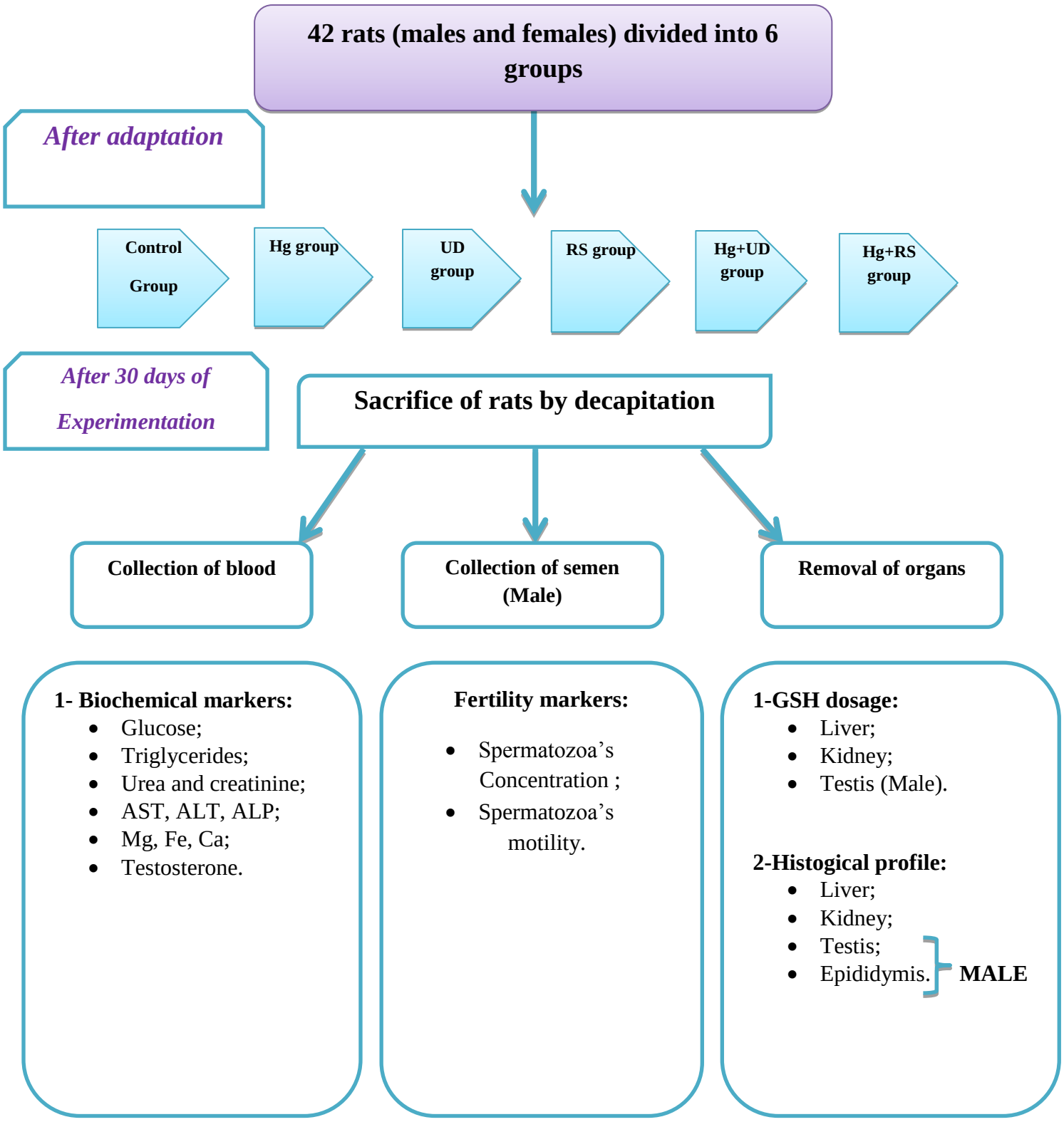


Figure N° 09: Summary of the experimental procedure.

2. Methods

2.1. Biochemical markers

Biochemical markers were measured by automated apparatus (**Diatron PICTUS 200**), except glucose. Testosterone concentration has been estimated by Electrochemiluminescence immunoassay (ECLIA) using the automated apparatus (**Cobas e 411**).

1. **Glucose:**

- **Principle:**

The determination of blood glucose was performed by a glucose meter that uses test strips. The latter are intended for use in vitro diagnostic for the test of blood glucose. They are designed to measure the glucose in the blood. The test strip contains the glucose-oxidase, an enzyme that oxidizes the glucose in the blood and which product of the acid D-gluconic and of hydrogen peroxide.

- **Operating Mode:**

The player turns on automatically by simple insertion of the test strip Accu- Chek (in the direction of the arrows, and up to the stop).

- The symbol of a gout flashes.
- Remove the drop of blood on the deposit zone orange of the test strip.
- The measurement is completed in approximately 5 seconds, and the result appears on the screen and the blood glucose is given in g/L.

2. **Triglycerides**

The dosage of triglycerides has been carried out by the enzymatic method colorimetric according to the technical data sheet of the Spinreact Kit (Spain).

- **Principle:**

The triglyceride incubated with the lipoprotein lipase (LPL), release the glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by the glycerol kinase and adenosine triphosphate (ATP). The glycerol-3-phosphate (G3P) is then converted by the glycerol phosphate dehydrogenase (GPO) in active ingredient dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). In the last reaction, the hydrogen peroxide (H_2O_2) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in the presence of peroxidase (POD) to give a red color.

The protocol therefore includes the following reactions:

$$\text{Triglycerides} + H_2O \xrightarrow{\textbf{LPL}} \text{Glycerol} + \text{Free Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\textbf{Glycerol kinase}} \text{Glycerol-3-Phosphate} + \text{Adenosine-5diphosphate}$$

$$\text{The glycerol-3-phosphate} + O_2 \xrightarrow{\textbf{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{p-Chlorophenol} \xrightarrow{\textbf{POD}} \text{Quinone} + H_2O$$

The intensity of the red color is proportional to the concentration of triglycerides in the sample (**Bucolo and David, 1973**).

- **Reagents :**

R 1: Buffer	**COOD pH 7,5** **p-Chlorophenol**	**50 mM/L** **2 mM/L**
R 2: Enzymes	Lipoprotein lipase (LPL) Glycerol kinase (GK) Glycerol-3-oxydase (GPO) Peroxydase (POD) 4-Aminophénazone (4-AP) ATP	150000 U/L 500 U/L 2500 U/L 440 U/L 0,1 mM/L 0,1 mM/L
Triglycerides CAL	Triglycerides aqueous (standard)	200 mg/dL

- **Work reagent:** dissolve the contents of one bottle of R2 in a vial of R1 and mix slightly. This reagent is stable for 6 weeks at 2-8°C or a week at 15-25 ^{O}C.

	Blank	**Buffer**	**simple**
Reagent of work (mL)	1,0	1,0	1,0
Buffer (µ.L)	--	10	--
Simple (µ.L)	--	--	10

3. Urea

The dosage of urea has been carried out by the enzymatic method colorimetric according to the technical data sheet of the Spinreact Kit (Spain).

- **Principle:**

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

$$\text{Urea} + H_2O \xrightarrow{\textbf{Urease}} (NH_4)_2 + CO_2$$

$$NH4 + \text{Salicylate} + NaClO \xrightarrow{\textbf{Nitroprusside}} \text{Indophenol}$$

The ions of ammonia forms responded with the salicylate and hypochlorite (NaClO) in the presence of the catalytic converter nitroprusside to form the green indophenol (**Kaplan, 1984**), according to the following reactions**.**

The intensity of the color formed is proportional to the concentration of urea in the sample.

- **Reagents :**

R1: Buffer	Phosphate pH 6.7	50 mmol/L
	EDTA	2 mmol/L
	Sodium salicylate	400 mmol/L
	Sodium nitroprusside	10 mmol/L
R2 : NaClO	Sodium hydrochlorite (NaClO)	140 mmol/L
	Sodium hydroxyde	150 mmol/L
R 3 : Enzymes	Urease	30000 U/L
Urea CAL	Urea aqueous (Standard)	50 mg/dL

- **Work reagent**: Dissolve a tablet of reagent 3 (enzymes) in a bottle of reagent 1 (buffer) and lightly mix. The reagent 2 (NaClO) is prepared for use.

- **Operating Mode:**

	Blanc	Buffer	simple
Reagent of work (mL)	1,0	1,0	1,0
Buffer (µ.L)	--	10	--
Simple (µ.L)	--	--	10

4. Creatinine

The assay of creatinine has been achieved by the kinetic method colorimetric according to the technical data sheet of the Spinreact Kit (Spain).

- **Principle:**

The test is based on the reaction of creatinine with the picrate of sodium as described by Jaffe. The creatinine reacts with the alkaline picrate forming a complex red. The time interval is chosen for the measures in such a way that it avoids interference with other components of serum. The intensity of the color is proportional to the concentration of creatinine in the sample (**Murray, 1984**).

- **Reagents:**

R1:Picric reagent	Picric acid	17,5 mM/L
R 2: Alcalin reagent	Sodium hydroxide	0,29 mM/L
Creatinine CAL	Creatinine aqueous (standard)	2 mg/dL

- **Work reagent**: Mix one volume of reagent 1 with a volume of reagent 2. The reagent is stable for 10 days at 15-25^{O}C.

	Blanc	Buffer	simple
Reagent of work (mL)	1,0	1,0	1,0
Buffer (µ.L)	--	100	--
Simple (µ.L)	--	--	100

5. ALT

The assay of aspartate aminotransferase has been achieved by the kinetic method according to the technical data sheet of the Spinreact Kit (Spain).

- **Principle:**

The alanine aminotransferase (ALT) also known as the glutamate-pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from the alanine at α-cetoglutarate forming glutamate and pyruvate. The pyruvate is reduced to lactate by lactate dehydrogenase (LDH) and the NADH formed according to the following reactions:

$$\text{Alanine} + \alpha\text{-cetoglutarate} \xrightarrow{\textbf{ALT}} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\textbf{LDH}} \text{Lactate} + NAD^{+}$$

- **Reagents :**

R 1: Buffer	TRIS pH 7,8	100 mM/L
	L-alanine	500 mM/L
R2: Substrate	NADH	0,18 mM/L
	Lactate deshydrogenase (LDH)	1200 U/L
	α-cetoglutarate	15 mM/L

- **Work reagent**: dissolve a tablet of R2 in a vial of R1 and mix slightly. This reagent is stable for 21 days at 2-8°C or 72 hours at 15-25^{O}C.

- **Operating mode:**

Work reagent (mL)	1.0
Sample (µL)	100

6. AST

The assay of aspartate aminotransferase has been achieved by the kinetic method according to the technical data sheet of the Spinreact Kit (Spain).

- **Principle**:

Aspartate aminotransferase (AST) also called the glutamate oxaloacetate transaminase (GOT) catalyses the reversible transfer of an amino group from the aspartate at α-cetoglutarate forming glutamate and oxaloacetate. The oxaloacetate is reduced to malate by malate dehydrogenase (MDH) and the NADH H according to the following reactions:

$$\text{Aspartate} + \alpha\text{-cetoglutarate} \xrightarrow{\textbf{AST}} \text{Glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^{+} \xrightarrow{\textbf{MDH}} \text{Malate} + NAD^{+}$$

- **Reagents :**

R 1: Buffer	TRIS pH 7,8	80 mM/L
	L-Aspartate	200 mM/L
R 2: Substrate	NADH	0,18 mM/L
	Lactate deshydrogenase (LDH)	800 U/L
	Malate deshydrogenase (MDH)	600 U/L
	α-cetoglutarate	12 mM/L

- **Work reagent:** dissolve a tablet of R2 in a vial of R1 and mix slightly. This reagent is stable for 21 days at 2-8°C or 72 hours at 15-25^{O}C.

- **Operating mode:**

Work reagent (mL)	1.0
Sample (µL)	100

7. ALP

The assay of alkaline phosphatase has been achieved by the kinetic method according to the technical data sheet of the Spinreact Kit (Spain).

- **Principle:**

The alkaline phosphatase (PAL) catalyses the hydrolysis of p-nitrophenyl phosphate at pH 10.4, freeing up the p-nitrophenol and the phosphate, according to the following reaction:

$$\text{ALT p-nitrophenyl phosphate} + H_2O \xrightarrow{\textbf{ALT}} \text{p-Nitrophenyl} + \text{Phosphate}$$

The rate of the decrease of the concentration in NADH+ H^+, measured by photometry, is proportional to the catalytic activity of alkaline phosphatase (**Wenger, 1984**).

- **Reagents :**

R1: Buffer	Diethanolamine (DEA) pH 10,4	1 mM/L
	Chloride de magnesium	0,5 mM/L
R 2: Substrate	P-Nitophenylphosphate (pNPP)	10 mM/L

- **Work reagent:** dissolve a tablet of R2 in a vial of R1 and mix slightly. This reagent is stable for 21 days at 2-8 °C or 72 hours at 15-25^{O}C.

- **Operating mode:**

Work reagent (mL)	1.2
Sample (µL)	20

8. Magnesium

The assay of magnesium has been achieved by the kinetic method according to the technical data sheet of the Spinreact Kit (Spain).

➢ **Principle**:

Magnesium forms a coloured complex when reacts with Magon sulfonate in alkaline solution.

The intensity of the color formed is proportional to the magnesium concentration in the sample (**Farrell et al., 1984**).

➢ **Reagents :**

R		
	Xylidyl Blue	0.1 mmol/L
	Thioglycolic acid	0.7 mmol/L
	DMSO	3000 mmol/L

9. Iron

The assay of iron has been achieved by the kinetic method according to the technical data sheet of the Spinreact Kit (Spain).

➢ **Principle**

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine a coloured complex:

$$\text{Transferrin } (Fe^{+3})_2 + e^- \xrightarrow{\textbf{Acid Ascorbic}} 2\ Fe^{+2} + \text{Transferrin}$$

$$Fe^{+2} \xrightarrow{\textbf{FerroZine}} \text{Coloured complex}$$

The intensity of the color formed is proportional to the iron concentration in the sample. **(Itano, 1978)**

➢ **Reagents :**

R 1 : Buffer	Acetate pH 4.9	100 mmol/L
R 2 : Reductant	Ascorbic acid	99.7%
R 3 :Color	FerroZine	40 mmol/L
IRON CAL	Iron aqueous primary standard	100 g/dL

- **Work reagent**: Dissolve the contents of one tube R 2 Reductant in one bottle of R 1 Buffer.

- **Mode operating:**

	WR blank	Standard	Sample blank	Sample
WR(mL)	1.0	1.0	1.0	1.0
R 3 (drops)	1	1	--	1
Distilled water (L)	200	--	--	--
Standard (L)	--	200	--	--
Sample (L)	--	--	200	200

10. Calcium

The assay of calcium has been achieved by the kinetic method according to the technical data sheet of the Spinreact Kit (Spain).

- **Principle**

The measurement of calcium in the sample is based on formation of color complex between calcium and *o*-cresolphtalein in alkaline medium:

$$Ca^{++} + o\text{-Cresolphtalein} \xrightarrow{OH^{+}} \text{Colored complex}$$

The intensity of the colour formed is proportional to the calcium concentration in the sample (**Farell et al., 1984**).

- **Reagents:**

R1: Buffer	Ethanolamine	500 mmol/L
R2: Chromogen	*0*-Cresolphatein 8-Hidroxyquinolein	0,62mmol/L 69mmol/L
CALCIUM CAL	Calcuim aqueous primary standard	10mg/dL

- **Work reagent:**

All the reagents are ready to use. To prepare monoreagent, mix according to this proportion: 50 vol. of R1 and 1 vol. of R2.

- **Mode operating:**

	Blank	Standard	Sample
R 1 (mL)	2.0	2.0	2.0
R 2 (drops)	1	1	1
Standard (L)	--	20	--
Sample (L)	--	--	20

2.2. Fertility profile

The spermogram has been realized according to the method of (**WHO, 1993**) by making a small incision at the epididymis level in order to obtain a drop of semen. One drop of sperm of nearly 1µl was added to 49 µl of physiological solution (0.9% NaCl) and then spermatozoa's concentration and motility were estimated.

- **Spermatozoa's concentration :**

The concentration of spermatozoa is measured by using a blade of counting (cell of Malassez). A drop of the same preparation filed on the blade of Malassez and covered with a coverslip, the counting is done in 5 fields of the blade to the magnification (x 40). The concentration of spermatozoa is calculated by the following method (**WHO, 1993**).

$$C\ (\text{number} \times 10^6\ /\text{ml}) = D \times V \times n / N$$

D: dilution factor (50 times)

V: volume of the blade of counting

n: number of sperm calculated in 5 fields.

N: number of small squares in the blade.

➢ **Spermatozoa's motility :**

A drop of semen is deposited on a cell of Malassez and then covered with a coverslip. The preparation is examined under optical microscope (**x40**). The field of observation is divided into 5 distinct zones to classify 100 sperm, and then we calculate the percentage of mobile sperm (**WHO, 1993**).

➢ **Testosterone**

The dosage of Testosterone has been carried out by applying the test of e Electrochemiluminescence (**ECLIA**) which is suitable for immunological assays using the automated apparatus (**Cobas e 411**).

- **Principle**

The test of this hormone depends of the competition by using a specific antibody directed against testosterone. The endogenous testosterone released by the action of the acid 8- anilino-naphthalene sulfonic (ANS) and of the norgestrel, enters into competition with the exogenous testosterone marked in the ruthenium to the binding sites of anti-testosterone antibody biotinylated.

- **Reagents:** are contained in a cabinet Elecsys-testosterone:

Microparticles lined Streptavidin	-One vial	6.5 ml
Microparticles lined Streptavidin	-Conservative	0,72 mg/mL
R1: antibodies anti-testosterone-biotin	- Antibodies (sheep) anti-testosterone marked to the biotin, ph7 - 2-Bromoestradiol (reagent of cytokine release) - Buffer pH 6.0 - Conservative	40ng/ml 20mmol/L 50mmol/L
R2: peptide testosterone	- Derived from testosterone brand in the ruthenium - Buffer pH 6.0 - Conservative	1.5ng/ml 50mmol/L

- **Operating mode:**

✓ **1st incubation**: 20 µl of sample are incubated with a monoclonal antibody anti-testosterone specific biotinylated. The binding sites of the antibody brand are occupied by the analyse content in the sample (as a function of its concentration).

✓ **2nd incubation**: Microparticles lined with streptavidin and derivatives of testosterone brand in the ruthenium are added to the reaction cup. The complex is fixed to the solid phase by a link streptavidin-biotin. "The reaction mixture is transferred in the measuring cell; the microparticles are maintained at the level of the electrode by a magnet.

The elimination of the free fraction is performed by the passage of Procell. A potential difference applied to the electrode triggers the production of luminescence which is measured by a photomultiplier.

The results are obtained using a calibration curve. The latter is generated, for the parser uses, by a calibration in 2 points and a reference curve stored in the bar code of the reagent.

2.3. GSH dosage

➢ **Preparation of homogenate:**

1 g of liver, kidney or testis of rats of different groups studied, has been used. After grinding and homogenization of tissues in the TBS (Tris 50 mm, NaCl 150 mM, pH 7.4) .We proceeded to a centrifugation of the cell suspension (9000 holes/min, 4°C, 15 min), and then the supernatant is aliquoted into tubes eppendorfs and then kept at (-20)°C pending to perform the determinations of the parameters of the oxidant stress.

➢ **Determination of the rate of reduced glutathione (GHS) at tissue:**

Glutathione is an intracellular thiol the most abundant present in all animal cells.

- **Principle**:

For the determination of glutathione, the colorimetric method by the reagent of Ellman (DTNB) is the commonest method (**Ellman, 1959**). The reaction is to cut off the molecule of acid 5.5 ' dithiodis-2-nitrobenzoique (DTNB) by the GHS, which frees the thionitrobenzoique acid (TNB) (figure) which presents an absorbance at 412 nm.

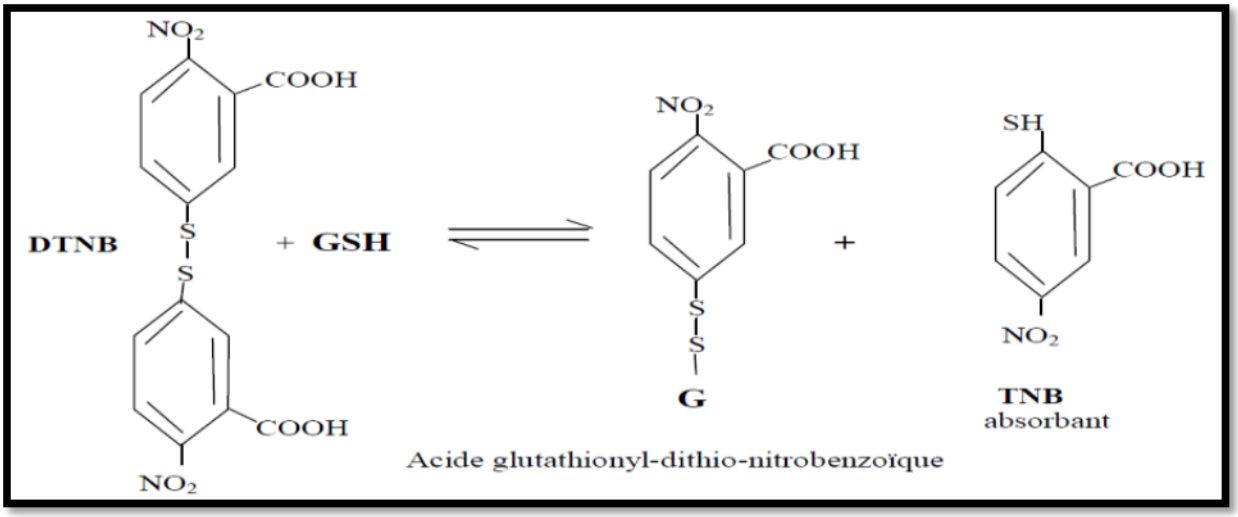


Figure N° 10: Glutathione and DTNB reaction.

- **Operating Mode**

- **C**ollect 0.8 ml of homogenate;
- **A**dd 0.2 ml of the solution with sulfosalicylic acid (0.25 %) and leave for 15 min in an ice bath;
- **C**entrifuge at 1000 tours/min for 15 min;
- **C**ollect 0.5 ml of the supernatant;
- **A**dd 1ml of buffer Tris-EDTA (containing 0.02 M EDTA, pH 9.6);
- **A**dd 0,025 ml of the acid 5,5 dithio-bis-2-nitrobenzoique (DTNB) to 0.01 M ;
- **L**eave for 5 min at an ambient temperature and read the optical densities at 412 nm against the reagent blank.

- **Calculation of the concentration**

The concentration is calculated of the GHS expressed in nanomoles per milligram of protein (nmol/mg prot) according to the following formula:

$$\mathbf{GSH} = \frac{\mathbf{OD \times L \times 1.525}}{\mathbf{13100 \times 0.8 \times 0.5 \times mg\ prot}}$$

- **OD**: Optical density ;
- **L**: Total volume of solutions used in the deproteinisation ;
- **1,525**: Total volume of solutions used in the dosing of the GHS at the level of the supernatant;
- **13100**: Coefficient of absorbance of the grouping -SH to 412 nm ;
- **0.8**: Volume of homogenate ;
- **0.5**: Volume of the supernatant.

- **Determination of total protein in the tissue level**
  - **Principle**

The tissue proteins were quantified according to the colorimetric method of **Bradford** (1976) who uses the Gloss Blue Coomassie G250 (BBC) as a reagent and the serum albumin of beef (BSA) as standard. The BBC reacts with the amino groups (-NH2) for protein to form a complex of blue color. The emergence of this color reflects the degree of ionization of the acid and intensity establishes the concentration of protein which is measured spectrophotometrically at 595nm.

- **Operating Mode:**

- **C**ollect 0.1 ml of the homogenate ;
- **A**dd 5 ml of brilliant blue Coomassie (BBC) (G250), as a reagent ;
- **S**hake and leave the mixture to ambient temperature during 5 min for the stabilization of the color ;
- **M**easure the absorbance of the sample at 595nm against the white containing distilled water in place of the homogenate. The optical density obtained is reported on the calibration curve (0 to 1 mg/ml of BSA) previously carried out in the same conditions.

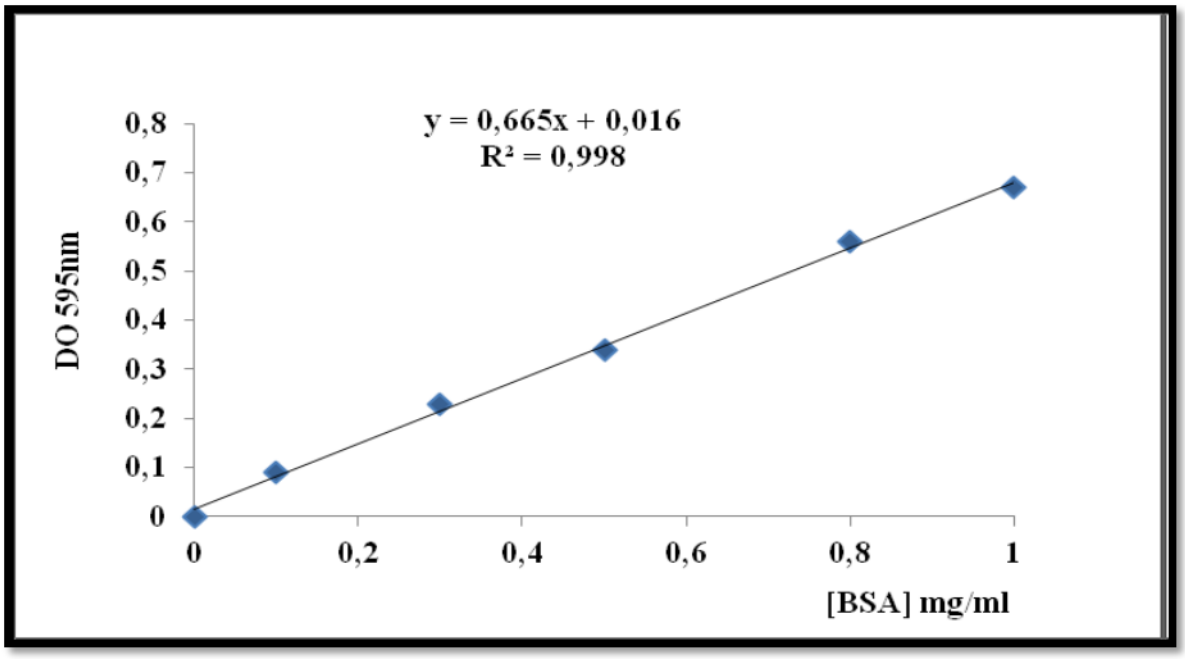


Figure N° 11: The calibration curve of BSA (1mg/ml) for the assay of proteins in the homogenates.

2.4. Histological profiles:

After sacrifice, liver, kidney, testis and epididymis were immediately collected and preserved in 10% neutral buffered formalin, where it examined according to the classical method of **Martoja Martoja (1967**) according to the following steps:

- **Fixing of the samples**

The fixing represents the time essential to the histological technique, it is designed to immobilise the structures in respecting their morphologies. The fixing is done in 10% neutral buffered formalin, fixer commonly used.

- **Dehydration**

The paraffin is not miscible in water; the sample must be fully dehydrated before the inclusion in the paraffin. The fixer is eliminated; the sample is rinsed with distilled water and then dehydrated by ethyl alcohol 700°, 900°, and 1000°.

The paraffin is not miscible with the alcohol used for the dehydration, for this we proceeded to a substitution by the toluene renewed 3 times (duration of the bathroom 30 minutes).

- **Inclusion**

The sample is included in paraffin that has been melted in an oven at 600 ^{0}C for 08 hours, the blocks is done using bars of Leuckard.

- **Cuts and colouring**

The blocks of inclusion are entries on a sample holder. The slices are made using a microtome; the series of slices are connected between them in the form of ribbon, which facilitates the replenishment of three-dimensional structures observed. The thickness of the slices is 4 to 7 µ.

The ribbons of slices obtained are pasted on glass slides degreased, posed on a heating plate (to lower temperature to the melting temperature of the paraffin wax). The heat allows the spreading of the ribbons of slices.

Coat the blades of glycealbumine, cover with distilled water and install the tapes above. The slices deparaffinise are coloured to: haemalum-eosin, dehydrate the preparation in two bathrooms of alcohol and fit by the deposit of a drop of Eukitt on which we place a coverslip. Finally, we proceeded to the observation at the optical microscope equipped with a camera.

The fitting is to attach a coverslip glass on histological sections after staining. This step allows the:

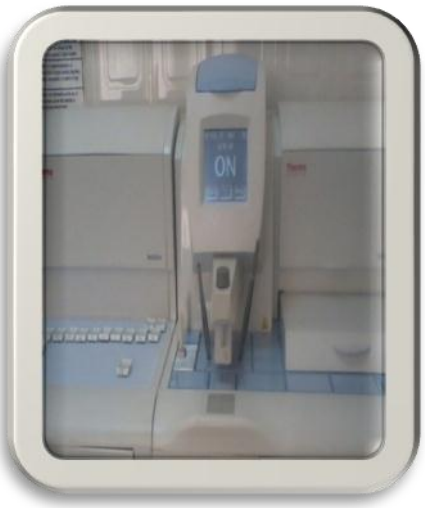
- **M**echanical protection of slices.
- **P**rotection of chemical dyes.

Dry the slides and then observe the optical microscope equipped with a camera.

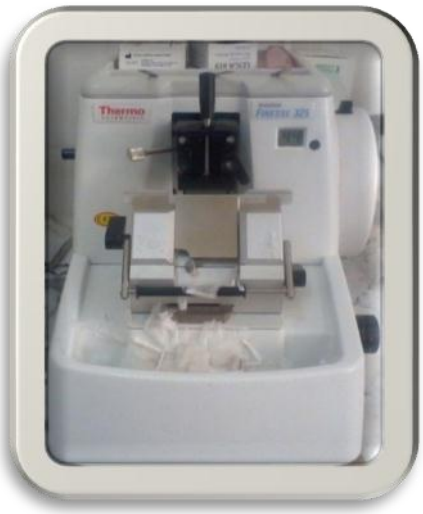
Fixation

Cassette

Circulator



Inclusion apparatus



Microtome

Deshydratation

Blocks of coloration

Colored blades

Figure N° 12. Materials and steps needed for the preparation of slides for the histological study.

3. Statistical analysis

The mean ± SEM values were calculated to determine the significance of intergroup. Each parameter was analysed separately using two ways analysis of variance (**ANOVA**). To find the difference between the groups, ***Student*** **'t'** test was used. ***P*** **<0.05** was considered to be significant.

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CHAPTER III:
RESULTS OF
BIOCHEMICAL MARKERS

RESULTS

1. The body weight

1.1. *Urtica dioica*

Final mean total body weights of **male** rats **(163.2±15.3 g)** after 30 days Hg exposure has decreased considerably compared to the initial weight **(182.2±23.5 g**) with a percentage of **(−10.44%).** Whereas, those of UD and Hg+UD mean total body weight have increased by **(+13.66%)** and **(+9%)**, respectively. However, the final total body weight of the control has risen by **(+16%)** after 30 days.

Final mean total body weights of **female** rats **(152.3±16.4g)** after 30 days Hg exposure has decreased considerably compared to the initial weight **(171± 33.8g)** with a percentage of **(−12.27%).** Whereas, those of UD and Hg+UD mean total body weight have increased by **(+15.11%)** and **(-5.42%),** respectively. However, the final total body weight of the control has risen by **(+16.35%)** after 30 days.

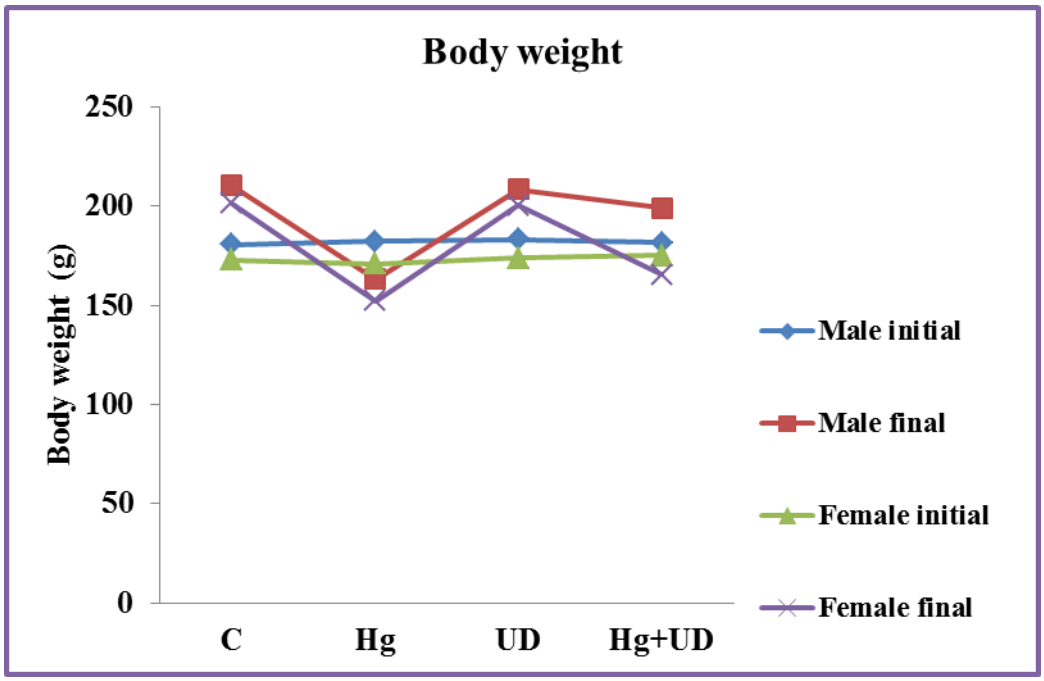

Figure N° 13: Total body weight variations in the groups treated with Hg and /or *U.dioica* for 30 days.

Body weight variations were summarised table **N°05**.

Table N° 05: Total body weight variations in the traited groups by Hg and/or *U.dioica* for 30 days.

	C		Hg		UD		Hg+UD	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Initial (g)	180.5±21,3	173± 27.7	182.2±23.5	171± 33.8	183.2±21.09	174± 22.2	181.6±20.3	175.2±12.6
Final (g)	210.3± 23$^{\#}$	201.3± 21$^{\#}$	163.2±15.3$^{*\#}$	152.3±16.4$^{*\#}$	208.3±16.6$^{\#}$	200.3±14.8$^{\#}$	199.2±23.1$^{*\#}$	165.5±20.3$^{*\#}$

***Significantly different when compared to the control;**

#Significantly different between groups.

1.2. *Raphanus sativus*

Final mean total body weights of **male** rats (**163.2 ± 15.3 g**) after 30 days Hg exposure has decreased considerably compared to the initial weight (**182.2 ± 23.5 g**) with a percentage of **(−10.44%**). Whereas, those of RS and Hg+RS mean total body weight have increased by **(+13.66%)** and **(+9%),** respectively. However, the final total body weight of the control has risen by **(+16%)** after 30 days.

Final mean total body weights of **female** rats (**152.3 ± 16.4 g**) after 30 days Hg exposure has decreased considerably compared to the initial weight (**171 ± 33.8 g**) with a percentage of **(−12.27%).** Whereas, those of RS and Hg+RS mean total body weight have increased by (**+22.8%)** and **(+6.5%**), respectively. However, the final total body weight of the control has risen by **(+16.35%)** after 30 days.

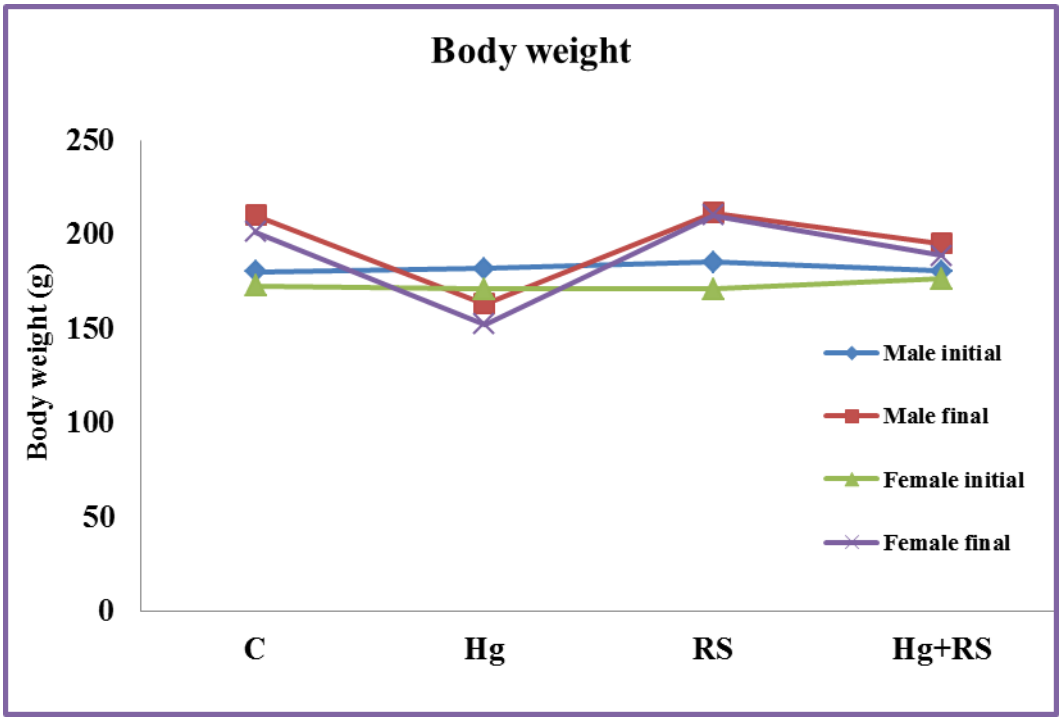

Figure N° 14: Total body weight variations in the groups treated with Hg and/or *R. sativus* for 30 days.

Body weight variations were summarised table **N°06**.

Table N° 06: Total Body weight variations in the traited groups by Hg and/or *R. sativus* for 30 days.

	C		Hg		RS		Hg+RS	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Initial (g)	180.5±21.3	173± 27.7	182.2±23.5	171± 33.8	185.3±22.1	171.3±26.4	180.6±19.6	176.5±21.9
Final (g)	210.3± 23$^{\#}$	201.3± 21$^{\#}$	163.2±15.3$^{*\#}$	152.3±16.4$^{*\#}$	211.6±18.9$^{\#}$	210.3±21.8$^{\#}$	195.2±24.6$^{*\#}$	188.8±21.9$^{\#}$

***Significantly different when compared to the control;**

#Significantly different between groups.

2. The absolute organs' weights

2.1. Urtica dioica

Our results indicate that there has been a significant increase in the absolute liver and kidney weight in Hg of male's rats comparing to the control group. Concerning the absolute kidney weight in males, there is a significant difference between all groups. Meanwhile, there has been a decrease in the absolute liver weight in female rats treated by Hg. On the other side, the supplementation of *Urtica Dioica* presents no significant difference between (Hg+ UD) group and the control.

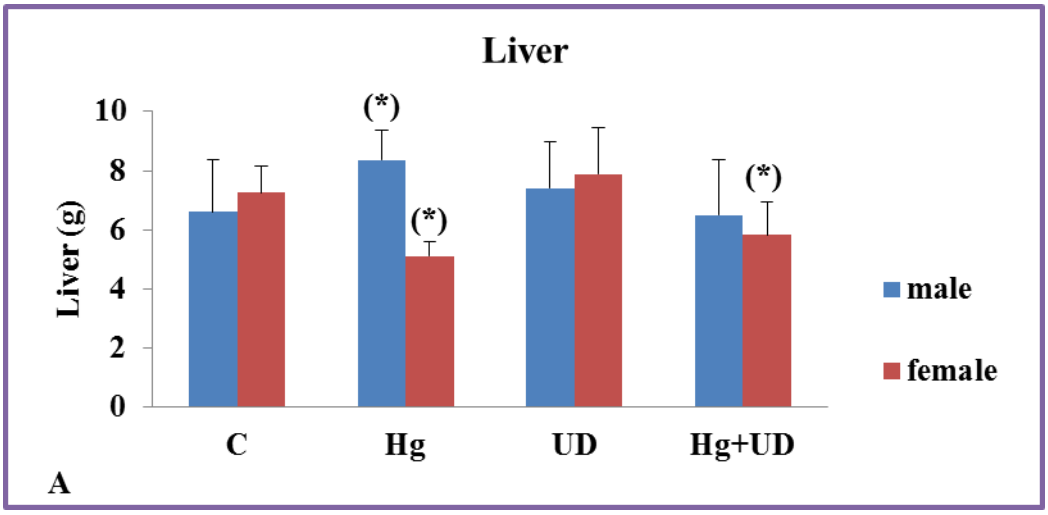

Figure N° 15: Absolute weight variations of liver in groups treated by Hg and/or *U. dioica* for 30 days.

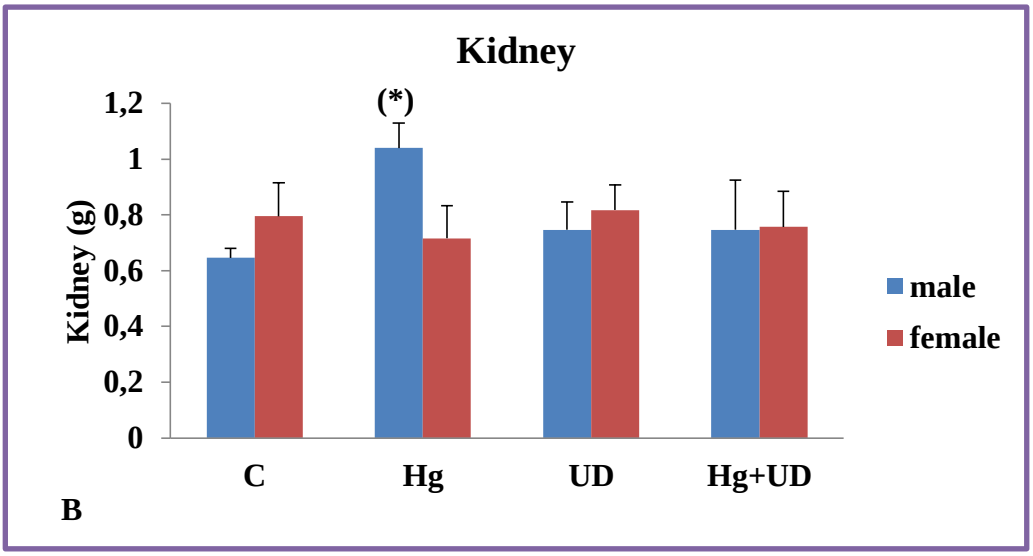

Figure N° 16: Absolute weight variations of kidney in groups treated by Hg and/or *U. dioica* for 30 days.

Organ's weight variations were summarised in table **N° 07**.

Table N° 07: Organ's weight variations in the treated groups by Hg and/or *U.dioica* for 30 days.

	C		Hg		UD		Hg+UD	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Liver(g)	6.6 ± 1.79	7.25 ± 0.92	8.35 ± 1.01*	5.12 ± 0.45*	7.40 ± 1.58	7.89 ± 1.55	6.49 ± 1.9	5.82 ± 1.11*
Kidney(g)	0.64 ± 0.03#	0.79 ± 0.12	1.04 ± 0.09*#	0.71 ± 0.11	0.74 ± 0.10#	0.8 ± 0.09	0.74 ± 0.17#	0.75 ± 0.12#

***Significantly different when compared to the control;**

#Significantly different between groups.

2.2. *Raphanus sativus*

Concerning organs, an increase of absolute liver and kidney weight of males treated by Hg was observed. In other side, there has been a decrease in the absolute liver weight of females treated by Hg. Contrary; the combined treatment with *R. sativus* has led to a slight increase in absolute weights of males' liver and kidney, accompanied with a significant decrease of females' liver weight.

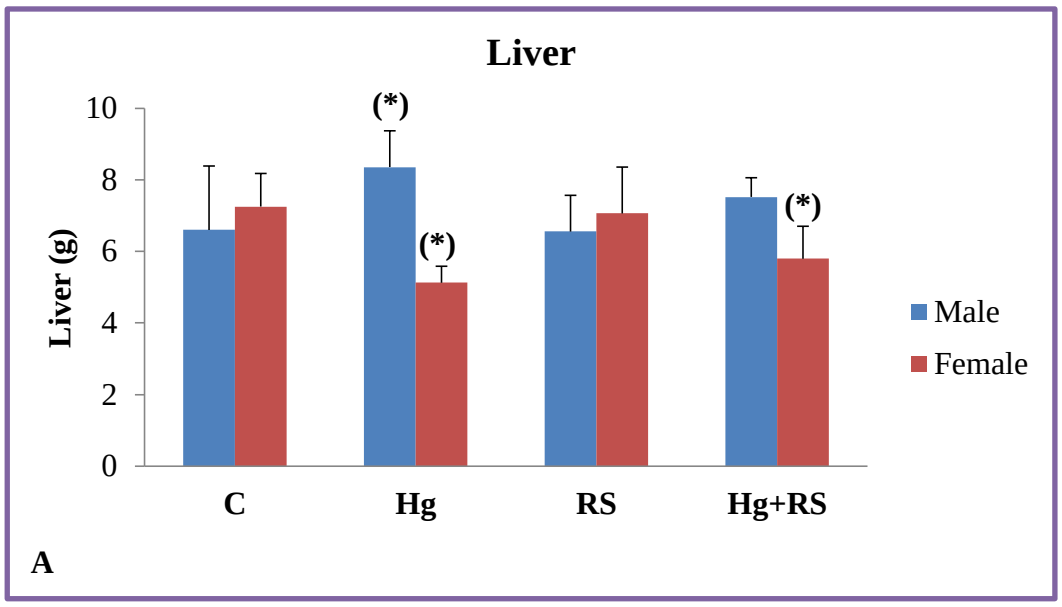

Figure N° 17: Absolute weight variations of liver in groups treated by Hg and/or *R. sativus* for 30 days.

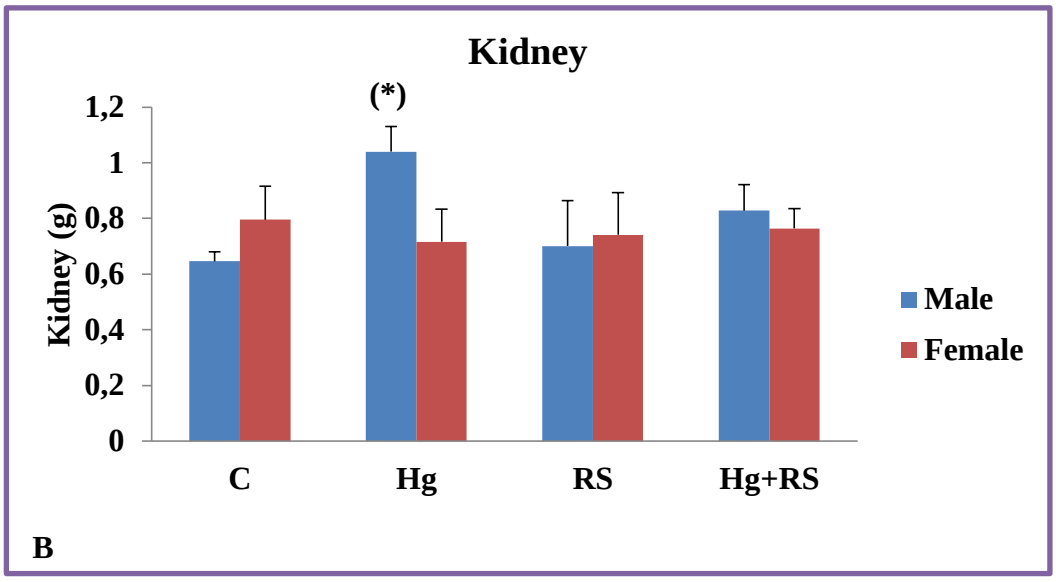

Figure N° 18: Absolute weight variations of kidney in groups treated by Hg and/or *R. sativus* for 30 days.

Organ's body weight variations were summarised in table **N° 08**.

Table N° 08: Organ's body weight variations in the treated groups by Hg and/or *R. sativus* for 30 days.

	C		Hg		RS		Hg+RS	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Liver(g)	6.6 ± 1.79	7.25 ± 0.92	8.35 ± 1.01*	7.06 ±1.29	6.56± 1.01	5.12 ± 0.45*	7.51±0.54	5.79 ±0.9*
Kidney(g)	0.64 ± 0.03#	0.79 ± 0.12	1.04 ± 0.09*#	0.741± 0.15	0.7 ±0.16#	0.71 ± 0.11	0.82± 0.09#	0.76±0.07

***Significantly different when compared to the control;**

#Significantly different between groups.

3. The biochemical markers

3.1. *Urtica dioica*

During the course of present investigations, it was observed that the treatment with Hg caused a significant increase in the concentration of glucose and triglycerides in male Wistar rats compared to the control, but no difference was recorded in both sexes of rats treated with Hg+UD and UD alone.

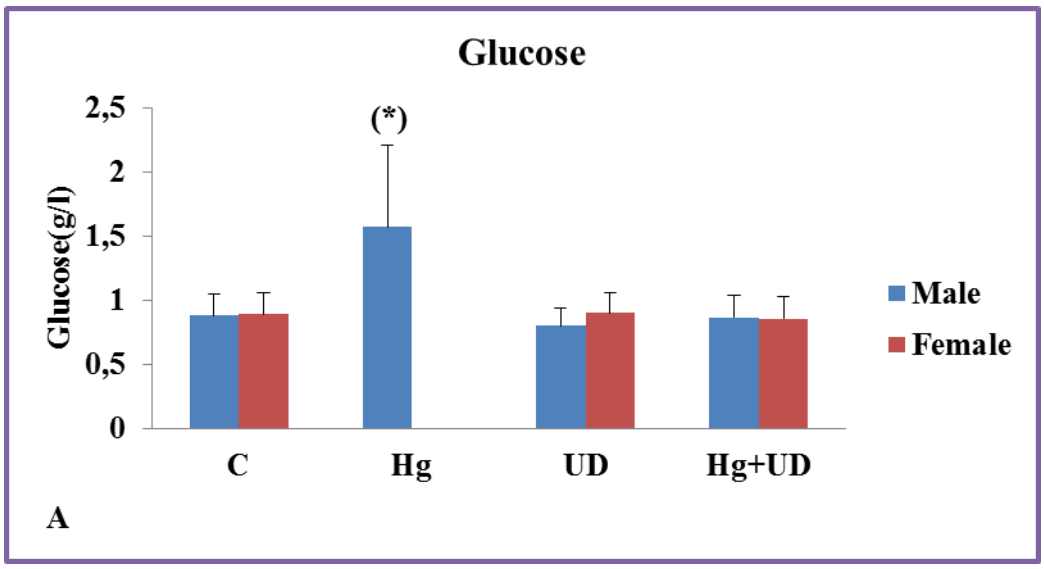

Figure N° 19. The effects of Hg and/or *U.dioica* on glucose level of rats after 30 days.

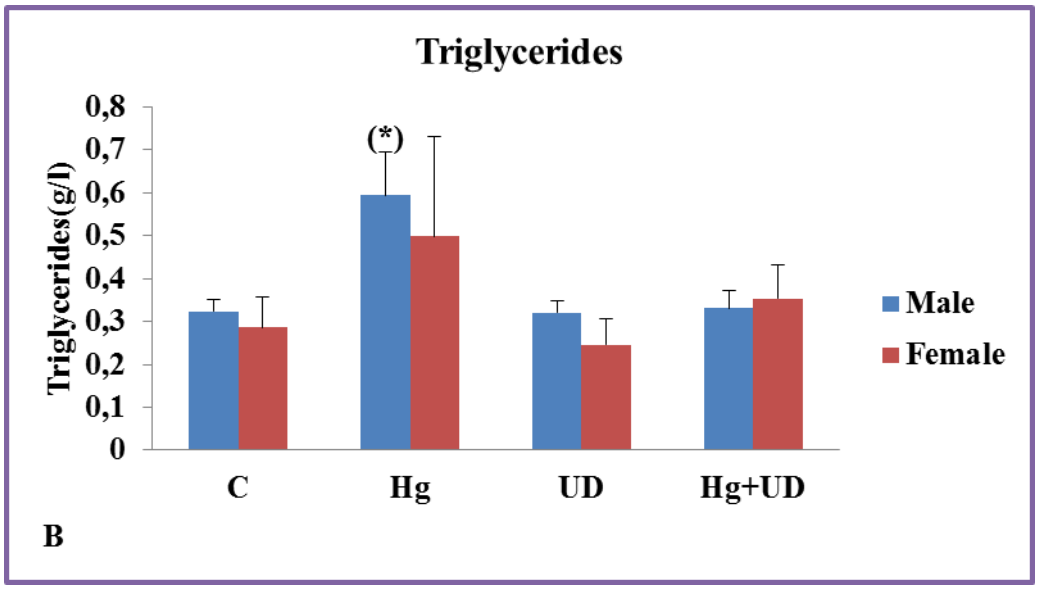

Figure N° 20. The effects of Hg and/or *U.dioica* on triglycerides level of rats after 30 days.

This study demonstrates in both sexes that the treatment of rats with Hg has led to a pronounced elevation of urea and creatinine concentration. The supplementation of rats with Hg+UD or *U.dioica* alone recorded no noticeable change of these markers. Besides, creatinine concentration shows a signification between all groups in both sexes.

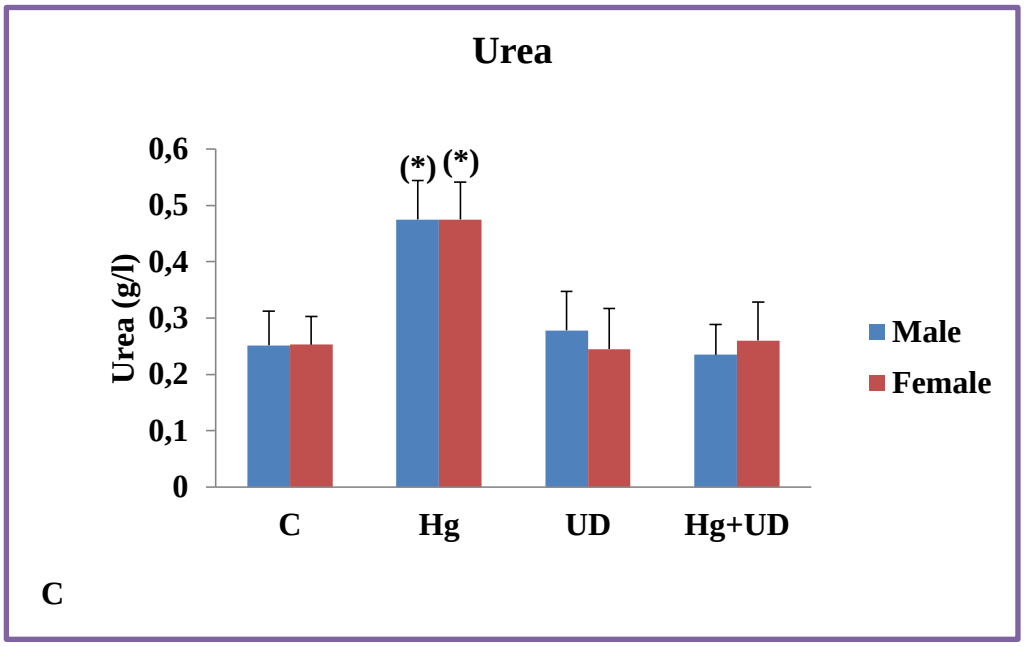

Figure N° 21. The effects of Hg and/or *U.dioica* on urea level of rats after 30 days.

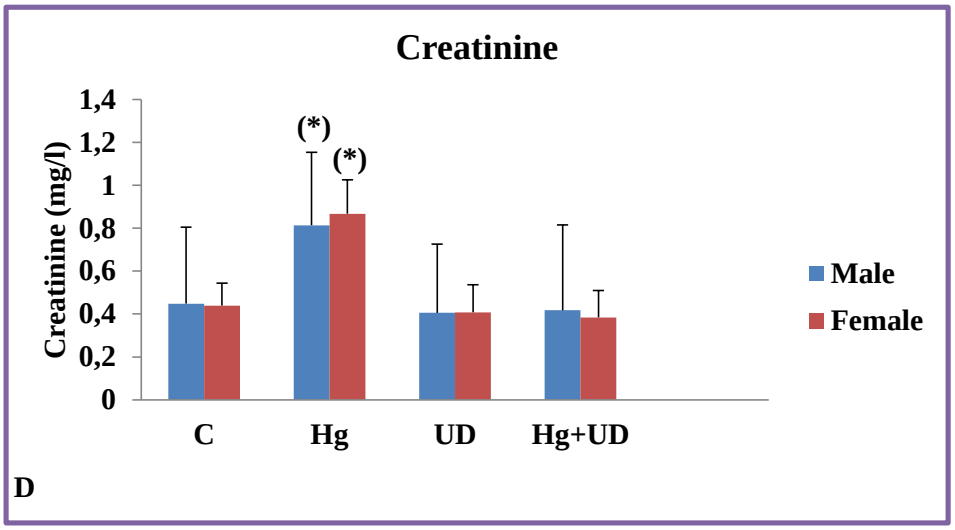

Figure N° 22. The effects of Hg and/or *U.dioica* on creatinine level of rats after 30 days.

These results indicate that the plasma AST, ALT and ALP activities were significantly increased by mercury in both sexes compared to the control group, but *U. dioica* has exerted its effectiveness towards the ALT and ALP only. Furthermore, ALP activity was significantly different between all groups only in female rats.

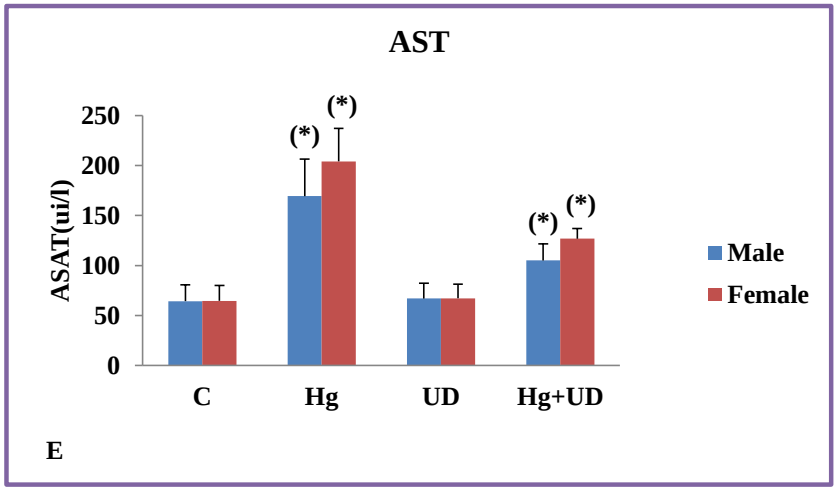

Figure N° 23: The effects of Hg and /or *U. Dioica* on AST activity of rats after 30 days.

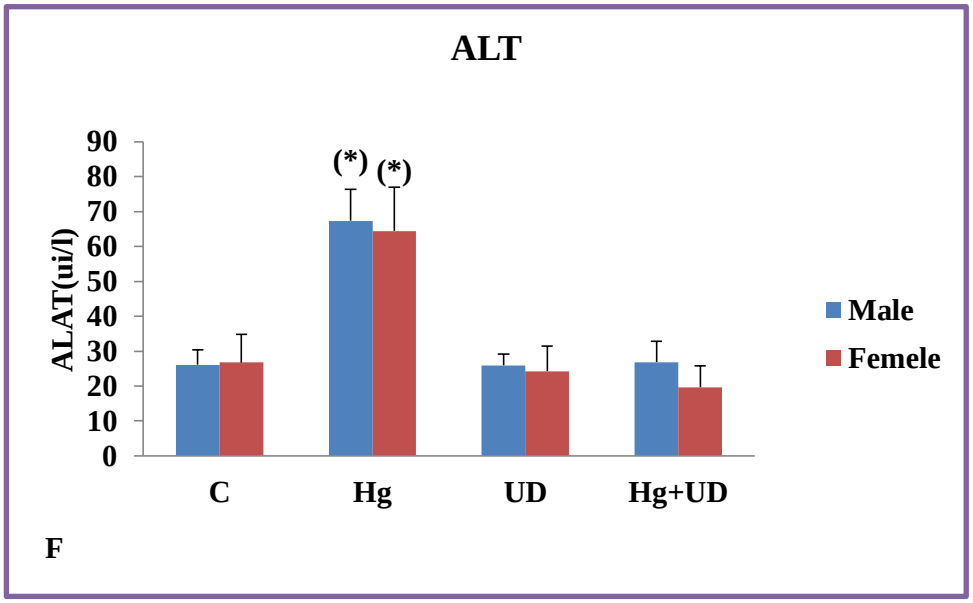

Figure N° 24: The effects of Hg and /or *U. Dioica* on ALT activity of rats after 30 days.

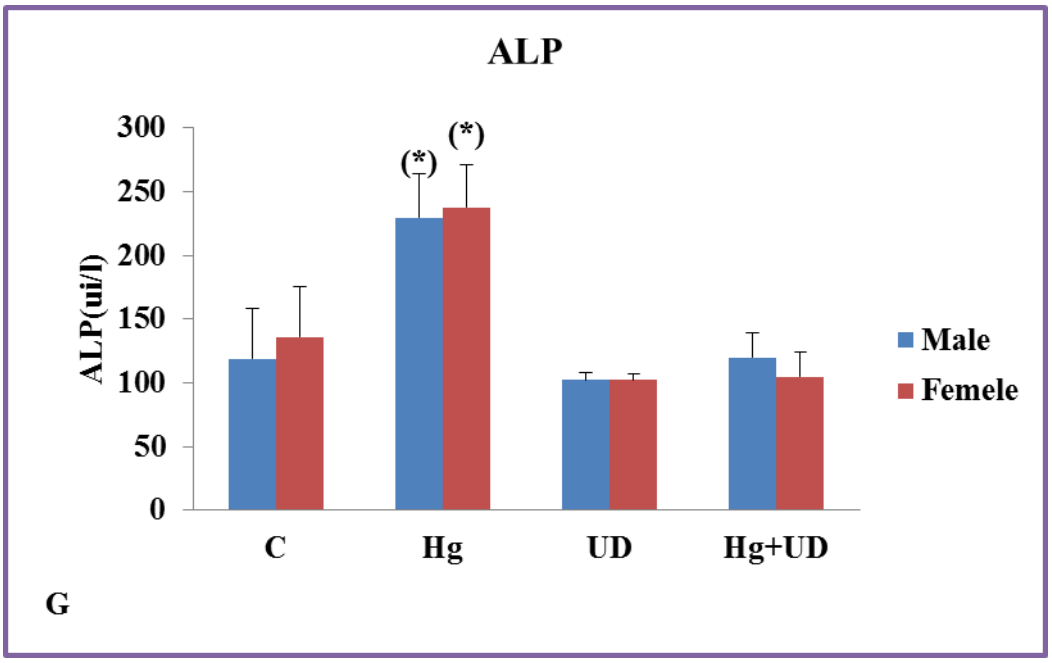

Figure N° 25: The effects of Hg and /or *U. Dioica* on ALP activity of rats after 30 days.

Moreover, results showed a significant decrease in the level of Mg, Fe and Ca in the Hg group. However, the treatment with *U. Dioica* and/or Hg prevented the decrease of Mg, Fe and Ca induced by Hg treatment compared with the control. Moreover; Mg and Ca concentrations were significantly different between all groups.

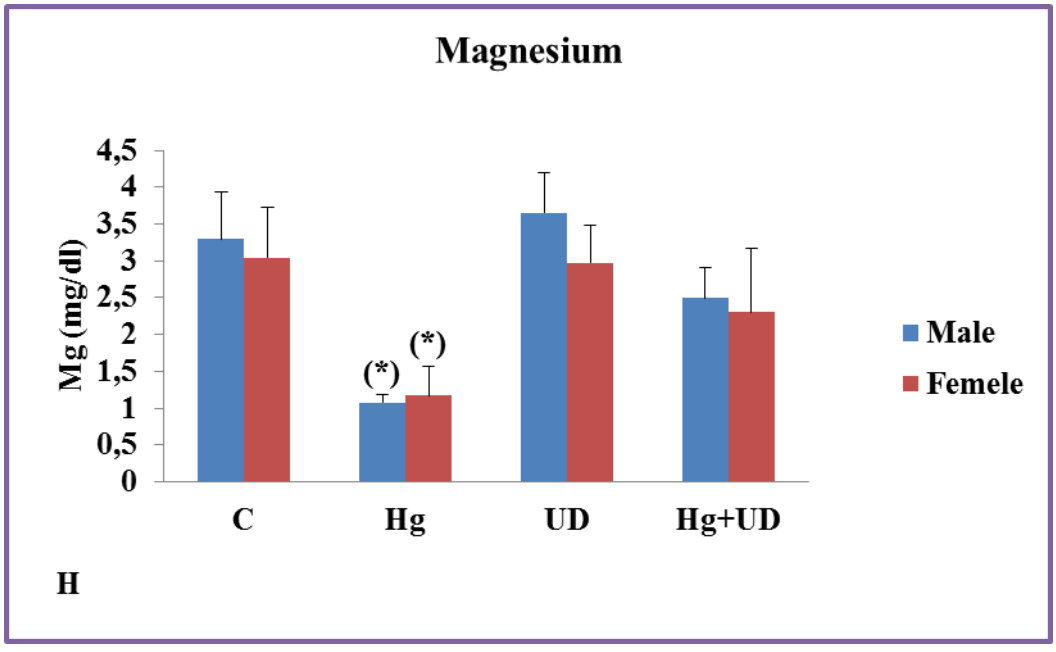

Figure N° 26: The effects of Hg and /or *U. Dioica* on Mg concentration of rats after 30 days.

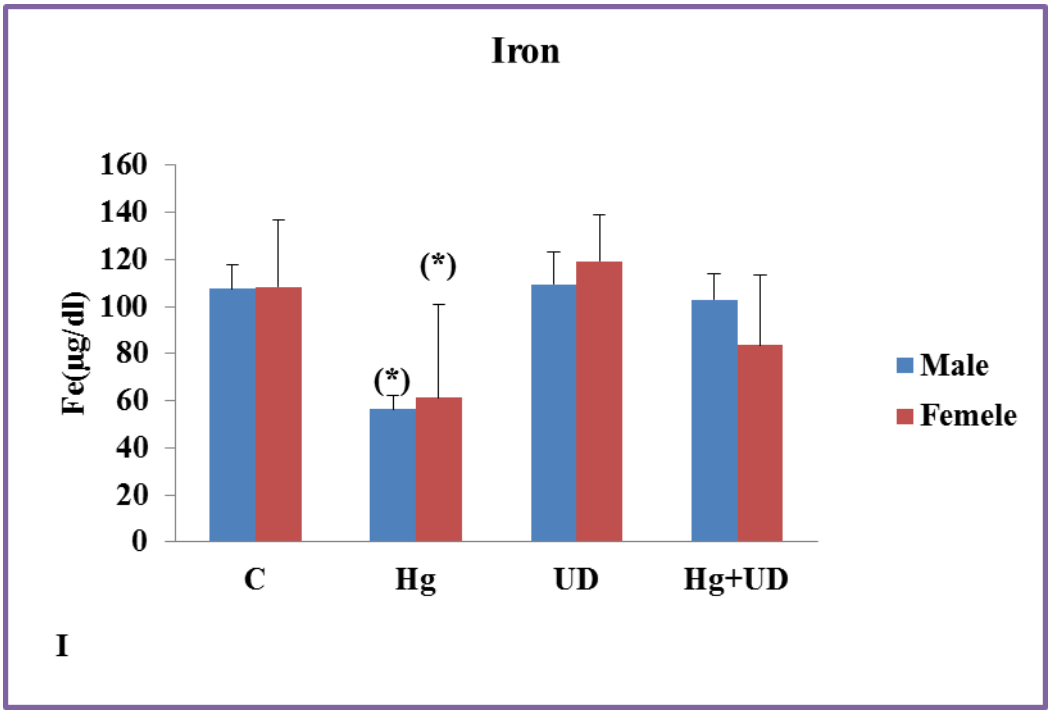

Figure N° 27: The effects of Hg and/or *U. Dioica* on Fe concentration of rats after 30 days.

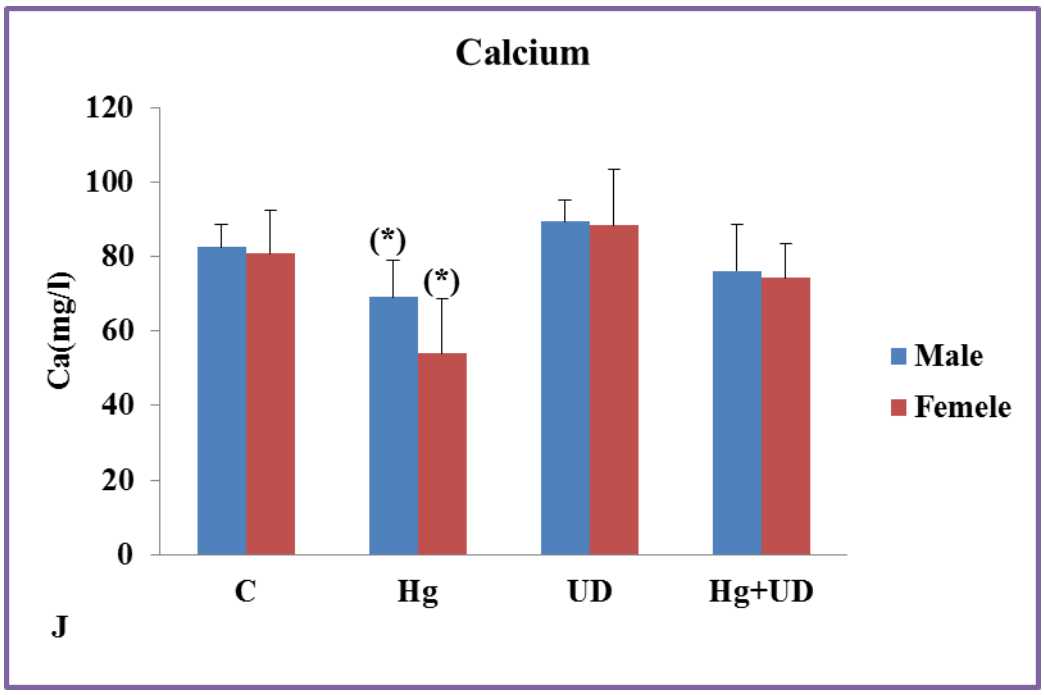

Figure N° 28: The effects of Hg and/or *U. Dioica* on Ca concentration of rats after 30 days.

Table N°09: The ameliorative Effect of *U.dioica* on biochemical markers of rats after 30 days Hg intoxication.

	C		Hg		UD		Hg+UD	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Glucose (g/l)	0.88±0.16	0.89±0.22	1.57± 0.64*	……..	0.8± 0.13	0.9± 0.15	0.86±0.16	0.850±.17
Triglycerides (g/l)	0.32±0.02	0.28±0.07	0.59±0.1*	0.49±0.23	0.32±0.02	0.24± 0.06	0.33±0.04	0.35±0.07
Urea (g/l)	0.25±0.06	0.25±0.05	0.47±0.06*	0.47±0.06*	0.27±0.06	0.24± 0.07	0.23±0.05	0.26±0.06
Creatinine (mg/l)	0.44±0.45#	0.43±1.05#	0.81±0.34*#	0.86±0.15*#	0.40± 0.32#	0.40±0.12#	0.40±0.39#	0.43±0.12#
AST (UI/L)	64.3±16.2	64.5±15.4	169.5±36.9*	204.2±33.2*	66.9±15.2	67.1±14.2	105.1±16.5*	126.9±10.2*
ALT (UI/L)	26.05±4.38	26.73±8.05	67.31±9*	64.4±12.6*	25.95±3.18	24.23±7.17	26.86±6.03	19.67±6.08
ALP (UI/L)	118.9±20.6	135.9±39.7#	229.4±20.8*	237.3±34.1*#	102.4±10.3	10.08±5.16#	120±15.4	104.6±19.2#
Mg (mg/l)	3.3±0.63#	3.04±0.67#	1.08± 0.1*#	1.17±0.38*#	3.65±0.54#	2.97±1.01#	2.49±0.53*#	2.3±0.52*#
Fe (µg/dl)	107.6±1.2	108.3±28.3	56.5± 5.68*	61.3±39.5*	109.5±13.7	119.3±19.6	102.9±11.1	83.7±29.5
Ca (mg/l)	82.6±6.07#	80.8±11.8	69.14±9.88*#	54±14.7*	89.42±5.63#	88.5±61.63	76.2±12.4#	74.37±9.14

Biochemical markers were summarised in table **N° 09**.

***Significantly different when compared to the control;**
#Significantly different between groups.

3.2. *Raphanus sativus*

The mercury treated males caused a significant elevation of plasma glucose and triglycerides when compared to the control. On the other hand, rats received *R. Sativus* alone or combined with Hg for 30 days did not show any negative effects.

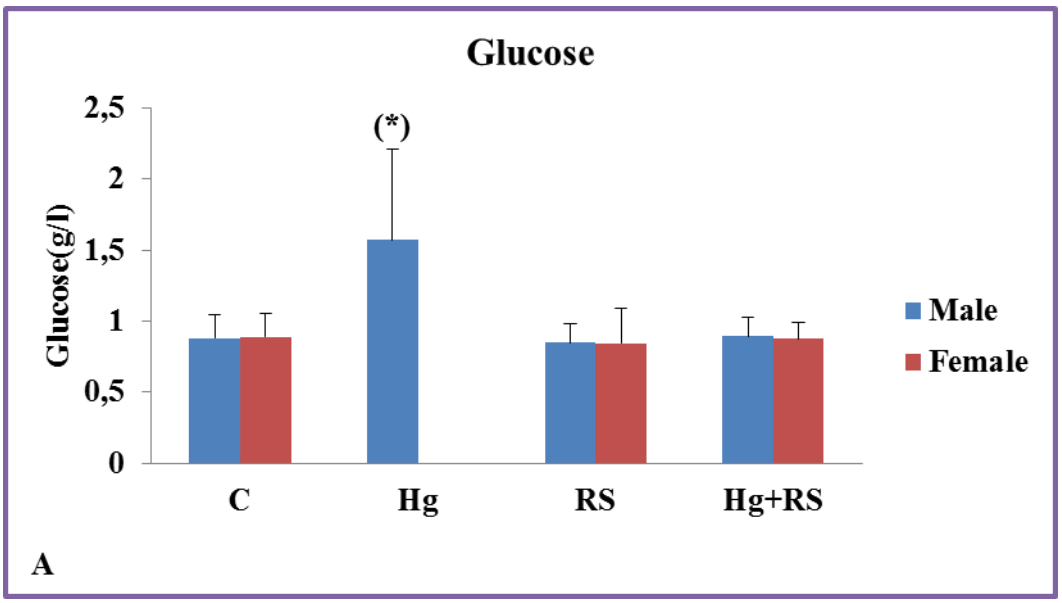

Figure N° 29. The effects of Hg and/or *R. sativus* on glucose level of rats after 30 days.

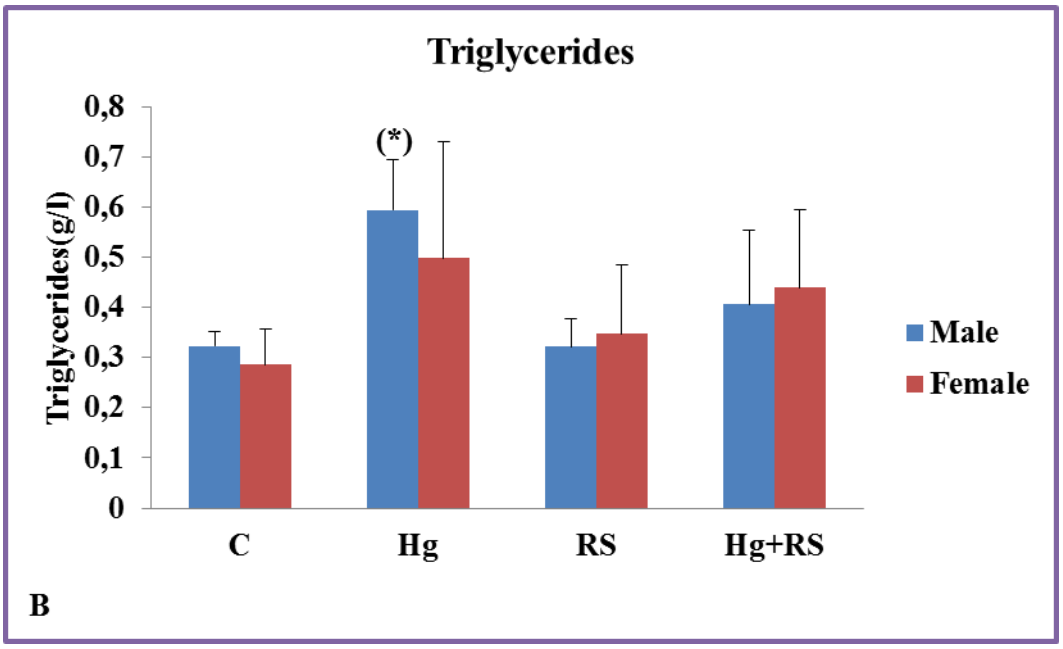

Figure N°30. The effects of Hg and/or *R. sativus* on triglycerides level of rats after 30 days.

Results demonstrate increased levels of plasma urea and creatinine in rats intoxicated by $HgCl_2$ in both sexes compared to the control. Fortunately, the supplementation of *R. sativus* corrected the increased urea and creatinine provoked by Hg. Besides, creatinine concentration shows a signification between all groups in both sexes.

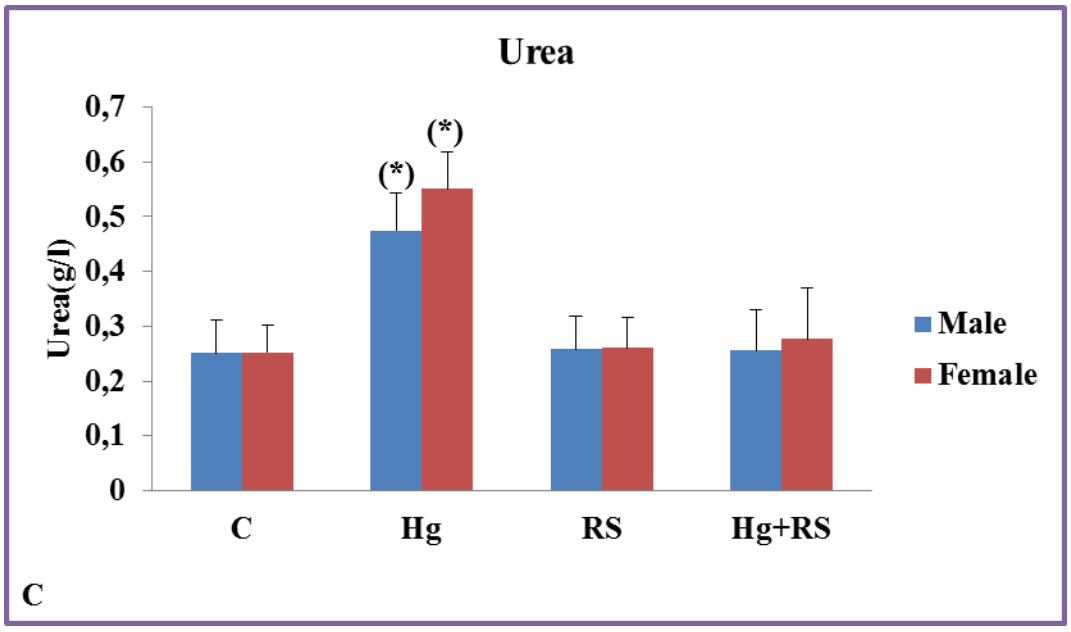

Figure N° 31: The effects of Hg and/or *R. Sativus* on urea level of rats after 30 days.

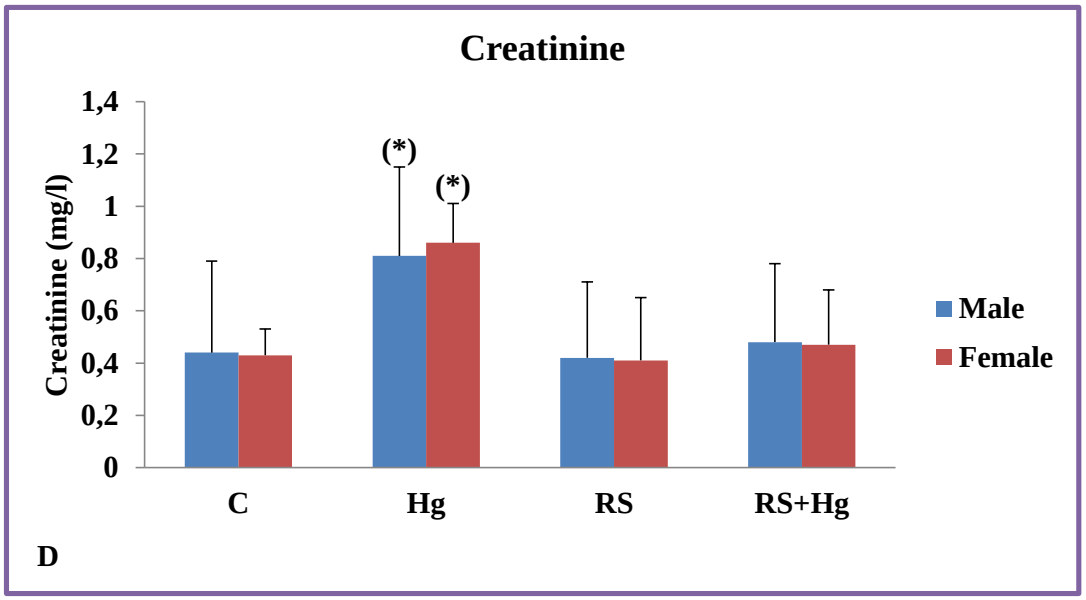

Figure N° 32: The effects of Hg and/or *R. Sativus* on creatinine level of rats after 30 days.

In the present result, a noticeable increase of AST, ALT and ALP activities has been observed in the group under Hg stress. However, the presence of *R. sativus* has suppressed the toxic effect, and the leakage of enzymes into the blood was decreased, except in the combined treatment (Hg+RS), only AST was significantly different than that of the control Furthermore, ALP activity was significantly different between all groups only in females.

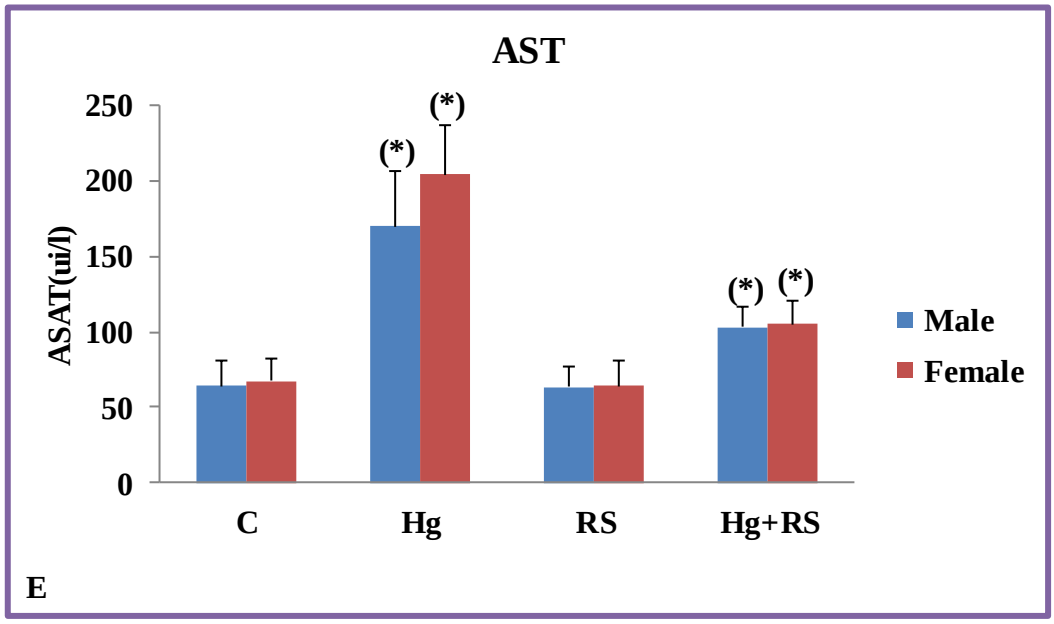

Figure N° 33: The effects of Hg and/or *R. Sativus* on AST activity of rats after 30 days.

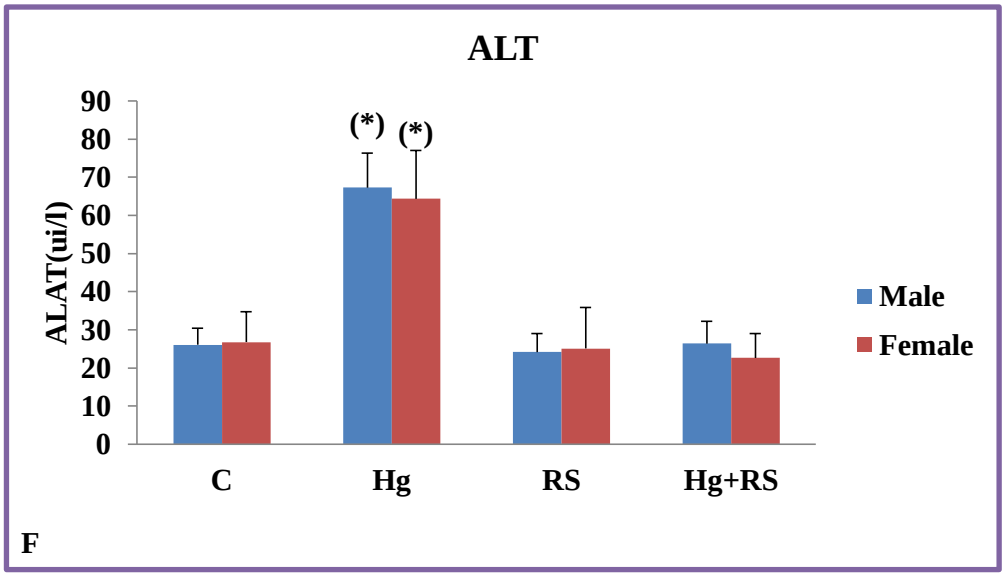

Figure N° 34: The effects of Hg and /or *R. Sativus* on ALT activity of rats after 30 days.

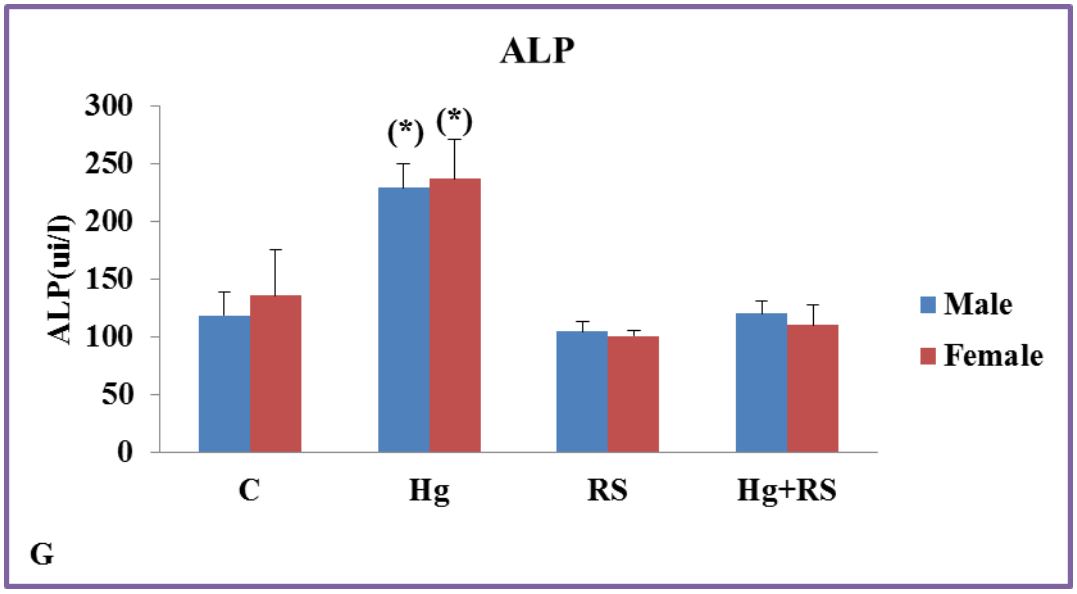

Figure N° 35: The effects of Hg and /or *R. Sativus* on ALP activity of rats after 30 days.

The levels of Mg, Fe and Ca were significantly decreased in rats exposed to Hg, whereas, no variation was noted concerning these minerals in the group treated with *R. sativus*. Nonetheless, in the combined group, there is a significant decrease in the Mg and Ca levels. Moreover; Mg and Ca concentrations were significantly different between all groups.

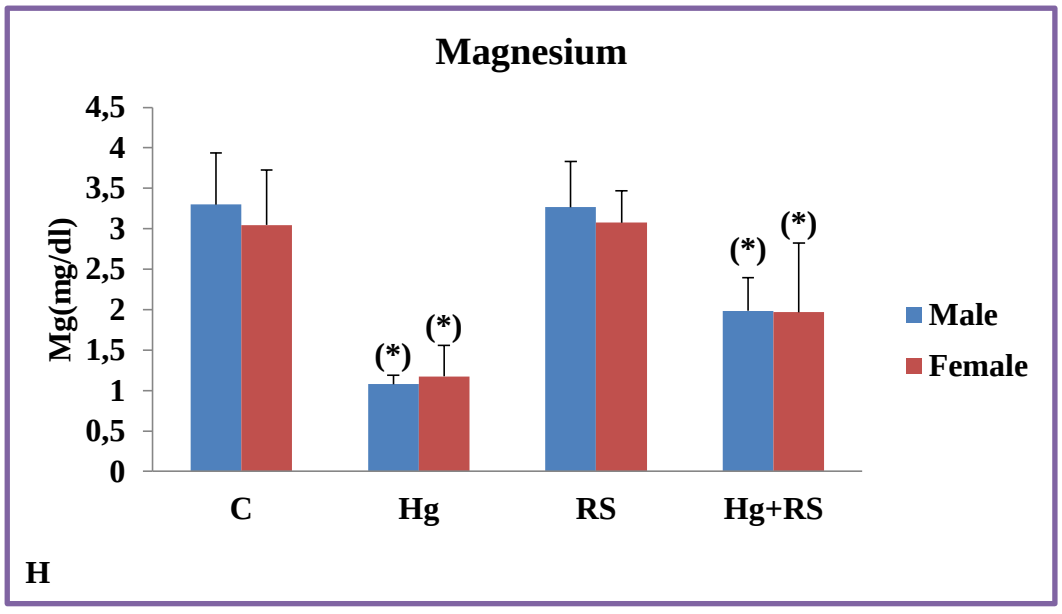

Figure 36: The effects of Hg and /or *R. Sativus* on Mg concentration of rats after 30 days.

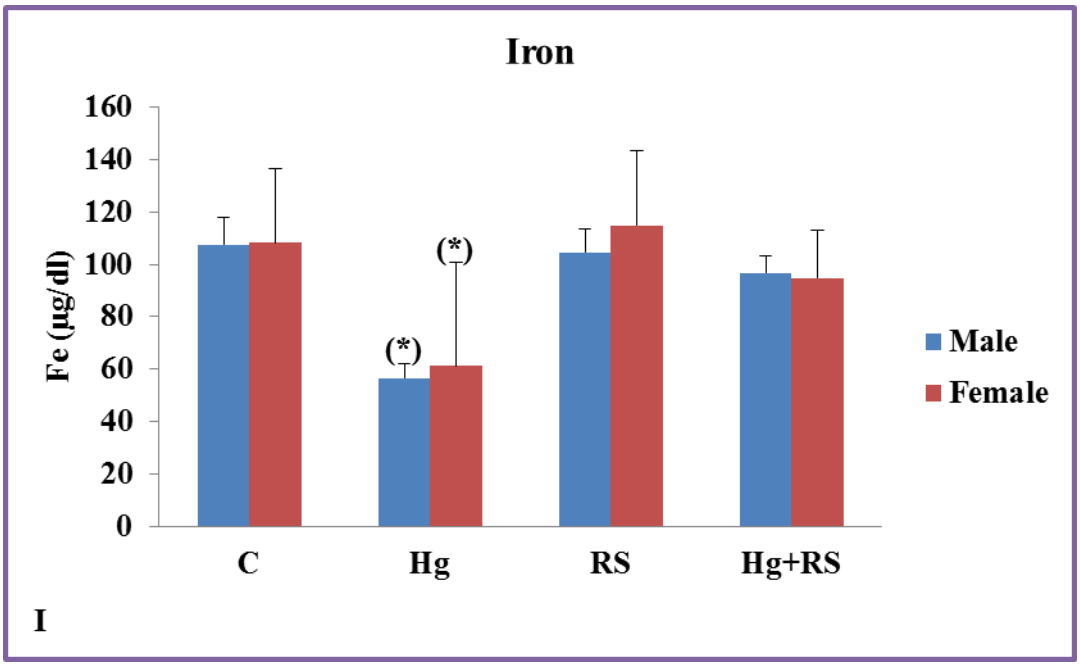

Figure N°37: The effects of Hg and /or *R. Sativus* on Fe concentration of rats after 30 days.

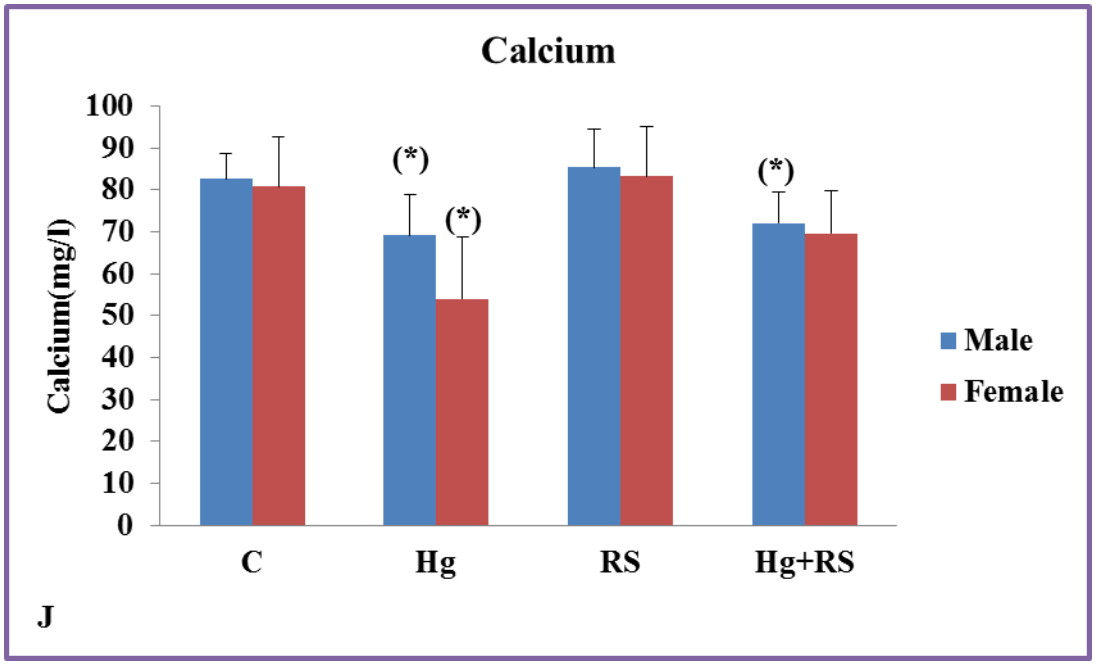

Figure N° 38: The effects of Hg and /or *R. Sativus* on Ca concentration of rats after 30 days.

Biochemical markers were summarised in table N° 10.

Table N°10: The ameliorative Effect of *R .sativus* on biochemical markers of rats after 30 days Hg intoxication.

	C		Hg		RS		Hg+RS	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Glucose (g/l)	0.88±0.16	0.89±0.22	1.57± 0.64*	……	0.84±0.13	0.84±0.24	0.89±0.13	0.87±0.11
Triglycerides (g/l)	0.32±0.02	0.28±0.07	0.59±0.1*	0.49±0.23	0.32±0.05	0.34±0.13	0.40±0.14	0.44±0.15
Urea (g/l)	0.25±0.06	0.25±0.05	0.47±0.06*	0.47±0.06*	0.25±0.05	0.26±0.05	0.25±0.07	0.27±0.09
Creatinine (mg/l)	0.44±0.45#	0.43±1.05#	0.81±0.34*#	0.86±0.15*#	0.42±0.29#	0.41±0.24#	0.48±0.3#	0.47±0.21#
AST (UI/L)	64.3±16.2	64.5±15.4	169.5±36.9*	204.2±33.2*	63.6±13.5	64.2±16.7	102.9±14.2*	105.2±15*
ALT (UI/L)	26.05±4.38	26.73±8.05	67.31±9*	64.4±12.6*	24.18±4.86	25.04±10.8	26.37 ±5.8	22.65±6.39
ALP (UI/L)	118.9±20.6	135.9±39.7#	229.4±20.8*	237.3±34.1*#	104.93±8.93	101.21±5.21#	120.4±11	110.8±17#
Mg (mg/l)	3.3±0.63#	3.04±0.67#	1.08± 0.1*#	1.17±0.38*#	3.26±0.56#	3.07±0.39#	1.98±0.41*#	1.96 ±0.85*#
Fe (µg/dl)	107.6±10.2	108.3±28.3	56.5± 5.68*	61.3±39.5*	104,53±9.18	114.9±28.6	96.59±6.55	94.85±17.9
Ca (mg/l)	82.6±6.07#	80.8±11.8	69.14±9.88*#	54±14.7*	85.44±9.08#	83.2±12	72.04±7.48*#	69.6 ±10.3

***Significantly different when compared to the control;**
#Significantly different between groups.

4. GSH

4.1. *Urtica dioica*

A significant decrease of hepatic GSH level was observed in males and females exposed to mercury, but its concentration was remarkably raised in the UD group, followed by Hg+UD group with less extent. Renal GSH concentration was significantly lower in the Hg exposed animals compared to the control and the UD group as well.

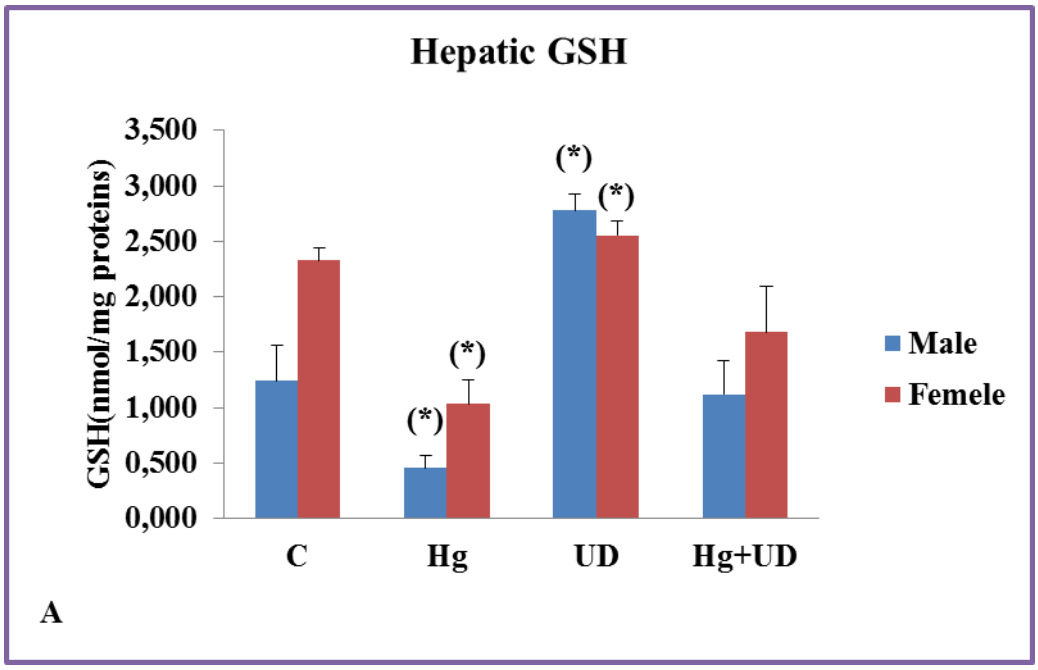

Figure N°39: The effects of Hg and /or *U. Dioica* on hepatic GSH of rats after 30 days.

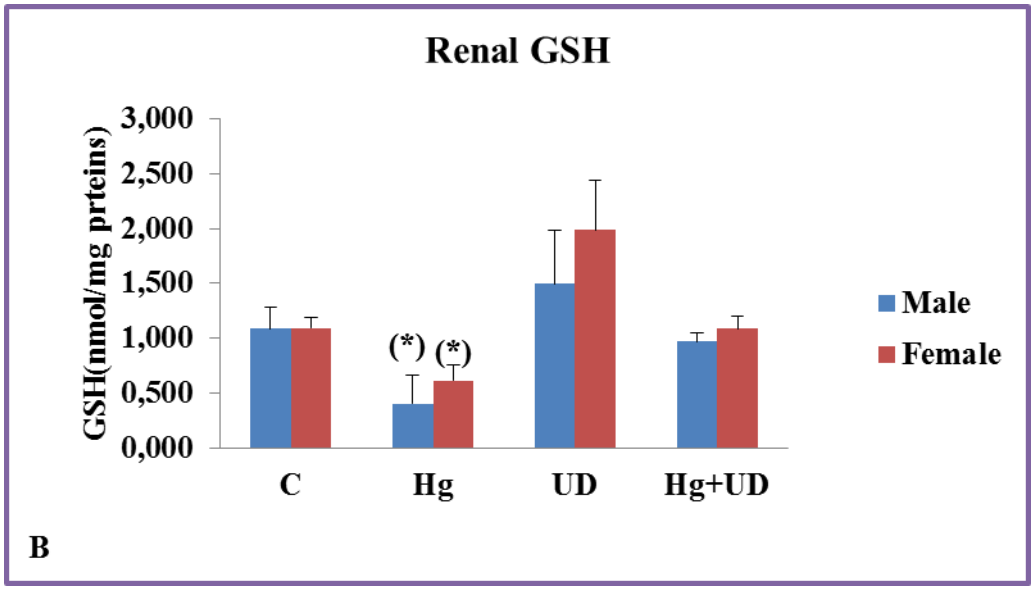

Figure N°40: The effects of Hg and/or *U. Dioica* on hepatic GSH of rats after 30 days.

GSH data was summarised in table **N° 11**.

Table N°11: The ameliorative effects of *U.dioica* on hepatic and renal GSH (nmol/mg protein) of rats after 30 days Hg intoxication.

	C		Hg		UD		Hg+UD	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Liver (nmol/mg prot)	1.24±0.11$^{\#}$	2.34±0.11$^{\#}$	0.45±0.22$^{*\#}$	1.14±0.22$^{*\#}$	2.78±0.14$^{*\#}$	2.58±0.14$^{*\#}$	1.06±0.41$^{\#}$	1.72±0.41$^{\#}$
Kidney (nmol/mg prot)	1.08±0.19	1.09±0.09	0.4±0.25*	0.61±0.14*	1.49±0.48	1.98±0.45	0,96±0,08	1.08±0.11

***Significantly different when compared to the control;**

#Significantly different between groups.

4.2. *Raphanus sativus*

The levels of hepatic and renal GSH are significantly decreased in Hg group compared to the control. Compared to the control, no observed variations were recorded in hepatic and renal GSH when supplemented with the *R. sativus* and also with (Hg+RS) in both sexes.

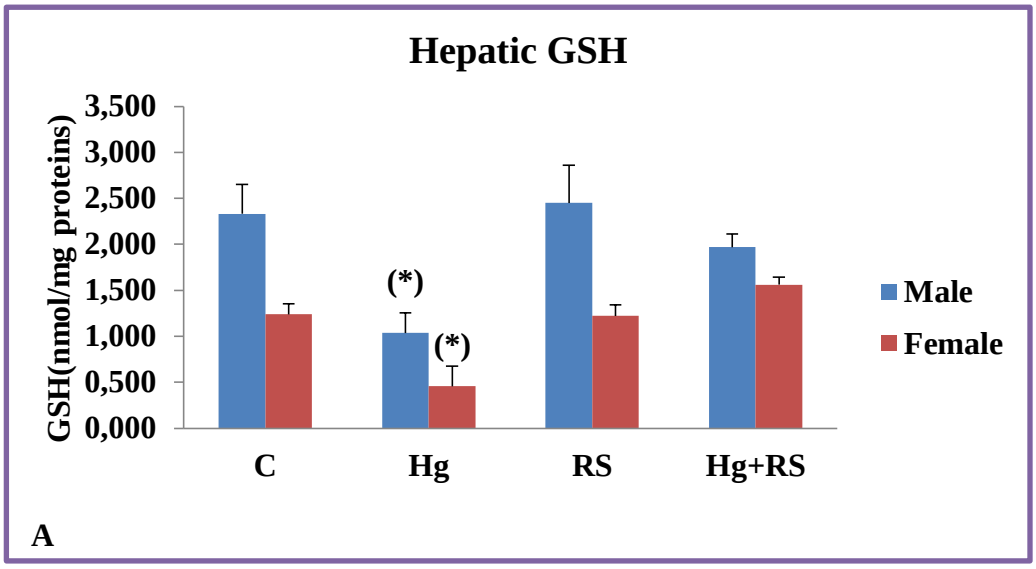

Figure N° 41: The effects of Hg and/or *R. Sativus* on hepatic GSH of rats after 30 days.

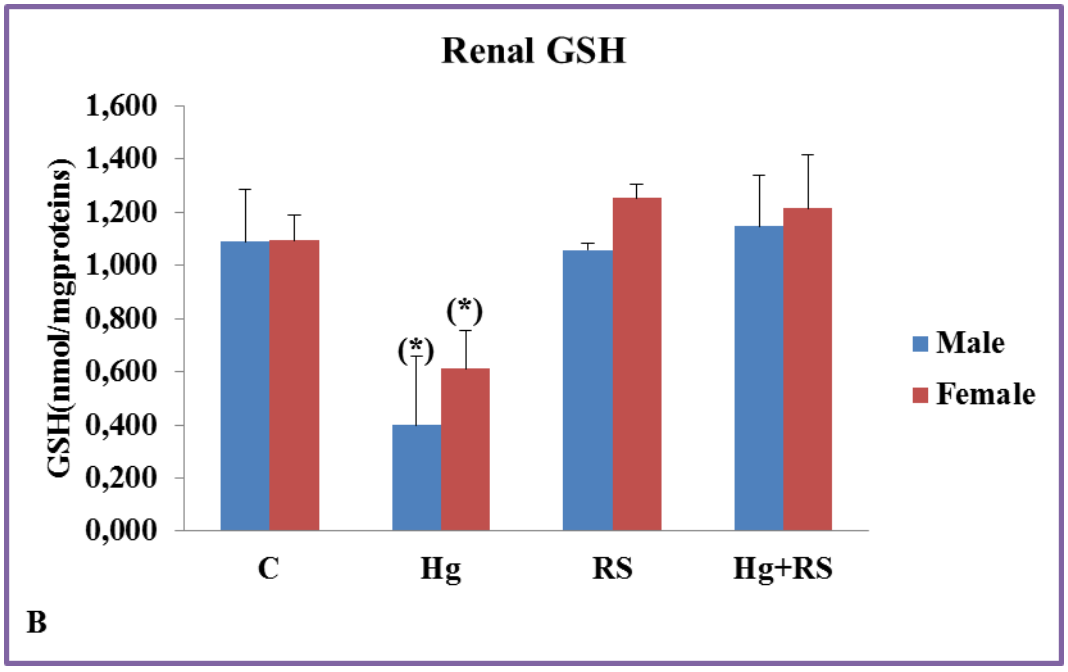

Figure N°42: The effects of Hg and/or *R. Sativus* on renal GSH of rats after 30 days.

GSH data are summarised in table **N°12**.

Table N°12: The ameliorative effects of *R. sativus* on hepatic and renal GSH (nmol/mg protein) of rats after 30 days Hg intoxication.

	C		Hg		RS		Hg+RS	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Liver (nmol/mg prot)	1.24±0.11#	2.34±0.11#	0.45±0.22*#	1.14±0.22*#	2.78±0.14*#	2.58±0.14*#	1.06±0.41#	1.72±0.41#
Kidney (nmol/mg prot)	1.08±0.19#	1.09±0.09	0.4±0.25*#	0.61±0.14*	1.05 ±0.02#	1.25± 0.05	1.14±0.19#	1.21 ±0.2#

***Significantly different when compared to the control;**

#Significantly different between groups.

5. Histological profile

5.1. Liver

Histological studies on liver tissues **(figure 43, 44)** following administration of $HgCl_2$ during 30 days showed remarkable histo-pathological changes in the liver of both females and males characterized by a congestion of the centrilobular vein and a destruction of liver cells at this dose; dissimilar to the control when observed. It has also been established that $HgCl_2$ treatment can cause liver injury and even failure in both animals and humans. These severe alterations affect liver function and can contribute to a malfunction of this organ.

Treatment with *U. dioica* and *R. sativus* alone or/and in a combination with Hg lowered the injury in liver tissues. This study proved that both *U. dioica* and *R. sativus* plants protects the liver and increase the antioxidant defence system in rats intoxicated with $HgCl_2$.

5. 2. Kidney

The representative pictures of histo-pathological examination in the kidney tissue are shown in figure **(figure 45, 46)**. Kidney sections from the control group and *U. dioica* and *R. sativus* rats showed intact histological structure of glomeruli and renal tubules. However, abnormalities in kidney of Hg treated rats were detected in renal tissues compared to the control. The main characteristic findings were the appearance of vacuolization and swelling in the endothelium and swelling in the lining epithelium of tubules. However, the co-administration of the *U. dioica* and *R. sativus* with Hg showed marked improvement in their histological structure in comparison to the Hg only.

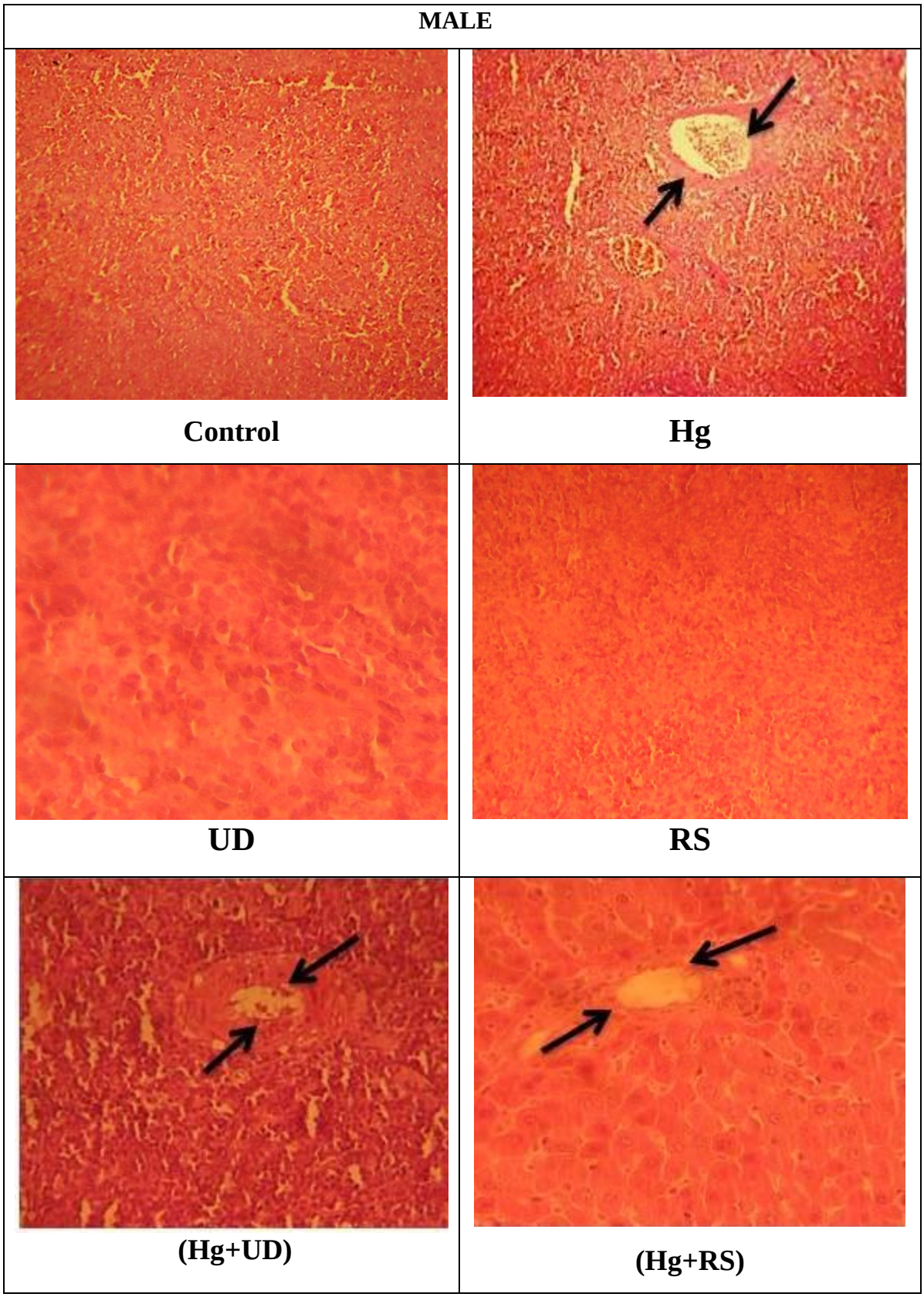

Figure N° 43. Histological profiles of male rats liver showing the control, the Hg ,the UD, the RS and the Hg+UD and Hg+RS groups after 30 days treatments (**x 250**).

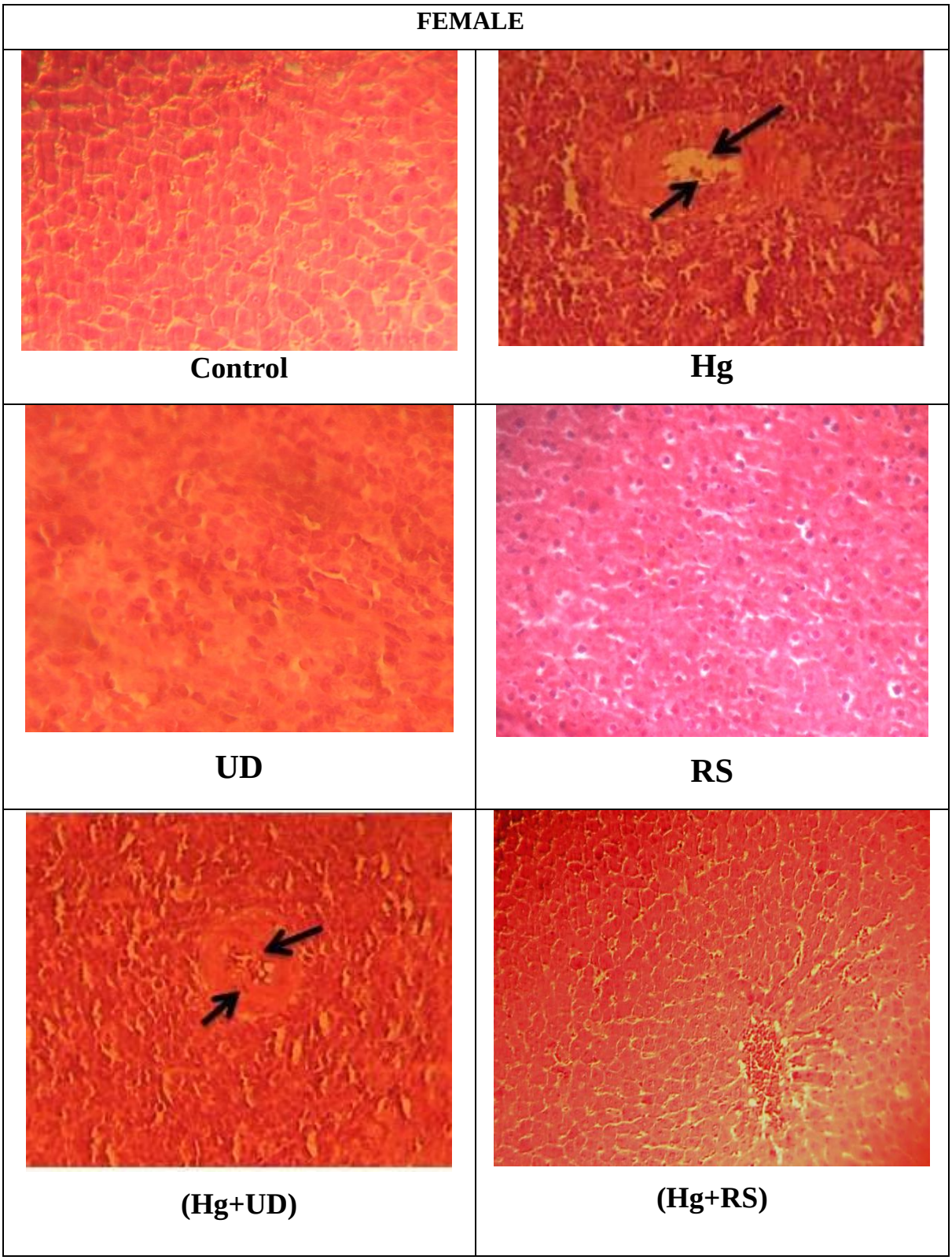

Figure N° 44. Histological profiles of female rats liver showing the control, the Hg ,the UD, the RS and the Hg+UD and Hg+RS groups after 30 days treatments (**x 250**).

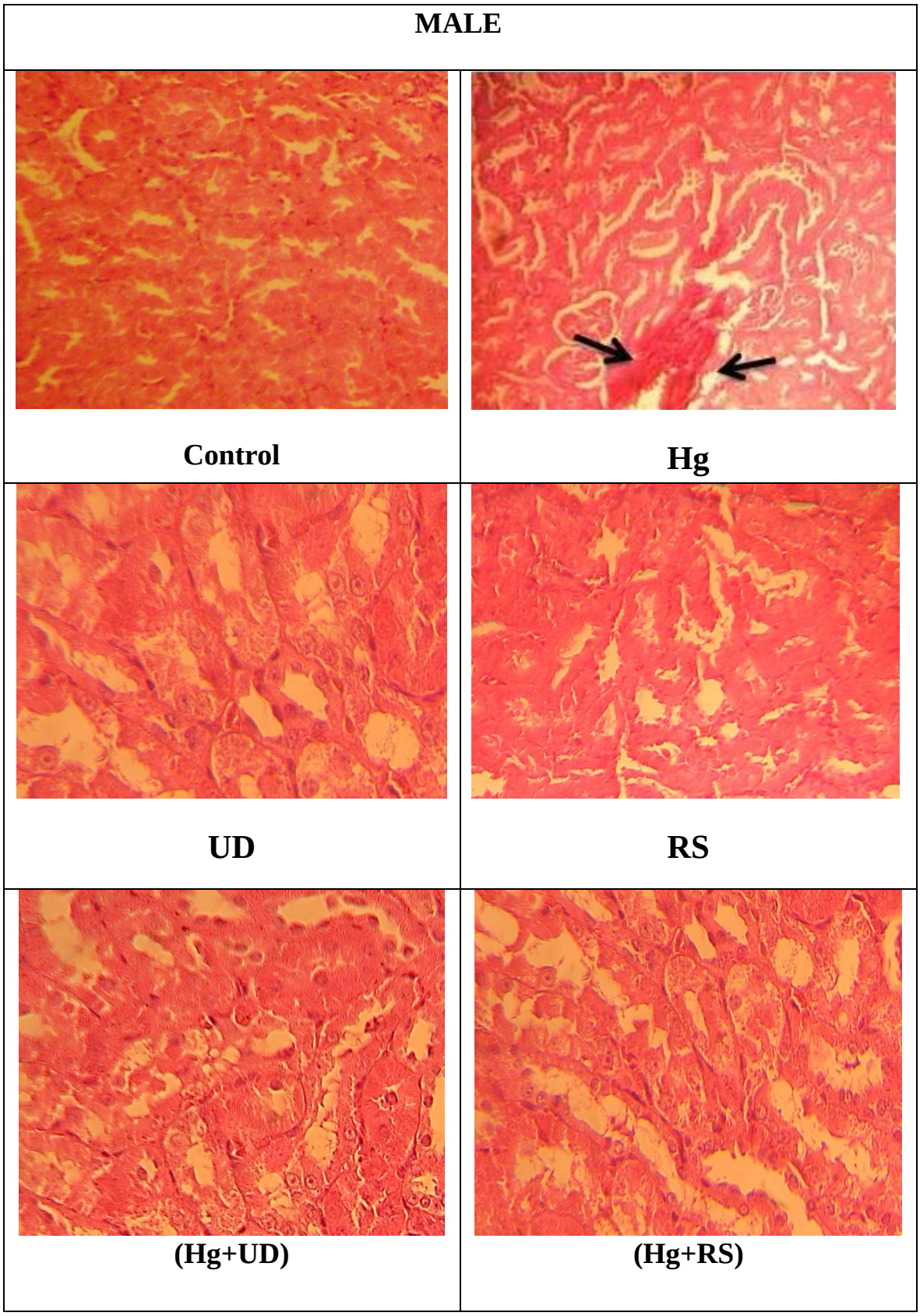

Figure N° 45. Histological profiles of male rats kidneys showing the control, the Hg ,the UD, the RS and the Hg+UD and Hg+RS groups after 30 days treatments (**x 250**).

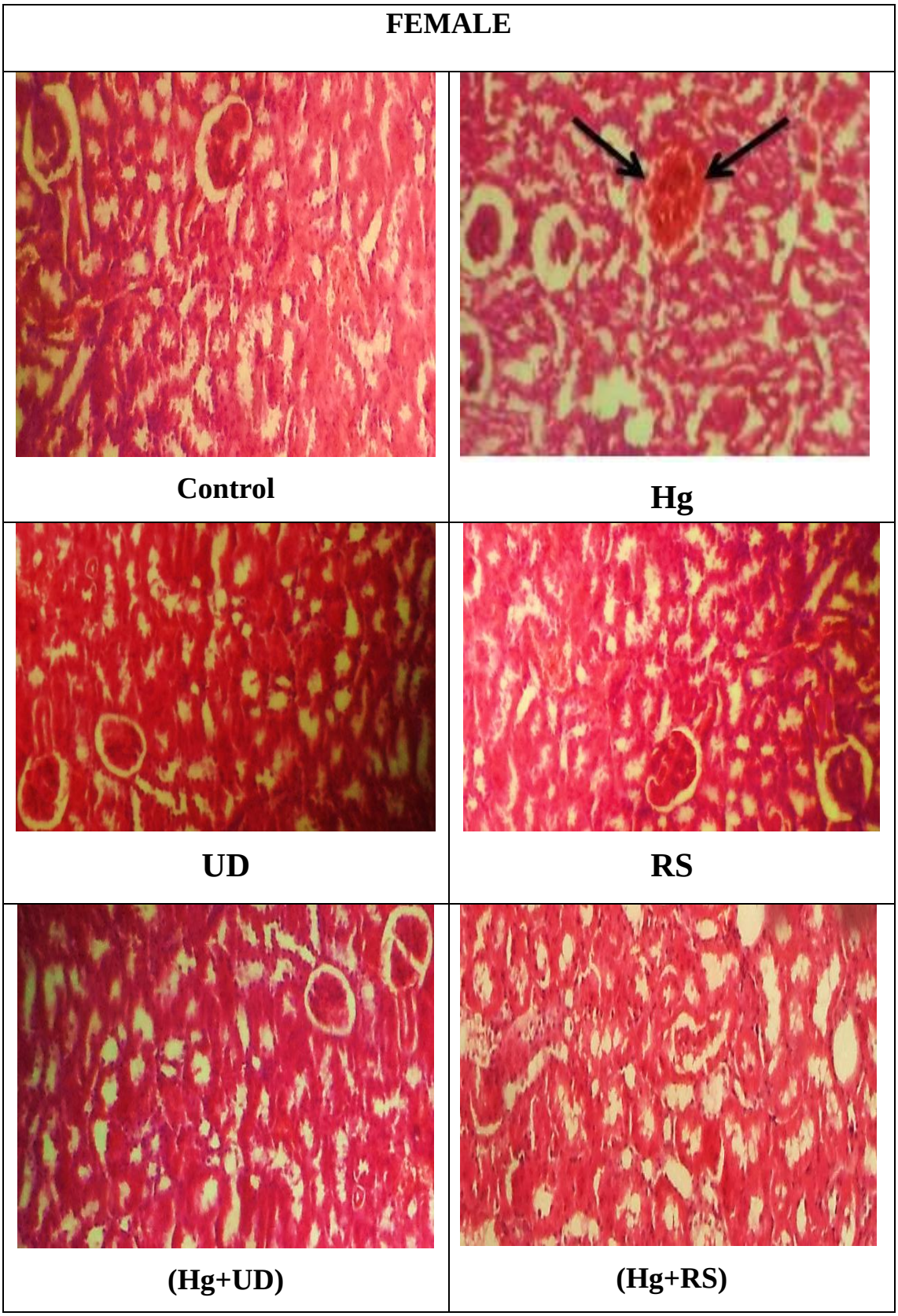

Figure N°46. Histological profiles of female rats kidneys showing the control, the Hg ,the UD, the RS and the Hg+UD and Hg+RS groups after 30 days treatments (**x 250**).

DISCUSSION

Body weight is frequently used for observing the growth of animals and its nutritional status (**Ndlovu *et al*., 2007**). In effect, the administration of Hg in rats showed a significant decrease of the total body weight in both males and females. This could be related to the decrease of the daily consumption of food and water, which is in accordance with the report of National Toxicology Program Working Group (**NTP, 1993**) that the exposure of rats to mercuric chloride reduced food and water intake leading to weight loss. Such result suggests that this metal presents adverse effects on rat body growth. This reduction is used as an indicator of the deterioration of the status of rats' general health. Heavy metals are well-known to disturb organ/body weight ratio when added to diet at toxic amounts (**Mahaffey *et al*., 1981**). Our results are supported by **Van Vleet *et al*. (1981)** who showed a marked decrease in body weight gain within the experimental period, induced by Cd and Pb administration.

Besides, in these experimental conditions, it was noted that the addition of *U. dioica* and *R. sativus* to rats treated by the Hg has caused an enhancement of the body weight. This could be due to the reduction of the formation of free radicals by the action of antioxidants presented in both plants. This results supported by **Yang *et al*. (2003)** and **Sayama *et al*. (2000)**, who found that green tea and body weight gain are associated in broilers and rats.

Organs' weights of liver and kidney were increased in males exposed to Hg. This is would be explained by the tissues hypertrophy caused by this metal. Contrary, a decrease in liver and kidney weights were noted in females. The increase of organ-body weight ratio is suggestion of inflammation or infection; whereas a decrease of this ration could be explained by cell constriction (**Moore and Dalley, 1999**). Our results are in accordance with that of **Nielsen and Andersen (1991)**, **Eto *et al*. (2007**) who found that the Methyl mercury produced a reduction in liver weight at 3.0 mg/kg body weight/day. Also **Klein *et al***. (**1972**) observed a decrease in liver weight of treated rats at high doses, correlating these data with low protein levels, glycogen depletion and changes in the adipose tissue structure. Though inorganic Hg form is considered as a nephrotoxic agent (**Clarkson, 1997**), it can pose renal deficiency, modifies body and organ weights of rats exposed to during the precocious phase of development (**Peixoto and Pereira, 2007**).

Glucose level in this study has increased in males exposed to Hg, but no change was recorded in rats treated with *U. dioica and R. sativus*. Previously, Hg intoxication was suggested to increase the energy supply to cope with the metal stress (**Margarat *et al*., 1999**). Interestingly, **Durczok *et al*. (2002)** demonstrated that glucose uptake was deeply affected in several tissues of neonatal rats exposed chronically to methylmercury chloride, confirming the existence of complex molecular mechanisms of Hg-induced dysglycaemia depending on the dose administered, the duration of exposure and the individual age. On the other hand, a considerable hypoglycemia was registered in $HgCl_2$ intoxicated rats, which was more obvious after 60 days exposure (**Merzoug *et al*., 2009**). It is suggested that Hg might damage endocrine function via its ability to inhibit the complex binding of hormone-receptor or through the inhibition of the enzymes; the pancreas is a vulnerable organ to the toxic effects of Hg. Insulin, that appear to be the most affected hormone by mercury, because it has many sulfur-binding sites which can bind Hg inducing then a dysregulation of blood glucose levels (**Chen *et al*., 2006**).

On the other hand, the treatment of diabetes with *U. dioica* has reduced blood sugar to its normal level (**Mehri *et al*., 2011; Namazi *et al*., 2012**) and had a hypoglycemic effect as well on pancreatic beta cells of hyperglycemic male rats (**Golalipour *et al*., 2007**). Moreover, when *U. dioica* mixed with various plants, the extract had antidiabetic activity (**Petlevski *et al*., 2001**). Similarly, it might be said that green tea and ginger extract have decreased the elevated glucose concentration in diabetic male rabbits **(Ahmed elkirdasy *et al*., 2015)**.

Fortunately, in our investigation the supplementation of *R. sativus* corrected the hyperglycemic status provoked by Hg. Most of plants contain glycosides, alkaloids, flavonoids, carotenoids, etc., that are often concerned by having antidiabetic effect. Root juice of red radish has been stated to reduce blood glucose levels in streptozotocin diabetic male rats (**Shukla *et al*., 2011**), which was attributed to the presence of ferulic acid in the roots of this plant (**Ohnishi *et al*., 2004**).

Triglycerides' concentration has risen remarkably in males Hg treated group, but has augmented slightly in female Hg group. However, it is not the case for the combined treatment of (Hg+UD) & (Hg+RS). Methylmercury had elevated serum triglycerides and cholesterol concentration in male rats, while the treatment with micronutrients (zinc and selenium) has reduced triglycerides by up to 77% and 52%,

respectively (**Joshi *et al.*, 2010**). Similarly, **Veena *et al.* (2010)** reported that the exposure of rats to Pb has significantly elevated serum cholesterol concentration. Dissimilar, in male rabbits, Hg has not affected the level of serum triglycerides and total cholesterol after 5 week's exposure (**Maarouf *et al.*, 2008**). Moreover, **Merzoug *et al.* (2009)** showed a significant decline of triglycerides in the treated male rats after 2 weeks Hg exposure. Lately, some research mentioned that Hg promotes cardiovascular disorders via metabolic changes, suggesting that blood cholesterol and triglycerides would consequently be involved in Hg-induced cardiovascular risks (**Mozaffarian, 2009**).

On the other side, *U. dioica* extract has shown an apparent effect on rats at doses of 100 and 300 mg/kg by reducing the levels of total cholesterol and LDL, accompanied with a remarkable decrease of liver enzymes and animal's weights when fed a high cholesterol diet (**Nassiri-Asl *et al.*, 2009**). The *U. dioica* leaves contain chlorophyll which was proved to have hypolipidemic effects (**Mahjoub *et al.*, 2012)**. Here, the supplementation of *R. sativus* to rats' clearly decreased triglycerides level comparing with the control group. Furthermore, the supplementation of black radish juice to lipid-rich diet resulted in a significant lowering of these parameters in male rats (**Lugasi *et al.*, 2005**). Moreover, total cholesterol level in liver homogenate was significantly reduced by administrating aqueous coriander extract (**Kansal *et al.*, 2012**), also, dandelion leaves are helpful by correcting triglycerides and cholesterol levels in female Pb rats (**Mansouri *et al.*, 2014**).

Urea and creatinine in this study have a remarkable elevation in male and female rats exposed to Hg. It is well known that inorganic mercury causes severe kidney damage after acute and chronic exposure (**Zalups, 1997**). Accordingly, serum urea was elevated after acute administration of $HgCl_2$ in mice which due to the toxic effects of Hg on kidney and liver cells (**Ghosh *et al.*, 2008**). Urea is the end-product of the amino acids catabolism; so its increase is an indicator of nephrotoxicity. Any increase in serum creatinine level could indicate a decreased excretion or a damaged renal function (**Lakshmi *et al.*, 2010**). So it can be said that urea and creatinine concentrations are the most traditional indicators for kidney function and renal structural integrity (**Ali *et al.*, 2001**). This elevation in creatinine and urea levels is in accordance with **Oriquat *et al.***

(2013), **Smaoui *et al.* (2000)** and **Necib *et al*. (2012**). Hg is able to accumulate in renal tissues and induces renal impairment (**Zalup *et al*., 1995**).

The supplementation of rats with Hg+UD recorded no noticeable change of these markers. In addition, flavonoids and the high potassium content of *U. dioica* contribute in the diuretic action, which allow the body to excrete much wastes including mercury, and that why stinging nettle is being used as a diuretic agent (**Roschek *et al*., 2009**). Moreover, the administration of methylmercury to male rats has led to an increase in urea serum and creatinine, while the co-administration of zinc and selenium has protected the animals from mercury poisoning remarkably (**Joshi *et al*., 2010**). Also, *Pistacia lentiscus* oil was beneficial to maintain normal serum urea level, which has been increased in male rabbits treated by mercury chloride for 37 days (**Maarouf *et al*., 2008**). Rabbits pretreated with *Juglans sinensis* were able to keep their regular urine volume than rabbits given mercury chloride alone. So, the increase of serum creatinine level by Hg was attenuated by *Juglans sinensis* pretreatment (**Ahn *et al*., 2002**).

In this study, no increased plasma urea and creatinine was recorded when rats were treated with *R. sativus* in both sexes. This is in agreement with the proposal of **Kumar *et al*. (2013**) that demonstrated a nephroprotection of *R. sativus* in gentamicin induced nephrotoxicity, which was mediated via its antioxidant effect in male Wistar rats. **Necib *et al*. (2012)** concluded that after sodium selenite has reduced the Hg toxicity in female Wistar rats.

Amino-transferases in the present study have increased remarkably in the plasma of rats intoxicated with Hg in two sexes. This elevation of these parameters has been supported by **Toshiko *et al*. (1998)**. ALT is a cytoplasmic enzyme found in very high amounts in the liver and an increase of this specific enzyme designates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function (**Aliyu *et al*., 2006**). Thus, the activity of AST and ALT enzymes in body fluid could be used as an indicator of tissues damage provoked by oxidative stress (**Svoboda, 2001**), which is certainly come from the damage of the plasma membrane through the fixing of Hg ions to its constituent proteins (**Recknagel *et al*., 1989**). Such enzymes are suggested to be sensitive indicators of tissues damage, since they are liberated from cells even when the magnitude of lesions is not sufficient for morphological detection (**Recknagel *et al*., 1989**). As a consequence, the significant modifications of plasma

AST and ALT activity could be the results of the hepatocellular injury or cellular apoptosis of liver, spleen or muscles (**Kaoud *et al*., 2011**). **Gutierrez *et al*. (1992)** found that Pb caused significant leakage of ALT and AST into the medium, which indicate cellular leakage and loss of functional integrity of hepatic membrane architecture made by trace metals.

In the current study, the presence of *U. dioica* and *R. Sativus* has removed such affect, by reducing the leakage of enzymes into the plasma. Thus, *U. dioica* leaves are known to contain minerals and other chelating agents which enable to reduce Hg toxicity (**Frank *et al*., 1998**). Furthermore, after five weeks, serum AST activity was higher in male rabbits intoxicated with mercury chloride, but in the *Pistacia lentiscus* oil rabbits combined with Hg, the activity of this enzyme was almost similar to the control (**Maarouf *et al*., 2008**). In male Swiss albino mice, serum AST and ALT were decreased by a single injection of mercuric chlorides after 40 days, meanwhile when Hg was given with the blue green algae *Spirulina fusiformis*, the enzymatic activities have not been affected (**Sharma *et al*., 2007**). Effectively, the treatment with *U. dioica* protected rats against Aflatoxin-induced hepatotoxicity, as evidenced by decreased AST and ALT activities (**Yener *et al*., 2009**). Besides, *U. dioica* treatment for 60 days has exhibited remarkable reduction in liver enzyme activities and also has increased the reduced antioxidant enzyme levels in tetrachloromethane-treated male rats (**Kanter *et al*., 2005**)**.** The present results are in line with the previous studies, which investigated the protective role of *U. dioica* (**Mitscher *et al*., 1997;Celik and Tuluce, 2007**).

On other side, current studies proved that *R. sativus* leaves shown a competitive efficacy of reducing the elevated transaminase under the paracetamol stress on albino rabbits (**Rukhsana and Mubasher, 2006**). Also, **Hussein *et al. (*2012)** demonstrated that plasma ALT and AST activity increased significantly in fish exposed to Hg. However, the addition of *Spirulina platensis* enhanced ALT and AST activity to be nearly as the control group. Our results are consistent with the recent finding of ALT, AST and ALP activities in male rats intoxicated with Pb (**Haleagrahara *et al*., 2010)**, while, vitamin C supplementation reversed these effects and attained the enzyme activities to normal levels because it has hepatic-protective property related to its antioxidative characteristic. The presence of dandelion has well-kept the activity of these enzymes, probably by reducing hepatic failure induced by Pb (**Mansouri *et al*., 2014**). Such herbs are assumed to motivate the elimination of bile, which increases the liver's ability to

clean up the plasma (**Bernard, 2011**). The results of the present study were also in accordance with those of **Kalantari *et al*. (2009)** who indicated that the treatment with radish extract has reduced the activity of AST and ALT in rats given carbon tetrachloride.

Alkaline phosphatise activity was clearly augmented by Hg intoxication in male and female rats. ALP is an enzyme found in many organs, especially liver, and can be liberated into the blood when parenchymal liver cells are damaged. It is reported that the treatment of rats by $HgCl_2$ at 5 mg / kg body weight for six months increased the activity of alkaline phosphatase in both sexes of rats and B6C3F1 mice (**NTP, 1993**). However, acute administration of $HgCl_2$ has caused many toxic effects on kidney and liver and it has been associated with increased serum ALP activity (**Ghosh *et al*., 2008**). In addition, **Rao *et al*. (2001**) have observed a reduction in enzyme activity of male mice exposed to $HgCl_2$ to (1.25 mg / kg / day) for 45 days. Meanwhile, **Hilmy (1981)** showed a diminution in ALP activity of *Aphanius dispar Rüpp* (Teleostei) from Red Sea following acute and long-term mercury exposure that it may be attributed to slow but constant inhibition of this enzyme by Hg ions. Therefore, it appears that the duration of exposure to $HgCl_2$ could affect organ function differently; the kidney and liver are the main sites of Hg deposition and therefore are target organs for its toxicity (**Kojima *et al*., 1989**).

However, in the group fed with *U. dioica* and *R. sativus*, no significant changes were reported. In Swiss albino mice, the efficiency of the blue green algae *Spirulina fusiformis* was noticeable to keep alkaline phosphatase activity within normal ranges against the inhibitory effect of mercury (**Sharma *et al*., 2007**). Additionally, both of *Spirulina fusiformis* and *Ocimum sanctum* have returned the enzymatic activity to their normal biochemical range (**Sharma *et al*., 2005**). **Dash *et al*. (2013)** and they showed that the high levels of serum ALT, AST, ALP and bilirubin in the toxicant males and females were the consequence of CCl_4-induced liver dysfunction, whereas the pretreatment with different doses of *Raphanus sativus* reversed the levels of these parameters towards normal.

Minerals levels (Mg, Fe and Ca) were significantly decreased in both sexes of rats exposed to Hg, whereas; no variation was noted in rats supplied with *U. dioica* and

R. sativus. This lowering effect was happened probably because mercury is known to cause lack of appetite (**Zahir *et al.*, 2005**), as well as, it is able to provoke many dysfunctions in the absorption of nutrients (**Farmanfarmaian *et al.*, 1989**). However, no changes in the levels of Mg, Fe and Ca have been reported in rats supplemented with *U. dioica* and *R. sativus* which might due to the high content of minerals and vitamins found in these medicinal plants. The nettle is nutritionally rich in minerals (iron, manganese, potassium, and calcium), carotenoids, and vitamins A, C and D also flavonoids (**Asgarpanah *et al.*, 2012**), as well red radish is a good source of many minerals (Fe, Ca, Se and Zn) and vitamins (A, K and C) **(Rayman, 2012).**

Glutathione is the main thiol antioxidant and the conjugating agent; it was known to bind electrophilic molecular species and free radicals intermediate. In the present investigation the GSH level showed a noticeable significant depletion following Hg exposure in different organs (liver, kidney and testis). Reduction in glutathione level results in the detoxification of peroxides formed by the increased of oxidative stress (**Verma *et al.*, 2001**). Such depletions may due to the high affinity of Hg to thiol groups, as results it disturbs GSH metabolism and damage cell functions (**Hultberg *et al.*, 2001**). Therefore, the metal–GSH conjugation manner is necessary in the elimination of toxic Hg into the bile. $HgCl_2$-induced oxidative stress is usually attributed to the formation of highly reactive hydroxyl radical (OH), the stimulator of lipid peroxidation and the sources of damage to the cell membrane (**Bharathi and Jagadeesan, 2012**). It has been reported that mercuric chloride administration decreases renal and hepatic GSH content and increases lipid peroxide formation (**Nath *et al.*, 1996, Jagadeesan and Sankarsami Pillai, 2007**).

In fact, most of the presented remedies help the healing or regeneration of the liver (**Neubauer *et al.*, 1998**). Beneficial remedies from medicinal plants are considered to be effective and safe for the alternative treatments for hepatotoxicity (**Ranawat *et al.*, 2009**). The co-administration of *U.dioica* with Hg has resulted in keeping glutathione at control level in males and females rats. The administration of *U. Dioica* extracts demonstrated an antioxidant and hepatoprotective effect against anxious stimulus by tetrachloromethane in Wistar rats (**Kanter *et al.*, 2005**). Many studies proved that the flavonoids and phenols are good antioxidant against Hg induced pathogenesis and also act as effective chelators for several toxic metal ions (**Farag *et al.*, 2013**). Numerous

active components in *Spirulina fusiformis* could promote the activity of ROS scavenging enzyme systems and protect against Hg induced renal damages (**Sharma *et al.*, 2007**). The co-administration of vitamin E with $HgCl_2$ resulted in maintaining of glutathione at control levels in male Wistar rats (**Rao and Sharma, 2001**). Besides, the reasons for such an effect of the *U. Dioica* infusions could be due to the presence of flavonoids in the plants which have strong antioxidant and metal-chelating properties and can therefore protect cells from free radicals (**Celik and Tuluce, 2007**). However, it has been suggested that antioxidants might help the treatment of Hg injuries (**Gado and Aldahmash, 2013**).

In our experimental work, we proved that the supplementation of red radish juice can improve hepatic, renal and testicular GSH level in in both sexes Hg treated rats; thus, the present study suggests that deleterious ROS or lipid peroxides responsible for $HgCl_2$ toxicity could be corrected by fresh juice of *R. sativus*, which in turn reflected in significant enhancement of hepatic, renal and testicular GSH as compared to $HgCl_2$ treated group. It has been reported that red radish has a high amount of total phenolics (**Stratil *et al.*, 2006**), and flavonoids (**Lugasi *et al.*, 2005**), as well as health benefits, including antioxidant activities (**Matsufuji *et al.*, 2007**). **Salah Abbès *et al.* (2008)** indicated that radish extract is rich on antioxidants. Radish seed can scavenge the free radicals in the cell constituents and defend liver from damages, due to its antioxidant property as source of vitamin C and sulfur containing compounds (**Naghibi *et al.*, 2003**). **Baek *et al.* (2008)** found that an oral administration of the sulfur radish extract in mice clearly reduced the liver failure. Additionally, the supplementation of red radish juice stopped the hepatic damage provoked by paracetamol treatment (**Popovic *et al.*, 1993**). Also, raw extract of *R. sativus* showed a hepatoprotective activity against zearalenone-induced peroxidative damage in female Balb/c mice (**Salah-Abbes *et al.*, 2008**).

Liver histology in this investigation has showed histological changes in males and female rats received Hg, where it characterized by a dilation of sinusoidal, a congestion of the centrilobular vein as well as a phenomenon of cellular necrosis (cytolyses) compared to the control. The changes of the liver histoarchitecture could mainly due to the toxic effect of mercury. Our results are in accordance with **Jadhaf *et al.* (2007)** who recorded an alteration in the standard levels of various blood chemical

markers correlated by the histological necrosis and liver degenerative changes of male rats exposed orally to $HgCl_2$ for 8 weeks. These variations were attributed to noxious effect of mercuric chloride on the endothelial cells lining blood vessels (**Mohamed, 2004).**

Ibegbu *et al*. (2014) showed congestion of hepatoportal blood vessels, congestion of central vein due to the toxic effect of mercuric chloride. It has been revealed that under conditions of liver toxicity by mercury, some cells become infiltrated and caused an increase in cell permeability resulting in the release of transaminases and ALP into the blood (**Kumar *et al*., 2005**), and it also damaged the hepatocytes (**Ibegbu *et al*., 2013**), which may become irreversible, leading to liver cirrhosis (**Quirino *et al*., 2012**).

The severe histopathological alterations of liver in the actual study were confirmed by the marked elevation of amino-transferases, indicating the impaired liver function. Furthermore, many researchers indicated that intoxication with Pb, Hg and Cd produced numerous hepatic injuries in experimental animals (**Jabeen and Chaudhry, 2011**). This supports the earlier reports on liver histopathological changes made by mercury intoxication (**Ketterer, 1998; Sharma *et al*., 2000**).

However, the examination of liver of groups (Hg+UD) and (Hg+RS) showed that the pathological changes had almost completely disappeared compared to Hg group. This could be associated with the ability of our medicinal herbal to eliminate the reactive oxygen species (ROS) once formed, via very rapid electron transfer system that stops lipid peroxidation (**El-Tohamy and El Nattat, 2010**).

Free oxygen radicals have a huge intermediary role in Ischemia-reperfusion injuries of several organs, including liver. Some researchers suggest that antioxidant molecules may show a protection from Ischemia-reperfusion injury. The *U. dioica* is recognized to be a strong antioxidant, reacting with free radicals. For this reason, it is predictable to be protective in hepatic rat injury (**Terzi *et al*., 2010**; **Mittman *et al*., 1990**). **Oguz *et al*. (2013**) concluded that *U. dioica* is helpful for liver regeneration after liver damage over oxidative stress, proliferation and apoptosis after partial hepatectomy in Wistar rats. **Kandis *et al*. (2010**) indicated that *U. dioica* has a protective effect on damage created during Ischemia-reperfusion in male Wistar rats.

A recent study has reported that vitamin E reversed Pb, Hg, Cd and Cu-induced liver damage (**Al-Attar, 2011**), and the addition of vitamin E declined the histopathological and biochemical alterations caused by Pb intoxication in female Sprague-Dawley albino

rats (**Sajitha *et al.*, 2010)**, because this **v**itamin E was confirmed to act as an antioxidant, and to stop the liver degeneration. The dilatations in the sinusoids were considerably reduced, and the hepatocytes maintained at normal structure in the co-exposed rats with Vitamin E and aluminium **(Kutlubay *et al.*, 2007).**

On the other side, raw extract of *R. sativus* showed a hepatoprotective activity against zearalenone-induced peroxidative damage in female Balb/c mice (**Salah-Abbes *et al.*, 2009**). This clearly demonstrates the fact that the anthocyanins of *R. sativus* have hepatic cell membrane stabilizing effect and cell regeneration ability, which is due to their anti-lipid peroxidation and/or free radical-scavenging activity. Silymarin is also known to exert its hepatoprotective action through a similar mechanism of action. Parenthetically, both these plants are polyphenolic compounds and belong to the flavonoids family. Thus, the hepatoprotective actions of the red radish anthocyanins fraction are due to their antioxidant and free radical scavenging characteristics common to flavonoids (**Dash *et al.*, 2013**).

Kidney histological study is one of the conformable tests for the nephrotoxic effect of mercury which reassures the formation of reactive oxygen species (ROS) and lipid peroxidation of the membrane lipid and protein denaturation. In this current research, kidneys of females and males Hg-treated rats after 30 days of Hg exposure showed severe necrosis compared to the control. Mercuric chloride is well known to accumulate in the renal cortex and disturb the morphology and function of kidney (**Greaves, 2000**). Oxidative stress has a dangerous role in the pathophysiology of numerous kidney diseases, and many complications are mediated by oxidative stress made by mercury toxicity (**Ghaima *et al.*, 2013**). The kidney is an organ highly susceptible to damage caused by ROS, likely due to the plenty of long-chain polyunsaturated fatty acids on the composition of renal lipids. Aminoglycosides are nephrotoxic agents; their nephrotoxicity is chiefly attributed to induction of ROS and depletion of antioxidant enzyme activities in kidney (**Alarifi *et al.*, 2012**). **Jadhav *et al.* (2007)** have observed degenerative and necrotic changes in the kidney of male rats exposed to mercury via drinking water. Normal morphology of the renal parenchyma with well-defined glomeruli and tissues was seen at lower dose of *Sidh Makardhwaj* added to mercury intoxication (**Sharma *et al.*, 2014**). These results agree with others who demonstrated kidney lesions induced by mercury (**Said *et al.*, 2008; Oda and El-Ashmawy, 2012)**.

Such lesions were attributed to the Hg direct toxic effect on the development and function of endothelial lining of renal blood vessels.

Conversely, the treatment with *U. dioica* and *R. sativus* showed an enhancement on the kidney architecture in two the sexes, Also, the pretreatment with silymarin reduced the intensity of the lesions. Wherein, kidneys showed focal areas of degenerative and necrotic tubular changes, mostly confined to the renal cortex and corticomedullary junction. Furthermore, slides from kidney section revealed the protective role of nettle in preventing the appearance of renal abnormal histological variations after treatment with Gentamicin in mice albino (**Moghaddam *et al*., 2010**). Also, **Kumar *et al*. (2013)** assumed that the nephroprotection showed by *R. Sativus* in gentamicin induced nephrotoxicity was mediated through its powerful antioxidant effect. The protective activity of nettle is possible due to its richness of phenols. Phenolic compounds have antioxidant properties by scavenging free radicals and active oxygen species such as single oxygen, free radicals and hydroxyl radicals (**Nale *et al*., 2012**). Also, the investigation done by **Toldy *et al*. (2005)** has proposed a neuro-protective property of nettle extract due to its antioxidant activity. There were no histopathological injuries detected in animals supplied with *Moringa oleifera* leaf extract, but the group given Hg had a destruction of the kidney cyto-architecture. This could be related to the hepatoprotective by the antioxidant properties possessed by *Moringa oleifera* leaf extract against Hg–induced oxidative stress in adult Wistar rats **(Ezejindu *et al*., 2014)**.

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CHAPTER IV:
RESULTS OF
REPRODUCTIVE PROFILE

RESULTS

1. Organ's weights

1.1. *Urtica dioica*

In males, a reduction in the testis and epididymis absolute weight of Hg group was seen compared to the control. In contrast, rats exposed to *U. dioica* had slight rise in absolute weights of these organs.

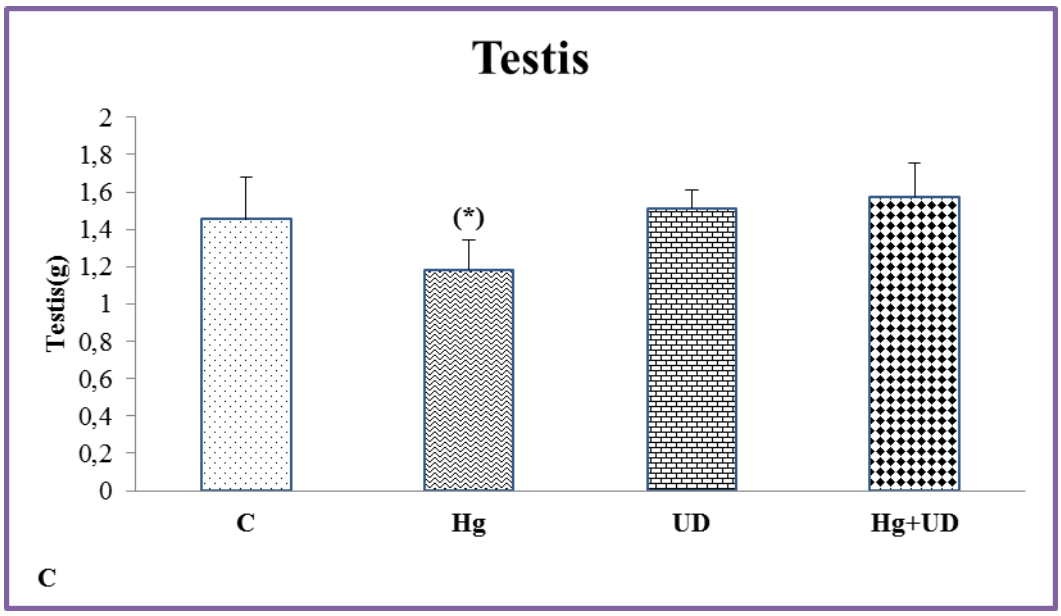

Figure N°47: Absolute weight variations of rats' testis in the groups treated by Hg and /or *U. dioica* for 30 days.

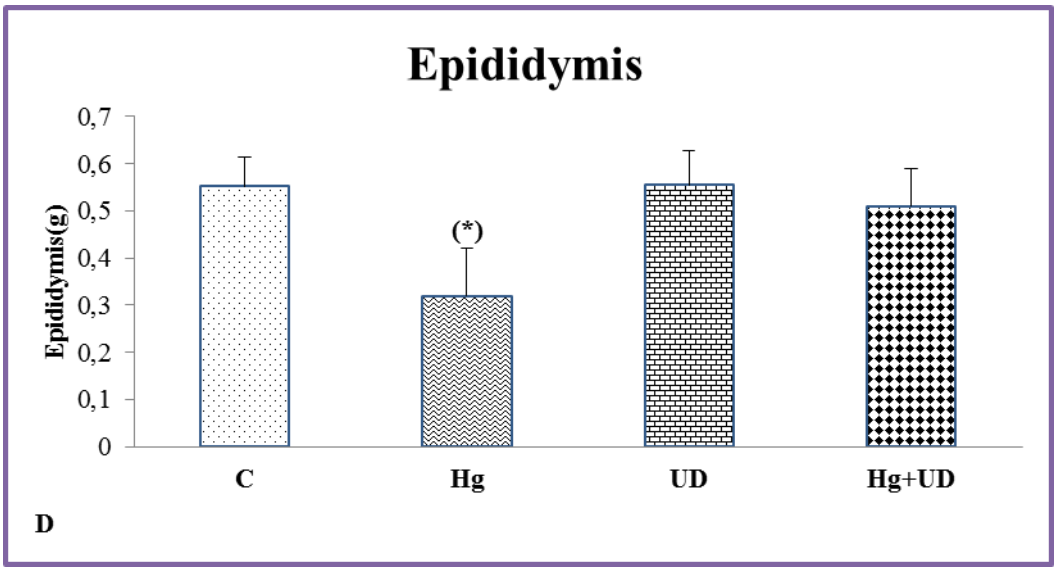

Figure N°48: Absolute weight variations of rats' epididymis in the groups treated by Hg and/or *U. dioica* for 30 days.

Testis and epididymis organ's absolute weight were summarised in table N° 13.

Table N° 13: The ameliorative effect of *U.dioica* on reproductive profile of male rats after 30 days Hg intoxication.

	C	Hg	UD	Hg + UD
Testis (g)	1.45 ±0.22	1.18 ±0.15*	1.51 ±0.09	1.57±0.18
Epididymis (g)	0.55±0.06	0.31 ±0.1*	0.55±0.07	0.51±0.07

***: Significantly different when compared to the control;**

#: Significantly different between groups.

1.2. *Raphanus sativus*

In male Wistar rats, we recorded a reduction in the testis and epididymis weight in Hg groups compared to the control group. Meanwhile, *R. sativus* addition presented an enhancement of weight of these organs comparing to the control group.

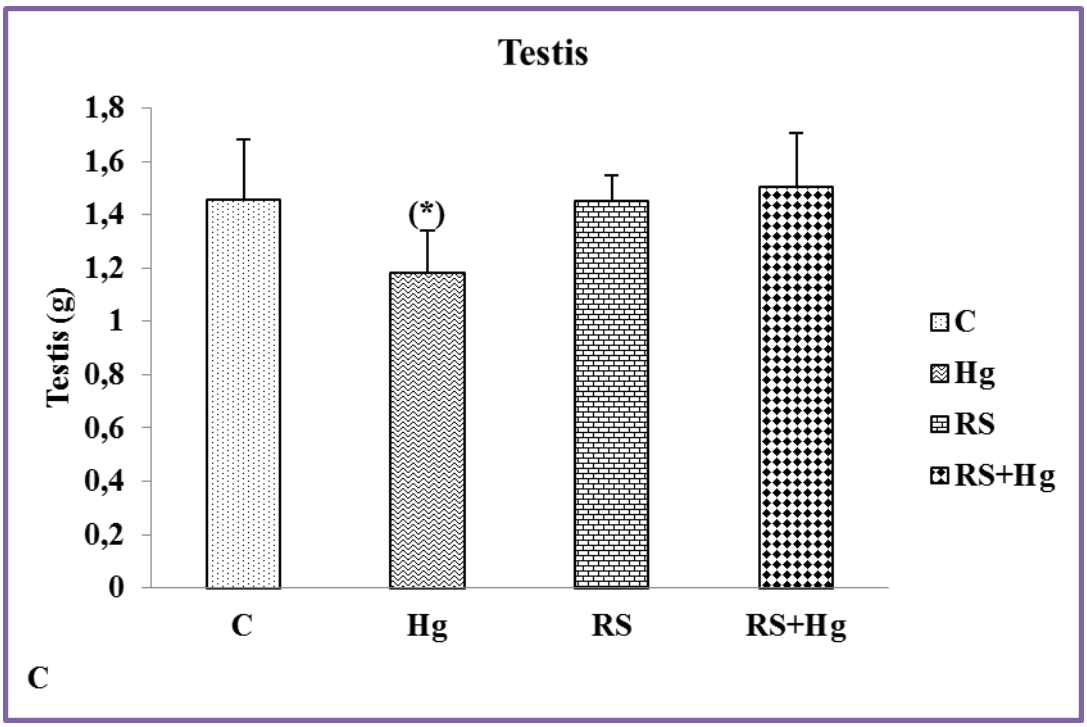

Figure N° 49: Absolute weight variations of rats' testis in the groups treated by Hg and /or *R. sativus* for 30 days.

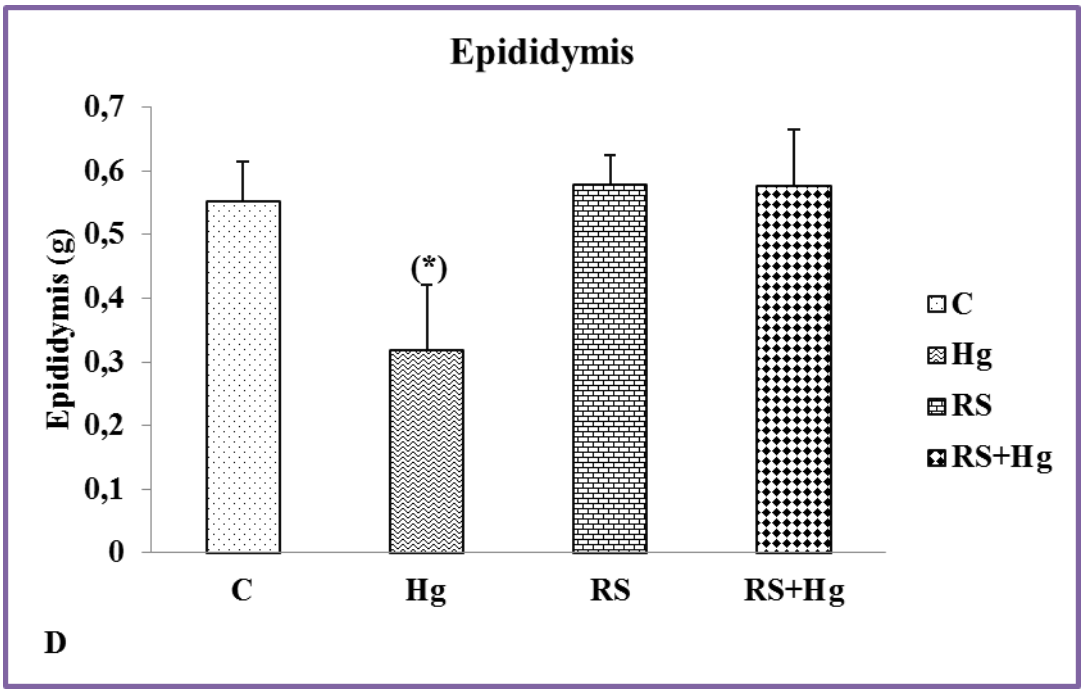

Figure N° 50: Absolute weight variations of rats' epididymis treated by Hg and/or *R. sativus* for 30 days.

Testis and epididymis organ's absolute weight were summarised in table **N° 14**.

Table N° 14: The ameliorative effect of *R. sativus* on testis and epididymis organ's weight of male rats after 30 days Hg intoxication.

	C	Hg	RS	Hg + RS
Testis (g)	1.45 ±0.22	1.18 ± 0.15*	1.45± 0.09	1.5± 0.2
Epididymis (g)	0.55±0.06	0.31 ±0.1*	0.57± 0.04	0.57± 0.08

***: Significantly different when compared to the control;**

#: Significantly different between groups.

2. The reproductive markers

2.1. *Urtica dioica*

Mercury group in the present study provoked pronounced reduction of the epididymal sperm concentration and motility. Concerning the motility, there is a significant difference between all groups. On other hand, a noticeable improvements in all sperm's parameters were recorded when *U.dioica* was added to rats.

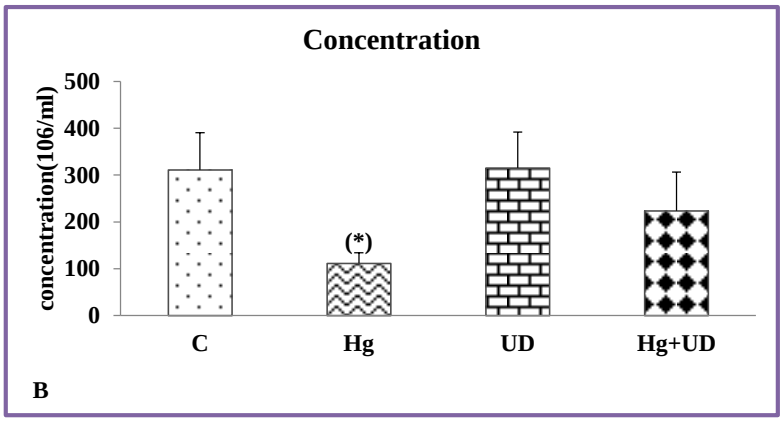

Figure N°51. The effects of Hg and /or *U. dioica* on spermatozoa's concentration of rats after 30 days.

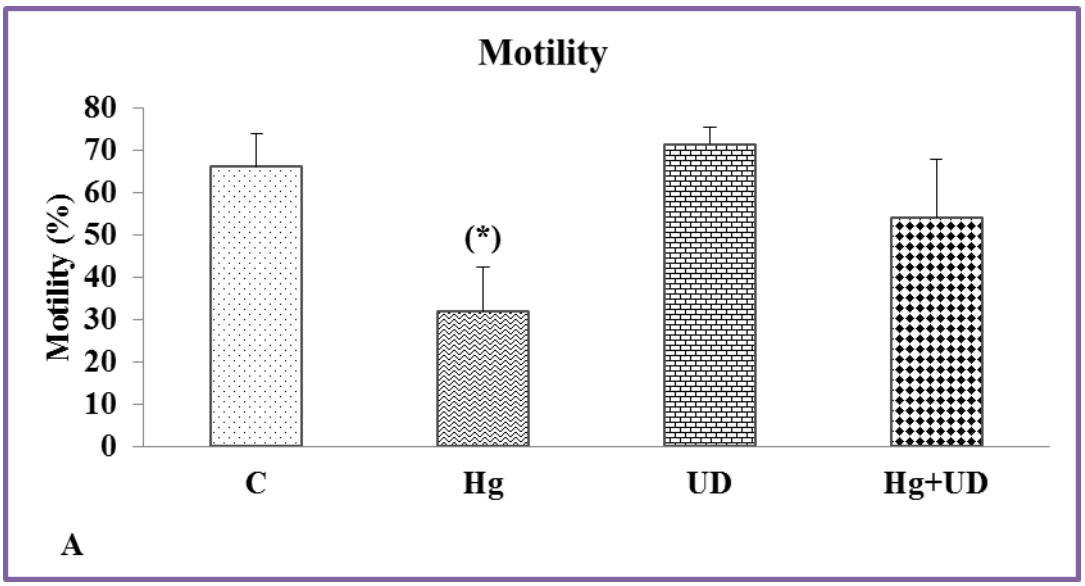

Figure N° 52. The effects of Hg and/or *U. dioica* on spermatozoa's motility of rats after 30 days.

Testosterone concentration showed clearly a significant decrease in the Hg group compared to the control. However, the treatment with *U.dioica* presented an improvement in the level of testosterone compared to the control group.

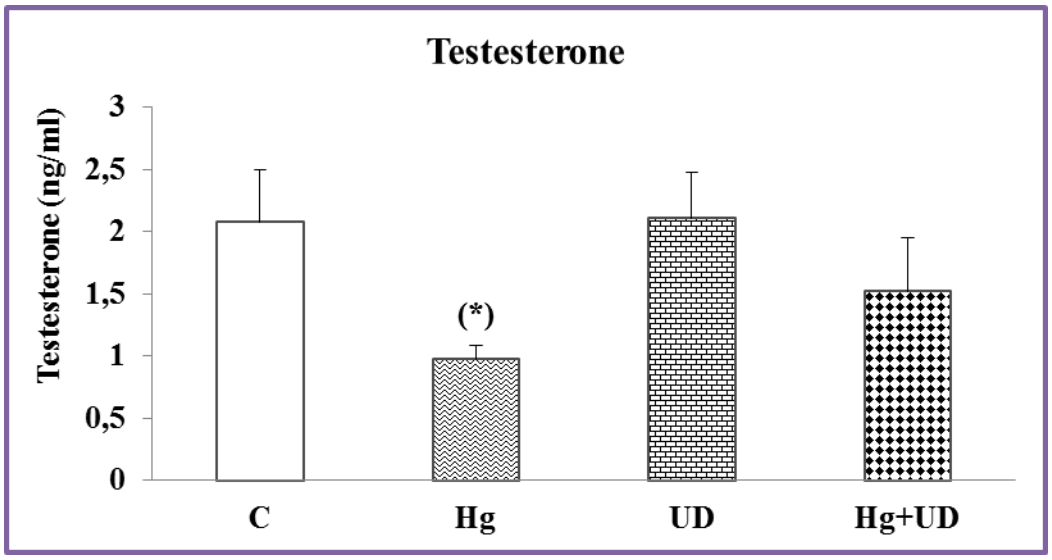

Figure N° 53. The effects of Hg and/or *U.dioica* on testosterone level of rats after 30 days.

Testicular GSH in rats stressed by mercury shows a decreased concentration comparing to the control group, however, the treatment with *U.dioica* raised significantly the concentration of testicular GSH toward control group. Besides, there is a difference between all the groups.

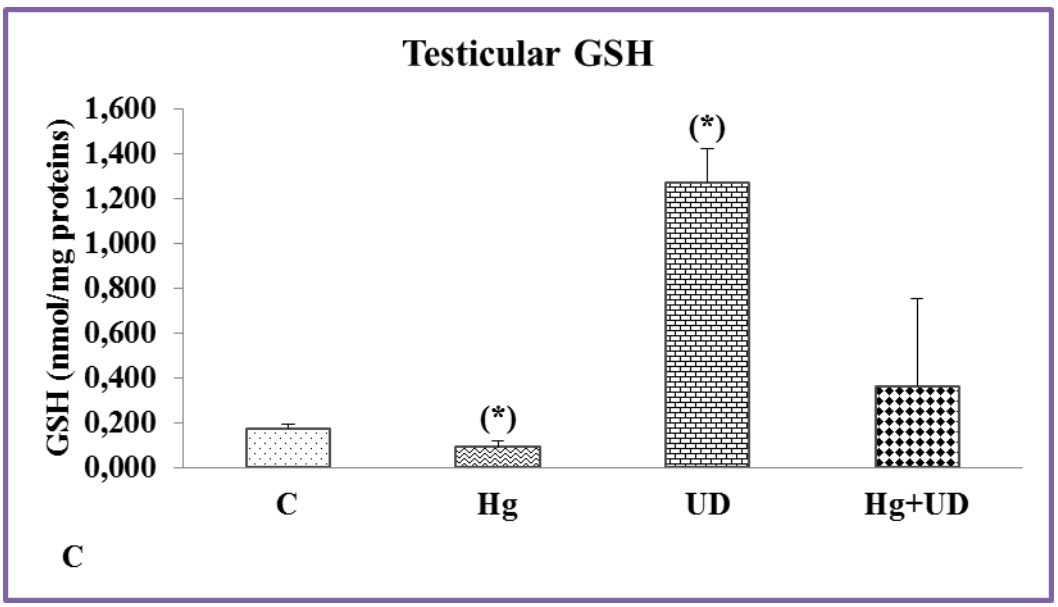

Figure N° 54. The effects of Hg and/or *U. dioica* on testicular GSH concentration of rats after 30 days.

The Reproductive markers were summarised in table N° 15.

Table N°15: The ameliorative effect of *U. dioica* on reproductive markers of male rats after 30 days Hg intoxication.

	C	Hg	UD	Hg + UD
Concentration (10^6)	311.3 ± 78.9	111.3 ± 23.1*	315.5 ± 76.3	223.5 ± 83
Motility (%)	66.05 ± 7.79#	31.9 ± 10.6*#	71.39 ± 4.17#	54.1 ± 13.9#
Testosterone (ng/ml)	2.07 ± 0.41	0.97 ± 0.11*	2..1 ± 0.36	1.52 ± 0.42
Testicular GSH (nmol/mg proteins)	0.17±0.01#	0.09±0.02*#	1.27 ±0.14*#	0.36 ±0.39#

*: **Significantly different when compared to the control;**

#: **Significantly different between groups.**

2.2. *Raphanus sativus*

Results show that the mean sperm concentration and motility decreased significantly in Hg group compared to control group. Also, there is a significant difference between all groups However, sperm concentration and motility of (RS) and (Hg+RS) groups were not statically different than that of the control.

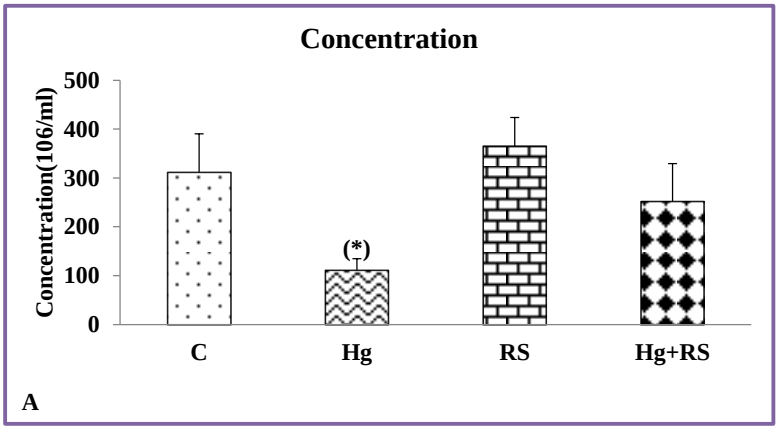

Figure N° 55. The effects of Hg and/or *R. sativus* on spermatozoa's concentration of rats after 30 days.

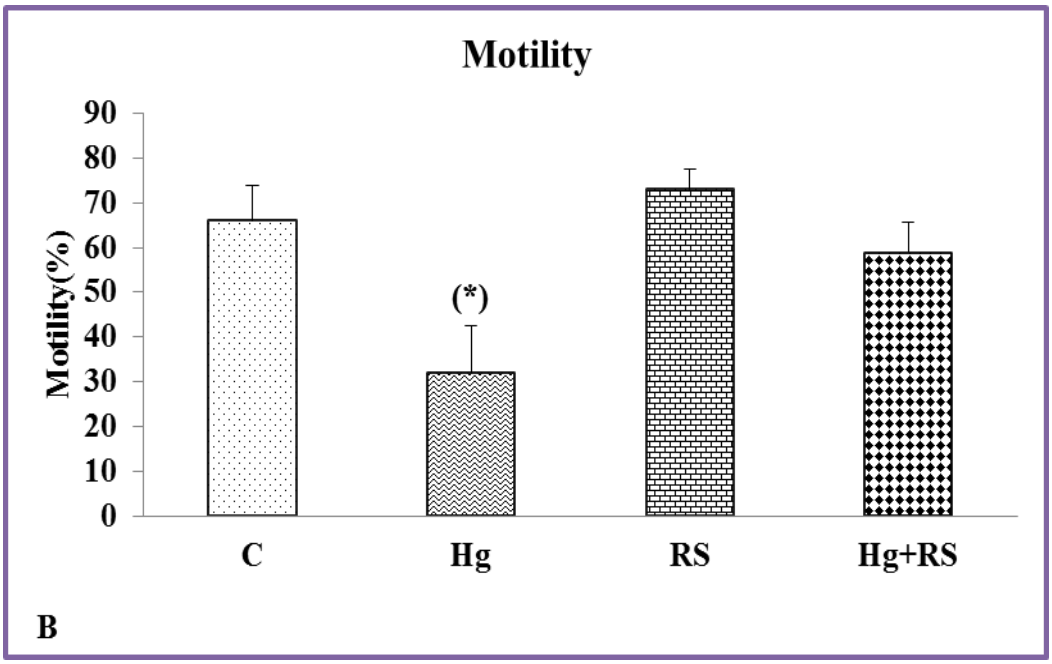

Figure N° 56. The effects of Hg and/or *R. sativus* spermatozoa's motility of rats after 30 days.

Testosterone concentration showed a clear significant decrease in the Hg group compared to the control. However, the treatment with *R. sativus* has presented a markedly normal level of testosterone compared to the control.

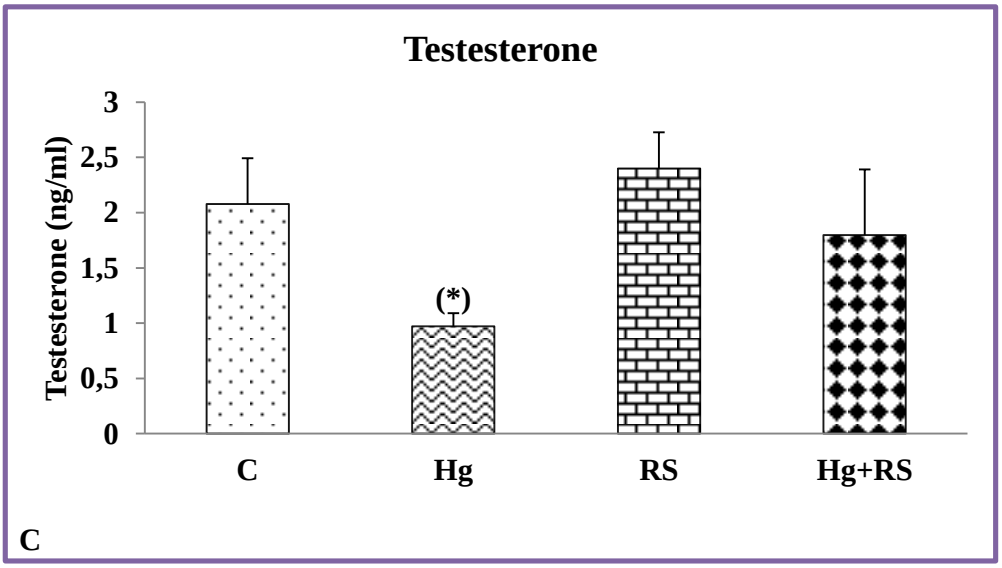

Figure N° 57. The effects of Hg and /or *R. sativus* on testosterone level of rats after 30 days.

Testicular GSH concentration was decreased significantly in the Hg group. Noticeably, males treated with *R. Sativus* alone showed a highly significant increase of testicular GSH concentration. GSH level of the (Hg+RS) was not different than that of the control. But, there is a significant difference between all groups.

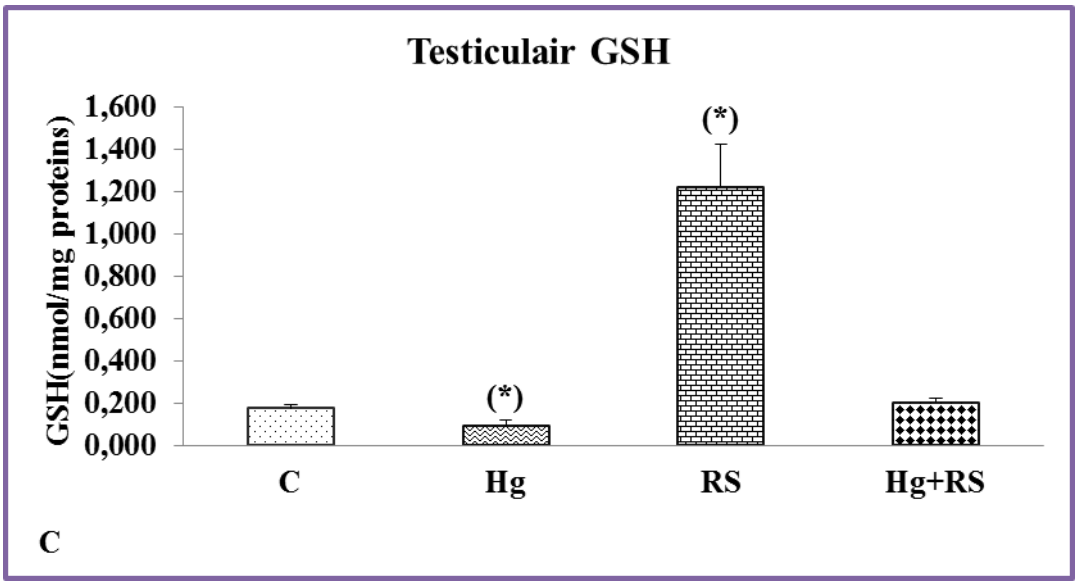

Figure N°58: The effects of Hg and/or *R. Sativus* on testicular GSH of rats after 30 days. Reproductive profile were summarised in table **N° 16**.

Table N° 16. The ameliorative effect of *R. sativus* on reproductive markers of male rats after 30 days Hg intoxication.

Groups	C	Hg	RS	Hg + RS
Concentration (10^6)	311.3 ± 78.9	111.3 ± 23.1*	365.6± 58	73.05± 4.44
Motility (%)	66.05 ± 7.79#	31.9 ± 10.6*#	73.05± 4.44#	58.86± 6.75#
Testosterone (ng/ml)	2.07 ± 0.41	0.97 ± 0.11*	2.39± 0.33	1.79±0.59
Testicular GSH (nmol/mg proteins)	0.17±0.01#	0.09±0.02*#	1.22± 0.2*#	0.2± 0.02#

*: **Significantly different when compared to the control;**

#: **Significantly different between groups.**

3. The Histological examinations

Figures (**59, 60**) illustrated the histological examination of testis and epididymis tissues of different groups. Microscopic assessment of male reproductive system in control group revealed normal seminiferous tubules, sperms with normal morphology, and concentration, as shown in Figures (**59, 60**). Figures demonstrate that $HgCl_2$ intoxication caused histological changes where spermatozoa were rarely seen in tubules, different degrees of degeneration in the lining spermatogenic tubules, and delocalization of seminiferous tubules. The histological changes were reduced remarkably in testis and epididymis in animals supplemented with *Urtica dioica* leaves and *Raphanus sativus* juice which showing sperm with normal morphology and concentration close to control group. UD and RS treated animals showed improved concentration of sperms and stabilization of organized seminiferous tubules and many newly formed spermatogenic cells arranged properly inside the tubules better than the control group. The results obtained from histological architecture were in consistency with the reproductive markers.

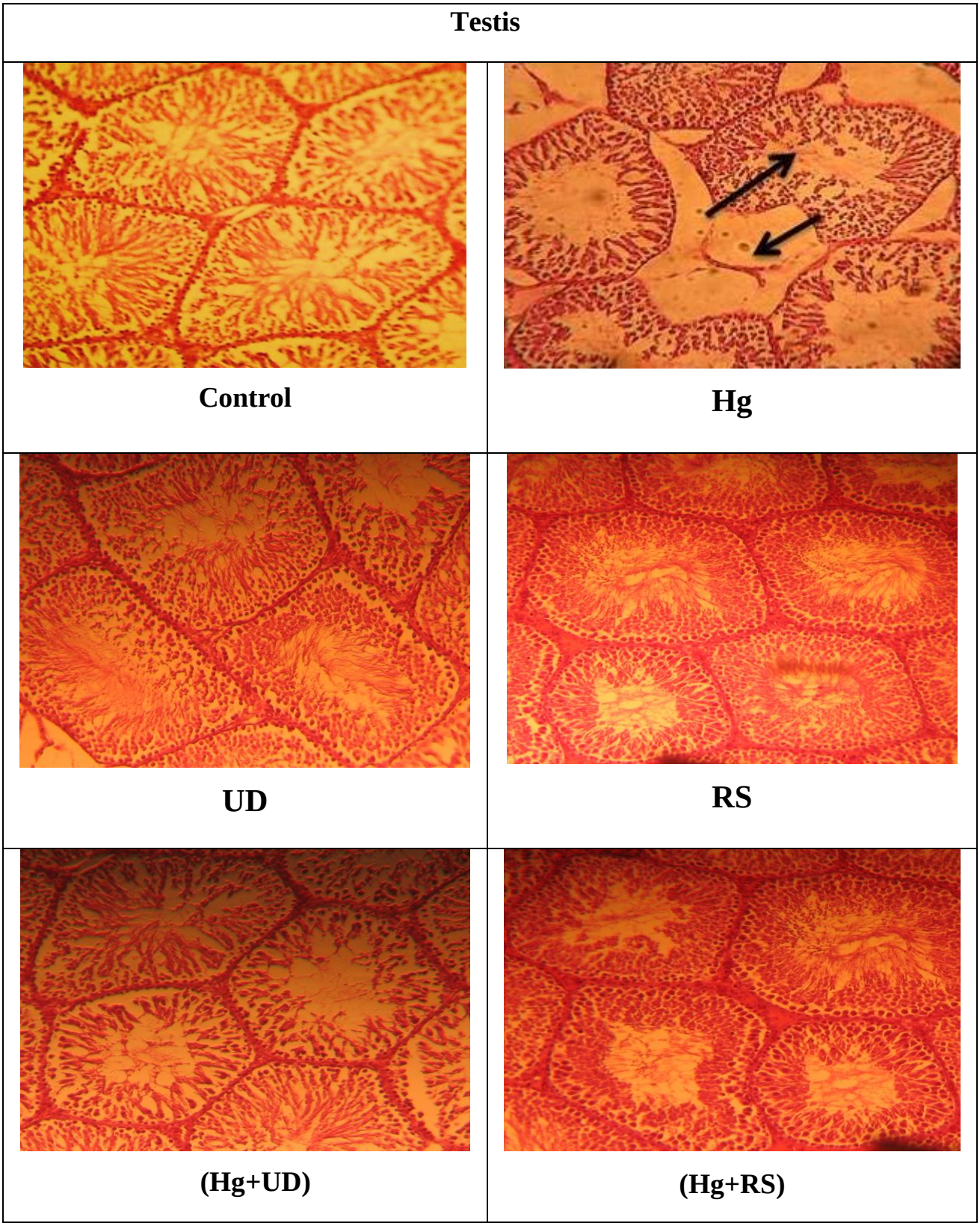

Figure 59. Histological profiles of male rats testis showing the control, the UD, the Hg and the Hg+UD groups after 30 days treatments (**x 250**).

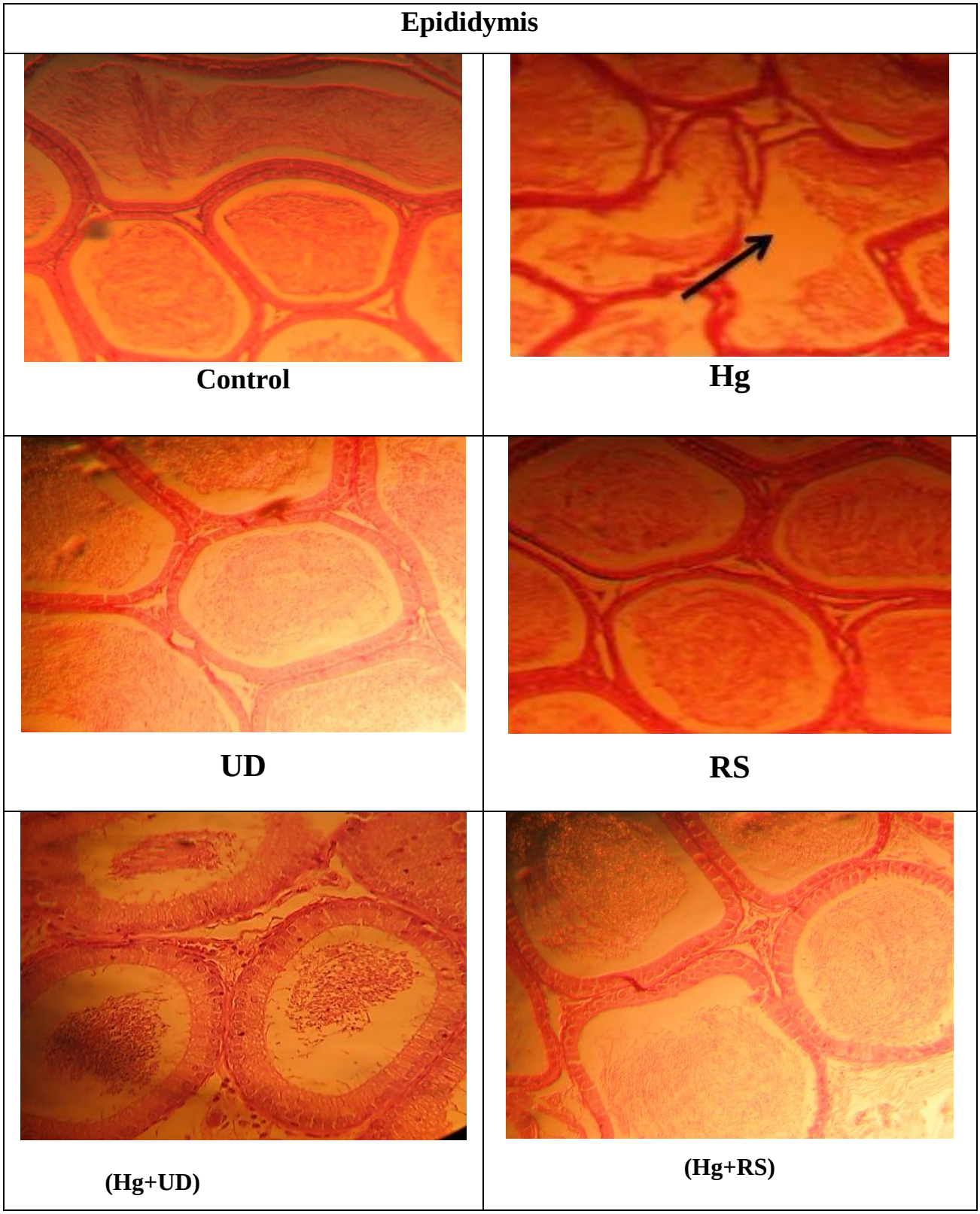

Figure 60. Histological profiles of male rats epididymis showing the control, the Hg, the UD, the RS and the Hg+UD and Hg+RS groups after 30 days treatments (**x 250**).

DISCUSSION

The weight of reproductive organs of male Wistar rats showed a diminution of testis and epididymis weight in the Hg group; such results are in line with **Al-Naimi *et al*. (2013**) who found a decreased testicular weight produced by the degenerative effect of mercuric chloride. The effect of $HgCl_2$ induced a decrease in sperm count by inhibiting the spermatogenesis, in parallel to a reduction in testis and epididymis weights. Adverse effects of $HgCl_2$ on rat testicular tissues have been reported with marked testicular spermatogenic degeneration at the spermatocyte level (**Chowdhury *et al*., 1986**). Mercury was reported to provoke alterations in testis along with biochemical changes (**Massanyi *et al*., 2007**). However, the increase in body weight and testicular weight in the supplemented group may be due to an increase in the intake of food and water, and also to the protective roles of compounds found in these plants against Hg toxicity. Thus, the observe elevation of the ratio organ-body weight (brain, kidney and liver) after supplying the leaves of *Chrysocoma ciliata* may be due to the functional capacity amelioration of these organs (**Ashafa *et al*., 2009**).

Reproductive markers have showed a reduction in the concentration and motility of epididymal sperm. It proves that male reproductive system is a target system to Hg exposure. Accordingly, another study revealed that sperm count and motility of the epididymis was markedly reduced by $HgCl_2$ (**Basu et Dickerson, 1996**). Such result is in agreement with that of **Moumen *et al*. (2011**). Thus, the epididymis is known to play an important role in providing the microenvironment for sperm maturation and storage. Consequently, the decrease in epididymal sperm number/motility might be explained on the basis that Hg has traversed the blood–testis barrier and gained access to germinal cells, leading to testicular functional inactivation (**Sharma *et al*., 1996**). Previously, it was found that oral exposure to mercuric chloride produced adverse effects on the reproductive performance of mice (**Khan *et al*., 2004**). Subsequently, mammalian spermatozoa are rich in poly unsaturated fatty acids and are thus very vulnerable to ROS attack; the later induced a decrease of sperm motility, presumably by a rapid loss of intracellular ATP, causing testicular damage (**De Lamirande and Gagnon, 1992**). Furthermore, it was reported that the exposure of Wistar rats to Mn induces a reduction of number of spermatids and spermatocytes in the seminiferous tubules (**Badou *et al*., 2013**). In addition, the study of **El-Tohamy and El-Nattat (2010)** proved that Pb treatment can impair male reproductive function by reducing the sperm count and volume.

Certainly, heavy metals are known to inhibit the enzymatic reactions by stopping the steroidogenesis process **(Smaoui *et al.*, 1997).** The epidemiological study carried out in Hong Kong had demonstrated that the infertility of both men and women are linked to the high levels of environmental mercury (**Dickman *et al.*, 1998**).

However, a noticeable improvement in all sperm's markers was observed when animals were treated with Hg+UD and Hg+RS, which suggests the protective roles played by these medicinal herbs. Additionally, the supplementation of essential *S. aromaticum* oil to rats previously poisoned by Mn, has helped to regenerate the majority of seminiferous tubules and interstitial cells, and has filled lights seminiferous tubules with spermatozoa (**Badou *et al.*, 2013**). Recently, **Shodehinde and Ganiyu Oboh** (**2013**) indicated that polyphenols' rich-red radish might act as great antioxidants that can inhibit lipid peroxidation, preventing testes from the oxidative stress. Furthermore, vitamin C was reported to ameliorate the harmful effect of Pb in rabbits, by improving sperm motility, reproductive function, and decreasing the production of ROS (**El-Tohamy, 2010**). Additionally, vitamin E, glutathione, zinc and selenium are considered as beneficial for the treatment of male infertility (**Sinclair, 2000**). Accordingly, *U. dioica* contains many minerals and vitamins, especially vitamin C and E, as well as flavonoids (**Asgarpanah *et al.*, 2012**), which can act as an antioxidants to counteract Hg intoxication. In fertile individuals, sperm motility levels, especially progressive sperms, are directly related to the reproductive capability. Moreover, *U. dioica* can act as an anti-oxidant and improve the sperm cells quality and count by the elevation of the anti-oxidant in comparison with the nicotine group (**Golalipour *et al.*, 2011**). Furthermore, it appears that *U. dioica* supplementation could improve the quality of spermatozoa and inhibits nicotine-induced toxic effects on sperm parameters in male mice (**Jalili *et al.*, 2014**). Many studies revealed that animals receiving vitamin E alone or combined with $HgCl_2$ had sperm markers similar to the normal values (**Basu and Dickerson, 1996**).

Testosterone level in this study has been negatively affected by mercury after one month exposure. In fact, mercury can react with the sulfhydryl's groups of proteins, which could inhibit enzymes, interrupt the synthesis of hormones and other cell components. Additionally, since Leydig cells are the only source of testosterone production; mercury intoxication had possibly has moderated their functions. However, oxidative stress is a result of the imbalance between ROS and antioxidants in the body, which mercury is responsible on this status, leading, to spermatozoa's damage, deformity and finally provoking male infertility

(**Agarwal *et al.*, 2008**). Similarly, **Moumen *et al.* (2011)** revealed that the exposure of domestic rabbit *Oryctolagus Cuniculus* to mercury chloride during six weeks caused a marked perturbation in testosterone level. Additionally, Pb has induced a reduction in testosterone concentration in male rabbits (**El-Tohamy, 2003);** such decrease could be explicated by the toxic effects of metals that have exhausted the testosterone plasma concentration. Generally, heavy metals are suspected to cause hormonal dysregulation through the neuroendocrine system that can disrupt the secretion of androgens from Leydig cells or from Sertoli cells (**Jensen *et al.*, 2006**). On the other hand, the level of plasma testosterone has not been affected in workers chronically exposed to mercury vapour **(Abdennour *et al.*, 2001**).

Contrary, the supplementation of rats with UD alone showed no significant change in testosterone level. The co-administrated vitamin E with $HgCl_2$, had protected reproductive markers, possibly by reducing Hg absorption through the digestive tract, and possibly reduced Hg level in testis and epididymis (**Rao and Sharma, 2001**). The observed reduction in plasma testosterone level found in this study agrees with the result of **EL-Boushy *et al.* (2000) and Nagwa *et al.* (2008).** The decreased testosterone could be a result from the direct damage of Hg on Leydig cells. Even so, the addition of *Raphanus sativus* rats showed a pronounced enhancement in the testosterone level. Additionally, supplementation of the aqueous extract of *Piper guineense* demonstrated a significant increase in the level of plasma testosterone (**Mbongue *et al.*, 2005**). Moreover, A noticeable correction in all sperm's markers were recorded when vitamin C and Date palm were supplemented to rabbit diet (**Moumen *et al.*, 2011**). Thus, it can be concluded that the repro-protective actions of red radish is due to their antioxidant and free radical scavenging properties common mainly to flavonoids.

The glutathione plays an important detoxifying of trace metals because it rich in SH group. Glutathione is a tripeptide existed in most cells, responsible for hydrophilic xenobiotics conjugation. GSH helps numerous physiological functions including protection of cells from reactive oxygen species (ROS), detoxification of exogenous compounds, and amino acid transport (**KojimaYuasa *et al.*, 2005; Mendoza-Co´ zatl *et al.*, 2005**). Sulfhydryl group of glutathione is essential for its antioxidant activity against many forms of ROS in cells (**Cnubben *et al.*, 2001**). Much of the pathology is linked with the reduction in intracellular GSH concentration (**Rouach *et al.*, 1997**). Probably glutathione is the most important protective mechanism for free radical scavenging and inhibition of xenobiotics attack on cellular macromolecules (**Cnubben *et al.*, 2001**). Testicular GSH concentration in

our research was decreased significantly in the Hg group. Noticeably, males treated with *U.dioica* and *R. Sativus* alone showed a highly significant increase of testicular GSH concentration. But, GSH level of the (Hg+UD) and (Hg+RS) was not different than that of the control. But, there is a significant difference between all groups. **Al-Attar (2011)** demonstrated that Cd, Hg and Pb had significant decrease on the levels of GSH in kidney and testis tissues. The addition of the juice of *R. sativus* and the infusion of *U.dioica*, have improved testicular GSH level in the Hg exposed animals, which in turn reflected a significant enhancement of testicular GSH as compared to $HgCl_2$ treated group. It has been reported that red radish has a high amount of antioxidants (**Stratil *et al.*, 2006**) including anthocyanins which found in red radish with the strongest antioxidant power out of 150 flavonoids (**Elliott *et al.*, 1992**). Also, the leaves and stem of *R. sativus* have showed antioxidant and radical scavenging activity (**Beevi *et al.*, 2010**). Moreover, antioxidants present in *U. dioica* infusion might have a defensive antioxidant and metal-chelating properties against Hg tissue injuries (**Celik and Tuluce, 2007**). Furthermore, the pretreatment of Wistar rats with *R. sativus* prevented the depletion of GSH level and helped the conversion of oxidized glutathione (GSSG) into reduced GSH by the reactivation of hepatic glutathione reductase enzyme after the CCl_4 exposure (**Singh *et al.*, 2009**). Similarly, **Turguta *et al.*** (**2006**) reported that the administration of the antioxidant vitamin E together with aluminium resulted in the recovery of malondialdehyde (MDA) and GSH levels in Al administered Balb-c mice.

The histological examinations of testis & epididymis have demonstrated some lesions in the testis which is certainly attributed to effect of mercury on endothelial cells of small vessels leading to its damage which give rise to increased capillary permeability, this lead to vascular escape of fluid and blood plasma into interstitial compartment, causing oedema, a decrease in capillary blood flow, ischemia and testicular degeneration (**EL-Boushy *et al.*, 2000)**. The testicular and epididymis histo-architecture of the mercury-treated animals from this study showed marked alterations characterized by the presence of different degrees of degeneration in the lining spermatogenic tubules. The lesions in the testis are attributed to the effect of Hg on endothelial cells, leading to its damage and testicular deformation **(EL-Boushy *et al.*, 2000).** Moreover, these overall changes in the testicular and epididymis histological profiles had been aggravated by Hg after the generation of ROS and the impairment of several cell membrane components. Recent studies indicated that the exposure to heavy metals produced testicular damage, which led to spermatogenic arrest (**Massanyi *et***

***al.*, 2007; Burukoğlu and Bayçu, 2008; Moumen and Abdennour, 2008; Al-Mansour, 2009; Obianime and Roberts, 2009**). These authors proved the existing relation between the degenerative statuses observed in the reproductive organs of Hg-treated animals and the serum biochemical data and oxidative stress markers.

Interestingly, rats supplied with a combination of Hg+UD and Hg+ RS have a homogeneous and normal testicular and epididymal tissue's structure. Effectively, it seems that the reproductive organs have been protected by *U. dioica* supplementation. Since this plant contains various minerals such as iron and vitamins A, which are identified for regulating the differentiation of epithelial cells, it seems that the parietal cells of sperm tubules in groups receiving the extract of *U. dioica* were rapidly differentiated and released from the tubules (**Breininger *et al.*, 2005**). This is why the diameter of seminiferous tubules have increased and filled with sperms in the presence of *U. dioica* extract.

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CONCLUSIONS & PERSPECTIVES

CONCLUSIONS & PERSPECTIVES

The objective of this research is to explore, on the one hand, the oxidative stress induced by a popular heavy metal "mercury", and on the other hand, to test the beneficial effects of two Algerian plants "Stinging nettle ***Urtica dioica*** & Red radish ***Raphanus sativus***".

After 30 successive days of treatment, biometric, biochemical and fertility markers, in addition to reduced glutathione level (liver, kidney and testis) and the histological profiles (liver, kidney, testis and epididymis) were evaluated.

The obtained results allowed us to draw the following conclusions:

- The administration of **inorganic mercury** in rats, has engendered in both sexes:
  - A negative effect on rats' growth:
    - ✓ A decrease on the body weight during the period of treatment;
    - ✓ A disturbance of some absolute organ's weight (liver, kidney, testis and epididymis).
  - A disturbance of hepatic and renal functions in males and females rats:
    - ✓ Glucose, Triglycerides, Urea, Creatinine, ALT, AST and ALP were significantly raised;
    - ✓ Mg, Fe and Ca were significantly decreased;
    - ✓ Hepatic and renal GSH were noticeably declined;
    - ✓ Histological profiles showed some hepatic and renal glomerulus impairment.
  - A disturbance of reproductive profile
    - ✓ Concentration, motility, testosterone and testicular GSH were evidently reduced;
    - ✓ Histological profile revealed a marked degeneration of most seminiferous tubules in testis, with few sperms in the epididymis ducts.
- The supplementation of the two Algerian plants (***U. dioica*** & ***R. sativus***), has produced:
  - A positive effect on rat's growth; also, the absolute organ's weight;
  - A noticeable protection of plasma levels of Glucose, Triglycerides, Urea, Creatinine, AST, ALT and ALP;
  - A remarkable enhancement of the hepatic and renal GSH;
  - An amelioration of the histological architecture of liver and kidney tissues;
  - A clear enhancement of reproductive markers (concentration, motility, testosterone and testicular GSH);
  - An improved histological structure of testis and epididymis with the presence of important sperm numbers.

*I*n Perspective

It is interesting to profound these conclusions by:

- Extracting the bioactive substances responsible for detoxification and antioxidant properties of *U. dioica* and *R. sativus*.
- Studying the detoxifying roles of these plants at the cellular and molecular levels;
- Exploring the beneficial side of *U. dioica* and *R. Sativus* in boosting the defensive system and the fertility of males and females.
- Convincing people to go back to their honourable history, and the culture of using medicinal plants for the general prevention firstly, and to treat their diseases secondly.

RESEARCH ACTIVITIES

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RESEARCH ARTICLE
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Can *Urtica dioica* supplementation attenuate mercury intoxication in Wistar rats?

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Abstract

Aim: The objective of this study is to investigate the possible protective role of nettle *Urtica dioica* (UD) against Hg-induced toxicity.

Materials and Methods: A total of 28 rats were equally divided into four groups: the control, the Hg (0.8 g $HgCl_2$/kg in the diet), the UD (1.5 ml UD/rat by gavage), and the Hg+UD group. $HgCl_2$ was daily dissolved in distilled water and immediately mixed with the standard diet. A solution of daily infused fresh nettle leaves in boiling water (16 g in 25 ml) was obtained and then it was administrated by gavage. Biochemical and reproductive markers, in addition to glutathione (GSH) level (liver, kidney and testis) and the histological profiles (testis and epididymis) were evaluated after 1 month exposure.

Results: Compared to the control, the levels of glucose, triglycerides, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were significantly raised in the Hg group. In the latter group, the concentrations of Mg, Fe, and Ca were significantly decreased. Besides, Hg+UD group has only showed raised AST activity and reduced Mg level. Concerning the fertility markers, Hg has provoked a significant decrease in the spermatozoa's concentration and motility and in plasma testosterone level as well. Furthermore, hepatic, renal and testicular GSH concentrations have declined significantly in the Hg treated rat compared to the control. A remarkable enhancement of the GSH level was observed in all organs of the UD group. The histological examinations of the Hg group have revealed marked testicular degeneration of the most seminiferous tubules, and showed few sperms in the lumen of epididymis ducts. However, the Hg+UD rats have demonstrated an improved histological structure with the presence of important numbers of sperms in the lumen. In addition, a clear stabilization of organized seminiferous tubules and an increased sperms' numbers were noted in the UD supplemented rats.

Conclusion: Nettle leaves have not only played a clear protective role during Hg intoxication, but it also enhanced hepatic, renal and testicular GSH level of Wistar rats.

Keywords: biochemical markers, fertility, glutathione, mercury, *Urtica dioica*, Wistar rats.

Introduction

Mercury has been regarded as a priority pollutant by many international agencies [1], because it is widely used in different fields of human life. It has been known that mercury toxicity could provoke neurological, digestive, hematological, renal, respiratory, immune, and reproductive disorders, which are dependent on the dose, the chemical form and the exposure route [2-4]. In fact, mercury has a high affinity and a stable complex to sulfhydryl groups and other biomolecules which might disturb some structures as enzymes [5] and metabolic processes [6]. Consequently, oxidative stress was proposed as one of the most mechanisms of Hg pathological exposure [7].

Healing plants' extracts and their bioactive metabolites play important role in the cases of oxidative injuries, not only in the prevention of diseases, but also to treat them with proven efficacy. The stinging nettle *Urtica dioica* (UD) has a long history of therapeutic utilizations in folk medicine [8]. It is an annual herb that is widely distributed around the world [9], especially in the Mediterranean region. UD contains different beneficial compounds as minerals (iron, manganese, potassium, and calcium), vitamins (A, D and C), proteins, anti-oxidants, chlorophyll, carotenoids [10], flavonoids, fatty acids, and polysaccharides [11]. The aqueous and alcoholic extracts have been used for long time for the treatment of anemia [12], and applied as diuretic in the treatment of urinary, bladder and kidney dysfunctions [13]. Furthermore, its beneficial effects have been reported on inflammation, hypoglycemia, hypotension, benign prostatic hyperplasia [14], and liver failure [15] and acts as an antioxidant [16,17].

The objective of this study is to evaluate the beneficial use of UD, a local natural herb widely distributed, against chronic mercury intoxication of male Wistar rats, where some biochemical and fertility markers were investigated.

Materials and Methods

Ethical approval

The project of the PhD research program has been permitted by the Ethical Committee of Animal

Sciences at the University of Badji Mokhtar-Annaba before starting the experimental work.

Animals

Male adult Wistar rats weighting 165±10 g were obtained from Pasteur Institute, Algiers (Algeria). Animals were maintained in the Animal House of the Biology Department under controlled conditions, in which they subjected to the same conditions of light, humidity and temperature. Standard diet was supplied by "ONAB, rodent feed, Bejaia, Algeria" while food and water were provided *ad libitum*.

Experimental design

About 28 rats were divided into four equal groups: the control, the Hg (0.8 g Hg/kg diet), the UD (1.5 ml UD/rat by gavage), and the Hg+UD group (0.8 g Hg/kg diet+1.5 ml UD/rat). Daily inorganic mercury ($HgCl_2$) was dissolved in distilled water and then it was mixed with known quantity of diet.

The nettle UD was collected from clean area of Souk Ahras region (North-East Algeria) at the beginning of spring, and then it was identified by the department staff. Daily fresh leaves were infused in boiling water (16 g in 25 ml of distilled water) during 5 min to get a hot green solution, which was immediately filtered and left for about 15 min to be cooled, and then it was administrated to rats by gavage. Both mercury and UD solution were given at nearly 09:00 am for 7 days a week during a period of 30 consecutive days.

Blood sampling

Blood was collected from anaesthetized rats by puncture of the jugular vein. Blood samples were immediately collected in labeled polypropylene test tubes containing heparin for biochemical studies. Blood was then centrifuged at 4000 rpm/min for 15 min, and then the plasma biochemical markers were evaluated.

Weight assessment

Total body weight of each rat was measured weekly in the early morning over the experimental period of 1-month while food and water were measured daily at the same time.

Biochemical analysis

Plasma biochemical markers were measured by an automated apparatus (Diatron PICTUS 200) where commercial "Spinreact kits, Spain" were used. Plasma testosterone concentration has been estimated by electrochemiluminescence immunoassay method using the automated apparatus (Cobas e 411).

Fertility markers

The spermogram were realized according to the method of the World Health Organization [18] by making a small incision at the epididymis level to obtain semen. One drop of sperm of nearly 1 µl was added to 49 µl of physiological solution (0.9% NaCl), and then spermatozoa's concentration and motility were estimated.

Histological examination

After sacrifice, testis and epididymis were immediately collected and preserved in 10% neutral buffered formalin, where it was examined according to the classical method of Martoja and Martoja [19].

Dosage of glutathione (GSH)

After removing organs (liver, kidney and testes), about 1 g of each one was homogenized in 2 ml of phosphate buffered saline. Homogenates were centrifuged at 10.000 g for 15 min at 4°C, and the resultant supernatant was used for the determination of reduced GSH [20], modified by Jollow *et al*. [21]. Total GSH content was expressed as nanomoles of GSH a milligram of proteins.

Proteins estimation

Proteins were measured by the method of Bradford [22] using bovine serum albumin as a standard.

Statistical analysis

The statistical analysis was achieved using one-way analysis of variance to compare between all groups, followed by Student's t-test. The test was considered significant at $p<0.05$ level.

Results

Body weight

Final mean total body weights (182±2±23.5 g) after 30 days Hg exposure has decreased considerably compared to the initial weight (163.2±15.3 g) with a percentage of (−10.44%). Whereas, those of UD and Hg+UD mean total body weight have increased by (+13.66%) and (+9%), respectively. However, the final total body weight of the control has risen by (+16%) after 30 days. The data were presented in Figure-1.

Clinical observations

During the period of mercury administration some adverse neurological and behavioral changes were observed. Rats have lost appetite relatively and they were anxious at the beginning of the experiment, then they started to be quiet thereafter due to tiredness

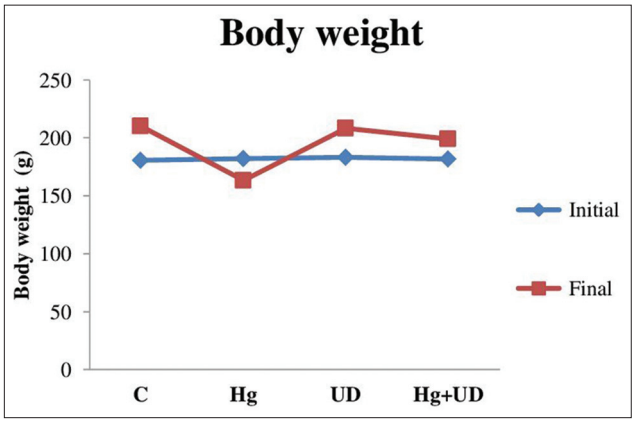

Figure-1: Initial and final total body weight (g) of males rats subjected to mercury and *Urtica dioica* during 30 consecutive days.

and muscle weakness, while the respiration rate has risen. Rats' unsteady walking is probably an indication of poor coordination. There was also a decrease in total body weight accompanied with a loss of skin hair in various body regions. Moreover, large fluctuation of food and water consumption was noticed, especially in the first few days. Contrary, the behavioral activities of the UD and the Hg+UD groups were comparable to the control.

Biochemical markers

Data are presented in Table-1. The mercury-treated rats caused a significant elevation in the level of glucose, triglycerides, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) when compared to the control rats. Contrary, results showed a significant decrease in the level of Mg, Fe, and Ca of the Hg treated group. In the combined treatment (Hg+UD), only AST and Mg levels were significantly different than that of the control. Creatinine, Mg and Ca concentrations were significantly different between all groups.

Fertility markers

Mean sperm concentration and motility were decreased significantly in rats exposed to mercury alone compared to the control. However, the concentration and motility of sperm cells were remarkably increased in both (UD) and (UD+Hg) groups in comparison with the control (Figures-2 and 3). Compared to the control, testosterone concentration has declined clearly in the Hg exposed group, but its decline was not statically significant in the combined treatment (Figure-4). The levels of fertility markers in the UD group were comparable to that of the control.

GSH

A significant decrease of hepatic GSH level was observed in rats exposed to mercury, but GSH concentration was remarkably raised in the UD group, followed by Hg+UD group with less extent (Figure-5). Renal GSH concentration was significantly lower in the Hg exposed animals compared to the control and the UD group as well (Figure-6). Accordingly, there was a significant reduction in testicular GSH level of the Hg group compared to the control, while its level was remarkably higher in the UD treated rats (Figure-7). A large fluctuation of testicular GSH concentration between individuals of the Hg+UD group was seen.

Histological profile

Figures-8 and 9 illustrated the histological examination of testis and epididymis tissues of different treatment groups. Microscopic assessment revealed the normal structure of seminiferous tubules, sperms with normal morphology and concentration in the control. The Hg has caused intoxication demonstrated by few numbers of spermatozoa in the tubules, different degrees of degeneration in the lining spermatogenic tubules, and delocalization of seminiferous tubules.

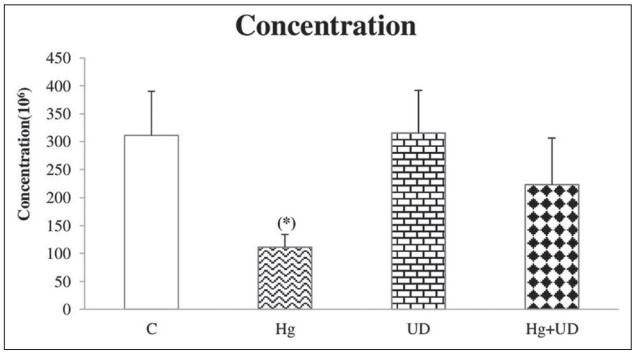

Figure-2: Spermatozoa's concentration (10^6) of males rats subjected to mercury and *Urtica dioica* during 30 consecutive days. * Significantly different when compared to the control.

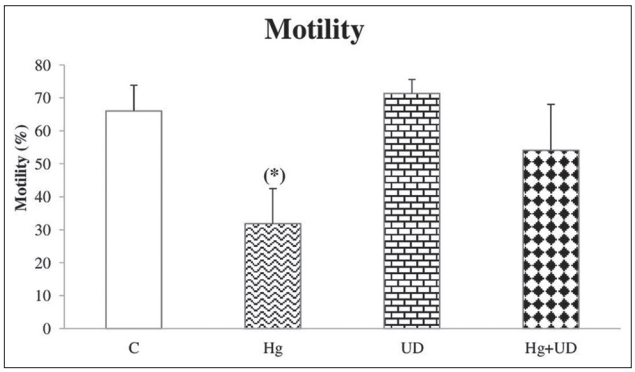

Figure-3: Spermatozoa's motility (%) of males rats subjected to mercury and *Urtica dioica* during 30 consecutive days. *Significantly different when compared to the control.

Table-1: Effect of UD on biochemical markers of rats after 30 days Hg intoxication.

Groups	Control	Hg	UD	Hg+UD
Glucose (g/L)	0.88±0.16	1.57±0.64*	0.80±0.10	0.86±0.16
Triglycerides (g/L)	0.32±0.02	0.59±0.10*	0.32±0.02	0.33±0.04
Urea (g/L)	0.25±0.06	0.47±0.06*	0.27±0.06	0.23±0.05
Creatinine (mg/g)	0.44±0.45#	0.81±2.64*#	0.40±0.73#	0.40±0.89#
AST (UI/L)	64.3±16.2	169.5±36.9*	66.9±15.2	105.0±16.5*
ALT (UI/L)	26.05±4.38	67.31±9*	25.95±3.18	26.86±6.03
ALP (UI/L)	118.9±20.6	229.0±20.8*	102.4±10.3	120.0±15.4
Mg (mg/dl)	3.30±0.63#	1.08±0.10*#	3.65±0.54#	2.49±0.53*#
Fe (µg/dl)	107.6±10.2	56.5±05.6*	109.5±13.7	102.9±11.1
Ca (mg/L)	82.60±6.07#	69.14±9.88*#	89.42±5.63#	76.20±12.40#

*Significantly different when compared to the control, #Significantly different between groups. AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, UD=*Urtica dioica*

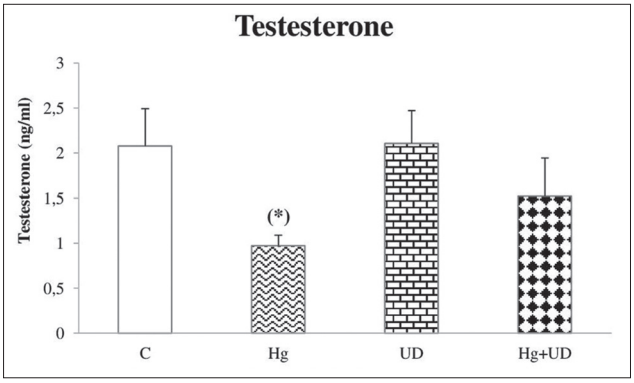

Figure-4: The concentration of testosterone (ng/ml) of male rats subjected to mercury and *Urtica dioica* during 30 consecutive days. *Significantly different when compared to the control.

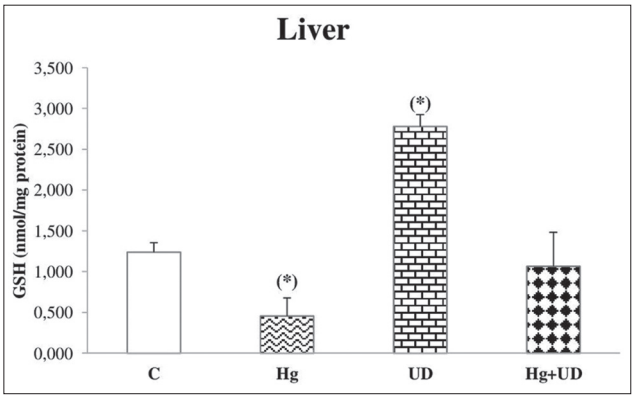

Figure-5: The level of hepatic glutathione (nmol/mg protein) of male rats subjected to mercury and *Urtica dioica* during 30 consecutive days. *Significantly different when compared to the control.

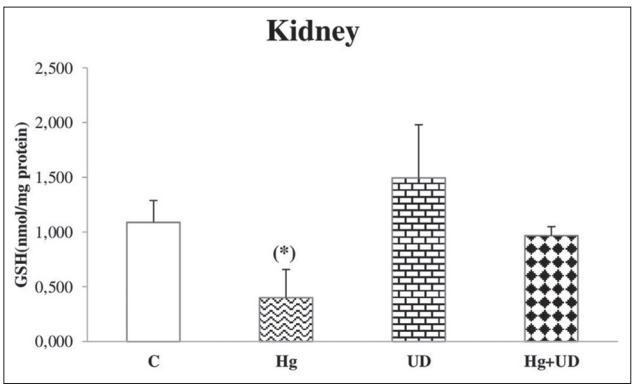

Figure-6: The level of renal glutathione (nmol/mg protein) of male rats subjected to mercury and *Urtica dioica* during 30 consecutive days. *Significantly different when compared to the control.

On the other hand, the toxic effects of mercury were reduced in testis of Hg+UD supplemented animals. In these circumstances, sperm with normal morphology, concentration and motility were observed and was close to the control group. Interestingly, rats supplied with a combination of Hg+UD or UD have a homogeneous and normal testis and epididymis tissue's structures. The UD treated group showed stabilization of organized seminiferous tubules and many newly formed spermatogenic cells arranged properly

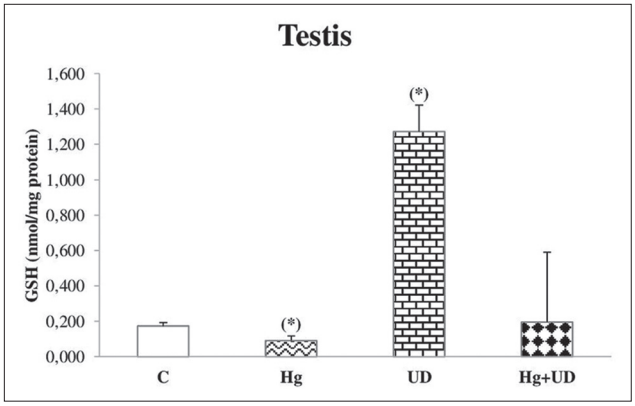

Figure-7: The level of testicular glutathione (nmol/mg protein) of male rats subjected to mercury and *Urtica dioica* during 30 consecutive days. *Significantly different when compared to the control.

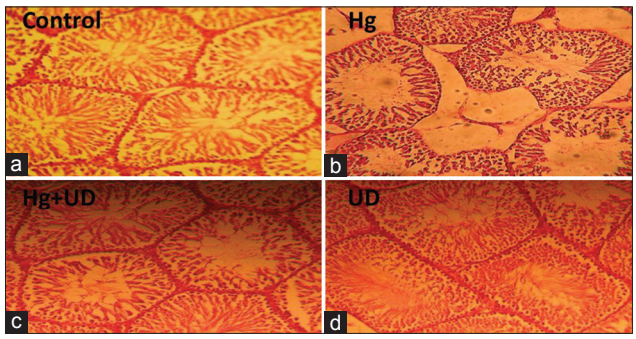

Figure-8: Histological profile of male rats testis showing the control showing normal histology, the *Urtica dioica* (UD), the Hg and the Hg+UD groups after 30 days treatment (×250), (a) Control: It has normal histological structure of active mature functioning seminiferous tubules associated with complete spermatogenic series, (b) Hg: It showed marked degeneration of most seminiferous tubules with absence of spermatogenic series in tubular lumen and a thickening of basal membrane, (c) Hg+UD: It showed normal histological structure of most seminiferous tubules, (d) UD: It showed and improved concentration of sperms and a clear stabilization of organized seminiferous tubules with many newly formed spermatogenic cells arranged properly in the tubules.

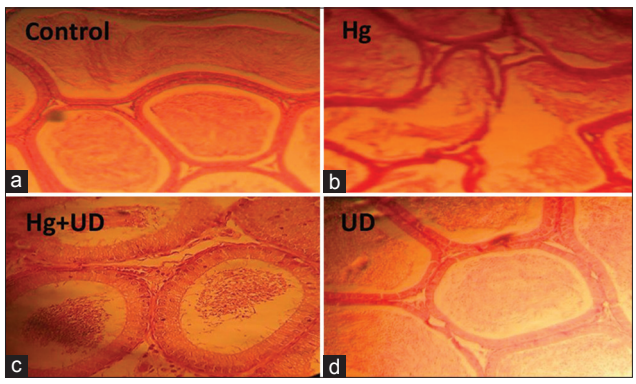

Figure-9: Histological profile of male rats epididymis showing the control, the Hg, Hg-*Urtica dioica* (UD) and UD the groups after 30 days treatment (×250), (a) Control: It showed normal histological structure, (b) Hg: It revealed few sperms in the lumen of epididymis ducts with irregular basement membrane disorganization and degeneration of some spermatogenic cells, (c) Hg+UD: It demonstrated nearly normal epididymis stucture with the presence of important numbers of sperms in the lumen, (d) UD: It showed an improved epididymis stucture with large numbers of sperms in the centre.

inside the tubules better than the control group. Results obtained from the histological architecture were inconsistency with the reproductive markers as well as the GSH status.

Discussion

The administration of Hg to rats showed a significant decrease of total body weight compared to the control. Such results might be related to the decrease of daily food and water consumption; which is in accordance with the report of National Toxicology Program Working Group [23]. Besides, in these experimental conditions, the presence of UD with Hg has caused an enhancement of rat body weight. Previously, UD antioxidants have been reported to eliminate free radicals produced [24] with no secondary effects [25].

In this study, the exposure of rats to Hg during 30 days has increased plasma glucose, but no difference was recorded in rats treated with Hg+UD. Previously, Hg intoxication was suggested to increase the energy supply to cope with the metal stress [26]. Interestingly, a remarkable hypoglycemia was registered in $HgCl_2$ intoxicated rats, which was more obvious after 60 days exposure [27]. In this case, it seems that the treatment of diabetes with UD has reduced blood sugar to its normal level [28,29], and had hypoglycemic effect as well as through improving insulin secretion of hyperglycemic rats [30].

The actual results show that the concentration of triglycerides raised remarkably in the Hg treated group, which is not the case for the combined treatment of Hg+UD. The UD leaves contain chlorophyll, which has been proved to have hypolipidemic effects [31]. The UD extract has shown an apparent effect on animal models at doses of 100 and 300 mg/kg by reducing the levels of total cholesterol and low density lipoprotein, accompanied with a remarkable decrease of liver enzymes and total body weight when fed a high cholesterol diet [32].

This study demonstrates that the treatment of rats with Hg has led to a pronounced elevation of urea and creatinine concentration. It is well known that Hg administration could accumulate in renal tissues [33], and elevate these markers [34-36]. On the other hand, the supplementation of rats with Hg+UD or UD alone showed no noticeable change of urea and creatinine. Therefore, flavonoids and the high potassium content of UD may contribute to the diuretic action, which allows the body to excrete wastes including mercury, and that why stinging nettle is being used as a diuretic agent [14].

Results indicated a significant increase in plasma AST and ALT activities by mercury, but UD has preserved normal level of AST activity only. This increase is certainly come from damaging the plasma membrane permeability through the fixing of Hg ions to its proteins [37]. The increased in liver enzymes during Hg intoxication was already supported by Sheikh *et al*. [38] and Ejebe *et al*. [39]. After 5 weeks exposure to mercury chloride, serum AST activity was higher in rabbits compared to the group supplemented with a combination of *Pistacia lentiscus* oil and Hg [40]. The treatment with UD effectively protected rats against Aflatoxin-induced hepatotoxicity, as evidenced by the decreased AST and ALT activities [41]. Furthermore, UD treatment for 60 days has exhibited remarkable reduction in the liver enzyme levels and also has increased the antioxidant enzyme activities in tetrachloromethane-treated rats [42]. The present results are in accordance with the reported results concerning the protective role of UD [43].

The results of this work indicated the clear effect of Hg on plasma ALP activity. This enzyme is liberated into the blood when parenchymal liver cells are damaged. It is reported that the treatment of rats by $HgCl_2$ for 6 months had increased the activity of ALP [23]. In addition, Rao and Sharma [44] have observed a reduction in mice ALP activity treated with $HgCl_2$ for 45 days. Contrary, no significant change was reported in the group fed with Hg and UD. Accordingly, the efficiency of blue-green algae *Spirulina fusiformis* was obvious to protect ALP activity from Hg toxicity in mice [45].

The current results showed a significant decrease in the levels of Mg, Fe and Ca of rats exposed to Hg. Meanwhile no variation was noted concerning these minerals in the group treated with Hg+UD. This lowering effect was happened probably because Hg is known to cause lack of appetite [46], as well as it is able to provoke many dysfunctions in the absorption of nutrients [47]. However, no changes in the levels of Mg, Fe and Ca was seen in rats supplemented with UD alone, possibly that is due to the high content of minerals and vitamins found in different parts of this herb [9], especially in the fresh leaves [48].

Mercury exposed rats of the present study indicated a reduction in the concentration and motility of epididymal sperm. Such results are in agreement with that of Pb on rabbit reproductive system [49]. Accordingly, another study revealed that epididymis sperm count and motility were markedly reduced by $HgCl_2$ [50]. The latter has led to spermatozoa's damage, human infertility [51] and affected mice reproductive performance [52]. Thus, the epididymis is known to play an important role in providing the microenvironment for sperm maturation and storage. Consequently, the decrease in epididymal number/motility of sperm could be explained on the basis that Hg has crossed the blood-testis barrier and gained access to germinal cells, leading to testicular dysfunction [53]. Mammalian spermatozoa are rich in polyunsaturated fatty acids which are very vulnerable to reactive oxygen species (ROS) attack; the latter induce a decrease of sperm motility, presumably by a rapid loss of intracellular adenosine triphosphate, causing testicular damage [54]. Meanwhile, noticeable improvements of all sperm's markers were recorded when UD was supplemented alone or combined with Hg, which

certainly suggests the protective roles played by this wild herb. Apparently, UD contains many minerals and vitamins, especially vitamin C and E, as well as flavonoids [11], which can act as antioxidants to counteract Hg toxicity. Furthermore, many studies revealed that animals receiving vitamin E alone or combined with $HgCl_2$ had sperm markers similar to the control values [49].

In this study, Hg has reduced testosterone concentration deeply after 1 month exposure. Hg intoxication may have affected testosterone synthesis at the level of Leydig cells. The supplementation of rats with UD alone or combined with Hg showed noticeable increase in testosterone level, which is in line with the results of mice exposed to $HgCl_2$ and vitamin E together [44]. The variation in testosterone level of this study totally agree with the result of El-Boushy *et al*. [55] and Nagwa *et al.* [56] who observed a reduction in plasma testosterone concentration under Hg exposure, but it was different than that of workers chronically exposed to mercury vapor [57].

The testicular and epididymal histoarchitecture of Hg-treated animals from this study showed marked alterations characterized by the presence of different degrees of degeneration in the lining of spermatogenic tubules. Such lesions are attributed to Hg effect on endothelial cells, leading to its damage and histological deformation [55]. These overall changes of the histological profiles were attributed to ROS formation leading to the impairment of several cell membrane components. The degenerative status observed is in support with the biochemical data and oxidative stress markers. Interestingly, rats supplied with a combination of Hg+UD or UD alone have a homogeneous and normal testicular and epididymal tissue structure. Since UD leaves contain various minerals as iron and vitamins A, which have been identified for regulating the differentiation of epithelial cells, it seems that the sperm cells in group receiving the extract of UD were rapidly differentiated and released from tubules [58], leading to increased diameters of seminiferous tubules.

In the present investigation, the GSH level showed a noticeable significant hepatic, renal and testicular depletion the following Hg exposure. These results were parallel to Sheikh *et al*. [38] and Syversen and Kaur [59] who reported that exposure to $HgCl_2$ were significantly decreased the reduced GSH and various antioxidants. Such depletions are likely related to the high affinity of Hg ions to thiol groups, as a result Hg could disturb cell functions [60]. It has been recommended that antioxidants could be valuable to the treatment of Hg injuries [61], therefore, the metal-GSH conjugation manner is necessary in the elimination of Hg into the bile. Hg was reported to cause remarkable exhaustion of GSH in the liver [62], testis [63] and other cell types [64]. Moreover, GSH-Hg complexes have been found in liver, kidney, and brain, and seemed to be the primary form in which Hg is transported and eliminated from the body [65]. On other hand, the co-administration of UD with Hg has resulted in keeping GSH at the control level. GSH is the main thiol antioxidant and the conjugating agent; it was known to bind electrophilic molecular species and free radicals intermediate. The supplementation of UD extracts demonstrated an antioxidant and hepatoprotective effect against anxious stimulus by tetrachloromethane [56]. Many studies proved that the flavonoids and phenols are good antioxidant against Hg-induced patho-toxicity and also act as effective chelators for several toxic metal ions [66]. Antioxidants present in UD infusion might have a defensive antioxidant and metal-chelating properties against Hg tissue injuries [43].

Conclusion

From this investigation, Hg has altered many biological markers of rats including GSH. The histological structure of liver, kidney and testes were also affected. However, the consumption of UD along with Hg has helped preventing such markers, especially spermatozoa's concentration and motility. Therefore, fresh nettle leaves are cheap natural protective herb that may play a beneficial role in the prevention of Hg intoxication.

Authors' Contributions

WS has carried out the experimental work in the AE laboratory and then she prepared the manuscript, while CA has checked the manuscript and polished English language. Both authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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