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Effect of *Alchemilla vulgaris* powder on the reproductive system and liver, spleen functions of female rats exposed to high dose of zinc sulfate in drinking water

A THESIS

SUBMITTED TO THE COUNCIL OF THE CLLEGE OF VETERINARY MEDICINE AT UNIVERSITY OF KERBALA AS A PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER SCIENCE OF VETERINARY MEDICINE PHYSIOLOGY

By

Shaimaa Kasem Mohammed

Supervised by

Asst. Prof.

Dr.Mehdi Abdul Khudhir Ali

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

" يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ "

صدق الله العظيم

(ايه رقم ١١ سورة المجادلة)

Supervisor Certification

I certify that this thesis entitled **"The protective effect of *Alchemilla vulgaris* on the reproductive system and some visceral organs (liver, spleen) of female rats exposed to high dose of zinc sulphate in drinking water"** was prepared under my supervision at the College of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Medicine/ Physiology.

Superviser

Asst.Prof.

Dr.Mehdi Abdul Khudhir Ali

The recommendation of the Department

In the view of the above recommendation, I forward this thesis for scientific discussion by the examining committee

Asst.Prof.

Dr. Kadhim Saleh Kadhim

Vice dean for scientific affairs and postgraduate

College of Veterinary Medicine

University of Kerbala

Dedication

I dedicate this study to my greatest bless in my life… Allah.

Thank you for being with me… my great mother.

To the symbol of sacrifice and my destiny in my life… my dear father.

Who paved for me the way and the help me... my lovely husband.

To the taste of the most beautiful moments with …my brothers and sister.

To whom who paved for me the way of science and knowledge…….my teachers

all love and gratitude to those who supported me in my study … my master friends.

Shaima'a

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SUMMARY

Summary

This study investigates the protective effect of the medicinal plant (*Alchemilla vulgaris*) against effect of high $ZnSO_4$ concentrations in the drinking water on the liver, spleen and ovaries of female rats during eight weeks period, and determined the beneficial effects of this medicinal plant on the study organs.

A 30 female albino rats, weighing about 150-250 G, the present study was conducted in the animal house in Pharmacy College at the University of Kerbala, Rats were distributed randomly in five cages, each cage contained six rats at room temperature, and supplied with water and diet. The groups were arranged as the following: Group1 (CO) control: six female rats were used, received only distilled water. Treatment 1 (T_1): six female rats, were received water with zinc sulfate ($ZnSO_4$) 1000 Mg/L only. Treatment 2 (T_2): six female rats were received water with zinc sulfate ($ZnSO_4$) 1000 Mg/L with *A. vulgaris* 200Mg/L powder. Treatment 3 (T_3): six female rats were used, received water with zinc sulfate($ZnSO_4$) 1000 Mg / L with *A. vulgaris* powder 300MG/L. Treatment 4(T_4): six female rats were used; give water with *A.vulgaris* powder 300Mg/L only. Substances were added to drinking water of animals. The results of the current study showed that serum ALT activity had a significantly($p\leq0.05$) increase in 1000 Mg/L $ZnSO_4$ with *A.V* 200Mg/L powder (T_2) group and *A.Vulgaris* powder 300Mg/L (T_4) group, but decreased in 1000 Mg/L $ZnSO_4$ with *A. vulgaris* powder 300 Mg/L group(T_3) when compared with control group (CO), while, the results revealed that there was decrease significant ($p\leq0.05$) in serum albumin concentration in T_1 and T_2 comparing with CO, T_3 and T_4. Serum globulin concentration were significantly ($p\leq0.05$) increased in T_4 group compared with control group CO. the results also, explained that a significant increase ($p\leq0.05$) in progesterone of the T_4 compared with control group, while other groups showed a significantly($p\leq0.05$) decrease compared with control group.

The estrogen concentration in T_4 group was significantly increased when compared with control group, whereas the estrogen concentrations were no significant ($p \leq 0.05$) differences between another groups. On the contrary, the results revealed that a significantly($p \leq 0.05$) increase in concentration of FSH hormone in T_3 group compared with control group CO. LH hormone concentration in T_3 group was significantly increased compared with control group, Administration 1000Mg/L of zinc sulfate caused a significantly ($p \leq 0.05$) drooping in RBCs numbers in T_1 (1000 Mg/L $ZnSO_4$), if compared with control group CO and other groups, in contrast to the results of RBC_S numbers in T_2 (1000Mg/L $ZnSO_4$ and 200Mg/L *A.V.*powder*)* which showed a significant($p \leq 0.05$) rising , if compared with other groups. In T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.V.*powder) treatment RBC_s numbers were significantly decreased ($p \leq 0.05$), if compare with CO. significant ($p \leq 0.05$) decrease in the results of HGB levels was found in T_3 group compared with control group CO and another groups. Also, the PLT levels increased significantly ($p \leq 0.05$) in T_2, T_3, T_4 comparing with control group. In T_2, T_3 & T_4 treatment groups WBC numbers respectively were increased significantly ($p \leq 0.05$), if comparing with control group CO, significant ($p \leq 0.05$) increased in the result of GRAN% was found in T_1, T_2, T_3 & T_4 respectively, when compared with control group CO. when administration 1000Mg/L of zinc sulfate caused no significant ($p \leq 0.05$) in LYM% in all treatments if compared with control group. the MID% significant ($p \leq 0.05$) increased in T_4 treatment when compared with control group CO. the result in of body weight significant ($P \leq 0.05$) decrease in in T_1 group at 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} weeks from experiment periods , respectively if compared with control group CO. The result in left ovary weight significantly ($P \leq 0.05$) increased in T_1 compared with control CO. in T_3 and T_4 treatment groups the index lift ovary weight, were increased significantly ($p \leq 0.05$), if comparing with control group CO. histological sections of liver tissue in 1000Mg/L $ZnSO_4$ groupT_1, central vein mild congestion, degeneration and narrow sinusoids, spleen tissue sever congestion of red pulp sinusoids with hemosiderin-laden macrophages and ovary tissue is normal and normal graffian follicle but reduce in numbers in started of experiment. In addition , the medicinal plants contain a high level of antioxidant constituents has been important role as an effective-

therapeutic approach for hepatic damages and increase in number normal growing follicles in ovaries. In Conclusion the study, used the medicinal plant for treatment most liver diseases better with low side effects by protective effects of these plant against excessive zinc, and improvement of liver and ovary functions.

List of Contents

List of Tables

List of Figures

List of Abbreviations

Albo	Albumin
ALT	Alanine transaminase
ANOVA	Analysis of variance….
Apo	Apolipoprotein….
AST.	Aspartate aminotransferase
A.V	*Alchemilla vulgaris*
°C	Celsius
D	Dose
Est	Estrogen hormone
FEDRIP	Federal research in progress
FSH	Follicle stimulating hormone
G	gram
GIT	Gastrointestinal tract
Glob	Globulin
GOT	Glutamatic-oxaloacetic transaminase

GPT	Glutamic pyruvic transaminase
GRAN	Granulocyte
H	Hormones
HB	Hemoglobin
HCT	Hematocrit
HM	Heavy metals
L	Litter
LD50	Lethal dose 50
LH	Luteinizing hormone
LYM	Lymphocyte
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MFF	Metal fume fever
MID	Multidimensional Inventory of Dissociation
Mg	Milligram

MT	Metallothionein
NO	Number
PLT	Platelet
Prog	Progesterone hormone
PPM	Per part million
RBC_s	Red blood cell
Std	Standard
T	Treatment
TP	Total protein
WBC	White blood cell
$ZnSO_4$	Zinc sulfate

CHAPTER ONE

INTRODUCTION

Introduction

Zinc is required for the function of animals, normal growth and development. Zinc is also important for the proper functioning of the immune system and for glandular, reproductive, and cell health. Abundant evidence demonstrates the antioxidant role of zinc **(*Sidhu, et al, 2004*)**. The metals are probably widely-known in the oldest toxic substances to the human and animals**(*El Okle, et al, 2014*)**. Heavy metals generally exist in the crust, rock, water, soil, atmosphere and biosphere, and some heavy metals in some environments may come from natural and anthropogenic sources.**(*Qadir, et al, 2014*)**. The metal ions essential and nonessential can be toxic when present in excess. Zinc ions, for instance, are used in biological systems as catalytic, structural and regulator components in a numberless of proteins. Because of the potential toxicity of metal ions, all living systems possess mechanisms to tightly regulate the distribution of metal ions and to minimize damage under conditions of excess metal supply **(*Tennstedt, et al, 2009*)**. Pollution of the aquatic environment with zinc and zinc compounds has become a serious health concern in recent years. This metal is introduced into the environment through various routes such as industrials effluents, agriculture pesticide runoff, domestic garbage dumps, and mining activities**. (*Abdel-Tawwab, et al, 2013*)**. Environmental pollution is the contamination of the ecosystem that causes disorder, instability, harm or discomfort to the physical systems or living organisms. Environmental factors have important links with infectious and non-infectious diseases of both acute and chronic nature, Metals toxicity depends upon the absorbed dose, the route of exposure and duration of exposure, i.e. acute or chronic. This can lead to various disorders and can also result in excessive damage due to oxidative stress induced by free radical

formation **(*El-Zwahry, et al, 2015*)**. Heavy metals as zinc, arsenic, cadmium, mercury and nickel. have been known to possess many adverse health effects; still, heavy metal pollution continues, and is even increasing in some parts of the world, in particular in less developed countries , Due to the uncontrolled industrialization, it has caused many kinds of the heavy metals accumulation in our organ tissue and inducing chronic toxicities **(*Chen, et al, 2009*)**. Increasing pollution due to heavy metals has become a serious environmental concern, also zinc concentration in water and soil has risen as a result of human activities such as mining or production of wastewater. High concentration of Zinc can affect the uptake of other nutrient elements such as Cu, Mn and Fe, and the deficiency of these elements may cause oxidative stress**(*Alkorta, et al, 2004*);(*Fernàndez, et al, 20014*);(*Singh, et al, 2016*)**.The occurrence of heavy metals in soil may be beneficial or toxic to the environment. Excess of metals may produce some common effects of individual metals on different plants, Zinc toxicity depends on pH, which controls the concentration of zinc in solution. High concentration of zinc can cause toxicity in plants. The general symptoms are curling, stunting of shoot and rolling of young leaves, death of leaf tips and chlorosis**(*Rout, et al, 2003*)**. **Lady's Mantle***(Alchemilla vulgaris)*The common lady's mantle of the *Rosaceae* family, a perennial herbaceous plant, is common throughout virtually the whole of Europe, along with a large proportion of the European territory of the USSR and Siberia, except for the most southern regions. Published data indicate that the above-ground part of the common lady's mantle contains a complex of diverse biologically active substances (BAS), dominated by phenolic substances (up to 9.6% depending on the developmental phase and site of plant collection): flavonoids, coumarols, and phenylcarbonic acids,as well as polysaccharides (up to 23%). The flavonoids consist mainly of lutein-

7-glucoside, luteolin, quercetin,apigenin, rutin, apigenin-7-glucoside and kaempferol**(*Smolyakova, et al, 2012*).** *A.v* used for astringent ,anti-hemorroidal and antidiarrheal properties. The plant infusion is used externally in the cases of wound healing and stomatitis(***Neagu, et al, 2015*)**.The plant extract exhibited different pharmacological roles, in addition to astringent, antidiarrheal and anti hemorroidal, act as diuretic, depurative, intestinal antiseptic, bacteriostatic and bactericidal, tonic, anti-arthritic and cancer deterrent**(*Hamid, et al, 2017*).**

Aims of study According to the above, this study was conduct on the protective effect of the medicinal plant (*Alchemilla Vulgaris*) against the effect of high $ZnSO_4$ concentrations in the drinking water on the liver, spleen and ovaries of females rat during 8 weeks period ,And determine the beneficial effects of this medicinal plant on the study organs.

CHAPTER TWO

REVIEW OF LITERATURE

2 Review of Literature

2.1 The Zinc Generally

Is classified as a group 11B post-transition metal. In biological systems, it exists as $Zn^{2+}$and is presented in all cells in the body. It is the second most abundant transition metal after iron and it is the only metal which appears in all enzyme classes as an structural ,regulatory or catalytic roles in many enzymes. It's an essential mineral of exceptional biologic and public health importance, also considers as an essential trace element for plants ,animals and microorganisms. It plays a role in immune function , protein and DNA synthesis and cell division ,wound healing ,normal growth and development***.(Egwurugwu, et al, 2013)***

200 enzymes or more requires zinc as a functional material and these enzymes affect most major metabolic processes in body , Despite the diversity of functions that zinc metallo-enzymes affect, correlations between loss of enzyme activity and characteristics of zinc-deficiency have proved unsuccessful***.(MacDonald, R. S., 2000)***

Zinc ions are crucial for multiple aspects of the immune system, including the normal developments, functions and differentiation of cells belonging to all types of immunity innate and acquired***.(Overbeck, et al, 2008)***

Also Zinc and it's compounds levels is very important for T cells, neutrophils, and natural killer cells functions. Oral zinc sulfate had been used for several skin diseases for many years with few side effects***.(Yazdanpanah,et al , 2011)***

In addition the zinc and it's compounds being an essential element and occurring in the environment at a natural background, chemical speciation of zinc in the environment may be relevant for biological processes and thus, for the assessment of potential risks, Zinc compounds also are used in dietary supplements, which consumers can buy over the counters***.(Bodar, et al, 2005)***

Also the zinc and it's compounds plays a role in synthesis and activity of insulin. We demonstrated previously that zinc depletion from insulin decreases its activity in rats***.(Roussel, et al, 2003)***

2.2 Toxicity of Zinc and it's compounds:

Although they are considers essential trace elements in all microorganisms , relatively nontoxic, ubiquitous in sub-cellular metabolism , but become toxic in high doses.It has been shown in men that zinc has an antioxidant effect and stabilizes cell membranes in low levels.***(Davinder, et al, 2007)***

Zinc and it's compounds toxicity from excessive ingestion is uncommon, but gastrointestinal disorders and diarrhea have been reported following ingestion of beverages standing in galvanized cans or from the use of galvanized utensils.***(Goyer, et al, 1996)***

The condition of zinc excess is less common than deficiencies and it is more prevalent in areas having galvanized plumbing in their residences , People that intentionally consume large doses of zinc and zinc compounds as a dietary supplement , the use of zinc lozenges to treat cold symptoms for over six weeks , and smoking cause overdose symptoms.***(Al-Habib, et al , 2013)***

At high doses, essential elements, such as copper(Cu), zinc (Zn), and selenium (Se), could also have toxic effects on kidneys and impair reproduction.(The relative solubility of zinc salts in aqueous solution varies widely: zinc sulfate and chloride are very soluble, zinc acetate is freely soluble, and zinc carbonate and oxide are practically insoluble .Solubility in aqueous solution should be strongly related to absorbability.This would explain why zinc absorption from the carbonate salt was significantly lower than from the sulfate or acetate salts, as assessed by post consumption plasma zinc concentrations.By using a similar approach, zinc was determined to be absorbed similarly from acetate and sulfate salts, but poorly from the oxide salt. Thus, zinc carbonate and zinc oxide are poor choices for zinc supplementation in humans. Gastric pH is also an important determinant of solubility because there may be some conversion of insoluble zinc salts to zinc chloride in the presence of gastric acid.***(Allen, L. H., 1998)***

Zinc sulfate, other zinc compounds like zinc oxide and zinc gluconate have effect on the gastrointestinal system. The gastrointestinal tract is directly affected by ingested zinc, before it is distributed through the body,multiple gastrointestinal symptoms after oral uptake of zinc have been reported. described several cases in which high zinc ingestion resulted from storage of food or drink in galvanized containers. Ingestion was caused by the moderately acidic nature of the food or drink, enabling the removal of sufficient zinc from the galvanized coating. The resulting

symptoms included nausea and vomiting, epigastric pain, abdominal cramps, and diarrhea.***(Plum, et al,2010)***

2.2.1 Effect of zinc and it's compounds on body weight and health:

Zinc is used in different figures as elemental zinc, zinc sulphate, zinc acetate, zinc chloride, zinc methionate, zinc citrate, zinc gluconate, zinc oxide, etc. In toxicity zinc and zinc compounds, the initial signs include reduced weight gain, reduced feed intake, bone resorption .in overdoses of zinc or toxic quantity for long periods of time the animal will suffer from diarrhea, internal hemorrhage, and even death. Also the increase of the cholesterol concentration in the body due to the toxicity of zinc in tissue organs such as liver, pancreas, and kidneys with damage of cells of these organs.***(Hussein, et al , 2012)***

Zinc toxicity can occur in both acute and chronic forms. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps and diarrhea. One case report cited severe nausea and vomiting within 30 minutes of ingesting 4 g of zinc gluconate (570 mg elemental zinc).Intakes of 150–450 mg of zinc per day have been associated with such chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipo_ proteins. Reductions in a copper-containing enzyme, a marker of copper status, have been reported with even moderately high zinc intakes of approximately 60 mg/day for up to 10 weeks.The doses of zinc used in the AREDS study (80 mg per day of zinc in the form of zinc oxide for 6.3 years,on average) have been associated with a significant increase in hospitalizations for genitourinary causes, raising the possibility that chronically high intakes of zinc adversely affect some aspects of urinary physiology.***(Ali H. , 2011)***

2.2.2 Effect of zinc and it's compounds on reproductive system:

Several Studies on rats have shown that excessive dietary zinc in these animals induces deficiencies of copper and iron, producing poor growth and anemia . In addition, zinc challenge with 2mg of zinc sulfate/kg could also cause congenital malformations in hamsters and zinc supplement of 30g/g resulted in harmful effects on the course of rat pregnancy . However, there have been alone sporadic reports on the toxicity of zinc and relatively little information is available from systemic observation of zinc toxic effects***.(Piao ,et al ,2003).***

Excess amount of zinc can cause system dysfunctions that result in impairment of growth and reproduction. The clinical signs of zinc toxicosis have been reported as vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane),liver failure, kidney failure and anemia.***(Duruibe, et al, 2007)***

Zinc contamination results from industrial smoke, with the most relevant compounds represented by Zn chloride, Zn chromate, Zn phosphur, Zn sulphate and Zn oxide. Contamination is also possible by use of zincate containers to heat milk and foods. Moreover, Zn is an important substance used in the fabrication process of several pesticides. It may enter the body either by enteral or respiratory way. It is easily absorbed in all tissues and rapidly diffuses. Zinc is excreted by feces, the bila etc.; it is co-secreted with insulin by the pancreas. In the presence of As, Zn toxic effects are 3-4-fold increased. so direct toxic effect of Zn salts act as endocrine disrupters. However, Zn, Cd and copper were able to potentiate the estradiol-induced response in a dose-dependent manner, thus indicating that Zn can act as a potential endocrine disrupter by modulating the estrogenic activity of endogenous hormones.(***Georgescu, et al, 2011)***

2.2.3 Effect of zinc and it's compounds on liver :

Liver sections of zinc treated rats revealed preservation of architecture and mild congestion in a number of vessels. Degenerative changes were infrequent in most sections. Mild to moderate RBC extravasations were present***.(Al-Jawad , et al , 2015)***.

Prolonged exposure to metals and metal compounds as zinc, would result in dysregulation of cellular pathways causing subsequent toxicity, also these compounds interfere with functions of liver and other organs in body. Recently, more attention and concern is given to metal compounds that have toxic effects at low levels of exposure than those that produce overt clinical and pathological signs and symptoms.***(Florea, et al, 2006)***.

Acute, high-dose oral exposure to zinc and its compounds generally results in gastrointestinal damage, with symptoms including nausea, vomiting, abdominal cramps, and diarrhea. Exposure levels resulting in these effects generally range from 2 to 8 g zinc/kg/day. Ingesting high levels of zinc for several months may cause anemia, pancreas damage, and decrease the level of high-density lipoprotein (HDL) cholesterol.***(Wang, et al , 2006)***

Zinc is considered to be relatively non-toxic, especially if taken orally. However, excess amount can cause system dysfunctions that result in impairment of growth and reproduction.The clinical signs of zinc toxicosis have been reported as vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane),liver failure, kidney failure and anemia***.(Duruibe, et al, 2007)***

2.2.4 Effect of zinc and it's compounds on blood(blood picture: RBC, WBC, Platelet):

Excessive zinc intake causes acquired copper deficiency that manifested hematologically by anemia and neutropenia and neurologically by myelopathy presenting with a spastic gait and prominent sensory ataxia . Whereas the most widely known effect of inhaling zinc-containing smoke is the so-called metal fume fever (MFF). Symptoms of this reversible syndrome begin generally a few hours after acute exposure and include fever, muscle soreness, nausea, fatigue, and respiratory effects like chest pain, cough, and dyspnea, it is not life threatening and the respiratory effects disappear within one to four days***.(Ali F., 2013)***.

In Adult zinc toxicity can occur from high intakes of zinc (>150mg/day) over a long period of time or from ingestion of >1 g of zinc) intravenous or supplementation feeding. Ingesting too much zinc at once can cause gastric distress and the typical signs and symptom that are often associated with food poisoning. High doses of zinc or zinc salts for long periods of time may leads to a decrease concentration of plasma lipoproteins and lower copper absorption.Decreased copper status may also inhibit the transport of iron and result in anemia.Although zinc-induced copper deficiency and the resulting anemia is serious, it occurs only after excessive zinc intake over a long period of time and is easily corrected by adjusting the intake levels of zinc and copper accordingly. Supplements of zinc and iron may also compete for absorption in the body.***(Fischer, et al, 2005).***

Zinc has decreased the Hb,erythrocyte and hematocrit levels significantly in both male and female.Leukocyte levels were also observed to decrease in the high – dose male mice. ***(AL-diwan,2010).***

Fortification of foods with iron in iron-deficiency anemia does not significantly affect zinc absorption. But large amounts of supplemental iron, greater than 25 mg, might decrease zinc absorption.Taking iron supplements between meals helps decrease its effect on zinc absorption. Several laboratory and human studies have

found that high levels of supplemental zinc taken over extended periods of time may result in decreased copper absorption in the intestine, and copper deficiency with associated anemia ***(Osredkar, et al, 2011)***.

Heavy metal intoxication as zinc also almost always reduces count of white blood cells, particularly lymphocytes, because increase in cortisol level which is responsible for a decrease in WBC, particularly in count of lymphocytes and their activity***.(Witeska, M. (2005)***

2.2.5 Effect of zinc and it's compounds on serum(proteins and enzymes):

Administration of zinc sulfate or zinc citrate with thioacitamide for 8weeks period leads to a significant decrease in (1.GPT and 2.GOT) and increase in (1.total protein and 2.albumin) which could be attributed to the antioxidant/ antiradicals and metal-chelating efficacy of this elements***.(Abo-Ghanema, et al, 2016).***

GIT ulcerations and burns following ingestion of toxic doses of zinc salt can precipitate an acute fall in hemoglobin and hematocrit levels and intravascular hemolysis may follow. Acute exposure to zinc sulfate for 1 week at a dose of _3 mg/kg/day resulted in anemia which could have been secondary to gastrointestinal hemorrhaging. Changes in serum serum ferritin, lipid profile and erythrocyte superoxide dismutase activity have been reported in a number of patients who have ingested high doses of zinc. Microcytic anemia and decreased blood platelets have been reported as a resulted of sustained of hands to zinc chloride solution.***(Nriagu, J., 2007)***

2.2.6 Effect of zinc and it's compounds on hormones:

Besides direct toxic effects of zinc, Zn salts act as endocrine disrupters. recent studies strongly suggest that some heavy metals may exert endocrine-disrupting activities in animals and human. Of these metals, Zn, Pb and Hg and As interfere with sex hormones and adrenal cortex hormones steroidogenesis to alter reproduction and sex differentiation. this may induce alterations in male and female fertility, may affect the function of the hypothalamo-pituitary-thyroid axis or the hypothalamo-pituitary-adrenal axis,and disrupt biosynthesis of steroid hormones***(Georgescu, et al, 2011).***

High levels of Zn in the liver and gonads may be factors causing the abnormal gonads in the Lake Van fish.***(Oğuz, et al, 2014).***

2.3 Drinking water pollutant

Environmental pollutants and their toxicity generate many problems worldwide. New pollutants stay emerging and pose severe health and systematic challenges. Water pollution is one of the biggest environmental issue causing serious problems to survive organisms. The removal of various toxic materials of water and wastewater has been the core importance of many scientists and researchers around the globe over the earlier decades***.(Uddin, et al, 2017);(Oehmen, et al, 2006).***

Generally, heavy metals are released from different natural (i.e., weathering, erosion of bed rocks, ore deposits and volcanic activities) and anthropogenic (i.e.,mining, smelting, industrial influx and agricultural activities)sources. They can contaminate the surface (river) and ground (spring, dug well and tube well) water that is used for domestic, agricultural and industrial purposes***.(Khan,et al, 2013);(Muhammad, et al, 2011);(Raikwar, et al, 2008).***

Generated industrial and urban wastewater often discharged into the receiving medium (seas, rivers, soils) without pretreatment, cause's environmental physical, chemical and biological quality degradation by several pollutants and generates many waterborne diseases. Among those pollutants, we notify mineral pollutants, like heavy minerals, where their density exceeds 5g/cm3, which are normally present in the environment as traces: zinc, copper, mercury, lead, cadmium, arsenic, nickel, cobalt, manganese. They have specific chemical properties that provide them toxicity to human beings as well as animal and vegetal kingdom living organisms, for which zinc is a necessary element. Zinc or zinc salts overdose can cause health problems, like stomach pains, skin irritation, illness, vomiting and anemia***.(Larakeb, et al , 2017)***

Pérez-Cadahía, et al, (2007) who identified the heavy metals, they are highly toxic substances at medium and especially at long term, because of the frequent accumulative processes in which they are involved.

2.3.1 The pollute drinking water with metals and heavy metals

The term heavy metals is often used without strict definition. Some metals, such as sodium, potassium, calcium, and magnesium, are essential for life, and are generally not thought of as heavy metals. The metals generally referred to as heavy metals include mainly lead, mercury, copper, cadmium, nickel, cobalt, chromium, manganese, zinc and selenium. Lead, mercury and cadmium are the most dangerous, HM are toxic at higher concentrations to flora and fauna, but interestingly some of them, such as zinc and selenium at trace metal concentrations are essential for normal body functions. HM toxicity depends upon the consumed dose, the route of exposure and duration of exposure, acute or chronic. This can lead to multiple disorders and can also follow in excessive damage due to oxidative stress caused by free radical production***(El-Zwahry, et al, 2015)***

Metals may affect the male reproductive system immediately, when they target specific reproductive organs, or indirectly when they work on the neuroendocrine system. Several factors can influence the health results of human exposure to metals. Toxic metals can interfere with the metabolism of necessary metals and reduce their concentration in the body or decrease their bioavailability.(***Pizent, et al, 2012)***,***(Mathur, et al , 2010)***

Also, the removal of heavy element contaminants from aqueous solutions is one of the most major environmental concerns because metals are bio refractory, and are toxic to various life forms***.(Rao, M. M, et al , 2008)***

HM contamination is potentially a significant problem in several community and agricultural areas because agrochemicals, including plant nutrients and fertilizers, can lead to dramatic increases in the concentrations of heavy metals in the water and soil. These metals have the potential to reach levels in the soil and then in the surface and groundwater that are adverse to human health.***(Wongsasuluk, et al, 2014)***

HM are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment.***(Baby, et al, 2010)***

2.4 Medicinal Plants

In ancient times, human beings have used plants in the treatment of many ailments because they seem to be low toxic it and hence less likely to causes side effects. Also, many of the available drug have been directly or indirectly derived from medicinal plant***.(Moradi, et al, 2016)***

The use of medicinal plants in a high level of antioxidant constituents has been importent role as an effective therapeutic approach for hepatic damages. often of the antioxidant compounds in a typical diet are derived from plant sources and belong to defferent classes of compounds as phenols, flavonoids, tannins, carotenoids and vitamins which play an important role in health protection from the risk of most diseases***.(El-Sayed, et al, 2017)***

Herbal remedies can be taken in most forms ranging from infusions of herbs or spices to decoction of flowers and leaves from them.***(Gulfraz, et al, 2011)***

Natural antioxidants from plants, the most prominent representatives of these compounds can protect the human body from free radicals***.(Sidiq, et al, 2018)***

Recent study we observed that herbs are a rich source of polyphenol compounds and their antioxidant activities are several times higher than those of vegetables and fruits. There was also a good correlation between the polyphenol content and the(ORAC) antioxidant activity of the herbs investigated, indicating that the polyphenol compounds are responsible for the free-radical scavenging capacity.***(Denev, et al, 2014)***

The uses of herbal medicines has been steadily increasing over the past decade to cure some of the disorders in human.***(Majid, et al, 2018)***

The Herbal medicines are now used by up to 50% of the Western population, in a substantial minority of instances for the treatment or prevention of digestive disorders. Herbal preparations contain many bioactive compounds as Flavonoids. There is clearly a need for greater education of patients and doctors about herbal therapy, for legislation to control the quality of herbal preparations, and in particular for further randomized controlled trials to establish the value and safety of such preparations in digestive and other disorders.***(Langmead, et al, 2001)[i](Vishal, R. (2013)[i](Bussmann, et al, 2010)[i](Bansal, et al, 2014)[i] (Pattanayak, et al, 2015)[i](Kumar, et al, 2013)***

Herbal drugs have importance and popularity in recent years because of their efficacy, safety and cost effectiveness. The association of medical plant with other plant in their habitat also have influences in some cases***(Kumari, et al, 2016).***

Herbs are not pharmaceutical medications. Herbs are part of whole plants, not isolated or not synthesized chemicals. Herbal effects have to do with the synergistic action of nature's formulation. Drugs and herbs are used differently but both can be extremely beneficial when used appropriately.***(Shinde, et al , 2012).***

Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumor activities. They have been reported to be a potential candidate to combat free radicals, which are harmful to our body and foods systems.***(Oviasogie, et al, 2009)***

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, In nature, these compounds are products extracted from plants and they are found in several parts of the plant (widely found in fruits, vegetables and certain beverages*).****(Di Carlo, et al, 1999);(Panche, et al, 2016)***

Flavonoids are reported to have antioxidant, anti-inflammatory and antiproliferative effects, which could explain possible involvement with development of diseases. However, many flavonoids are also considered endocrine disruptors.***(Ohlsson, et al, 2010);(Zendehbad, et al, 2014);(Kaya, et al, 2012)***

Antioxidants are the major plant products that play a role as anticancer agents by acting as reducing agents as well as reduced oxidative stress-related diseases.***(Al-Snafi, A. E. (2015)***

Herbal teas as *Alchemilla Vulgaris* are widely used in traditional medicine, Antioxidant effect is one of the major descriptive characteristics of organic and medicinal activity.***(Dimiņš, et al, 2013)***

Also , Flavonoids exhibit significant steroid hormone activity, and may have an effect in the modification of cancer risk by diet, or in cancer therapeutics and prevention.***(Zand, et al, 2000)***

They also have a regulatory role on different hormones as androgens, estrogen and thyroids hormone.***(Agrawal, A. D. (2011)***

2.4.1 Effect of medicinal plant on liver

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailment.***(Kumar, et al, 2012)***

Hepatic disease (Liver disease) is a term for a collection of conditions, disorder, disease and infections that affect the cellular or tissue's structures or functions of the liver. Also these causes various patho-physiological changes like cirrhosis, nonalcoholic hepatitis, hepatic steatosis, biliary cirrhosis, alcoholic hepatitis, liver cancer**(P.G. Scholar, et al, 2017)**

liver is considered as the major organ responsible for conducting various metabolic processes and due to it's highly exposed to the toxic effects of different xenobiotics result to different types of disorders and diseases namely as liver injury or hepatotoxicity.***(Said, et al, 2011)***

The treatment choices for various liver ailments for example chronic hepatitis, fatty liver and cirrhosis are still challenging.***(HINA, S. (2017)***

The liver dysfunction remains as one of the serious health problems but we do not have satisfactory antihepatotoxic drugs in the allopathic medical practice for serious liver diseases. So present a number of plants have shown to possess hepatoprotective properties by proving the antioxidant statues**(Govind, P., 2011);(Samani, et al, 2018).**

The primary goal of herbal approach to healthy liver is to enhance detoxification processes and help protection against further damage based on their ability for helping and promoting balance within the body and nourish the liver and related functions including digestive and bile secretion. **(Said, et al, 2011)**

Medicinal plants can neutralize or detoxify toxins and protect respiratory, urinary, hepatic and neural systems from the toxic effects of drugs and chemicals**(Al-Snafi, A. E. ,2015).**

Medicinal plants with high level of antioxidant constituents can be an effective therapeutic approach for treating hepatic damages, These antioxidants are usually classified as flavonoids and vitamins**(Ansari, I., & Maiti, D., 2018);(Ho, W. Y., 2012).**

Phenolic compounds in medicinal plant such as flavonoids can protect cells against reduced glutathione via increasing antioxidant enzymes' capability (such as glutathione peroxidase).These compounds, with antioxidant properties, can counteract free radicals in the environment and hence prevent their destructive effects. Flavonoids, as antioxidant, free radical- scavenging and anti_lipoperoxidant agents, are helpful for hepatoprotection**(Moradi, et al, 2016).**

2.4.2 Effect of medicinal plant on ovary hormones

World's population has Ever increasing severly depleted the natural resources and has forced mankind to develop new methods to fertility regulation . Though considerable progress has been made in the developments of effective methods of fertility control but most the methods developed include chemical formulation being non-herbal have many side effects. It has, therefore, become necessary to screened and use biologically active botanical substances as fertility-regulating agents which are safe and interfere with the natural patterns of reproduction.***(Sharma, et al, 2013)***

The imbalance of estrogen and progesterone may be due to a disruption of the normal feedback systems that control the hypothalamus-pituitary-ovary axis or to a dysfunction of any one of these glands (most commonly the ovaries). This is commonly considered to indicate a deficiency or failure of the corpus luteum .It may also be that the ovaries are functioning fine,but hepatic metabolism and excretion of estrogens is impaired. Also, elevated prolactin levels imply a degree of pituitary dysfunction or imbalance, especially a lack of sensitivity to the inhibitory messages. The elevated aldosterone, like FSH, implies a degree of pituitary disorder and lack of sensitivity to a rising water content of the body. The Stress acts by affecting hormone production and stimulating the secretion of a range of other hormones that interfere with the sex hormones: adrenocorticotropic hormone (ACTH), cortisol, the catecholamine-epinephrine and norepinephrine; aldosterone, a corticosteroid that causes renal sodium retention. Also, Obesity and excess adipose tissue in relation to lean body mass affect estrogen/progesterone ratios**.*(Deepashree C L and Shubha Gopal, 2013)***

Herbal Medicine can be used for hormone imbalances as pre menstrual tension, cramping, fluid retention, irritability, migraines, skin problems, irregular or heavy

menses, pelvic congestion, fibroids, endometriosis and cysts**. (Sağlık Bilimleri Dergisi, 1930)**

2.4.3 Effect of medicinal plant on blood

Herbal medicine can not only be used complimentarily to the medical treatment of the disease but can also be used as a safe alternative to the drugs in order to help resolve the outstanding issues causing the disease. Without continually disrupting hormonal functions and suppressing the body's normal cycles, as the drugs set out to do, a comprehensive herbal treatment plan can address not only the symptoms of pain, but can also reduce inflammation, improve overall immune health, help the body process and rid itself of harmful environmental toxins, as well as reduce the emotional and physiological side effects that often accompany the disease including stress and depression.***(Staeb, et al, 2016)***

A herbal formulation used to treat patients with sickle-cell anaemia complicated with jaundice, also recommended as a protective agent against liver damage due to chronic ingestion of alcohol***.(Ishola, et al, 2015)***

2.5 *Alchemilla Vulgaris*

2.5.1 Plant description

Alchemilla was first described by Linnaeus (1753) and contain species are represented by more than 1,000 species.***(Kaya, et al, 2012);(Sepp, S., & Paal, J. (1998)***

Lady's mantle, **or *Alchemilla,*** is an not common herbaceous member of the rose family Rosaceae. The insignificant flowers, which is lack eye-catching petals, it do not have much freatures like flamboyant cousins as roses (Rosa) and cinquefoils (Potentilla). Approximately 300 species of *Alchemilla* native to Europe and Asia. Most lady's mantles are mounded, clump-forming perennials with basal leaves arising from woody rhizomes. The palmately lobed to divided leaves are typically fan-shaped with small apical teeth. The long stalked grey-green to green leaves are often covered with soft hairs, which slow water drops on the surface and along the margins. The green to bright chartreuse flowers are small. ***(Al-osaj, S. L. (2016)***

Also, this plant grow on wet meadows in Europe, western Asia and North America. *Alchemillae* herba, are officially recognized as a pharmaceutical drugs in the European Pharmacopoeia.(***Duckstein, et al, 2012)*;*(Sarina, et al, 2014)***

This genus can easily be distinguished from the closely related genera by its leaves that radiate from a common point or with a fan shape and it has small flower without petals and appear in clusters. So, *Alchemilla* species are very similar to each other and they are indistinguishable in many cases without microscopic identification used.***(Türk, et al, 2011)***

It should be noted that Herba Alchemillae includes aerial parts of different species that are subsumed in Alchemilla vulgaris complex. herbalists collect all species of lady's mantles occurring within the natural populations Due to difficulties in the identification of the species them.***(DIMITROV, D. (2015)***

Also, it has been used as medicinal plant by local people in Turkey in north-east black sea region and it is represented by nearly 80 species in Turkey***.(Kaya, et al, 2012)***

Also, in ***2013*** suggested ***Akbulut, et al,*** this plant used in widely traditional and official medicine.

2.5.2 *Origin*

The common lady's mantle – *Alchemilla vulgaris* – of the family Rosaceae, a perennial herbaceous plant, is common throughout virtually the whole of Europe, along with a large proportion of the European territory of the USSR and Siberia, except for the many southern regions. Published data indicate in study that the airal part of the common lady's mantle contains a complex materials of diverse biologically active substances (BAS)***.(Smolyakova, et al , 2012)***

Krivokuća, et al, 2015*;*Falchero, et al, 2008*;*Nedyalkov, et al, 2014 who descriptions the species of ***Alchemilla*** have potent free radical scavenging activity, attributed to the phenolic compounds, tannins, and flavonoid glycosides present in her. Also, indicated ***Trendafilova, et al, 2011; Renda, et al, 2018*** for these compounds as (flavonoids and tannins) present in this plant are responsible for the pharmacological activity of Lady's mantle.

2.5.3 Scientific Classification of *Alchemilla Vulgaris plant:*

Kingdom : Plantae

Order : Rosales

Family : Rosaceae

Subfamily : Rosoideae

Tribe : Potentilleae

Genus : Alchemilla

Species : Vulgaris

Figure (2-1) of *Alchemilla Vulgaris (Al-osaj,(2016)*

2.5.4 Medicinal uses of *A.Vulgaris* in general:

A.vulgaris also used with astringent, anti-hemorroidal and anti-diarrheal properties. The plant infusion is used externally in the cases of wound healing and stomatitis.***(Neagu, et al, 2015)*** ***(Trendafilova, et al, 2017)***

Also, added ***Delcheva, et al, 2016*** herba *Alchemillae* is characterized by astringent, anti-inflammatory, styptic, and epithelium recovery effects.

The aerial parts of this plant have possess antioxidant activity. Thought to this activity has been arise from the phenolic compounds of the extract such as flavonoids.***(Condrat, et al, 2010)***

A.Vulgaris *complex* is the commercial herbal- mixture of *Alchemila* species, herb plant with well documented medicinal application.***(Nikolova, et al, 2012)***

The species of ***Alchemilla*** have been used against dysmenorrhea, acute diarrhoea and menorrhagia in Bulgarian folk medicine, also they have been used as wound healer and anti-inflammatory in Sweden. the Aerial parts of some species are used as menstrual regulator, diuretic, constipant, tonic, emmenagogue, wound healer and for bronchitis, menstrual pain and rheumatoid arthritis in Turkish folk medicine, as well as, due to these species are rich in flavonoids, tannins and phenolic acids which are proved to be responsible for the some of the pharmacologic activities, so, Its used orally in mild and nonspecific diarrhea and gastrointestinal disorders.***(Renda, et al, 2017);(Vitkova, et al, 2013)***

2.5 Flavonoid's:

Flavonoids are polyphenol compounds, which have 15 carbon atoms and are structural formula C6 – C3 –C6, fig.(2-2). It is widely distributed in vegetables, tree bark, grains, roots and flowers, thus constitutes an important part of food.***(Cartea, et al, 2011)***

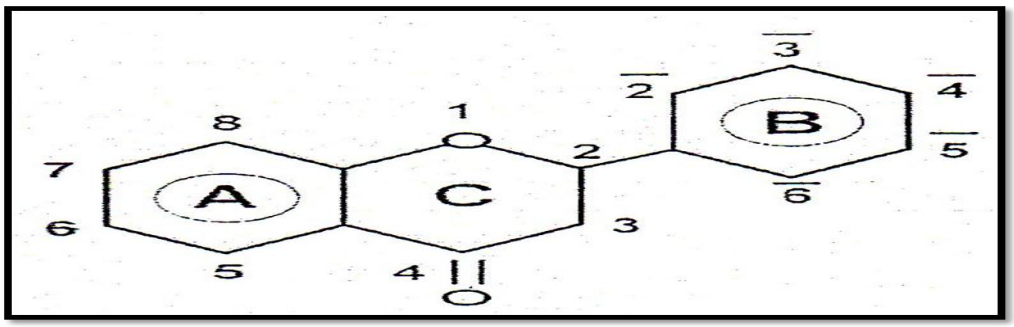


Figure (2-2): General chemical composition of flavonoid ***(Cartea, et al., 2011)***

Zhishen, et al, 1999 who descripted Phenolic compounds like flavonoids act as antioxidant activity and their effects on health and human nutrition are considerable. The mechanisms of action of flavonoids are through chelating process or scavenging and which act as free radical terminators.

2.6 Spleen

The spleen is an organ a dark red into blue, located generally in left cranial abdominal cavity. It is an elongated organ roughly triangular in the cross section.

The spleen functions of are centering on the systemic circulations. it's lack afferent lymphatic, and comprised of two morphologically and functionally, red and white pulps. The red is the blood filter (removes foreign materials and effete and damaged RBC. also, It site for storage iron, platelets and erythrocytes. In the rodents, particularly in fetal and neonatal animals it is a site of hematopoiesis.

Also, this organ conceder the largest second lymphoid organ contained 1\4 of the body's lymphocyte and begans the immune response to blood borne antigen. the white pulp surrounds the central arterioles and which this function is charged to it. composed the white pulp is 3 sub-compartments: the per arteriolar lymphoid sheath, the marginal zone and follicles. ***(Cesta, M. F. (2006)***

2.6.1 Effect of *A.V* on the body and digestive system:

In folk medicine, lady's mantle was also used to soo the infections of the mucous membranes of mouth and throat. Aerial part of the plant which is used medicinally, traditionally used for skin irritations, wounds. Also, used for treating blood sugar control diseases. It is registered in European Pharmacopoeia 6.0 as a medicinal plant and it has been claimed to exhibit a variety of pharmacodynamics activities. The tannins in this species are responsible for the pharmacological activities such as antimicrobial. ***(Altameme, et al, 2015); (Makau, et al, 2013);(Karatoprak, et al, 2018)***

Also, is regarded as safe by the German Commission even at high doses without known adverse effects. Deeply rooted in Arabic medicine, this plant has been used for treating obesity, inflammation and gastrointestinal pain . The amines of this plant are mainly the tannins reported to increase the metabolic action in cold environments and the flavonoids reported to regulate digestive enzymes and Beside metabolic stimulation it have cardioprotective effects. ***(Said, et al, 2011); (Kiselova, et al, 2006);(Said, et al, 2009)***

2.6.2 Effect of *A.Vulgaris* on the liver:

The primary goal of herbal plant to healthy liver is to enhance detoxification processes and act protection against further damage according to their ability for enhancing and produce balance within the body and nourish the liver and related functions including digestive enzymes and bile secretion. ***(Said, et al, 2011)***

There is evidence of a hepatoprotective activity of A. vulgaris on the polyphenolic and flavonoids compounds of plant leaves, which have potent antioxidant properties.***(El-Hadidy, et al, 2019);(*Afshar, *et al, 2015)***

2.7 Ovaries

The paired ovaries of the female rat are like the grape structures that varies in appearance and size, this dependent on the stage of the oestrous cycle, the cortex contain numerous follicles at different stages of development. In sexually mature rats.

Reproduction is a key and the most complicated biological process in existence and maintaining of species. Humanity used the power of herbs to suppress or promote fertility Consummation of herbs can positively influence improving the menstrual cycle in women ,strengthening endometrium, improving blood supply and circulation of uterine and ovary, promoting growth and development of follicle**.**

(Kádasi, et al, 2012)

The paired ovaries in female rats are like the grape structures in shape, but vary in gross appearance and size, itis depending on the stages of the oestrous cycle. the surface is covered a single layer of modified peritoneal mesothelium, the ovarian surface epithelium (OSE), that supports the ovary by which is continuous with the broad ligament (mesovarium)

Primordial follicle –is earliest stage of follicular growing, it is form during early fetal development and are typically located within the peripheral cortex, direct beneath the tunica albuginea, and followed by these stages (primary, secondary, tertiary and Graafian follicles.

Then, luteinisation, This termed mean the process Following extrusion of the secondary oocyte from the Graafian follicle, the thecal cells and granulosa of the

follicle remnant undergos hypertrophy and, to a lesser extent. , this process occurs under the effect of luteinising hormone (LH) and prolactin hormone, the 2 major luteotrophic hormones in rodents. the basement membrane is degeneration accompaning Luteinisation by of separating the zona granulosa and theca interna , and interfering of the post-ovulatory follicle by blood vessels from the theca interna.

The result is mature corpus luteum ("yellow body") formed is structure a large eosinophilic that obscure the ovarian cortico-medullary junction or may bulge out from the surface ovarian , this dependent on its location..***(Umer, et al, 2017)***

estrogens: major product of ovary: act on Growth of the uterine muscles, Development of endometrial lining and other functions.

Progesterone considered a precursor of the estrogens, androgens, and adrenocortical steroids, Ovary (corpus luteum) and Placenta (during pregnancy) mainly sources of this hormone in female main functions of progesterone Hormone replacement therapy and Hormonal contraception.

So, FSH and LH are secreted first in small amounts …… small quantity of estrogen is produced…… breast development, alteration of fat distribution, growth spurt and later epiphysial closure.

After about one year later ….. sufficient amount of estrogen is produced ….. endometrial changes and periodic bleeding.

FSH causes enlargement of variable number of follicles (vesicular follicles) each containing an ovum.

After 5 to 6 days ……. one follicle begins to develop more rapidly.

Under the influence of LH ….. multiplication of granulosa cells of the rapidly growing follicle …… synthesis and release of increasing amounts of estrogens …….. inhibition of FSH release …… regression of the other smaller and less mature follicles.

The structure of this mature follicle is an ovum surrounded by a fluid-filled antrum and lined by granulosa and theca cells.

The peak of estrogen secretion is reached just before the mid cycle.

At that time the granulosa cells …… begin to secrete progesterone***.(Umer, et al, 2017)***

2.8 Effect of *A.V* on Ovary hormones and blood:

A.Vulgaris is favorite for a gynecologist. So, the aerial parts of this plant are used to heal inflammation of female reproductive tracts, including maintaining to stop minor bleeding and to treat wounds. Also, it is in Libyan folk that this medicine is applied in urinary diseases. Moreover, it is also used to treated ovarian infections in women as well as for the treatment of internal bleeding and treat vaginal diseases as uterine and abdominal relaxations after birth and repeated abortions***.(EDRAH, S. M. (2017)***

ERGENE, et al, 2010 who proved this plant is mainly used in treatement gynecological diseases, also considered to regulate the glandular activities of uterine and reduce bleeding. Also, in Canada reported used this plant against retained placenta, In France, used it treated menopausal complaints.

It is used for the adaptation to the hormonal levels of women body in case of menopause.***(Eshak, al et, 2018)***

A.vulgaris is useful in a variety of female problems such as menstrual disorders including excessive menstruation and menopause, as an aid during conception, in the prevention of miscarriages, and act to the body heal after childbirth. ***(Saad, et al, 2008); (Özbilgin, et al, 2010)***

The Testo such as alchemilla (ladies mantle) is reported to be a progesteronic herb. also, presentd to be helpful in correcting a heavy menstrual flow and other cases irregularity, and can be the reason for regularization of menstrual cycle. because contain it salicylates, anti-inflammatory, astringents, analgesic compounds, probably the cause for relief in dysmenorrhoea and associated constellation of symptoms.***(Parven, S. (2015)***

CHAPTER THREE

MATERIALS AND METHODS

3 Materials and Methods

3.1 Lists of Materials and Equipment's

The materials and equipment's of the study were summarized in the tables (3-1) & (3-2) respectively.

Table (3-1) list of materials

No. of item	Materials	Source
1	Buffered formalin 10%	BDH England
2	Zinksulfat-7-hydrat	Ph.France
3	Alchemilla vulgaris powder	Local market - Iraq
4	Ethylene Diamine Tetra Acetic Acid Disodium salt (EDTA)	Merck company, Germany
5	Eosin hematoxylin stain	Merck – Germany
6	Histological microtome	Germany
7	Kit for AST + ALT	Spectrum , Egypt
8	Kit for FSH , LH, Est. and Prog.	Biobase, koria
9	Kit for total protein, Albumin and globulin	Spinreact, Spain
10	Paraffin wax	Local market - Iraq

Table(3-2) list of equipment

No. of item	Equipment's	Source
1	Centrifuge	England
2	Deep freezer	Germany
3	EDTA tubes	China
4	Electric sensitive balance	Mettler, Switzerland
5	Eppendrof tubes	China
6	Gel tubes	China
7	Histokinate	Leitz - Germany
8	HPLC	Shimadzu, Germany
9	Light Microscope	Olympus – Japan
10	Manual plastic drinkers (5L)	Local market - Iraq
11	Manual plastic feeder (38cm)	Local market - Iraq
12	Mechanical Balance	Germany
13	Micro-hematocrit centrifuge	Germany
14	Slide and cover slide	Chine
15	Spectrophotometer	Unico , TM USA
16	Thermometer	Local market - Iraq
17	Water Bath	Japan

3.1.1 Experimental animals:

A total 30, 2-3 months old, apparently healthy, female albino rats initially weighing about 150_250 G were used in this study, The present study was conducted in the animal house in pharmacy college/ university of kerbala, Rats were kept randomly in 5 cages in a rate of six rats per cage at room temperature and supplied with standard diet and water.

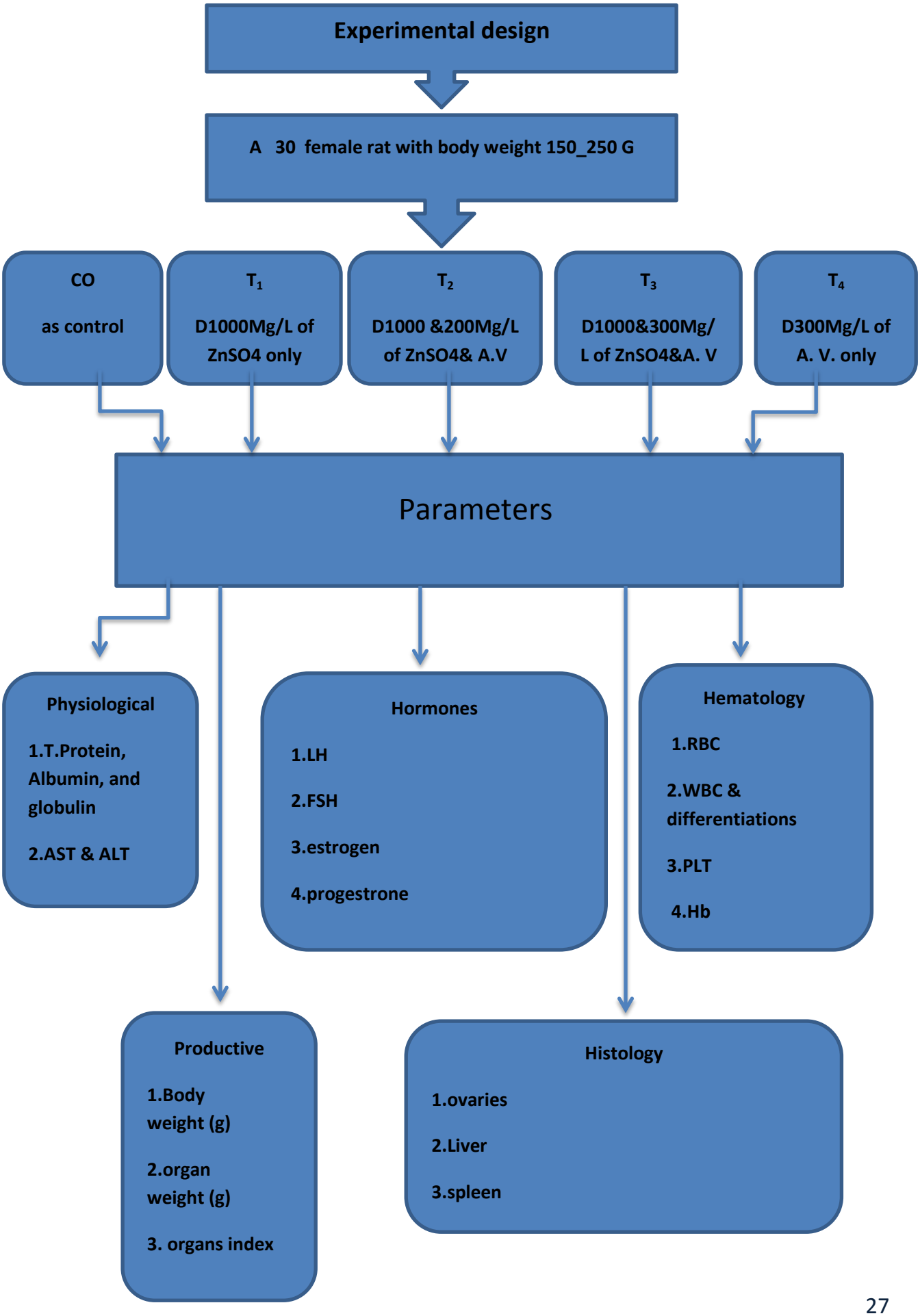
3.1.2. Experimental design:

The groups were arranged as the following:

Were divide into five groups (six rats for each treatment):

1. Group 1(CO) control: 6 female rats were received, distill water uses.
2. Treatment 1(T_1): 6 female rats were received, water with zinc sulfate ($ZnSO_4$) 1000 Mg / L only.
3. Treatment 2(T_2): 6 female rats were received, water with zinc sulfate ($ZnSO_4$) 1000 Mg / L with 200Mg/L *A. vulgaris*.
4. Treatment 3(T_3): 6 female rats were received, water with zinc sulfate($ZnSO_4$) 1000 Mg / L with 300Mg/L *A. vulgaris*.
5. Treatment 4(T_4): 6 female rats were received, water with 300Mg/L *A. vulgaris* only.

Added the substances with drinking water of animals.


Experimental design
A 30 female rat with body weight 150_250 G
CO
as control
T1
D1000Mg/L of ZnSO4 only
T2
D1000 &200Mg/L of ZnSO4& A.V
T3
D1000&300Mg/ L of ZnSO4&A. V
T4
D300Mg/L of A. V. only
Parameters
Physiological
1.T.Protein, Albumin, and globulin
2.AST & ALT
Hormones
1.LH
2.FSH
3.estrogen
4.progestrone
Hematology
1.RBC
2.WBC & differentiations
3.PLT
4.Hb
Productive
1.Body weight (g)
2.organ weight (g)
3. organs index
Histology
1.ovaries
2.Liver
3.spleen

3.2 *A. vulgaris* powder source:

Alchemilla vulgaris powder from Asst.proff. dr.Ibrahim Al-Jubour at head of pharmocognosy and medicinal plants department of pharmacy collage/University of Mustansiriyah /Baghdad. Figure (3_1) *A. Vulgaris* powder, mixed this powder with a small amount of distill water, after the homogeneity is complete, the mixture is mixed with the amount decided of distilled water.

Figure (3_1) *A. Vulgaris* powder

3.3 Blood collection:

At the end of the experiment period, the rats were anesthetized by using xylazine and ketamine (one from each cage randomly), Blood was collected by cardiac puncture. first portion of blood sample were puts into dry and clean tube with anticoagulant (1mg EDTA/5ml blood) and immediately using for estimations of some blood picture by using automatic blood counter apparatus .other part of blood The serum was carefully separated into dry clean gel tubes and kept frozen till analysis at-20°C.

3.4 Organs extraction:

Then take studied organs from these animals (ovaries, liver and spleen) with take balance them and put in formalin 10% then sent to hospital for postmortem and applied preparation of tissue shredding.

They were placed in a Bowen solution for 24 hours for the purpose of installation. The samples were then washed in 10% ethyl alcohol and placed in small glass containers marked with the animal number and the animal's own number for routine tissue work.

3.5 Methods

3.5.1 Parameters of study:

Analysis blood picture Automatically in apparatus in laboratory analysis begins when a well-mixed whole blood sample is placed on a rack in the analyzer. The instrument utilizes flow cells, photometers and apertures in order to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood ***Wheeler, L. A. (1998).***

3.6 Estimation of serum proteins concentrations:

3.6.1 Measurement of total protein concentration:

Total protein was measured by using special kit (Spinreact, Spain) in alkaline medium , protein gives an intensive violet- blue complex with cooper salt , color intensity is proportional to the total protein concentration, this method reported by **(Young, 1995) . Appendix(1)**

3.6.2 Measurement of Albumin concentration:

Albumin concentration in serum was estimated by colorimetric kit (Spinreact, Spain), Albumin at slightly acid PH and presence of bromcresol green, change the color from yellow – green to green – blue as indicator **(Young, 1995). Appendix(2)**

3.6.3 Measurement of Globulin concentration:

The estimation of total globulin concentration was carried out directly by **Young, (1995)** equation.

globulin concentration (g/dL) = serum total protein – serum albumin

3.7 Estimation of Liver enzymes concentrations:

3.7.1 Measurement of AST concentration:

Aspartate aminotransferase activity was measured using a special kit (Spectrum AST, Egypt) according to (**Young , 1990)** method , and flowing reaction . **Appendix(3)**

3.7.2 Measurement of ALT concentration:

Alanine aminotransferase activity in serum was measured using a special kit (Spectrum AlT, Egypt) according to (**Young , 1990)** method , and flowing reaction . **Appendix(4)**

3.8 Hormones concentrations:

Measurement of LH, FSH, estrogen and progestrone concentrations by special ELIZA kits (Biobase, Koria).In **appendix(5) and (6)**

3.9 Histological Technique:

Ovaries, liver and spleen of each animal were quickly removed and prepared for histological study according to ***Mescher method (2010)*** with aid of the light microscope.

3.10 Statistical Analysis:

Data was analyzed as one-way (ANOVA) using the general linear model (GLM) procedure to (SPSS 22.0) software ***(Delwiche, et al, 2012).*** Four treatment means were separated using a "protected" Duncan`s analysis in level (0.05).

CHAPTER FOUR

RESULTS

4 Results

4.1 Biochemical parameters:

4.1.1 Liver enzymes:

The effects of zinc sulfate and *Alchemilla vulgaris* on serum Aspartate Transaminase (AST), Alanine Transaminase (ALT) activity in female rats are represented in table (4_1)

It can be seen that serum ALT activity had a significantly($p \leq 0.05$) increased in 1000 Mg/L $ZnSO_4$ with 200Mg/L *A.V* (T_2) group and 300Mg/L *A.Vulgaris* (T_4) group, but decreased in 1000 Mg/L $ZnSO_4$ with 300 Mg/L *A. vulgaris* group(T_3) if compared with control group (CO), table(4_1).

Table 4_1: The effect of 1000Mg/L $ZnSO_4$ and *A. vulgaris* on liver enzymes ALT and AST

Parameter / Treatment	ALT(U/L)	AST(U/L)
CO(control group)	45.50 ±4.27	182.33± 15.43
T_1(1000 Mg/L $ZnSO_4$)	44.83 ±3.78	182.17 ±9.09
T_2(1000Mg/L$ZnSO_4$& 200Mg/L *A.v.)*	50.33 ±4.31	176.67 ±29.43
T_3(1000Mg/L$ZnSO_4$ and 300Mg/L *A.V*)	39.66 ±1.85	192.83 ±15.08
T_4(300Mg/L *A.Vulgaris*)	54.16 ± 4.61	185.00 ± 5.50

4.1.2 Serum Proteins:

The results revealed that there was decrease significant ($p \leq 0.05$) in serum albumin concentration in T_1 and T_2 comparing with CO, T_3 and T_4.

Serum globulin concentration results in table (4_2) were increased significantly ($p \leq 0.05$) in T_4 group compared with control group CO.

Table (4_2) Effect of *A.Vulgaris* on serum proteins in female rats exposed to 1000Mg/L $ZnSO_4$.

Parameters / Treatments	T.Protein(g/dl)	Albumin(g/dl)	Globulin(g/dl)
CO(control group)	6.38±0.43	4.25±0.24	2.71±0.34
T_1(1000Mg/L$ZnSO_4$)	6.28±0.66	3.82±0.22	2.66±0.33
T_2(1000Mg/L $ZnSO_4$ and200Mg/L *A.V.*)	6.05±0.38	3.79±0.13	2.91±0.23
T_3(1000Mg/L $ZnSO_4$ and300Mg/L *A.V.*)	6.03±0.33	4.35±0.36	2.60±0.45
T_4(300Mg/L *A.V.*)	6.50±0.46	4.34±0.30	3.00±0.37

4.1.3 Hormones

The data on table (4_3) pertaining to Progesterone, Estrogen, Follicular stimulating hormones (FSH) and Luteinizing hormone (LH)

The results explained that a significant increase ($p \leq 0.05$) in progesterone of the T_4 compared with control group, but the results other groups showed a significantly($p \leq 0.05$) decrease compared with control group.

The estrogen concentration in T_4 group was significantly increased when compared with control group, whereas the estrogen concentrations were no significant ($p \leq 0.05$) differences between another groups.

On the contrary, the results revealed that a significantly($p \leq 0.05$) increase in concentration of FSH hormone in T_3 group compared with control group CO.

LH hormone concentration in T_3 group was significantly increased compared with control group, table (4_3)

Table (4_3) Effect of *A.Vulgaris* on hormones in female rats exposed to 1000Mg/L $ZnSO_4$.

parameter / Treatment	Progesterone (Pg/ml)	Estrogen (Pg/ml)	FSH (Pg/ml)	LH (Pg/ml)
CO(control group)	28.78±5.70	50.05±7.73	0.10±0	0.14±0.01
T_1(1000Mg/L$ZnSO_4$)	11.21±3.15	52.59±9.16	0.13±0.007	0.19±0.01
T_2(1000Mg/L $ZnSO_4$ and 200Mg/L *A.V.)*	10.99±1.53	49.85±2.68	0.11±0.01	0.17±0.01
T_3(1000Mg/L $ZnSO_4$ and300Mg/L *A.V.*)	17.62±6.87	48.93±8.63	1.32±0.29	1.17±0.26
T_4(300Mg/L *A.V.)*	44.35±11.86	69.11±16.42	0.12±0.009	0.15±0.04

4.1.4 Blood Parameters

4.1.4.1 Red Blood Cells, Hemoglobin and Platelets

Table (4_4) represents the mean values of RBC_s, HGB, and PLT levels in control and treated groups

Administration 1000Mg/L of zinc sulfate caused a significantly ($p \leq 0.05$) drooping in RBCs numbers in T_1 (1000 Mg/L $ZnSO_4$), if compared with control group CO and other groups, in contrast to the results of RBC_S numbers in T_2 (1000Mg/L $ZnSO_4$ and 200Mg/L *A.V.)* which showed a significant($p \leq 0.05$) rising , if compared with other groups.

In T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.V.*) treatment RBC_s numbers were significantly decreased ($p \leq 0.05$), if compare with CO.

Significant ($p \leq 0.05$) decrease in the results of HGB levels was found in T_3 group compared with control group CO and another groups.

Analysis of data in table (4_4) also indicated that PLT levels increased significantly ($p \leq 0.05$) in T_2, T_3, T_4 comparing with control group.

Table (4_4) : the effect of zinc sulphate and *A. Vulgaris* on RBC, HGB & PLT of the study(Mean±SE)

Parameter / Treatment	$RBC_s(x10^6/mm^3)$	HGB(g/dl)	PLT%
CO(control group)	6.44± 0.47	13.05± 1.49	514.5± 93.4
T_1(1000 Mg/L $ZnSO_4$)	6.001± 0.10	12.71± 1.19	546.8± 94.51
T_2(1000Mg/L$ZnSO_4$ and200Mg/L *A.V)*	7.08± 0.22	13.25± 0.43	636.0± 76.56
T_3(1000Mg/L$ZnSO_4$ and300Mg/L *A.V)*	5.56± 0.43	10.92± 0.87	712.5± 65.13
T_4(300Mg/L *A.V)*	6.51± 0.18	11.90± 0.34	640.7± 83.95

4.1.4.2 White Blood Cells and differentiations

Table (4_5) represents the mean values of WBC_s, GRA%, LYM% and MID% levels in control and treated groups

In T_2, T_3 & T_4 treatment groups WBC numbers respectively were increased significantly ($p \leq 0.05$), if comparing with control group CO.

Significant ($p \leq 0.05$) increased in the result of GRAN% was found in T_1, T_2, T_3 & T_4 respectively, when compared with control group CO.

Administration 1000Mg/L of zinc sulfate caused no significant ($p \leq 0.05$) in LYM% in all treatments if compared with control group.

The MID% significant ($p \leq 0.05$) increased in T_4 treatment when compared with control group CO.

Table (4_ 5): the effect of zinc sulphate and *Alchemilla Vulgaris* on WBC, GRAN%, LYM% & MID% of the study(Mean±SE)

Parameters / Treatment	$WBC_s(10^9/1)$	GRA%	LYM%	MID%
CO control group	5.91± 0.61	4.31±1.93	89.37±2.07	6.41± 0.45
T_1(1000Mg/L$ZnSO_4$	5.42± 0.47	8.59±0.63	87.01±1.89	6.05± 0.68
T_2(1000Mg/L$ZnSO_4$and 200Mg/L *A.V)*	7.45± 0.46	6.88±0.93	85.87±2.23	6.28± 1.01
T_3(1000Mg/L$ZnSO_4$& 300Mg/L *A.V*)	7.50± 1.77	9.64±1.88	84.49±3.49	6.76± 1.60
T_4(300Mg/L*A.V*)	6.80± 1.98	6.02±2.71	86.41±2.69	7.86± 0.52

4.2 Performance:

4.2.1 Effect of zinc sulfate and *A.V* and their combination on weekly live body weight, organs and index weight:

The result in table (4_6) showed significant (P≤0.05) decrease in body weight in T_1 group at 1st, 2nd, 3rd, 4th and 5th weeks from experiment periods , respectively if compared with control group CO.

Table (4_ 6) Effect of *A.Vulgaris* and zinc sulphate on body weight in female rats

Weeks / Treat.	1st week(g)	2nd week(g)	3rd week(g)	4th week(g)	5th week(g)	6th week(g)	7th week(g)	8th week(g)
CO	182.5± 18.10	196.6± 20.5	210.0± 19.66	210.0± 17.07	210.0± 15.59	198.0± 12.20	197.5± 11.98	178.3± 15.89
T_1	165.0± 13.41	174.1± 15.88	182.5± 15.90	187.5± 9.72	194.1± 13.80	197± 15.29	196.25 ±18.52	168.33 ±11.66
T_2	188.3 ± 15.89	199.1 ± 15.99	196.67 ± 15.2	198.3 ± 10.05	196.6 ± 7.60	193 ± 11.57	191.25 ±15.46	170±17 .55
T_3	195.8 ± 11.57	204.1 ± 11.06	205.0 ± 11.03	207.5 ± 10.85	206.6 ± 10.54	206 ± 11.33	196.25 ±10.07	188.33 ±6.006
T_4	182.5± 17.78	195.8 ± 17	201.6 ± 15.20	206.6 ± 14.86	210.0 ± 12.71	198 ± 9.96	192.50 ±9.68	188.33 ±15.89

4.2.2 Organs weight

Table (4_7) was showed spleen weight significantly (P≤0.05) increased in T_1, T_3 & T_4 groups, respectively compared with control CO.

The result in table(4_7) showed no significant (P≤0.05) in right ovary weight in all groups if compared with control group CO.

Table (4_7) was showed left ovary weight significantly (P≤0.05) increased in T_1 compared with control CO.

Table (4_7) Effect of *A.Vulgaris* and zinc sulphate on study organs weight in female rats

Organs / Treat.	LIVER g	SPLEEN g	RIGHT OVARY g	LIFT OVARY g
CO	5.83 ± 0.21	0.93 ± 0.05	0.14± 0.02	0.16±0.007
T_1	5.57 ± 0.24	1.11 ± 0.38	0.14± 0.01	0.25 ± 0.12
T_2	5.55 ± 0.22	0.71 ± 0.04	0.12± 0.01	0.12 ± 0.01
T_3	6.06 ± 0.21	1.03 ± 0.14	0.13 ±0.01	0.13 ± 0.01
T_4	5.78 ± 0.24	1.13 ± 0.09	0.12± 0.01	0.12 ± 0.01

4.2.3 Index weight of organs

In T4 and T5 treatment groups the mean values of index lift ovary weight, were increased significantly ($p \leq 0.05$), if comparing with control group CO, the index lift ovary weight in T_1 group and T_2 group no significant when compare with control group CO.

Table (4_ 8) Effect of *A.Vulgaris* and zinc sulfate on index study organs weight in female rats

Organs / Treat.	LIVER	SPLEEN	RIGHT OVARY	LIFT OVARY
CO	0.06± 0.11	0.06 ± 0.01	0.43± 0.04	2.83±0.016
T_1	0.11 ± 0.05	0.07 ± 0.01	0.51± 0.18	3.03± 0.26
T_2	0.06± 0.004	0.06± 0.003	0.35± 0.02	3.00± 0.25
T_3	0.06± 0.004	0.06± 0.003	0.43± 0.08	3.16± 0.16
T_4	0.06± 0.004	0.06± 0.003	0.43± 0.08	3.16± 0.16

4.4 Histological study:

4.4.1 The histological changes in liver:

The histological sections obtained from liver of female rats in the control group (CO) stained with (E&H) shows normal parenchymal tissue, normal architecture of central vein and normal sinusoidal. As figure (4_1).

On other hand, histopathological sections of liver tissue in 1000Mg/L $ZnSO_4$ groups that induced oxidative stress (T_1), figure (4_2), shows central vein congestion, feathery degeneration and narrow sinusoids.(stain H&E).(X40).

But the result of histopathological changes in T_2(1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) treatment has shown mild return back normal hepatocyte with little congestion and significant degeneration section (mild response). figure (4_3).

While the results in histpathological changes in T_3(1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*) treatment has shown return back normal regular hepatocyte with mild degenerative and no necrosis , (mild response). figure (4_4).

The liver tissue treated with 300 Mg/L *A.Vulgaris* (T_4) shows normal parenchymal tissue with no inflammation with mild degeneration, figure (4_5) .

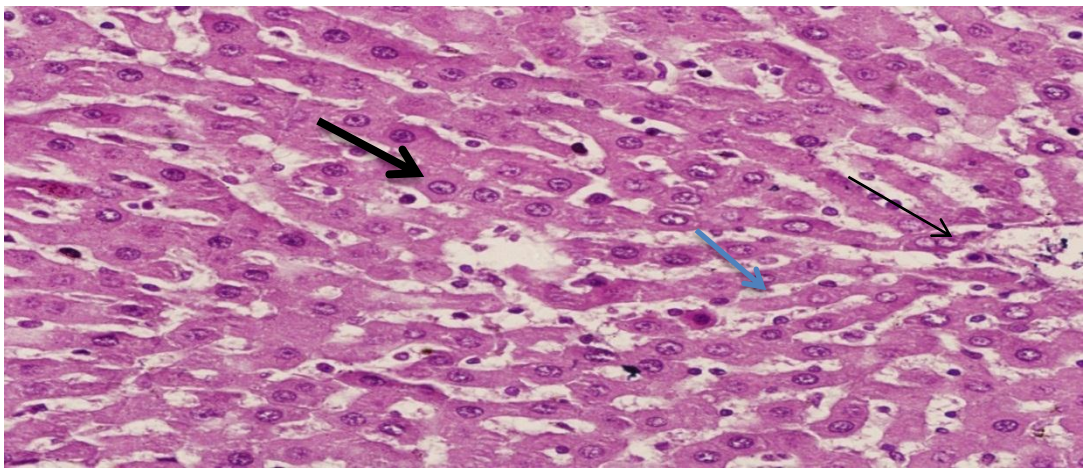


Figure (4_1) liver in female rats of control group(T1). Shown normal hepatocytes (thick arrow), normal central vein (thin arrow) and normal sinusoidal(blue arrow). (stain H&E).(X40).

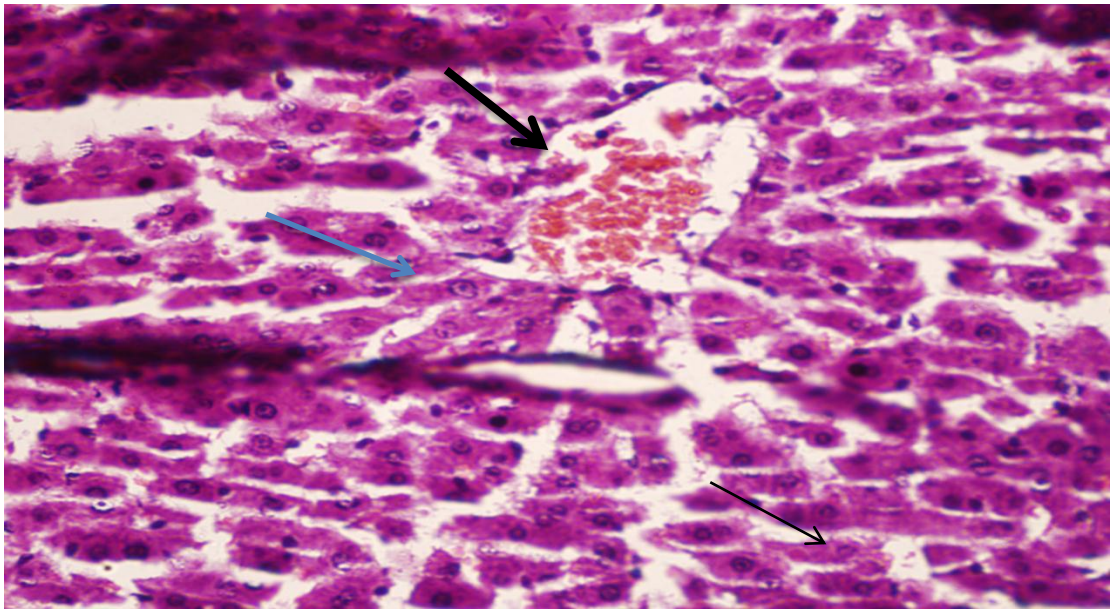


Figure (4_2) liver in female rats with 1000Mg/L ZnSO4 (T_1)group, shows central vein congestion (thick arrow) , feathery degeneration (thin arrow) and narrow sinusoids (blue arrow) .(stain H&E).(X40)..

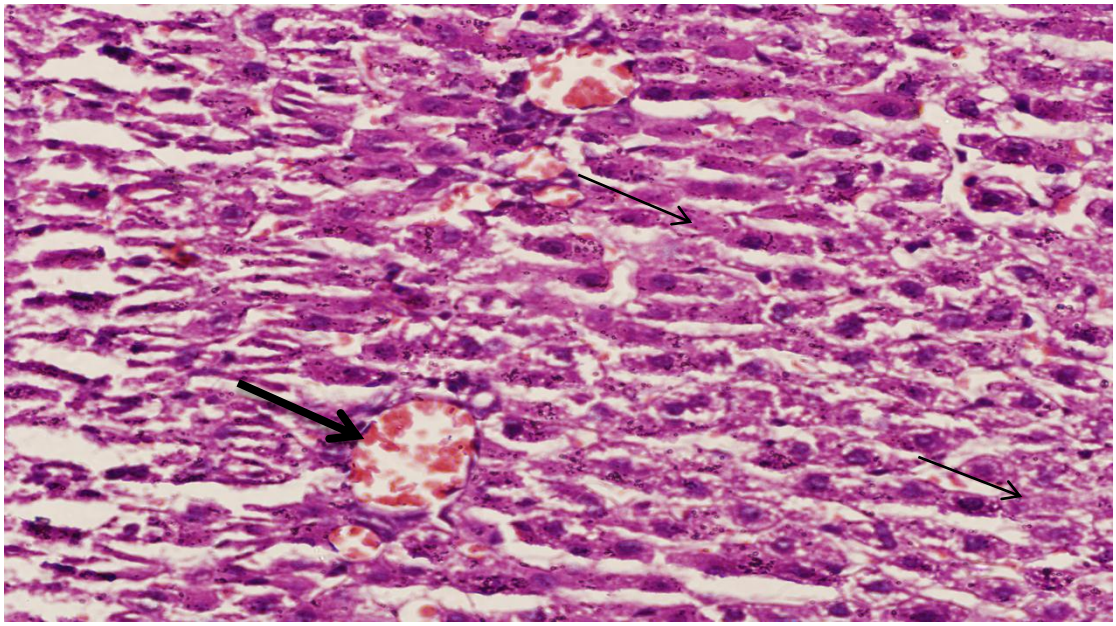


Figure (4_3) liver in female rats treated with 1000Mg/L $ZnSO_4$ and 200Mg/L *A.Vulgaris* (T_2) group, shows mild congestion (thick arrow) and mild degeneration (thin arrow) .(stain H&E).(X40).

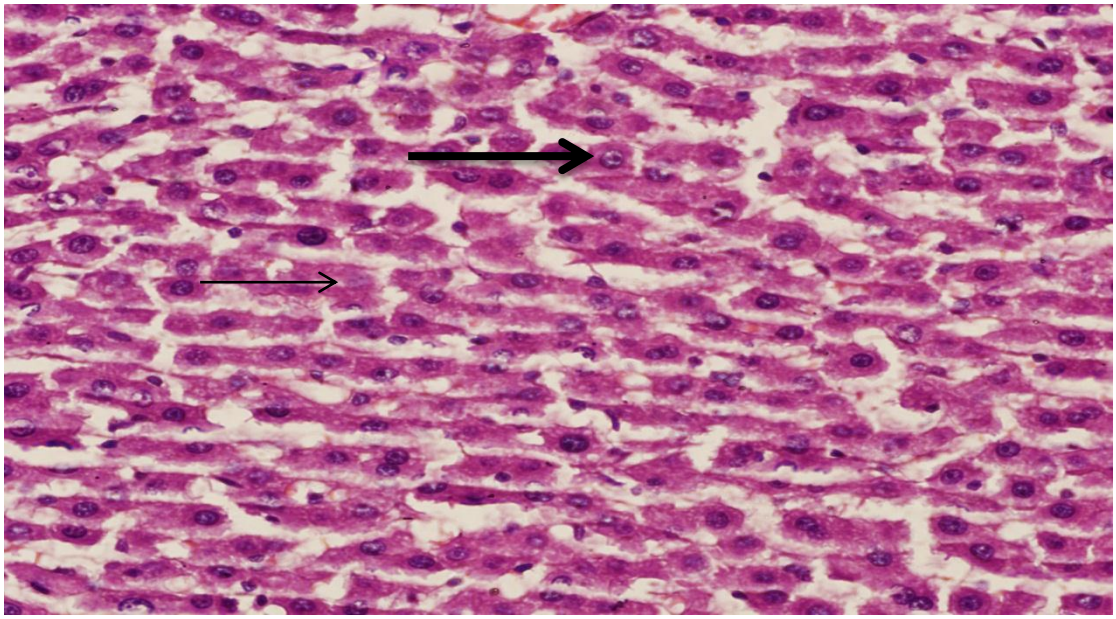


Figure (4_4) liver in female rats treated with 1000Mg/L $ZnSO_4$ and 300Mg/L *A.Vulgaris* (T_3) group, shows normal hepatocytes (thick arrow) with mild degeneration (thin arrow) .(stain H&E).(X40).

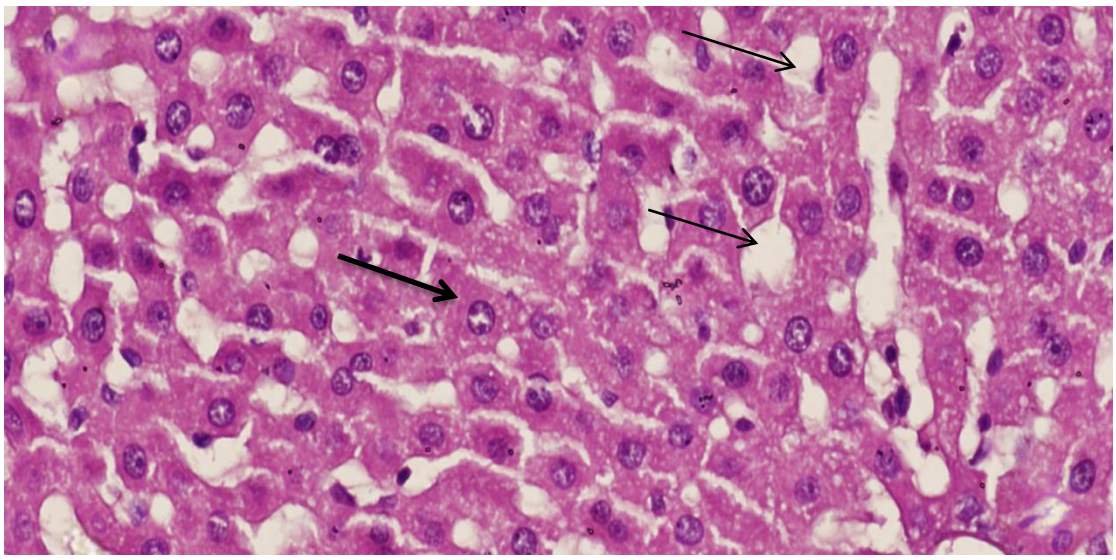


Figure (4_5) liver in female rats treated with 300Mg/L *A.Vulgaris* (T_4) group, shows regular hepatocytes(normal hepatocytes)(thick arrow) and steatosis (fatty change) (thin arrow).(stain H&E).(X40).

4.4.2 The histological changes in Spleen:

The histological sections obtained from spleen of female rats in the control group (CO) stained with (E&H) shows normal tissue, normal red pulp , normal white pulp and normal central artery. As figure (4_6).

On other hand, histopathological sections of spleen tissue in 1000Mg/L $ZnSO_4$ groups that induced oxidative stress (T_1), figure (4_7) showed sever congestion of red pulp sinusoids with hemosiderin-laden macrophages.

But the result of histopathological changes in T_2(1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) treatment has shown sever hemorrhage in red pulp and hemosiderin-laden macrophage in white pulp. figure (4_8).

While the results in histpathological changes in T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*) treatment has shown mild congestion with significant number of hemosiderin-laden macrophage .figure (4_9).

The spleen tissues treated with 300 Mg/L *A.Vulgaris* (T5) shows normal red pulp and white, figure (4_10).

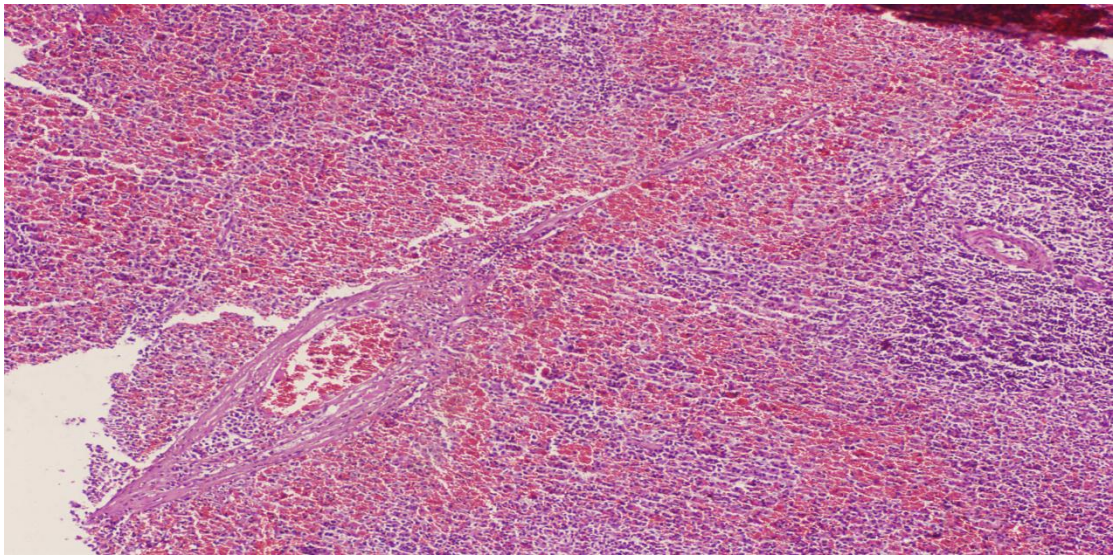


Figure (4_6) spleen in female rats in control (CO) group, shows normal tissue, normal red pulp , normal white pulp and normal central artery.(stain H&E).(X40).

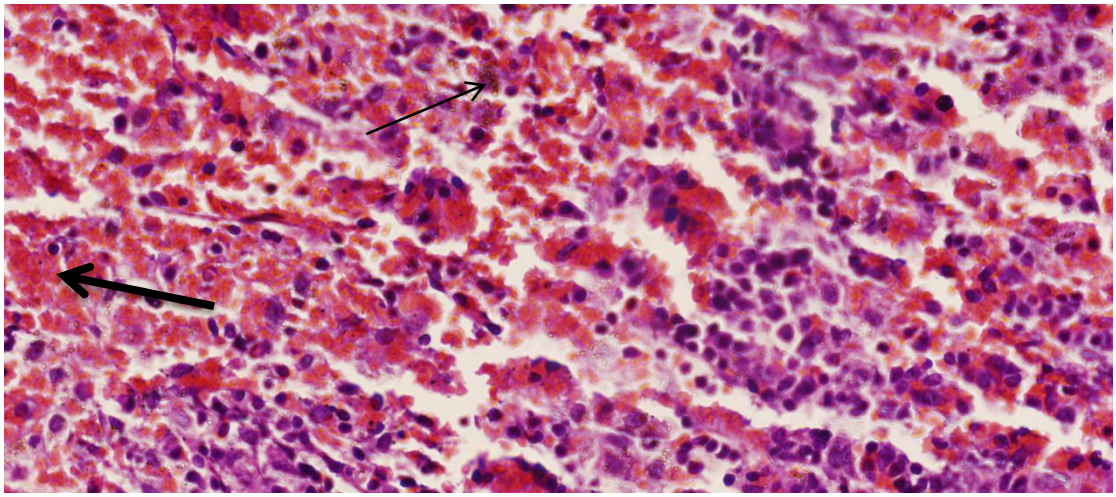


Figure (4_7) spleen in female rats with 1000Mg/L $ZnSO_4$ (T_1) group, shows sever congestion of red pulp sinusoids (thick arrow) with hemosiderin-laden macrophage (thin arrow) .(stain H&E).(X40).

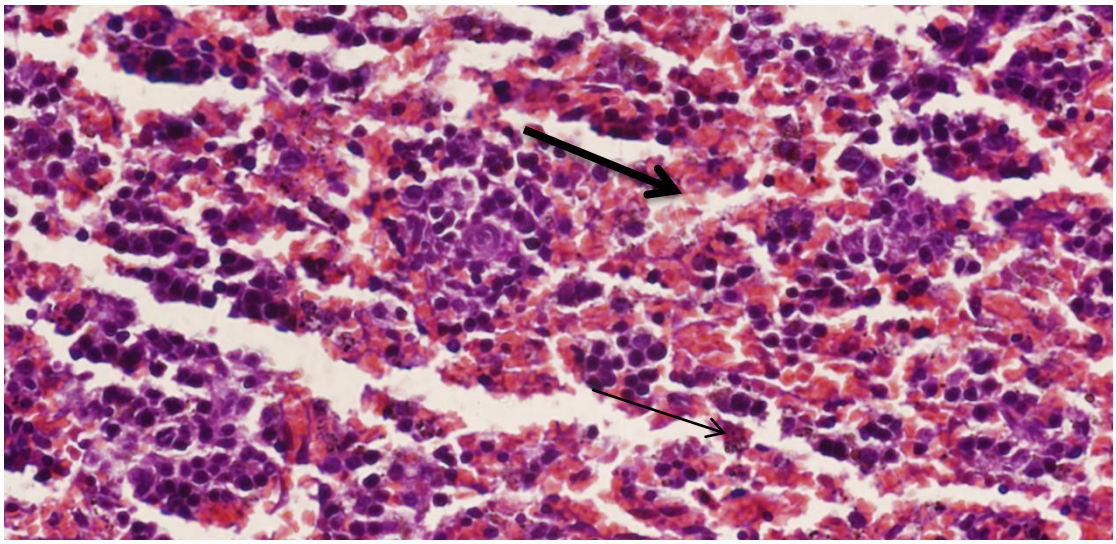


Figure (4_8) spleen in female rats treated with 1000Mg/L $ZnSO_4$ and 200Mg/L *A.Vulgaris* (T_2) group, shows sever hemorrhage (thick arrow)and hemosiderin-laden macrophage (thin arrow) in white pulp .(stain H&E).(X40).

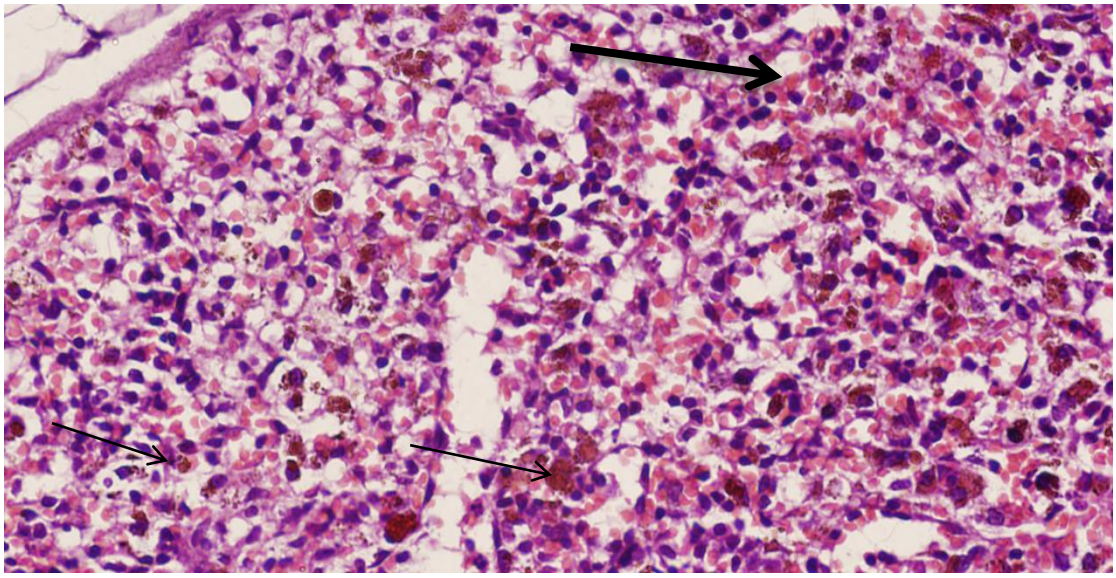


Figure (4_9) spleen in female rats treated with 1000Mg/L $ZnSO_4$ and 300Mg/L *A.Vulgaris* (T_3) group, shows mild congestion (thick arrow) with number of hemosiderin-laden macrophage (thin arrow) in white pulp.(stain H&E).(X40).

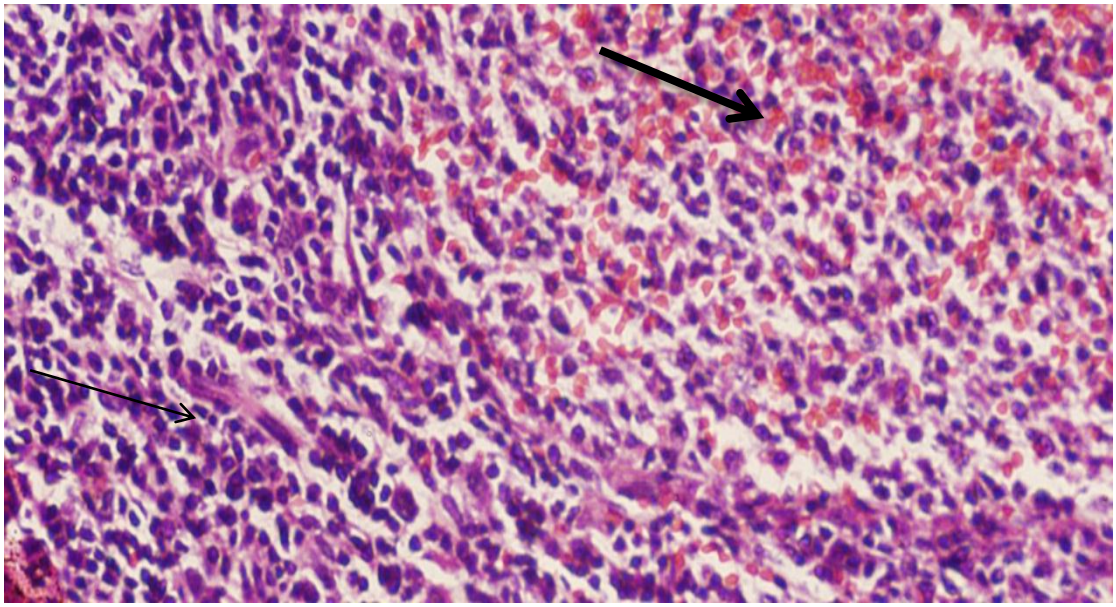


Figure (4_10) spleen in female rats treated with 300Mg/L *A.V* (T_4) group, shows normal red pulp (thick arrow) and normal white pulp (thin arrow) (normal tissues).(stain H&E).(X40).

4.4.3 The histological changes in ovary:

The histological sections obtained from ovary of female rats in the control group (CO) stained with (E&H) shows normal ovarian tissue. As figure (4_11).

On other hand, histopathological sections of ovary tissue in 1000Mg/L $ZnSO_4$ group (T_1), figure (4_12) showed graffian follicle.

But the result of histopathological changes in T_2 (1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) treatment has shown normal graffian follicles. figure (4_13).

While the results in histpathological changes in T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*) treatment has shown normal mature graffian follicles. figure (4_14) .

The ovary tissues treated with 300 Mg/L *A.Vulgaris* (T_4) shows significant increase in number normal primordial growing follicles. figure (4_15).

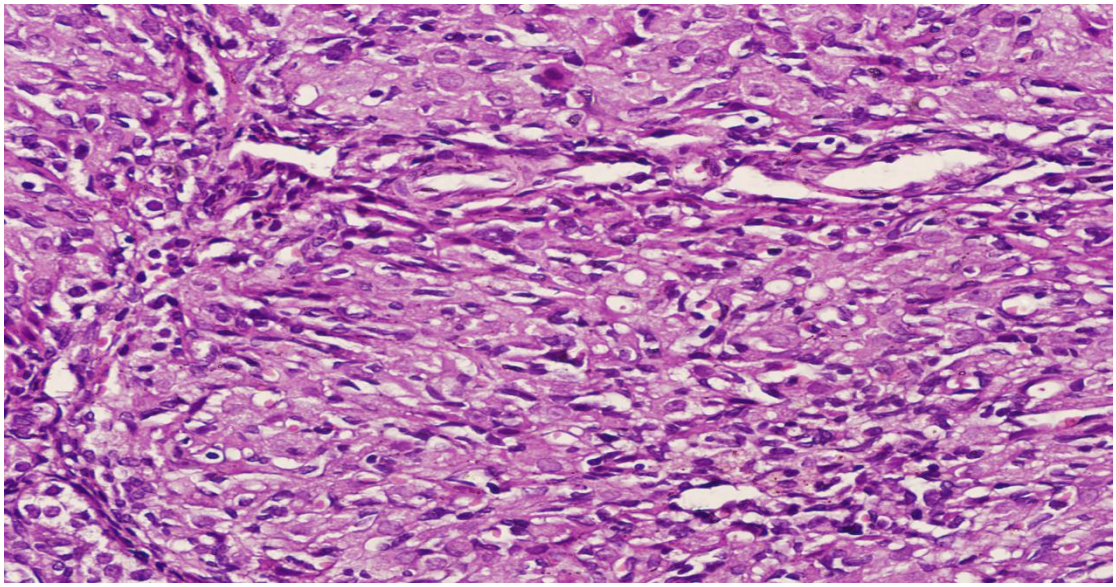


Figure (4_11) ovary in female rats of control group(CO). Shown normal ovarian tissue. (stain H&E).(X40).

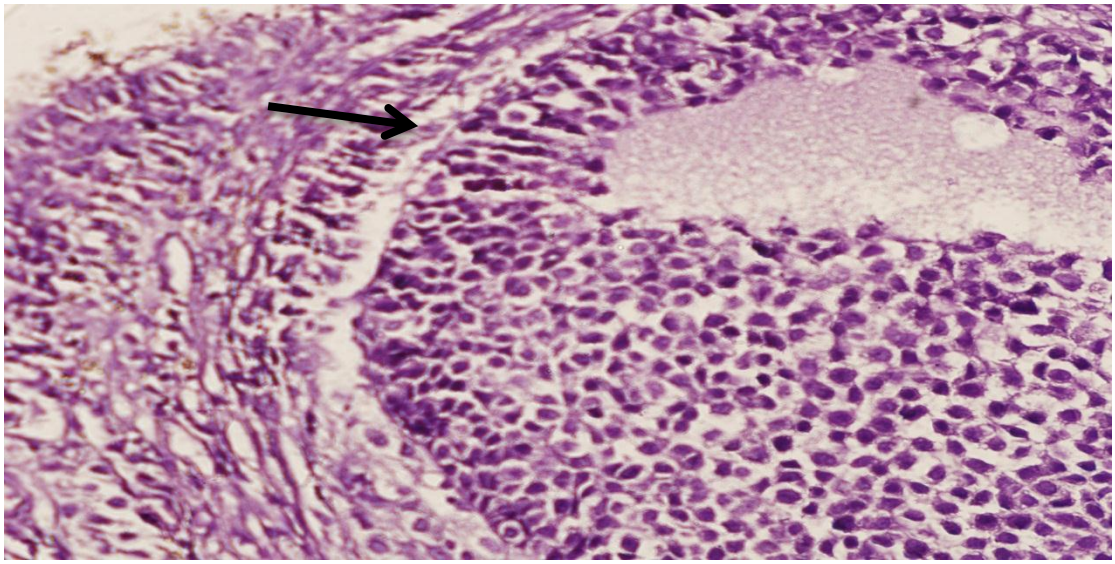


Figure (4_12) Ovary in female rats of T_1(1000Mg/L $ZnSO_4$) group. Shown graffian follicle . (stain H&E).(X40).

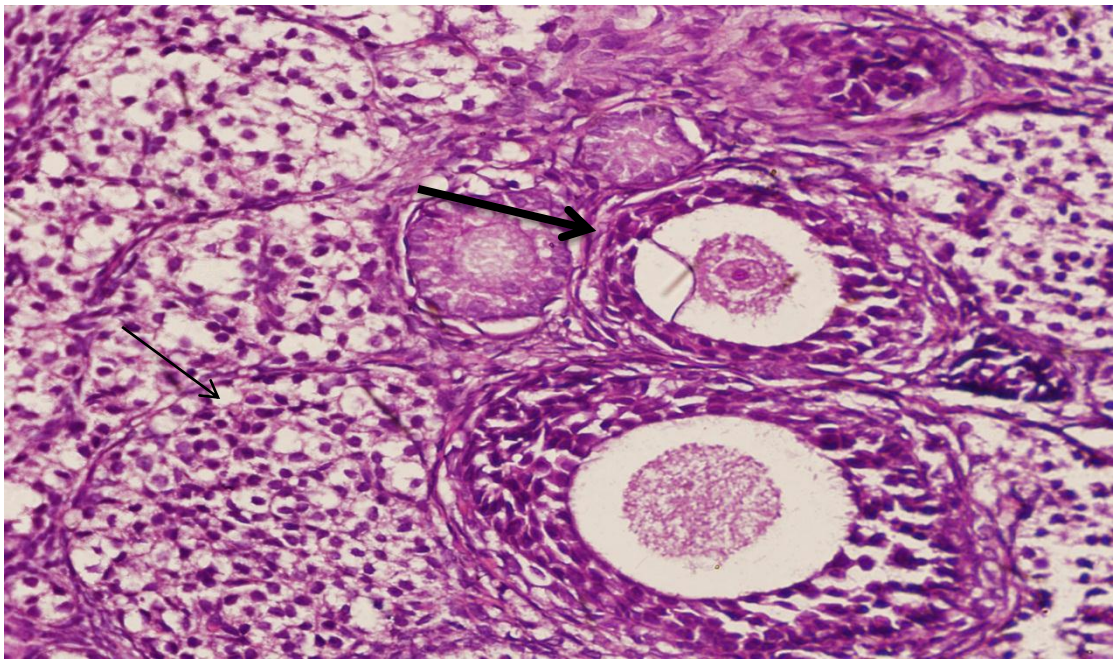


Figure (4_13) Ovary in female rats of T_2(1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) treatment has shown normal growing follicles. (stain H&E).(X40).

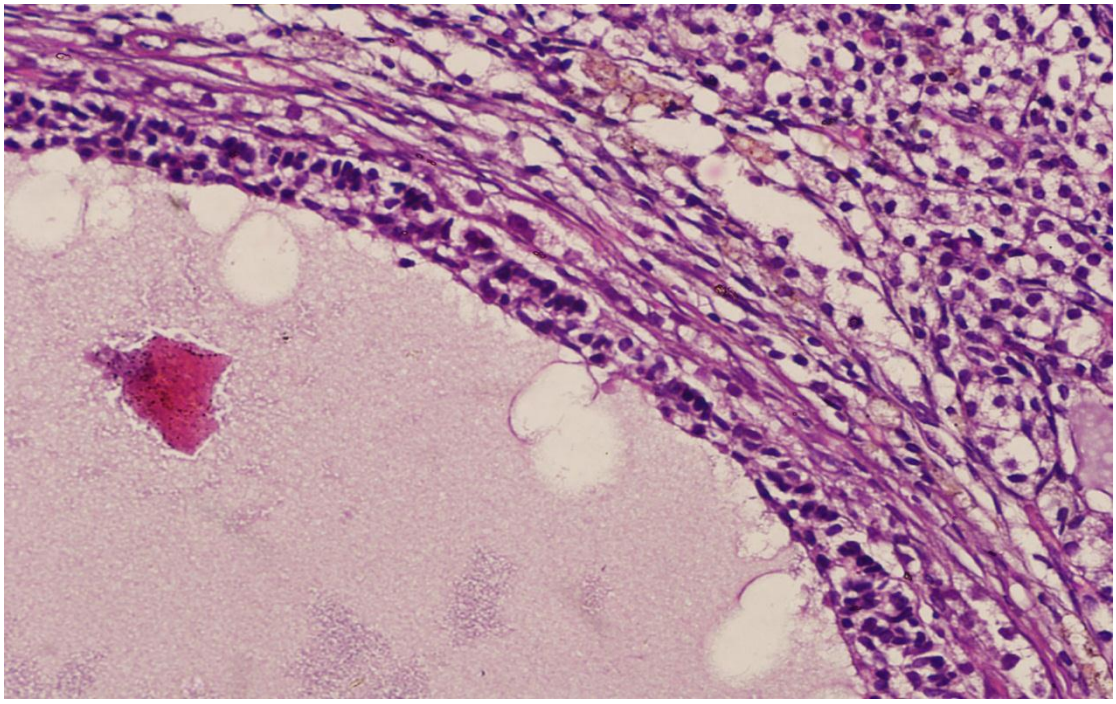


Figure (4_14) Ovary in female rats of T_2 (1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) treatment has shown normal mature graffian follicle. (stain H&E).(X40).

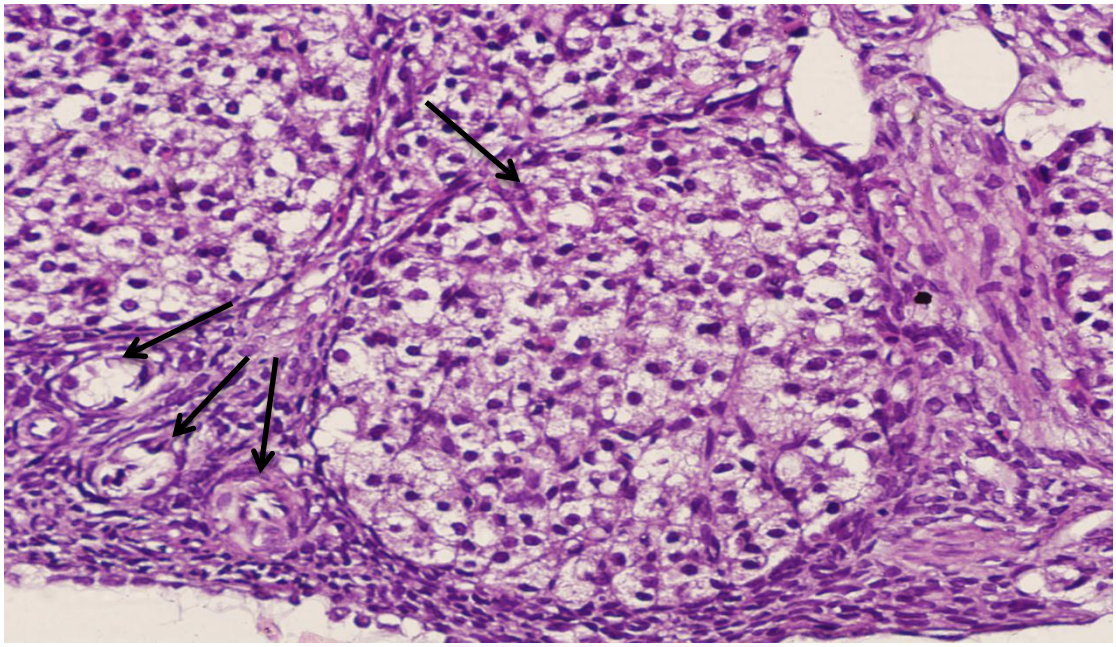


Figure (4_15) Ovary in female rats treated with 300Mg/L *A.Vulgaris* (T_4) group, shows normal growing follicles .(stain H&E).(X40).

CHAPTER FIVE

DISCUSSION

5 Discussions

5.1 Biochemical parameters:

5.1.1 Liver enzymes

The result of the present study indicated a significantly differences in ALT in T_2, T_3 and T_4 groups, as Noticed decreasing of ALT activity in T_3 (1000Mg/L $ZnSO_4$ with 300 Mg/L *A.Vulgaris*) group , but showed increasing of ALT activity in T_2(1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) and T_4 (300 Mg/L *A.Vulgaris*), but not change in T_1 (1000Mg/L $ZnSO_4$) group in activity enzyme in table (4_1).

Liver enzymes are used to assess liver damage, disturbance of liver enzymes is probably due to the presence of free radicals that leading to release of a marked quantity of these enzymes into the serum after hepatocytes damage ***(Choudhary and Devi, 2014).***

Elevation in serum liver enzyme (ALT) a reflection of radical mediated lipid peroxidation of liver cell membrane, release of liver enzymes from cytosol can occur secondary to cellular necrosis with membrane damage, causing elevation in serum levels of these enzymes ***(Sosnowski, et al., 2012).***

The primary goal of herbal approach to healthy liver is to enhance detoxification processes and help protection against further damage based on their ability for helping and promoting balance within the body and nourish the liver and related functions including digestive and bile secretion. ***(Said, et al, 2011)***

Flavonoids one of the antioxidant components that inhibits oxidative stress, and prevent hepatoxicity by prevent lipids peroxidation and pore formation in liver cells membranes , hence

return back the levels of AST and ALT to the normal ***(Hamden, et al., 2009).***

The result of liver enzyme (ALT) in T_3 group in our study are agreement with many previous indicated to the role of antioxidetive effect of flavonoids compound in medicinal plant, against free radical accumulation by excessive zinc supplementation with drinking water may be this plant rich with phenolic compounds , flavonoids and tannins, on activity of liver enzyme and improvement of the liver functions in experimental animals. Such as ***(Samani, et al, 2018);(Said, A. M., et al, 2011);(Ansari, I., & Maiti, D. (2018);(Ho, W. Y., et al, 2012),*** but not agreement with our results in histopathologicals sections of liver in T_4 treatments in figure (4_2), (chapter four)

5.1.2 Serum Proteins:

The results of this study showed no any different of total protein in all treatments, table (4_2) .

Thus, the results revealed that there was no significant ($p \leq 0.05$) but decrease in mean value of serum albumin concentration in T_1 and T_2.

Free radical produce throughout oxidative stress are able to damage the peptide back bone of protein. this may be also lead to miss - folding and depression of protein ***(Khudair, 2010).***

In T_1 results because effect of free radicals produced after ZnSO4 exposure may mediated protein oxidation and degradation of albumin leading to it's depletion ***(Roche et al., 2009).***

In T_2 no responses of the liver cells for this concentration of medicinal plant (200Mg/L *A.V*) against toxicity of zinc sulfate.

But, in T3 group there is evidence of a hepatoprotective activity of A.vulgaris by the polyphenolic and flavonoids compounds of this plants leaves, which have potent antioxidant properties in this concentration (300Mg/L) where led to return normal activity of albumin***.(El-Hadidy, et al, 2019);(Afshar, et al, 2015)*** I can considered this dose it is effective dose

Serum globulin concentration results in table (4_2) were significantly ($p \leq 0.05$) increased in T_4 group.

This result return to same cause in albumin activity in T_3 group when treated with 300Mg/L of *A.v* in drinking water.

5.1.3 Hormones:

The results explained that a significantly increase ($p \leq 0.05$) in progesterone of the T_4, but the results other groups T_1, T_2, and T_3 showed a significantly ($p \leq 0.05$) decrease.

The increment of estrogen and decrement of progesterone in T_1 (imbalance between them) due to the zinc interfere with hormone (estrogen) receptors which capable increase estrogen in blood, my results are agreemented with ***(Georgescu, et al, 2011);(Oğuz , et al, 2014)***

Also, the improvement of ovary hormones may be attributed to increase activity of enzymes led to increase production of hormones ***(Eshak , et al, 2018);(Özbilgin, et al, 2015)***

The estrogen concentration in T_4 group was significantly increased due to the regulator effect of medicinal plant the photoestogenocity compounds.

On the contrary, the result revealed that a significantly($p \leq 0.05$) increase in concentration of LH and FSH hormones in T_3 group, table (4_3).

Sağlık Bilimleri Dergisi, 1930 stated the role of herbal medicine in hormone imbalances, these herbs act on function of regulatory mechanisms such as the endocrine system, that control hormones. also, may assist in regulating, this cycle by encouraging a normal, healthy balance of estrogen and progesterone in women. so, may assist in maintaining a healthy balance of LH and FSH hormones in the body, as well as stimulating the secretion of progesterone in the luteal phase of a woman's cycle..

As I pointed out in chapter four (results chapter) effect of the herbal plant (*A.Vulgaris*) on the hormones in increase numbers of primoredial growing in ovary.

5.1.4 Blood Parameters

5.1.4.1 Red Blood Cells, Hemoglobin and Platelets

In table(4_4) , When administration 1000Mg/L of zinc sulfate caused a significantly($p \leq 0.05$) drooping in RBCs numerous in T_1(1000 Mg/L $ZnSO_4$) and T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*) groups.

The excessive zinc led to copper deficiency through lower absorption in small intestine that result in anemia , these results agreement with ***(Rawi, et al, 2015);(Fischer, et al, 2005);(AL-diwan, et al, 2010).***

Plum, et al, 2010 stated the mechanism by which copper deficiency causes anemia is based on the requirement of copper for several enzymes

involved in iron transport and utilization and, therefore, in heme synthesis.

In contrast to the results of RBC_S numbers in T_2(1000Mg/L $ZnSO_4$ and 200Mg/L *A.Vulgaris*)which showed a significant ($p \leq 0.05$) rising .

A herbal formulation used to treat patients with sickle-cell anaemia complicated with jaundice. Through antioxidant action of compounds in this plant against oxidation reaction through ingestion high doses of zinc sulfate as**. *(Ishola, et al, 2015)***

Also, indicated that the percent of PLT levels significantly ($p \leq 0.05$) increased in T_2 (1000Mg/L $ZnSO_4$ and 200Mg/L *A.Vulgaris*), T_3(1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*), and T_4(300Mg/L *A.Vulgaris*) treatments.

There, the aerial parts of this plant (*A.V*) are used to heal inflammation of female reproductive tracts, including maintaining to stop minor bleeding and to treat wounds. Also, it is in Libyan folk that this medicine is applied in urinary diseases. Moreover, it is also used to treated ovarian infections in women as well as for the treatment of internal bleeding and treat vaginal diseases as uterine and abdominal relaxations after birth and repeated abortions**.*(EDRAH, S. M. (2017)***

Some the experts considered this plant (*A.V*) to be good to treatments the wounds because its have coagulation action(blood clotting). ***(Al-osaj, S. L. (2016).***

It is used to heal inflammations in mouth, bleeding of the nose, furuncules and gynecological diseases. this plant is also considered to regulate the glandular activity of uterine and reduce bleeding**.*(ERGENE, et al, 2010)***

5.1.4.2 White Blood Cells and differentiations:

In T_2, T_3 and T_4 treatment groups the numbers of WBC were increased. Table (4_5).

This mean in the inflammatory states increased in numbers of inflammatory cells(WBC) in blood in these treatments T_2, T_3 and little increment in T_4 group due to use this plant in them .

Flavonoids are an important class of natural products; particularly, they are belonging to a class of plant secondary metabolites having a polyphenolic structure, In nature, these compounds are products extracted from plants and they are found in several parts of the plant (widely found in fruits, vegetables and certain beverages***).(Di Carlo, et al, 1999);(Panche, et al, 2016)***

Flavonoids are reported to have antioxidant, anti-inflammatory and antiproliferative effects, which could explain possible involvement with development of diseases. However, many flavonoids are also considered endocrine disruptors.***(Ohlsson, et al, 2010);(Zendehbad, et al, 2014);(Kaya, et al, 2012)***

Also, added ***Delcheva, et al, 2016*** herba *Alchemillae* is characterized by astringent, anti-inflammatory, styptic, and epithelium recovery effects.

5.2 Performance:

5.2.1 Effect of zinc sulfate and *A.V* and their combination on weekly live body weight, organs and index weight:

The result in table (4_6) showed significant ($P \leq 0.05$) decrease in body weight in T_1 group at 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} weeks from experiment periods, respectively if compared with control group CO.

These results are in agreement with ***Lucia, et al, 2010*** who indicated the role of oxidative stress induced by zinc sulfate on body weight in female rats.

High zinc concentrations may cause weight loss in the animals. through decreased food intake by way of inhibits appetite, so decreased body weight.

Sidiq, et al, 2018 explained cause high body weight in T_3 (1000Mg/L ZnSO4 with 300 Mg/L *A.Vulgaris*) group when used this herbal plant as protective effect against oxidative stress of zinc sulfate in this experiment.

5.2.2 Organs weight:

Table (4_7) was showed spleen weight non significantly ($P \leq 0.05$) increased the mean values in the (T2) , (T4)and (T5) groups.

In T_1 treatment the increment in size of spleen because presence free radicals which produced from oxidative stress toxicity of zinc sulfate in drinking water led splenomegaly, this result agreement with ***Khan, et al, 1999.***

While the increment in size of spleen in T_3 and T_4 groups return to the increasing body weight of these treatments.

5.2.3 Index weight of organs:

In T_3 and T_4 treatment groups the mean values of index lift ovary weight, were increased. table (4_8)

This result return to increase activity of ovary in these animals and increase the primordial follicles.

Used herbal medicine in different times during the menstrual cycle in women. This treatment is utilized to help restore balance to hormonal fluctuations. as this plant where act on increased the numbers of growing primordial follicles in ovaries, so, this led to increase in weight ovary, this result shows in chapter four, this results agreement with ***Yarnell, E., & Abascal, K. (2009)***

5.3 Histological study:

5.3.1 The histological changes in liver:

In figure (4_2), the results of the present study showed histopathological changes in the liver tissues of female rats treated with 1000Mg/L $ZnSO_4$ was characterized by central vein mild congestion, degeneration and narrow sinusoids.

The heavy metal toxicity as zinc is their ability to bind strongly to oxygen and induce oxidative stress, through produce free radicals ***(Thapa, et al, 2012)***

In a study by ***Plum, et al, 2010*** when ingested of 150 mg zinc sulfate tablets, leads to appear symptoms as: abdominal cramps, nausea, and vomiting.

While in figure (4_3), the result of histopathological changes in T_2 (1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) treatment has shown mild return back normal hepatocyte with little congestion and significant degeneration section (mild response).

Also, in figure (4_4), the results in histpathological changes in T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*) treatment has shown return back normal regular hepatocyte with mild degenerative and no necrosis , (mild response).

The liver tissue treated with 300 Mg/L *A.Vulgaris* (T_4) shows normal parenchymal tissue with no inflammation and normal architecture of central vein with mild degeneration, figure (4_5).

The use of medicinal plants in a high level of antioxidant constituents has been important role as an effective therapeutic approach for hepatic damages. Often of the antioxidant compounds in a typical diet are derived from plant sources and belong to different classes of compounds as phenols, flavonoids, tannins, carotenoids and vitamins which play important roles in health protection from the risk of most diseases. This no agreement with ***(El-Sayed, et al, 2017)*** due to present little congestion in some cells of liver.

Natural antioxidants from plants, the most prominent representatives of these compounds can protect the human body from free radicals***.(Sidiq, et al, 2018)***

5.3.2 The histological changes in Spleen:

The histopathological sections of spleen tissue in 1000Mg/L $ZnSO_4$ groups that induced oxidative stress (T_1), figure (4_7) showed sever congestion of red pulp sinusoids with hemosiderin-laden macrophages.

In a study by ***Samman and Roberts, 1990*** leads ingestion tablets containing 150 mg of zinc sulfate, to appear symptoms in GIT.

But the result of histopathological changes in T_2(1000Mg/L $ZnSO_4$ with 200Mg/L *A.Vulgaris*) treatment has shown mild hemorrhage in red pulp and hemosiderin-laden macrophage. figure (4_8).

While the results in histpathological changes in T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*) treatment has shown mild congestion with significant number of hemosiderin-laden macrophage .figure (4_9).

The spleen tissues treated with 300 Mg/L *A.Vulgaris* (T_4) shows normal red pulp and white, figure (4_10).

5.3.3 The histological changes in ovary:

The histopathological sections of ovary tissue in 1000Mg/L $ZnSO_4$ (T_1) group

At high doses, necessary elements, as copper, zinc, and selenium, could also have toxic effects on kidneys and impair reproduction***(Allen, L. H., 1998)***

But this results no agreement with ***FEDRIP 2003*** stated No histological alterations in the testes or ovaries were noted in mice fed zinc sulfate (1,110 mg zinc/kg/day) for 13 weeks.

While the results in histpathological changes in T_3 (1000Mg/L $ZnSO_4$ with 300Mg/L *A.Vulgaris*) treatment has shown normal mature graffian follicles and normal primordial growing follicles .figure (4_14).

This results returned to the different doses of medicinal plant in T_2 and T_3 treatments, in 200Mg/L of A.V .not response while the dose of this plant in 300Mg/L A.V. effected dose (good response).

The presence of phenol compounds makes the resistance to diseases in human and plants. Tannins are similarly recognized as antimicrobial agents; additionally, it has potential to prevent the development of microorganisms by precipitating microbial protein, as well, by inhibiting of the growth of several Microorganisms such as bacteria, fungi, and it also has physiological properties such as anti-parasitic, anti-secretolytic and anti-phlogistic effects. Consequently, because of the good results of applications against the human pathogens, these plants may preferably be used as medications.***EDRAH, S. M. (2017)***

The ovary tissues treated with 300 Mg/L *A.Vulgaris* (T_4) shows normal ovary tissue with significant increase in number normal primordial growing follicles. figure (4_15).

So, when used this medicinal plant in concentration 300Mg/L act as a stimulates the function of ovary in increase produce the primordial growing follicles in these experimental animals, this agreement with ***EDRAH, S. M. (2017).***

CHAPTER SIX

CONCLUSIONS & RECOMMENDATION

6.1 CONCLUSIONS

From results obtained from present study, it was concluded that:

1- The effective dose of *A. Vulgaris* powder against oxidative stress in female rats was found to be equal 300 Mg/L water to improve antioxidant status and ovary response.

2_Treatment with *A.Vulgaris* powder minimize the toxic effect of zinc sulfate on function and histological structure of ovary , liver and spleen.

Addition of *A.Vulgaris* enhance some hematological parameters.

3_ The toxic dose of zinc sulfate in drinking water for eight weeks induces oxidative stress.

6.2 Recommendations

From through the results of this present study, it can be recommended that:

1_ *A. Vulgaris* powder at concentration 300Mg/L water in drinking water for improvement ovary fuction through increase the primordial follicles in numbers.

2_uses the Zinc and it's compounds carefully because have toxic effect on biological organs in high concentrations and long period exposure in drinking water or other routes.

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Appendix (1)

Measurement of total protein concentration

Principle:

Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity of the color formed is proportional to the total protein concentration in the sample.

REAGENTS

R Biuret	Sodium potassium tartrate Sodium iodide Potassium iodide Copper (II) sulphate Sodium hydroxide	15 mmol/L 100 mmol/L 5 mmol/L 5 mmol/L 1000 mmol/L
T PROTEIN CAL	Bovine albumin primary standard 7 g/dL	

SAMPLES

Serum or heparinized plasma1:

Stability of the sample: 1 month at refrigerator (2-8ºC).

PROCEDURE

1. Assay conditions:

Wavelength: . . . . 540 (530-550) nm

Cuvette: . . . . . .1 cm. light path

Temperature: . . . . . .37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1,0	1,0	1,0
Standard(Note 1,2,3) (µL)	--	25	--
Sample (µL)	--	--	25

4. Mix and incubate 5 min at 37ºC or 10 min at room temperature.

5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS:

$$\frac{(\mathbf{A})\ (\mathbf{Sample}) - (\mathbf{A})\ (\boldsymbol{Blank})}{(\boldsymbol{A})(\boldsymbol{Blank}) - (\boldsymbol{A})(\boldsymbol{standard})}$$ **x 7(Standard conc.) = g/dL of total protein in the sample**

Appendix (2)

Measurement of Albumin concentration:

Quantitative determination of albumin mg/dl:

Principle:

Albumin in the presence of bromcresol green at a slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the albumin concentration in the sample

Reagents

R	Bromcresol green pH 4,2	0,12 mmol/L

Samples

Serum or plasma, free of hemolysis1: Stability 1 month at 2-8ºC or

1 week at 15-25ºC.

Performance characteristics

Measuring range: From detection limit of 0,0349 g/dL to linearity limit of

6 g/dL.

If the results obtained were greater than linearity limit, dilute the sample

1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay(n=20)		Inter-assay (n=20)	
Mean (g/dL)	5,00	3,71	4,56	3,07
SD	0,02	0,02	0,28	0,18
CV (%)	0,47	0,55	6,20	5,90

Sensitivity: 1 g/dL = 0,2003 A.

Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient (r)2: 0,99169.

Regression equation: y= 1,045x – 0,028.

The results of the performance characteristics depend on the analyzer used.

Appendix (3)

Measurement of AST concentration:

Assay Principle

The reactions involved in the assay are as follows :

The amino group is enzymatically transferred by AST present in the sample from L-asparate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate.

L – Aspartate + 2- Oxoglutarate $\xrightarrow{\text{AST}}$ Oxaloacetate + L-Glutamate .

AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2.4 – dinitrophenylhydrazine.

Reagents

Regent 1 (R1 Buffer)

Phosphate buffer	100	mmol/L
L-aspartate	100	mmol/L
2- Oxoglutarate	5	mmol/L
Sodium Hydroxide	140	mmol/L
Sodium Azide	12	mmol/L

Harmful (Xn) : R20 / 22 L: Harmful by inhalation and if swallowed

System Parameters

Wavelength	546 nm (530-550nm)
Optical path	1 cm
Assay type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1 : 60
Temperature	37 ºC and 20 – 25 ºC
Zero adjustment	Reagent or Sample blank
Sensitivity	7 U/L
Linearity	89 U/L

Procedure

1. Measurement against Reagent Blank

Pipette into test tubes

Reagent	Sample	
R1 (buffer)	0.5 ml	0.5 ml
Sample	-------	100 µl
Distilled water	100 µl	------

Mix, and incubate for exactly 30 minutes at 37 ºC

R2	0.5ml	0.5ml

Mix, and incubate for exactly 20 minutes at 20-25 ºC

Sodium hydroxide	5.0ml	5.0ml

Calculation :

Obtain the AST activity from the following table :

Absorbance	U/L	Absorbance	U/L
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76

0.090	31	0.170	89

Appendix (4)

Measurement of ALT concentration:

Assay Principle

The reactions involved in the assay are as follows :

The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.

L – Alanine + 2- Oxoglutarate $\xrightarrow{ALT}$ pruvate + L-Glutamate .

ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2.4-dinitrophenylhydrazine

Reagents

Regent 1 (R1 Buffer)

Phosphate buffer	100	mmol/L
DL-Alanine	200	mmol/L
2- Oxoglutarate	6	mmol/L
Sodium Azide	12	mmol/L

Reagent 2 (R2)

2.4 dinitrophenylhydrazine 2.0 mmol/L

(C) – Corrosive contains caustic materials

R35 Causes severe burns

R41 Risk of serious damage to eyes.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 After contact with skin, wash immediately with plenty of soap and water.

For further information, refer to the Alanine aminotransferase reagent material safety data sheet.

System Parameters

Wavelength	546 nm (530-550nm)
Optical path	1 cm
Assay type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1 : 60
Temperature	37 ºC and 20 – 25 ºC
Zero adjustment	Reagent or Sample blank
Sensitivity	4 U/L
Linearity	94 U/L

Procedure

1. Measurement against Reagent Blank

Pipette into test tubes

	Reagent	Sample
R1 (buffer)	0.5 ml	0.5 ml
Sample	-------	100 µl
Distilled water	100 µl	------

Mix, and incubate for exactly 30 minutes at 37 ºC

R2	0.5ml	0.5ml

Mix, and incubate for exactly 20 minutes at 20-25 ºC

Sodium hydroxide	5.0ml	5.0ml

Mix, measure absorbance of specimen against reagent blank at 546 nm after 5 minute .

2. Measurement against sample Blank

	Sample blank	Sample
R1 (buffer)	0.5 ml	0.5µl
Sample	-----	100 µl

Mix and incubate for exactly 20 minutes at 20-25 ºC

Sodium hydroxide	5.0ml	5.0 ml

Mix, measure absorbance of specimen against sample blank at 546nm after 5 minutes

Calculation :

The ALT activity in the serum can be determined from the following table :

Absorbance	U/L	Absorbance	U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

Appendix (5)

In the FSH ELISA Assay Kit, the essential reagents required for an immune-enzymatic assay include high affinity and specificity antibodies (enzyme-linked and immobilized) with different and distinct epitope recognition, in excess, and native antigen.

In this procedure the immobilization takes place during the assay at the surface of a micro-plate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti FSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, reation results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex .

The interaction is illustrated by the following equation:

Ka

EnzAb(p) + AgFSH + BtnAb(m) ⇄ EnzAb(p)- AgFSH- BtnAb(m)

K-a

BtnAb(m) = biotinylated monoclonal antibody (Excess quantity)

AgFSH = native FSH antigen (variable quantity)

EnzAb(p) = enzyme labeled policlonal antibody (Excess quantity)

EnzAb(p)- AgFSH- BtnAb(m) = antigen-antibodies sandwich complex

Ka = rate constant of association

K-a = rate constant of disassociation.

Simultaneously the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

This interaction is illustrated below:

^{Enz}Ab(p)-AgFSH-BtnAb(m) + streptavidincw □ immobilized

Complex

Streptavidincw = streptavidin immobilized on well

Immobilized complex = antibodies-antigen sandwich bound.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by a washing step.

The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

By using several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Reagents and Materials Supplied in the Kit

1. FSH Calibrators (6 vials, 1 mL each)

CAL0	**REF DCE002/1006-0**
CAL1	**REF DCE002/1007-0**
CAL2	**REF DCE002/1008-0**
CAL3	**REF DCE002/1009-0**
CAL4	**REF DCE002/1010-0**
CAL5	**REF DCE002/1011-0**

2. FSH Control (1 vial, 1 mL)

Control concentration is Lot-specific and it is indicated on the Certificate of Analysis

REF DCE045/1003-0

3. Conjugate (1 vial, 12 mL)

Antibody anti FSH conjugated with Horseradish peroxidase (HRP)

Antibody anti FSH conjugated with Biotine

REF DCE002/1002-0

4. Coated Microplate (1 breakable microplate)

Streptavidin adsorbed on microplate

REF DCE002/1003-0

5. TMB Substrate (1 vial, 15 mL)

H2O2-TMB 0.26 g /L *(avoid any skin contact)*

REF DCE004-0

6. Stop Solution (1 vial, 15 mL)

Sulphuric acid 0.15 mol/L *(avoid any skin contact)* **REF DCE005-0**

7. 10X Conc. Wash Solution (1 vial, 50 mL)

Phosphate buffer 0.2M **REF DCE054-0**

Reagents necessary not supplied

Distilled water.

Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm)

Appendix (6) Estimation of Luteinizing Hormone (LH) Concentration (ng/ml)

Measurement of serum gonadotropin (LH) concentration is generally regarded as valuable tool in the diagnosis of homeostasis of fertility regulation via the hypothalamic–pituitary–gonad axis; kit was used (Monobind Inc. lake forest CA 92630, USA).

Principle of the test

The Monobind (LH) ELISA is based on the principle of competitive enzyme immunoassay; the essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

1-LH -enzyme conjugate solution was prepared by diluting 1ml of LH enzyme conjugate with 11ml of total LH conjugate buffer in a suitable container.

2-Wash solution was prepared by diluting 20ml of concentrated wash solution with 980ml of distilled water to final volume of 1000ml.

3-Substrate solution was mixed solution A and B.

4- Desired number of microplate wells was secured in the holder.

5- The serum (50 µl) and adding LH enzyme conjugate solution of the standard and treated group were dispensed into the assigned wells (all samples were run in duplicate concurrently so that all conditions of testing were the same).

6- LH enzyme conjugate solution (100µl) was added to all well.

7- Microplate thoroughly was gently mixed and covered for 20-30 seconds. It is important to have a complete mixing in this step.

8- Microplate was incubated for 60 minutes at room temperature.

9- Contents of the wells were drawn by manual plate washer and the wells were rinsed 3 times with diluted wash solution (300µl per well).

10-100 µl of substrate solution was added to each well.

11- Microplate was incubated for 15 minutes at room temperature.

12-The enzymatic reaction was stopped by adding 50µl of stop solution to each well.

13-The absorbance (OD) of each well was determined at 450nm with a

microplate reader.

الخلاصة

هدفت هذه الدراسة إلى دراسة التأثير الوقائي للنباتات الطبية (عشبة عباءة السيدة) ضد سمية كبريتات الخارصين، وكذلك الآثار المفيدة من هذا النبات الطبي على الاعضاء المدروسة وتأثير تركيزات عالية من كبريتات الخارصين في مياه الشرب على الكبد والطحال والمبايض على إناث الجرذان خلال فترة ثمانية أسابيع ،. تم استخدام ٣٠ من اناث الجرذان المبكرة النضج ، من عمر شهرين إلى ثلاثة أشهر ، بصحة جيدة ، تتراوح اوزانها بين ١٥٠-٢٥٠ غرام ، وقد أجريت هذه الدراسة في البيت الحيوان في كلية الصيدلة / جامعة كربلاء ، وكانت الجرذان توزع بشكل عشوائي في أقفاص بمعدل ستة جرذان لكل قفص في درجة حرارة الغرفة ومزودة بنظام غذائي وماء. تم ترتيب المجموعات على النحو التالي:تم تقسيمها عشوائيا إلى خمس مجموعات (ستة جرذان لكل معاملة): المجموعة الاولى مجموعة التحكم: تم استخدام ستة من إناث جرذان ،يستخدم الماء المقطر. المجموعة الثانية (تي١) فيها ستة من أناث الجرذان ،استخدمت فيها المياه الحاوية على ١٠٠٠ ملغرام/لتر من كبريتات الخارصين. المجموعة الثالثة (تي٢) فيها ستة من أناث الجرذان ، والمياه المستخدمة معها ١٠٠٠ملغ / لتر من كبريتات الخارصين مع ٢٠٠ملغ/لتر من مسحوق عشبة عباءة السيدة. المجموعة الرابعة (تي٣) فيها ستة من أناث الجرذان ، والمياه المستخدمة معها ١٠٠٠ملغ / لتر من كبريتات الخارصين مع ٣٠٠ملغ/لتر من العشبة. المجموعة الخامسة (تي٤) فيها ستة من أناث الجرذان ،والمياه المستخدمة معها ٣٠٠ملغ/لتر من العشبة فقط. تمت إضافة المواد بمياه الشرب للحيوانات.اظهرت نتائج الدراسة الحالية أن نشاط انزيم ALT في مصل الدم كان له زيادة معنوية ($p\leq0.05$) في المجموعة الثانية التي اعطيت١٠٠٠ ملغرام/لترمن كبريتات الخارصين مع ٢٠٠ ملغرام/لتر من مسحوق العشبة(T2) ومجموعة ٣٠٠ملغرام/لتر من مسحوق العشبة(T4) ، ولكن انخفض تركيز هذا الانزيم في المجموعة الرابعة (T3) التي اعطيت١٠٠٠ ملغرام/لتر من كبريتات الخارصين مع٣٠٠ملغرام/لتر من العشبة بالمقارنة مع مجموعة السيطرة (CO) ، في حين أظهرت النتائج أن هناك انخفاض معنوي ($p\leq0.05$) في تركيز الزلال في مصل الدم T1 و T2 مقارنة مع CO، T3 و T4. كذلك زيادة معنوية في تركيز الجلوبيولين في الدم بشكل ملحوظ ($p\leq0.05$) في مجموعة T4 مقارنة مع مجموعة السيطرة CO. كما اضهرت النتائج زيادة في تركيز الاستروجين في مجموعة T4 بشكل معنوي عند مقارنته بمجموعة السيطرة ، بينما لم تكن تركيزات الاستروجين ذات فروق معنوية (p00.05) بين المجموعات الأخرى. على العكس من ذلك ، كشفت النتائج أن زيادة ملحوظة ($p\leq0.05$) في تركيز

هرمون FSH في مجموعة T3 مقارنة مع مجموعة التحكم CO.وزيادة تركيز هرمون LH في المجموعة T3 مقارنة مع مجموعة التحكم ، ١٠٠٠ملغرام/لتر من كبريتات الخارصين تسبب في انخفاض معنوي ($p\leq0.05$) في عدد كريات الدم الحمراء في (T1) ، إذا ما قورنت بمجموعة التحكم CO والمجموعات الأخرى ، على العكس من ذلك اضهرت نتائج عدد الكريات الحمراء في (T2) ارتفاعًا ملحوظًا ($p0.05$) ، إذا ما قورنت بمجموعات أخرى. في (T3) انخفضت أعداد كريات الدم الحمراء بشكل ملحوظ ($p\leq0.05$) ، إذا ما قورنت مع CO.كذلك انخفاض ملحوظ ($p\ 0.05$) في نتائج مستويات الهيموكلوبين في مجموعة (T3) مقارنة مع مجموعة السيطرة CO والمجموعات الأخرى. أيضًا زادت مستويات الصفائح الدموية بشكل ملحوظ ($p\leq0.05$) في T2 و T3 و T4 مقارنة مع مجموعة التحكم.و في المجموعات T2، T3 & T4 على التوالي ، زادت أعداد الكريات البيضاء بشكل ملحوظ ($p\leq0.05$) ، إذا ما قورنت بمجموعة السيطرة CO ، كذلك وجد زيادة كبيرة ($p\leq0.05$) في نتيجة الخلايا الحبيبية البيضاء في T1 و T2 و T3 و T4 على التوالي ، عند مقارنتها بمجموعة السيطرة CO. زادت نسبة MID٪ ($p\leq0.05$) في مجموعة T4 عند مقارنتها مع مجموعة التحكم CO ، مما أدى إلى انخفاض كبير في وزن الجسم ($P\leq0.05$) في المجموعة T1 في الأسابيع الأولى والثانية والثالثة والرابعة والخامسة على التوالي من فترات التجربة ، إذا ما قورنت بمجموعة السيطرة CO. اضهرت النتائج زيادة في وزن المبيض الأيسر بشكل ملحوظ ($P\leq0.05$) في T1 مقارنة مع CO في مجموعات العلاج T3 و T4 وزاد مؤشروزن المبيض الى وزن الجسم بشكل معنوي ($p\leq0.05$) ، إذا ما قورنت بمجموعة التحكم ، من نتائج المقاطع النسيجية للكبد في المجموعة (T1) ،اضهرت احتقان خفيف في الوريد المركزي ، اضمحلال وتضخم الجيوب الكبدية الضيقة ، واحتقان قطع النسيج في الطحال من الجيوب اللبية الحمراء مع الضامة المحملة بالنوسفيدين مع الضامة الحمضية والنسيجية المبيضية ,لكن قل من أعداد الجريبات المبيضية في بداية التجربة. بالإضافة إلى ذلك ، وبسبب امتلاك هذه النباتات الطبية على مستوى عال من مكونات مضادات الأكسدة وكذلك لها دور مهم باعتبارها فعالة النهج العلاجي للأضرار الكبدية وزيادة عدد بصيلات النمو الطبيعية في المبايض.و في الختام ، استخدمت الدراسة النباتات الطبية لعلاج معظم أمراض الكبد بشكل أفضل مع آثار جانبية منخفضة من خلال الآثار الوقائية لهذه النباتات ضد الخارصين الزائد ، وتحسين وظائف الكبد والمبيض.

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب البيطري

تأثير مسحوق عشبة عباءة السيدة على الجهاز التناسلي ووظيفة الكبد والطحال لأناث الجرذ المعرضه لجرع عالية من كبريتات الخارصين في مياه الشرب

رسالة

مقدمه إلى مجلس كلية الطب البيطري/ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في علم الفسلجة والأدوية البيطرية والكيمياء الحياتية

من قبل

شيماء قاسم محمد حلو

بكالوريوس طب وجراحة بيطرية

كلية الطب البيطري/جامعة كربلاء

بأشراف

أ.م. د. مهدي عبد الخضر علي عزيز

دكتوراه في علم الفسلجة الحيوانية

١٤٤١هـ ٢٠٢٠م