الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي و البحث العلمي

University of Ferhat Abbas Setif 1

Faculty of Life and Nature Sciences

جامعة فرحات عباس، سطيف 1

كلية علوم الطبيعة و الحياة

DEPARTMENT OF BIOCHEMISTRY

N° ...../SNV/**2023**

Thesis

Presented by

BETTIHI Sarra

Submitted for the fulfillment of the requirements for the degree of

Doctorate of Sciences

In Biology

OPTION: Biochemistry

Theme

The toxic effect of the total alkaloids extract of the seeds of *Peganum harmala* L. on ovogenesis, embryology, and fertility in rat Albinos Wistar.

Discussed on / /

jury

President	**DJIRAR Nacer**	**Pr. UFA Setif-1**
Supervisor	**BOUZIDI Abdelouahab**	**Pr. UFA Setif-1**
Co-supervisor	**MAHDEB Nadia**	**MCA. Setif-1**
Examiner	**SOBHI Widad**	**Pr. Constantine**
	MOSBAH Asma	**MCA. Constantine 1**
	MENNANI Achour	**MCA. M'sila**

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty ALLAH for granting me good health, peace, strength and patience to complete my study.

I would like to express my most sincere gratitude to my supervisor, Professor ***BOUZIDI ABDELOUAHAB*** *and his wife* ***MAHDEB NADIA****. I am so grateful to him especially for encouraging me to achieve this study, all the support and encouragement you have given me, and that you've always had time to listen to my ideas and questions. they are not only my mentors at work but also a friends during the past years. I would never have been able to finish my work and dissertation without your help. I would also like to thank, Professor* ***KHABAR ARBI****, for his encouragement and motivation during my work.*

I would like to express my gratitude to all my colleagues at the Department of Biochemistry for creating a nice and friendly atmosphere in work. Special thanks to ***BOUCHEFFA SALIHA, SEDANI SAID, MAAMACH WALID, KARICH SEHAM****, for their great help.*

My sincere gratitude goes to Pr. ***DJARAR NACER*** *of the university of Sétif for having agreed to chair the thesis defense jury, as well as to Pr.* ***SOBHI WIDAD*** *from the university of Constantine,* ***MESBAH ASMA*** *from the university of Constantine too, and* ***MENNANI ACHOUR*** *from the university of M'sila for agreeing to judge this work.*

Finally, Great thanks to my friends for giving me unforgettable memories, encouragement and motivation during my work on this thesis.

BETTIHI

Dedication

All of the letters will be unable to find the words that they should...

express our gratitude, love, respect, and recognition.

So there you have it:

I dedicate this thesis...

To my cherished ***father***

To my cherished ***mother***

No dedication, dear parent, could express the depth of my feelings for you; your selfless sacrifices and devotion inspired me.

To my husband ***Tahar***

For the love and affection that binds us together.

I can't thank you enough for your unwavering support over the years.

You have always encouraged me, incited me to do better, and your support has enabled me to realize the long-awaited dream.

With my best wishes for success, prosperity, and happiness, I dedicate this work to you.

I beseech Almighty ALLAH to keep our bond strong and to grant all of our wishes.

To my children, ***Baraa****,* ***Roaya****, and* ***Alaa****.*

To my brothers ***Yassine****,* ***Yasid, AbdGhani****, and* ***Salaheldine****,*

To my sisters ***Samah****,* ***Nassma,*** *and* ***Nadjet***

I could never imagine life without you,

You mean a lot to me, you are who ensure

their role as it should be, I will never forget their

encouragement and support throughout my studies,

I esteem all of you very much and I love all of you very much.

To my dear friends…

SARRA

الملخص

تهدف هذه الدراسة الى تبيان الأثر السمي لمستخلص القلويدات الكلية لنبات *Peganum harmala* L. على كل من تكون الجريبات والإباضة، والتكون الجنيني، والإخصاب. يعتبر نبات *Peganum harmala* L. من النباتات الطبية و المعروف محليًا باسم الحرمل، والتي تستخدم بذورها في الطب التقليدي كعامل مدر للطمث والإجهاض بالإضافة إلى استعمالاتها الدوائية المختلفة. نتج عن استخلاص القلويدات الكلية من البذور ما يقارب عن 1.820± 0.358غ لكل 100غ (وزن/وزن) في هيئة مسحوق أحمر داكن. تم تصنيف نبتة الحرمل ضمن النباتات متوسطة السمية حسب النتائج المتحصل عليها من دراسة الجرعة القاتلة DL_{50} لذكور وإناث الجرذان و التي قدرت بالقيمتين 164.43مغ/كغ و159.953مغ/كغ على التوالي. أظهرت دراسة سمية المستخلص الكلي للقلويدات على المبايض و الهرمونات عند إناث الفئران المعالجة بالحقن تحت السفاقي لجرعة 3.99 مغ/كغ/يوم (DL_{50}40/1) لمدة ثلاثة أشهر زيادة معنوية في الوزن النسبي لكل من الكلية والقلب والرئتين والدماغ والمبايض، و زيادة معنوية في PLA، تغير معنوي في ALAT و ASAT و البيليروبين الكلي وغير المباشر، واليوريا، وزيادة معنوية في هرموني FSH و الإستروجين. نتج عن دراسة سمية المستخلص الكلي للقلويدات على إناث الجرذان أثناء فترة الحمل عن طريق الحقن تحت السفاقي لجرعة 7.99 مغ/كغ/يوم (DL_{50}20/1) ارتفاع معدلات الحمل المؤكدة بنسبة 90-100%، بالإضافة الى زيادة معنوية في وزن الجسم، و تغير الوزن النسبي للمبايض بشكل معنوي في جميع المجاميع المعالجة. بين التحليل تغير معنوي في العديد من المقاييس البيوكيميائية في المجاميع المعالجة، و أظهر التحليل الهرموني تغيرا معنويا في مستويات FSH و البروجسترون و الاستروجين في المجموعات المعالجة مقارنة بالمجموعة الشاهدة. أظهرت نتائج تأثير المستخلص الكلي للقلويدات فرقاً معنوياً في مجموع الاجنة، و نسبة وفياتها، و ارتفاع معنوي في وزنها، كما بينت انخفاضًا ملحوظًا في عدد نقاط التعشيش، و نسبة الأجنة غير المتطورة في المجموعات المعالجة. أما في ما يخص دراسة الخصوبة لذكور الجرذان و المعالجة بجرعة 4.110 مغ/كغ/يوم (DL_{50}40/1) لمدة 90 يومًا وتزاوجها مع إناث عذراء في نهاية فترة المعالجة ، فقد بينت النتائج عدم وجود أعراض مرضية للإناث خلال فترة الحمل أو تسجيل حالات الوفاة، مع تغير أوزانها معنويا خلال فترة الحمل. أما في ما يخص التحليل البيوكيميائي و الهرموني فقد أظهر تغيرات معنوية في مستويات البيليروبين الكلية وغير المباشرة و الكرياتينين و FSH و LH، كما كشفت الدراسة عدم وجود فرق في عدد الإناث الحوامل، و لوحظ اختلاف معنوي في عدد اللأجسام الصفراء ومواقع التعشيش، وأوزان وعدد وفيات الأجنة. نقترح بناءا على النتائج المتحصل عليها أن المستخلص الكلي للقلويدات من البذور و حسب الجرعة المستخدمة في كل دراسة يمكن أن يحفز تكوين الجريبات والإباضة عن طريق زيادة إفراز الهرمونات التناسلية FSH و الاستروجين، كما يمكن أن يتسبب في تسمم الحمل، التطور الجنيني، مما يؤدي الى الإجهاض.

الكلمات المفتاحية: *Peganum harmala* L.، القلويات، الحمل، اضطراب تكوين البويضات، التسمم الجنيني، الإجهاض، المؤشرات البيوكيمياء، الهرمونات.

Abstract

The aim of this study is to evaluate the toxic effect of the total alkaloid extract of the seeds of *Peganum harmala* L. on ovogenesis, embryology, and fertility in rat Albino Wistar. *Peganum harmala* L. (Zygophyllaceae) is a medicinal plant known locally as Harmel. Traditional medicine has used the seeds as an emmenagogue and abortifacient agent, as well as for other pharmacological effects. The extraction was yielding approximately 1,820±0,358 per 100g (w/w) of a dark red powder. Depending LD_{50} (164.43mg/kg. and 159.953mg/kg) of total extract in male and female rats classified this plant as moderately toxic. The hormonal imbalance, ovarian toxicity, and oogenesis disruption in treated female rats by total alkaloids extract for three months of daily IP administration of 3.99 mg/kg/day ($1/40LD_{50}$) show significant increases in the relative weight of the kidney, heart, lungs, brain, and ovaries. The study shows a substantial increase in PLA, and significant changes in ALAT, ASAT, total and indirect bilirubin, and serum blood urea. The hormonal analysis shows that FSH and Estrogen have increased significantly. The results in maternal toxicity by daily IP administration of 7.99 mg/kg/day ($1/20LD_{50}$) of total alkaloids extract summarized in confirmed pregnancy rates were high (90-100%), maternal body weight and weight gain changes were statistically significant in all pregnant. Precisely, the relative weight of ovaries was significantly changed in all the treated groups. Serum's biochemical parameters were changed significantly in the group treated compared to the control group. The hormonal analysis shows considerable changes in FSH, Progesterone, and Estrogen levels in treated groups. The results show a significant difference in fetus weight, the number of implantations, and total resorbed litter. For fertility evaluation, the male rats were treated IP with 4.110 mg/kg/day ($1/40DL_{50}$) of the total alkaloid extract for 90 days and mated with a virgin female at the end of the study period, the results show no maternal morbidity and mortality with a normal behavior during the gestational period. The maternal body weight was changed significantly in all pregnancy rates. Serum's biochemical analysis shows significant changes in dams. The hormonal analysis shows significant changes in FSH and LH. The results revealed no difference in the number of pregnant females, and a significant difference in corpus luteum number, implantation sites, resorption, and fetal body weights. We can hypothesize that the total extract in the seeds, at the dose used, can stimulate folliculogenesis and ovulation by increasing gonadotropin hormone secretion (FSH and Estradiol), but it has negative effects on maternal toxicity, embryonic development, and abortion.

Keywords: *Peganum harmala* L., Alkaloids, Pregnancy, Oogenesis disruption, Maternotoxicity, Abortion, Serum biochemical, Hormones.

Résume

Le but de cette étude est d'évaluer l'effet toxique de l'extrait des alcaloïdes totaux des graines de *Peganum harmala* L. sur l'ovogenèse, l'embryologie et la fertilité chez le rat Albino Wistar. *Peganum harmala* L. (Zygophyllaceae) est une plante médicinale connue localement sous le nom de Harmel. La médecine traditionnelle a utilisé les graines comme emménagogue et agent abortif, ainsi que pour d'autres effets pharmacologiques. L'extraction a donné environ 1 820 ± 0,358 pour 100 g (p/p) d'une poudre rouge foncé. Selon la DL_{50} (164,43 mg/kg et 159,953 mg/kg) de l'extrait total chez les rats mâles et femelles, cette plante a été classée comme une plante modérément toxique. Le déséquilibre hormonal, la toxicité ovarienne et la perturbation de l'ovogenèse chez les rats femelles traitées par l'extrait d'alcaloïdes totaux pendant trois mois d'administration IP de 3,99 mg/kg/jour ($1/40DL_{50}$) montrent des augmentations significatives du poids relatif des reins, du cœur, poumons, cerveau et ovaires. L'étude montre une augmentation substantielle du PLA et des changements significatifs de l'ALAT, de l'ASAT, de la bilirubine totale et indirecte et de l'urée sanguine sérique. L'analyse hormonale montre que la FSH et les œstrogènes ont augmenté de manière significative. Les résultats de la toxicité maternelle par administration IP quotidienne de 7,99 mg/kg/jour ($1/20DL_{50}$) d'extrait d'alcaloïdes totaux résumés dans les taux de grossesse confirmés étaient élevés (90-100 %), les modifications du poids corporel maternel et du gain de poids étaient statistiquement significatives chez toutes enceintes. Précisément, le poids relatif des ovaires a été significativement modifié dans tous les groupes traités. Les paramètres biochimiques du sérum ont été significativement modifiés dans le groupe traité par rapport au groupe témoin. L'analyse hormonale montre des changements considérables dans les niveaux de FSH, de progestérone et d'œstrogènes dans les groupes traités. Les résultats montrent une différence significative dans le poids du fœtus, le nombre d'implantations et la portée totale résorbée. Pour l'évaluation de la fertilité, les rats mâles ont été traités IP avec 4.110 mg/kg/jour ($1/40DL_{50}$) de l'extrait alcaloïde total pendant 90 jours et accouplés avec des femelles vierges à la fin de la période d'étude, les résultats ne montrent aucune morbidité maternelle et la mortalité avec un comportement normal pendant la période de gestation. Le poids corporel maternel a changé de manière significative dans tous les taux de grossesse. L'analyse biochimique du sérum montre des changements significatifs chez les mères et l'analyse hormonale montre des changements significatifs dans la FSH et la LH. Les résultats n'ont révélé aucune différence dans le nombre des femmes enceintes et une différence significative dans le nombre de corps jaunes, les sites d'implantation, la résorption et le poids corporel des fœtus. On peut émettre l'hypothèse que l'extrait total dans les graines, à la dose utilisée, peut stimuler la folliculogenèse et l'ovulation en augmentant la sécrétion d'hormones gonadotrophines (FSH et Estradiol), mais il a des effets négatifs sur la toxicité maternelle, le développement embryonnaire et l'avortement.

Mots clés : *Peganum harmala* L., Alcaloïdes, Grossesse, Perturbation de l'ovogenèse, Maternotoxicité, Avortement, Sérum biochimique, Hormones.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

AChEl: Acetylcholinesterase inhibitor.

ACTH: adrenocorticotropic hormone.

ALAT: Alanine Amino-transferase.

ALP: Plasma Alkaline Phosphatase.

ASAT: Aspartate Amino-transferase.

CBG: cortisol binding globulin.

CCAAT: cytosine-cytosine-adenosine-adenosine-thymidine..

CL: Corpora luteal.

CNS: centre nervous system.

CRH: Corticotropin releasing hormone.

DHEA: dehydro-epiandrosterone.

DL_{50}: dose lethal de 50%.

DNA: Deoxyribonucleic acid.

EPS: Serum protein electrophoresis.

ER: estrogen receptor that they bind.

F: follicles.

fl: femtoliter.

FSH: follicle stimulating hormone.

GammaGT: Gamma Glutamyl-transferase

Gd: gestational day.

GnRH: gonadotropin releasing hormone.

GRA%: Granulocyt neutrophil.

H&E: hematoxylin and eosin.

Hb: Hemoglobin.

hCG: Human chorionic gonadotropin.

HCS: human chorionic somatomamotropin.

HCT%: Hematocrit.

HCT: Hematocrit.

HDL-c: high density lipoprotein.

HIV: human immunodeficiency virus.

HPL: placental lactogen.

HDL-c: HDL-cholesterol.

HSP: heat shock protein.

K: Number of doses.

LDL-c: LDL-cholesterol.

LH: luteinizing hormone.

LDL: Low Density Lipoprotein.

LPCR: Large Platelet Concentration Ratio

LPCR: Platelet Concentration Ratio

LRH1: liver receptor homologue1.

LYM g/l: lymphocyte

LYM%: Absolute Lymphocyte count

MAO: monoamine oxidase.

MAOI: monoamine oxidase inhibitors.

MCHC: mean corpuscular of Hb concentration

MCV: mean corpuscular volume

MDW: Red distribution width

MID%: Large Mid-sized cell

MPV: mean platelet volume

PDI: platelet distribution index

PPAR γ: peroxisome proliferator-activated receptor γ.

PR: progesterone receptor.

PR-A: progesterone receptor type A.

PR-B: progesterone receptor type B.

PTC: platelet total concentration,

RBC: Red Blood Cell

SEM: standard error of the mean.

SF1: steroidogenic factor1.

SHBG: sex hormone binding globulin.

StAR : steroidogenic acute regulatory

T3: triiodothyronine

T4: thyroxine

TGF-β: transforming growth factor-β.

THBG: thyroid hormone binding globulin.

TLC: thin layer of chromatography.

TSH: Thyroid stimulating hormone.

UCP: Uncoupling proteins;

WBC: white blood cell

Zitch: Streptozotocin-induced diabetic.

TABLE OF CONTENTS

SECOND CHAPTER.

MATERIAL AND METHODS

THIRD CHAPTER: RESULTS

FOURTH CHAPTER. DISCUSSION

General introduction

General introduction

For thousands of years, herbal medicine has been used for primary health care by 80% of the world's population. Herbal remedies have recently gained popularity as a dietary supplement for disease prevention and as an alternative/complementary medicine. A wide range of herbal medicines are widely available on the market worldwide and because of the increased use of herbal products, the safety and efficacy of herbal medicine have become a public health concern and an adverse health effects associated with herbal products could be attributed to both herbal medicine's inherent toxic effects and toxicities caused by adulterants/contaminants. The growing body of evidence regarding the side effects of herbal medicine has highlighted the demand and importance of toxicological studies for herbal products. In addition, toxicology constitutes an essential role in the development of herbal medicines, with the advancements of analytical techniques and molecular technology, coupling with the conventional test systems (Woo et *al*., 2012).

Peganum harmala L. (Zygophyllaceae), also known as "Harmal," is a plant that grows naturally in semiarid and pre-desertic regions, and it is widely distributed in North Africa and the Middle East. The seeds of this plant were used as a powder, decoction, maceration, or infusion in Algerian traditional medicine for fever, intestinal pain, diarrhea, subcutaneous tumors, and abortion, and it is widely used as a remedy for other various health conditions.

P. harmala seeds include β-carboline alkaloids, such as harmine, harmaline, harmol, and harmalol, as well as quinazolines, which are responsible for the toxicological and pharmacological effects of the plant (Herraiz et *al*., 2010).

All parts of the plant were reported to be toxic and severe intoxication occurs, digestive and nervous syndromes have been reported in domestic animals after consumption of a sublethal amount of the plant, and physical symptoms of poisoning appeared in a narcotic state interrupted by occasional short periods of excitement. Abortion is frequent in animals that digest this plant clearly warning, for this reason therapeutic use to pregnant women should be prohibited. Overdoes of *P. harmala* can cause hallucinations and neurosensorial syndromes, as well as bradycardia, nausea, and vomiting. In addition, the toxicity has been reported in humans, with poisoning symptoms similar to those seen in domestic animals (Kuete, 2014).

The objective of this study is to show the richness of *P. harmala* in metabolites side effects and to determine their toxic properties at the dose used on folliculogenesis and ovulation, as well as the adverse effects on maternotoxicity, embryonic development, and abortion. To this end, in this study, we first presented some knowledge in the bibliography section on the botanical characteristics of the studied plant, their chemical compositions. Secondly, The experimental side consisted of three main parts, the first was phytochemical extraction of the major alkaloids from the seeds and simple phytochemical screening using the chromatographic technique. The second aspect is devoted to evaluate the toxic effects of total alkaloid extract on the folliculogenesis and ovulation, maternotoxicity, embryonic development, and abortion by evaluate its effects on hematological, biochemical, and hormonal parameters related to structure and function in the conditions of oogenesis, embryology, and abortion.

FIRST CHAPTER: BIBLIOGRAPHIC SYNTHESIS

I. Presentation on *Peganum harmala* L.

I.1. Generalities on *Peganum harmala* L.

Peganum harmala L. (Harmel) medicinally is an important perennial flowering herbaceous or shrubs of Zygophyllaceae family. It has various common names, including harmala, Syrian rue, wild rue, Armal, and Africa rue. *P. harmala* is widely distributed geographically throughout the tropics, subtropics, warm temperate zones, and has been introduced into America and Australia (Kubitzki, 2011; Marwat and Rehman, 2011). It has been traditionally used for medicinal purposes as a remedy against syphilis, fever, hysteria, malaria, neuralgia, parkinsonism, rheumatism, colic, asthma and eye complaints (Abdelfattah et *al*., 1995).

I.2. Etymology of the *P. harmala*

P. harmala the genus name, **peganum**, is the classical Greek name for a species of rue, and its common English name which is rue came because of a resemblance to rue, although the two names are not related. The species name, **harmala**, derives from the Lebanese town hermel (Mars, 2009).

I.3. The position systematic of *P. harmala* in botanical classification.

P. harmala is classified in several different ways, depending on its molecular phylogenetical and morphological data (Decraene and Smets, 1996). In the old time, the plant belonged to the family of Zygophyllaceae according to Ambasta (1986) and Stewart (1972), but recently the family has been placed in the family of Nitrariaceae according to Sheahan and Chase (1996).

The taxonomy of *P. harmala* accords to many authors (Soni et *al*., 2013; Mashreghi, 2012; Chase, 2009; Haston and *al*., 2009; Takhtajan, 2009; Stewart, 1972) is:

- Kingdom: Plantae.
- Subkingdom: Tracheophyta (Vascular plants).
  - Phylum: Magnoliophyta (Flowering plants).
  - Superdivision: Spermatophyta (Seed plants).
  - Division: Angiosperms.

- Clade: Eudicots.

  Clade: Core Eudicots.

  Clade: Super-rosids.

  Clade: Rosids.

  Clade: Fabids.

  - Class: Magnoliopsida- Dicotyledons.
    - Super Order: Sapindales.
    - Order: Zygophyllales.
      - ❖Family: Zygophyllaceae.
      - ❖Genus: Peganum.
      - ❖Specie: harmala.

I.4. Morphological characteristics of *P. harmala*

P. harmala is a small wild-growing flowering herb, and it is growing between a late spring to early autumn whereas in India it has been reported from March to October, and it blossoms time between June and August. Despite the long period of life, the plant is not usually grazed because its bitter taste and a strong deterrent odor which repel animals (Quattrocchi, 2012; Kubitzki, 2011).

P. harmala has a bushy appearance, with a spread of 120 cm and a height of around 100 cm in mature plants, the stem is stiff and straight, highly branched in habit, angled above and glabrous, its contains also a layer of cuticle on epidermis, Parenchymatous and sclernchymatous cortex and beneath the epidermis, there is the Photosynthetic mesophyl tissues of leaf which consists of stake, spongy tissues and the vascular bundles in rings and conserving tissues of the water around the vascular bundles. The roots are deep tape. The leaves of the herb are simple, opposite, stipulates and bright green of 4 to 8 cm long, they are sessile irregularly and pinnatisectly dissected deeply into 3 to 5 cm long, 2 to 3 mm broad, linear-lanceolate or sub-elliptic in shape, with acute segments (figure 1) (Bibi et *al*., 2015; Patel et *al*., 2012; Chopra et *al*., 1960).

The plant is also characterized by solitary, bisexual, veined white or yellowish white flowers of 2 to 2.5 cm across. The filiform pedicle of plant is 1.2 cm long, there are 4 to 5 narrow linear sepals and 5 oblong or oblong-elliptic, sub-equal petals. In general, the flowers contain 15 stamens arranged in five outer pairs opposite the petals, and five inner stamens,

opposite the sepals. The filament is 4 to 5 mm long, with the anther longer than the filament, and dorsi-fixed, the ovary is 8 to10 mm long, the upper 6 mm being triangular or three-keeled. The fruit is small spherical, leathery capsule of around 6 to 10 mm, depressed at the apex and accompanied the persistent calyx. Capsule is green when unripe and orange-brown at maturity and it has three chambers, which open by three valves at the apex to release the seeds which are blackish-brown, triangular, of 2 to 4 mm long (figure 1) (Bibi et *al*., 2015; Boullard, 2001; Decraene and Smets., 1996).

I.5. Geographical distribution of the *P. harmala*

P. harmal as wild-growing flowering plant is abundantly distributed in North-West India, Central Asia, Middle East and North Africa; its growing is widely and spontaneously throughout semi-arid and pre-deserted regions of south-east Morocco, Tunisia, Libby, Syria, and Egypt especially in the coastal region from Sallum to Rafa. The plant is also widespread in Pakistan, Iran and Turkey and it has been introduced into Australia and America by the farmers. Since then it has spread invasively to Arizona, California, Montana, Nevada, Oregon, Texas, and Washington. In Algeria, *P. harmal* is common in the area of fairly level high ground (high plateau), in the northern and southern Sahara and in the mountains of the central Sahara (Shao et *al*., 2013; Soliman et *al*., 2012; Goel et *al*., 2009; Hemmateenejad et *al*., 2006; Boullard, 2001; Lamchouri et *al*, 2000; Decraene and Smets., 1996).

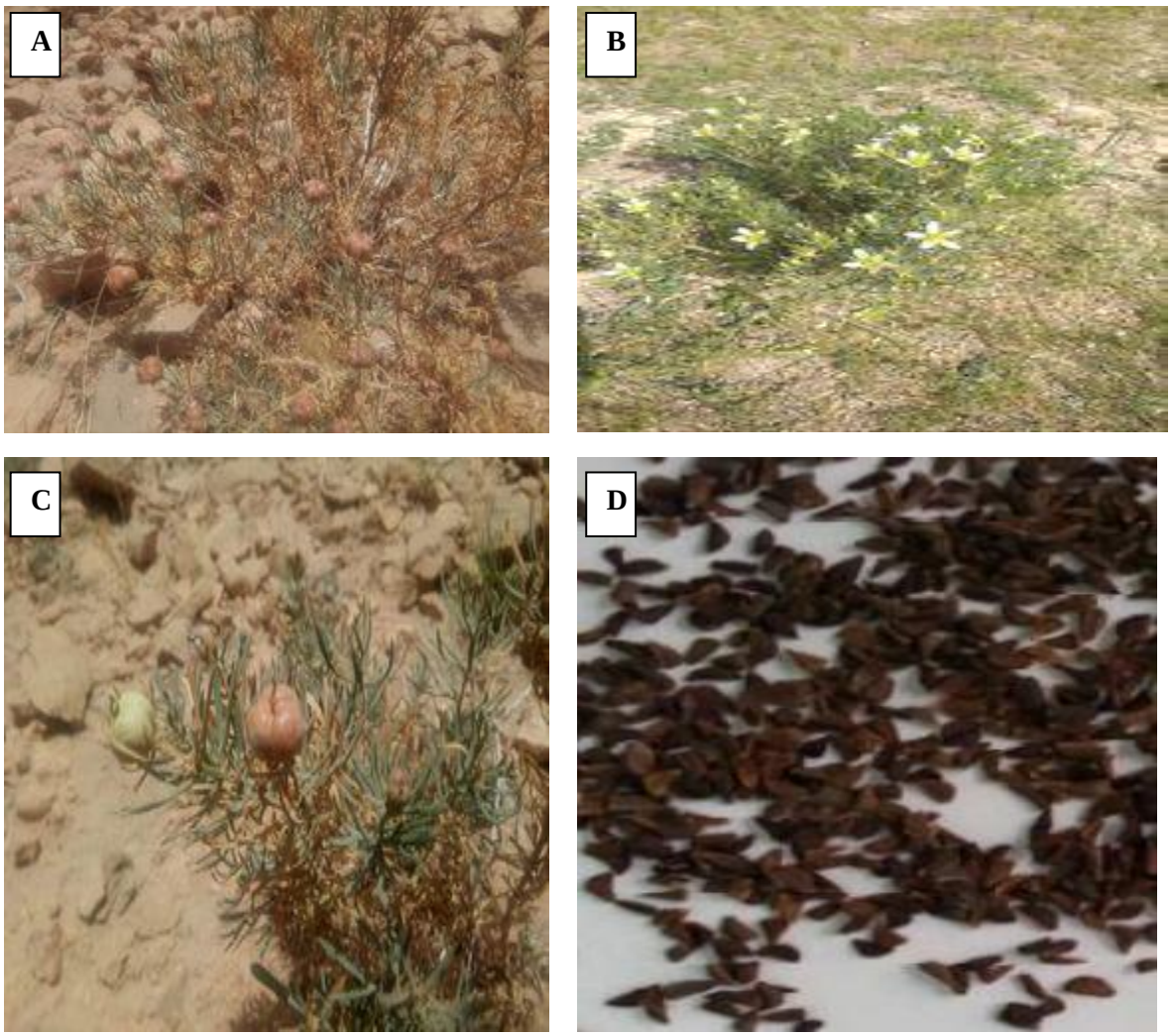

Figure1: *P. harmala*; the flower in (A) and (B), the fruit in (C), and the seeds in (D).

I.6. The common names of *P. harmala*

P. harmal has various common names according to the areas of distribution, some of these names are Armel, Syrian rue, Pègano, Harmal, African rue, Harmala, Hoama, Soma, Harmel gemeine syrische, steppenraute, Ruta di-siria, Harmel peganum, Peganum, wild rue, Espand (Mars, 2009; Wagstaff, 2008).

I.7. Traditional usages of *P. harmala*

In antiquity, *P. harmal* is used as a poison, and all the parts of the plant are used traditionally as powder, decoction, maceration, or infusion to treat a number of diseases such as a vermifuge, rheumatism, asthma and remedy for ocular affections. The plant is also used as abortifacient, emmenagogue, galactogogue, soporifics, diuretic agent and it is useful in weakness of muscles and brain. It have been claimed to possess hypothermic and

hallucinogenic properties and so-called aphrodisiac substance. In ethno-medicinal, peoples use a different parts of the plant for many therapeutic purposes, such as the root which is used to treat the nervous disorders and the fruits and seeds have digestive, nauseant, emetic, antispasmodic, hypnotic, narcotic and uterine stimulant activities. The powder of the seed is recommended as an anthelmintic agent and the decoction of the seeds and the leaves are given in laryngitis, colic, dysmenorrhea, hiccup, hysteria, neuralgia. In Turkey and Iran, the red dye obtained from the seeds is widely used for dying ornate rugs (Goel et *al*., 2009; Sultana, 1987).

In ancient times, the dried capsules from the plant are used as a talisman to protect against the "evil eye" and the seeds burned to produce incense (scented smoke) and a dense smoke during Zoroastrians rituals and still used until today. the seeds also are roasted and pulverized to obtain a fine powder, called techepakchiatzen taken alone or smoked with other ingredients to obtain narcotic effects in India, or curing persons suffering from mental disorders, but in Iran the smoke of its seeds is traditionally used as disinfectant and in modern western culture, it is often used as an analog of *Banisteriopsis caapi* to create the Ayahuasca (a psychoactive infusion or decoction prepared from *Banisteriopsis caapi*) (Monsef et *al*., 2004; Apostolico et *al*., 2016; Kuete, 2014, Frison et *al*., 2008).

I.8. The symptoms, and the prognosis of the intoxication by *P. harmala*

- **Human toxicity of *P. harmala***

There are several reports in the literature indicating a great variety of pharmacological effects of medicinal herbs such as harmel. It is widely used to treat health disorders, mainly because it is readily available and cheaper than modern medicines but unfortunately, this herb can leads to critical conditions and even death by its adverse effects. A several cases of toxicity by harmal have been already reported for many applications such as emmenagogue, hypermenorrhae, constipation, the abortion, and activate childbirth through ingestion of crushed seeds, which lead to the quick appearance of clinical manifestations of intoxication include helmet headache, dizziness, nausea, tingling of extremities, tinnitus ringing, visual hallucinations, digestive disorders, abdominal pains follow bilious vomiting, tremor of limbs and facial muscles, convulsions, hypothermia and bradycardia, neurological disorders such as euphoria, kidney disorders such as anuria and in severe cases, paralysis, intense asthenia,

centre nervous system (CNS) depression, dyspnea, as well as arterial hypotension. (Djafer et *al*., 2017; Hammiche et *al*., 2013; Moshiri et *al*., 2013; Achour et *al*., 2012)

- **Animal toxicity of *P. harmala***

All parts of plant are thought to be toxic and the poisoning by *P. harmal* concerns all domestic animals such as cattle, camels, donkeys, sheep and horses especially in dry seasons, when forage is shortage. The physical symptoms of poisoning are similar to what has been reported for humans cases and the abortion is frequently occurs. The animal appears in a narcotic state and usually, the nervous syndromes are predominant; where represented in excitability followed by muscular trembling and stiffness, prostrate with hyper-salivation, suffer anorexia and accelerated breathing. Standing is impossible and the animal goes into recumbency. The animal refuses nourishment and digestive disorders manifested in vomiting, diarrhea, frequent urination, hypothermia, dyspnea, mydriasis and the death follows within two days after the onset of signs of intoxication (kour et *al*., 2016; Kuete, 2014; Asgarpanah et Ramezanloo., 2012)

In general, the problem of poisoning by this herb is more intense by the fact that there is no accurate information available because very few cases are reported and it is also true that in a number of plant poisoning cases; the treatment is practically the same because there is no a specific antidote therapy. However, the manifestations are dose dependent, prognosis of these intoxications is not serious and transient in most cases and can be managed successfully, but consuming an overdose of it accidentally, intentionally, or for suicidal attempt may cause mortality. The treatment is control of vital signs, where it represented in the evacuation of the gastric contents, completed by a gastric lavage, must intervene as quickly as possible. The symptomatic treatment of central neurological manifestations should be started as soon as the neurological signs occur, and before convulsions occur, renal cleansing should be accelerated (Berdai et *al*., 2014; Gupta. 2016; Mohammadi et *al*., 2016).

II. The screening of photochemical aspect.

P. harmala is classified among the best-known species to contain different types of bioactive metabolites from its seeds, leaves, flowers, stems and roots, such as alkaloids, steroidal components, fatty acids, flavonoids, volatile oils, anthraquinones, amino acids, protein, polysaccharides and mineral elements. Among these compounds, the alkaloids,

mostly β-carbolines were found to be the main substances, and quinazoline derivatives in the second level (Li et *al*., 2017; Shao et *al*., 2013; Kaskoos et *al*., 2014).

Alkaloids represent a large family of structurally complex natural products displaying a wide range of biological activities. Traditionally, alkaloids are defined as secondary metabolites and heterocyclic nitrogen compounds biosynthesized generally from amino acids (Eguchi et *al*., 2019; Mazid et *al*., 2011; Springob and Kutchan. 2009; Pallardy, 2008; Aniszewski, 2007; Saxton et *al*., 1971).

II.1. β-Carboline alkaloids from *P. harmala*

The β-carbolines are very important natural and synthetic indole alkaloids with different degrees of aromaticity, some of which are widely distributed in nature, including various plants, foodstuffs, insects, mammalians as well as human tissues and body fluids. These compounds are of great interest due to their diverse biological activities (Apostolico et *al*., 2016, Cao et *al*., 2007).

These alkaloids comprising a tricyclic pyrido [3,4-*b*] indole ring structure or an indole nucleus and a pyridine ring (figure 2), at different levels of unsaturation. They can be divided into three structural groups, depending upon their degree of ring saturation:

- Harman, harmine, and harmol are all completely aromatic harmane derivatives or aromatic β-Carbolines with a fully unsaturated pyridine ring.
- The dihydro or harmalane derivatives represented in harmalan, harmaline and harmalol.
- The tetrahydro derivatives represented in tetrahydroharmine and tetrehydro-harman (Glennon et *al*., 2000).

Generally, the pyridine nitrogen atom is characterized by a more basic character than the acidic indolic nitrogen (Kukula-Koch and Widelski., 2017; Shao et *al*., 2013; Kuete, 2014). Harmine, harmaline, and tetrahydroharmine are the main β-carbolines substances were found and received the most attention (Sobhani et *al*., 2002).

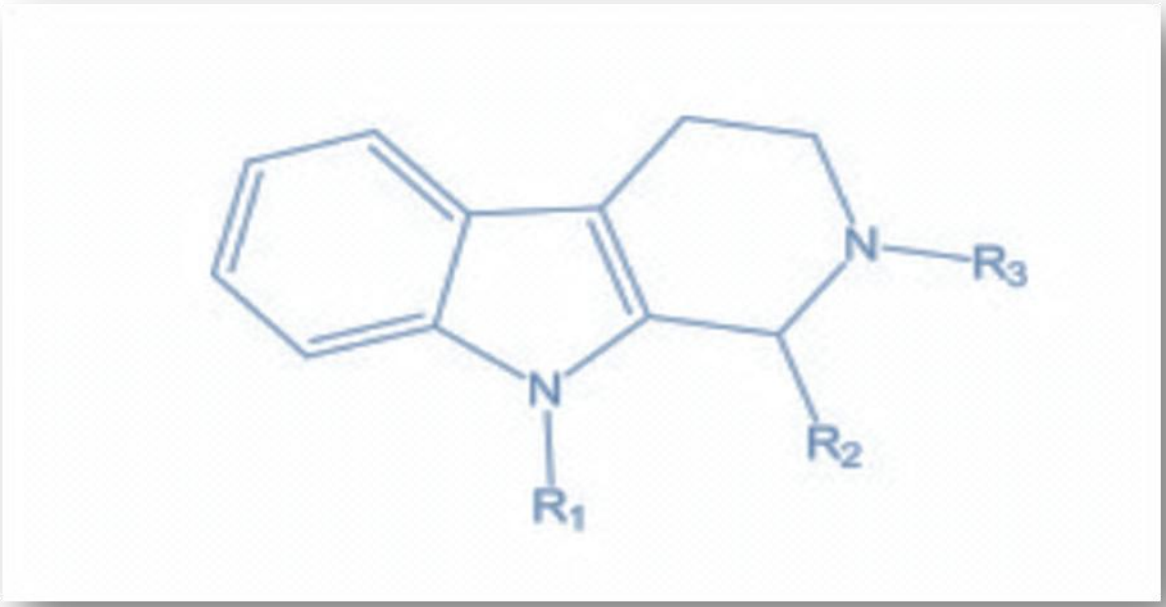

Figure 2. Chemical structures of β-carboline alkaloid skeleton (Kukula-Koch and Widelski, 2017.).

II.1.1. Physic-chemicals properties of the β-carboline alkaloids.

β-Carbolines alkaloids crystallize easily and have moderately high melting points. Generally, they are optically inactive and forms colorless prisms in many solvents, except tetrahydro bases have an asymmetric carbon, only in one case was optical activity noted in the tetrahydroharmine. The bases in which the pyridine nucleus is at least partly dehydrogenated reveal a strong fluorescence in UV-light. Even the tetrahydro bases generally reveal this property in consequence of air oxidation. The properties of the naturally occurring alkaloids, as well as those of several important artifacts, are shown in table 1 according to Manske (1965) et Casal (2015).

Table 1: The properties of the β-carboline alkaloid (Manske., 1965).

Alkaloids	Formula	Melting point
Harmaline	C13H14ON2	238
Harmalol	C12H12ON2	212
Harman	C12H10N2	238
Harmidine	C13H14ON2	257
Harmidol	C12H12ON2	259
Harmine	C13H12ON2	256
Harmol	C12H10ON2	32 1
N-Methyltetrahydroharmol	C13H16ON2	268
Norharman	C11H8N2	198
Tetrahydroharman	C12H14N2	180
Tetrahydroharmine	C13H16ON2	199
Tetrahydroharmol	C12H14ON2	268
Tetrahydronorharman	C11H12N2	207
2-Methyltetrahydro-2-carboline	C12H14N2	216
1,2-Dirnethyltetrahydro-2-carboline	C13H16N2	112

- **Harmaline**

Harmaline also known as harmidine is the 4, 9-dihydro-7- methoxy-1-methyl-3H-pyrido [3,4-*b*] indole, and it is the main alkaloid of P. harmal. It crystallizes in colorless or pale yellow prisms and is optically inactive. This component is slightly soluble in water, alcohol and ether, quite soluble in hot alcohol and dilutes acids. Its hydrochloride dehydrate is moderately soluble in water and alcohol, when crystallizes as yellow needles (figure 3) (Kubitzki, 2011).

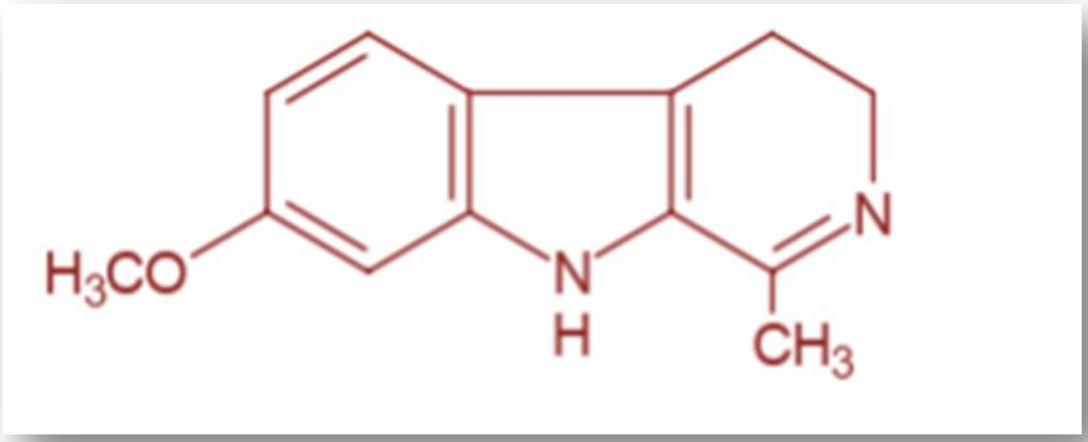

Figure 3. Chemical structure of Harmaline (4, 9-dihydro-7- methoxy-1-methyl-3H-pyrido [3, 4-*b*] indole) (Glennon et *al*., 2000)

- **Harmine**

Harmine or banisterine is an indole alkaloid with the 7-Methoxy-1-methyl-9H-pyrido [3, 4-b] indole ring structure that is characterized by slightly soluble in water, alcohol or ether. It is optically inactive and forms colorless rhombic prisms from methanol and its Saluted salts show a deep blue fluorescence (figure 4) (Mahmoudian et *al*., 2002).

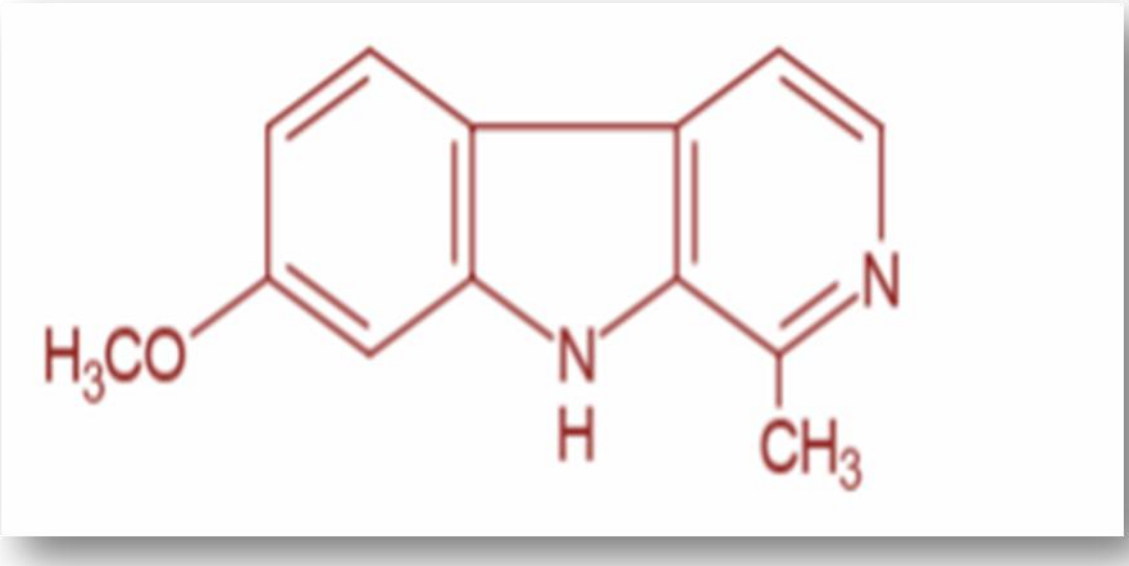

Figure 4. Chemical structure of harmine (indole alkaloid with the 7-Methoxy-1-methyl-9H-pyrido [3, 4-b] indole) (Glennon et *al*., 2000).

- **Harmalol**

Harmalol is 4, 9-dihydro-1-methyl-3H-pyrido [3, 4-*b*] indol-7-ol. It is crystallized to the trihydrate in water and it is freely soluble in hot water, acetone or chloroform but only sparingly soluble in benzene. Harmalol is unstable when exposed to air and its methyl ether is harmaline (figure 5) (Mahmoudian et *al*., 2002).

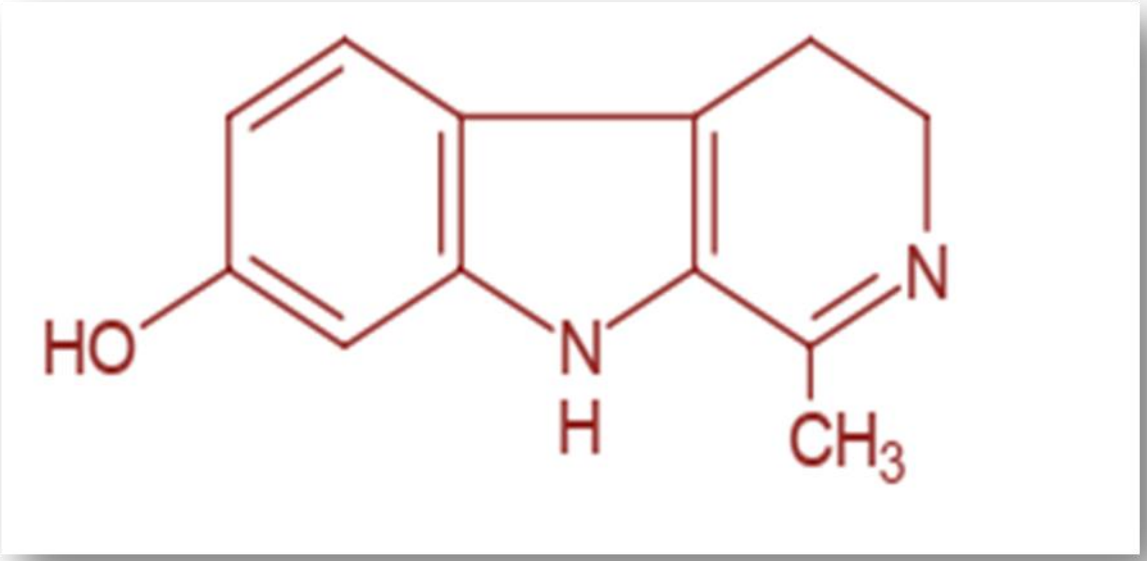

Figure 5. Chemical structure of harmalol (4, 9-dihydro-1-methyl-3H-pyrido [3, 4-*b*] indol-7-ol) (Glennon et *al*., 2000).

- **Harman**

Harman or 1-Methyl-3, 4-dihydro-beta-carboline is crystallized from several organic solvents as colorless prisms. It is readily soluble in methanol, alcohol, acetone, chloroform, or ether but only moderately so in hot water. It dissolves in mineral acids and exhibits a blue-violet fluorescence (figure 6) (Mahmoudian et *al*., 2002).

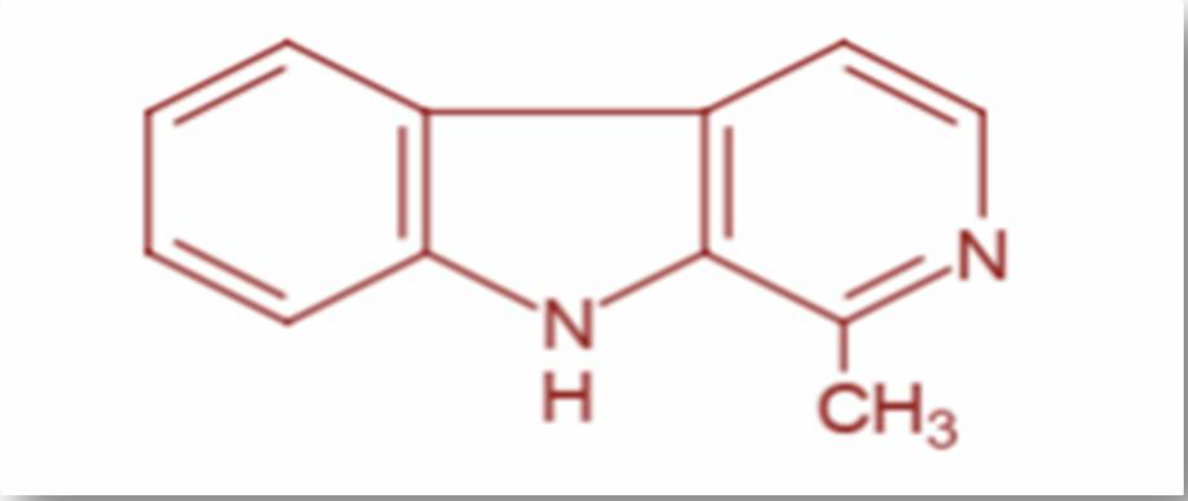

Figure 6. Chemical structure of harman (1-Methyl-3, 4-*dihydro-beta*-carboline) (Glennon et *al*., 2000).

II.1.2. Occurrence of β-carboline alkaloids

So-called harmala alkaloids or beta-carboline were first found in *P. harmala*, together with other chemical congeners globally designated, but these alkaloids present also in plants of many other families as it's shown in the table 2. Moreover these alkaloids are detected in bacteria, algae, fungi, marine bryozoans and insects too. Precisely, harman and norharman have been identified in tobacco smoke and coffee as exogenous sources, as well as identified in several edible plants (wheat, rice, maize, soy, mushrooms, grapes), in alcoholic beverages (wine, beer), in cooked meat and fish etc... In addition norharman and harman are also identified in human tissues and body fluids; including heart, kidney, liver, urine, red blood cells, cerebrospinal fluid, and brain tissues, and their presence is the result of endogenous synthesis in the human. (Herraiz, 2017; Emilija et *al*., 2018; Casal, 2015; Manske, 1965).

II.2. Quinazoline Alkaloids from *P. harmala*

Quinazoline alkaloids, specifically Pyrroloquinazolines, are the building blocks for around eighty naturally occurring alkaloids identified from plants such as *Adhatoda vasica*, *Sida cordifolia*, *Daemonorops draco*, *P. harmala*, and *Galega officinalis*, as well as microbes and mammals (table2). The most important of these alkaloids are vasicine, vasicinone, deoxyvasicinone, and a new chemical known as peganine glycoside, which was proposed by Herraiz *et al*., (2007), despite that vasicine was the first one identified

and isolated from Adhatoda vasica in 1888 (Herraiz et *al*., 2017; Bergman, 1983; Liljegren, 1968).

P. harmala is one of the best-known species for quinazoline alkaloids. Its seeds have been extensively researched and found to contain a variety of quinazoline alkaloids, including vasicinone, which has been isolated as a bioactive natural product. This alkaloid has long been used in traditional medicine as a treatment for colds, asthma, and bronchitis. It also drew a lot of interest because of its various pharmacological and biological effects (Filali et *al*., 2019).

Table 2: Plants and their contained alkaloid (Manske, 1965).

Plant	Alkaloid
Peganum harmala Zygophyllaceae (rutaceae)	harmine harmaline harmalol harmidine
Symplocos racemosa roxb. Symplocaceae (styracaceae) *Sickingia rubra* k. schum. *(Arariba rubra* mart.)	harman (loturine) harmine (aribine)
Rubiaceae *Eeagnus angustifolia* l. Eleagnaceae	tetrahydroharman tetrahydroharmol n-methyltetrahydroharmol
Banisteria caapi spruce Malpighiaceae	harmine harmaline tetrahydroharmine
Cabi paraensis ducke	Harmine
Malpighiaceae *Banisteriopsis inebrians*morton	*Harmine*
Malpighiaceae Leptactina densijora*hook. f.*	*Tetrahydroharmine*
Rubiaceae *Passijorain carnuta*l. Passifloraceae	Tetrahydroharman *harmine* harman harmol
*Zygophyllum fabago*l. zygophyllaceae (first isolation of harmol)	*Harmine* *harman* *harmol*
*Arthrophytum leptocladum*popov (chenopodiaceae)	4-methyl- and 3,4-dimethyl 3,4,5,6-tetrahydro-4-carboli
*Petalostylis labicheoides*r. br. (leguminosae)	Tetrahydroharman
*Strychnosme linoniana*baill. (loganiaceae)	n,-methylharman
*Calligonum minimum*lipski *(*polygonaceae*)*	tetrahydroharman (calligonine) base, clzhlonz base, cxzh140nz

- **Vasicine** ($C_{13}H_{15}ON_2$)

It is the oldest known quinazoline alkaloid, it was first isolated from the fresh leaves of *Adhatoda vasica* and it was the predominant one in the crude total alkaloids of this plant, later vasicine was identified in flowers and stems of *P. harmala* under the name of peganine. Chemically the salts of vasicine are obtained as crystals (Mahmoudian et *al*., 2002).

- **Vasicinone** ($C_{11}H_{10}O_2N_2$)

It is the product of the autooxidation of vasicine. Its concentration in the plant's crude is low, but when vasicine is gradually converted to vasicinone, its concentration rises. The base crystallizes into colorless crystals. With mineral acids, the alkaloid produces crystalline salts. (Liu et *al*., 2015; Li et *al*., 2018).

- **Deoxypeganine** or **deoxyvasicine** ($C_{11}H_{12}N_2$)

It is 1,2,3,9-tetrahydropyrrolo[2,1-*b*]quinazoline hydrochloride quinazoline alkaloids from *Peganum harmala* L. (Herraiz et *al*., 2010).

II.3. β-carbolines and the quinazoline Profile in *P. harmala*

The total alkaloid content of *P. harmala*, and its seeds is varied between 3.92 and 7% of the dried weight, and the main alkaloid components are β-carboline (Harmaline, Harmine, Harmalol, Harmol and tetrahydroHarmine) and derivatives of quinazoline (peganine, deoxypeganine and the peganine glucoside), which their concentration and distribution highly increased during development, maturing and drying process of plant. The qualitative profiles of β-carboline alkaloids highly varied depending on each part of the plant. For example, the flowers had no appreciable presence of β-carbolines, stems showed low levels of harmol and harmine and leaves only contained harmine while harmol mainly occurring in roots. In contrast, the coatings of the dried seeds, the dried Seeds and Whole green fruits (capsules) mainly contained large amounts of β-carboline such as Harmaline exclusively accumulated in these parts, harmine and low levels of Harmalol, and Tetrahydroharmine (Li, 2018; Bensalem, 2014; Fathizada et *al*., 2007; Kartal et *al*., 2003; Hassani and El Hadek, 1999).

The qualitative profiles of quinazoline alkaloids (vasicine, deoxyvasicine, and deoxypeganine) in *P. harmala* varied with tissue and seed development. Peganine (vasicine) was detected in significant concentrations in dry seeds, immature capsules, green fruits, leaves,

and stems and roots. Deoxypeganine (deoxyvasicine) was discovered in immature and green fruits, as well as trace amounts or extremely low amounts in dried seeds. Peganine glycoside was found in large concentrations in dried seeds (Herraiz et *al*., 2017; Herraiz et *al*., 2010; Sobhani et *al*., 2002).

II.4. Pharmacology and therapeutic effects of β-carboline alkaloids and quinazolines

The previous research has revealed that β-carboline and quinazolines alkaloids in P. harmala play a crucial role in pharmacology and therapeutic effects.

II.4.1. Abortifacient activity

The quinazoline alkaloids (vasicine and vasicinone) in *P. harmala* seeds are thought to be responsible for their abortifacient effect. It has been claimed that these substances stimulate the uterus, presumably through the release of prostaglandins (Mahmoudian et *al*., 2002 Shapira et *al*., 1989).

II.4.2. Acetylcholinesterase inhibitor (AChEl) or Anti-cholinesterase

Alzheimer's disease is treated with cholinesterase inhibitors. Deoxypeganine from harmala seeds has been shown to exhibit reversible cholinesterase inhibitor action. It inhibits acetylcholinesterase and monoamine oxidase, preventing the degradation of acetylcholine and dopamine, and is thus thought to be beneficial in the treatment of Alzheimer's dementia (Singh, 2003).

II.4.3. Antileishmanial activity

Leishmaniasis, caused by Leishmania spp, has been identified as a serious public health issue. Sand-flies spread Leishmania parasites by ingesting the parasite in its amastigote stage in macrophages and subsequently inoculating the promastigote stage into additional hosts. It has been discovered that of *P. harmala* seeds extract suppresses the growth of promastigote forms of *Leishmania major*, the parasite that causes *Cutaneous Leishmaniasis* (Yousefi et *al*., 2009).

II.4.4. Antimicrobial activity

Various extracts from *P. harmala* seeds were tested in vitro against numerous antibiotic-resistant infections and selected protozoa, obtained from poultry. All bacteria and protozoa were shown to be inhibited by *P. harmala* seed extract. The potential role of the known four β-carboline alkaloids in crude extracts of *P. harmala* was also investigated, for their antimicrobial activity. For all bacteria, the action of pure alkaloids was harmane, then harmaline, then harmalol, then harmine, however for protozoa, it varied depending on the microbe. It is determined that harmala or its alkaloids might be employed to manage antibiotic-resistant bacteria and protozoa isolates (Arshad et *al*., 2008).

II.4.5. Antinociceptive activity

The effect of *P. harmala* seed extract on formalin-induced pain response in mice was investigated by Monsef et *al*., (2004), the results showed that the pain response was significantly reduced by alkaloid extract. Harmaline was the predominant antinociceptive agent found in the harmala alkaloid extract.

II.4.6. Antiplasmodial and vasorelaxant activities

Malaria, caused by Plasmodium parasites, is one of the most common infectious disorders in many tropical and temperate countries. Because harmine and harmaline have previously been shown to have antiparasitic action against various parasites, their antiparasitic impact on the Plasmodium parasite was investigated. Harmine, harmaline, vasicinone, and deoxyvasicinone were isolated from the seeds of *P. harmala* using bioassay-guided purification. Harmine and harmaline showed modest antiplasmodial efficacy against Plasmodium falciparum in vitro. Vasicinone, a quinazoline alkaloid, demonstrated vasorelaxant efficacy against phenylephrine-induced contraction of isolated rat aorta (Astulla et *al*., 2008).

II.4.7. Antitumor effect

The alkaloidic fraction of a methanol extract of *P. harmala* seeds was examined in vitro on three tumor cell lines of Uncoupling proteins (UCP); UCP-Med and Med-mek carcinomas, as well as UCP-Med sarcomas. During the first 24 hours of interaction, proliferation was considerably inhibited at all tested dosages. After 24 hours, there was cell lysis, which progressed to total cell death within 48 to 72 hours, depending on the measured dose. As a

result, *P. harmala* alkaloids have substantial antitumor potential and may be effective as a new anticancer therapeutic (Lamchouri et *al*., 2000).

II.4.8. Antiviral potential

P. harmala seed extract offers a novel therapeutic option for viruses as well as an altogether new means of interrupting the viral life cycle. In human immunodeficiency virus (HIV) inhibition tests, it causes a significant reduction in HIV activity and demonstrates prospective therapeutic utility (Belzil, 2006).

II.4.9. Human monoamine oxidase inhibitors

P. harmala alkaloids are monoamine oxidase inhibitors (MAOI) for a limited period of time (MAOIs). An MAOI works by inhibiting a key enzyme in the human body, monoamine oxidase (MAO), which is important for functions in the brain and throughout the body. The alkaloids of *P. harmala* inhibit biogenic amine from attaching to the active site of the MAO molecule and undergoing deamination.

Harmala alkaloids inhibit the protective enzyme MAO until their action is reversed and MAO activity is reduced. The quantitative inhibition of MAOA by seed extracts has been linked to harmaline and harmine. The significant suppression of MAOA by *P. harmala* seeds containing β-carbolines should contribute to the plant's psycho-pharmacological and toxicological effects, and might be the foundation for its purported antidepressant properties (Herraiz et *al*., 2010).

III. The reproductive system

III.1. Structure of ovaries

The female gonads are the ovaries, which are responsible for the growth and release of oocytes as well as the production of a number of steroid and protein hormones that affect other organs in the body. The ovaries are paired pelvic organs that are attached to the uterus via the fallopian tubes, which are located near the lateral wall of the pelvis and anterior to the rectum on either side of the uterus. A short peritoneal fold connects each ovary to the posterior surface of the broad ligament, the ipsilateral uterine cornu through the utero-ovarian ligament, and the lateral pelvic wall through the fundibulo pelvic ligament. In an adult, they are about 4 cm long and weigh 5 to 8 g, though these sizes vary a lot depending on your age

and reproductive status. Early in reproductive life, the ovary has a smooth pink-white surface that becomes convoluted and gyriform with age (Boorman et *al*., 2018).

On cross sectioning, thin-walled follicular cysts filled with clear fluid, as well as yellow corpora lutea and white corpora albicantia, are frequently found at the periphery. The ovarian arteries, which emerge straight from the aorta slightly under the renal arteries, give blood to the ovaries. On the right, venous drainage goes into the inferior vena cava, while on the left, it goes into the renal vein. The ovary has a large number of follicles (figure 7). The vast majority are primordial follicles or immature oocytes. The quantity of oocytes with which the lady was born was simply handed to her. They develop and mature one at a time during her reproductive life. There are millions of primordial follicles in the fetal ovary, with around 400 000 during menarche (onset of menstrual bleeding). A single layer of granulosa cells inside a basement membrane surrounds each primary oocyte structurally. Many of these primordial follicles will develop into mature oocytes over a woman's reproductive life, between adolescence and menopause, one of which will be released at ovulation each month. The maturation of a primordial follicle into a mature oocyte (figure 7) begins the process of follicle recruitment or the selection of one primordial follicle for future development, which takes several months. Follicle selection is known to be independent of gonadotropins; new research has linked the anti-müllerian hormone as a follicular response inhibitor to follicle stimulating hormone (FSH), but otherwise little is known about the process. As the primordial follicle grows, the single layer of cells around it separates to form the granulosa cell layer (figure 7). The theca layer is formed as stromal cells grow around the exterior of the follicle as it grows. Although the burst follicle fills with blood and appears as a red haemorrhagic mass in the ovary shortly after ovulation, these granulosa and theca cells remain actively secretory and make up the corpus luteum. The corpus luteum progressively regresses through apoptosis to generate the corpus albicans, a scar like enclosure (figure 7) (Weidner et *al*., 2009).

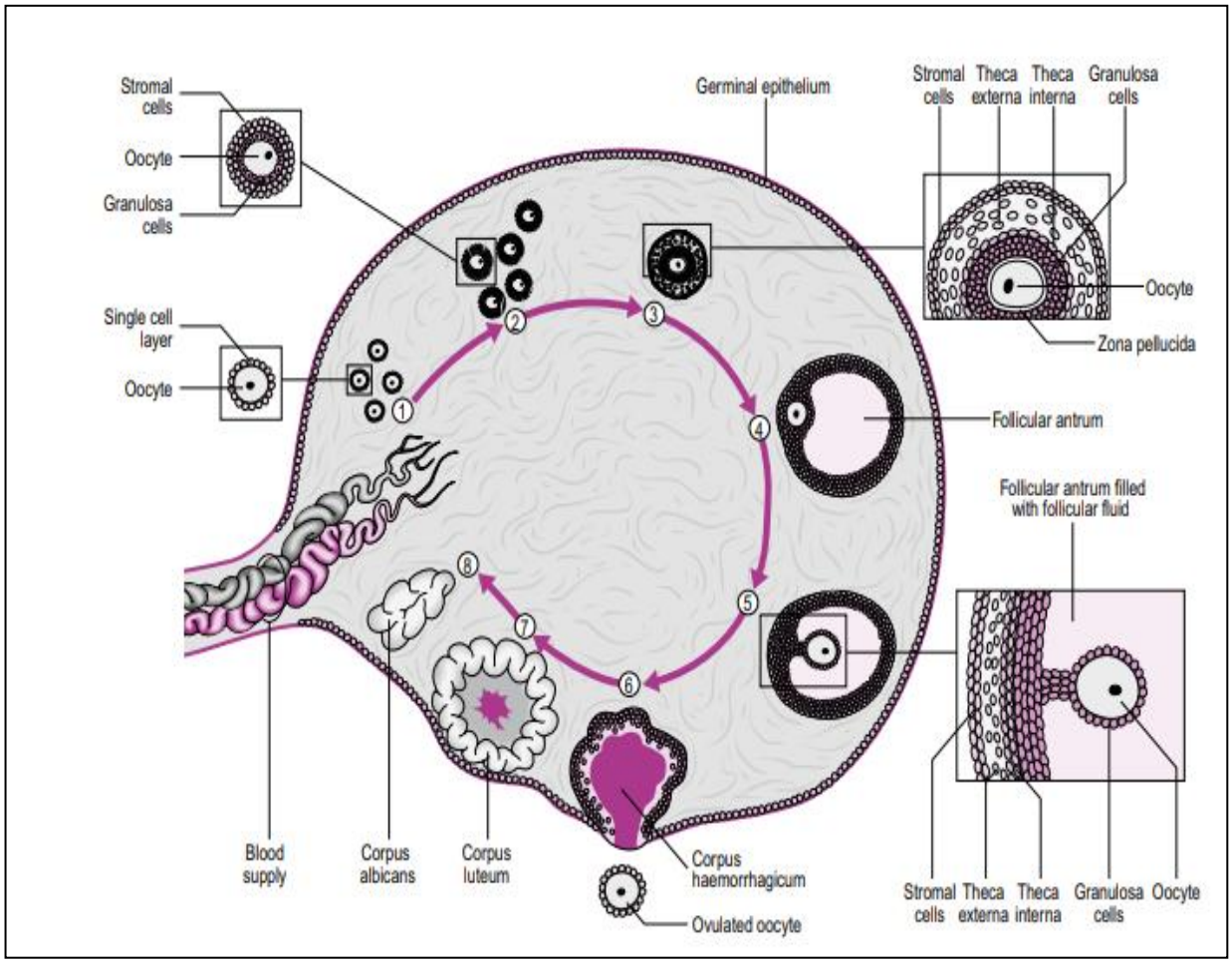

Figure 7. The ovary's follicular development stages. (1) A single cell layer surrounds an egg in a primordial follicle. (2) The cells divide to produce a stromal layer that surrounds the granulosa cells during the commencement of follicular development. (3) The creation of the theca cells, which reside between the granulosa and stromal cells, continues follicular development. The zona pellucida surrounds the oocyte. (4) The follicular antrum forms, which is full with follicular fluid. (5) A mature oocyte is suspended in follicular fluid and linked to the granulosa cell layer by a stalk. (6) The antrum fills with blood when the follicle ruptures to release the egg (the moment of ovulation), forming a corpus haemorrhagicum, which develops into the corpus luteum (7). The creation of the scar-like corpus albicans is caused by the corpus luteum's regression (8). The entire cycle depicted here takes several months to complete. The growing follicles are not depicted to scale; for example, a follicle at stage 2 is roughly 20 mm in diameter, but a mature follicle at stage 5 is 250 times bigger, at 5 mm, and plainly visible to the naked eye (Hinson et *al*., 2010).

III.2. Ovarian hormones

The ovary has two functions: Oocyte production and hormone release. Steroids (oestrogens, progesterone, and androgens), as well as peptides (inhibin, activin and relaxin), are secreted. Oestrogens and progesterone play a vital role in uterine endometrial lining maintenance and pituitary hormone release negative feedback control. The ovarian peptide hormones, in contrast to the steroid hormones, were identified relatively recently and their roles are less well known. These steroid and peptide hormones are secreted by the cells of the developing follicle, the theca cells and the granulosa cells, as well as by the corpus luteum (figure 8) (Leung and Armstrong et *al*., 1980).

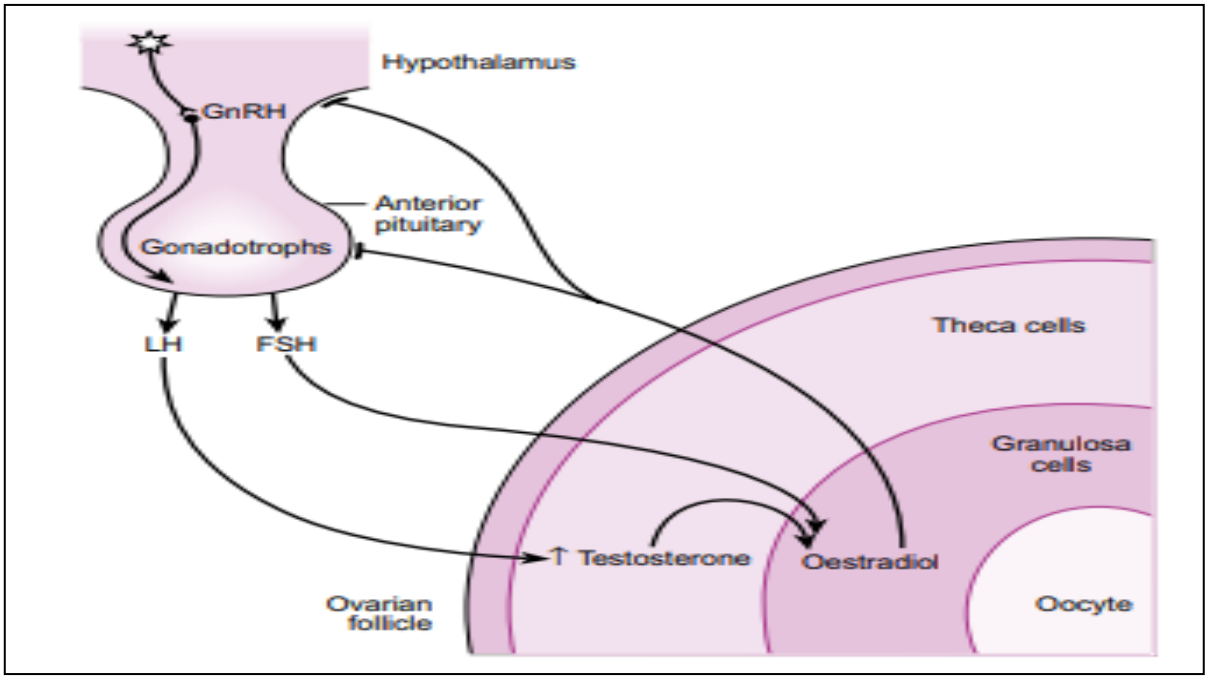

Figure l8. Hormonal control of steroidogenesis in the ovarian follicle. The pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus stimulates release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the gonadotroph cells of the anterior pituitary. LH receptors are located on the theca cells and LH binds to these receptors, stimulating the secretion of androgens, particularly testosterone. The testosterone is converted to oestradiol in granulosa cells. Levels of the enzyme that catalyse this reaction, aromatase, are increased by the action of FSH on the granulosa cells. Oestradiol exerts a negative feedback effect on the hypothalamus and pituitary (Hinson et *al*., 2010).

III.2.1. Estrogens

Estradiol-17β is the most potent estrogen produced by the ovary, and the ovary is its principal source in non-pregnant, premenopausal women. Most of this hormone is produced by the dominant follicle destined to ovulate, and other follicles in the same cohort produce mainly androgens and undergo atresia before reaching maturity. Estradiol is produced by the cooperation between follicular granulosa and theca cells and requires stimulation by both FSH and LH (two-cell, two-gonadotrophin theory): LH promotes theca cells to secrete androgens, which diffuse to the granulosa cells where FSH promotes aromatization to estrogen. Growing follicles also produce the protein hormone inhibin B, which is a component of the negative feedback loop on pituitary function and is a clinical indicator of the size of the follicle reserve. When inhibin and estrogen levels rise in the follicular phase of the cycle, blood FSH declines, allowing only one follicle (or sometimes two) to survive to ovulation. In the absence of follicles after menopause, inhibin levels decline to near zero. Steeply rising estrogen from the dominant follicle triggers a positive feedback response from the pituitary by increasing both the pulsatility of GNRH secretion and sensitivity of pituitary cells. The surge of LH secretion triggers ovulation approximately 36 hours later, and the collapsing follicle is remodeled to form a corpus luteum (figure 9) (Gosden, 2017).

III.2.2. Progesterone

Progesterone is released mostly by the corpus luteum's granulosa lutein cells, which are produced by granulosa cells after the LH surge (figure 9). Progesterone is the predominant hormone of pregnancy, and after week 8, the placenta takes over as the primary source of progesterone from the corpus luteum. Several steroids have similar properties and are together classified as the ‘progestogens’. These include 17α-hydroxyprogesterone and pregnenolone as well as progesterone itself (figure 10) (Hinson et *al*., 2010).

III.3. Transport and metabolism of estrogen and progesterone

Ovarian steroids are carried in the bloodstream via carrier proteins. 60% of oestrogen is linked to sex hormone binding globulin (SHBG), with the other 40% attached loosely to albumin or in the free form. Progesterone does not have an unique carrier protein, however it is usually attached to cortisol binding globulin (CBG), and albumin when it circulates. The concentration of SHBG in blood is regulated by steroid hormones, being increased by

oestrogen and decreased by testosterone. Women therefore have around twice as much SHBG in their blood as men (Hinson et *al*., 2010).

In common with other steroids, the ovarian steroids are metabolized in the liver to less active steroids, typically oestrone and oestriol, and excreted in the urine. A proportion of the oestradiol is conjugated in the liver and excreted in bile salts. There is entero-hepatic recycling of steroids by which they are conjugated by the liver and excreted into the gut, where they are de-conjugated and re-absorbed into the circulation (Hinson et *al*., 2010).

III.4. Cellular actions of estrogens

Estrogen has a specific estrogen receptor that they bind to (ER). This receptor is divided into two types: ER α, which is encoded by a gene on chromosome 6, and ER β, which is encoded by a gene on chromosome 14. While some tissues exhibit both types of receptors, others preferentially express one. The uterus, liver, and heart, for example, express the α subtype, while the ovaries, central nervous system, prostate, and gastrointestinal tract express the β subtype. Both ER subtypes belong to the nuclear receptor family.

These receptors, like the androgen receptor, are linked to a chaperone protein called heat shock protein in the cytoplasm of the target cell. The heat shock protein dissociates when oestrogen binds to the receptor, and the oestrogen–receptor complex is translocated to the nucleus. The receptor attaches to the oestrogen response element on Deoxyribonucleic acid (DNA) in dimers, either homodimers (two ER units joining or two ER units joining) or heterodimers (one each and unit). This complex attracts co-activators, which bind to each other and allow gene transcription to occur(figure 10) (Santi et *al*., 2018).

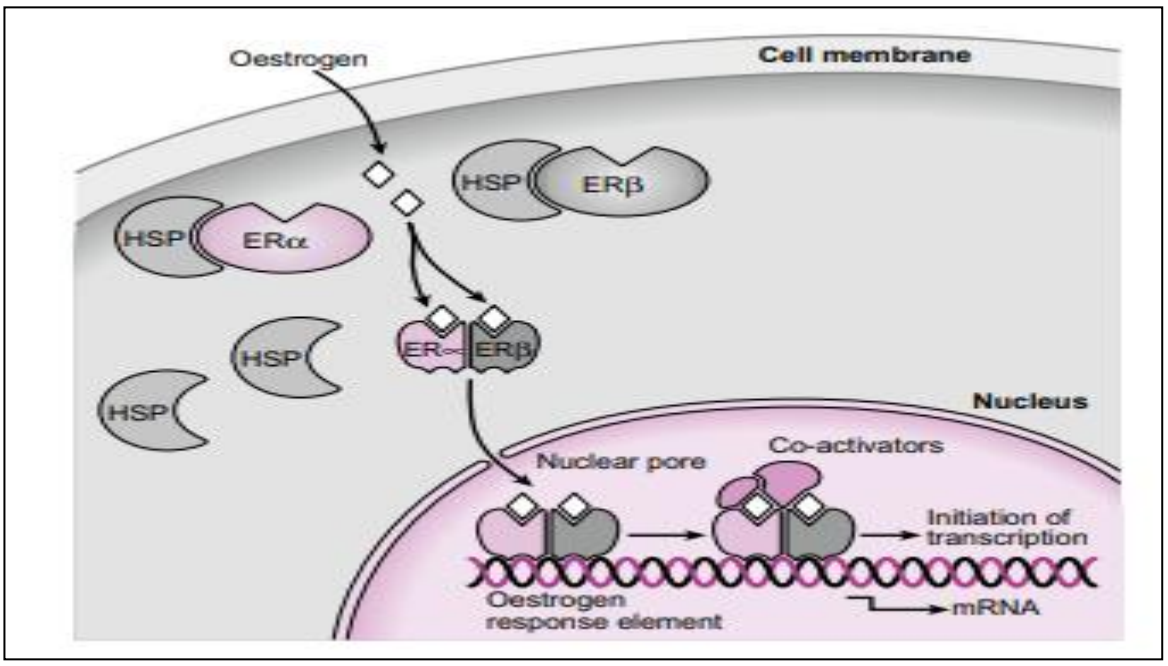

Figure 9. Cellular actions of estrogens. The receptors for oestrogens (ER) are located in the cytoplasm of target cells. In the absence of estrogen, the receptors are associated with heat shock protein (HSP) which dissociates in the presence of estrogen. There are two forms of ER, α and β, which can form either homodimers (α-α or β-β) or heterodimers (α-β). When estrogen binds, the hormone receptor complex moves into the nucleus and binds to the estrogen response element on DNA. The complex attracts co-activators to form an initiation complex which then allows transcription to proceed (Hinson et *al*., 2010).

III.5. Physiological actions of estrogens

Estrogens have a wide range of effects on secondary sex characteristics, metabolism, bone, and the brain in the body. Oestrogens play a role in the development and maintenance of a woman's secondary sexual characteristics, such as breast development. They promote the expansion of the breast tissues and pigmentation of the areoles throughout adolescence and pregnancy. They maintain the structure of the vaginal mucosa and increase cervical mucus production, which maintains the vaginal lubrication going. During the menstrual cycle, oestrogens have a permissive role in increasing ovarian follicle growth and promoting uterine development by encouraging endometrial cell proliferation (figure 10). In the brain, oestrogens act to increase libido. It is also thought that they play a role in the process of memory formation and enhance neural repair follow inginjury. Oestrogens are very important for bone health, particularly during the pubertal period when they stimulate closure of the epiphyses in both boys and girls. In addition, oestrogens are thought to be protective against

cardiovascular disease. This may be a result of their actions on the liver to reduce circulating cholesterol, but the mechanism of this effect is not fully understood (Hinson et *al*., 2010).

III.6. Actions of progesterone

Progesterone acts by binding to a specific progesterone receptor (PR) which has some similarities with the glucocorticoid receptor. The progesterone receptor is divided into two isoforms, Although they are all encoded by the same gene, they each have their own transcription start point., resulting in progesterone receptor type B (PR-B) being larger than progesterone receptor type A (PR-A). Oestrogens control the expression of the progesterone receptor, whereas progesterone inhibits oestrogen's proliferative effects via PR-A. As a result, progesterones are almost usually used in conjunction with estrogen therapy, such as the oral contraceptive pill and hormone replacement therapy.

The major function of progesterone is to keep a pregnancy going. Progesterone is necessary for preserving the structure of the uterus in order for the embryo to implant, and it plays an important part in pregnancy. Blocking progesterone synthesis or action is a good way to end a pregnancy. Progesterone is thought to operate as a thermogenic steroid, raising body temperature. This feature can be used to determine a woman's fertile period each month, as there is a modest but consistent rise in body temperature after ovulation, which coincides with increased progesterone output. (figure 9 D) (Hindmarsh and Geertsma., 2017).

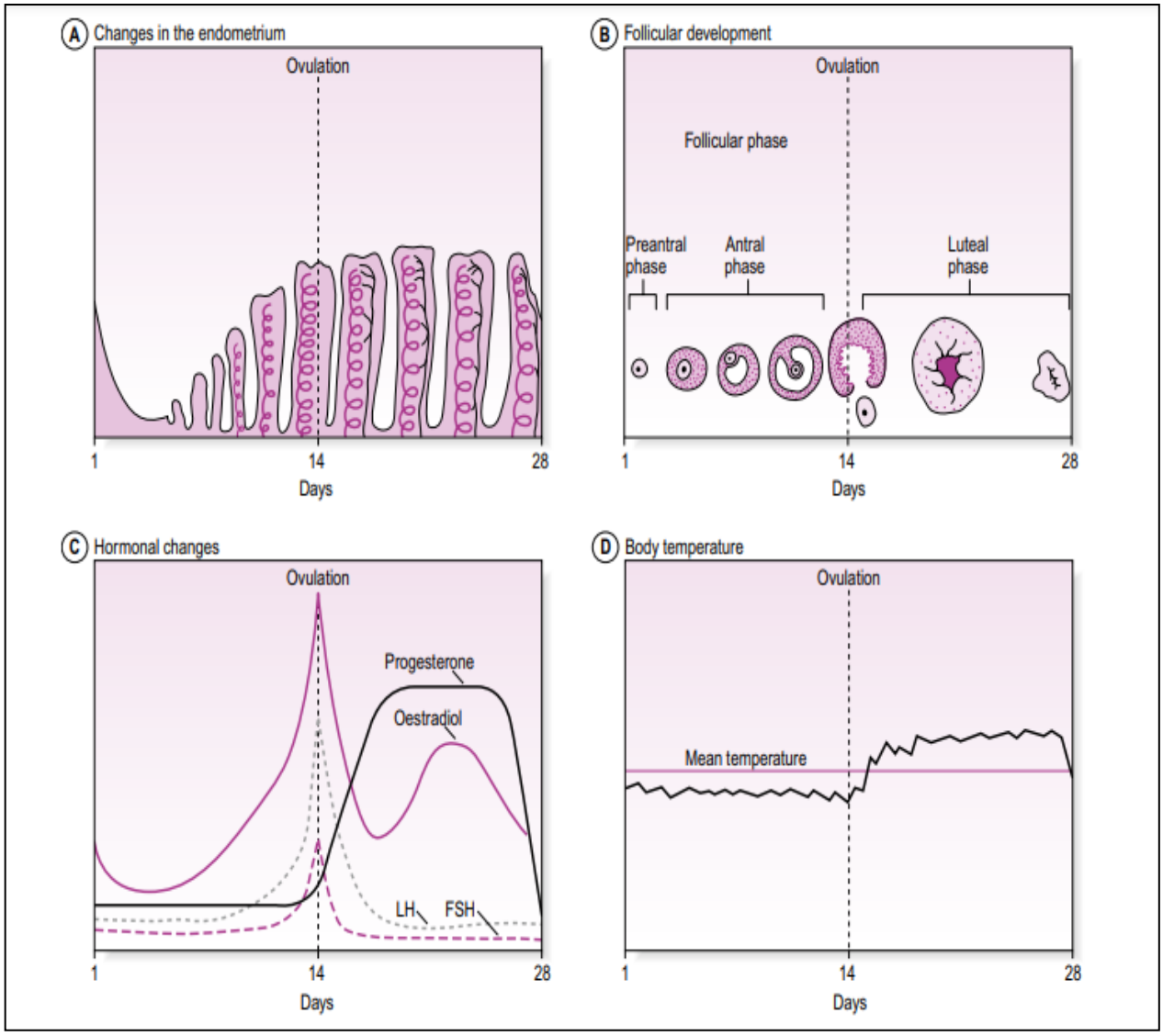

Figure 10. The menstrual cycle is a period of time in which a woman's body The days of the menstrual cycle are traditionally counted from the first day of menstruation (day 1). In a regular 28-day cycle, ovulation occurs on day 14. The proliferative phase (the time between menstruation and ovulation) lasts a variety of times, and ovulation does not always happen on day 14. The secretory phase, on the other hand, exhibits little variation in length. (A) Endometrial changes during the menstrual cycle. (B) During the menstrual cycle, the stages of follicular development. (C) Hormonal changes during the menstrual cycle; oestradiol levels peak just before ovulation. (D) Body temperature fluctuations during the menstrual cycle. Under the influence of progesterone, basal body temperature rises after ovulation and remains higher than average during the secretory phase, until decreasing to slightly below average temperature with the commencement of menstruation (Hinson et *al.*, 2010).

III.7. Androgen secretion by the ovaries

Androgens, the traditional male sex steroids, are produced by the ovary, but these hormones also have significant functions in women. To begin with, they are required for oestrogen production: the enzyme aromatase converts androgens to oestrogens in both the ovary and adipose tissues. Second, androgens play a key role in the formation and maintenance of pubic and axillary hair, as well as in regulating sex drive (libido).

Testosterone is the most powerful androgen in both men and women. The ovaries produce the majority of circulating testosterone in women, but the balance is produced through the conversion of adrenal androgens. Women have larger quantities of the plasma binding protein SHBG than men, hence their circulating testosterone concentration is significantly lower. Androstenedione and dehydro-epiandrosterone (DHEA), which are less potent androgens generated by the ovaries and adrenals, contribute significantly to the overall amount of circulating androgen. Excessive androgen production by the ovaries (or adrenals) causes a degree of masculinization and disruption of the normal menstrual cycle (Hinson et *al*., 2010).

III.8. Ovarian peptide hormones

Regulatory peptides, inhibin and activin, produced by the Sertoli cells in the testis, are also produced in women in the ovaries. In addition, the ovaries produce a third peptide hormone, which is relaxin.

- **Inhibin**

It is a glycoprotein that is released by the developing follicle's granulosa and theca cells, and has a role in inhibiting FSH secretion. Inhibin may have a role in follicle selection, according to certain reports. Inhibin levels may also be an early marker of the onset of menopause (Hinson et *al*., 2010).

- **Activin**

Activin is a member of the transforming growth factor-β (TGF-β) peptide family. It was once considered to be a possible reproductive hormone, but it is now have a larger role in the inflammatory response. The endometrium also produces high levels of activin, which contributes in the growth of the endometrial during the menstrual cycle. Clinically, activin

might be used as a prognosticator in women undergoing ovulation stimulation as part of assisted conception (Hinson et *al*., 2010).

- **Relaxin**

Relaxin was first identified in the 1920s. It is now known that there are seven members of the relaxin family of peptides, with a range of different roles. Relaxin stimulates follicular development and oocyte maturation, and may have a role in implantation of the embryo. It is known to have an important role in parturition and has a number of other effects outside the reproduction system, including an antifibrotic action in wound healing (Hinson et *al*., 2010).

III.9. Physiological regulation of ovarian function

III.9.1. Follicle-stimulating hormone.

Follitropin or FSH is a heterodimeric glycoprotein hormone produced by the anterior pituitary and its release into the blood is under the control of the hypothalamic hormone, GnRH. FSH is a factor of development and reproductive functions to regulate the production of germ cells. In both adult fertile males and females, FSH mediates spermatogenesis and folliculogenesis. For example, in females, FSH stimulates granulosa cell proliferation and antral follicle growth in the ovaries, cells that are critical to produce high quality oocyte maturation or oogenesis and eventual ovulation of the oocyte into the oviduct where it can be fertilized by spermatozoa, the male gamete. In the male FSH stimulates Sertoli cell proliferation in the testis, cells that are critical for proper formation of haploid sperm cells, engendering robust spermatogenesis at puberty (Cohen et Dias., 2019; Furman, et Schriefer, 2007; Miller, 2003).

III.9.2. Luteinizing hormone (LH).

Luteinizing hormone (LH) is an hypophyseal glycoprotein produced by the pituitary gland under the trend of the hypothalamus. Its secretion is pulsative manner except preceding ovulation when a massive release of LH (LH surge) occurs to trigger ovulation. LH is heterodimer consisting of two subunits: α- and β-subunits. The α-subunit is common between LH, FSH, and consists of 92 amino acids. The β-subunit is distinct and hormone-specific, which allows the different biological actions of each hormone. The LH β-subunit has 120 amino acids and one to two sialic acid residues, giving it its shorter half-life of

approximately 20 min. In males, LH drives the testes to make testosterone whereas in females, LH involved in both follicular maturation and corpus luteum function. During the follicular phase, effects of LH must be considered according to the stages of follicular development: in the early follicular phase, LH acts through specific receptors, constitutively present on thecal cells, for stimulating androgen production (Padmanabhan et *al* ., 2018; Hindmarsh et *al*., 2017; Hugues et Durnerin., 2000).

III.9.3. Hormonal control of ovarian function

Two essential gonadotropins, LH and FSH, released by the anterior pituitary gonadotroph cells under the supervision of GnRH from the hypothalamus, regulate ovarian activity (figure 8). The release of these hormones is pulsatile in both men and women, with the amplitude and frequency of pulses fluctuating during the menstrual cycle. LH is secreted in pulsatile fashion under control of GnRH, but modulated by a negative feedback effect of oestradiol and progesterone, and responding to a positive feedback in midcycle leading to the LH surge responsible for ovulation. FSH control is more complicated since it is partly under the control of GnRH, but partly independent of this. As well as the negative feedback from oestradiol and progesterone, there is a further negative feedback from inhibins and a positive stimulating effect of activin.

Inhibins are dimeric glycoprotein hormones from the ovary suppressing FSH by a direct effect on the pituitary; activins are dimers which act mainly at a local level in paracrine or autocrine fashion. Activins are in turn activated by follistatin, an activin-binding third gonadal peptide (Norris et James., 2021; Padmanabhan et *al*., 2018).

III.10. The menstrual cycle.

The menstrual cycle is a multi-endocrine process that picks and develops a follicle in the ovaries while preparing the uterus for prospective fertilized egg implantation. There are two stages to the cycle: follicular and luteal. The first day of the cycle is the first day of menstruation: the shedding of the uterine lining as a result of the failure of a fertilized egg to implant. Ovulation marks the end of the follicular period. The luteal phase begins the day after ovulation and ends the first day of menstruation. The "ideal" cycle lasts a lunar month: 28 days, with a typical range of 25–30 days. Each phase of a 28-day cycle lasts 14 days. Variability in menstrual cycle duration is caused mostly by the efficiency of the follicular phase and its related hormones (Hedayat et Lapraz, 2019; Gosden, 2007).

III.10.1. The follicular phase or the proliferative phase

The proliferative phase explains the changes that occur in the uterus when the follicle matures in the ovary before to ovulation. The endometrium proliferates and develops a rich blood supply under the influence of oestrogens released by the follicle. This is known as endometrial 'decidualization.' During this stage, the targeted follicle finishes maturing within the ovary before releasing the egg. Gonadotropin release from the pituitary gland increases oestrogen production by the developing follicle. Normally, increased oestrogen levels would prevent further gonadotropin release, but this negative feedback process is stopped during the late follicular phase, and there is a surge in LH production just before ovulation (figure 9C). At this time, immediately per- ovulation, the follicle presses against the ovarian wall, generating a protrusion known as the stigma (Hedayat et Lapraz., 2019).

III.10.2. The LH surge and ovulation

- **The LH surge**

As mid-cycle approaches, the level of circulating estradiol increases dramatically. This is followed by a significant LH increase and, to a lesser extent, an FSH surge, which causes the dominant follicle to ovulate. During each menstrual cycle, one follicle ovulates and gives rise to corpus luteum. In women, LH or its surrogate, Human chorionic gonadotropin (hCG), is required to trigger the rupture of the mature follicle. It has been postulated that enhanced local prostaglandin production in the follicle mediates the ovulatory impact of LH (Bulun et *al*., 2019).

- **Ovulation**

Ovulation is characterized by rapid follicular expansion followed by follicle protrusion from the surface of the ovarian cortex. Following this, the follicle ruptures and an egg-cumulus complex is extruded into the peritoneal cavity. Ovulation or follicular rupture happens 34 to 36 hours following the commencement of the LH surge. The rupture is preceded by the elevation of a conical stigma on the surface of the projecting follicle. The ovum and antral fluid are expelled gently rather than explosively when this stigma ruptures.

Ovulation requires a variety of transcriptional regulators downstream of the LH receptor. Following the LH surge, PR levels quickly increase in the preovulatory follicle's mural granulosa cells. The generation of proteases acting locally on protein substrates in the

basal lamina by LH or PR may play a significant role in stigma development and follicular rupture. Plasminogen activator levels, in particular, rise in the follicle before to rupture. The proteolytic digestion of the follicular wall, which is required for follicular rupture, may be aided by plasminogen activator–mediated conversion of plasminogen to plasmin. Endothelin 2, peroxisome proliferator-activated receptorγ (PPARγ), cytosine-cytosine-adenosine-adenosine-thymidine (CCAAT)/enhancer-binding protein-β, liver receptor homologue 1 (LRH1), steroidogenic factor 1 (SF1), and nuclear receptor–interacting protein 1 have all been implicated in ovulation or follicular rupture in mouse studies (Melmed et *al*., 2019).

III.10.3. The luteal phase or the secretive phase

The luteal phase is dominated by progesterone's effects. After ovulation, the burst follicle from which the ovum was liberated creates a corpus haemorrhagicum, which is created by bleeding into the damaged follicle. This grows into the corpus luteum, which has a 14-day lifespan (unless the ovum is fertilized, in which case the corpus luteum persists). In the absence of a fertilized ovum, the corpus luteum degenerates (a process known as luteolysis) and ceases secreting progesterone. This reduction in progesterone production leads the endometrium to disintegrate and menstruation to begin. The luteal phase is also known as the "secretory phase.". This refers both to the secretion of progesterone from the corpus luteum and to the secretion of a clear fluid by the endometrium during this phase (Hedayat and Lapraz., 2019).

III.11. The endocrinology of pregnancy

III.11.1. The placenta

When an ovum is released and fertilized by a spermatozoon, pregnancy occurs. Fertilization can occur in either the fallopian tubes or the uterus. The conceptus becomes embedded in the uterine endometrium and establishes a blood supply via the placenta. The fetus and the mother are genetically distinct individuals linked via the placenta, which is maternal rather than fetal tissue. By delivering blood flow to the fetus, the placenta oversees the delivery of nutrients to the baby. Throughout pregnancy, the placenta is also the most essential endocrine tissue. Throughout the first trimester of pregnancy, it sends hormonal signals to the corpus luteum, preventing luteal regression and maintaining progesterone and oestrogen secretion. It also metabolizes maternal hormones and regulates the growing fetus' endocrine environment (Zakowski and Geller., 2020).

III.11.2. Hormone secretion by the placenta

Human chorionic gonadotropin (hCG) is the main hormone generated by the growing placenta during the first weeks of pregnancy (figure 11). hCG is structurally extremely similar to LH and plays a vital role in preserving luteal function and inhibiting the natural regression of the corpus luteum, which results in an infertile menstrual cycle. The placenta secretes a variety of steroids, but unlike other steroid-secreting tissues, it does not require the steroidogenic acute regulatory (StAR) protein to transport cholesterol across the mitochondrial membrane (Dattani and Gevers., 2016).

The placenta replaces the corpus luteum as the primary source of progesterone after around 8 weeks of gestation, however the corpus luteum remains throughout pregnancy. The release of progesterone from the placenta increases throughout pregnancy and is essential to maintain the pregnancy. Progesterone is thought to calm the myometrium (the muscular lining of the uterus), preventing contractions and fetal evacuation. This is accomplished in part by suppressing the expression of oxytocin receptors. Progesterone may also have an effect on appetite and energy levels. Oestrogens (oestrone, oestradiol, and oestriol) are another kind of steroid produced by the placenta, and their levels grow throughout pregnancy (figure 11). This is due to a complicated interaction with the fetal adrenal gland, which processes early steroid molecules from the placenta (Dattani and Gevers., 2016).

The placenta also secretes testosterone, which increases in concentration throughout pregnancy and reaches 10 times pre-pregnancy levels at term, as well as a placental lactogen (hPL), also known as human chorionic somatomammotropin (hCS), which stimulates breast growth during pregnancy. HPL is closely connected to growth hormone and prolactin, and it also works to counteract the effects of insulin, which may increase the delivery of nutrients to the fetus. During pregnancy, hPL secretion rises. Late in pregnancy, the placenta also generates CRH, a hypothalamus hormone that helps to indicate the end of pregnancy (Dattani and Gevers., 2016).

III.11.3. Non-placental hormones and binding proteins in pregnancy

In addition the hormones released by the placenta during pregnancy, there are several additional significant impacts on the endocrine system. Other important effects on the endocrine system. Thyroid hormone synthesis increases throughout the first trimester of pregnancy and then plateaus, but thyroid hormone binding globulin (THBG) concentration

increases as well, thus free thyroxine levels stay constant. During pregnancy, cortisol levels rise to three times pre-pregnancy levels at term, whereas adrenocorticotropic hormone (ACTH) production stays steady. Thyroid stimulating hormone (TSH) levels diminish throughout the first trimester and subsequently recover to pre-pregnancy levels, but LH and FSH levels from the anterior pituitary are relatively low throughout pregnancy. The secretion of growth hormone is continuous, but prolactin levels rise progressively throughout pregnancy (Hinson et *al*., 2010).

III.11.4. The feto–placental unit

The fetoplacental unit serves three important functions: it serves as a source of protein and steroid hormones that are delivered to the maternal circulation; it serves as a selective barrier that determines the nature of communication and interaction between the maternal and fetal endocrinological and physiological systems; and it participates in the control of fetal growth, development, endocrine function, and parturition (birth).

The fetal and maternal placenta have independent circulatory systems that exchange nutrients, gases, and waste materials in the blood pools of the maternal section of the placenta, Blood depleted of oxygen and nutrients exits the fetus via the umbilical arteries and enters the chorionic villus capillaries. Blood in these capillaries is oxygenated by, obtains nutrients from, and excretes wastes to the maternal blood before returning to the fetus via the umbilical vein. Through the maternal veins, oxygen-depleted blood exits the maternal blood pool. Thus, fetal oxygenated blood is delivered by veins rather than arteries from the moment blood flow is established three weeks after conception. Oxygen-depleted blood leaves the maternal blood pool through the maternal veins. Thus, from the time blood flow is established at 3 weeks following fertilization, fetal oxygenated blood is carried by veins rather than arteries (and oxygen-poor blood by arteries rather than veins), a reversal from the situation after birth when the neonatal circulatory system becomes functional (Norman et *al*., 2015).

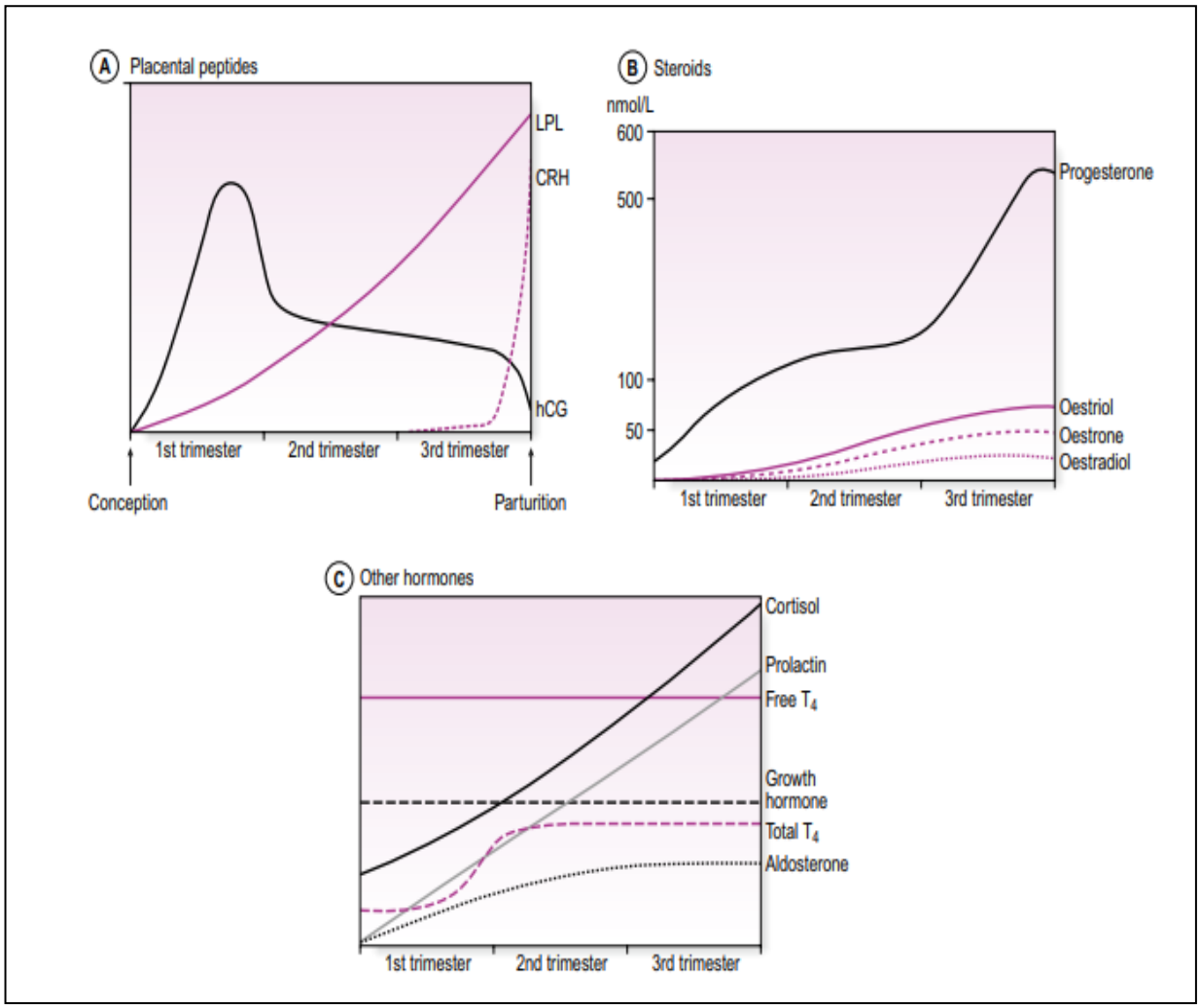

Figure 11. Changes in the plasma concentrations of different hormones during pregnancy. (A) Placental peptides. HCG is the major peptide secreted in the first trimester, and peaks at around 10 weeks' gestation. Levels of hPL, in contrast, rise gradually throughout gestation, peaking just before parturition. Corticotropin releasing hormone (CRH) is the third placental peptide, and is a hormone of late pregnancy, increasing only about 3 weeks before parturition. (B) Steroid hormones. Progesterone is the major steroid hormone of pregnancy, with levels increasing throughout gestation, peaking just before parturition and falling sharply afterwards. Of the other steroids, oestriol is the major oestrogen. Levels of all the oestrogens rise gradually during pregnancy. Testosterone concentration, not shown here, also increases throughout pregnancy. (C) Other hormones. Levels of the adrenal hormones, cortisol and aldosterone, increase during pregnancy, with aldosterone reaching a plateau during the third trimester. Total thyroxine concentration increases during the first trimester, although there is no change in free thyroxine (T4) or free triiodothyronine (T3). Levels of prolactin, from the anterior pituitary, increase throughout pregnancy, but there is no change in growth hormone secretion(Hinson et *al*., 2010).

SECOND CHAPTER: MATERIAL AND METHODS

1. Plant Collection and identification

The seeds of *P. harmala* were collected in august from the Touama region in Bordj Bou Arreridj (northeast of Algeria) characterized by a dry, semi-arid climate. The plant and its seeds were identified on the basis of its morphological characteristics (Chopra et *al*., 1960) (figures12). Before the extraction, the seeds were dried in the obscurity at room temperature (20 to 25°C) for more than a month, followed by grinding separately in a coffee grinder at the time of the extraction.

Figure 12: The seeds of *P. harmala*.

2. The extraction of the total alkaloid from seeds

According to Bruneton (1999) method, *P. harmala* alkaloid extract is obtained using liquid-liquid extraction, which is based on different solubility of the alkaloids in two-compartment between acid and basic nature. the extraction is outlined in figure13. Briefly, the first step was starting by grinding around 100 g of the seeds, and was agitated continuously within 250 ml of petroleum ether for 2 hours to eliminate the maximum of fixed oils. After the filtration, the crude of the seeds was wetted by 20 ml of NH_4OH (0.5N) for 8 h. Secondly, the result was washed out by 250 ml of chloroform ($CHCl_3$) by using a Soxhlet apparatus for

4 h (this time allowed to get 5 cycles). The organic extract (free alkaloids, lipophilic impurities, and other organic components) was shaked three times with 100 ml of aqueous sulphuric acid (0.5N), then the acid extract which was contained the alkaloids in salts forma was treated with a volume of NH_4OH (0.5N) to liberate the free alkaloids. The third step in this extraction was the lavage of liquid obtained in previous step by 100 ml of diethyl ether ($(CH_3CH_2)_2O$) three times, and was dehydrated by sodium sulfate (Na_2SO_4). Finally, the result was evaporated to obtain a red crude of total alkaloids.

3. The qualitative analysis of the total alkaloids by the thin layer of chromatography assay (TLC)

TLC is a physicochemical separation process that employs a stationary or adsorbent phase and a mobile phase. TLC is not adequate to identify the products, but it may offer information about the nature of the elements in the extract throw the appearance of staining in the presence of the appropriate reagent (Randerath, 1971).

According to Kurt's method with some modifications, (1971), and before starting the toxicity studies, we confirmed the presence of alkaloids in the total extract using TLC test. The test was carried out using silica gel (gel plates Alugram sell G/UV254 20×20) procured from Macherey–Nagel, Germany. A few quantity of the total alkaloids powder was dissolved in methanol, and was applied to the gel plate, in the same time the extraction chamber was saturated by methanol, chloroform, and ammonia (78.5:20:1.5) solvent system as a mobile phase. TLC spots containing alkaloids were visualized by spraying Dragen-Dorff reagent on plate.

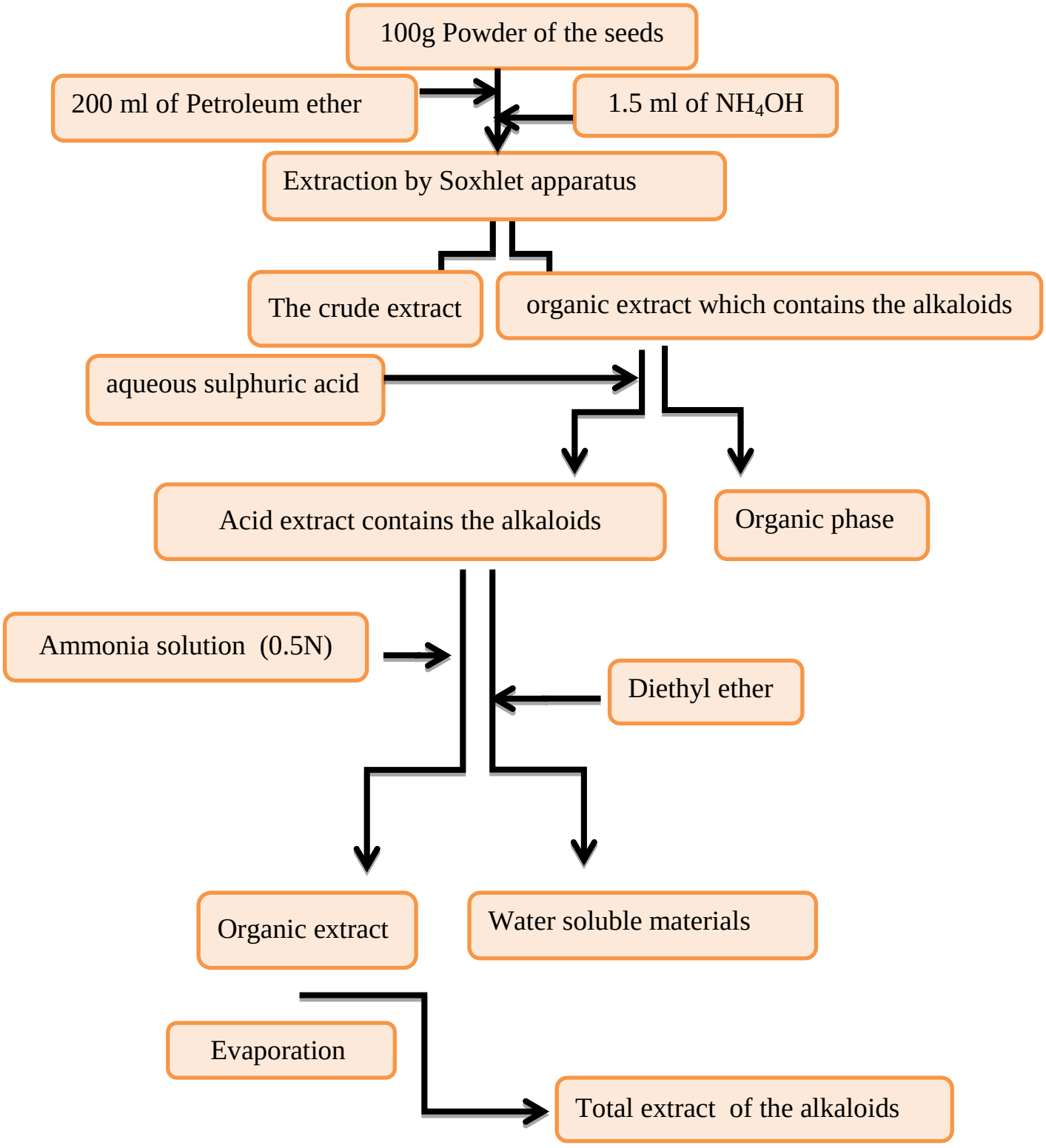

Figure 13. Outline of the extraction procedure for alkaloids (Bruneton, 1999).

4. Animal material

Wistar rats of both sexes, and weights of 160 to 200g, were purchased from Pasteur Institute (Algiers – Algeria). Rats were housed in stainless steel wire mesh cages up to 6 per cage, in standard condition, with free access to tap water and food *ad libitium*, the animals are acclimated in the animal room at the faculty of Natural Sciences and Life at Ferhat Abbes university for three weeks prior to the experimentation. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the scientific council of the Faculty of Natural Sciences and Life of the University Ferhat Abbas, Setif – Algeria.

5. Evaluation of the toxicity studies of acute, chronic ovarian, embryo-toxicity, and fertility activity

5.1. Evaluation of the acute toxicity (DL_{50}) of total alkaloid extract from the seeds

5.1.2.Acute Toxicity design

Total alkaloids extract of *P. harmala* seeds was dissolved in 10 µl of methanol and completed by physiological water until 1 ml, and administered intraperitoneally.

Fifty-four adult Wistar rats of both sexes, weights of 160 to 200g were used to evaluate the medium lethal dose (DL_{50}). The rats were weighed, and divided into groups of 6 animals, one group of each sex is a control group. The treated groups of both sexes were treated with simple application and successively with the following doses: 155, 160, 165, 170, and 175 mg/kg body weight. The extract dose was administered once and the control animals were kept in similar conditions without treatment. DL value, upper and lower confidence limits and slope value were calculated according to Litchfield and Wilson, (1949) method.

5.1.3. Clinical observations

The animals were observed 30 min after dosing, followed by hourly observation for 8h, and once a day for the next 14 days. All observations were systematically recorded with individual records being maintained for each animal. Surviving animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance, injury, pain, and signs of illness were conducted daily during the period.

5.2. Evaluation of the ovarian toxicity of total alkaloids extract of *P. harmala*

5.2.1. Chronic ovarian toxicity design.

The DL_{50} of the total extract of the alkaloids in female rats was calculated by the graphic method of Litchfield and Wilcoxon, (1949), and was defined as 159.95 mg/kg of body weight.

Thirty mature female Wistar rats at the age of three months and a body weight of 180 to 200 g were used. The females were divided into two groups, following: a control group of ten female rats; and an experimental group of twenty female rats. The treated group was received intraperitoneally a volume of 0.5 ml/100g of 1/40 DL_{50} of total alkaloids extract which is equal to 3.99 mg/kg, every day for three months.

5. 2. 2. Clinical observations.

The female rats were weighted once a week for three months and physical signs, behavior, and survival of female rats were closely observed and recorded. In the end of the test period, rats of both groups were anatomized and venesected after diethyl ether anesthesia.

5. 3. Evaluation of embryo-toxicity of total alkaloid extract of *P. harmala*

5. 3. 1. Experiment Animals and husbandry.

Fifteen proven fertile adult males and forty virgin or nulliparous healthy young female Albinos Wistar rats of the age of 10 weeks and weighing 170–200g, were obtained from pet store of biology faculty.

For **husbandry**, three females were placed into a cage with a male rat overnight. The next morning successful mating was confirmed by the presence of sperm in the vaginal smear, (Jahnke, 1999) and the following 24 hours were designated as day 0 of gestation (GD0). Confirmed-mated females were assigned to treatment groups by stratified randomization (9/group in the screening study), so that mean body weight on GD0 did not differ among treatment groups in the study. Maternal body weight, for confirmed pregnant females used in these study, was ranged from 170 to 200 g on GD0. Confirmed-mated females were individually housed, and the pregnancy was verified by weight gain and abdominal palpation.

5. 3. 2. Dosage and Treatment.

Dose formulations were prepared by dissolving 1/20 DL_{50} mg which is an equal to 7.99 mg of total alkaloids extract in10 micro-liter of methanol and one milliliter of normal saline. The test dose was prepared daily prior to use. In the morning, the rats were received 7.99 mg/kg/day of total extract by intraperitoneal administration. The pregnant rats received the treatment from GD0 to GD6 designed to be the group treated for seven days, GD0 to GD15 of treatment is a group treated for two weeks, and a group treated for three weeks from GD0 through GD19. Control rats was received only an equivalent volume of vehicles.

5. 3. 3. Maternal Toxicity Evaluations

- ✓ **Clinical Signs and Body Weight**

Throughout pregnancy, pregnant females were observed daily for mortality, morbidity, general appearance, behavior, and clinical condition at least once/day from GD0 to GD20. Maternal body weights (g) were recorded on the mornings of GD0 to GD20, and immediately before the sacrifice in GD20.

- ✓ **Gross Findings and Organ Weights**

On GD20, all pregnant females were euthanized and sacrificed by an overdose of ether and were exsanguinated via the aorta in the early morning. A complete gross postmortem (Thoracic and abdominal cavities) examination was performed. The absolute and relative weights of the brain, lungs, liver, spleen, kidneys, heart, and ovaries were recorded. In addition, Pregnancy status was confirmed by uterine examination. Uterine contents were examined to determine the number of implantation sites (all of live pups and dead fetuses), pre/post-implantation, resorptions, dead fetuses, and live fetuses. Uteri is checked very well to record any implantation sites which might have undergone very early resorption (Jahnkle, 1999). According to Toyin et *al*, (2014), the following reproductive parameters were computed from these data:

- ✓ Number of implantations.
- ✓ Number of corpora lutea.
- ✓ Percentage mating success = (number mated/number paired) x100.
- ✓ Quantal pregnancy = (number pregnant/number mated) x100.
- ✓ Fertility index = (number pregnant/number paired) x100.

- ✓ The post-implantation loss is calculated by determining the ratio of dead to total implants from the treated group compared to the ratio of dead to total implants from the vehicle/solvent control group.
- ✓ Pre-implantation loss is calculated as the difference between the number of corpora lutea and the number of implants, or as a reduction in the average number of implants per female in comparison with control mating.
- ✓ Implantation index= (total number of implantation sites/number corpora lutea) x 100.
- ✓ Resorption index= (total number of resorption sites/total number of implantation sites)x100.
- ✓ Pre-implantation loss= (number of corpora lutea- number of implantations/number of corpora lutea) x 100.
- ✓ Post-implantation loss = (number of implantations -number of live fetuses/number of implantations) x 100.

5. 3. 4. Embryo/fetal evaluations

On GD20, ovaries were observed for any abnormalities, and all fetuses were dissected from the uterus counted, weighed, and examined for external morphological abnormalities before placing them in 10 % formaldehyde.

The following parameters of abortion/implantation were recorded and/or computed according to Toyin et *al*, (2014):

- ✓ Number of live and dead fetuses.
- ✓ Survival ratio=(number of live fetus/(number of live + dead fetus)) x100.
- ✓ Number of rats that aborted.
- ✓ Percentage aborted=(number of rats that aborted/number of rats used)x100.

5. 4. Evaluation of the fertility activity of total alkaloids extract of *P. harmala*

5. 4. 1. Animals and husbandry

Fertility was estimated in adult male rats after a treatment period of three months. Male rats were exposed to a total alkaloids extract of *P. harmala* and mated to untreated virgin females. Following mating, the females are euthanized in the gestation period (Bataineh et *al*., 2007).

5. 4. 2. Experimental Group Composition, Dosage and Treatment.

Twenty Adult male and thirty virgin female Wistar rats were used in the test of fertility, weighing about 165 to 190 g and 175 to 190 g respectively.

5. 4. 3. Experimental Design

Male rats were divided into control group of ten rats received a vehicle, and a group of twenty rats were treated intra-peritoneally by the dose of $1/40DL_{50}$ which is equal to 4.110 mg/kg/day of the total alkaloids extract of *P. harmala*'s seeds for 90 days, this period covers one spermatogenic cycle of 60 days. At the end of the experimental period, the males mated with a virgin female overnight. The next morning successful mating was confirmed by the presence of sperm in the vaginal smear (Jahnke, 1999), and the following 24 hours were designated as day 0 of gestation (GD0). Confirmed-mated females were placed in separate cages, and the pregnancy was confirmed by weight gain and abdominal palpation. The pregnant females were kept until the end of their pregnancy period or GD20. During the course of the treatment period, pregnant rats were observed daily for any abnormal behavior. The body weights of the pregnant animals were recorded every 7 days. At 20 days of gestation, pregnant female rats were killed under ether anesthesia.

5. 4. 4. Maternal Toxicity Evaluations

- ✓ **Clinical Signs and Body Weight.**

Throughout pregnancy, pregnant females were observed daily for mortality, morbidity, general appearance, behavior, clinical condition, and * signs of toxicity at least once/day on GD0 through 20. Maternal body weights (g) were recorded on the mornings for GD0 and GD20, and immediately before the sacrifice on GD20.

- ✓ **Gross Findings and Organ Weights**.

On GD20, all pregnant females were euthanized and sacrificed by an overdose of ether and were exsanguinated via the aorta in the early morning. A complete gross postmortem (Thoracic and abdominal cavities) examination was performed. The absolute and relative weights of the brain, lungs, liver, spleen, kidneys, heart, and ovaries were recorded. In addition, Pregnancy status was confirmed by uterine examination. Uterine contents were examined to determine the number of implantation sites (sum of live pups and dead fetuses),

pre/post-implantation, resorptions, dead or resorbed fetuses, and live fetuses. Uteri which checked very well to record any implantation sites which might have undergone very early resorption (Jahnkle, 1999). According to Toyin et *al*, (2014), the reproductive parameters were computed.

5. 4. 5. Embryo/fetal evaluations

On GD20, ovaries were observed for any abnormality. All fetuses were dissected from the uterus counted, weighed, and examined for external morphological abnormalities before being placed in 10 % formaldehyde. The parameters of abortion/implantation were recorded and/or computing.

6. Serum biochemical, hormonal, histology examination of the toxicity studies.

- ✓ **Serum biochemical examination**

Blood samples were taken from the orbital sinus of rats by hematocrit, and were centrifuged at 3000 rpm for 10 minutes within 1 hour of collection. After serum isolation, all the hepatic parameters (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma glutaryl-transferase (GammaGT), alkaline phosphatase (PAL), total, direct, and indirect bilirubin), renal parameters (blood urea nitrogen, creatinine, glucose, and monogrammed Na/K/Cl), and hematologic parameters (RBC, Hb, HCT, MPV, MCV, MCHC, MDW, WBC, LYM%, PDI, PTC, LPCR, MID%, GRA%).

- ✓ **Serum hormonal examination**

The density of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and progesterone hormones was measured by ELISA.

7. Histopathology study

The main organs of each animal namely, the liver, kidney, and brain, as well as the target organ of this study, the ovaries were excised and fixed in 10% formaldehyde. Following the fixation, the organs were cut into small pieces and placed in 70% ethanol. Tissues were dehydrated by using different concentrations of alcohol in the following sequence: 95% ethanol for 30 min (twice); 100% ethanol for 30 min (twice); 100% xylene (twice). Tissue was subsequently embedded in paraffin. Five-µm sections were cut using a microtome, were mounted on glass slides, and stained with hematoxylin and eosin (H&E).

8. Statistical data of the toxicity studies

- ✓ **Ovarian toxicity**

Data were expressed as means±standard error of the means (SEM) for the studied variable. Statistical analysis was performed using the Systat Software, Inc. SigmaStat Executable (SigmaStat® 3.5). The statistical significant differences between groups were validated with a one-way analysis of variance (ANOVA) followed by a post hoc Tukey test. A probability value of <0.05 was considered significant.

- ✓ **Embryo-toxicity**

Data were then analyzed using ANOVA to assess the differences among the means. When statistically significant P-values were obtained with ANOVA, the data were analyzed using Tukey's post hoc multiple comparison test. A P-value of <0.05 was considered statistically significant for each group of animals contained 9 rats, with 11–18 fetuses per rat.

- ✓ **Fertility test**

Statistical measurements were expressed as means ± standard error of the means (SEM) for the studied variable. Statistical analysis was performed using the Systat Software, Inc. SigmaStat Executable (SigmaStat®3.5). The unit for statistical measurement was the pregnant female. For each statistical comparison, the significance was reported as P<0.05. Nonparametric tests applied to continuous variables included the post hoc Tukey test; ANOVA by ranks for among-group differences, and two-way analysis was used for all parameters, except that maternal and fetal body weight parameters, were used to identify significant dose-response trends.

THIRD CHAPTER: THE RESULTS

1. The extraction yield

The alkaloids of *P. harmala* seeds were extracted using Bruneton, (1999) protocol which allowed us a yielding approximately 1.820±0.358 per 100g (w/w).

2. Qualitative analysis of total alkaloids

The presence of alkaloids in the obtained extract was confirmed by qualitative analysis using a Thin-layer chromatography method with silica gel and Dragendorff reagent (Pascual *et al*., 2002). The plate revealed the presence of four spots (figure 14).

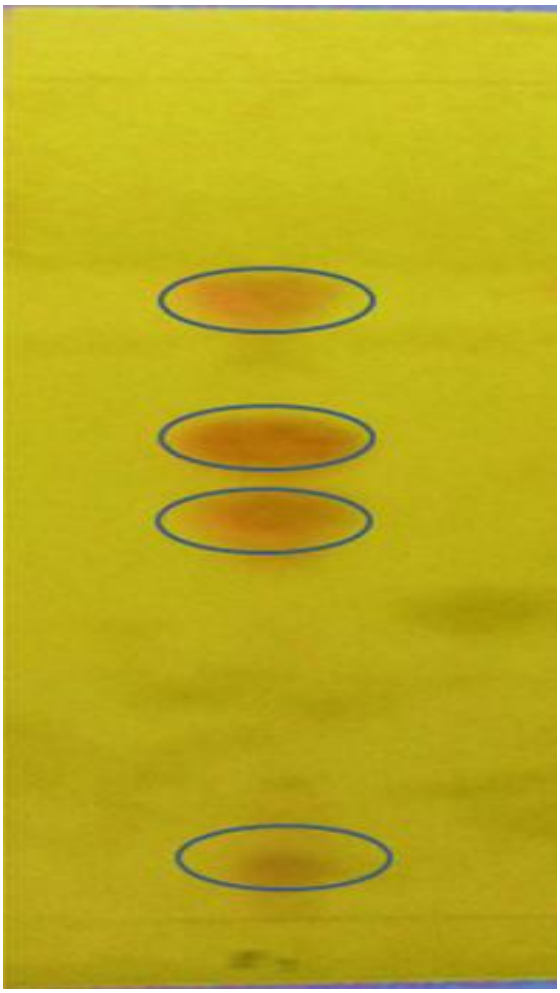

Figure 14. Thin-layer chromatography of the total alkaloids extract of from the seeds, mobile phase: methanol, chloroform, and ammonia (78.5:20:1.5).

3. The toxicity investigations of acute, chronic, embryo toxicity, and fertility test findings

3.1. DL_{50} of a total alkaloids extract

3.1.1. Behavioral changes and poisoning symptoms

We recorded a clinic behavioral card, characterized by acute symptoms with all doses used. Rats become hyperactive soon after exposure, with strong convulsion, agitation, and tachycardia. The difficulty in breathing, leg paralysis, and hyperactivity was observed in the first 30 minutes. Subsequently, the rats were dead. Recording the death was in the first 10 minutes to 24 hours, until 5 days, and surviving animals stayed showing abnormal performance in comparison to the control groups such as a decrease in locomotor activity for both sex, drowsiness, and anorexia.

3.1.2. Estimation of DL_{50} by the Litchfield and Wilson's method

Tables 3 and 4 show the values DL_{50} following intra-peritoneal injection to different groups of rats, as well as the mortality percentage and dose function. The curve in figure 15 is created using the results from table 3, which indicates the DL_{50} of the extract in male rats, which is 164.43 mg/kg. We also determined the LD_{50} value for female rats, which is 159.953mg/kg based on table 4 and figure 16.

Table 3. DL_{50} total alkaloids extract in male albinos Wistar rats.

Dose mg/kg	N° of Dead	Observed effect		Attend effect		≠Ce%	X^2
		%	Probit	%	Probit		
160	0/6	4.9	3.35	16.07	4.031	11.17	0.1
165	3/6	30	5.00	50.10	5.00	20.1	0.2
170	5/6	50	5.97	83.70	5.97	33.7	0.7
175	6/6	99.1	7.37	97.4	6.94	1.7	0.01

- $\sum X^2 = 1.01$, theorical value of X^2 for p=0.05 correspond to n [Number of degrees of freedom= K (Number of doses)-2].
- probit4=log dose correspondent 2.203, lethal dose of 16%=159.58mg/kg
- probit5=log dose correspondent 2.216, lethal dose of 50%= 164.43mg/kg
- probit6=log dose correspondent 2.230, lethal dose of 84%= 169.82mg/kg
- $\sum X^2 = 1.01$.
- $X^2_{Theo} = 5.99 \geq X^2_{expir}e = 1.01$. We concluded that the experience was accepted.
- S=1.0.
- N' = 18.
- Radical du N' was 4.242.
- ∫dl50%= 1.973.
- The range of the lethal dose was between 83.340 and 324.42 or [83.340, 324.42].

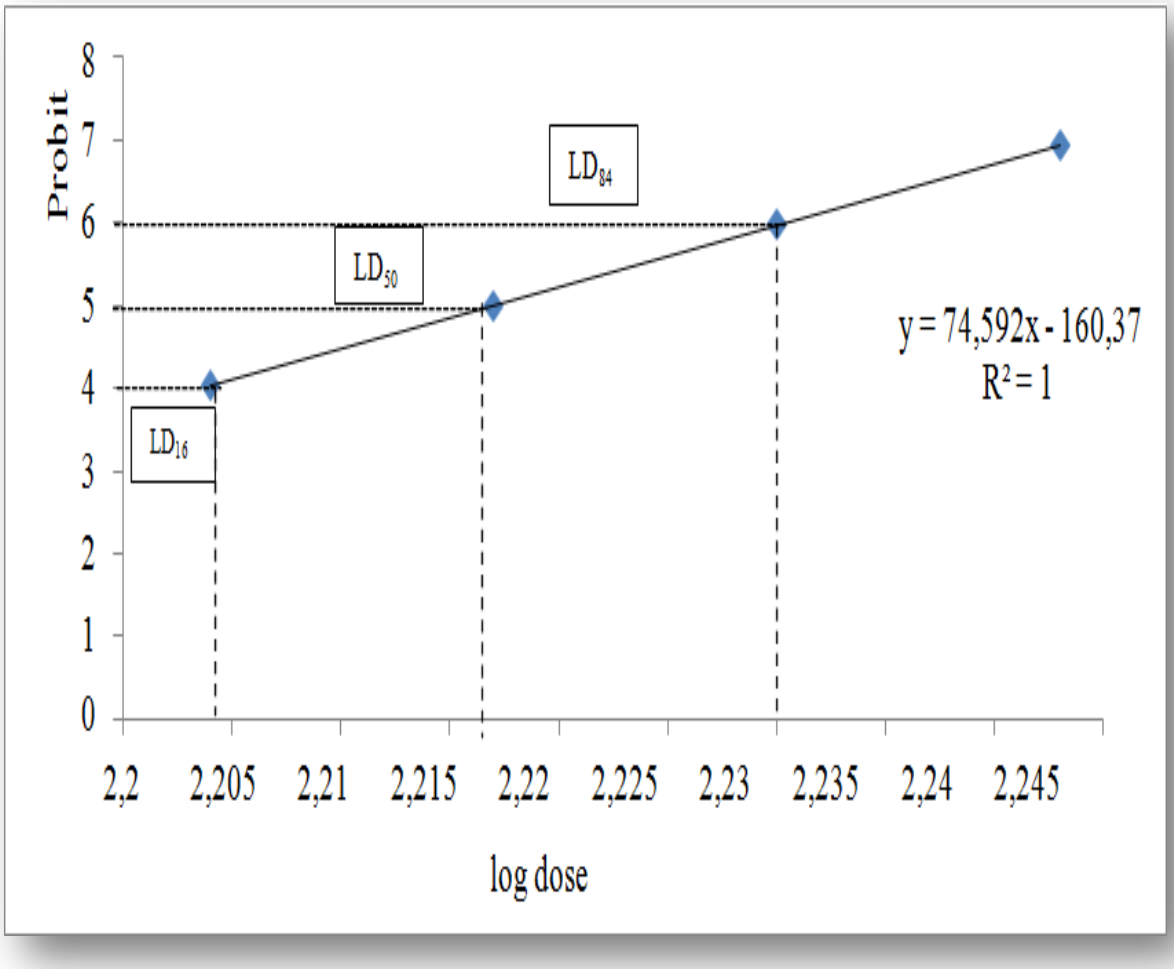

Figure 15. Curve characterizing the dose-response relationship for the determination of lethal parameters in male rats treated by simple application with the total alkaloids of *P. harmala* (LD_{50}= 164.43mg/kg).

Table 4. The DL_{50} of total extract in albino Wistar female.

Dose mg/kg	**N° of Dead**	**Observed effect**		**Attend effect**		**≠ce%**	**X^2**
		%	**Probit**	**%**	**Probit**		
155	**0/6**	**8.0**	**3.61**	**19.72**	**4.47**	**11.72**	**0.1**
160	**3/6**	**30**	**5.00**	**43.5**	**4.83**	**13.5**	**0.07**
165	**4/6**	**40**	**5.43**	**67.9**	**5.46**	**27.9**	**0.3**
170	**5/6**	**50**	**5.97**	**83.1**	**5.95**	**33.1**	**0.7**
175	**6/6**	**97.6**	**6.98**	**92.5**	**6.43**	**5.1**	**0.03**

- $\sum X^2 = 1.2$, theorical value of X^2 for p=0.05 correspond to n [Number of degrees of freedom= K (Number of doses)-2].
- Probit 4= log dose correspondent 2.177, lethal dose of 16%=150.3mg/kg
- Probit 5= log dose correspondent 2.204, lethal dose of 50%=159.953mg/kg
- Probit 6= log dose correspondent 2.231, lethal dose of 84%=170.203mg/kg
- $\sum X^2 = 1.2$
- $X^2_{Theo} = 7.82 \geq X^2_{expire} = 1.2$ l'expérience acceptable
- S= 1.06
- N' = 24
- Radical du N' was 4.898.
- ∫dl50%= 1.86
- Interval of lethal dose l of 50% was [88.86, 287.91]

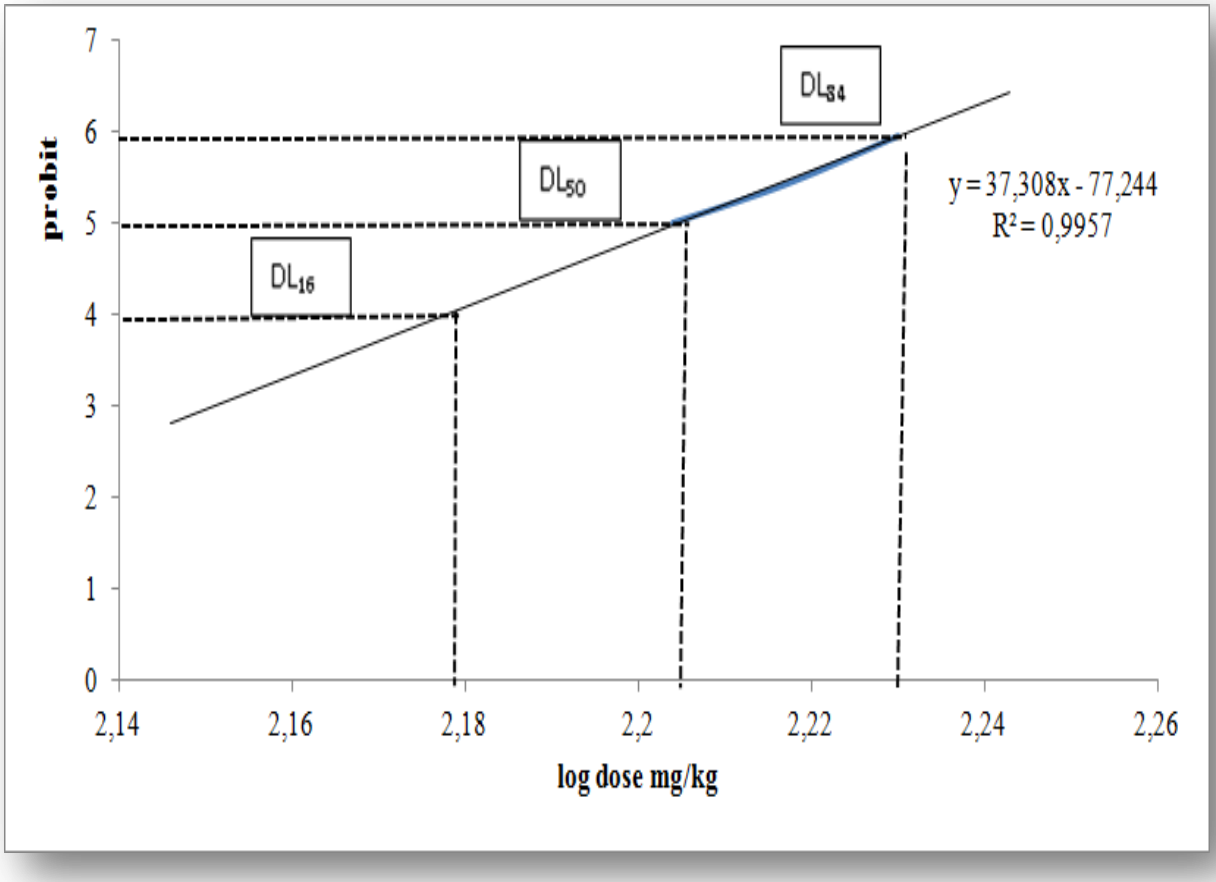

Figure 16. Curve characterizing the dose-response relationship for the determination of lethal parameters in female rats treated by simple application with the total alkaloids of *P.harmala* (LD_{50}= 159.953 mg/kg).

3.2. The oogenesis toxicity finding

- **General signs and weight variation**

The findings of a daily intra-peritoneal injection of total alkaloids extract at 1/40 of DL_{50} showed no clinical evidence of toxicity or mortality after 90 days of therapy and steadily raised of body weight in the experimental female rat compared to the control group (figure 17). Figure 18 shows that the relative organ-weight increased statistically substantially in the experimental group for the kidney, heart, lungs, brain, and ovaries, in contrast to the liver and spleen, which showed no significant relative organ-weight increases.

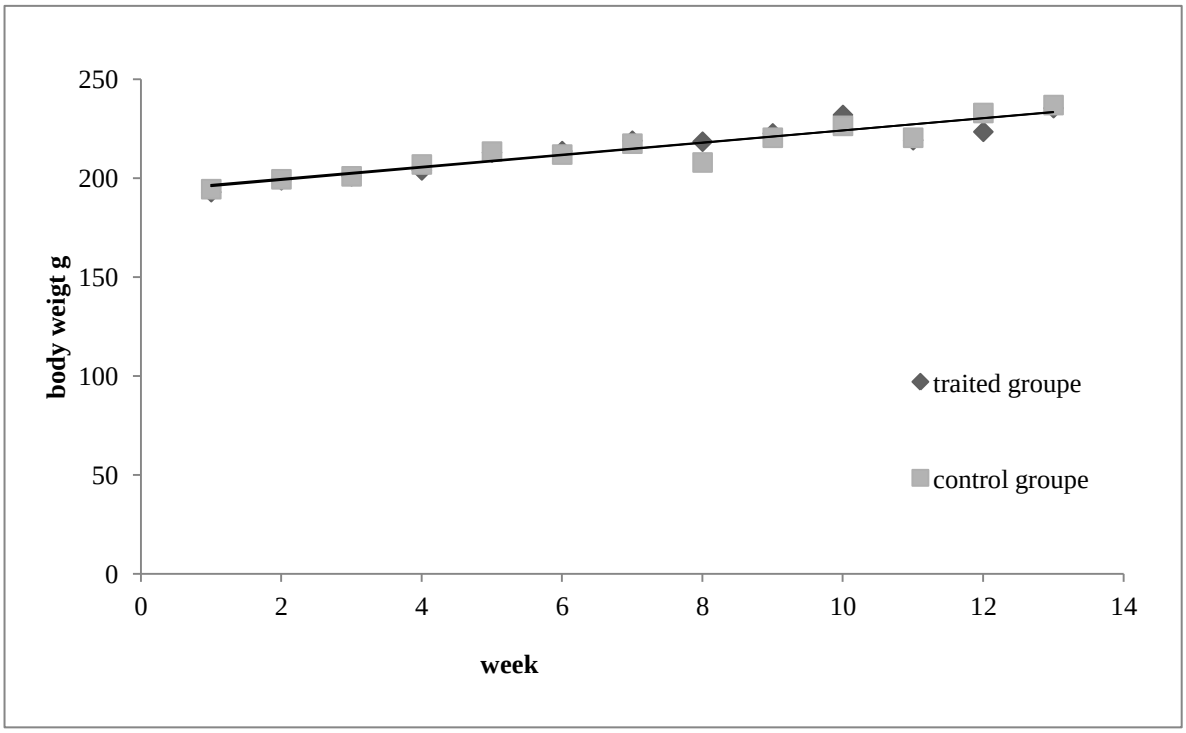

Figure 17. Mean body weight of female rats receiving 1/40 of DL_{50} of *P.* harmala extract for 90 days. Values are expressed as mean ± SEM (*n* = 10 for control group, and *n* = 20 for treated group). **P* values < 0.05 were considered significant difference compared to control.

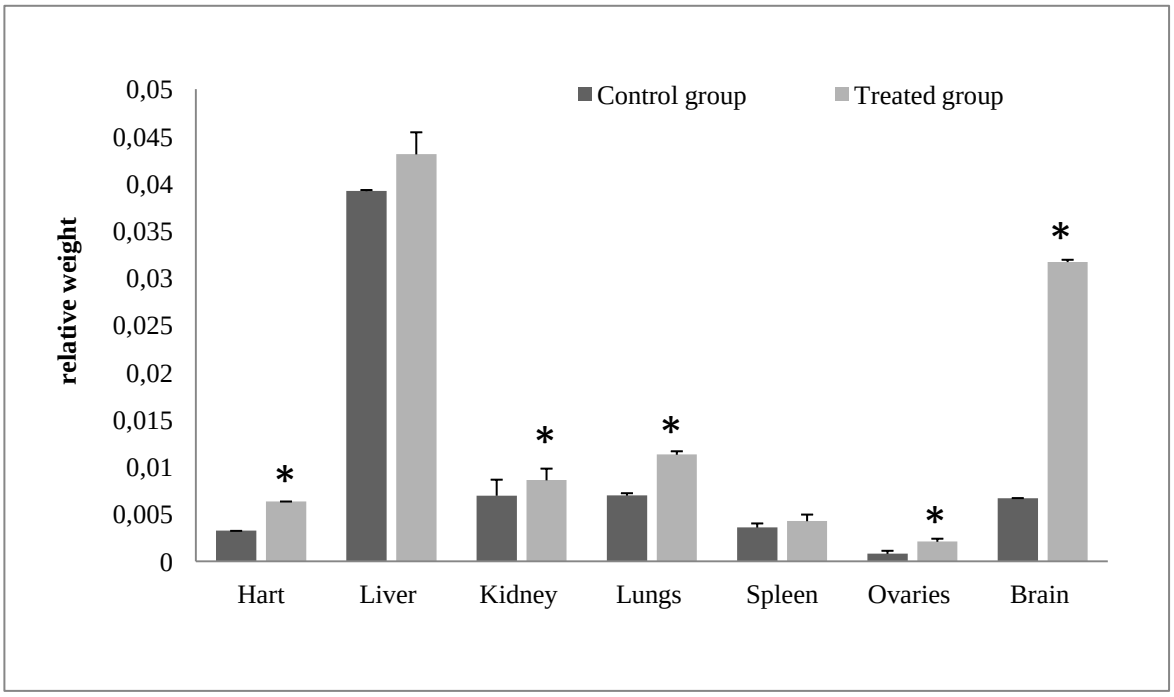

Figure 18. Relative weight changes during 90-day intraperitonial administration of the total alkaloids of *P. harmala* (Mean± SEM, Significantly different from control).

- **Serum's biochemical and hormonal parameters**

The results demonstrate that PLA activity increased significantly, but ALAT and ASAT activity decreased significantly. Furthermore, no alteration in GammaGT was observed (figure 19). In treated group, there was a substantial decrease in total bilirubin and indirect bilirubin but no significant change in direct bilirubin in comparison to the control group (figure 20).

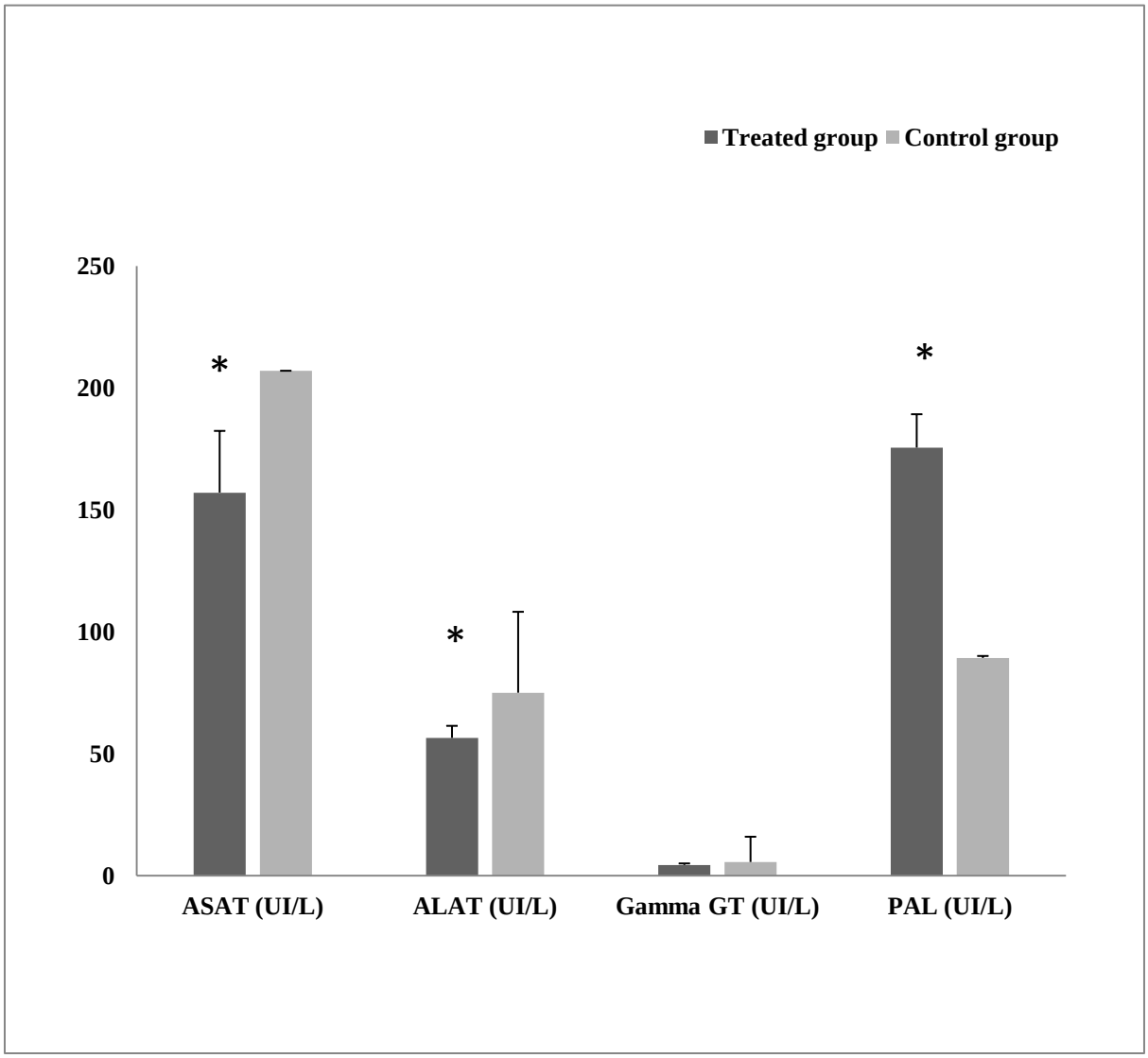

Figure 19. The chronic effect of total alkaloids of *P. harmala* on serum biochemical parameters (Mean± SEM, Significantly different from control, P<0.05).

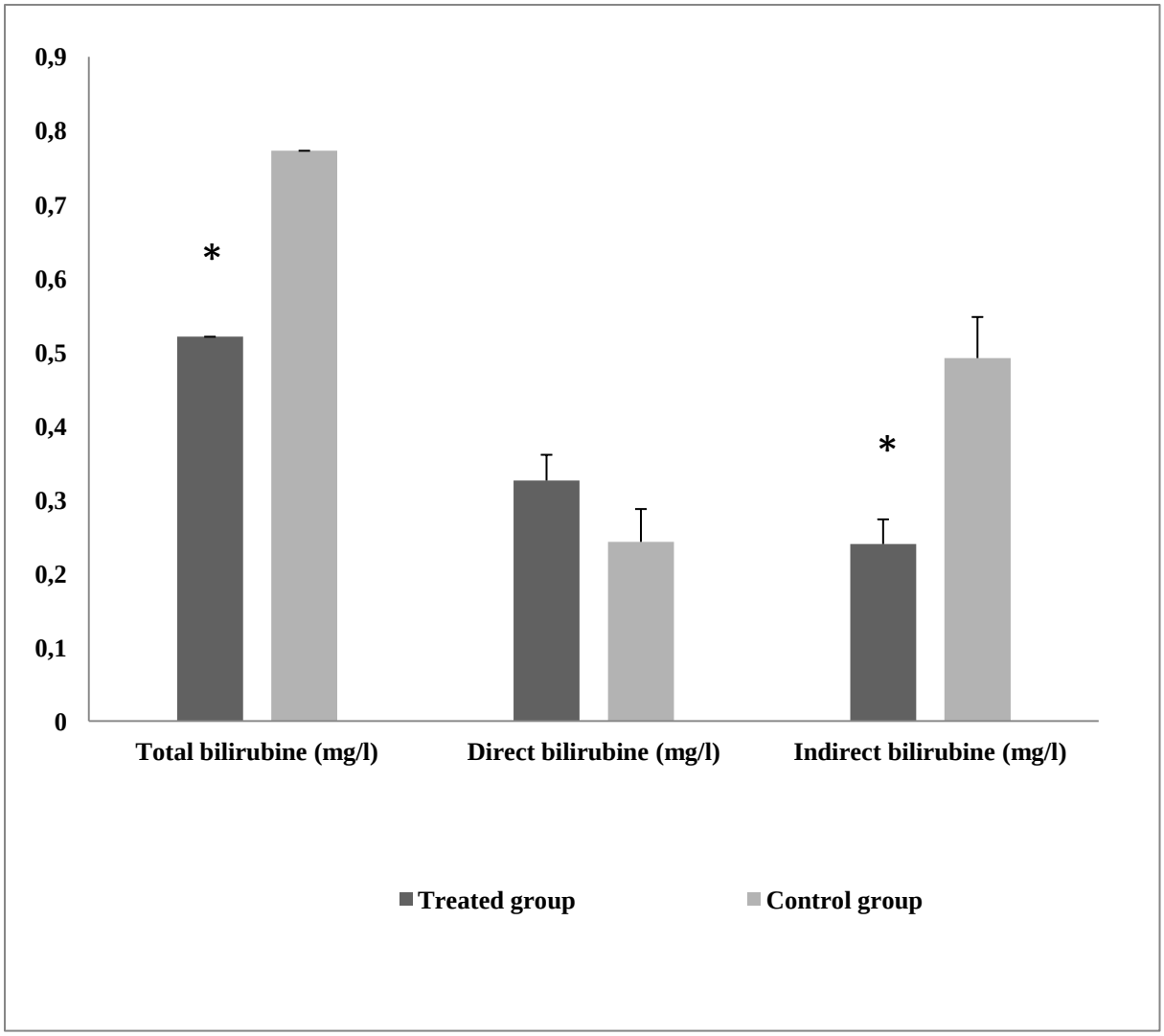

Figure **20.** The chronic effect of total alkaloids of *P. harmala* on total bilirubin, direct and indirect bilirubin of female rats (Mean± SEM, Significantly different from control, P<0.05).

As demonstrated in figure 21, the kidney function parameters namely, glucose did not exhibit any significant changes in glucose, but there was a substantial drop in serum urea and creatinine in the experimental group compared to the control group. The hormonal data demonstrated a significant rise in the concentrations of FSH and Estrogen, but no change in the concentrations of LH and progesterone in the experimental group compared to the control group, as shown in figures 22 and 23.

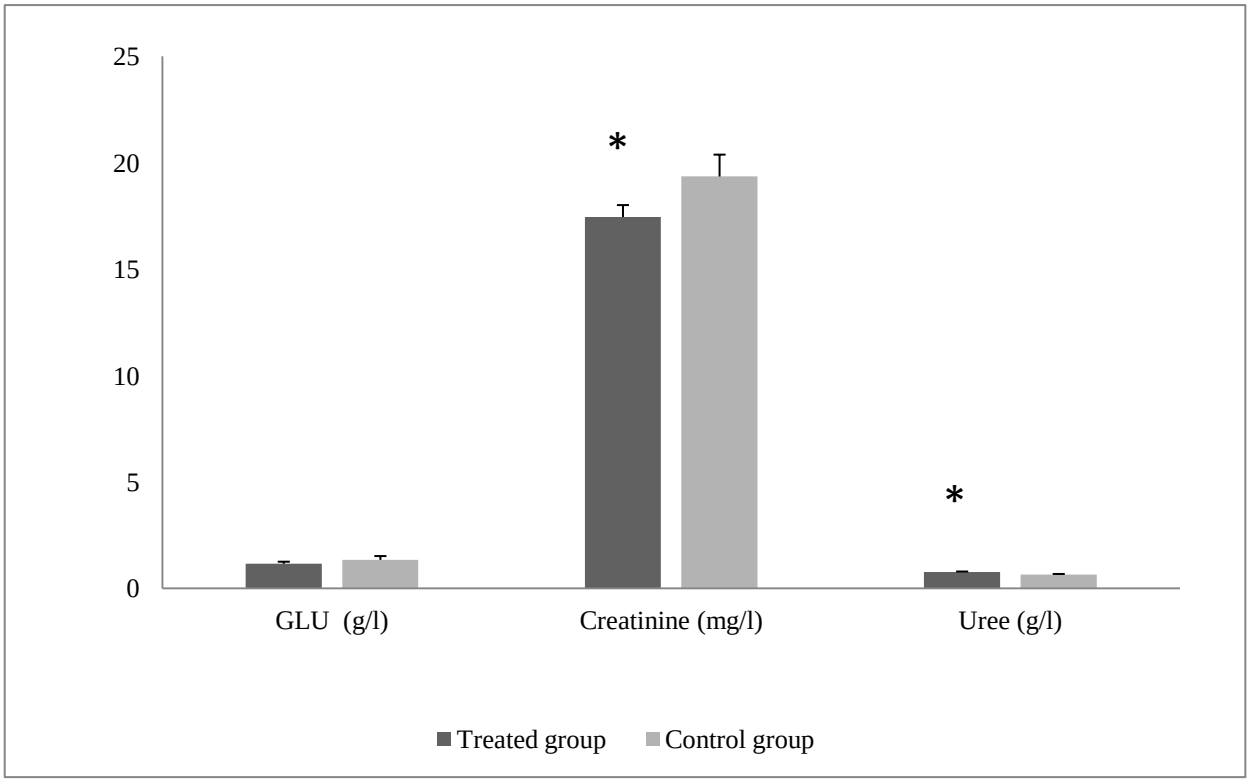

Figure 21. Effect of total alkaloids extract of harmala with GLU, Creatinine and Urea of female rats. (Mean± SEM, Significantly different from control, P<0.05.

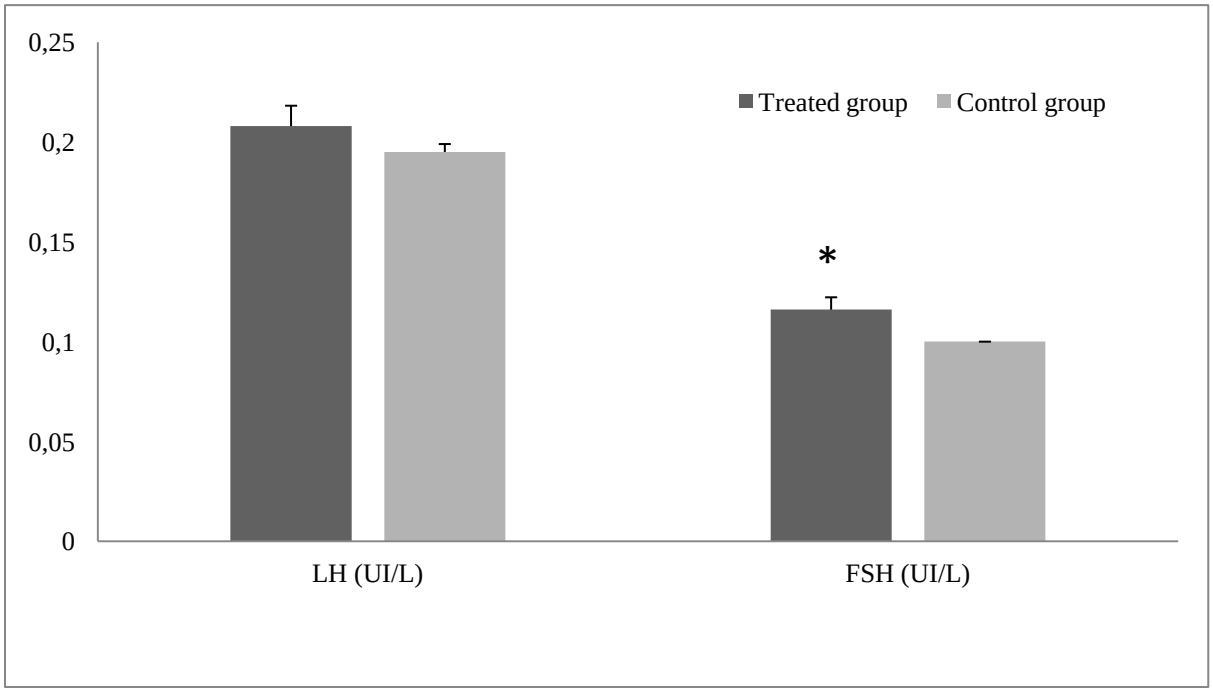

Figure 22. *E*ffect of total alkaloids extract of *P.harmala* LH and FSH of female rats. Values (Mean± SEM, Significantly different from control, P<0.05).

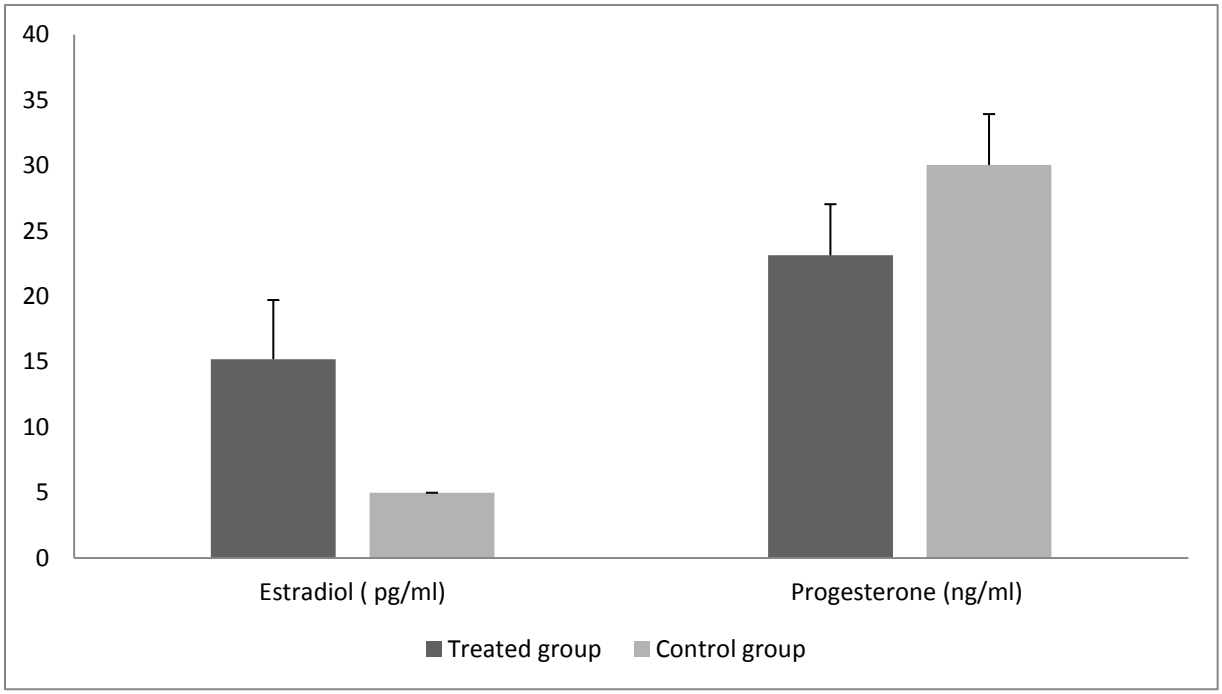

Figure 23. Effect of total alkaloids extract of *P. harmala* Progesterone and Estradiol of female rats. (Mean± SEM, Significantly different from control, P<0.05).

- **Hematological parameters**

The hematological parameters demonstrated a significant increase of Red Blood Cell (RBC), Mean corpuscular hemoglobin concentration (MCHC), a decrease in mean corpuscular volume (MCV), and increase in White blood cell number (WBC), platelet total concentration (PTC), lymphocyte (LYM g/l), and Red distribution width (MDW). While Hematocrit (HCT), Hemoglobin concentration (Hb), Large Platelet Concentration Ratio (LPCR), Mid-sized cell (MID%), and Absolute Lymphocyte count (LYM%) remained within physiological range during the treatment period (90 days). In addition, mean platelet volume (MPV), GRA% and index distribution of palette (PDI) were not affected by the used dose (table 5).

Table 5. Effects of 1/40 DL_{50} of total extract on hematological parameters in female rats.

	RBC 10*12/L	**Hb g/dl**	**HCT%**	**MPV**	**MCV fl**	**MCHC g/dl**	**MDW fl**	**WBC $10*^{9}$/ L**	**LYM %**	**PDI fL**	**PTC %**	**LPCR %**	**MID %**	**GRA %**
Treated group	7.244 ± 0.0626*	13.3 ± 0.124	39.221 ± 0.421	5.81 ± 0.0371	54.147* ± 0.435	33.925 ± 0.185*	33.453 ± 0.355*	8.08* ± 1.031	73.95 ± 2.151*	9.235 ± 0.0494	0.387* ± 0.0155	3.567 ± 0.173	8.171 ± 0.525	17.89 ± 1.703
Control group	6.803 ± 0.169	12.8 ± 0.304	38.666 ± 1.209	5.733 ± 0.0467	56.722 ± 0.61	33.277 ± 0.358	33.033 ± 0.45	3.633 ± 0.257	74.733 ± 1.233	9.111 ± 0.0639	0.276 ± 0.0162	3.655 ± 0.167	8.611 ± 0.526	16.655 ± 0.851

RBC: red blood cell; Hb: hemoglobin; HCT%: Hematocrit; MPV: mean platelet volume; MCV: mean corpuscular volume MCHC: mean corpuscular of Hb concentration; MDW: Red distribution width; WBC: white blood cell; LYM%: Lymphocyte count; PDI. platelet distribution index; PTC: Platelet total Concentration; LPCR: Platelet Concentration Ratio; MID**%**; Large Mid-sized cell; GRA%: Granulocyt neutrophil. Values are expressed as Mean± SEM, Significantly different between treatment group and control group (* $P<0.05$).

- **Histological study**

Histological examination sections revealed a regular hepatic parenchyma in A and B as a control groups, while in treated group the section showed sinusoidal inflammation and lympho-plasmocyte inflammation around the centro-lobulaire of vein in sections C and D, necrosis and congestion in the centro-lobulaire vein in sectione C. In section D, the centro-lobule vein was full of red blood cells with the presence of granuloma inflammation well-limited by lymphocytes, plasmocytes, polynuclear neutrophils, eosinophils, and phagocytize cells (figure 24). The kidney sections B and C showed a typical renal parenchyma with usual glomerular morphology, congestive follicles surrounded by endothelial cells, their lumina filled by red blood cells and both proximal and distal tubules were normal. In addition, kidney's section C indicates the existence of lympho-plasmocyte inflammation areas (figure 25). Histological sections of the ovaries showed the presence of various stages of follicular development and corpora luteal, and a significant number of secondary follicles and corpora luteal with a significant large size in comparison to control ovaries (figure 26).

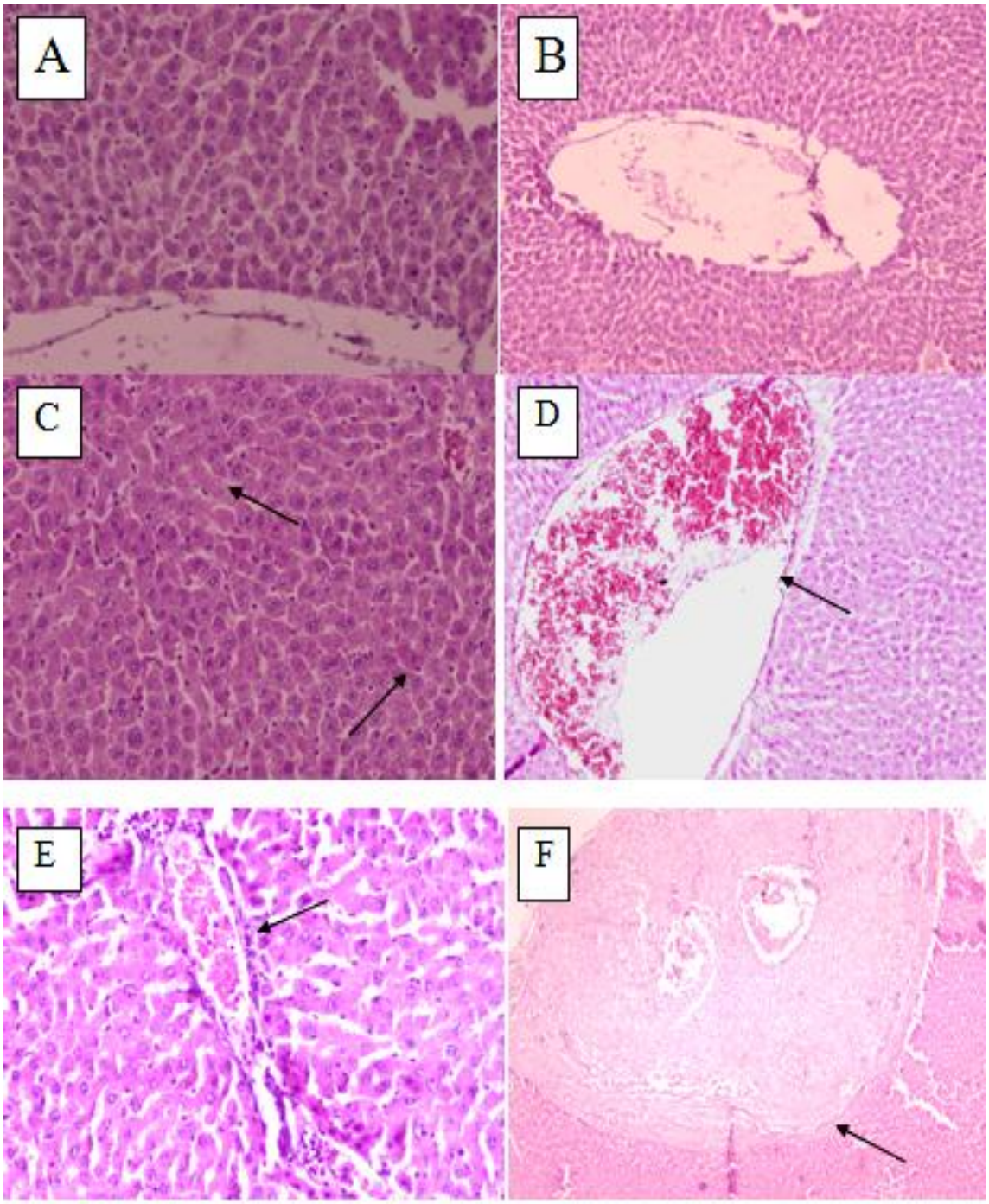

Figure 24. Histopathological photomicrographs of the liver in the experimental groups: Control group (A,B); treated group with the total extract of the seeds of *P. harmala* (C, D, E, F) (H&E, ×100).

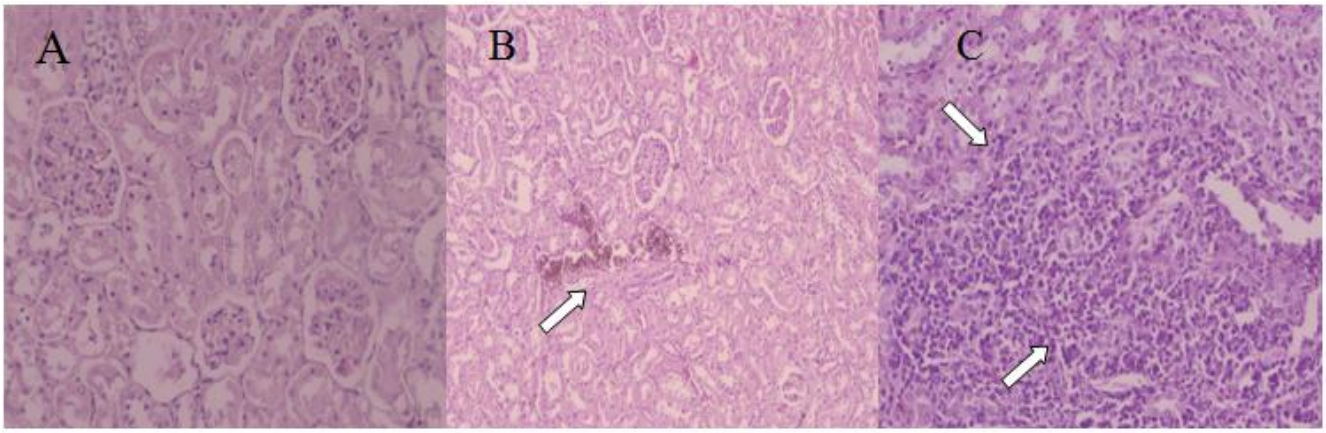

Figure 25. Histopathological photomicrographs of the kidney in theexperimental groups: Control (A); treated group with the total extract of the seeds of *P. harmala* (B, C) (H&E, ×100).

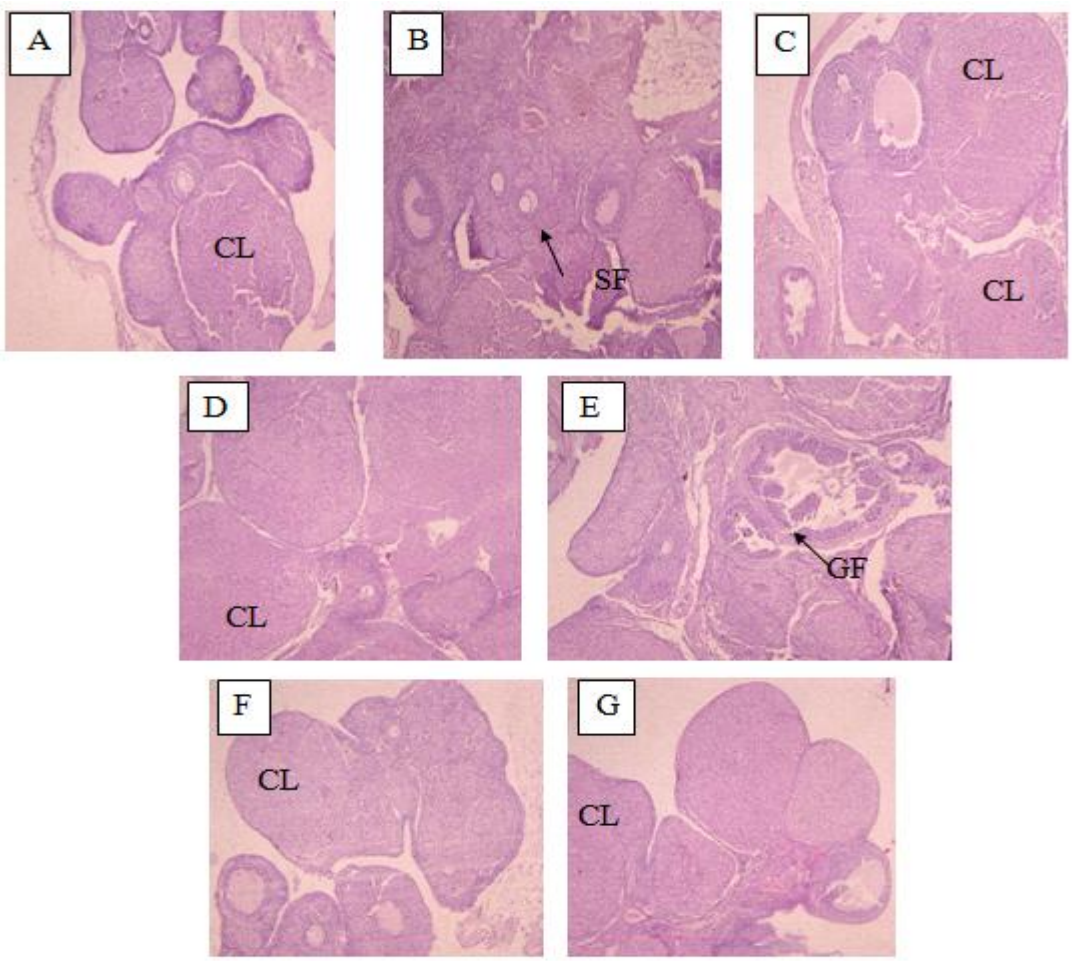

Figure 26. Histopathological photomicrographs of the ovaries in the experimental groups: treated group (A, B, C, D, E); Control (F, G). Images A, B, C, D, E showed the morphological aspect of the ovaries with huge number of Corpora luteal (CL) and follicles (SF, GF) in different stages of maturation and a significant large size of the Corpora luteal (CL) in comparison with the control group (H&E, ×400).

3.3. Effect of total alkaloids extract on embryo-toxicity and fertility

3.3.1. Pregnancy detection

Mating is indicated by the presence of sperm in the vaginal smear or the detection of a vaginal plug. In rats, the presence of sperm in a vaginal smear is a good predictor of pregnancy. The first day of gestation is the day where sperm is discovered in a vaginal smear. The fetuses may be palpated after 10 days of gestation, however palpation is more accurate after day 12. The abdominal growth is noticeable by day 13 of gestation, while mammary development and nipple enlargement can be seen on day 14.

Successful mating was confirmed by the presence of sperm in the vaginal smear (figure 27) the following morning and this day was considered as day 1 of pregnancy. The incidence rate of pregnancy was high 100% in both groups; control and mated females.

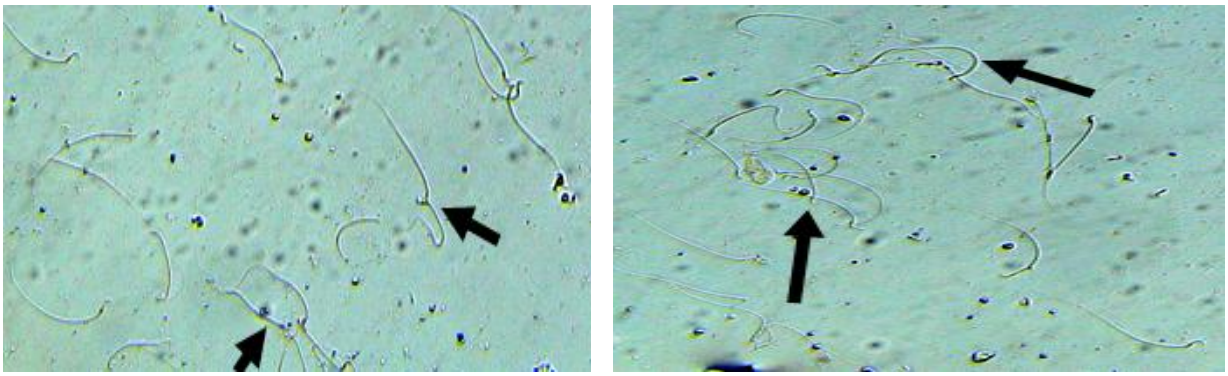

Figure 27. The presence of sperm in the vaginal smear of female rat.

3.3.2. Effect of the total extract on embryo-toxicity

3.3.2.1. Maternal toxicity, Abortion

- **Clinical Signs and Body Weight**

In maternal and developmental toxicity, confirmed pregnancy rates were 90-100% for all treated groups (table 6) and no maternal morbidity/mortality was observed in the dams of the control, treated groups for seven days, two weeks, and three weeks. On the other hand, a decrease in locomotor activity, paralysis, dyspnea, depression, and hypothermia were identified in dams in treated groups by total extract in the first two hours after the treatment,

and the signs were stayed only in the first seven days of the treatment period for all the treated groups.

Upon measuring the maternal body weight and weight gain, a significant changes were observed in all pregnant rates during pregnancy, either these changes were an increase or a decrease in treated groups of three stages of gestation (seven, two, and three weeks). In addition, there were a significant differences in maternal weight gain during pregnancy for the group treated by 1/20DL_{50} mg/kg/day of total alkaloid extract (table 6).

Table 6. Dam body weight changes of pregnant rats.

Parameters	**1/20 DL_{50} of total extract (mg/kg/day)**			
	Group control	Group treated for seven days	Group treated for two weeks	Group treated for three weeks
Maternal pregnancy status				
No. of rats mated	10	9	9	10
No. of dams	10	8	8	9
Maternal body weight (g)				
Gd 1	215±5.137	171.66±5.713	169.88±4,392	178.33±2.205
Gd 7	220±4.249	178.88±8.489*	209.44±4.522	183.88±3.977
Gd 14	224.44±5.429	189.44±5.429*	227.22±4.867*	179.44±5.234
Gd 21	233.88±5.122	196.11±5.122*	252.77±8.544*	198±6.388*

Gd: gestational day. Values are presented as means±SD. the ANOVA; Tukey. A; Includes all dams pregnant at sacrifice; *$P < 0.05$; Dunnett's test.

The results showed no significant changes in relative weights of liver, hart and brain in all experimental groups, while the relative weight of kidney in treated group for seven days and three weeks by total extract was increased significantly in comparison to the control. The relative weight of lung was significantly increased in the group treated for seven day and two weeks. Only in group treated for seven days, the spleen relative weight was significantly increased. Precisely the relative weights of ovaries were significantly changed in all the treated groups by the total extract compared to the control group (figure 28).

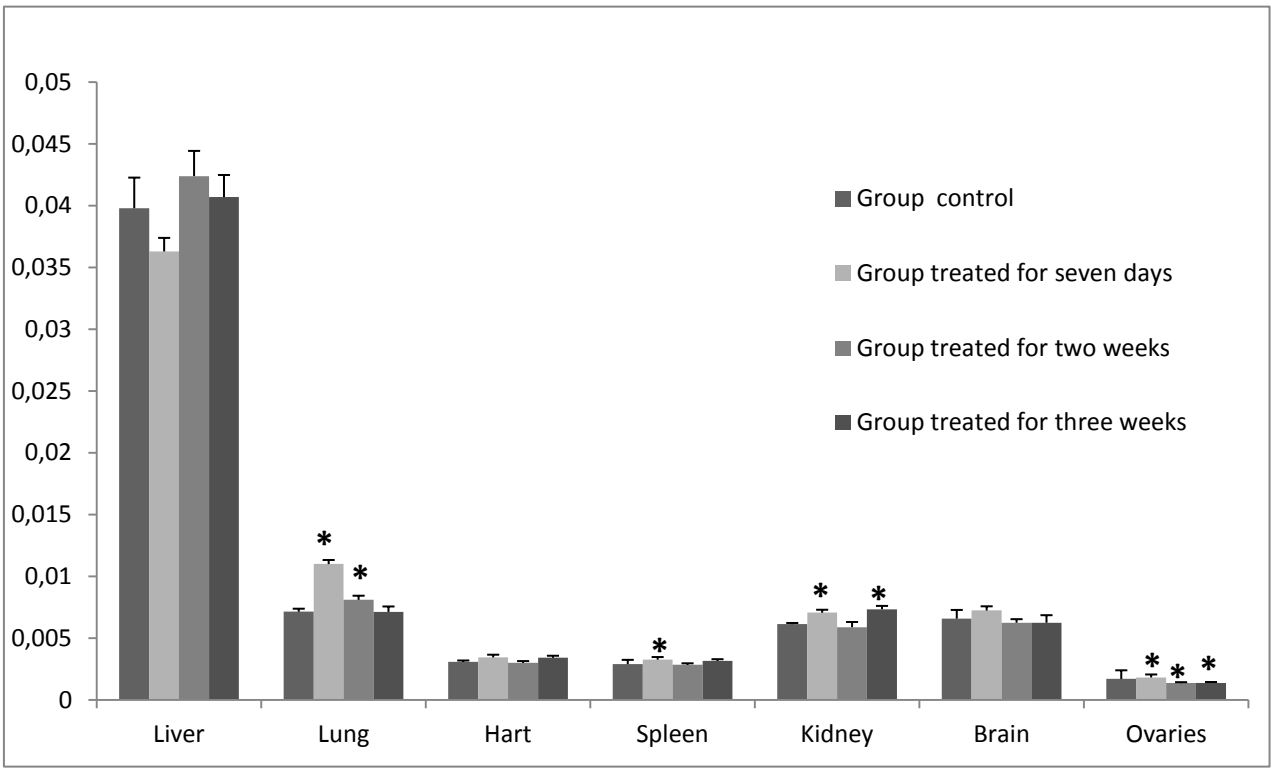

Figure 28. Relative organs weights of pregnant rats. Values are presented as the means±SEM, * significant differences at P<0.05.

- **Serum biochemical and hormonal analyses.**

To judge functional abnormalities in the dams, we analyzed serum biochemical values at the end of the gestational period. There were no statistically significant differences in the ASAT and PLA concentrations between treated groups and the control, while ALAT and gamma GT concentrations showed a significant changes only in the treated group for seven days (figure 29 and 30), in addition, a significant changes in the total and indirect bilirubin levels were observed in all treated groups in comparison to the control (figure 31). The renal parameters showed a significant change in glucose in the group treated for two weeks, blood urea nitrogen in the group treated for three weeks, and creatinine in the group treated for seven days (figure 32). The blood ionogram analysis show no significant changes in potassium (K), but there was a significant change in sodium (Na) levels in the groups treated for seven days and two weeks, while the levels of chloride (Cl) was changed significantly in the treated group for two weeks (figure 33).

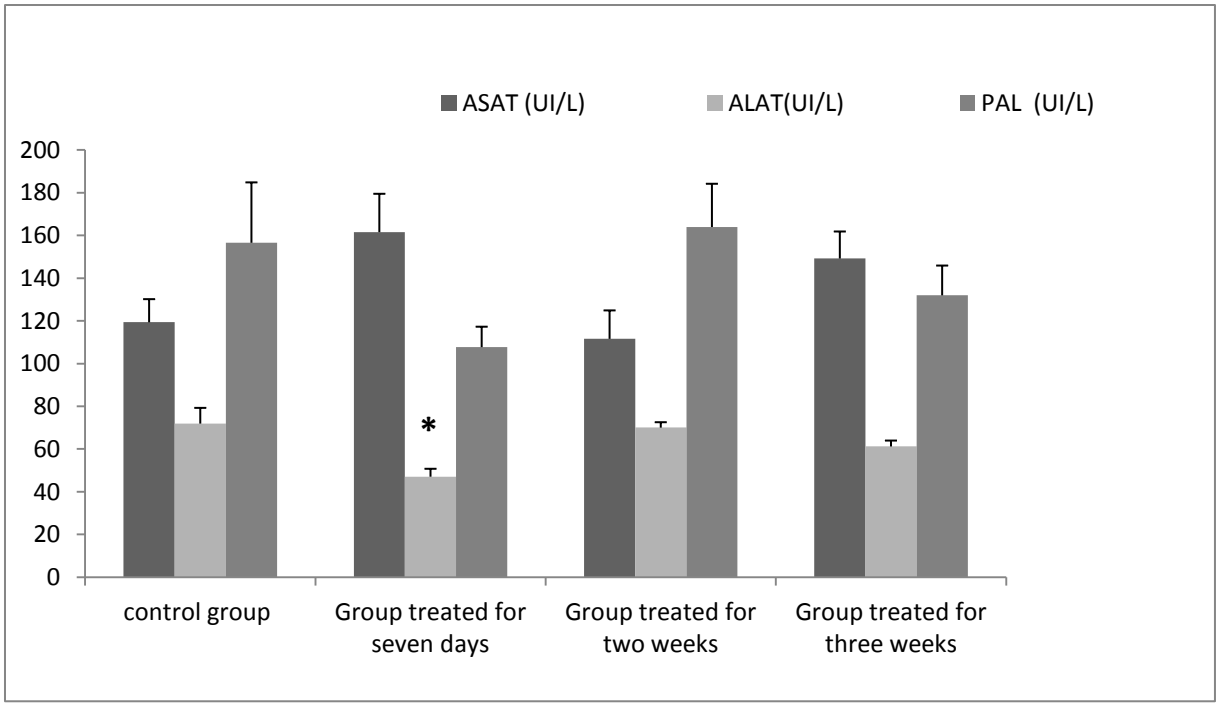

Figure 29. ASAT, ALAT, and PAL values of maternal serum for treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05 compared to the control group.

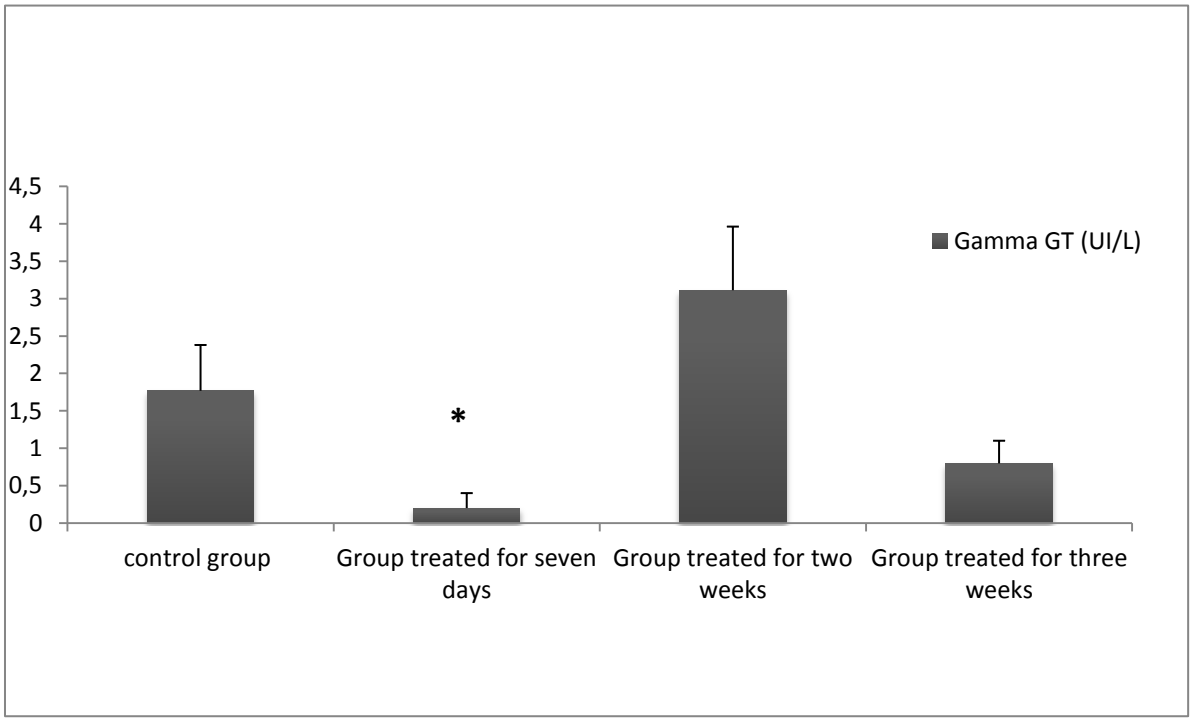

Figure 30. Maternal serum biochemical values of Gamma GT in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05 compared to the control group.

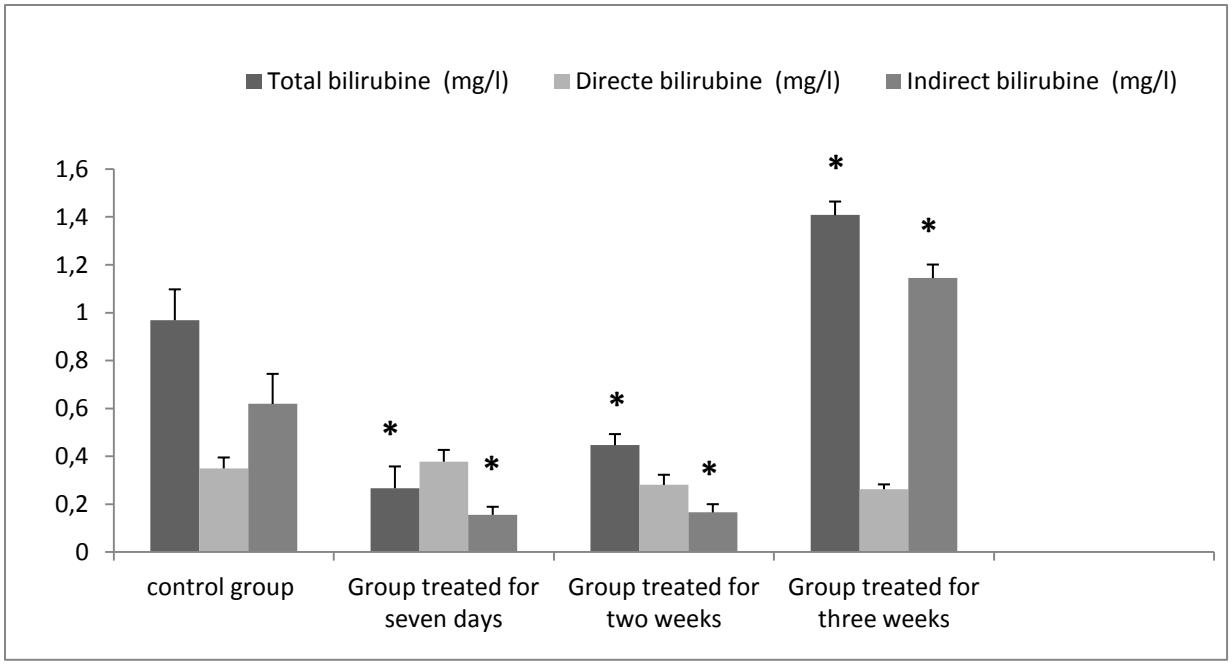

Figure 31. Total, direct and indirect bilirubin values of maternal serum in treated groups and the control. Values are presented as the means±SEM, *significant differences at P<0.05 compared to the control group.

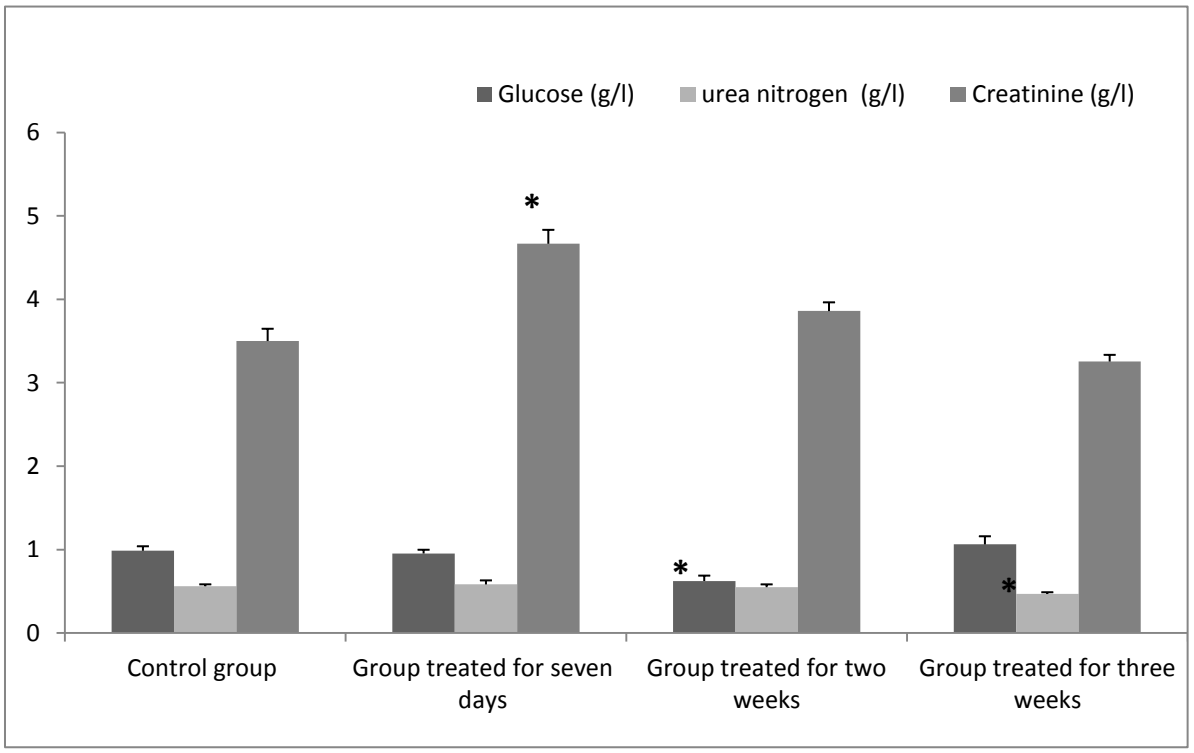

Figure 32. Glucose, blood urea nitrogen, and creatinine values of maternal serum in treated groups and the control. Values are presented as the means±SEM, *significant differences at P<0.05 compared to the control group.

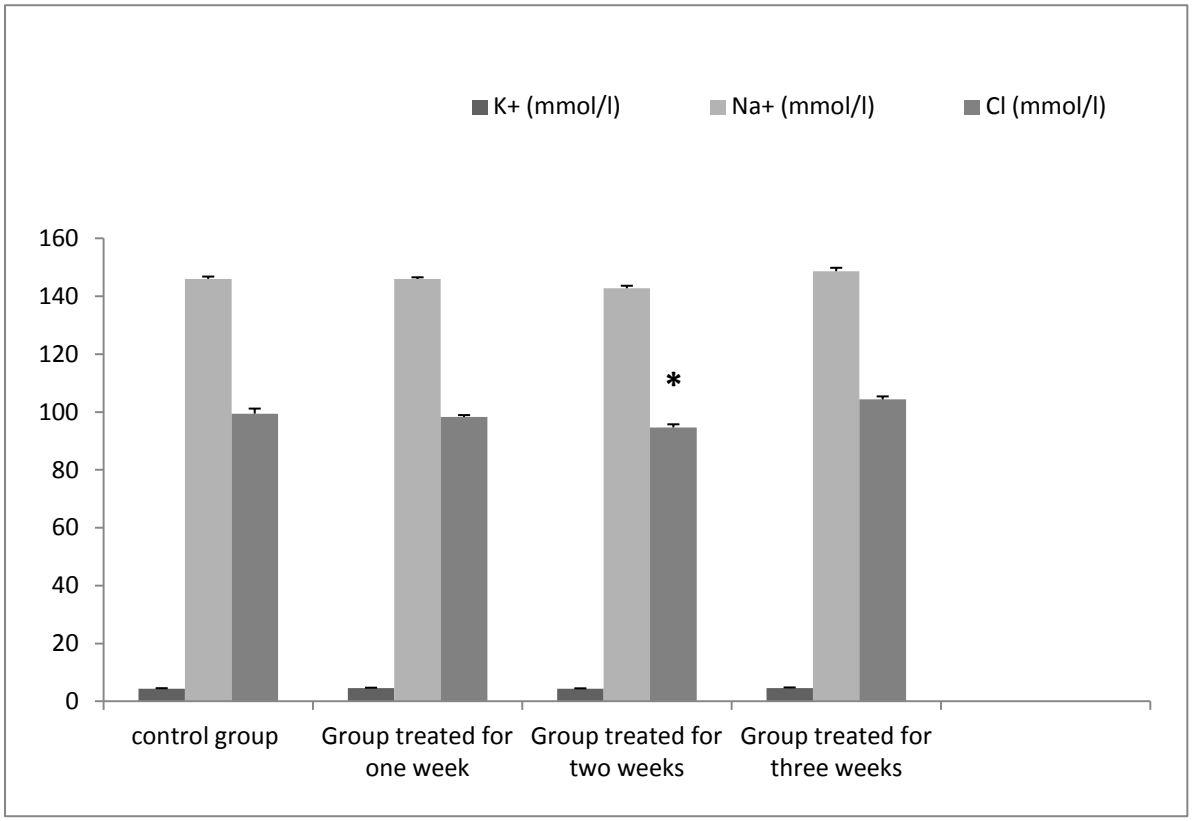

Figure 33. Blood ionogramme k^+, Na+, and Cl^- values of maternal serum in treated groups and the control. Values are presented as the means±SEM, *significant differences at $P<0.05$ compared to the control group.

The hormonal analysis showed a significant decrease in FSH levels in treated group for seven days and two weeks (figure 34). Progesterone level was decreased significantly in treated groups for seven and three weeks, and was increase significantly in treated group for two group, however the levels of Estrogen were changed significantly only in treated group for three weeks (figure 35). There was no changes in the LH levels between groups and the control (LH level was less than of 0.1 mUI/ml).

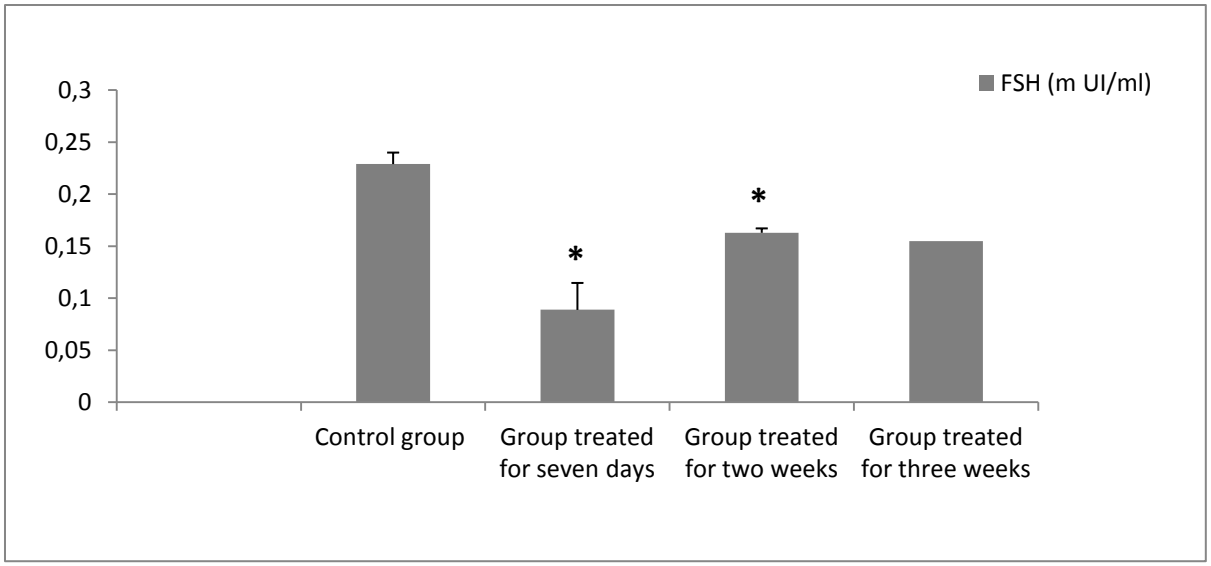

Figure 34. FSH values of maternal serum in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05 compared to the control group.

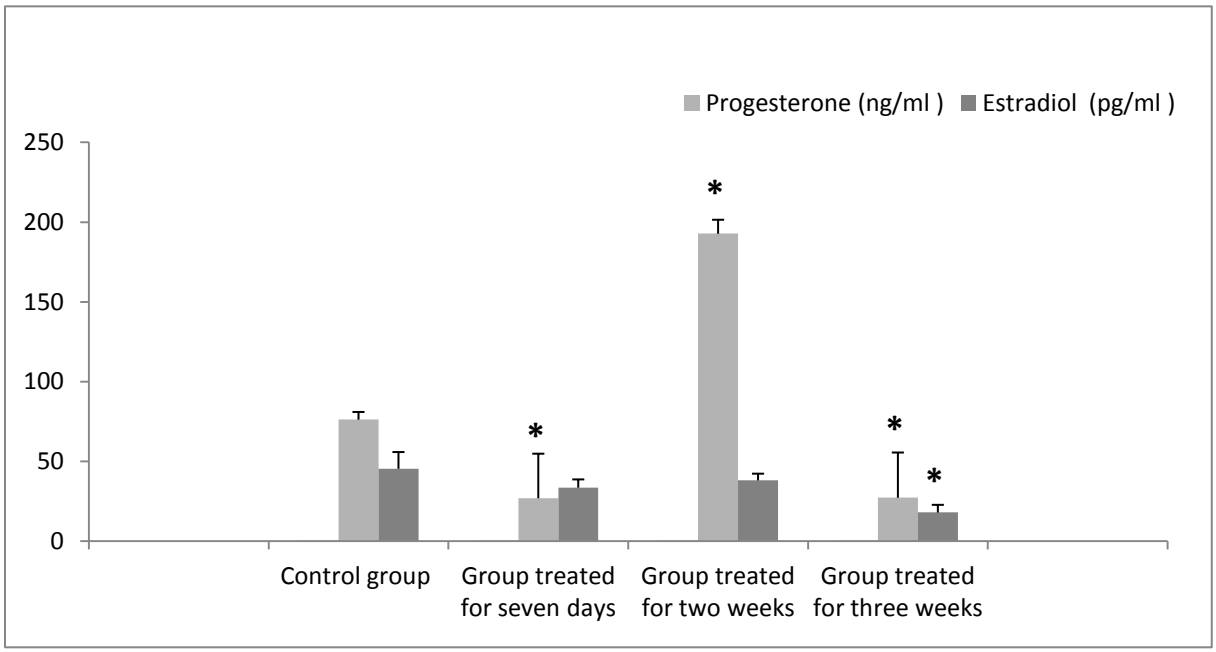

Figure 35. Progesterone and Estradiol values of maternal serum in treated groups and the control. Values are presented as the means±SEM,*significant differences at P<0.05 compared to the control group.

- **Hematological analyses**.

The blood parameters showed a significant changes between the control group and the treated groups in all the parameters as we can see in table 7.

Table 7. Effects of 1/40 DL_{50} of total extract on hematological parameters in female rats.

Parameter	RBC10*6/mm3	Hb g/dl	HCT%	MPV fl	MCV fl	MCHC g/dl	RDW	WBC10*3/mm3	Platelet
Control group	**7.004±0.314**	**12.957±0.495**	**37.514±1.48**	**53.743±0.854**	**5.929±0.115**	**34.729±0.218**	**29.257±0.934**	**5.573±0,642**	**657.574±112.54**
Treated group for seven days	**7.634±0.195**	**13.344±0.301**	**42.944±1.034***	**56.278±0.376***	**7.762±0.127***	**31.078±0.145***	**26.578±0.805***	**6.284±0.314**	**907.903±125.96***
Treated group for two weeks	**5.501±0.588***	**10.3±1.034***	**29.589±3.12***	**54.122±0.548**	**6.044±0.0818**	**35.656±0.908**	**29.733±0.685**	**5.671±0.837**	**749.111±73.209**
Treated group for three weeks	**6.831±0.184**	**12.613±0.265**	**36.825±0.929**	**53.925±0.497**	**6.188±0.0811**	**34.25±0.241**	**29.325±0.666**	**6.891±0.955**	**679.908±98.44**

RBC: red blood cell; Hb: hemoglobin; HCT%: Hematocrit; MPV: mean platelet volume; MCV: mean corpuscular volume MCHC: mean corpuscular of Hb concentration; RDW: Red Cell Distribution Width. WBC: white blood cell. Values are expressed as Mean± SEM, Significantly different between treatment group and control group (* $P<0.05$).

3.3.2.2. Embryo/fetal evaluations.

Table 8 summarizes the reproductive and developmental data for pregnant rats treated with 1/20 DL_{50} mg/kg/day total alkaloids extract on gestational days 1 through 20. It is important to mention that the results in the treated group for seven days were significant in the entire study in comparison to the control and the other treated groups. The results showed a substantial difference in entirely resorbed litters and the frequency of fetal deaths in the group treated for three-weeks. There were no significant variations in the number of corpora lutea between the treated groups and the control group; however, the fetal weight in treated group for two weeks was significant. The results showed a significant decrease in the number of implantations, an increase in pre- and post-implantation loss rates, no developed fetus with only implantation sites in both uterine horns of all treated dams. As a main result in the materno-toxicity, the total alkaloid extract had an abortive effect according to the significant deference of corpora lutea and the number of fetuses.

Table 8. Dam Cesarean section observations and fetal weights

Parameters	**1/20 DL_{50} of total alkaloid (mg/kg/day)**			
	Group control	**Group treated for seven days**	**Group treated for two weeks**	**Group treated for three weeks**
Maternal pregnancy status				
No. corpora lutea/dam	11.222±0.364	11.00±0.840	11.333±0.687	11.778±0.619
Total implant sites				
No. implantation sites/dam	8.5±0.719	3.00±0.00*	9.625±0.498	9.40±0.872
Pre-implantation loss (%)[A]	24.25	72.72*	15.07	20.19
Post-implantation loss (%)[B]	13.76	100*	14.28	19.14
No. resorptions/dam	0.778±0.434	0.429±0.429*	1.222±0.401	4.333±1.453*
No. fetal life/dam	7.33±0.989	00±00*	8.25±0.701	7.6±0.872
No. fetal death/dam	0.778±0.434	0.429±0.429*	1.222±0.401	4.333±1.453*
Fetal weight (g)	1.074±0.167	-	1.555±0.054*	1.76±0.442

A: Values are presented as means ± SD. *significant differences at $p<0.05$. **B**: Pre-implantation loss (%) = [(No. of corpora lutea - No. of implantation sites)/ No. of corpora lutea] ×100. **C:** Post-implantation loss (%) = [(No. of implantation sites - No. of live embryos)/ No. of implantation sites] ×100.

3.4. Effect of the total alkaloids extract on fertility activity.

3.4.1. Maternal toxicity and abortion

- **Clinical Signs and Body Weight.**

No maternal morbidity and mortality were observed in the dams of the control and pregnant females, with a normal behavior during the gestational period. The maternal body weight and weight gain statistically were significant in all pregnant rates, as it is shown in table 9. The absolute weight was decreased only in first week. In addition, the maternal weight gain was decreased significantly in treated groups for seven days and two weeks.

Table 9. Body weight changes of pregnant rats.

Parameter	Group control	Mated females with treated males
Maternal pregnancy status		
No. of rats mated	10	9
No. of dams	10	9
Maternal body weight (g)		
Gd 1	215±5.137	184±2.082
Gd 7	220±4.249	204±2.867*
Gd 14	224.44±5.429	218,5±6.283
Gd 21	233.88±5.122	225±6.236

Gd: gestational day. A values are presented as means ± SEM (g). * Significant difference at $p<0.05$.

The results showed no significant changes for the relative weights of the liver, kidney, heart, brain, spleen, and ovaries, while the relative weight of lungs in the females mated with treated males was increased significantly in comparison to the control (figure 36).

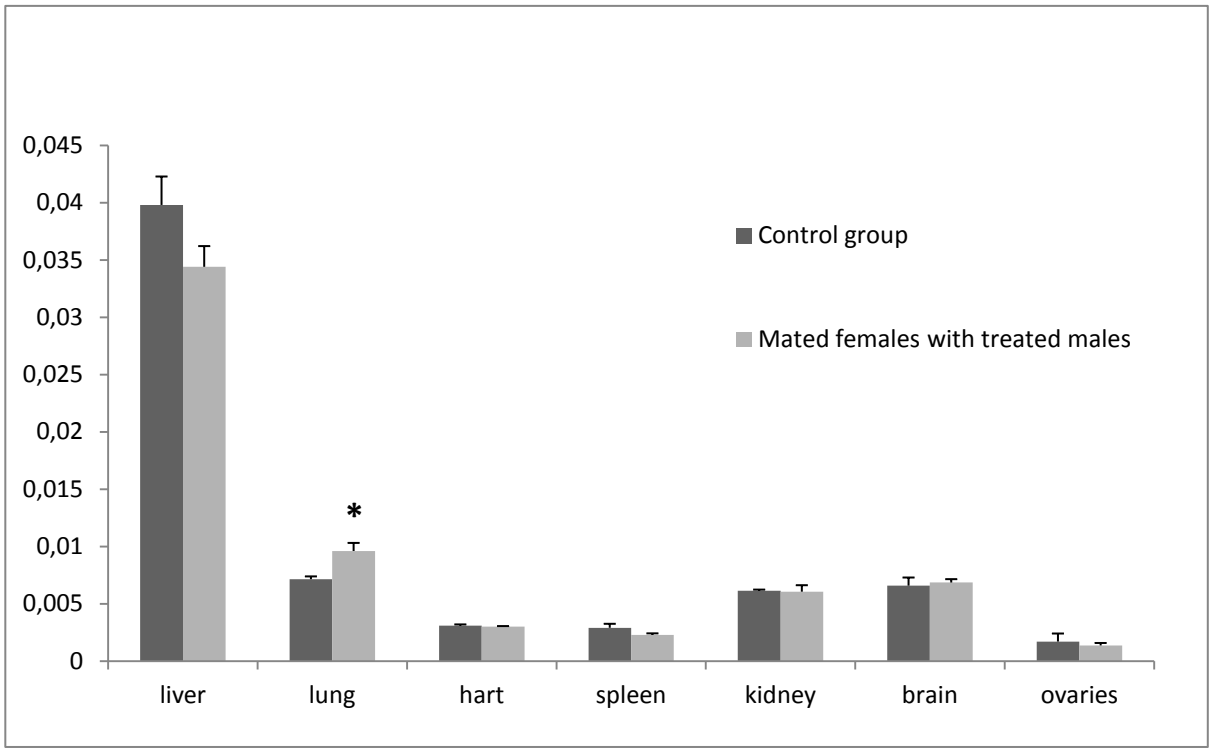

Figure 36. Maternal relative organs weights of treated groups. Values are presented as the means±SEM, * significant differences at P<0.05.

- **Serum biochemical and hormonal analysis.**

Serum biochemical analysis showed no statistically significant differences for ASAT, ALAT, and PLA concentrations between groups and the control, while gammaGT concentrations was increased significantly (figure 37 and 38). A significant changes in total and indirect bilirubin levels were observed for mated females with treated males in comparison to the control (figure 39). The renal parameters showed a significant change for creatinine levels and no changes for glucose, and blood urea nitrogen (figure 40). The blood ionogramme analysis showed no significant changes in potassium (K), but the levels of sodium and chlorure were changed significantly (figure 41).

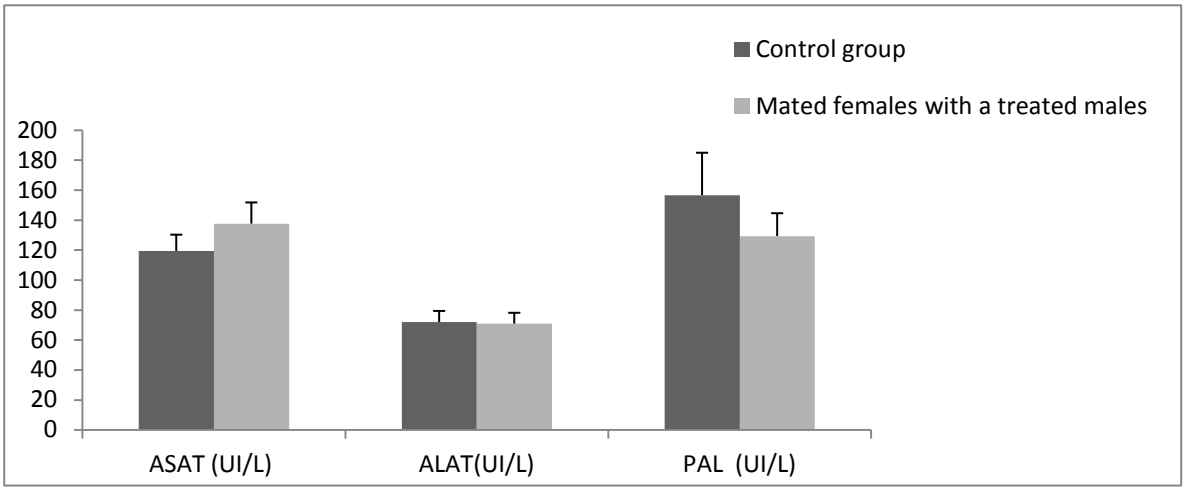

Figure 37. Maternal serum biochemical values of ASAT, ALAT and PAL in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05.

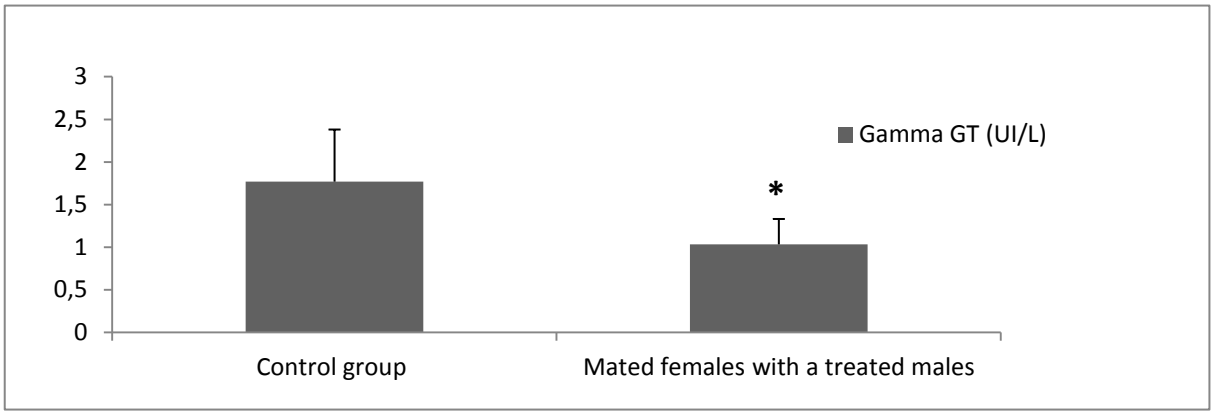

Figure 38. Maternal serum biochemical values of Gamma GT in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05.

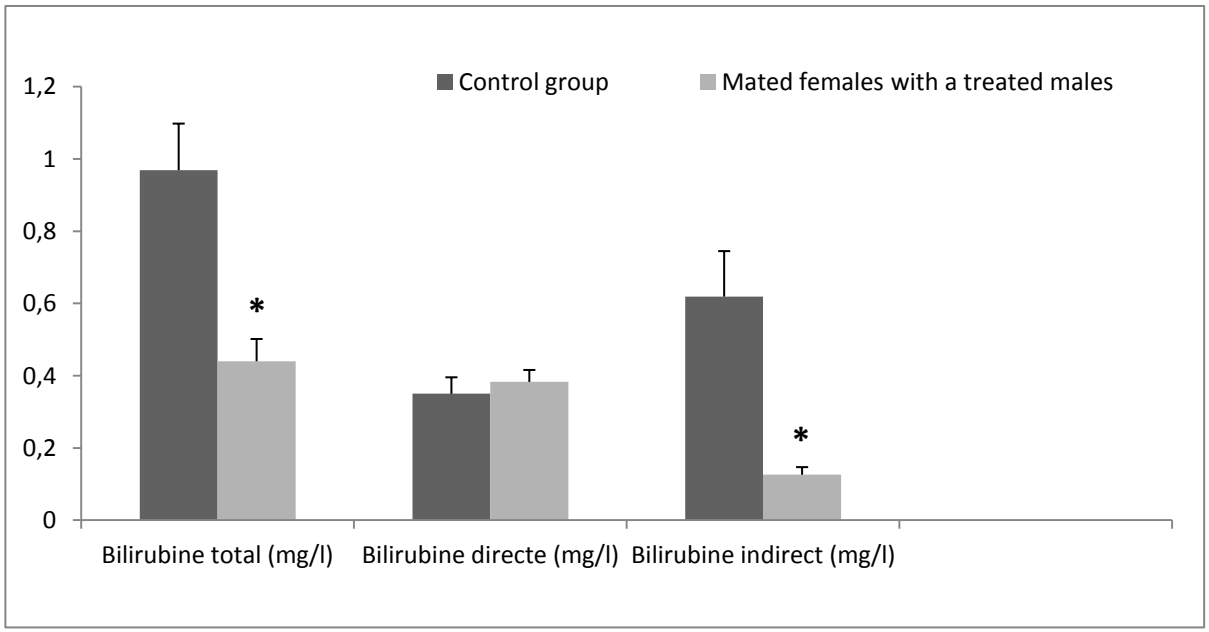

Figure 39. Maternal serum biochemical values of total, direct and indirect bilirubine in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05.

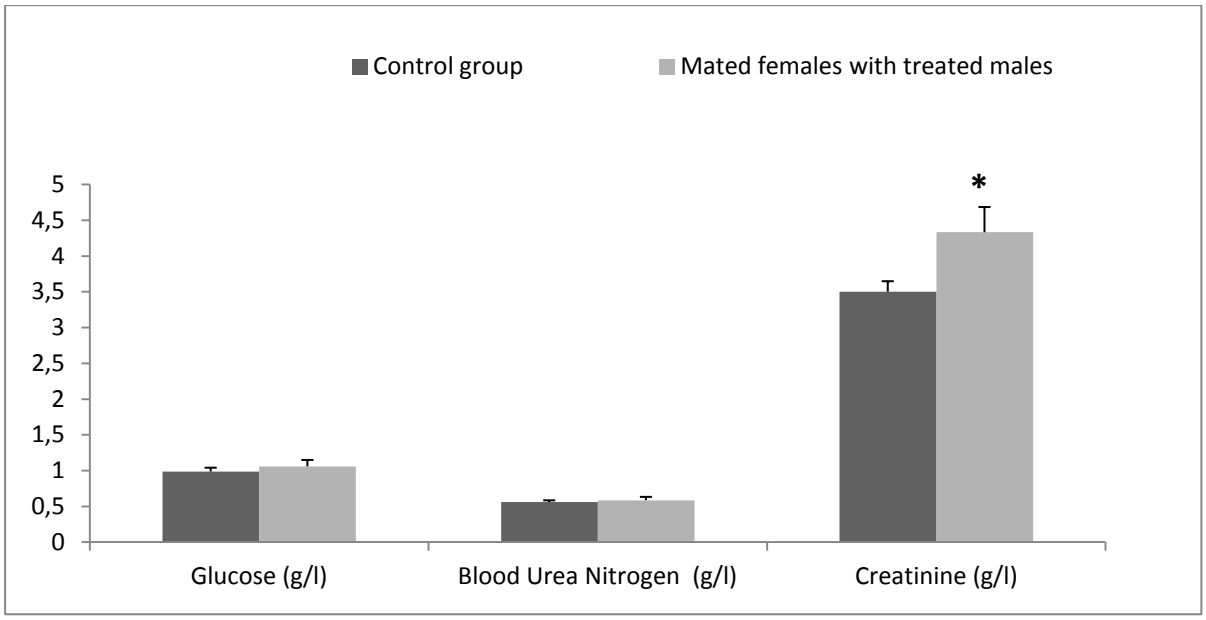

Figure 40. Maternal serum biochemical values of glucose, blood urea nitrogen and creatinine in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05.

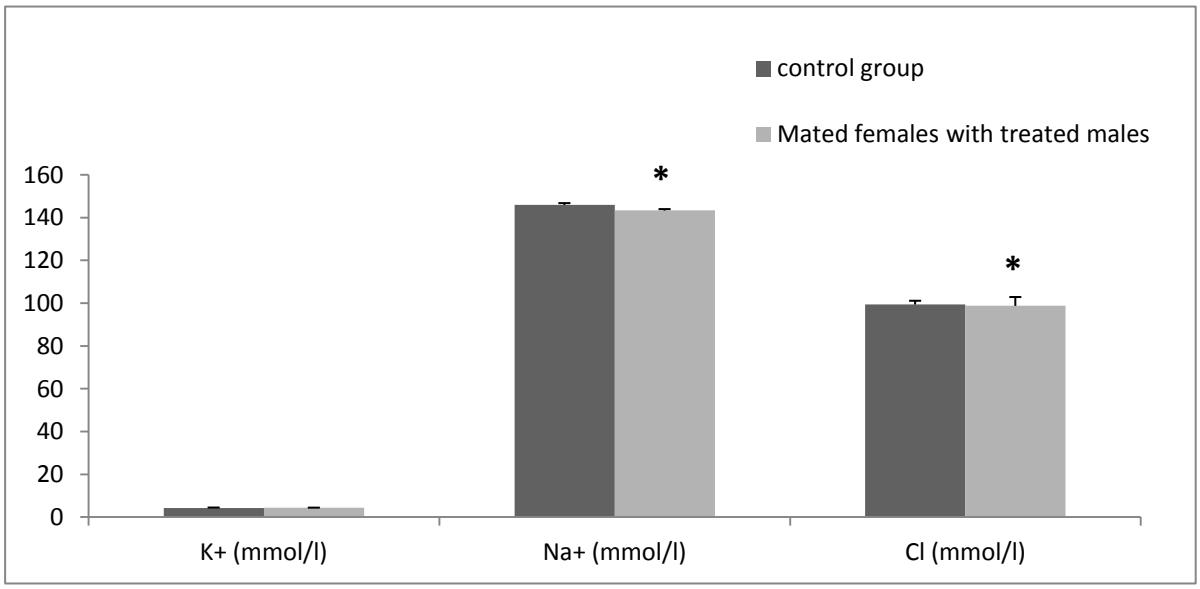

Figure 41. Maternal serum biochemical values of blood ionogramme k^{+}, Na+ and Cl^{-} in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05.

The hormonal analysis showed a significant changes in FSH and no changes in Progesterone, and Estrogen levels as shown in figure 42. Additionally, LH level was less than of 0.1 mUI/ml for mated females group with treated males and the control.

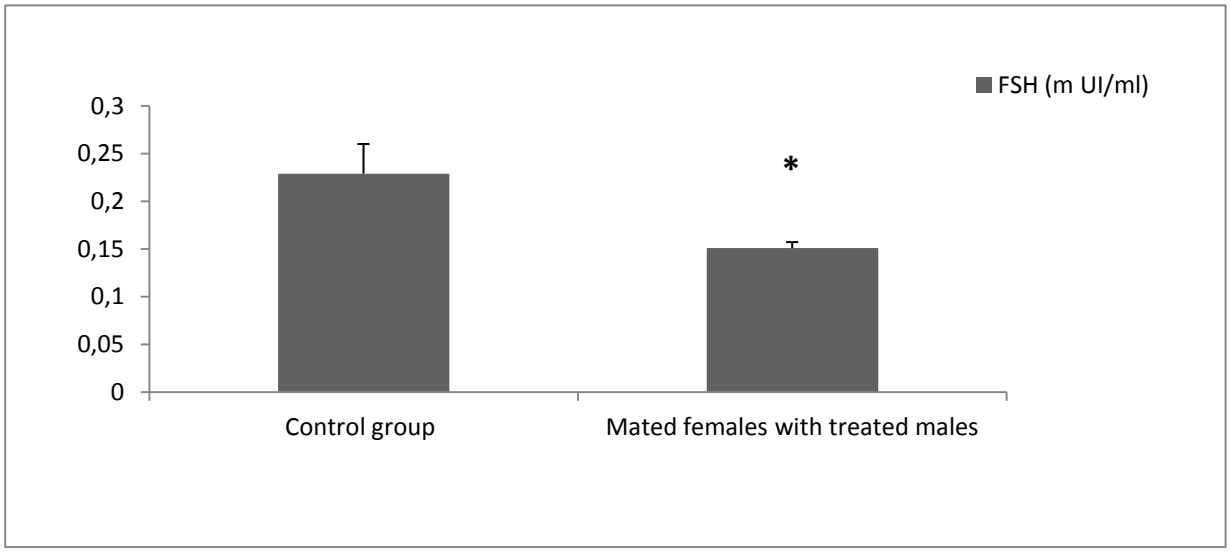

Figure 42. Maternal hormonal values of FSH in treated groups and the control. Values are presented as the means±SEM, * significant differences at P<0.05.

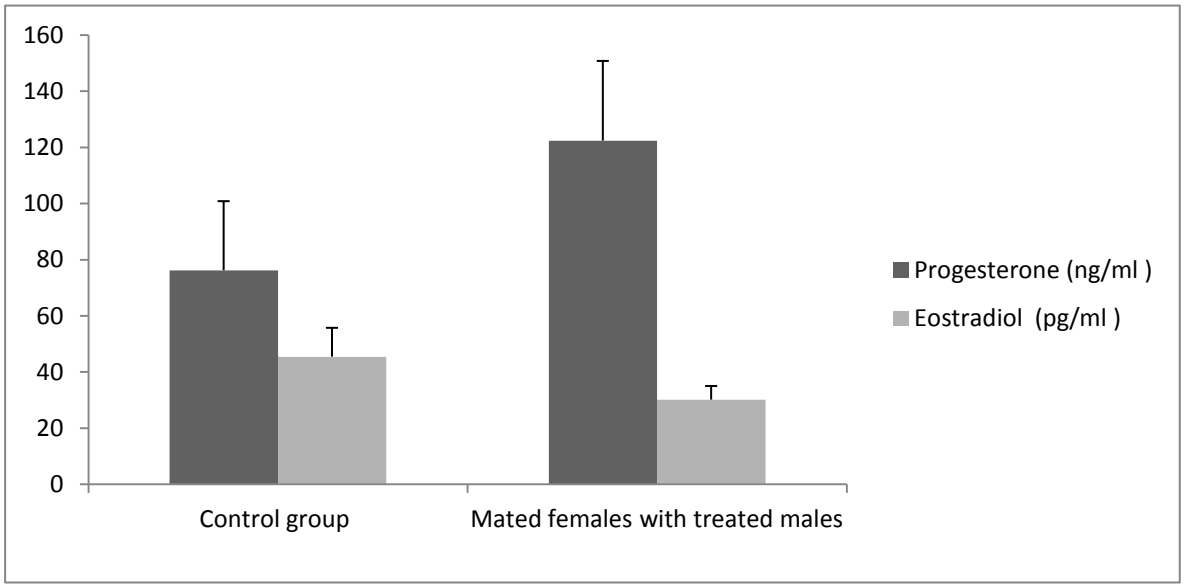

Figure 43. Maternal hormonal values of Progesterone and Estradiol in treated groups and the control. Values are presented as the means±SEM,*significant differences at P<0.05.

- **Hematological analyses.**

The blood parameters showed no significant changes between the control group and the mated females with males as we can see in table 10.

Table 10. Blood parameters changes in female rats mated by treated males.

Parameter	RBC10*^{6}/mm3	Hb g/dl	HCT%	MPV fl	MCV fl	CCMH g/dl	RDW	WBC10*3/mm3	Platelet
Control group	7.004±0.314	12.957±0.495	37.514±1.48	5.929±0.115	53.743±0.854	34.729±0.218	29.257±0.934	5.573±0.642	657.574±112.547
Mated females with a treated males	5.628±0.906	10.5±1.634	30±4.808	6.143±0.236	46.688±6.688	35.229±0.529	28.571±0.421	5.286±1.005	574.5±137.668

RBC: red blood cell; Hb: hemoglobin; HCT%: Hematocrit; MPV: mean platelet volume; MCV: mean corpuscular volume; MCHC: mean corpuscular of Hb concentration; RDW: Red Cell Distribution Width; WBC: white blood cell. Values are expressed as Mean± SEM, Significantly different between treatment group and control group (* $P<0.05$).

3.4.2. Embryo/fetal evaluations.

The fertility study for pregnant rats mated by treated males revealed no difference in the number of pregnant females, but a significant difference in corpora lutea number, implantation sites, pre- and post-implantation loss rates, resorption, fetal deaths, and fetal body weights were observed, as it is shown in the table11.

Table 11: Caesarean section data of pregnant rats and fetal weights. Values are presented as means± SEM.

Parameter	Group control	Group of mated females with treated males
Maternal pregnancy status		
No. corpora lutea	11.222±0.364	12±0.866*
No. implantation sites	8.5±0.719	9.4±0.371*
Pre-implantation loss (%)[A]	24.25	21.66*
Post-implantation loss (%)[B]	13.76	25.53*
No. fetal life	7.33±0.989	7.00±0.707
No. fetal death	0.778±0.434	2.5±1.041*
Fetal weight (g)	1.074±0.167	1.293±0.093*

A: Pre-implantation loss (%) = [(No. of corpora lutea - No. of implantation sites)/ No. of corpora lutea] ×100. B**:** Post-implantation loss (%) = [(No. of implantation sites - No. of live embryos)/ No. of implantation sites] ×100.

3.5. Histological study for embryology and the test of fertility

- **Liver**

The main lesions in histological sections of liver were sinusoidal inflammation and lympho-plasmocyte inflammation around the centrilobular vein with regular hepatic parenchyma (D,F,J), necrosis (C,F,I,J)and blood congestion (D,E,F), the centrilobular of the vain was full of red blood cells (G,H), and granuloma inflammation well-limited by lymphocytes in the sections of embryology and the fertility test (figure 44).

- **Kidney**

The kidney sections showed a typical renal parenchyma with usual glomerular morphology, congestive follicles surrounded by endothelial cells, their lumina filled with red blood cells. In addition, kidney's section indicates the existence of lympho-plasmocyte inflammation areas (figure 45).

- **Ovaries**

Histological sections of the ovaries showed the presence of various stages of follicular development and corpora lutea. A significant number of secondary follicles and developed corpora luteal with a significant large size in comparison with control ovaries (figure 46).

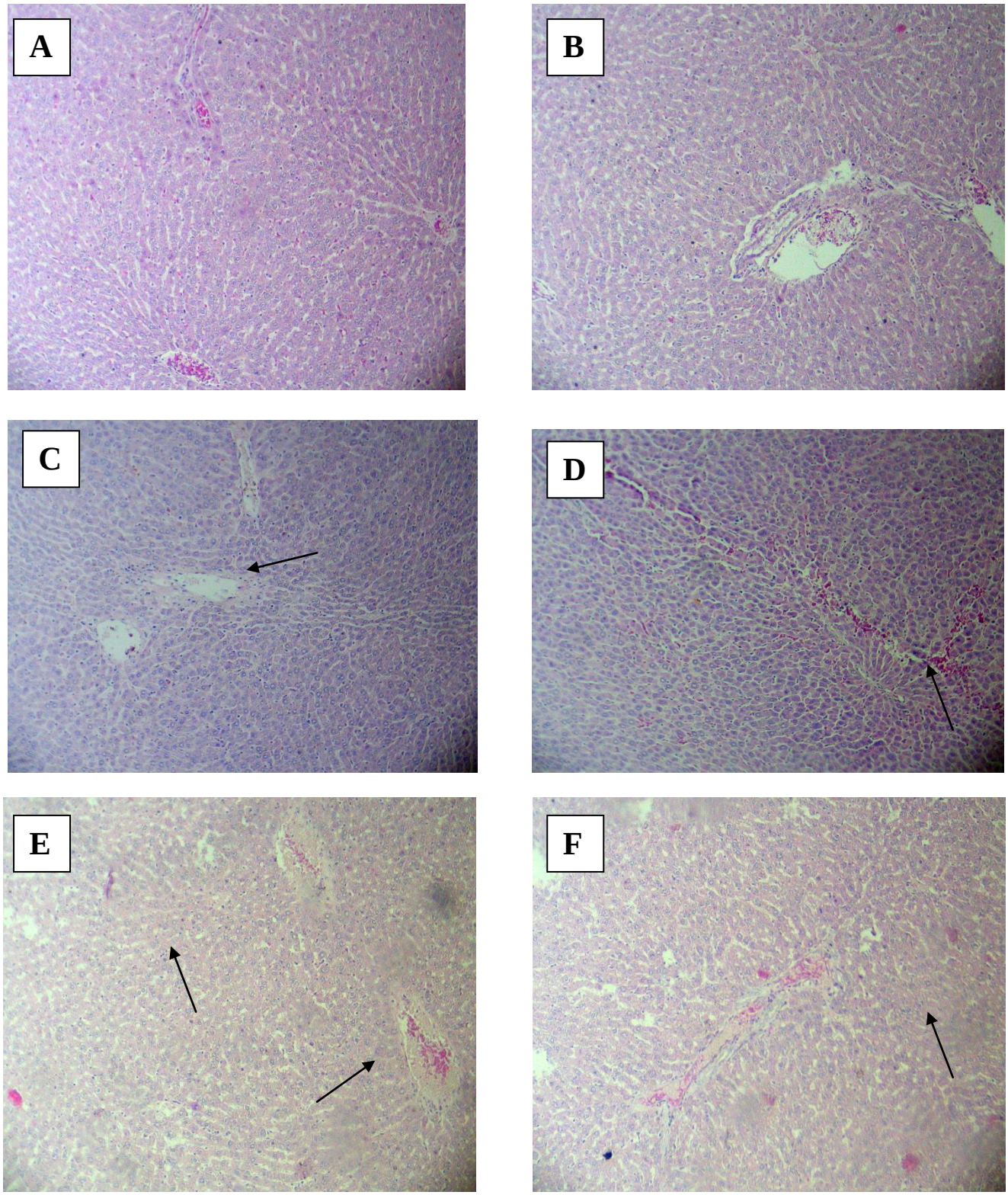

Figure 44. Histopathological sections of liver; Control (A,B); treated group for seven days (C, D), two weeks (E, F), three weeks (G,H), and mated females with treated males (H&E, ×100).

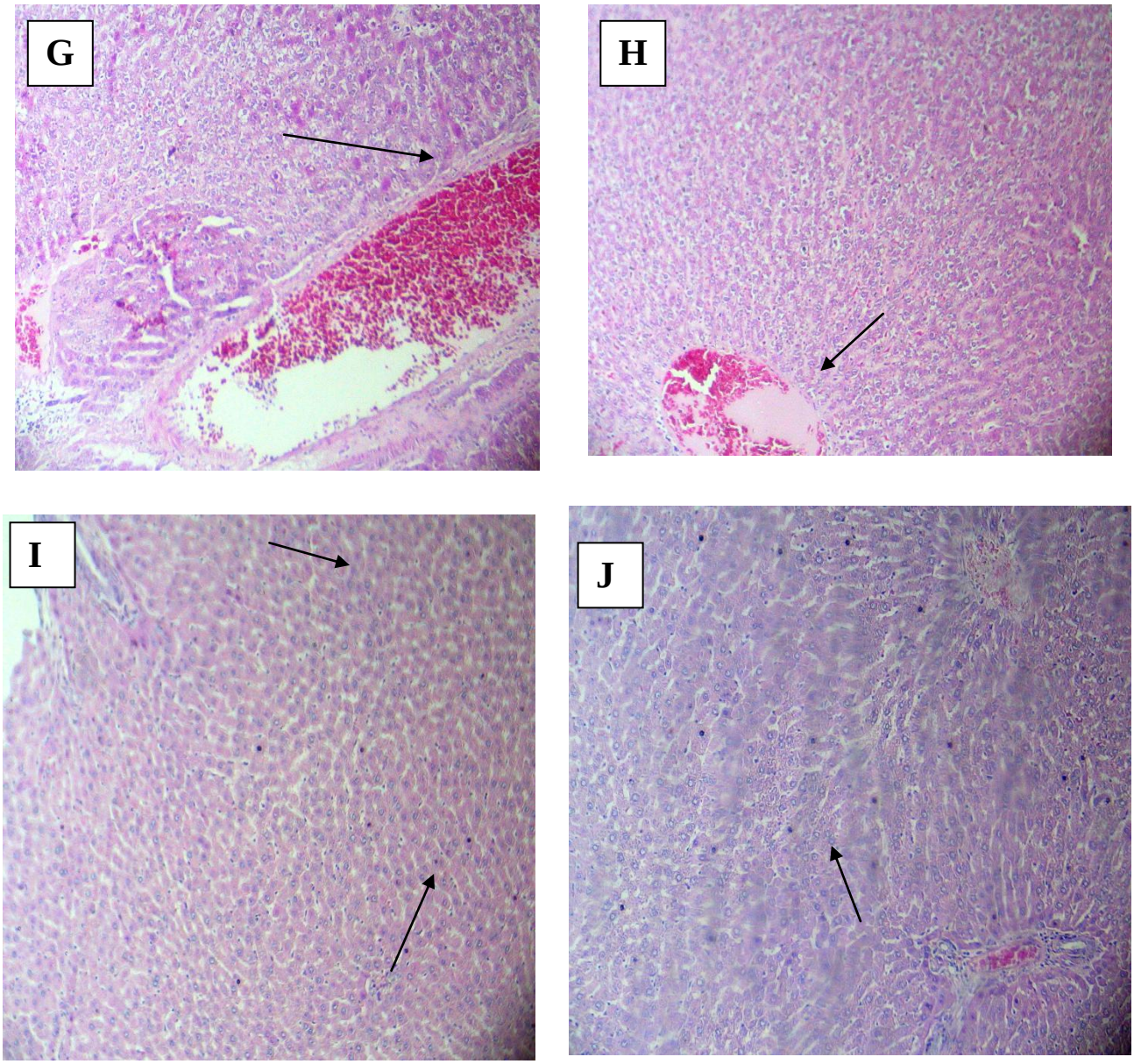

Figure 45. Histopathological sections of liver; Control (A,B); treated group for seven days (C, D), two weeks (E, F), three weeks (G,H), and mated females with treated males (H&E, ×100).

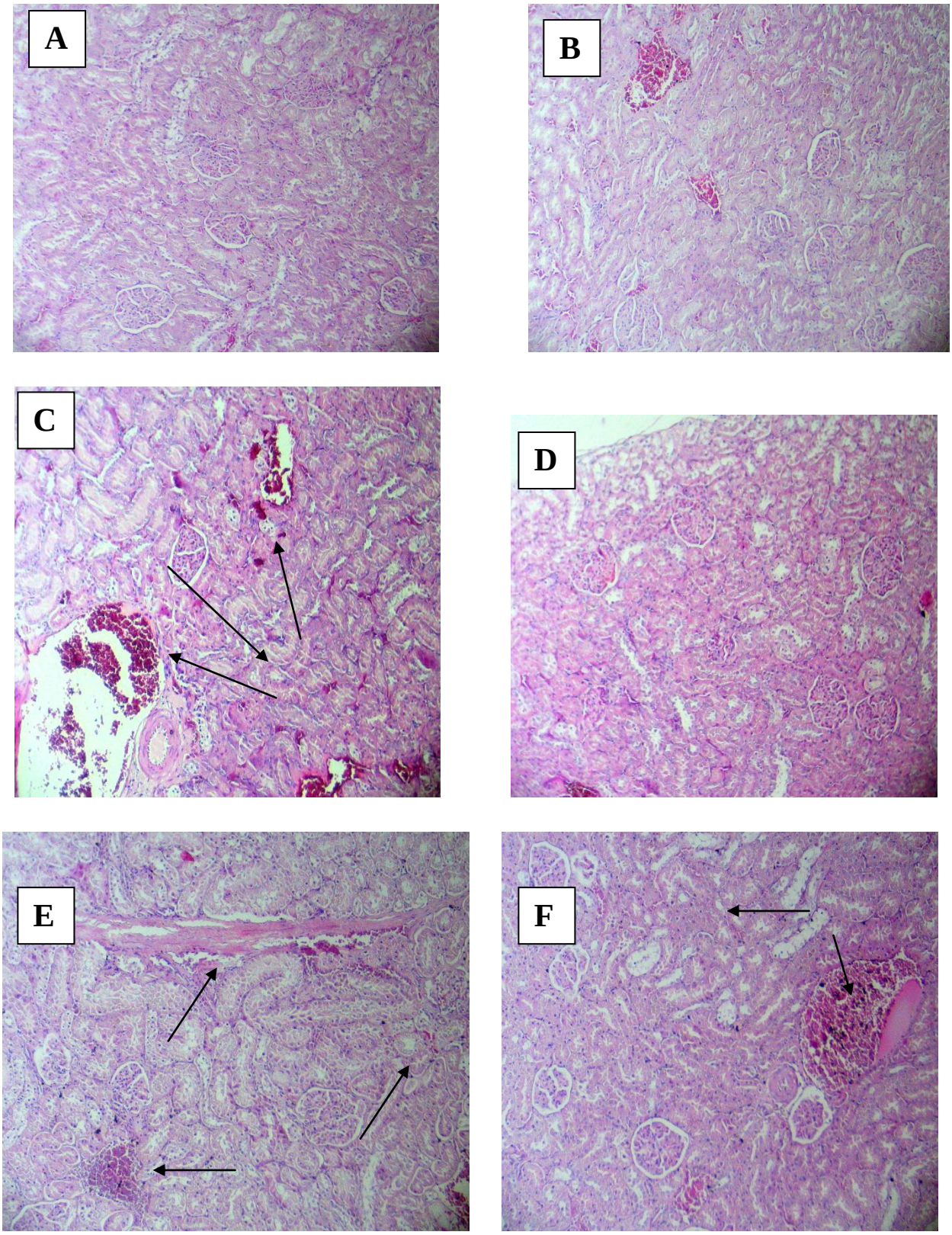

Figure 46. Histopathological sections of the kidney in the experimental groups: Control (A,B); treated group for seven days (C, D), two weeks (E, F), and tree weeks (G,H) (H&E, ×100).

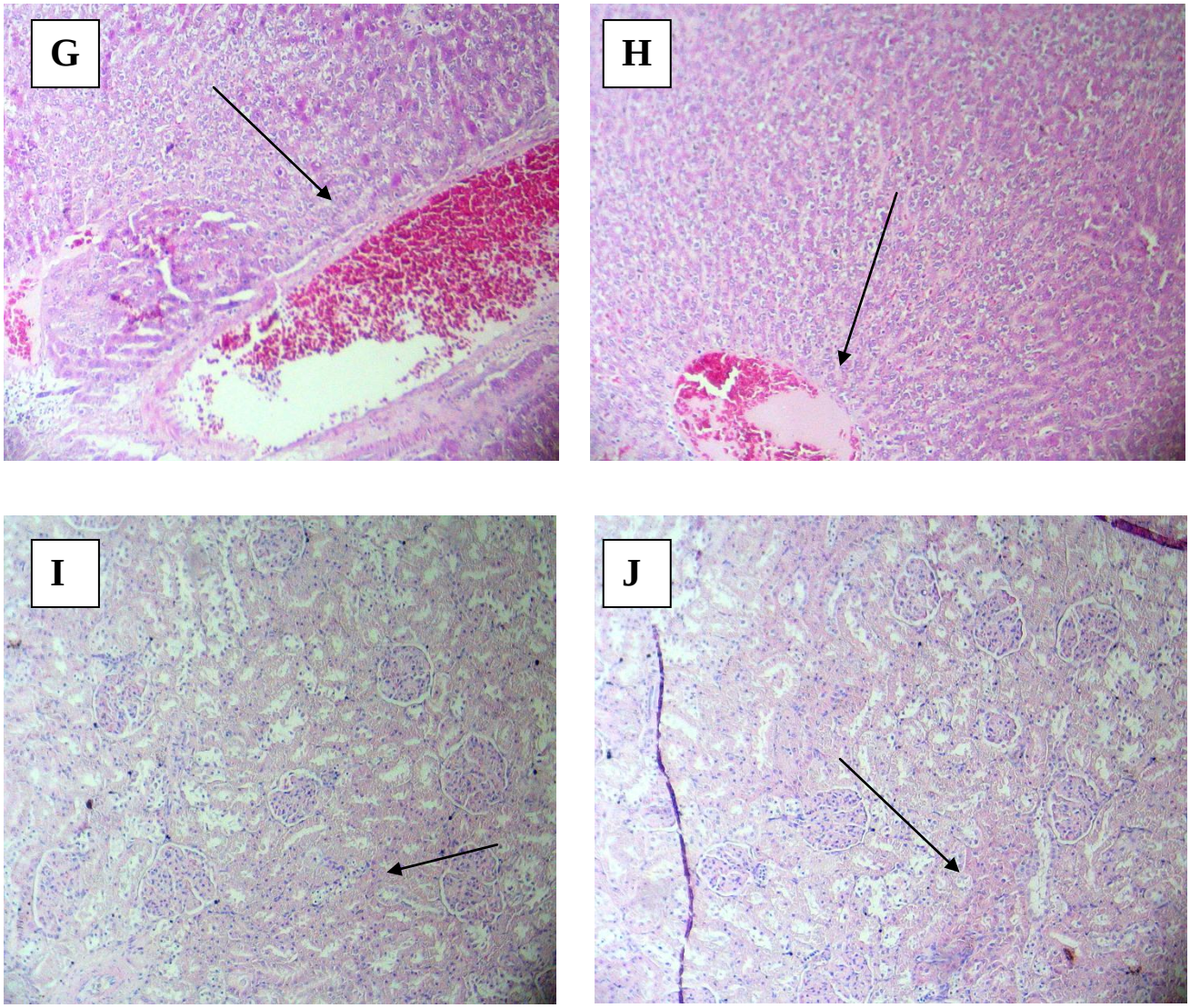

Figure 25. Histopathological sections of the kidney in the experimental groups: Control (A,B); treated group for seven days (C, D), two weeks (E, F), three weeks (G,H), and), and mated females with treated males (H&E, ×100).

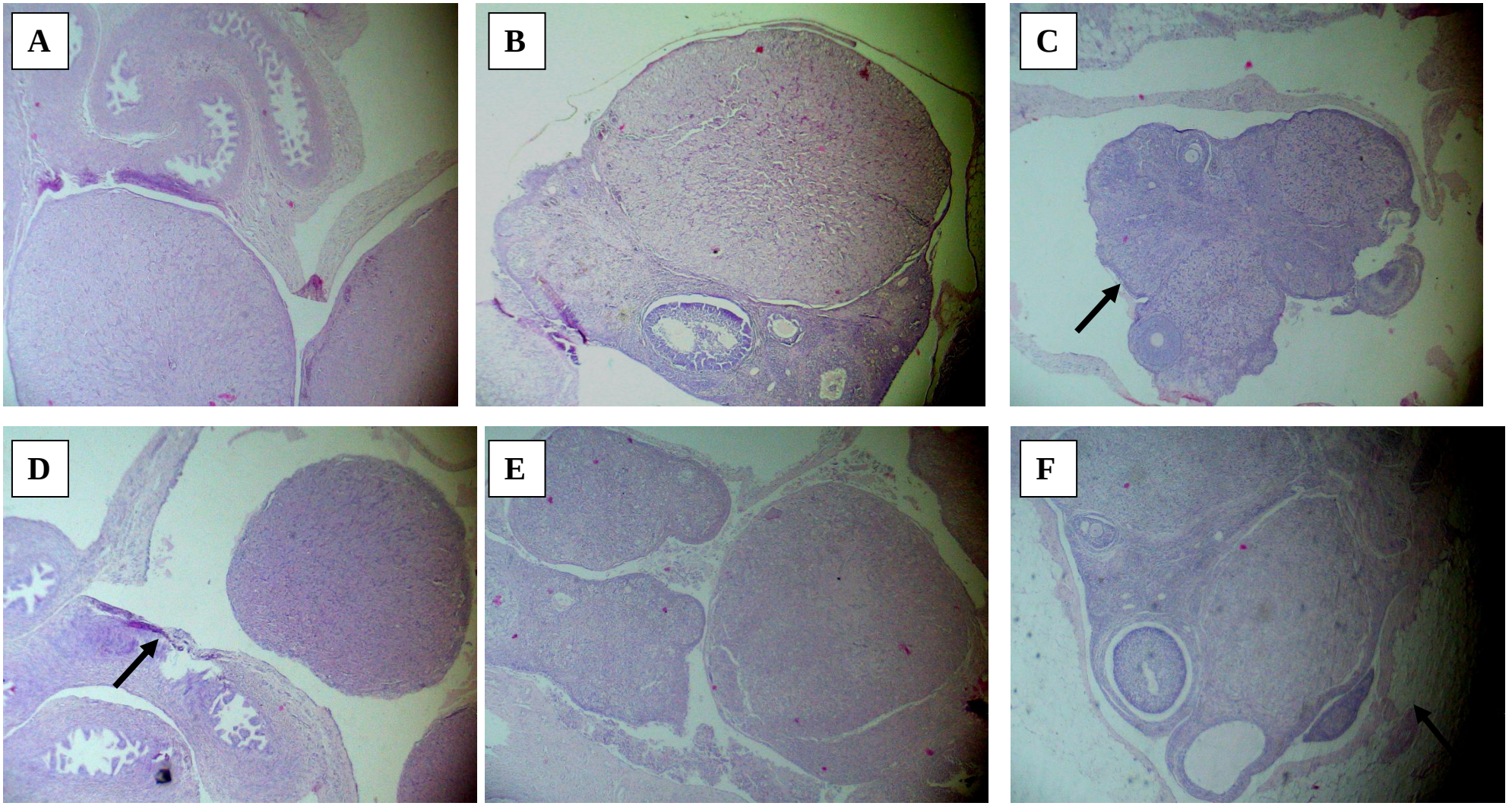

Figure 48. Histopathological sections of the ovaries in the experimental groups: Control (A,B); treated group for seven days (C, D), two weeks (E, F), three weeks (G,H), and), and mated females with treated males . the sections show the morphological aspect of the ovaries with huge number of Corpora luteal (CL) and follicles (F) in different stages of maturation and a significant large size of the Corpora luteal (CL) in comparison with the control group(H&E, ×100).

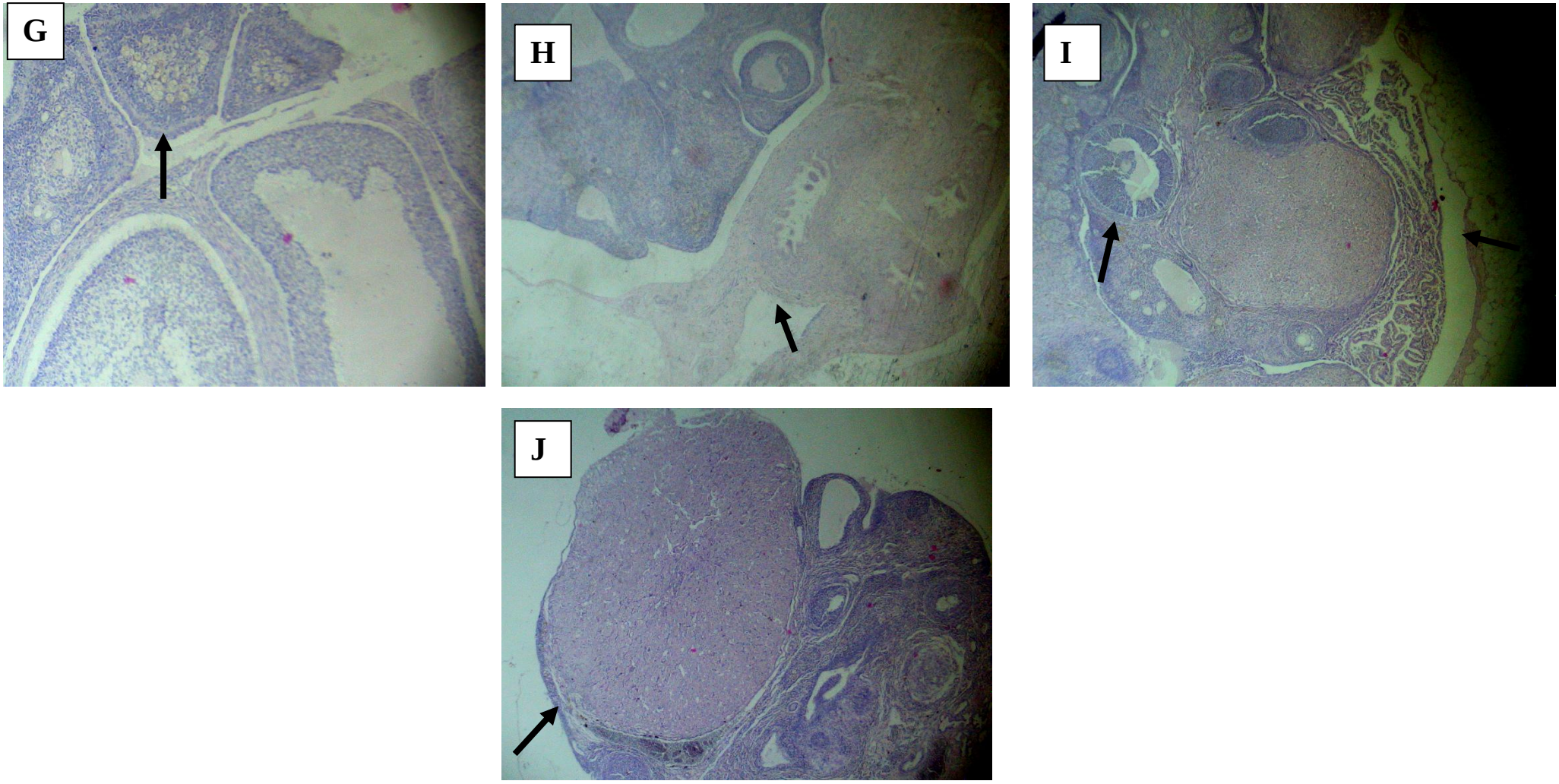

Figure 49. Histopathological sections of the ovaries in the experimental groups: Control (A,B); treated group for seven days (C, D), two weeks (E, F), and three weeks (G,H), and), and mated females with treated males. the sections show the morphological aspect of the ovaries with huge number of Corpora luteal (CL) and follicles (F) in different stages of maturation and a significant large size of the Corpora luteal (CL) in comparison with the control group(H&E, ×100).

FOURTH CHAPTER:

DISCUSSION

P. harmala is identified by descriptive data of its morphological characteristics according to Chopra et *al* (1960), and Bruneton (1999). It is known as "harmel", and it is one of the most used medicinal herbs for ritual, protective and therapeutic purposes (Bellakhdar, 1997). The seeds mainly have long been used as narcotics, anthelmintics, antispasmodics, and used against rheumatism and asthma (Bellakhdar, 1997). The plant grows naturally in different regions in Algeria. All herbalists sell the plant, and people use it for many therapeutic purposes without know a risk of overdose, and many incidents of poisoning in animals and humans have previously been described (Mahmoudian et *al*., 2002). *P. harmala* includes β-carboline alkaloids, such as harmine, harmaline, harmol, and harmalol, as well as quinazolines, which are responsible for the toxicological and pharmacological effects of the plant (Kashimov et *al*., 1971; Herraiz et *al*., 2010).

In this study, the total alkaloids was extracted from *P. harmala* seeds using a liquid-liquid extraction, which resulted in a brick red extract with a yield of 1.82g. This yield is consistent with that reported by Mahdeb et *al*. (2020) and Guergour et *al.* (2017) on the same species. The qualitative TLC analysis of the total alkaloids extract of *P. harmala* seeds revealed the presence of numerous spots after Dragendorff's reagent revelation. The presence of four spots that most likely corresponded to β-carboline alkaloids (harmine, harmaline, harmalol, harmol, and harman, tetrahydroharmine), but may be the two of these spots are harmine and harmaline as a majority alkaloids in the extract according to many others such as Mahmoudian et *al*., (2002). In addition, these results are compatible with those of Herraiz et *al*. (2010) who reported that harmine and harmaline accumulate in dry seeds at 4.3–5.6%, respectively.

In 1927, DL_{50} test was created to standardize biologically dangerous drugs. It was incorporated into the routine toxicological protocol for other classes of chemical compounds, and it is now a part of almost all governmental guidelines governing the toxicological testing of chemicals. DL_{50} value, which is defined as the statistically determined dose that, when provided in an acute toxicity test, is expected to kill 50% of the treated animals in a particular period, and it is currently used to classify compounds (Allouni et *al*., 2017). In addition, DL_{50} is useful for predicting the lethal dose in humans as well as the symptomatology of poisoning following acute overdosing. The lethal doses of total alkaloids extract from seeds of *P. harmala* determined in Wistar rats in both sexes were 164.43 mg/kg and 159.95 mg/kg for males and females respectively, and according to the values obtained and the classification of

Hodge and Sterner chemical substances with an DL_{50} between 50 and 500 mg/kg body weight determined is considered as a moderately toxic product (Lu, 1992).

P. harmala is an alternative medicine, traditionally used for managing many diseases (Bruneton, 1999). The assumption that herbal preparations/remedies are safe and effective has influenced the indiscriminate consumption of such remedies, where these remedies can be administered for a long period of time without considering the dose or concentration that will bring about toxic side effects (Bouzidi et *al*., 2011). Many studies have shown the toxic effect and pharmacologic uses of *P. harmala* seeds as the main part of the plant because of its richness on β-carboline alkaloids such as harmaline, harmine, and harman as bio-affective molecules and their potential effects (Litchfield and Wilcoxon, 1949; Hamden et *al*., 2008; Owen et *al*., 2000). Based on the previous information, we support our current study to evaluate the chronic toxicity of *P. harmala* in an animal model. We suggest that *P. harmala* can affect the reproductive system and the oogenesis in female rats, with evaluate the general chronic toxicity of total alkaloids extract, throw hormonal, biochemical, and histo-pathological data. Daily intraperitoneal administration of total alkaloid extract with a dose of 1/40 of DL_{50} did not show any obvious clinical abnormalities or death recorded after three months of treatment. The change in body weight may be is a reflection of toxicity or a marker of the negative effects of toxic ingredients in herbal extract (Li et *al*., 1995), but in the ovogenesis toxicity, the results show a steadily increase, no significant dose-related changes in body weight in the experimental group compared to the control group for the entire treatment period, we might suggest that growth and food consumption showed no obvious relationships with the doses used and no adverse effect, contrarily to the finding of AL-Jborreyand Al-Shahwany (2017), in the study of the herbal alcoholic extract of the seeds of *P. harmala* for two weeks, reported a decrease in body weight after being treated with many high doses, probably because of the activity of the alkaloids to reduce cholesterol, triglycerides and Low Density Lipoprotein (LDL), and the mechanism efficacy of diverse medicinal herbs to reduce serum cholesterol level. These result could be attributed to the different concentration of the alkaloid (harmine, harmaline, and harmol) in both studies.

Generally, any change in the relative weight of organs indicates that it is the target organ of toxicity. So in our study, the relative weight of organs was increased significantly for the kidney, heart, lungs, brain, and ovaries. In contrast, the finding of Al-Asmari et *al*., (2014) showed no significant relative organ-weight changes observed in the sub-chronic toxicity of total alkaloid extracts from seeds evaluated after 28-day oral administration.

Measurement of biochemical and blood parameters such as the activities of enzymes and substances in tissues, and body fluids plays an important role in abnormalities investigation, diagnosis, and toxicity (Wang et *al*., 2015; Wang et *al*., 2015; Rahmat et Hajighasemi, 2020). In addition, when there is a predominant elevation of the ALAT, and ASAT (mitochondria enzyme), and increased in their activities in plasma reflects severe tissue injuries, and the increase in ALAT activity mainly is indicate that the liver injury at a dose used as a reflection of lesions in hepatocellular and not cholestatic or intrabiliary in origin (Malomo, 2000, Larrey, 2002, Wanga et *al*., 2019). Consistent with this, our results showed a significant decrease in ALAT and ASAT, and a significant increase in PLA, and no change in GammaGT , While the most studies recorded an increase in enzymes, and only a few of them recorded a decrease of these parameters (AL-Jborrey et Al-Shahwany, 2017). Furthermore, there were a significant decrease in total bilirubin, and indirect bilirubin. Similarly to, the results of the effect of hydroalcoholic extract of *P. harmala* seeds on Streptozotocin-induced diabetes in rats for 4 weeks were showed a remarkable decrease in ALAT, ASAT, GammaGT, and bilirubin levels in comparison with control rats that make the extract of the plant useful in the treatment of diabetes and hypolipidemic activities. In contrast, some of the harmful effects of diabetes may relate to hepatic dysfunction which is revealed in liver function tests such as ALAT, ASAT, GammaGT, and bilirubin. These tests change after diabetes induction and are modified after treatment and may be corrected by the decrease in the activities of these enzymes, which reflects that *P. harmala* extract possesses liver damage recovering or hepato-protective effect (Awe and Banjoko, 2013). On the contrary, the presence of the alkaloid can lead to the same hepatotoxicity in the liver of animals (Banaee et *al*., 2011; Mostakim et *al*., 2015). In other respects, the results mean that the herbal extract of *P. harmala* has harmful effects, especially in high doses, this indicates that there were disturbances in the metabolism of carbohydrates, proteins, and fats after a period of treatment of the animals with a plant extract (Lamchouri et *al*., 2002). This result was confirmed in a study of the effect of total alkaloid extracts from seeds of *P. harmala* in female rats at a high dose, which reported a reduction in total bilirubin and an increase in ALAT, PLA, and Glu, as well as the possibility of liver damage due to the high relative liver weight. However, the microscopic histopathological examination of the liver did not show any remarkable pathological changes (Lamchouri et *al*., 2002).

The renal profile parameters, serum urea and creatinine were significantly changed, indicating that the kidney's excretory function may be impaired. In line with this, our study

showed a significant decrease in serum urea and creatinine in the female experimental group compared to the control group. In contrast, in the findings of Ahmed et *al*., (2013) and Kim et *al*., (2010) in toxicity of *P. harmala* extract were revealed the failure and renal pathological changes throw the higher level of serum urea with or without nephrotoxicity. According to the results of Komeili et *al.*, (2016) and Awe and Banjoko, (2013) in the study of ethanol extract effect of *P. harmala* orally administrated for 4 weeks in diabetic male rats reported anti-diabetic properties, which resulted in a remarkable decrease in glucose in male treated by Streptozotocin-induced diabetic. Contrary to the results of our study, Lamchouri et *al.*, (2002) shows in his study about the effect of total alkaloid extracts from seeds of *P. harmala*, particularly at a dose of 150 mg/kg in female rats, demonstrated a significant alterations in blood glucose and lipid metabolism in the liver, but they could be recovered after four weeks of drug withdrawal, indicating that the toxicity was reversible. This reflects that alkaloids showed hypoglycemic properties.

Blood offers an important profile to study the toxicological influence on animal tissues. Different blood parameters are often subjected to change depending upon stress conditions and various other toxic factors, and the alterations in some blood parameters could be associated with the nature of the toxicants in the studies. So the decrease in hematological variables (RBC, Hb, and PCV) may be due to hemolysis and shrinkage of RBC, leading to a significant decrease in hematocrit value which results in anemia, and the increase rate of the breakdown or the reduce rate of formation of RBCs might also be responsible for the reduction in RBC count (Rahmat et Hajighasemi, 2020). In addition, This increase breakdown may also be attributed to hemodilution resulting from impaired osmoregulation across the gill epithelium, or an appreciable decline in hematopoiesis. WBCs are important cells in the immune system because of their main defensive function, and react immediately to any change in medium caused by a toxicant. So the increase in WBC count occurred as a pathological response since these WBCs, play a great role during infection by stimulating the hemopoietic tissues and the immune system by producing antibodies and chemical substances working as a defense against infection (Rahmat et Hajighasemi, 2020).

Our data showed a significant increase in MCHC, MDW, MID, PTC, GRA, LYM, and WBC, and a decrease in MCV concentrations, contrarily to Lamchouri et *al*, (2002), in his study about the sub-chronic effect of total alkaloid extract by oral administration of many doses in both sexes, the hematological parameters (WBC, RBC, Hb, HCT, MCV, MCH,

MCHC, Lym) were showed no significant changes, the conclusion of this study, showed that the extract did not affect the hemopoietic system by the dose used.

Generally, the reproductive system is under hormonal control through the hypothalamus/pituitary/gonad axis (HPG axis), which regulates its various functions of folliculogenesis, ovulation, luteinization, luteolysis, pregnancy, and parturition. In the other hand, toxicants tend to interfere with this balance, making it irregular that ultimately affecting the fertility of the organism (Kaneko, 2000). The hypothalamus is the source of the gonadotropin-releasing hormone to produce LH and FSH from the anterior part of the pituitary gland, and the central organ for the endocrine regulation and reproductive function. In general, LH and FSH act directly on the gonads to start and promote gametogenesis or oogenesis, and play an essential role in maintaining the integrity of the reproductive organs (Ochei et Kolhatkar, 2000). LH activates the growth of corpus luteum, ovulation, and also the release of progesterone in women. In our study, the levels of FSH and Estrogen were significantly changed, which are similarly to the finding of Lamchouri et *al*, (2000). According to Qazan, (2009), Berdai et *al*., (2014), Wan et *al*., (2013), and Kaneko, (2000), total alkaloid extract can disrupt hormonal balance probably throw the direct effect of these alkaloids on the hypothalamus/pituitary/gonad axis or on the feedback, this can suggest that the alkaloids are infertility agent.

Histopathological study of ovaries showed a significant number of secondary follicles and corpora lutea with a significant size. These results may suggest that the total alkaloid extract by the dose used could stimulate folliculogenesis and ovulation, throw stimulating the secretion of gonadotropin hormones FSH, estradiol. Contrarily to Shapira et *al*., (1987) report, he mentioned that the absence of adverse histopathological findings suggests that the effect of methanol extracts on the estrous cycle, as well as on the reproduction rate, can be attributed to a direct effect on targets associated with the reproductive system.

A toxicant usually induces more than one type of effect in a dose-dependent manner. For example, induction of malformation is almost invariably associated with increased embryonic death and an increased incidence of less severe structural changes. Given an effect on one endpoint, secondary investigations for possible associations should be considered (the nature, scope, and origins of the substance's toxicity should be characterized). The characterization should also include the identification of dose-response relationships to facilitate risk assessment, this is different from the situation in first-pass tests where the

presence or absence of a dose-response assists discrimination between treatment-related and coincidental differences (Health Canada Detection of Toxicity to Reproduction for Medicinal Products, Guidance for Industry ICH Topic S5A, 1996).

An embryotoxicity study was conducted to investigate potential adverse effects of total alkaloids extract on pregnant dams or maternotoxicity, embryonic development, and the abortion by inter-peritoneal repeated dose of $1/20DL_{50}$ mg/kg/day on three stages of gestation from GD1 to GD7, GD1 to GD14, and through 21 days. The obtained results showed no mortality of dams whether the toxicity signs were observed as a daily decrease in locomotor activity, paralysis, depression, and hypothermia in first two hours until three to four days after the treatment. The clinical presentation of this study is nearly similar to the case report of Azizi et *al*., (1998), pregnant rats were treated by harmaline and harmine treatment with different inter-peritoneal concentrations, that was resulted in toxic symptoms including decreased daily activity and severe tremor was developed with a time. According to Yuruktumen et *al*. (2008), in the study of toxic effect of *P. harmala* co-ingested with other drugs or foods was revealed the same clinical signs. In human cases of *P. harmala* intoxication, precisely women (Frison et *al*., 2008; Yuruktumen et *al*., 2008) and animals most signs were hypotension (Marwat et *al*., 2011). These intoxications are presented with nervous and digestive system symptoms commence by excitability and progress to muscle trembling and stiffness (Mahmoudian et *al*., 2002). According to Moshiri et *al*., (2012), these symptom are related to β–carbolines, especially Harman, but in another hand, the MAO inhibitory by harmaline can induce hypertension crisis in high doses. Neurological presentations are prominent in all cases of harmal toxicity similar to our study.

Maternal body weight during the gestation period was used to assess maternal toxicity. The change in maternal body weight and weight gain in our study was contrary to the study Shapira et *al*., (1989), where he treated the dams by methanol and acetone extracts of the epigeal parts of *P. harmala* administered orally for 30 days, which resulted in the absence of weight change, and he suggested that the stability was not due to malnutrition and/or to toxification. In addition, the report of Adaay, (2014), showed that the changes in the body weight of dams treated with 5mg/kg of harmine and harmaline were negligible, while dams treated with 10mg/kg of both alkaloids showed an increase in their body weight. None of the two dosages of harmine and harmaline used in this investigation were lethal to the dams, on the other hand, animals treated with 10mg/kg of harmine and harmaline consumed more food

than the controls. The results revealed that the total alkaloids extract during pregnancy had a minimally maternotoxic effect.

To judge the functional abnormalities in the dams, we analyzed serum biochemical values at the end of the gestational period. Briefly, ALAT, GammaGT concentrations, and indirect bilirubin levels showed a significant changes. The renal parameters showed a significant change in glucose, Blood Urea Nitrogen, creatinine, Na, and Cl. The hormonal analysis shows a significant decrease in FSH, Progesterone, and Estrogen. In contrast to the case reported by Pranzatelli and Snodgrass, (1987), rats treated with *P. harmala* showed tremors and convulsions with normal biochemical lab tests. on the other hand, chronic oral administration of aqueous extract of *P. harmala* for 3 months to rats increased transaminase levels according to Marwat et *al*., (2011) and moshiri et *al*., (2012). According to the study of Komeili et *al*., (2016) about the effect of the hydroalcoholic extract of *P. harmala* seeds for 4 weeks in the Streptozotocin-Induced diabetic (Zitch) rats, the result showed an increase in blood glucose as well as changes in lipid profile in comparison with normal rats, while the treatment by hydroalcoholic extract showed a significantly decrease in the levels of glucose, triglyceride, cholesterol, and LDL-cholesterol (LDL-c) and increase in the level of HDL-cholesterol (HDL-c) in diabetic rats Zitch. These findings are similar to the issue of Singh et *al*., (2008), which showed that ethanolic extract of *P. harmala* showed a significantly decrease in blood glucose levels in normal and diabetic rats at doses of 150 and 250 mg/kg of body weight. We should notice that Zargari et *al*., (2013), demonstrated that *P. harmala* has been traditionally used to treat diabetes in the folk medicine of some parts of the world.

In mammals, embryo/fetal development occurs within the maternal organism, where mothers receive the initial exposure to chemicals while embryos and fetuses are exposed secondarily through the placenta, a maternal-fetal unit. Moreover, if chemicals undergo biotransformation after being absorbed, the embryos and fetuses are exposed not only to the chemical itself, but also to metabolites produced in the maternal organism (Paumgartten, 2010). Our results show a significant difference in totally resorbed litters, the number of fetus death, and a decrease in the fetus weight with a remarkable decrease in the number of implantations, the increase of pre-and post-implantation loss rates, and undeveloped dead fetuses. These results are nearly similar to the result of Shapira et *al*., (1989) in the study of the effect of methanol and acetone extracts of the epigeal parts of *P. harmala* administered orally for 30 days, The methanol extracts at doses used showed a significant decrease in body weight in fetuses size, even there were no changes in the physical and nutritional status of the

animals and no adverse toxicological effects. According to the report of Merzouki et *al*., (2000) and Bellakhdar, (1997), the infusion of mixed leaves and fruits of *P. harmala* or decoction of leaves power orally administered provoked an abortion in pregnant women. Here we can suggest that the total alkaloids extract considered as anti-fertility, anti-implantation, and abortion agent according to our results and other reports.

The mechanism of abortion is not clear but according to Sepulveda and Robinson, (1974), probably attributed to harmine and harmaline throw inhibiting the sodium-dependent transport, elevate the production of hypothalamic norepinephrine and serotonin according to Queshi et *al*., (1979), and the abolition of inhibitory synaptic transmission according to Sokolove and Roth, (1978). Abortifacient activity has been reported only for vasicine alkaloids, which have a uterine stimulatory effect, apparently through the release of prostaglandins (Gupta et *al*., 1978; Zutshi et *al*., 1980). These findings are nearly similar to the case report of Shapira et *al*., (1989), which reported that the methanolic extract reduced the number of living pups and increased the number of resorption, and the litter size. On another side, the study of Fathiazada et *al*, (2006) revealed that the hydro-alcoholic extract of *P. harmala* seeds on spontaneous rhythmic contractions of isolated rat uterine and endometrial free preparations exhibited a significant spontaneous contractions of the uterus and stripped myometrium relative to the solvent control. In order to determine the mechanism of the extract of *P. harmala* on abortion, the extract produced a uterotonic effect in calcium-free solution in the presence of KCl. This finding showed that the serum protein electrophoresis (EPS) may increase calcium influx through voltage-dependant calcium channels and the effects of *P. harmala* on rat uterus were not dependent on prostaglandins (Fathiazada et *al*., 2006).

The main interest of fertility studies and early embryonic development is to assess the toxic potential of a toxic compound on the female estrous cycle, tubal transport, implantation, and development of pre-implantation stages of the embryo, as well as on the functional effects of male fertility (Loomis and Hayes , 1996). The results of the aforementioned rat study consistently showed no maternal morbidity and mortality were observed in mated females with normal behavior during all the gestational period and maternal body weight and weight gain were changed significantly. The reductions in maternal body weight gain during pregnancy, which is one of the commonly used indicators of maternal toxicity in developmental toxicity studies, were not necessarily associated with embryo lethality and teratogenicity (Francisco and Paumgartten, 2010).

Reduced litter size at birth may be due to a reduced ovulation rate (corpora lutea count), higher rate of pre-implantation deaths, higher rate of post-implantation deaths, or immediate post-natal deaths. In the other hand, these deaths may be the consequence of an earlier physical malformation that can no longer be observed due to subsequent secondary changes. Particularly for effects with a natural low frequency among controls, discrimination between treatment-induced and coincidental occurrence is dependent upon association with other types of effects (Shapira et *al*., 1989). Contrary to the result of the report of Azizi et *al*., (1998), where harmaline and harmine were injected intraperitoneally into pregnant rats in different days of pregnancy from the 1^{st} – 23^{rd} showed no different toxic symptoms in treated rats. They observed no abortion in rats that received l-80 μg/Kg of alkaloids and all rats delivered live births on time with no obvious abnormalities. Therefore, it is concluded that, because of observed toxicity which can be lethal, pregnant women should not use seeds or extract of espand (Azizi et *al*., 1998).

Indeed, the embryotoxicity study of Adaay, (2014) on of harmaline and harmol effects given intraperitoneally to pregnant rats on days 1^{st}-14^{th} of gestation revealed that the average number of implantation sites and live fetuses per dam was within the control range in the different dose levels of the two compounds. Fetal death or resorption increased in the treated groups, this increase was statistically significant in the groups treated with 10mg/kg of harmaline and harmol. A decrease in fetal body weight was evident in group treated by 5 and 10mg/kg of harmaline and harmol in comparison with the control group. The fetal mortality and the decrease in body weight indicate that harmaline and harmol or their metabolites cross the placenta fast enough to reach the embryo. Adaay, (1994) suggested that some harmala alkaloids (harmaline and harmol or their metabolites) may induce embryolethality in different the stage of gestation.

CONCLUSION AND PERSPECTIVES

Conclusion

The seeds of *Peganum harmala* L. have been used in traditional medicine as an emmenagogue and abortifacient agent as well as various pharmacological effects. This study's goal was to assess the toxicity of the total alkaloid extract from *P .harmala* seeds on ovulation, embryogenesis, and fertility in rat Albino Wistar. The results are as follows

- LD_{50} of total alkaloids seeds in male and female rats classified this plant as moderately toxic.
- Acute toxicity of total alkaloids of *P. harmala* seeds symptoms are recorded in excitability, increased rhythm cardiac, convulsions, tremors, arriving until death. These signs due to the direct action of alkaloids on the central nervous system.

The hormonal unbalance, ovarian toxicity, and oogenesis disruption in treated female rats by total alkaloids extract after three months of daily IP administration shows:

- Significant increases in the relative weight of the kidney, heart, lungs, brain, and ovaries. A significant increase in PLA, significant decreases in ALAT, ASAT, total and indirect bilirubin, and serum blood urea. The hormonal analysis shows that FSH and Estrogen have increased significantly.
- Histological studies of the liver, kidney, and ovaries show sinusoidal inflammation and lymph-plasmocyte inflammation around the Centro-lobular vein, congestive follicles surrounded by endothelial cells, and the presence of a significant number and a huge size of secondary follicles and corpora lutea respectively.

In the maternal and developmental toxicity during pregnancy, the results summarized in:

- high confirmed pregnancy rates, a decrease in locomotor activity, paralysis, and hypothermia. Maternal body weight and weight gain changes were statistically significant in all pregnant.
- Ovarian relative weight changed substantially.
- The ALAT and gamma GT concentrations significantly change for the group treated for seven days. Significant changes in the total and indirect bilirubin levels were observed in all treated groups.
- FSH, progesterone and Estrogen levels were significantly changed in the treated groups.

- The fetus weight, total resorbed litters, and the number of fetus deaths were Significant changed.

For fertility Evaluation, the Male rats were treated IP with total alkaloid extract for 90 days and mated with a virgin female; at the end of the study period, the results show:

- No maternal morbidity and mortality in the female rats with normal behavior during the gestational period.
- The maternal body weight and weight gain statistically significant changes were in all pregnant rates.
- Serum biochemical analysis shows significant changes in the total and indirect bilirubin levels were observed. The renal parameters showed a significant change in creatinine levels, significant changes in FSH and LH, and significant differences in corpora lutea number, implantation sites, pre-and post-implantation loss rates, resorption, fetal deaths, and fetal body weights were observed.

Total alkaloid extract from *P.harmala* seeds at the dose used could stimulate folliculogenesis and ovulation by increasing gonadotropin hormones FSH and Estradiol secretion and has adverse effects on maternotoxicity, embryonic development, and abortion.

The perspectives

Despite the attempt to assess as many biological activities of plants as possible selected medicinal products, there are still several aspects that can be exploited as future prospects citing as an example:

- No better than working on a pure bioactive molecules, so the first thing should do is isolate the molecules involved in the different activities.
- Test the different extracts on other experimental models.
- study the mechanism of ovogenesis, embryology and abortion by different herbal preparations.

LIST OF REFERENCES

List of References

A

Abdel-Fattah AM, Matsumoto k' Gammaz HA, Watanabe H. 1995. **Hypothermic effect of harmala alkaloid in rats: Involvement of serotonergic mechanism.** Pharmacology Biochemistry and Behavior**,** 52:2, 421-426.

Achour S, Saadi H, Turcant A, Banani A, Mokhtari A, Soulaymani A, Soulaymani Bencheikh R. 2012. *Peganum harmala L* poisoning and pregnancy: two cases in Morocco. Medcine et santé tropicales, 22: 84-86.

Adaay MH. 1994. Some observations on the reproduction toxicity of the aqueous extract of *Peganum harmala* seeds. Journal of Fitoterapia, 65: 214-218.

Adaay MH. 2014. Embryotoxicity of harmaline and harmalol hydrochloride on pregnant rats. Mater. Environment Science journal, 5:1:127-132.

Ahmed H, Abu El Zahab H, Alswiai G. 2013. Purifcation of antioxidant protein isolated from *Peganum harmala* and its protective effect against CCl4 toxicity in rats. Turkish Journal of Biology, 37, 1: 39–48.

Al-Asmari AK, Al-Elaiwi AM, Athar MT, Tariq M, Al Eid A, Al-Asmary SM. 2014. Review Article A Review of Hepatoprotective Plants Used in Saudi Traditional Medicine. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine; 842-890.

AL-Jborrey M.H, Al-Shahwany A.W. 2017. Histopathological Effect of *Peganum harmala*; Alcoholic Extract of Seeds on Liver and Kidney in Mice. Biomedicine and Biotechnology. 5, 1: 1-5.

Allouni R, Guergour H, MahdebN, Omrane M, Bouzidi A. 2017. Acute Toxicity Study of the Total Alkaloids of *Ruta montana* in Male Mice. International Journal of Pharmacognosy and Phytochemical, 17; 9;8: 1060-1066.

Ambasta SP. 1986. The useful Plants of India. Publication and information directorate, CSIR, New Delhi, 60-70.

Aniszewski T. 2007. Secrets of life (Chapter 1: Alkaloid chemistry, biological significance, applications And ecological role). 1st Edition. Elsevier, 1st 8,53, 62, 111- 114, 148.

Apostolico I , Aliberti L , Caputo L, Feo VD , Fratianni F , Nazzaro F , Souza LF , and Khadhr M. Chemical Composition, Antibacterial and Phytotoxic Activities of Peganum harmala Seed Essential Oils from Five Different Localities in Northern Africa. Molecules 2016, 21, 1235; doi:10.3390/molecules21091235.

Apostolico I, Aliberti L, Caputo L , Feo VD, Fratianni F , Nazzaro F, Souza L F , KhadhR M. 2016. Chemical Composition, Antibacterial and Phytotoxic Activities of *Peganum harmala* Seed Essential Oils from Five Different Localities in Northern Africa. Molecules, 21 (9): 1235.

Arshad N, Zitterl-Eglseer K, Hasnain S, Hess M. 2008. Effect of Peganum harmala or its beta-carboline alkaloids on certain antibiotic resistant strains of bacteria and protozoa from poultry. Phytotherapy research, 22(11):1533-8.

Artal FJC. 2015. Bioactive Nutraceuticals and Dietary Supplements in Neurological and Brain Disease (Chapter 23 - Adverse Neurological Effects Caused by the Ingestion of Plants, Seeds, and Fruits). Prevention and Therapy, 215-219

Asgarpanah J, Ramezanloo F. 2012. Chemistry, pharmacology and medicinal properties of *Peganum harmala* L. African Journal of Pharmacy and Pharmacology, 6(22):1573-1580.

Astulla A, Zaima K, Matsuno Y, Hirasawa Y, Ekasar Wi, Widyawaruyanti A, Zaini N C, Morita H. 2008. Alkaloids from the seeds of *Peganum harmala* showing antiplasmodial and vasorelaxant activities. Journal of Natural Medicines**,** 22(11):1533-8.

Awe EO and Banjoko SO. 2013. Biochemical and haematological assessment of toxic effects of the leaf ethanol extract of *Petroselinum crispum* (Mill) Nyman ex A.W. Hill (Parsley) in rats. BMC Complementary and Alternative Medicine, 13:75.

Azizi A, Akbari-javar H, Salehi MH, Jalali N. 1998. Evaluation of toxic alkaloids of seeds of *Peganum harmala* L. as an abortion agent in pregnant rat. Pathophysiology journal, 5:1001:94.

B

Bataineh HN, Bataineh ZM, Daradka H. 2007. Short-term exposure of female rats to industrial metal salts: Effect on implantation and pregnancy. **Reproductive Medicine and Biology.** 6;6(3):179-183.

Belzil C, 2007. Therapeutic Potential for Inhibition of HIV Activation. Lethbridge Undergraduate Research Journal, 2 (2).

Bensalem S, Soubhye J, Aldib I, Bournine L, Nguyen NT, Vanhaeverbeek M, Rousseau A, Boudjeltia KM, Sarakbi A, Kauffmann JM, Nève J, Prévost M, Stévigny C, Benabdesselam FM, Bedjou F, Antwerpen PV, Duez P. 2014. Inhibition of myeloperoxidase activity by the alkaloids of *Peganum harmala* L. (Zygophyllaceae). Journal of Ethnopharmacology, 154: 361-369

Berdai M.A, Labib S, Harandou M. 2014. *Peganum harmala* L. intoxication in a pregnant woman. Case Rep. Emerg. Med, 1–3.

Berdai MA, Labib S, Harandou M. 2014. Abortion Induced by *Peganum Harmala* Ingestion in a Pregnant Woman: A Case Report and Literature Review. International Journal of Medical Toxicology and Forensic Medicine, 9(3):165-170.

Bergman J. 1983. The Alkaloids (Chapter 2: the quinazolinocarboline alkaloids). Academic Press, XXI: 34.

Bibi H, Afzal M, Kamal M, Sohail IU, Khan SM, Sher AA, Ziaulhaq IU, Ali A, Khan SA, Rahman IU. 2015. Morphological and Anatomical Characteristics of Selected Dicot Xerophytes of District Karak, Khyber Pakhtunkhwa, Pakistan. Middle-East Journal of Scientific Research, 23 (4): 545-557.

Boorman G, Suttie A, Leininger J, Eustis S, Elwell M, Bradley A, MacKenzie W. 2018**.** Boorman's Pathology of the Rat**, 2^{ed},** 523-536.

Boorman G, Suttie A, Leininger J, Eustis S, Elwell M, Bradley A, MacKenzie W. 2018**.** Boorman's Pathology of the Rat**, 2^{ed},** 523-536.

Boullard B. 2001. Plantes médicinales du monde réalités et croyances. Paris, 395.

Bouzidi A, Mahdeb N, Kara N. 2011. Toxicity studies of alkaloids of seeds of *Datura stramonium* and synthesis alkaloids in male rats. Medicinal Plants Research journal, 4: 3421-3431.

Bruneton J. 1999. Pharmacognosie, phytochimie, plantes médicinales. Paris, 973-783.

Bruneton J. Pharmacognosie. Phytochimie, Plantes médicinales. 3^{ed}. Lavoisier, Paris, TEC & DOC, 973-783.

Bulun SE. 2019. Physiology and Pathology of the Female Reproductive Axis (Chapter 17). Williams Textbook of Endocrinology, 14^{ed}, 574-641**.**

C

Cao R, Peng W, Wang Z, Xu A. 2007. beta-Carboline alkaloids: biochemical and pharmacological functions. Current Medicinal Chemistry;14(4):479-500.

Casal S. 2015. Neuroactive β-Carbolines Norharman and Harman in Coffee. Coffee in Health and Disease Prevention, 737-743.

Chase MW, Reveal JI. 2009. A phylogenetic classification of the land plants to accompany APG III. Botanical Journal of the Linnean Society, 161: 122–127.

Chase MW. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society, 141: 399–436.

Chase MW. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering Plants: APG III. Botanical Journal of the Linnean Society, 161: 105–121.

Chopra IC, Abrol BK, Handa. 1960. The pharmacological aspects; In: Medicinal Plants of the Arid Zones. Paris, 11–54.

Christenhusz MJM, Byng JW. 2016. The number of known plants species in the world and its annual increase. Phytotaxa, 261(3): 201–217.

Cohen BD, Dias JA. 2019. Follitropin; Reference Module in Biomedical Sciences. https://doi.org/10.1016/B978-0-12-801238-3.99542-4

D

Dattani MT, Gevers EF. 2016. Endocrinology of Fetal Development (Chapter22). Williams Textbook of Endocrinology, 13^{ed}, 849-892.

Decraene LPR; Smets EF. 1996. The morphological variation and systematic value of stamen pairs in the Magnoliatae. Feddes Repertorium, 107, 1-2.

Djafer R, Akil Dahdouh S, Boukachabia R, Megueddem M. 2017. A propos d'un cas d'intoxication mortelle par le harmel (*Peganum harmala* L.). Phytothérapie, 15(5):288–289.

E

Eguchi R, Ono N, Morita AH, Katsuragi T, Nakamura S, Huang M, Altaf-Ul-Amin M, Kanaya S. 2019. Classification of alkaloids according to the starting substances of their biosynthetic pathways using graph convolutional neural networks. BMC Bioinformatics, 20:380.

Emilija V, Wenhao X, Blaine P, Wong WE, Jennyn H, Oviedo CA, Julia H, Mannuel P. Overview of Epidemiological Data on Smoking and Preclinical and Clinical Data on Nicotine (Chapter 10 - Other Compounds From Tobacco With Potential Impact on Neurodegenerative Diseases). Nicotine and Other Tobacco Compounds in Neurodegenerative and Psychiatric Diseases, 83-97.

F

Fathiazada F, Azarmib Y, Khodaiea L. 2006. Pharmacological Effects of *Peganum harmala* Seeds Extract on Isolated Rat Uterus. Iranian Journal of Pharmaceutical Sciences Spring, 2(2): 81-86.

Fazal H, Ahmad N, Abbasi BH. 2013. Identification, Characterization, and Palynology of High-Valued Medicinal Plants. The Scientific World Journal, 9: 1-10

Filali I, Jelassi A, Ben Jannet H. 2019. New Bioactive Esters and Phosphonates Semisynthesized From (±)-Vasicinone: An Alkaloid Isolated From *Peganum harmala*. Natural Product Communications, 14(12): 1–9.

Frison G, Favretto D, Zancanaro F, Fazzin G, Ferrara SD. 2008. A case of b-carboline alkaloid intoxication following ingestion of *Peganum harmala* seed extract. Forensic Science International journal, 179: 37–43.

Furman BL, Schriefer J. 2007. XPharm: The Comprehensive Pharmacology Reference, 1-5.

G

Glennon R A, Dukat M, Grella B, Hong S, Costantino L, Teitler M, Smith C, Egan C, Davis K, Mattson MV. 2000. Binding of beta-carbolines and related agents at serotonin (5-HT(2) and 5-HT(1A)), dopamine (D(2)) and benzodiazepine receptors. Drug Alcohol Depend, 1;60(2):121-32.

Goel N, Singh N, Saini R. 2009. Efficient in vitro multiplication of Syrian rue (*Peganum harmala* L.) using 6-benzylaminopurine pre-conditioned seedling explants. Nature Science, 7(7): 129–134.

Gosden R.G. 2007. Encyclopedia of Gerontology, 2^{ed}, 151-159

Guergour H, Allouni R , Mahdeb N, Bouzidi A. 2017. Acute and Subacute Toxicity Evaluation of Alkaloids of *Peganum harmala* L. in Experimental Mice. International Journal of Pharmacognosy and Phytochemical Research, 9(9); 1182-1189.

Gupta OP, Anand KK. Ghatak BJR, Atal CK. 1978. Vasicine alkaloid of *Adhatoda vasica*, a promising uterotonic abortifacient. Indian Journal of Experimental Biology, 16(10): 1075-1077.

Gupta PK. 2016. Fundamentals of Toxicology; Essential Concepts and Applications (Chapter 26 - Toxic effects of poisonous plants). Elsevier, 293-303.

H

Hamden K, Masmoudi H, Ellouz F, Elfeki A, Carreau S. 2008. Protective effects of *Peganum harmala* extracts on thiourea-induced diseases in adult male rat. Journal of Environmental Biology, 29, 1:73–77.

Hamden K, Masmoudi H, Ellouz F, Elfeki A, Carreau S. Protective effects of *Peganum harmala* extracts onthiourea-induced diseases in adult male rat. Journal of Environmental Biology. (2008). 29:173-77.

Hammiche V, Merad R, Azzouz M. 2013. Plantes toxiques à usage médicinal du pourtour méditerranéen. Springer. Paris, 137.

Hassani LMI, El-Hadek M. 1999. Analyse de la composition de l'huile de *Peganum harmala* L. (Zygophyllaceae), Acta Botanica Gallica, 146(4): 353-359.

Haston E, Richardson JE, Stevens PF, Chase MW, Harris DJ. 2009. The Linear Angiosperm Phylogeny Group (LAPG) III: a linear sequence of the families in APG III. Botanical Journal of the Linnean Society, 161: 128–131.

Health Canada Detection of Toxicity to Reproduction for Medicinal Products Guidance for Industry. ICH Topic S5A. 1996.

Hedayat KM, Lapraz GC. 2019.Advanced Concepts for the Treatment of Complex Clinical Conditions **(**Chapter 4 - Regulation of the menstrual cycles**)**, 3, The Theory of Endobiogeny, 3, 69-87.

Hemmateenejada B, Abbaspoura A, Maghami H, Mirib R, Panjehshahinba MR. 2006. Partial least squares-based multivariate spectral calibration method for simultaneous determination of beta-carboline derivatives in *Peganum harmala* seed extracts. Analytica Chimica Acta, 575: 290–299.

Herraiz T, González D, Ancín-Azpilicueta C, Arán JV, Guillén H. 2010. β-Carboline alkaloids in *Peganum harmala* and inhibition of human monoamine oxidase (MAO). Food and Chemical Toxicology, 48: 839–845.

Herraiz T, Guille´n H, Galisteo J. *N*-Methyltetrahydro β-carboline analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin are oxidized to neurotoxic β-carbolinium cations by heme peroxidases. Biochem. Biophys. Res. Commun. (2007). 356:118–123.

Herraiz T, Guillén H, Arán VJ, Salgado A. 2017. Identification, occurrence and activity of quinazoline alkaloids in *Peganum harmala*. Food and Chemical Toxicology, 103:261-269.

Herraiz T. 2007. Identification and Occurrence of β-Carboline Alkaloids in Raisins and Inhibition of Monoamine Oxidase (MAO). Journal of Agricultural and Food Chemistry, 55:8534–8540.

Hindmarsh PC, Geertsma K. 2017**.** A Comprehensive Guide (Chapter 25 - Other Hormones and Their Roles). Congenital Adrenal Hyperplasia, 309-325.

Hinson J, Raven P, Chew S. 2010. hormonal control of reproduction part ii: female reproductive system. The endocrine system, 2ed, 99-113.

Hugues JN, Cedrin-Durnerin I . 2000. Role of luteinizing hormone in follicular development and corpus luteum function. Gynécologie Obstétrique & Fertilité, (10) 28, 738-744.

J

Jahnke G, Marr M, Myers C, Wilson R, Travlos G. 1999. Price C Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. toxicological sciences journal, 50:2:271-9.

K

Kaneko H. Clinical biochemistry of domestic animals. San Diego: Academic. (1989).

Kartal M, Altun M.L, Kurucu S. 2003. HPLC method for the analysis of harmol, harmalol, harmine and harmaline in the seeds of *Peganum harmala* L. Journal of Pharmaceutical and Biomedical Analysis, 31: 263-269.

Kashimov HN, Teclezhenetskaya MV, Sharakhimar NN, Yunasov SY. 1971. The dynamics of the accumulation of alkaloids in *Peganum harmala.* Chemistry of Natural Cornpounds journal, 3:364-365.

Kaskoos RA. 2014. Physico-chemical Parameters, Phytochemical Screening and Antioxidant Activity of Seeds of *Peganum harmala* Collected from Iraq. Asian Journal of Biomedical and Pharmaceutical Sciences; 4(28), 20-24.

Kim YS, Song MY, Park JD, Song KS, Ryu HR, Chang HK. 2010. Subchronic oral toxicity of silver nanoparticles. Particle and Fibre Toxicology; 7:(1); 20.

Komeili G, Hashemi M, Bameri-Niafar M. 2016. Evaluation of antidiabetic and antihyperlipidemic effects of *Peganum harmala* seeds in diabetic rats. Hindawi Publishing Corporation Cholesterol journal, 1-6.

kour GD, Singh DC, Chaubey S, Tewari RC. 2016. Therapeutic value of medicinal plants of Arid zone w.s.r. to Zygophyllaceae. International Journal of Ayurvedic and Herbal Medicine 6 (1): 2082 – 2092.

Kubitzki K. 2011. The famillies and genera of vascular plants.Germany: 5: 417-431.

Kuete V. 2014. Toxicological Survey of African Medicinal Plants (Chapter 22: Physical, Hematological, and Histopathological Signs of Toxicity Induced by African Medicinal Plants). Elsevier, 1ed, 635-657

Kukula-Koch WA, Widelski J. 2017. Pharmacognosy; Fundamentals, Applications and Strategies (Chapter 9 - Alkaloids). Elsevier, 163-165, 182.

Kurt R. 1971. Chromatographie sur couches minces, 2sd ed. Gauthiers Villars, Paris. **398.**

L

Lamchouri F , Settaf A, Cherrah Y, Hassar M,. Zemzami M, Atif N, Nadori EB, Zaid A, Lyoussi B. 2000. In vitro cell-toxicity of *Peganum harmala* alkaloids on cancerous cell-lines. Fitoterapia, 71: 50–54

Lamchouri F, Settaf A, Cherrah Y. 2002. Experimental toxicity of *Peganum harmala* seeds. *Annales Pharmaceutiques Francaises*, 60, 2: 123–129.

Larrey D. 2002. Epidemiology and individual susceptibility to adverse drug reactions affecting the liver. Seminars in Liver Disease, 22:145–155.

Leung PCK and Armstrong DT. 1980. Interactions of Steroids and Gonadotropins in the Control of Steroidogenesis in the Ovarian Follicle. Annual Review of Physiology, 42:71-82.

Li GW, Liang PG, Pan GY. 1995. Radioprotective effect of α-carboline and its carboline analogues. Acta Pharmacol Sinica, 30:715-717.

Li S, Cheng X, Wang C. 2017. Review A review on traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the genus Peganum. Journal of Ethnopharmacology, 203: 127-162.

Li S, Wang K, Gong C, Bao Y, Qin N, Li D, Li Z, Bai J, Hua H. 2018. Cytotoxic quinazoline alkaloids from the seeds of *Peganum harmala*. Bioorganic & Medicinal Chemistry Letters, 28: 103–106.

Li SP, Wang YW, Qi SL, Zhang YP, Deng G, Ding WZ, Ma C, Lin QY, Guan HD, Liu W, Cheng XM, CH Wang. 2018. Analogous b-Carboline Alkaloids Harmaline and Harmine Ameliorate Scopolamine-Induced Cognition Dysfunction by Attenuating Acetylcholinesterase Activity, Oxidative Stress, and Inflammation in Mice. Frontiers in Pharmacology, 9:346.

Li Y, Adur M. K, Hale B. J, Ross J. W. 2018. Regulation of Oogenesis by Noncoding RNAs. Encyclopedia of Reproduction, 2nd ed. Volume3. Pp: 190-196.

Li Y, He Q, Du S, Guo S, Geng Z, Deng Z. 2018. Study of Methanol Extracts from Different Parts of *Peganum harmala* L. Using 1H-NMR Plant Metabolomics. Hindawi Journal of Analytical Methods in Chemistry, 1-9.

Liljegren DR. 1968. The biosynthesis of quinazoline alkaloids of *Peganum harmala* l. Phytochemistry, 7: 1299-1306.

Litchfield J.T and Wilcoxon F. 1949. A simplified method of evaluating dose-effect experiments. Journal of pharmacology and experimental Therapeutics, 96, 2: 99-113.

Liu W, Wang Y, He D, Li S, Zhu Y, Jiang B, Cheng X, Wang Z, Wang C. 2015. Antitussive, expectorant, and bronchodilating effects of quinazoline alkaloids (±)-vasicine, deoxyvasicine, and (±)-vasicinone from aerial parts of *Peganum harmala* L. Phytomedicine, 22:1088–1095

Loomis TA and Hayes WA. 1996. Loomis's Essentials of Toxicology (Chapter13: Toxicologic testing methods). 4th Ed, Elsevier, 189-222.

Lu FC. 1992. Toxicologie : données générales, procédures d'évaluation, organes cibles, évaluation du risque Paris, Masson,1, (XIV) : 361.

M

Mahdeb N, Bettihi S, Ghedjati N, Bouzidi A. 2020. Evaluation of Acute Toxicity of the Alkaloids of *Peganum harmala* L Seeds in Albino Wistar Female Rats. Advances in Pharmacology and Pharmacy, 8(2): 24-30.

Mahmoudian M, Jalilpour H, Salehian P. 2002. Toxicity of *Peganum harmala*: Review and a Case. Iranian Journal of Pharmacology & Therapeutics, 1(1):1-4.

Malomo AO. 2000. Toxicological implications of certriaxone administration in rats. Nigerian Journal of Biochemistry and Molecular Biology, 1533–1538.

Manske RHF. 1965. The Alkaloids Chemistry and Physiology (the indole alkaloids). Academic Press, New York, London, I I I.

Mars B. 2009. The Desktop Guide to Herbal Medicine: The Ultimate Multidisciplinary Reference to the Amazing Realm of Healing Plants, in a Quick-study, One-stop Guide: Easyread Super Large, 18ed, 3: 191-193.

Marwat SK, Rehman Fu. 2011. Medicinal and pharmacological potential of harmala (*Peganum harmala* L.) seeds. Nuts and Seeds in Health and Disease Prevention (Chapter 70). Press, 585-599.

Mashreghi M. 2012. The Effect of *Peganum harmala* and *Teucrium Polium* Alcoholic Extracts on Growth of Escherichia coli. Jundishapur journal of microbiology, 5(3): 511-515.

Mazid M, Khan TA, Mohammad F. 2011. Role of secondary metabolites in defense mechanisms of plants. Biology and Medicine, 3 (2): 232-249.

Melmed S. 2020. Physiology and Pathology of the Female Reproductive Axis. Williams Textbook of Endocrinology, 589-663.

Melmed Shlomo MB ChB, MACP, in Williams Textbook of Endocrinology, 2020. Physiology and Pathology of the Female Reproductive Axis. **j- Williams Textbook of Endocrinology (Thirteenth Edition)** Physiology and Pathology of the Female Reproductive Axis

Merzouki AC, Ed-derfoufi BF , Molero Mesa CJ. 2000. Short communication Hemp (Cannabis sativa L.) and abortion. Ethnopharmacology journal, 73:501–503.

Miller WL. 2003**.** Encyclopedia of Hormones **(**Follicle-Stimulating Hormone (FSH)**)**. 645-652.

Mohammadi RS, Bidaki R, Mirdrikvand F, Yazdi SNM, Anari PY. 2016. *Peganum Harmala* (Aspand) Intoxication; a Case Report. Spring, 4(2): 106–107.

Monsef HR., Ghobadi A, Iranshahi M, Abdollahi M. 2004. Antinociceptive effect of Peganum harmala L. alkaloid extract on mouse formalin test. Journal of Pharmacy and Pharmceutical Sciences, 7: 65–69.

Moshiri M, Etemad L, Javidi S, Alizadeh A. 2013. *Peganum harmala* intoxication, a case report. Avicenna Journal Phytomedecine, 3(3): 288–292.

Mostakim G.M, Zahangir M, Mishu M.M, Rahman K, Islam M.S. 2015. Alteration of Blood Parameters and Histoarchitecture of Liver and Kidney of Silver Barb after Chronic Exposure to Quinalphos Hindawi Publishing Corporation Journal of Toxicology, 10, 1155; 415984.

N

Norman AW, Henry HL. 2015**.** Hormones of Pregnancy, Parturition and Lactation. Hormones **(**Chapter 14**)**, 3ed, 297-320.

Norris DO, James DA. 2021**.** Vertebrate Endocrinology (Chapter 10 - The endocrinology of mammalian reproduction**),** 6**ed**, 315-369 .

Ochei J, Kolhatkar A. Medical laboratory science. Theory and practice. 2nd ed. New Delhi: Tata McGraw- Hill. (2000). Pp: 331–349.

Owen R, Giacosa A, Hull WE, Haubner R, Spiegeehalder B, Bartsch H. The antioxidant/anticancer potential of phenolics compounds isolated from olive oil. Eur. J. Cancer 2000;36:1235-1247.

P

Padmanabhan V, Puttabyatappa M. 2018. Hypothalamus–Pituitary–Ovary Axis. Encyclopedia of Reproduction 2ed**,** 2, 121-129.

Pallardy SG. 2008. Physiology of woody plants. Elsevier, 3ed, 244-253.

Pascual ME, Carretero ME, Slowing KV, Villar A. 2002. Simplified Screening by TLC of Plant Drugs . Pharmaceutical Biology :4 :2, 1744-5116.

Patel K, Gadewar M, Tripathi R, Prasad SK, Patel DK. 2012. A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid ‘‘Harmine’’. Asian Pacific Journal of Tropical Biomedicine, 2(8): 660–664.

Paumgartten FJR. 2010. Influence of maternal toxicity on the outcome of developmental toxicity studies. Toxicology and Environmental Health journal, Part A: Current Issues, 73(13-14): 944-951.

Q

Qazan W.S. 2009. The effect of low levels of dietary *Peganum harmala* L. and Ballotaundulata or their mixture on chicks. **Journal of Animal and Veterinary Advances**, 8:1535–1538.

Quattrocchi U. 2012. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology CRC press. Pay Florida USA, 5: 2817.

Queshi Z, Akhtar MS, Malik, ZA. 1975. Norepinephrine and 5-hydroxytryptamine contents of hypothalami of rats treated with harmidine hydrochloride. Planta Medica journal, 35:235- 241.

R

Rahmat N, Hajighasemi F. 2020. Effect of *Peganum Harmala* Seeds Extract on Nitric Oxide in U937 Monocytes and Macrophages. International Journal of Medical Laboratory, 7, 4: 312-320.

Randerath K. 1971. Chromatographie sur Couches Minces. Edition Gauthier-Villars, Paris, 337-339.

S

Santi D, Casarini L, Marshall GR, Simoni M. 2018. Encyclopedia of Endocrine Diseases (Follicle-Stimulating Hormone (FSH). 2ed, 2: 149-156.

Saxton JE. 1971. The Alkaloids, a specialist periodical report. Burlington House, London, 1: 96-102, 150-199.

Sepulveda FV, Robinson JWL. 1974. Harmaline, a potent inhibitor of sodium-dependent transport. Biochimica et Biophysica Acta, 373:527-531.

Shao H, Huang X, Zhang Y, Zhang C. 2013. Main Alkaloids of *Peganum harmala* L. and Their Different Effects on Dicot and Monocot Crops. Molecules, 18: 2623-2634.

Shapira Z, Terkel J, Egozp QY, Nyskad A, Friedman J. 1989. Abortifacient potential for the epigeal parts of *Peganum harmala*. Ethnopharmacology journal, 27 (3): 319 – 325.

Sheahan MC, Chase MW. 1996, A phylogenetic analysis of Zygophyllaceae based on morphological, anatomical and rbcL DNA sequence data. Botanical Journal of the Linnean Society, 122: 279–300.

Singh AB, Chaturvedi JP, Narender T, Srivastava AK. 2008. Preliminary studies on the hypoglycemic effect of *Peganum harmala* L. seeds ethanol extract on normal and streptozotocin induced diabetic rats. Indian Journal of Clinical Biochemistry, 23:4:391–393.

Sobhani A M, Ebrahimi SA, Mahmoudian M. 2002. an in vitro evaluation of human dna topoisomerase i inhibition by peganum harmala l. seeds extract and its β-carboline alkaloids. The Iranian Journal of Pharmaceutical Research,5(1):19-23.

Sokolove PG, Roth SH. 1978. Effect of harmaline on the crayfish stretch receptor: Blockade at a GABA-mediated inhibitory synapse. Neurophunnacology journal, 17:729-731.

Soliman AM, Abu-El-Zahab HS, Alswiai GA. 2012. Efficacy evaluation of the protein isolated from *Peganum harmala* seeds as an antioxidant in liver of rats. Asian Pacific Journal of Tropical Medicine, 285-295

Soliman AM, Abu-El-Zahab HS, Alswiai GA. 2012. Efficacy evaluation of the protein isolated from *Peganum harmala* seeds as an antioxidant in liver of rats. Asian Pacific Journal of Tropical Medicine, 285-295.

Soni PD, Upadhyay SU, Upadhyay UM. 2013. A Review On Commiphora Myyrha. International Journal of Pharmaceutical Sciences, 4 (3):190.

Springob K and Kutchan TM, Osbourn AE and Lanzotti V. 2009. *Plant-derived Natural Products(*69. Introduction to the Different Classes of Natural Products*)*. Springer Science and Business Media.

Springob K, Kutchan TM. 2009. Introduction to the Different Classes of Natural Products. Karin Springob and Toni M. Kutchan. Plant-derived Natural Products, PP

Stewart RR. 1972. Flora of West Pakistan. Publisher Fakhri Printing Press Publisher, 434.

Sultana N. 1987. Studies in the derivatives of harmine series of alkaloids and a reinvestigation of the chemical constituents of *Datura metal* and *Brophyllum Pinnatum*. Phd thesis. H.E.J. Research institute of chemistry. Karachi, 1–170.

T

Takhtajan A. 2009. Flowering Plants. Springer. Congress, Petersburg, Russia, 2ed: 391- 395.

Toyin YM, Olakunle AT, Adewunmi AM. 2014. Toxicity and beneficial effects of some african plants on the reproductive system. Toxicological Survey of African Medicinal Plants journal, 445-492.

W

Wagstaff DJ. 2008. international posnous plants checklist- art evidence based reference. London. CRC Press, 294.

Wan LO, Yong HW, Boon KB, Keong YS. 2013. Updates on antiobesity effect of garcinia origin HCA. **Evidence-Based Complementary and Alternative Medicine**, 1:1-10.

Wang C, Zhang Z, Wang Y, He X. 2015. Cytotoxic indole alkaloids against human leukemia cell lines from the toxic plant *peganum harmala*. Toxins, 7:4507-4518.

Wang Y, Wang C, Jiang C, Zeng H, He Z. 2015. Novel mechanism of harmaline on inducing G2/M cell cycle arrest and apoptosis by up-regulating Fas/FasL in SGC-7901 cells. Scientific reports. Scientific RepoRts, 5: 18613.

Wanga Y, Wanga H, Zhanga B.L, Zhanga Y, Shengc Y, Denga G. 2019. Subchronic toxicity and concomitant toxicokinetics of long-term oral administration of total alkaloid extracts from seeds of *Peganum harmala* Linn: A 28-day study in rats. Journal of Ethnopharmacology, 238.

Weidner N. Weiss LM. Richard J. Suster CS. 2009. Modern Surgical Pathology (CHAPTER 39-Ovaries), 2ed, 2,1356-1408

Woo CSJ, Lau JSH, El-Nezami H. 2012. **Advances in Botanical Research** (Chapter 10 - Herbal Medicine: Toxicity and Recent Trends in Assessing Their Potential Toxic Effects), 62: 365-384.

Yousefi R, Ghaffarifar F, Asl AD. 2009. The Effect of Alkanna tincturia and Peganum harmala Extracts on Leishmania major (MRHO/IR/75/ER) in vitro. ***Iranian Journal of Parasitology,*** 4 (1): 40-47.

Yuruktumen A, Karaduman S, Bengi F, Fowler J. 2008. Syrian rue tea: a recipe for disaster. Clinical Toxicology journal, 468:749-52.

Z

Zakowski MI, Geller A. 2020**.** The Placenta: Anatomy, Physiology, and Transfer of Drugs**.** Chestnut's Obstetric Anesthesia, 4, 56-76.

Zargari M, Mikaili P, Aghajanshakeri S, Asghari M. H, Shayegh J. 2013. Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. Pharmacognosy Reviews, 7(14):199–2013.

Zutshi U, Rao PG, Soari A, Gupta OP, Atal CK. 1980. Absorption and distribution of vasieine, a modern uterotonic. Planta Meddca journal, 40(40):373-377.

Turkish Journal of Agriculture - Food Science and Technology, 10(10): 2067-2075, 2022
DOI: https://doi.org/10.24925/turjaf.v10i10.2067-2075.5491

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Evaluation of Maternal Toxicity in Rats Exposed to the Total Extract of the Alkaloids in the seeds of *Peganum harmala* L. during Pregnancy

Sarra Bettihi[1,a,*], Nadia Mahdeb[1,b], Abdelouahab Bouzidi[1,c]

[1]*Department of Biochemistry, Faculty of natural sciences and life, University Ferhat Abbas, Setif 19000, Algeria*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 07/09/2022
Accepted : 29/09/2022

Keywords:
Peganum harmala L
Alkaloids
Pregnancy
Maternal toxicity
Serum biochemistry

ABSTRACT

Peganum harmala L. (Zygophyllaceae) known locally as harmel is a medicinal plant. In traditional medicine, its seeds have long been used for therapeutic purposes because of their richness in β-carboline alkaloids. This study aimed to evaluate the maternal and developmental toxicity during pregnancy by daily IP administration of 7.99 mg/kg/day (1/20 DL_{50}) of total alkaloids extract in P.harmala' seeds. The results summarized in confirmed pregnancy rates were high 90-100%, decreased locomotor activity, paralysis, and hypothermia. Maternal body weight and weight gain changes were statistically significant in all pregnant. Precisely, the relative weight of ovaries was significantly changed in all the groups treated. The ALAT and gamma GT concentrations show a significant change in the group treated for seven days. Significant changes in the total and indirect bilirubin levels were observed in all treated groups. The hormonal analysis showed a significant decrease in FSH levels in a treated group for seven days and two weeks, Progesterone levels were increased significantly in treated groups for seven and three weeks and increased significantly in a treated group for two groups, however, the levels of Estrogen were changed significantly only in the treated group for three. The results show a significant difference in total resorbed litters and the number of fetus deaths in the group treated for three weeks. The fetus weight in the group treated for two weeks was significant. The results show a significant decrease in the number of implantations and an increase in pre-and post-implantation loss rates, and there were no developed live or dead, and no resorbed fetuses in all treated dams, there were only implantation sites in both uterine horns. The total extract of the alkaloids in the seeds of P. harmala has adverse effects on maternotoxicity, embryonic development, and abortion.

[a] *sarra_bettihi@outlook.fr* *https://orcid.org/0000-0002-8692-1972*
[b] *nmahdeb@yahoo.com* *https://orcid.org/0000-0003-1568-8752*
[c] *bouzidiab@yahoo.fr* *https://orcid.org/0000-0002-4370-0600*

This work is licensed under Creative Commons Attribution 4.0 International License

Introduction

Folk medicine has a lengthy history of evolution over time, which is a combination of many different cultures (Uysal et al., 2021; Akgül et al., 2022). Presently, internet access is the most essential and inexhaustible resource of all knowledge about ethnobotanical plants since it is simple to utilize (Kına et al., 2021; Pehlivan et al., 2021). Furthermore, the most significant advantage of herbal medication is its inexpensive cost in comparison to modern pharmaceuticals, as well as the confidence in minimal adverse effects (Pu et al., 2017; Sevindik et al., 2017; Mohammed et al., 2022), which put people at risk of the poisoning by these plant materials intentionally or accidentally (Hamdy et al., 2017; Al-Tohamy et al., 2018). *Peganum harmala* L. (Zygophyllaceae) known locally as harmel is a plant commonly found in Algeria. It occupies the stations of arid hillsides, dry uncultivated fields, and earthy steppes (Jahandiez and Maire, 1932). In traditional medicine, its seeds have long been used as narcotics, anthelmintics, antispasmodics, and in some cases against rheumatism and asthma (Siddiqui et al., 1988; Bellakhdar, 1997). This use of seeds is due to their richness in β-carboline alkaloids, the most important are harmine, harmaline, harmol, and harmalol as has already been reported for the first time. By Goebel, 1841 (Merck Index, 1989) and taken up by many authors. Its pharmacological interest is no longer to be demonstrated: it exhibits antiviral activity (Rashan and Adaay, 1989), abortive in rats (Shapira et al., 1989; Nath et al., 1993; Adaay 1994), and exhibits toxicity even in humans (Ben Salah et al., 1986). In the present paper, we summarized the maternal and developmental toxicity of total extract of the alkaloids in the seeds of P. harmala in experimental animals during Pregnancy. Few studies have reported significant findings regarding the developmental toxicity of alkaloids in humans. Available data from animal experiments may indicate the cause for concern. unless otherwise stated.

Materials and Methods

Plant Collection and Identification

P. harmala seeds were harvested in August from the Touama area in Bordj Bou Arreridj (northeast Algeria), which has a dry, semi-arid environment. The plant and its seeds were identified based on morphological features (Chopra et al., 1960) (Figure 1). Before extraction, the seeds were air dried at ambient temperature (20 to 25°C) for more than a month, then ground individually in a coffee grinder during the extraction.

Figure 1. The seeds of *Peganum harmala* L.

Extraction of the Total Alkaloids

The complete alkaloid extract in the seeds of P. harmala was extracted by using the Soxhlet extraction technique of Bruneton, (1999) (with slight adjustments), and the presence of alkaloids in the extract was confirmed using Thin Layer of Chromatography (TLC) as described by Bouzidi et al., 2011.

Experiment Animals and Husbandry

Fifteen proven fertile adult males and forty virgin or nulliparous healthy young female albinos wistar rats, 10 weeks of age and weighing 170–200 g, were obtained from our colony in the Ferhat abbes university

For husbandry, three females were placed into a cage with a male rat overnight. The next morning successful mating was confirmed by the presence of sperm in the vaginal smear (Jahnke, 1999), and the following 24 hours were designated as day 0 of gestation (GD0). Confirmed-mated females were assigned to treatment groups by stratified randomization (9/group in the screening study), so that mean body weight on GD0 did not differ among treatment groups in the study. Maternal body weights, for confirmed pregnant females used in these studies, ranged from 170 to 200 g on GD0. Confirmed-mated females were individually housed were housed individually and the Pregnancy was verified by weight gain and abdominal palpation.

Dosage and Treatment

Dose formulations were prepared by dissolving 1/20 DL_{50} mg witch equal to 7.99 mg of total extract of alkaloids of the seeds of P. harmala in10 micro-liter of methanol and one milliliter of normal saline. The suspension has subjected to agitation for 3 minutes to obtain a more homogenous and dispersed suspension. The test dose was prepared daily prior to use. In the morning, time-mated rats were administered a dose volume of 7.99 mg/kg/ day of total extract of the alkaloids daily by intraperitoneal injection. The pregnant rats received the treatment from GD0 to GD6 designed to be the group treated for seven days, GD0 to GD15 of treatment a group treated for two weeks, and a group treated for three weeks from GD0 through GD19. Control rats received an equivalent volume of vehicles alone.

Maternal Evaluations

Clinical Signs, Body Weight

Throughout pregnancy, mated females were observed daily for mortality, morbidity, general appearance, behavior, and clinical condition at least once/day on GD0 through 19, females were observed for clinical condition and signs of toxicity at daily dosing, and generally at first to 8 hours thereafter. Maternal body weights (g) were recorded on the mornings of GD0 and GD20, and immediately after the following sacrifice on GD20. On GD20, females were observed for the clinical condition at weighing and again at scheduled termination.

Gross Findings, Organ Weights

On GD 20, all time-mated females were euthanized and sacrificed by an overdose of ether and were exsanguinated via the aorta in the early morning. A complete gross postmortem (Thoracic and abdominal cavities) examination was performed. The absolute and relative weights of the brain, lungs, liver, spleen, kidneys, heart, and ovaries were recorded. In addition, Pregnancy status was confirmed by uterine examination. Uterine contents were examined to determine the number of implantation sites (sum of live pups and dead fetuses), pre/post-implantation, resorptions, dead or resorbed fetuses, and live fetuses. Uteri which observed very well to record any implantation sites which might have undergone very early resorption (Jahnkle, 1999). the reproductive parameters were computed According to Toyin et al, (2014).

Serum Biochemical Examination

Blood samples were taken from their orbital sinus by hematocrit, and were centrifuged at 3000 rpm for 10 minutes within 1 hour of collection. Using a biochemical analysis is important to estimate alterations in organ function. After serum isolation, all the hepatic (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutaryl-transferase, alkaline phosphatase (PAL), total, direct, and indirect bilirubin), renal parameters (blood urea nitrogen, creatinine, glucose, and monogrammed Na/K/Cl), and hematologic parameters (RBC, Hb, HCT, MPV, MCV, MCHC, MDW, WBC, LYM, PDI, PTC, LPCR, MID, GRA)

Serum Hormonal Examination

The density of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and progesterone hormones was measured according to the ELISA in the laboratory.

Statistical Analysis

The unit for statistical measurement was the pregnant female or the litter. For each statistical comparison, the significance was reported as $P<0.05$. Nonparametric tests applied to continuous variables included the Tukey; one-way analysis of variance (ANOVA) by ranks for among-

group differences for a significant $P<0.05$, and two-way analysis was used for all parameters, except that maternal and fetal body weight parameters, were used to identify significant dose-response trends.

Results

Pregnancy Detection

The presence of sperm in the vaginal smear or observation of a vaginal plug indicates the occurrence of mating. On the other hand, detection of sperm in vaginal smear is an excellent predictor of pregnancy in rats. The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. After 10 days of gestation, the fetuses can be palpated, but palpation is more accurate after day twelve. By day thirteen of gestation, the abdominal enlargement is visible, and mammary development and nipple enlargement can be observed on day 14 of gestation. Successful mating was confirmed by the presence of sperm in the vaginal smear (Figure 2) the following morning and this day was considered day 1 of pregnancy. The rate pregnancy rate was high 100% in the both groups; control and mated females.

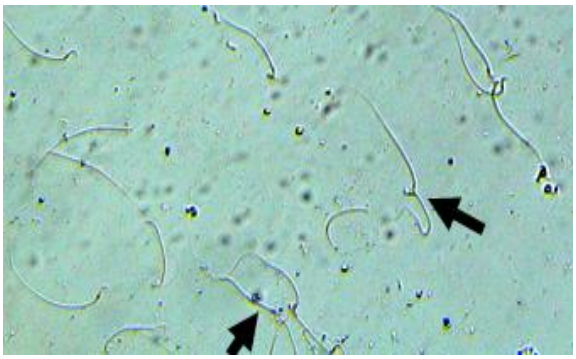

Figure 2. The presence of sperm in the vaginal smear

Maternal toxicity, Abortion

Maternal Evaluations; Clinical Signs and Body Weight

In the maternal and developmental toxicity by repeated intra-peritoneal administration of 1/20 DL_{50} mg/kg/day of total extract of the alkaloids in the seeds of P. harmala, confirmed pregnancy rates were high 90-100% for all groups (Table 1) and no maternal morbidity/mortality was observed in the dams of the control, treated groups for seven days, two weeks, three weeks. On the other hand, decreased locomotor activity, paralysis, dyspnea, depression, and hypothermia were identified in dams of treated groups by total extract in the first two hours after the treatment and only continued in the first seven days of the gestational period.

Upon measuring the maternal body weight and weight gain statistically significant changes were observed in all pregnancy rates during pregnancy whether these changes were an increase or a decrease between the control group and treated groups for seven, two, and three weeks. In addition, there were statistically significant differences in maternal weight gain during pregnancy for the group treated with 1/20 DL_{50} mg/kg/day of total extract (Table 1).

The results show that the relative weights of the liver, heart, and brain were no significant changes in all experimental groups, while the relative weight of kidneys in the groups of females treated for seven days and three weeks by the total extract was significantly increased, but not in the other group in comparison to the control, for the relative weight of lung a the significant increased was observed in the group treated for seven day and two weeks and only in group treated for seven days has a significant increased in spleen relative weight. Precisely the relative weight of ovaries was significantly changed in all the groups treated with the total extract compared to the control group (Figure 3).

Serum Biochemical and Hormonal Analyses

To judge functional abnormalities in the dams, we analyzed serum biochemical values at the end of the gestational period. There were no statistically significant differences in the Aspartate ASAT and PLA concentrations between the treated groups and the control, while the ALAT (Figure 4) and gamma GT concentrations show significant changes in the group treated for seven days only (Figure 5). Significant changes in the total and indirect bilirubin levels were observed in all treated groups in comparison to the control (Figure 6). The renal parameters show a significant change in glucose, blood urea nitrogen and creatinine levels in the group treated for two weeks, the group treated for three weeks and the group treated for seven days respectively (Figure 7). The blood ionogram analysis show no significant changes in the potassium (K), but the levels of sodium (Na) were change significantly in the groups treated for seven days and two weeks, while the levels of chloride (Cl) changed significantly in the group treated for two weeks (Figure 8).

The hormonal analysis shows a significant decrease in FSH levels in the treated group for seven days and two weeks as shown in Figure 9, Progesterone levels increased significantly in treated groups for seven and three weeks and increase significantly in the treated group for two groups, however, the levels of Estrogen were changes significantly only in the treated group for three weeks as shown in Figure 10. There were no changes in the LH levels between the groups and the control.

Hematological Analyses

The blood parameters demonstrated significant changes between the control group and the treated groups in all the parameters as we can see in Table 2.

Embryo/Fetal Evaluations

Table 3 summarizes the reproductive and the findings for the pregnant treated rats with 1/20 DL_{50} mg/kg/day of total extract of the alkaloids on gestational days 1 through 20. The results show a significant difference in total resorbed litters and the number of fetus deaths in the group treated for three weeks only. There were no significant differences between the treated groups related to the number of corpora lutea and between the control and treated groups, the fetus weight in the group treated for two weeks was significant. A remarkable result in all the studies was in the group treated for seven days, Thus the results show a significant decrease in the number of implantations, an increase in pre-and post-implantation loss rates, and there were no developed live or dead, and no resorbed fetuses in all treated dams, there were only implantation sites in both uterine horns.

Table 1. Dam body weight changes of pregnant rats

Parameters	1/20 DL $_{50}$ of total extract (mg/kg/day)			
	Group control	Group treated for seven days	Group treated for two weeks	Group treated for three weeks
Maternal pregnancy status				
No. of rats mated	10	9	9	10
No. of dams	10	8	8	9
Maternal body weight (g)[A,B]				
Gd 1	215±5.137	171.66±5.713	169.88±4,392	178.33±2.205
Gd 7	220±4.249	178.88±8.489*	209.44±4.522	183.88±3.977
Gd 14	224.44±5.429	189.44±5.429*	227.22±4.867*	179.44±5.234
Gd 21	233.88±5.122	196.11±5.122*	252.77±8.544*	198±6.388*

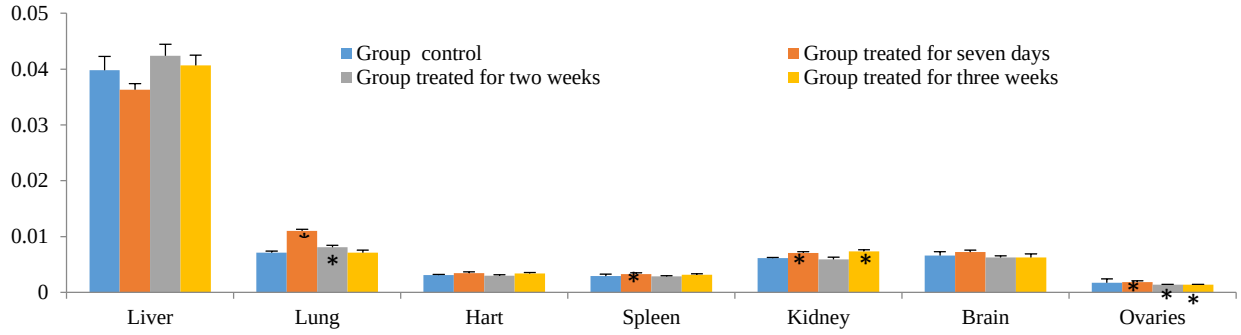

Figure 3. Maternal relative weights of pregnant rats. Values are presented as the means±SEM, * significant differences at P<0.05.

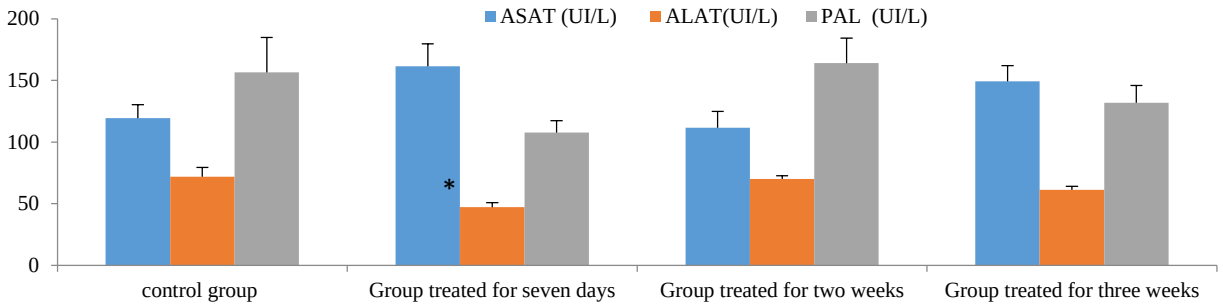

Figure 4. Maternal serum biochemical values of ASAT, ALAT and PAL in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

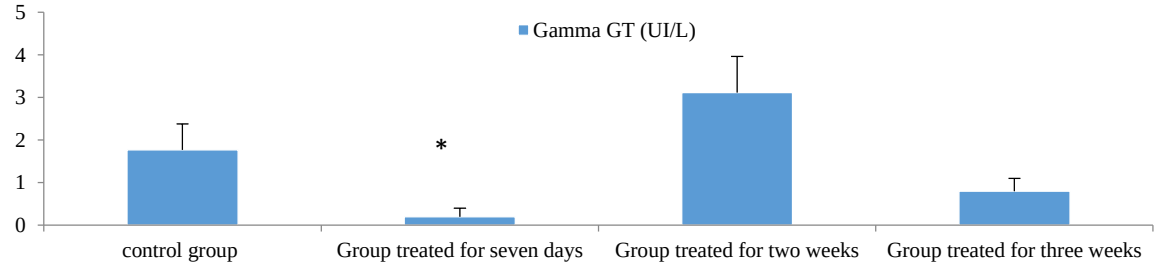

Figure 5. Maternal serum biochemical values of Gamma GT in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

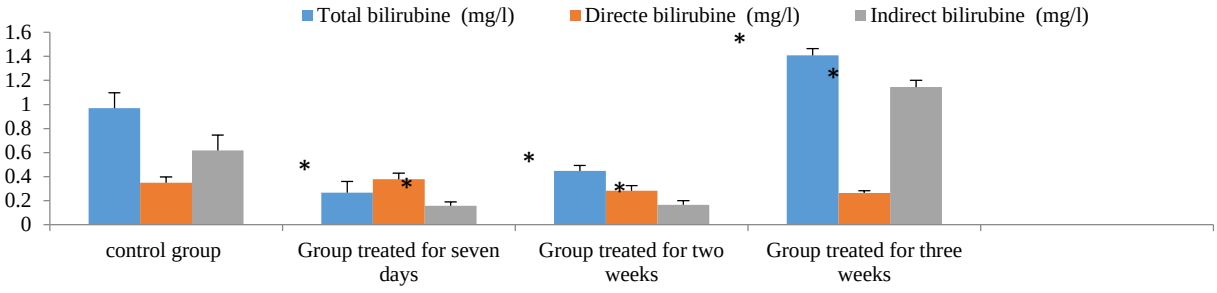

Figure 6. Maternal serum biochemical values of total, direct and indirect bilirubine. Values are presented as the Means±SEM, * significant differences at P<0.05.

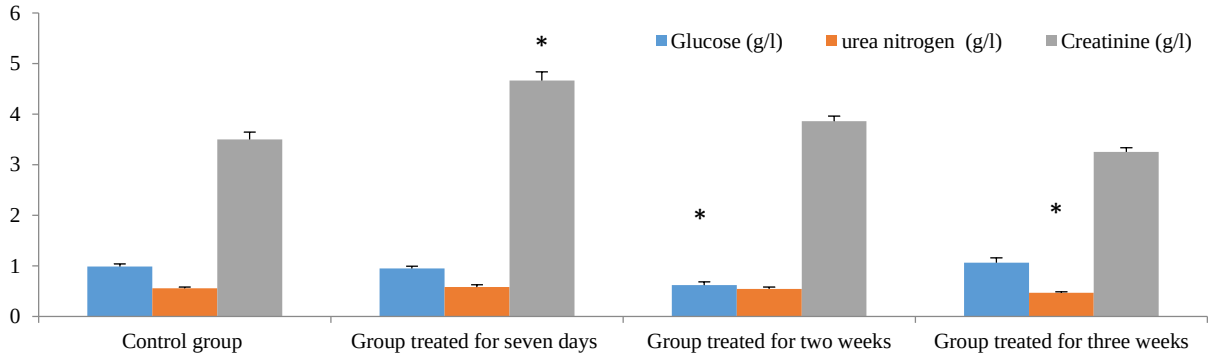

Figure 7. Maternal serum biochemical values of glucose, blood urea nitrogen and creatinine in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

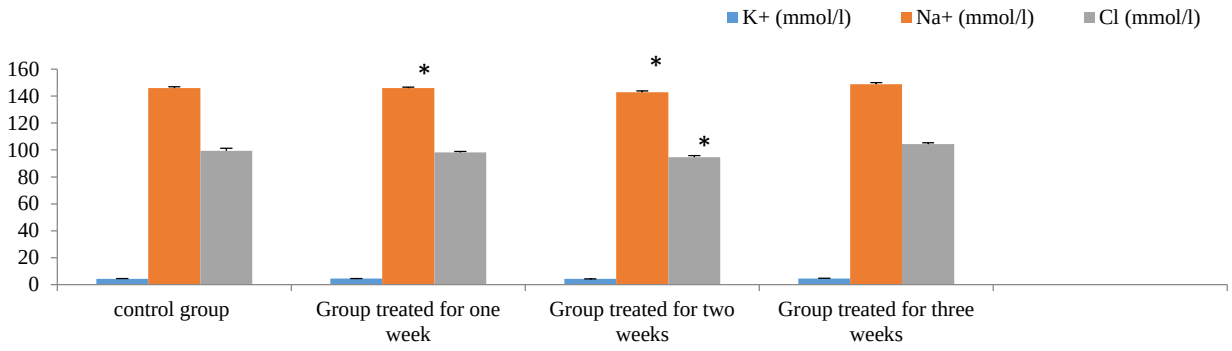

Figure 8. Maternal serum biochemical values of blood ionogramme k+, Na+ and Cl- in pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

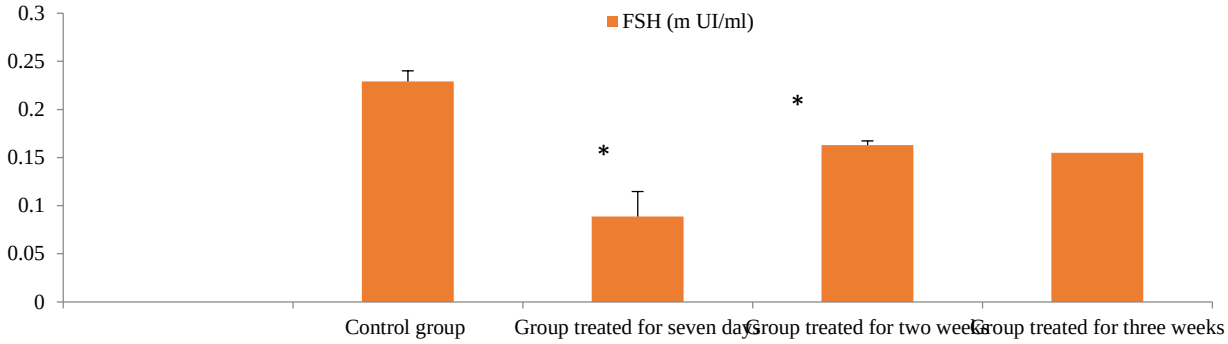

Figure 9. Maternal hormonal values of FSH in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

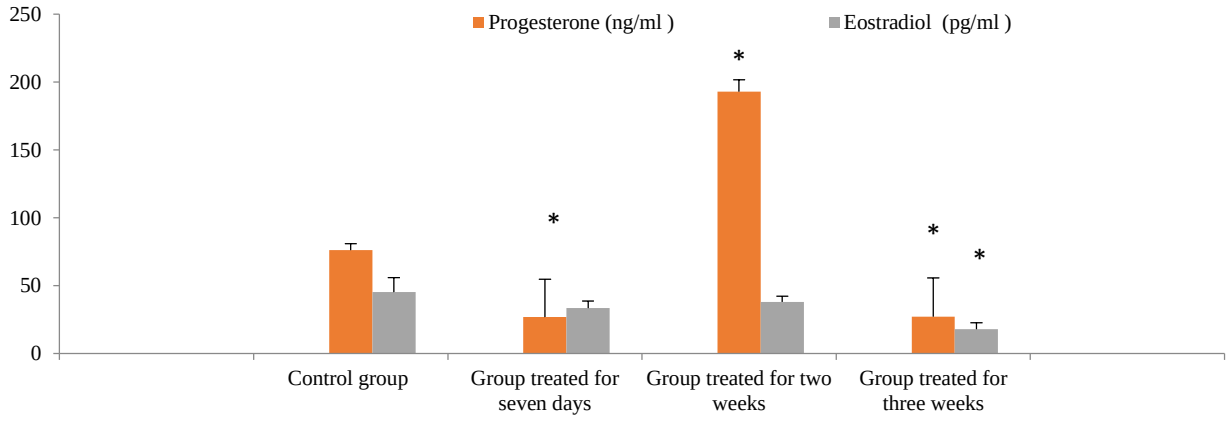

Figure 10. Maternal hormonal values of Progesterone and Estradiol in of pregnant rats. Values are presented as the Means±SEM,*significant differences at P<0.05.

Table 2. Effects of 1/20 DL50 of total extract in the seed of *Peganum harmala* on hematological parameters in female rats.

P	RBC10*6/mm^3	Hb g/dl	HCT %	MPV fl	MCV fl
C	7.004±0.314	12.957±0.495	37.514±1.48	53.743±0.854	5.929±0.115
7D	7.634±0.195	13.344±0.301	42.944±1.034*	56.278±0.376*	7.762±0.127*
2W	5.501±0.588*	10.3±1.034*	29.589±3.12*	54.122±0.548	6.044±0.0818
3W	6.831±0.184	12.613±0.265	36.825±0.929	53.925±0.497	6.188±0.0811
P	MCHC g/dl	RDW	WBC10*3/mm3	Platelet	
C	34.729±0.218	29.257±0.934	5.573±0,642	657.574±112.54	
7D	31.078±0.145*	26.578±0.805*	6.284±0.314	907.903±125.96*	
2W	35.656±0.908	29.733±0.685	5.671±0.837	749.111±73.209	
3W	34.25±0.241	29.325±0.666	6.891±0.955	679.908±98.44	

P: Parameters; C: Control group; 7D: Treated group for seven days; 2W: Treated group for two weeks; 3W: Treated group for three weeks

Table 3. Dam Cesarean section observations and fetal weights

Parameters	1/20 DL $_{50}$ of **total extract** (mg/kg/day)			
	Group control	Group treated for seven days	Group treated for two weeks	Group treated for three weeks
Maternal pregnancy status				
No. corpora lutea/dam	11.222±0.364	11.00±0.840	11.333±0.687	11.778±0.619
Total implant sites				
No. implantation sites/dam	8.5±0.719	3.00±0.00*	9.625±0.498	9.40±0.872
Pre-implantation loss (%)^A	24.25	72.72*	15.07	20.19
Post-implantation loss (%)^B	13.76	100*	14.28	19.14
No. resorptions/dam	0.778±0.434	0.429±0.429*	1.222±0.401	4.333±1.453*
No. fetal life/dam	7.33±0.989	00±00*	8.25±0.701	7.6±0.872
No. fetal death/dam	0.778±0.434	0.429±0.429*	1.222±0.401	4.333±1.453*
Fetal weight (g)	1.074±0.167	-	1.555±0.054*	1.76±0.442
No. stunted fetuses				

A: Values are presented as means ± SD.,*significant differences at P<0.05; B: Pre-implantation loss (%) = [(No. of corpora lutea - No. of implantation sites)/ No. of corpora lutea] ×100; C: Post-implantation loss (%) = [(No. of implantation sites - No. of live embryos)/ No. of implantation sites] ×100.

Discussion

A toxicant usually induces more than one type of effect in a dose-dependent manner. For example, induction of malformation is almost invariably associated with increased embryonic death and an increased incidence of less severe structural changes. Given an effect on one endpoint, secondary investigations for possible associations should be considered, (the nature, scope, and origins of the substance's toxicity should be characterized). The characterization should also include the identification of dose-response relationships to facilitate risk assessment; this is different from the situation in first-pass tests where the presence or absence of a dose-response assists discrimination between treatment-related and coincidental differences (Health Canada Detection of Toxicity to Reproduction for Medicinal Products, Guidance for Industry ICH Topic S5A, 1996). An Embryotoxicity study was conducted to investigate potential adverse effects of total extract of the alkaloids of P. harmala. on pregnant dams or maternotoxicity, embryonic development, and the abortion by inter-peritoneal repeated dosing of 1/20 DL_{50} mg/kg/day on three stages of gestation from GD1 to GD7, GD1 to GD14 and through 21 days.

The results obtained showed no mortality of dams whether the toxicity signs were observed as a daily decrease in locomotor activity, paralysis, depression, and hypothermia first two hours and continued for the first few days of the treatment period only. The clinical presentation of this study is nearly similar to the case report of Azizi et al., (1989), in which Harmaline and Harmine treatment by different inter-peritoneal concentrations in pregnant rats, resulted in toxic symptoms including decreased daily activity and mild tremor were seen that progressed to restlessness, severe tremor (Azizi et al., 1989). particularly when P. harmala co-ingested with other drugs or foods such as the case reported by Yuruktumen et al. (2008) toxic effects of other cases presented 3-4 hours after ingestion of the plant. In human cases of P. harmala intoxication precisely in women (Frison et al., 2008; Yuruktumen et al., 2008) and animals (Marwat et al., 2011) most signs were hypotension. This symptom is related to beta–carbolines, especially Harman. in another hand, the MAO inhibitory by harmaline can induce hypertension crisis in high doses. These intoxications are presented with nervous and digestive system symptoms. Nervous system presentations commence with excitability and progress to muscle trembling and stiffness (Mahmoudian et al., 2002). Animals also have hypothermia, dyspnea, and mydriasis. Neurological presentations are prominent in all cases of harmal toxicity. Our case had tremors as others. It seems that these are related to harmaline effect (Moshiri et al., 2012). Maternal body weight during the gestation period was used to assess maternal toxicity. The change in maternal body weight and weight gain in our study is contrary to the study of treated dams with methanol and acetone extracts of the epigeal parts of P. harmala administered orally for 30 days which suggests that the absence of weight change is not due to malnutrition and/or to toxification (Shapira et al., 1989). But our results are nearly similar to the report of Adaay, 2014, the changes in the body weight of dams treated with 5mg/kg of harmine

and harmaline were negligible, whereas dams treated with 10mg/kg of both alkaloids showed an increase in their body weight. None of the two dosages of harmine and harmaline used in this investigation was lethal to the dams. The daily food intake of dams given was not affected. On the other hand, animals treated with 10mg/kg of harmine and harmaline consumed more food than did the controls. The results revealed that the total extract of the alkaloids during pregnancy is minimally maternotoxic. To judge the functional abnormalities in the dams, we analyzed serum biochemical values at the end of the gestational period. Briefly, ALAT, gamma GT concentrations, and indirect Bilirubin levels show significant changes. The renal parameters show a significant change in glucose, Urea Nitrogen, creatinine, Na, and Cl were changed significantly, The hormonal analysis shows a significant decrease in FSH, Progesterone, and Estrogen. In contrast to the case report by Pranzatelli and Snodgrass, 1987, rats treated with P. harmala showed tremors and convulsions with normal biochemical lab tests (Pranzatelli and Snodgrass, 1987). However, chronic oral administration of aqueous extract of P. harmala for 3 months to rats increased transaminase levels according to Marwat et al., 2011 and Moshiri et al., 2012. According to the study by Komeili et al., 2016 about the effect of the hydroalcoholic extract of P. harmala seeds for 4 weeks in the Streptozotocin-induced diabetic (Zitch) rats which showed an increase in blood glucose as well as changes in lipid profile in comparison with normal rats, while the treatment by hydroalcoholic extract showed a significantly decreased in the levels of glucose, triglyceride, cholesterol, and LDL-c and increased the level of HDL-c in diabetic rats Zitch. These findings are similar to the issue of Singh et al., 2008) which showed that ethanolic extract of P. harmala seed significantly decreased blood glucose levels in normal and diabetic rats at doses of 150 and 250 mg/kg of body weight. P. harmala has been traditionally used to treat diabetes in folk medicine in some parts of the world (Moloudizargari et al., 2013). In mammals, embryo/fetal development occurs within the maternal organism, where mothers receive the initial exposure to chemicals while embryos and fetuses are exposed secondarily through the placenta, a maternal-fetal unit. Moreover, if chemicals undergo biotransformation after being absorbed, the embryos and fetuses are exposed not only to the chemical itself but also to metabolites produced in the maternal organism (Paumgartten, 2010). Our results show a significant difference in totally resorbed litters, the number of fetus death, and a decrease in the fetus weight with a remarkable decrease in the number of implantations, the increase of pre-and post-implantation loss rates, and undeveloped dead fetuses. These are nearly similar to the result of the effect of methanol and acetone extracts of the epigeal parts of P. harmala administered orally for 30 days, The methanol extracts at doses used appeared to produce a dose-dependent significant decrease in body weight in fetuses size. No change in the physical and nutritional status of the animals and no adverse toxicological effects were observed (Shapira et al., 1989). According to the report of Merzouki and his collaborators, the infusion of mixed the Leaves and fruits of P. harmala and administered orally provoke an abortion, mentioned also that midwives of Casablanca in morocco used P. harmala seeds are toxic, and their abortive power by a decoction of a handful of the seed is taken orally to provoke abortion (Marzouki et al., 2000; Bellakhdar, 1997). Here we can suggest that the total extract of the alkaloids of P. harmala can be considered an anti-fertility, anti-implantation, and abortion agent according to our results and the other reports. It was important to mention in the report of Shapira et al., 1987, that the absence of adverse histopathological findings suggests that the effect of methanol extracts on the estrous cycle, as well as on reproduction rate, can be attributed to a direct effect on targets associated within the reproductive system, and are not due to malnutrition and/or to toxification. Chemically, the epigeal parts of P. harmala are known to contain various β-carbolines such as harmaline, harmine, quinazoline alkaloids, vasicine, and vasicinol (Kashimov et al., 1971) with a wide spectrum of biological activities. The effects attributed to harmine and harmaline are the inhibition of sodium-dependent transport (Sepulveda and Robinson, 1974), elevation in the production of hypothalamic norepinephrine and serotonin (Queshi et al., 1979), and the abolition of inhibitory synaptic transmission (Sokolove and Roth, 1978). Abortifacient activity has been reported only for vasicine alkaloids, which have been found to have a uterine stimulatory effect, apparently through the release of prostaglandins (Gupta et al., 1978; Zutshi et al., 1980). Currently, we are in the process of isolating vasicine, vasicinone, deoxivasicinon, harmine, and harmaline, after which it will be possible to evaluate separately the specific effects of each of these alkaloids on the reproductive system. These findings are nearly similar to the case report of Shapira et al., 1989, have reported that The methanolic extract reduced the number of living pups and increased the number of resorption. This extract at doses used produced a decrease in litter size. It is believed that quinazoline alkaloids (vasicine and vasicinone) are responsible for the abortifacient activity of P. harmala extracts. It has been reported that these chemicals have a uterine stimulatory effect, apparently through the release of prostaglandins (Gupta et al., 1978; Zutshi et al.1980; Mahmoudian et al., 2002; Kuete, 2013), but on another side, the study of Fathiazada et al, (2006) revealed that the effects of hydro-alcoholic extract of P. harmala seeds on spontaneous rhythmic contractions of isolated rat uterine were tested on the isolated uterus and endometrial free preparations. The extract of P. harmala seeds was found to exhibit significant spontaneous contractions of the uterus and stripped myometrium relative to the solvent control. After recording the pattern of uterus tissue spontaneous motility, in order to determine the mechanism of the extract of P. harmala seed's pharmacological effects, atropine, indomethacin, or prazosin was added to the organ baths. Pretreatment with atropine in both the whole uterus and in the stripped myometrium preparations had no effects on the response to cumulative dosage of extract of P. harmala' seeds. Calcium-free solution decreased uterus contractions. In calcium dose-response curves, extract of P. harmala seeds in some concentrations produced a uterotonic effect in calcium-free solution in the presence of KCl. This finding showed that EPS may increase calcium influx through voltage-dependant calcium channels and the effects of P. harmala on rat uterus are not dependent on prostaglandins, these contractions are related to external calcium (Fathiazada et al., 2006). Reduced litter size at

birth may be due to a reduced ovulation rate (corpora lutea count), higher rate of pre-implantation deaths, higher rate of post-implantation deaths, or immediate post-natal deaths. In turn, these deaths may be the consequence of an earlier physical malformation that can no longer be observed due to subsequent secondary changes, and so on. Particularly for effects with a natural low frequency among controls, discrimination between treatment-induced and coincidental occurrence is dependent upon association with other types of effects (Shapira et al., 1989). Contrary to the result of the report of (Azizi et al., 1989), where Harmaline and Harmine were injected intraperitoneally into pregnant rats, injections on different days of pregnancy from 1st – 23rd days showed no different toxic symptoms in treated rats. They observed no abortion in rats that received l-80 µg/Kg of alkaloids and all rats delivered live births on time with no obvious abnormalities. Therefore, it is concluded that, because of observed toxicity which can be lethal, pregnant women should not use seeds of espand (Azizi et al., 1989). But the embryotoxicity study of Adaay, 2013 on the effects of harmaline and harmol given intraperitoneally to pregnant rats on days 1st-14th of gestation revealed that the average number of implantation sites and live fetuses per dam was within the control range in the different dose levels of the two compounds. Fetal death or resorption was increased in the treated groups in comparison with the control group, this increase was statistically significant in the groups treated with 10mg/kg of harmaline and harmol. A decrease in fetal body weight was evident in the 5 and 10mg/kg groups of harmaline and harmol in comparison with the control group. Two fetuses from the group treated with 5mg/kg of harmol were abnormal, in one of them the toes of the left leg were missing, and two toes emerged from an abnormal position at the leg, the other fetus showed missing toes and femur of the right hand. The fetal mortality and decrease in their body weight indicate that harmaline and harmol or their metabolites cross the placenta at a fast enough rate to reach concentrations toxic to the embryo. It has been suggested that some harmala alkaloids harmaline and harmol or their metabolites may induce embryo-lethality which varies with the stage of gestation. (Adaay, 2013)

Conclusion

In the present study, The total extract of the alkaloids in the seeds of P. harmala has adverse effects on maternal toxicity, embryonic development, and abortion.

Acknowledgment

The authors wish to thank colleagues Faculty of Natural Sciences and Life, University + valuable suggestions in improving earlier version of manuscript.

References

Adaay MH. 2014. Embryotoxicity of harmaline and harmalol hydrochloride on pregnant rats. Mater. Environment Science journal, 5:1:127-132.

Adaay MM. 1994. Some observations on the reproduction toxicity of the aqueous extract of Peganum harmala seeds. J. Fitoterapia, 65: 214-218

Akgül H, Mohammed FS, Kına E, Uysal İ, Sevindik M, Doğan M. 2022. Total Antioxidant and Oxidant Status and DPPH Free radical activity of *Euphorbia eriophora*. Turkish Journal of Agriculture-Food Science and Technology, 10(2): 272-275.

Al-Tohamy R, Ali SS, Saad-Allah K, Fareed M, Ali M, El-Badry A. 2018. Phytochemical analysis and assessment of antioxidant and antimicrobial activities of some medicinal plant species from Egyptian flora. Applied Biomedicine journal, 6: 289–300.

Azizi A, Akbari-javar H, Salehi MH, Jalali N. 1998. Evaluation of toxic alkaloids of seeds of *Peganum harmala* L. as an abortion agent in pregnant rat. Pathophysiology journal, 5:1001:94.

Bellakhdar J. 1997. La pharmacopée marocaine (Médecine arabe et ancienne et savoirs populaires). Ibis Press. Saint Etienne, 746.

Ben Salah N, Amamou M, Jerbi Z, Ben Salah F, Yacoub M. 1986. Un cas de surdosage en *Peganum harmala* L. Toxicologie Clinique et Expérimentale journal, 6: 319-322.

Bouzidi A, Mahdeb N, Kara N. 2011. Toxicity studies of alkaloids of seeds of *Datura stramonium* and synthesis alkaloids in male rats. Medicinal Plants Research journal, 4: 3421-3431.

Bruneton J. 1999. Pharmacognosie. Photochimie, Plantes médicinales. 3rd ed. Lavoisier, Paris, TEC & DOC, 973-783.

Budavari S. 1989. An Encyclopedia of chemicals, drugs and biological, 1606.

Chopra IC, Abrol BK, Handa. 1960. The pharmacological aspects. In: Medicinal Plants of the Arid Zones, Chopra IC, Abrol BK, Handa L, Paris R, Dillemann G (eds.) UNESCO Publications, Paris, 11–54.

Fathiazada F, AzarmibY, Khodaiea L. 2006. Pharmacological Effects of *Peganum harmala* Seeds Extract on Isolated Rat Uterus. Iranian Journal of Pharmaceutical Sciences Spring, 2:2: 81-86.

Frison G, Favretto D, Zancanaro F, Fazzin G, Ferrara SD. 2008. A case of b-carboline alkaloid intoxication following ingestion of *Peganum harmala* seed extract. Forensic Science International journal, 179: 37–43.

Gupta OP, Anand KK. Ghatak BJR, Atal CK. 1978. Vasicine. alkaloid of Adhatoda vasica, a promising uterotonic abortifacient. Indian journal Exp Biol, 16:1075-1077.

Hamdy AA, kaseem HA, Awad EA, EI-Kady SM, Bento MT, Doyaguez EG, Jemeno ML, Lall AA, Hussein AA. 2017. In-vitro evaluation of certain Egyptian traditional medicinal plants against *Propionibacterium acnes.* South African Journal of Botany, 109:90-95.

Health canada detection of toxicity to reproduction for medicinal products, guidance for industry ICH topic S5A. 1996.

Jahandiez E, Maire R. 1932. Catalogue des plantes du Maroc (Spermaphytes et Ptéridophytes); Dicotylédones et Archichlamydées. Minerva. Alger, 2:557.

Jahnke G, Marr M, Myers C, Wilson R, Travlos G. 1999. Price C Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. toxicological sciences journal, 50:2:271-9.

Kasbimov HN, Teclezhenetskaya MV, Sharakhimar NN, Yunasov SY. 1971. The dynamics of the accumulation of alkaloids in *Peganum harmala.* Chemistry of Natural Cornpounds journal, 3:364-365.

Kına E, Uysal İ, Mohammed FS, Doğan M, Sevindik M. 2021. In-vitro antioxidant and oxidant properties of *Centaurea rigida*. Turkish Journal of Agriculture-Food Science and Technology, 9(10): 1905-1907.

Komeili G, Hashemi M, Bameri-Niafar M. 2016. Evaluation of antidiabetic and antihyperlipidemic effects of *peganum harmala* seeds in diabetic rats. Hindawi Publishing Corporation Cholesterol journal, 7389864:6.

Kuete V. 2014. Physical, Hematological, and Histopathological Signs of Toxicity Induced by African Medicinal Plants Toxicological Survey of African Medicinal Plants. 1st ed, 235-257.

Mahmoudian M, Jalilpour H, Salehian P. 2002. Toxicity of *Peganum harmala*. Iranian Journal of Pharmacology & Therapeutics, 1:1-4.

Marwat SK, Rehman F. 2011. Medicinal and pharmacological potential of harmala (*Peganum harmala* l.) seeds. Nuts and Seeds in Health and Disease Prevention- Chapter 70. Press, 585-599.

Merzouki AC, Ed-derfoufi BF , Molero Mesa CJ. 2000. Short communication Hemp (Cannabis sativa L.) and abortion. Ethnopharmacology journal, 73:501–503.

Mohammed FS, Kına E, Uysal İ, Mencik K, Dogan M, Pehlivan M, Sevindik M. 2022. Antioxidant and Antimicrobial Activities of Ethanol Extract of *Lepidium spinosum*. Turkish Journal of Agriculture-Food Science and Technology, 10(6): 1116-1119.

Moloudizargari M, Mikaili P, Aghajanshakeri S, Asghari M. H, Shayegh J. 2013. Pharmacological and therapeutic effects of Peganum harmala and its main alkaloids. Pharmacognosy Reviews, 7:14:199–2013.

Moshiri M , Etemad L, Javidi S, Alizadeh A. 2013. *Peganum harmala* intoxication, a case report. Avicenna Journal of Phytomedicine Received, 288-292.

Nath D, Sethi N, Srivastava R, Jain Ak, Singh Rk. 1993. Study on the teratogenic and antifertility activity of *Peganum harmala*. Fitoterapia journal, 64:321-324.

Paumgartten FJR. 2010. Influence of maternal toxicity on the outcome of developmental toxicity studies. Toxicology and Environmental Health journal, Part A: Current Issues, 73:13-14:944-951.

Pehlivan M, Mohammed FS, Şabik AE, Kına E, Dogan M, Yumrutaş Ö, Sevindik M. 2021. Some Biological activities of ethanol extract of *Marrubium globosum*. Turkish Journal of Agriculture-Food Science and Technology, 9(6): 1129-1132.

Pranzatelli MR, Snodgrass SR. 1987. Harmala alkaloids and related betacarbolines: a myoclonic model and antimyoclonic drugs. Exp Neurol journal, 96: 703- 719.

Pu H, Li X, Du Q, Cui H, Xu Y. 2017. Research progress in the application of chinese herbal medicines in aquaculture: a review. animal nutrition and feed science. Engineering journal, 3:5:731-737.

Queshi Z, Akhtar MS, Malik, ZA. 1975. Norepinephrine and 5-hydroxytryptamine contents of hypothalami of rats treated with harmidine hydrochloride. Planta Medica journal, 35:235- 241.

Rashan IJ, Adaay MH. 1989. In vitro antiviral activity of the aqueous extract from the seeds of *Peganum harmala* L. Fitoterapia journal, 60: 365-367.

Sepulveda FV, Robinson JWL. 1974. Harmaline, a potent inhibitor of sodium-dependent transport. Biochim Biophys Acta journal, 373:527-531.

Sevindik M, Akgul H, Pehlivan M, Selamoglu Z. 2017. Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. Fresen Environ Bull, 26(7): 4757-4763.

Shapira Z, Terkel J, Egozp QY, Nyskad A, Friedman J. 1989. Abortifacient potential for the epigeal parts of *Peganum harmala*. Ethnopharmacology journal, 27: 319 – 325.

Siddiqui S, Khan, OY, Faizi S, Siddiqui BS. 1988. Studies in the chemical constituents of the seeds of *Peganum harmala*: Isolation and structure elucidation of two ß-carboline lactams, harmalanine and harmalacidine. Heterocycles journal, 27:1401-1410.

Singh AB, Chaturvedi JP, Narender T, Srivastava AK. 2008. "Preliminary studies on the hypoglycemic effect of *Peganum harmala* L. seeds ethanol extract on normal and streptozotocin induced diabetic rats. Indian Journal of Clinical Biochemistry, 23:4:391–393.

Sokolove PG, Roth SH. 1978. Effect of harmaline on the crayfish stretch receptor: Blockade at a GABA-mediated inhibitory synapse. Neurophunnacology journal, 17:729-731.

Toyin YM, Olakunle AT, Adewunmi AM. 2014. Toxicity and beneficial effects of some african plants on the reproductive system. Toxicological Survey of African Medicinal Plants journal, 445-492

Uysal İ, Mohammed FS, Şabik AE, Kına E, Sevindik M. 2021. Antioxidant and Oxidant status of medicinal plant *Echium italicum* collected from different regions. Turkish Journal of Agriculture-Food Science and Technology, 9(10): 1902-1904.

Yuruktumen A, Karaduman S, Bengi F, Fowler J. 2008. Syrian rue tea: a recipe for disaster. Clinical Toxicology journal, 468:749-52.

Zutshi U, Rao PG, Soari A, Gupta OP, Atai CK. 1980. Absorption and distribution of vasieine, a modern uterotonic. Planta Meddca journal, 40:373-377.

الملخص

تهدف هذه الدراسة الى تبيان الأثر السمي لمستخلص القلويدات الكلية لنبات *Peganum harmala* L. على كل من تكون الجريبات والإباضة، والتكون الجنيني، والإخصاب. يعتبر نبات *Peganum harmala L.* من النباتات الطبية و المعروف محليًا باسم الحرمل، والتي تستخدم بذورها في الطب التقليدي كعامل مدر للطمث والإجهاض بالإضافة إلى استعمالاتها الدوائية المختلفة. نتج عن استخلاص القلويدات الكلية من البذور ما يقارب عن 1.820 ± 0.358غ لكل 100غ (وزن/وزن) في هيئة مسحوق أحمر داكن. تم تصنيف نبتة الحرمل ضمن النباتات متوسطة السمية حسب النتائج المتحصل عليها من دراسة الجرعة القاتلة DL_{50} لذكور وإناث الجرذان و التي قدرت بالقيمتين 164.43مغ/كغ و159.953مغ/كغ على التوالي. أظهرت دراسة سمية المستخلص الكلي للقلويدات على المبايض و الهرمونات عند إناث الفئران المعالجة بالحقن تحت السفاقي لجرعة 3.99 مغ/كغ/يوم (DL_{50}40/1) لمدة ثلاثة أشهر زيادة معنوية في الوزن النسبي لكل من الكلية والقلب والرئتين والدماغ والمبايض، و زيادة معنوية في PLA ، تغير معنوي في ALAT و ASAT و البيليروبين الكلي وغير المباشر، واليوريا، وزيادة معنوية في هرموني FSH و الإستروجين. نتج عن دراسة سمية المستخلص الكلي للقلويدات على إناث الجرذان أثناء فترة الحمل عن طريق الحقن تحت السفاقي لجرعة 7.99 مغ/كغ/يوم (DL_{50}20/1) ارتفاع معدلات الحمل المؤكدة بنسبة 90-100%، بالإضافة الى زيادة معنوية في وزن الجسم، و تغير الوزن النسبي للمبايض بشكل معنوي في جميع المجاميع المعالجة. بين التحليل تغير معنوي في العديد من المقاييس البيوكيميائية في المجاميع المعالجة، و أظهر التحليل الهرموني تغيرا معنويا في مستويات FSH و البروجسترون و الاستروجين في المجموعات المعالجة مقارنة بالمجموعة الشاهدة. أظهرت نتائج تأثير المستخلص الكلي للقلويدات فرقاً معنوياً في مجموع الاجنة، و نسبة وفياتها، و ارتفاع معنوي في وزنها، كما بينت انخفاضًا ملحوظًا في عدد نقاط التعشيش، و نسبة الأجنة غير المتطورة في المجموعات المعالجة. أما في ما يخص دراسة الخصوبة لذكور الجرذان و المعالجة بجرعة 4.110 مغ/كغ/يوم (DL_{50}40/1) لمدة 90 يومًا وتزاوجها مع إناث عذراء في نهاية فترة المعالجة ، فقد بينت النتائج عدم وجود أعراض مرضية للإناث خلال فترة الحمل أو تسجيل حالات الوفاة، مع تغير أوزانها معنويا خلال فترة الحمل. أما في ما يخص التحليل البيوكيمياني و الهرموني فقد أظهر تغيرات معنوية في مستويات البيليروبين الكلية وغير المباشرة و الكرياتينين و FSH و LH، كما كشفت الدراسة عدم وجود فرق في عدد الإناث الحوامل، و لوحظ اختلاف معنوي في عدد اللأجسام الصفراء ومواقع التعشيش، وأوزان وعدد وفيات الأجنة. نقترح بناءا على النتائج المتحصل عليها أن المستخلص الكلي للقلويدات من البذور و حسب الجرعة المستخدمة في كل دراسة يمكن أن يحفز تكوين الجريبات والإباضة عن طريق زيادة إفراز الهرمونات التناسلية FSH و الاستروجين، كما يمكن أن يتسبب في تسمم الحمل، التطور الجنيني، مما يؤدي الى الإجهاض.

الكلمات المفتاحية: *Peganum harmala L.*، القلويات، الحمل، اضطراب تكوين البويضات، التسمم الجنيني، الإجهاض، المؤشرات البيوكيمياء، الهرمونات.

Abstract

The aim of this study is to evaluate the toxic effect of the total alkaloid extract of the seeds of *Peganum harmala* L. on ovogenesis, embryology, and fertility in rat Albino Wistar. *Peganum harmala* L. (Zygophyllaceae) is a medicinal plant known locally as Harmel. Traditional medicine has used the seeds as an emmenagogue and abortifacient agent, as well as for other pharmacological effects. The extraction was yielding approximately 1,820±0,358 per 100g (w/w) of a dark red powder. Depending LD_{50} (164.43mg/kg. and 159.953mg/kg) of total extract in male and female rats classified this plant as moderately toxic. The hormonal imbalance, ovarian toxicity, and oogenesis disruption in treated female rats by total alkaloids extract for three months of daily IP administration of 3.99 mg/kg/day (1/40LD_{50}) show significant increases in the relative weight of the kidney, heart, lungs, brain, and ovaries. The study shows a substantial increase in PLA, and significant changes in ALAT, ASAT, total and indirect bilirubin, and serum blood urea. The hormonal analysis shows that FSH and Estrogen have increased significantly. The results in maternal toxicity by daily IP administration of 7.99 mg/kg/day (1/20LD_{50}) of total alkaloids extract summarized in confirmed pregnancy rates were high (90-100%), maternal body weight and weight gain changes were statistically significant in all pregnant. Precisely, the relative weight of ovaries was significantly changed in all the treated groups. Serum's biochemical parameters were changed significantly in the group treated compared to the control group. The hormonal analysis shows considerable changes in FSH, Progesterone, and Estrogen levels in treated groups. The results show a significant difference in fetus weight, the number of implantations, and total resorbed litter. For fertility evaluation, the male rats were treated IP with 4.110 mg/kg/day (1/40DL_{50}) of the total alkaloid extract for 90 days and mated with a virgin female at the end of the study period, the results show no maternal morbidity and mortality with a normal behavior during the gestational period. The maternal body weight was changed significantly in all pregnancy rates. Serum's biochemical analysis shows significant changes in dams. The hormonal analysis shows significant changes in FSH and LH. The results revealed no difference in the number of pregnant females, and a significant difference in corpus luteum number, implantation sites, resorption, and fetal body weights. We can hypothesize that the total extract in the seeds, at the dose used, can stimulate folliculogenesis and ovulation by increasing gonadotropin hormone secretion (FSH and Estradiol), but it has negative effects on maternal toxicity, embryonic development, and abortion.

Keywords: *Peganum harmala* L., Alkaloids, Pregnancy, Oogenesis disruption, Maternotoxicity, Abortion, Serum biochemical, Hormones.

Résume

Le but de cette étude est d'évaluer l'effet toxique de l'extrait des alcaloïdes totaux des graines de *Peganum harmala* L. sur l'ovogenèse, l'embryologie et la fertilité chez le rat Albino Wistar. *Peganum harmala* L. (Zygophyllaceae) est une plante médicinale connue localement sous le nom de Harmel. La médecine traditionnelle a utilisé les graines comme emménagogue et agent abortif, ainsi que pour d'autres effets pharmacologiques. L'extraction a donné environ 1 820 ± 0,358 pour 100 g (p/p) d'une poudre rouge foncé. Selon la DL_{50} (164,43 mg/kg et 159,953 mg/kg) de l'extrait total chez les rats mâles et femelles, cette plante a été classée comme une plante modérément toxique. Le déséquilibre hormonal, la toxicité ovarienne et la perturbation de l'ovogenèse chez les rats femelles traitées par l'extrait d'alcaloïdes totaux pendant trois mois d'administration IP de 3,99 mg/kg/jour (1/40DL_{50}) montrent des augmentations significatives du poids relatif des reins, du cœur, poumons, cerveau et ovaires. L'étude montre une augmentation substantielle du PLA et des changements significatifs de l'ALAT, de l'ASAT, de la bilirubine totale et indirecte et de l'urée sanguine sérique. L'analyse hormonale montre que la FSH et les œstrogènes ont augmenté de manière significative. Les résultats de la toxicité maternelle par administration IP quotidienne de 7,99 mg/kg/jour (1/20DL_{50}) d'extrait d'alcaloïdes totaux résumés dans les taux de grossesse confirmés étaient élevés (90-100 %), les modifications du poids corporel maternel et du gain de poids étaient statistiquement significatives chez toutes enceintes. Précisément, le poids relatif des ovaires a été significativement modifié dans tous les groupes traités. Les paramètres biochimiques du sérum ont été significativement modifiés dans le groupe traité par rapport au groupe témoin. L'analyse hormonale montre des changements considérables dans les niveaux de FSH, de progestérone et d'œstrogènes dans les groupes traités. Les résultats montrent une différence significative dans le poids du fœtus, le nombre d'implantations et la portée totale résorbée. Pour l'évaluation de la fertilité, les rats mâles ont été traités IP avec 4.110 mg/kg/jour (1/40DL_{50}) de l'extrait alcaloïde total pendant 90 jours et accouplés avec des femelles vierges à la fin de la période d'étude, les résultats ne montrent aucune morbidité maternelle et la mortalité avec un comportement normal pendant la période de gestation. Le poids corporel maternel a changé de manière significative dans tous les taux de grossesse. L'analyse biochimique du sérum montre des changements significatifs chez les mères et l'analyse hormonale montre des changements significatifs dans la FSH et la LH. Les résultats n'ont révélé aucune différence dans le nombre des femmes enceintes et une différence significative dans le nombre de corps jaunes, les sites d'implantation, la résorption et le poids corporel des fœtus. On peut émettre l'hypothèse que l'extrait total dans les graines, à la dose utilisée, peut stimuler la folliculogenèse et l'ovulation en augmentant la sécrétion d'hormones gonadotrophines (FSH et Estradiol), mais il a des effets négatifs sur la toxicité maternelle, le développement embryonnaire et l'avortement.

Mots clés : *Peganum harmala* L., Alcaloïdes, Grossesse, Perturbation de l'ovogenèse, Maternotoxicité, Avortement, Sérum biochimique, Hormones.