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Studing the Effect of Hydroxychloroquine Drug and *Artemisia herba-alba* Extract in Sex and Thyroid Hormones and Some Biochemical Parameters of Laboratory Male Mice

A Thesis

Submitted to the Council of the College of Science/University of Misan as Partial Fulfillment of the Requirements for the Master Degree in Biology

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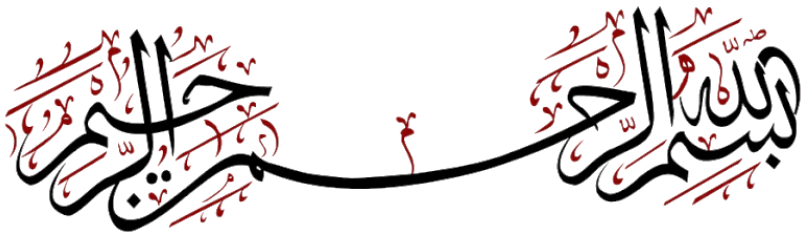

﴿ الَّذِي جَعَلَ لَكُمُ الْأَرْضَ مَهْدًا وَسَلَكَ لَكُمْ فِيهَا سُبُلًا وَأَنْزَلَ مِنَ

السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ أَزْوَاجًا مِنْ نَبَاتٍ شَتَّىٰ ﴾

صدق الله العلي العظيم

(سورة طه, الآية ٥٣)

Supervisor 's Certificate

We certify that this thesis entitled **"Effect of hydroxychloroquine and *Artemisia herba alba* on some hormones and biochemical parameters in laboratory male mice**

"has been prepared under our supervision at the College of Science, University of Misan; as a partial fulfillment of the requirements for the degree of Master of Biology

Signature

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Recommendation of Head of Biology Department

I view of the available recommendations; I forward this thesis debate by the examining committee.

Signature

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Date: / /2022

Dediction

- **To……**
- **To ……whom Allah sent as mercy to the**
- **Worlds ……Prophet Mohammed**
- **To ……imam of our time ……AL-Mahdi Almuntazar**
- **To the candle that melted to lighten my road ……My parents**
- **To those who sacrificed themselves to protect us …… Our martyrs**
- **To the roses that perfumed my life ……My brothers**
- **To the one who supported me……My family and my friends**
- **With my love…… *ream***

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Summary

This study had been conducted in the animal house of the department of biology at the college of science / University of Misan by choosing (104) male albino mice from 10/2/2021 to 14/8/2021. Twenty four (24) mice were be used to measure the LD50 of the plant extract, further eighty (80) mice were randomly be divided into Four (4) groups, each group consisting of twenty (20) mice (18 of them are killed and 2 were left for mating). Our aim was to evaluation the effect of oral administration to hydroxychloroquine and Artemisia aqueous extract on reproductive hormones and thyroid hormones. In addition, its effect on some biochemical parameters which had included: liver function tests, kidney function tests, measuring the value of C-reactive protein, as well as evaluating their effect on body weight, organ weights and fertility criteria.

The study was divided into groups depending on the type of oral administration into:

1- The control group (group A) which received 0.2 ml of normal saline orally.

2-The hydroxychloroquine group (group B) received 0.2 ml hydroxychloroquine twice daily orally at a concentration of 400 mg/kg for the first day and 200 mg/kg for the remainder of the treatment period.

3-Hydroxychloroquine + *Artemisia herba alba* extract group (group C) which received orally 0.2 ml of hydroxychloroquine in the morning and 0.2 of Artemisia herba alba extract in the evening.

4-*Artemisia herba alba* extract group (group D) which received 0.2 ml of the extract at a concentration of 8000 mg/kg two doses daily orally.

The Results of the Current Study Showed:

According to the Groups

1- A significant($P<0.05$) decrease in the concentration of follicle stimulating hormone (FSH) and testosterone in group B and a significant($P<0.05$) increase in its concentration in group D . A significant ($P<0.05$) increase in the concentration of luteinizing hormone (LH) was observed in the group B and a significant ($P<0.05$)decrease in its concentration in C and D groups.

2-There was no significant($P>0.05$) change in TSH concentration in all treated groups, a significant ($P<0.05$)increase in T3 and a significant ($P<0.05$) decrease in T4 in group B, and a significant($P<0.05$) decrease in the concentration of T3and T4 in C and D groups.

3- Significant ($P<0.05$)increase in AST, ALT, and ALP in group B , also there was a significant($P<0.05$) increase in AST and ALP in C and D groups.

4-Significant ($P<0.05$)increase in the concentration of urea in B group and significant($P<0.05$) decrease in urea of C group, there was no significant ($P>0.05$)change in the concentration of creatinine in the treated groups compared to the control group.

5-There was no significant ($P>0.05$)change in the value of C-reactive protein in all treatment groups.

According to the Periods

1-There was no significant (P>0.05)change in FSH in all treatment groups after 6,12 days, while there was a significant (P<0.05) decreased in its value in all treatment groups after 18 days compared to the control group.

A Significant(P<0.05) decreased in LH in C and D groups after 6, 12 and 18 days, while there was no significant (P>0.05)change in LH value in group B in all periods.

A significant (P<0.05)decreased in the concentration of testosterone in the group B after 6, 12 and 18 days. While, there was no significant (P>0.05)difference in its value in the other groups in all periods compared to the control group.

2-There was no significant (P>0.05)change in TSH after 6, 12 and 18 days in all treatment groups. A significant (P<0.05)decreased in T3 concentration in C and D groups after 6 and 12 days, while there was a significant (P<0.05)increased in T3 concentration in group B after 12 and 18 days compared to control. A significant (P<0.05)decreased in T4 concentration in C and D groups after 6 and 18 days, as well as significant (P<0.05) decrease in T4 concentration in group D in all periods.

3-A significant(P<0.05) increased in AST values in all treatment groups in all periods compared to the control group, a significant (P<0.05)increased in ALT concentration in group B in all periods and there was a significant (P<0.05) increased in ALT in the B and C groups after 18 days compared to the control group. A significant (P<0.05) increased in ALP in B and C groups in all periods compared to the control group, and its values increased in Artemisia extract group compared to the control group after 6 and 18 days of administration.

4-A significant(P<0.05) increased in urea concentration in group B and a significant (P<0.05) decreased in urea value in group C for all periods and a significant(P<0.05) increased in it's concentration in the group D after 18 days as will as there is no significant (P>0.05)change in the serum creatinine value in all treatment groups compared to the control group and for all periods.

5-There was no significant(P>0.05) change in C-reactive protein in all treatment groups and for all periods compared to the control group.

The Effect of Administration on Body weight ,Organs weight and Fertility Included:

1-Significant(P<0.05) decrease in body weight in the group B and a significant (P<0.05)increased body weight in group C.

2- In the group B there was significant (P<0.05)decreased in the weight of the heart was observed after 12 and 18 days of administration . A reduction in the weight of the liver and intestine after 6 and 18 days of administration, and there was no significant (P>0.05) change in the weight of the stomach compared to the control group . A significant(P<0.05) decreased in the weight of the spleen after 12 days and a significant(P<0.05) increased in the weight of the kidney after 6 days and a significant (P<0.05)decreased in it's weight after 18 days.

In group C there was no significant (P>0.05)change in the weights of the heart, liver, intestines, stomach, spleen and kidneys. As for the group treated with Artemisia extract, it showed a significant (P<0.05) decreased in the weight of the heart after 12 days and a decrease in the weight of the liver and intestine after 18 days, and this group did not showed a significant (P>0.05) change in the weight of the stomach , spleen and kidneys.

3-A significant($P<0.05$) decrease in the number of sperms in the treated groups compared to the control group, as well as a significant($P<0.05$) decreased in the fertility and pregnancy rates in B group ,a non significant ($P>0.05$)difference in the number of births in all groups and a reduction in birth weights in B group.

List of Contents

List of Tables

List of Figure

List of Abbreviations

Abbreviation	Meaning
ACEO	*Artemisia campestris* essential oil
ALP	Alkaline phosphates
ALT	Alanine aminotransferase
AST	Aspartateaminotransferase
CCl4	Carbon tetrachloride
CPF	chlorpyrifos
CRP	C-reactive protein
CYP	Cytochrom p
CQ	Chloroquine
ESR	Erythrocyte sedimentation rate
FSH	Follicle stimulating hormone
GnRH	Gonadotropin Releasing Hormone
Group A	Control group
Group B	Hydroxychloroquine group
Group C	Hydroxychloroquine + Artemisia extract group
Group D	Artemisia extract group
HCS	Hydroxychloroquine sulfate
HCQ	Hydroxychloroquine
LDH	Lactate dehydrogenase
LH	Luteinizing Hormone
NADH	Nicotinamide adenine dinucleotide
PCOS	Polycystic ovaries syndrome
RF	Rheumatoid arthritis
ROS	Reactive oxygen species
SARS	Severe Acute Respiratory syndrome
SLE	Systemic lupus erythemaosus
TSH	Thyroid Stimulating Hormone
T3	Tri-Iodothyronine
T4	Thyroxin
TT	Total Testesterone
WHO	World Health Organisation

Chapter One

Introduction

1-1 Introduction

Hydroxychlouroquine (HCQ) is an antimalarial agent. It is a hydroxyl derivative of chloroquine, belongs to the group of 4-aminoquinolines, which are weak bases that are completely absorbed from the gastrointestinal tract and are metabolized in the liver to pharmacologically active products by the enzyme CYP450 and its isoforms (Browning,2014;Stokkermans and Trchonas,2020).

HCQ is a broad-spectrum treatment. In addition to being used against malaria, HCQ is also used alone or in combination with other agents to treat autoimmune diseases, including lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome (Browning,2014).Hydroxychloroquine also acts as an anti-cancer cell by inhibiting the autophagy process(Shi *et al*.,2017).

It is also used against infectious diseases such as acquired immunodeficiency syndrome and influenza, and it has also been used recently against the Covid 19 virus (Abena *et al*,2020).

HCQ has many effects including anticoagulant, lipid lowering, antidiabetic, reducing cardiovascular risk (Costedoat-Chalumeau *et al*.,2014).

Hydroxychloroquine has many reasons why it is a broad-spectrum treatment, the most important of which are its immunomodulatory effects, which include proteolysis, phagocytosis, chemotaxis, reduction of proinflammatory cytokines, blocking of Tand B cell receptors(Ruiz-Irastorza and Khamashta,2008;Ben-Zvi *et al*,2012).

In addition to the wide use of HCQ, it has many advantages, including its low cost and speed of absorption, and has few side effects (Rainsford *et al*,2015;Ponticelli and Moroni,2017)

Themost common side effects of hydroxyclouroquine are digestive symptoms, itching and skin changes.In addition cardiotoxicity and non-

reversible retinopathy where prolonged use of hydroxychloroquine in large doses leads to retinal damage (Srinivasa *et al*.,2017;Abdulaziz *et al*., 2018).

Hydroxychloroquine is less toxic than its counterpart chloroquine, but there are some studies that have shown that hydroxychloroquine leads to elevation of liver enzymes and causes some cases of acute hepatotoxicity (Sunkara *et al*,2018;Falcão *et al*,2020)

The use of hydroxychloroquine does not affect the kidneys, as it is associated with a reduced risk of developing chronic kidney disease(Wu *et al*,2018), reduces proteinuria, and supports kidney function(Tang *et al*,2021).

Medicinal herbs are considered a local heritage of great importance, as it is a source of vital compounds used in traditional nutritional and therapeutic systems ,due to the diversity of plants of therapeutic importance and their great spread, they are of interest to researchers all over the world (Harsha *et al*.,2002).

Where there is evidence of increased demand for medicinal plant (Kotnis *et al*.,2004). The traditional medicinal plant treatments are highlighted due to their low probability of causing side effects, unlike industrially produced chemicals ,so the search for their medicinal and therapeutic properties has become mandatory(Wu.,2005).

Artemisia, belonging to the Asteraceae family, is considered one of the most widespread genera, as 500 species among various plants belong to the Artemisia genus. It spreads in Asia, America and Europe and is widely used for various purposes, the most important of which are therapeutic and nutritional uses and its use as a spice(Riggins and Seigler,2012).

Among the main chemicals of the genus are flavonoids, terpenoids, glycosides, coumarins, caffeolinic acids, sterols and acycline (Kshirsagar and Rao.,2021).

This genus has been used since ancient times in traditional medicine, and one of its most important uses is a pain reliever, an antipyretic, a treatment for abdominal pain, a treatment for high blood pressure, diabetes, an anti-malarial, an insecticide, an antimicrobial, an anticoagulant, a treatment for digestive disorders(Liu *et al*.,2004 ,Ahuja *et al*.,2011).

The types of the artemisia are also used as anti-fever, gastric activists and heart alarm, treatment hepatitis and memory improvement (Guarrera.,2005).It is also used in the treatment of indigestion, colic, diarrhea, headache, colds, bladder and kidney disorders, and is used as a laxative (Thring and Weitz, 2006).Some types of wormwood are used to treat dysmenorrhea and premenstrual syndrome(Garcia and Adams .,2005).

It is also used in the treatment of various inflammatory diseases. Because it contains flavonoids and antioxidant products, in addition to Artemisin, it has had an effect in the treatment of cancer cells as well as the treatment of inflammatory diseases, including viruses, fungi and bacteria (Ferreira *et al* .,2010).

Artemisia herba alba widely used to treat various diseases, and among these uses is their use by diabetic patients because of its anti-diabetic effect and also has a protective effect on the live ,administration of *Artemisia herba alba* extract reduced AST,ALT and glucose levels in diabetic rats (Farhad *et al*,2013).

It also possesses kidney protective effects in rats dosed with alloxan. Administration artemisia extract reduced the generation of alloxan-induced free radicals .The extract also strengthened the antioxidant defense system and reduced the sensitivity of the kidneys to oxidative stress (Sekiou *et al*,2021).

1-2- Aims of the study:

This study aimed to evaluated the administration effects of hydroxychoroquine ,Artemisia extract and both on some physiological parameters in male mice, which includ:

1-Reproductive hormones (LH , FSH and Testosterone).

2-Thyreid hormnone (TSH, T3 and T4).

3-Biochemical Parameters (Liver function, tests, kidney function tests and C-Reactive probein),

4-Measuring the body weight and weight of internal organs, and

5- Fertility criteria (sperm count,number and weight of births).

Chapter Two

Literature Review

2-1 Hydroxychloroquine (HCQ)

2-1-1 Historical Background

Hydroxychloroquine (HCQ) is an antimalarial drug, and it is one of the synthetic analogs of chloroquine and differs from it by a group of hydroxyl (Taherian *et al*.,2013). The anti-malarial properties were known by the Incas in the seventeenth century, when they discovered the beneficial properties of the bark of the chinchona tree against malaria because it contains quinine(Wallace,2001).

In 1891, the drug quinine, which is derived from the bark of the chinchona tree, took first place as a natural and effective antimalarial compound, then some modifications were made to produce quinacrine (Al-Bari,2015). In 1934, chloroquine was first synthesized as the first antimalarial drug with a completely different composition from the natural antimalarial. Where Hans Andersag and his colleagues converted quinacrine by replacing its acridine ring with a quinolone ring and produced the chloroquine compound under the name Sesochin(Krafts *et al*.,2012; Taherian *et al*.,2013)

Due to some of the toxic properties of chloroquine, there was a need to find a more effective and less toxic analog. In 1946, Alexander Surrey and Henry Hammer synthesized hydroxychloroquine by adding a hydroxyl group to the main compound of chloroquine,which reduced the toxicity of chloroquine to the third of the original molecule(Price *et al* .,2014).

2-1-2 Chemical Structure of Hydroxychloroquine

The drug is composed of the elements such as oxygen, chlorine, carbon and nitrogen. These elements form the molecular formula of hydroxychloroquine, which is C18H26CIN30. Hydroxychloroquine has a structure similar to chloroquine ,but differs from it by ,the presence hydroxyl group in its N-ethyl side chain as shown in figure (1) (Browning .,2014).

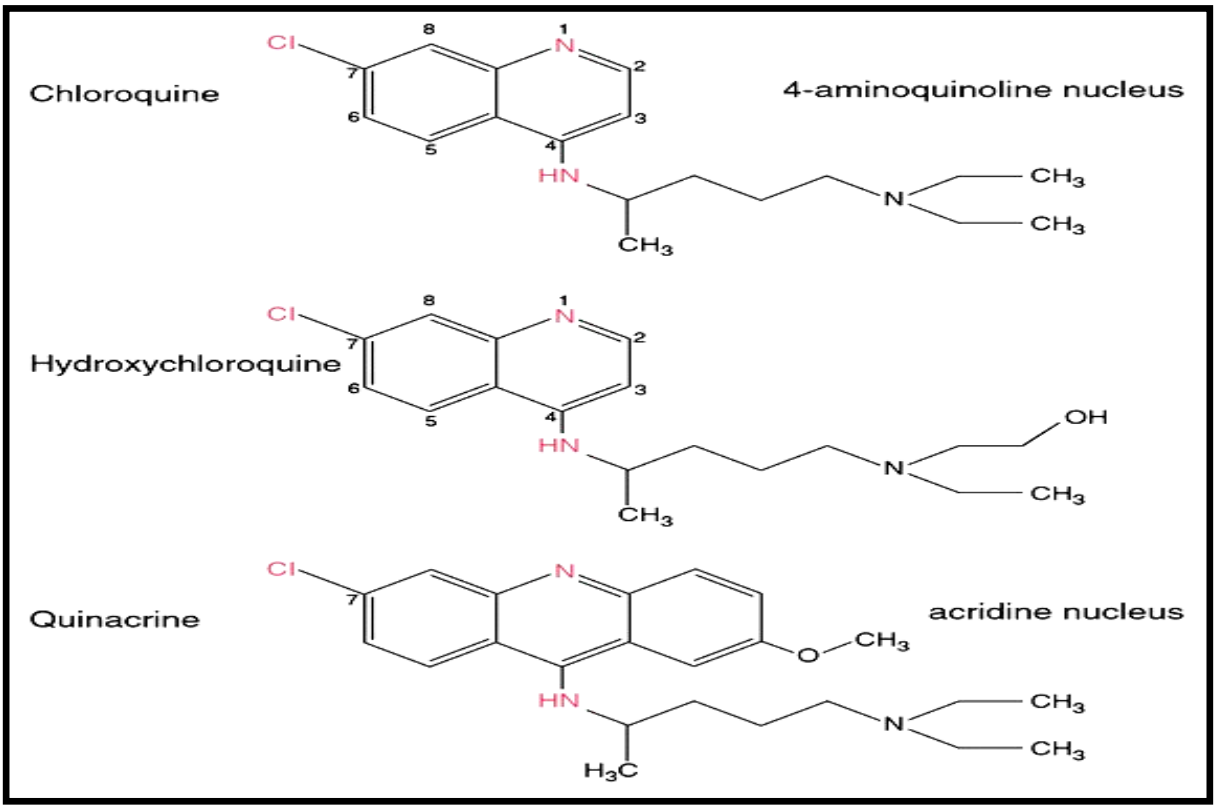


Figure (1):Chemical Structure of Hydroxychloroquine (Browning ,2014)

2-1-3 Metabolism of Hydroxychloroquine

Hydroxychloroquine is metabolized in the liver ,via the enzymes that be responsible for the metabolism of most drugs .Cytochrome p (CYP450) and its isoforms play a critical role in hydroxychloroquine metabolism (Paniri *et al*.,2020). Hydroxychloroquine undergoes several changes including dealkylation by CYP450 isoforms and converting them to active metabolites (Projean *et al*., 2003). Studies have indicated that the CYP450 isoforms involved

in hydroxychloroquinemetabolism are CYP3A4/5, CYP2C8 and CYP2D6 (Li *et al*., 2003; Projean *et al*., 2003).

CYP enzymes catalyze the dealkylation of hydroxychloroquine to pharmacologically active metabolites, and Hydroxychloroquine as been documented to be metabolized by the CYP3A,2D6 and 2C8 systems to yield three active metabolites: desethylhydroxychloroquine, desethylchloroquine, and bisdesethylhydroxychloroquine (Kalia and Dutz ,2007; Bauman and Tisdale,2020)

2-1-4 Side Effect of Hydroxychloroquine

Hydroxychloroquine side effects include : gastrointestinal disturbances such as vomiting, diarrhea, nausea, stomach pain and cramps and loss of appetite(Ruiz-Irastorza *et al*.,2010). It also causes skin problems such as itching, rashes, dry skin, alopecia and emergency pimples, these symptoms startafter four weeks from taking the treatment and disappear after a period of leaving it (Sharma *et al* ,2020) ,.

The drug-induced ringing in the ears, nervousness, mood changes, headache, dizziness, loss of balance, weight loss and hair loss. Prolonged use of hydroxychloroquine causes retinopathy, eye problems and blurred vision(Yam and Kwok.,2006; Wolfe and Marmor.,2010).

2-1-5 Current Clinical Uses of Hydroxychloroquine

Hydroxychloroquine was first used as an effective treatment against malaria, then in the treatment of rheumatic and autoimmune diseases such as rheumatoid arthritis, lupus erythematosus and Sjögren's syndrome, also used in the skin diseases such as cutaneous porphyria and chronic ulcerative stomatitis, and it acts as an antitumor and treatment of cancerous diseases (Shukla and Shukla.,2019).

As well, it was used in cardiovascular diseases, acts as an antioxidant and has a role in lowering blood sugar, has an anti-inflammatory effect .It is used to treat Q fever, and has an anti- bacterial, anti-fungal and anti-viral effect, also it was previously used in the treatment of people with human immunodeficiency virus (HIV)and influenza virus (Al-Bari 2015; Plantone and Koudriavtseva.,2018; Della Porta*et al*.,2020).

It has been assumed that hydroxychloroquine and chloroquine are targeted agents ,for corona virus infection since the outbreak of severe acute respiratory syndrome (SARS) in 2003 (Savarino *et al*.2003 ; Vincent *et al* .,2005) ,and as recently as 2020, hydroxychloroquine has returned to the spotlight due to its antiviral activity and has been suggested as an antiviral treatment for Covid-19 (Conforti *et al*.,2020 ;Gautret *et al*.,2020).

2-1-6 Mechanisms Action of Hydroxychloroquine

The mechanisms of action of hydroxychloroquine are variety. If it is used as a treatment for malaria, it has been proven that accumulates in lysosomes, leading to acidification ,this activity interferes with the ability of the parasite to break down hemoglobin, which prevents its growth and reproduction(Ben-Zvi *et al*.,2012).

As for its role in suppressing cancer cells, itsimportance comes from inhibiting autophagy, thus the death of cancer cells that depend on autophagy to survive (Shi *et al*.,2017).

The mechanism action of hydroxychloroquine against Covid-19 has not been fully elucidated, but it is assumed that due to the high pH inside acidic cellular organelles such as endosomes and lysosomes, which inhibits viral integration with its cellular receptors and thus prevents itsentry into host cells, and prevents binding to terminal glycosylation, which is the receptor targeted by SARS Cov 2(Meyerowitz *et al*.,2020) Hydroxychloroquine acts as alkalizing the

highly acidic lysosome,in the case of infections, it reduces the release of cytokines such as interleukin and tumor necrosis factor (Shukla and Shukla.,2019).

2-1-7 Effect of Hydroxychloroquine on

2-1-7-1 Reproductive Hormones

A large number of foreign compounds can disrupt the functional diversity of the male reproductive system and interfere with the complexity of its hormonal regulation resulting in male fertility failure (Creasy,2001) .

The toxic effects of anti-inflammatory and immunosuppressive drugs on the gonads, including hydroxychloroquine, have rarely been studied. However, there are experimental animal studies that report that these drugs may impair male and female fertility fecundity (Østensen *et al*,2006)

Studies have shown that the administration of synthetic, semi-synthetic and quinine-derived antimalarial compounds in rats can alter the cellular function of the testes, hormonal balance, sperm development and thus may cause male infertility (Izunya *et al*.,2010; Farombi *et al*., 2012).

The study was done by Elgndy *et al*.,(2017) showed that hydroxychloroquine has slight orchial toxic effects ,does not cause impairment in male rats' fertility,also they noted avery slight decreased in testosterone and a significant decrease in luteinizing hormone (LH) and folliclestimulatinghormone (FSH) levels.

Wu *et al*.,(2015) noted the abnormal reproductive hormones improved after 6 months of taking hydroxychloroquine, as testosterone levels increased in the blood compared to their pre-treatment values in a patient with primary Sjogren's syndrome ,they also recorded a significant decrease in the level of LH

and FSH, on the contrary, the levels of gonadotrophins did not decreased among the males receiving hydroxychloroquine therapy.

In a study carried out by Khezri *et al* (2007), they used 40 rats and divided them into four groups and gave 3 different doses ,the first,second and third group received a specific dose of hydroxychloroquine by intraperitoneal injection, while the fourth group received distilled water ,and the results showed that there was no difference in FSH and LH levels among groups, while at the maximum level of the drug(third group) testosterone showed a significant decrease ,in addition to that the researchers indicated that the decrease in, because they noticed that it decreased in the third group that received the highest dose.

2-1-7-2 Thyroid Hormones

The thyroid gland is an important butterfly-shaped organ in the endocrine system that secretes a hormone T4 and T3 which play important roles in development and growth (Soundarrajan and Kopp,2019).

The level of thyroid hormone production is under the control of thyroid stimulating hormone (TSH) which is released from the pituitary and regulated TSH by the hypothalamus (Mullur *et al*,2014).

Thyroiditis and autoimmune thyroid disease lead to hypothyroidism, which leads to reduced production of thyroid hormones T3 and T4 Hence, increased or excessive secretion of TSH (Evered *et al*,1973).

Autoimmune thyroid disease is associated with a number of rheumatic disorders including Sjögren's syndrome, lupus, and rheumatoid arthritis,and all of these diseases used hydroxychloroquine as one of the effective drugs in treating them ,researchers noted that hydroxychloroquine and corticosteroids may help control autoimmune thyroiditis in systemic lupus erythematosus (SLE) patients with hypothyroidism(Chen and Liou,2005).

In some women with homozygous rheumatoid arthritis , they took HCQ at a dose of 400 mg/day for 4 weeks, it was observed that it increased T3 and T4 values and decreased TSH values, after hydroxychloroquine was stopped they values returned to normal (Barbosa *et al*,2001).

In another study by Amichai *et al*.,(2007) they was noted that thyroid function tests were normal in women with rheumatoid arthritis after taking HCQ 400mg/day for a year.

2-1-7-3 Liver Function Enzymes

In a study by Lzci-Cetinkaya *et al*.,(2020) aimed at evaluating demographic, clinical and laboratory outcomes, treatments provided, and the effects of treatments on liver tests for patients diagnosed with COVID-19, no statistically significant increase was observed in aspartate aminotransferase (AST), alanine aminotransferase(ALT),total bilirubin, direct bilirubin, lactate dehydrogenase(LDH), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) values in the hydroxychloroquine group.

Hydroxychloroquine is concentrated in the liver and has been shown increased adiponectin levels and in an animal study it has been shown improved hepatic steatosis (Qiao *et al* .,2019) .

A study by Abdel Galil (2015) that included lupus erythematosus patients, observed that the level of liver enzymes increased in a patient with lupus erythematosus.

The above study aimed to find out the cause of hepatic degeneraion ,acut toxic hepatitis was diagnosed, the levels of enzymes ALT and AST which increased tenfold,and the researcher noted that the liver enzymes returned to their normal state after stopping taking the suspected drug hydroxychloroquine.

An increase in the levels of liver enzymes AST, ALT occurred in two patients when taking hydroxychloroquine to treat infection with Covid 19 virus,

and the increase in ALT reached grade 2 according to the classification of (CTCAE)After completion of treatment with HCQ, AST and ALT levels decreased in both patients (Chen *et al*., 2020).

2-1-7-4 Kidney Function Tests

Pons-Estel *et al* .,(2009) demonstrated that hydroxychloroquine delays the onset of renal damage caused by lupus nephritis .Patients who received hydroxychloroquine showed less frequency of glomerulonephritis and had lower disease activity. After controlling for confounding factors, hydroxychloroquine protected against complete kidney damage.

Gómez-Guzman *et al*.,(2014) study the effect of hydroxychloroquine on kidney disease caused by lupus erythematosus, they administered the drug to a group of mice suffering from lupus erythematosus ,which noted that hydroxychloroquine treatment of SLE mice partially reduced glomerular lesions and almost completely prevented extracapillary and interstitial tubular lesions and urinary protein excretion in mice with systemic lupus erythematosus was increased compared controls and significantly decreased with hydroxychloroquine treatment.

Furthermore, Gao *et al*.,(2017) reported the HCQ which improved the proteinuria in patients with IgA nephropathy. Hydroxychloroquine reported that hydroxychloroquine improves appears to be effective in these autoimmune diseases.

2-1-7-5 C reactive protein

In a study conducted by Scott *et al*.,(1989) on 101 rheumatoid patients who used a combination therapy with slow-acting antirheumatics, 27 patients took gold/hydroxychloroquine, 32 patients took gold/placebo, for a period of 12 months, and 42 patients withdrew.Over time, the levels ofC-reactive protein and rheumatoid factor (RF) showed a significant decreased in patients who received treatment for 12 months, and the decreased was greater in patients who received gold/hydroxychloroquine.

In a study by Xu *et al*.,(2020) they noted the height value of C-reactive protein (CRP) in patients with covid-19, they have taken HCQ 400mg/A day, after 11 days researchers noted that CRP was still high.

In another study by Grandolfo *et al*.,(2020) they was observed that C-reactive protein was high in patients who took HCQ 400 mg/day for 20 days and after withdrawal HCQ laboratory tests were normal after 1-2 Months.

2-1-7-6 Body Weight

Hydroxychloroquine causes loss of appetite and therefore can cause weight loss(Ochsendorf and Runne,1991). In a study that included cancer patients . It has been used HCQ to act as an autophagy inhibitor, it was used at a dose of 600 mg/kgtwice dailyAmong the observed effects were stomatitis, nausea, loss of appetite and weight loss(Rangwala *et al*., 2014)

Elgndy.,(2017) also observed that after hydroxychloroquine was administered to male rats with dose 124 mg/kg for 6 weeks, it caused loss of appetite and weight loss.Another study , also showed that the use of hydroxychloroquine against mice exposed to a high diet for 6 weeks leads to a reduction in the weight of mice(Qiao *et al*.,2019).

2-2 Herbal Medicine

Medicinal plants, which are underestimated, have great medical importance due to the many effective principles that nature has provided during millions of years of evolution (Kashirsagar and Rao , 2021). These plants have phytochemicals which in turn have far reaching, biologically active and beneficial effects and provide protection for plants from insects, viruses, bacteria and other harmful organisms ,these phytochemicals either alone or with a mixture of several compounds affect multiple pathways at the same time to produce the desired pharmacological effect (Batiha *et al.*,2020).

Many medicinal plants or herbs are revered in ancient medical traditions (Chinese medicine, Ayurveda, Native Americans, etc.) due to their therapeutic benefits and about 40% of modern medicines are derived from plants (Rao *et al* .,2012;Parasuraman *et al* ., 2014; Barkat *et al*., 2021).

Plants were used in ancient times to treat some infectious diseases, and some of them are now effective treatments for many diseases. Due to the side effects and resistance shown by pathogenic microorganisms to antibiotics and most available drugs, extracts and bioactive compounds isolated from plant species have received great attention especially those used in herbal medicine (Batiha *et al*., 2020).

The World Health Organization (WHO) estimates that 80% of the world's population currently uses herbal medicines for healthcare (Parasuraman *et al* ., 2014). In general, these drugs are considered free from side effects (Bodhisattwa *et al*., 2011).

Medicinal plants have been used since ancient times to treat diseases. Medicinal plants are the backbone of medicine (Zulfiker *et al*., 2010).

2-3 The Genus of Artemisia

Artemisia is a herbaceous perennial, woody, plant of the Asteraceae family. It has many types that spread in most parts of the world, including wild, medical, industrial, and ornamental, and it reaches 500 species(Mohamed *et al*.,2010).

2-3-1 The Types of Artemisia and its Spread Areas

Artemisia is one of the largest and most widespread genera of the family asteraceaeitis a diverse genus consisting of more than 500 varied species and is exists in temperate regions of Europe, Asia and North America(Bora and Sharma, 2011).Among the most famous types are *A.herba alba* , *A. absinthium*, *A.annua* ,*A. dracunculus*, *A. afra* ,and *A.vulgaris* (Kashirsagar and Rao , 2021).

2-3-2 General Description of Artemisia:

Artemisia is a perennial wild shrub with large branches and compound leaves, reaching a height of about 40 cm ,the roots are stiff and erect, raised from the bottom,the first leaf carriers are oval and spherical in shape,dicotyledonous with elongated lobe, dioecious, with simple branchingdecorate their seated ends with 2-4 flowers for each one as shown in figure (2) (Mohamed *et al*.,2010).

Figer (2): Artemisia herba alba (Mohamed *et al*.,2010)

2-3-3 Taxonomy of Artemisia herba alba:

Kingdom : Plantae

Subkingdom : Tarcheobionata

Superphylum :Spermatophyta

Phylum : Magnoliophta

Class : Magnoliopsida

Subclass : Asteridae

Order : Asterales

Familia : Asteraceae

Subfamilia : Asteroideae

Tribus : Anthemideae

Subtribus : Artemisiinae

Genus : Artemisia

Species : *Artemisia herba alba Asso* (Caratini, 1971).

2-3-4 The Chemical Composition of Artemisia

Artemisia contains many compounds, including glycosides such as santonin, absinthin, and artemisin. Also it contains alkaloids, saponins, tannins, coumarins, and flavonoids. In addition, Artemisia contains essential oils, which contain many active compounds.Also, flavonoids are found in all types of artemisia , and their type and percentage vary from one type to another such as sesquiterpenelactones,and germacranolides(Mohamed *et al*.,2010; Bora and Sharma,2011).

Most types of Artemisia contain artemisinin and it is considered essential and has great medical importance ,it is an indoperoxide sesquiterpene lactone, effective against multidrug-resistant malaria, it is found in large quantities in the species Artemisia annua (Liu *et al* .,2011).

2-3-5 The Medicinal Importance of Artemisia

The Artemisia used to treat many diseases such as diabetes by reducing blood sugar (hypoglycemia),regulating the heart beat and in some cases of liver disorders ,other medical uses for artemisia in antipyretic , stimulate gastric glands ,antitoxin and treatment of some skin diseases and Cancer (Pereira.,2019).

Artemisia has many anti-activities including antioxidant activity, antibacterial, antiviral and antiparasitic activity, and has a role in neurodegeneration and has an anti-fibrotic effect (Rao *et al*., 2012).

Artemisia species exhibit powerful anti-inflammatory effects. Several sesquiterpenes which derive from Artemisia and its derivatives including artemisinin, artesunate …etc , particular attention is paid to its role in preventing inflammation. Using animal models, Artemisia species were used to treat inflammatory conditions, including rheumatoid arthritis ,systemic lupus erythematosus, multiple sclerosis and allergic disorders(Shi *et al*., 2015)

Artemisia has a malaria-killing effect.The first clinical trial of Artemisia extract on human patients with malaria was conducted in 1972. After that experiment,the active compound in Artemisia extract was isolated and identified as Artemisinin(Reiter *et al* ., 2014).

Artemisin has an anti-cancer effect, as much evidence has proven that artemisinin and its derivatives possess cytotoxic effects against lots types of human cancer cells in both *in vitro* and *in vivo* animal experiments (Ho *et al* .,2014; Reiter *et al*., 2015; Fröhlich *et al*., 2017).

2-3-6 Toxicity of Artemisia

AL-Dabhawi (2005) when conducting an experiment on mice reached to determine the toxic dose LD50 of the herb and was equal to 5.5 g/kg body weight of mice .

As noted by Mukinda and Syce (2007) when they tested the toxicity of *Artemisia afra* on rodents ,where they noticed that single intraperitoneal injections of *Artemisia afra* extract (1.5-5.5 g/kg) resulted in a systematic dose-dependent increase in mortality, behavioral adverse effects and mortality were dose independent. The LD50 after acute intraperitoneal was 2.45g/kg and orally 8.96g/kg.

Also, in the same experiment, they observed that when giving oral doses as of much as 1g/kg, the mice survived for 3 months.

Muto *et al* (2003) performed a 13-week repeat-dose toxicological study of artemisia extract in both sexes of Wistar Hannover rats. Rats were divided into 4 groups, each group consisted of 10 males and 10 females, and were given water containing 0, 0.125, 0.5, or 2% artemisia extract. All rats had survived at the end of the study and no changes indicating obvious toxicity attributable to artemisia extract treatment were observed in body weights, blood and blood biochemistry assays, organ weights, and histopathological examinations.

2-3-7 Effects of Artemisia Extract

2-3-7-1 Reproductive Hormones

Testosterone is a C-19-17-hydroxy steroid that belongs to the group of androgens. Testosterone is produced by Leydig cells and the rest is produced by the adrenal cortex (Kokilavani *et al*., 2014) .There are three hormones that play a key role in the formation of sperm and the production of testosterone,these hormones are FSH and LH which are produced in the pituitary gland and testosterone ,FSH effected directly on sperm cells and Sertoli cells to accelerate spermatogenesis by absorbing testosterone. At the same time, LH increases testosterone production and allows final differentiation and acts on Sertoli cells(Thibault and levasseur,2001).

Artemisia has a positive effect on the cells of the reproductive system, especially Leydig cells due to the antioxidant effect of artemisia . Flavonoids activate the production of steroids by Leydig cells (Cormier *et al* .,2017) .

Recent experiments have confirmed that the compounds found in the Artemisia plant have antioxidant properties and block the effect of oxygen-free radicals(Ghlissi*et al* .,2018).oxygen-free radicals and reactive oxygen species disrupt biological functions and are implicated in many pathological conditions, including male infertility, especially when there is no balance between the

activities of reactive oxygen species and the antioxidant defense systems (Riley and Behrman ,1991;Aprioku,2013)

Research has shown that Artemisia extract has an effect because it contains phytosterols that reduce the activity of the5-alpha enzyme, and thedeficiency in this enzyme reduces the plasma concentration of dehydrotestosterone, Therefore, phytosterols reduce tissue sensitivity to androgens and reduce the activity of androgens, including testosterone, by inhibiting aromatase and 5-alpha reductase,it has also been shown that phytosterols reduce precursors of testosterone synthesis by lowering cholesterol (Opoku-Acheampong *et al*.,2015).

Artemisia, through its role in increasing the activity of antioxidant enzymes and decreasing the level of inflammatory cytokines in the blood, may lead to a change in serum levels of pituitary-ovarian hormones in rats with polycystic ovaries, and also causes the return of serum levels of LH and testosterone to normal after it was elevated due to the syndrome Polycystic ovaries, thus resuming ovulation, so some Artemisia compounds reduce PCOS(Sadoughi*et al*.,2017).

2-3-7-2 Thyroid Hormones

Artemisia extract contains flavonoids as mentioned by Khlifi *et al*.,(2013)Flavonoids are polyphenolic compounds produced by plants and consumed for medicinal and food purposes.Excessive intake of some flavonoids is associated with hypothyroidism(Goncalves *et al*,2017).

Experimental data showed that many flavonoids inhibit the activity of thyroid hormones, thus reducing the levels of thyroid hormones T3,T4and thus increasing TSH .It causes an enlarged thyroid gland(Dos Santos *et al*.,2011).

Other research has proven that Artemisia extract has thyroid regulating effect (Méndez-Del Villar *et al*.,2016;Zarezade *et al*,2018)which leads to an increase in T4 and T3 levels .

In study included adult male laboratory rats with hypothyroidism that suffer from high TSH level and low thyroid hormones T3 and T4 ,dosing these rats with Artemisia dracunculus extract at a dose of 100, 200, 300 mg/kg led to a reduction in the levels of TSH and increase in the levels of T3 and T4, the decrease greater in the dose 300 mg/kg (Mohammdi *et al* .,2020).

2-3-7-3 Liver Function Enzymes:

The liver is a large and necessary organ found in vertebrates . It has many functions, include detoxification, protein synthesis, production of biochemical substances needed for digestion,glycogen storage , red blood cell lysis production of hormones (Thapa and Walia, 2007).

Livertransaminases AST (spartate transaminase) and ALT (alanine aminotransferase) are biomarkers of liver damage in a patient with a certain degree ofother sources that are not impaired in liver function including transaminases. AST and ALT are the two liver enzymes interested in amino acid metabolism (Kabir *et al*., 2008; Lee *et al*., 2012).

ALT is detected in the kidneys, heart, and muscles, and much more concentrated in the liver than in other body tissues ,while AST is found in the highest concentration in the heart compared to other body tissues such as the liver, muscles and kidney,as forALP is found in the mucosal epithelium of the small intestines and convoluted tubules of the kidneys, bones, liver, and placenta (Gowda *et al*., 2009; Modaresi *et al*., 2011)Both AST and ALT are naturally present in serum at low levels, and released into the blood in greater amounts when hepatocytes are damaged (Nyblom *et al*., 2004; Aragon and Younossi, 2010).

Therefore, a change in the proportion of these enzymes indicates a defect in the liver , when a defect occurs in the liver, these enzymes are excreted in large quantities into the blood(Aragon and Younossi., 2010).

Gilani and Janbaz (1995) studied the effect of alcoholic and aqueous extract *A.absinthium* against hepatotoxicity caused by CCl4 and acetaminophen after the oral dose of extract by dose 500 mg/kg body weight of mice twice daily protects the liver from the effects of these compounds and reduces the level of AST and ALT ,while the aqueous extract dose 85 mg /kg of body weight did not occur any effect in the level of liver enzymes AST and ALT in rabbits group.

No significant changes in liver parameters (AST, ALT and ALP) were observed in the three groups of male rats that were dosed with the extract*Artemisia deserti* extract may have been shown to have no effect on liver function(Yazdani *et al*., 2013).

While, Rezaei *et al*(2013), studied the role of the alcoholic extract *Artemisia aucheri* against thioacetamide-induced hepatotoxicity in male albino rats after administration 100,200 and 300 mg /kg for 21 days,the results showed that the extract worked to protect the liver and reduce the enzymes

2-3-7-4 Kidney Function Tests

kidneys are the primary organ for the removal and excretion of vital foreign substances, including medicines, from the body. In addition, electrolytes and water balance are regulated by the kidneys. Urea and creatinine are waste products of protein metabolism that are excreted by the kidneys. Increased urea and creatinine are a sign of kidney damage. Although urea concentration has increased due to dehydration, medication and diet (Ene-ojo *et al*., 2013), Creatinine is a product of creatine secreted by the kidneys and the amount of this

biochemical compound in the blood is commensurate with the rate of glomerular filtration (So and Thorens, 2010).

Zeggwagh *et al*.,(2014) studied the effect of aqueous extract of Artemisia on the cardiovascular system and kidney function in normal rats, they were showed that the intravenous injection of the aqueous extract of Artemisia at a dose 50,100 and 200 mg/kg may cause an increase in urine excretion increased in sodium and potassium excretion,while they showed no change in glomerular filtration.

Astudy done by Irshaid *et al*.,(2012) showed that the oil extract of Artemisia has a significant role in protecting the heart, liver and kidneys in rats induced with diabetes, which is due to the strong antioxidants that induced insulin secretion and reduced glucagon secretion in rats induced with diabetes, which has Positive effect on the performance of the functions of various body tissues, especially the blood, heart, kidneys and liver, and resistance to the toxic action of alloxan .

Jayasimha *et al*., (2011) reported that the methanol extract of *A. absinthium* leaves at different concentrations (100, 250 and 500 mg/kg) produced significant hypoglycemic activity, moreover, the extract of this plant significantly reduced the levels of urea and creatinine in diabetic mice.

2-3-7-5 C-reactive Protein

Measurement of C-reactive protein is one of the most important indicators of inflammation, and CRP plays a role in removing altered cells and bacteria and has more complex immune functions(Rhodes *et al* ,2011).

In study that included 159 rheumatoid arthritis patients with elevated CRP and RF, they were given Artemisia ennua extract with leffunomide for 48 weeks. The results showed improvement in the wormwood extract + lefflunomide group, and low results were obtained for C-reactive protein,

erythrocyte sedimentation rate ESR and serum rheumatoid factor (RF) after 48 weeks of starting treatment compared to the control group (Yang *et al*.,2017).

2-3-7-6 Body Weight

In a study done by Muto *et al* (2003) , in which a group of adult Wester Hanover rats (6 weeks old) were used , both sexes were used in this experiment. The rats were given *Artemisia absenthium* extract for 13 weeks with 3 different concentrations at the end of the experiment they were not observed changes indicating the presence of toxicity on body weights , due to the artemisia extract .In a study conducted by Mukinda and Syce (2007) to test the toxicity of African wormwood by using mice , they were given an oral dose of 1 g / kg for 3 months and at the end of the experimenthas never noted any changes in the weights of the members , indicating that Artemisia is not toxic when giving it sharply.

On the other hand Choi *et al* (2013) conducted their study on 5 - week - old male C57BL / 6J mice , they administerated the alcoholic extract of *Artemisia iwayomogi* . They pointed out that the Artemisia extract may prevent obesity caused by a high fat diet or metabolic disorder , this means that artemisia extract may have a weight - reducing and anti - obesity effect , possibly by downregulating the expression of genes associated with lipogenesis .

Also , in a study conducted on 24 rats C57BL/ 6J with an average weight of 23 g and aged 7 weeks , they have dosed with *Artemisia annua* extract. The researchers noted that artemisia extract inhibited the level of lipids ,and the weight gain was significantly less in the group treated with the extract , which indicates the anti - obesity effect (Baek *et al* ., 2015).

Chapter Three

Materials and Methods

3-1 Materials:

3-1-1 Laboratory Animals

This study was conducted from 10/2/2021 to 14/8/2021, white laboratory male mice of BALB/C dynasty were used in this study, obtained from and raised in the animal house of the Department of Biology College of Sciences/ University of Misan, under controlled conditions in terms of temperature 20-25º C and lighting cycle 12 hours light / 12 hours dark, for the duration of the study and the mice were placed in Plastic cages of standard sizes (30 * 12 * 11) cm manufactured by Kajeen company, Iran, and the cages were spread with sawdust, as they were changed weekly,It was fed using the ration consisting of a group of substances shown in the table (3-1) .

Table (3-1): The feed ingredients according to (Jawad,1996)

No	Substances	Percentage
1	Flour	75%
2	Animal protein	15%
3	Vegetable protein	6%
4	Milk	2%
5	Minerals and vitamins	1 gm/kg

3-1-2 Animal Husbandry

Laboratory mice were mated by placing an adult male with four adult females of 10-12 weeks of age in the cages used in the study and under the conditions referred to in paragraph (3-1-1). Sixteen days after mating, the pregnant females were isolated in single cages and left undisturbed. Until birth, then the newborns are weaned four weeks after birth, where they are isolated

from their mothers in special cages according to sex until they are used in laboratory experiments related to the subject of the study (AL- Maliki,2000).

3-1-3 Apparatus

The apparatus and equipment used in this study are listed inthe table (3-2)

Table (3-2): The Apparatus and Equipment used with their Producing Companies and Countries.

Apparatus	Manufacture company	Country
Centrifuge	Beckman	England
Cobas c 111	Roche	Germany
Cobas e 411 analyzers (disk system)	Roche	Germany
Distillation unit	WB2800	Germany
Electrical balance	Kerm	Germany
Electric grinder	Hermile	Germany
Hemocytometer	Hermile	Germany
Incubator	Binder	USA
Light microscope	Olympus	Japan
Magnetic stirrer	Daihan labtech	Korea
Refrigerator	LG	USA
Sensitive balance	DENVER	Germany

3-1-4 Kits

The kits that used in this study are summarized in table (3-3):

Table (3-3): The kits that are used with their producing companies and countries

Kits	Company	Country
Alanine Aminotransferase (ALT)	Roche	Germany
Alkaline Phosphatase (ALP)	Roche	Germany
Aspartate Aminotransferase (AST)	Roche	Germany
Blood urea	Roche	Germany
Creatinine	Roche	Germany
C reactive protein	Roche	Germany
Follicular Stimulating Hormone (FSH)	Roche	Germany
Luteinizing Hormone (LH)	Roche	Germany
Thyroid Stimulating Hormone (TSH)	Roche	Germany
Thyroxine (T4)	Roche	Germany
Total Testosterone (TT)	Roche	Germany
Triiodothyronine (T3)	Roche	Germany

3-1-5 Instruments

The instruments employed in this study are summarized in table (3-4).

Table (3-4): The instruments used with their producing companies and countries

Instruments	Company	Country
Beaker	ISOLAB	Germany
Conical flask	ISOLAB	Germany
Chloroform	Scharlau	Spain
Cylinder	ISOLAB	Germany
Eosin	BDH	England
Funnel	ISOLAB	Germany
Gel tube	AFMA	Cordan
Gloves	Broche	China
Medical gauze	Kardelen	Turkey
Normal Saline Solution	Fresenius Kabi	Germany
Oral gavage	Hebson	India
Petri dishes	Citioglas	China
Plain tube	AFMA	Jordan
Plastic cage	Kajeen	Iran
Plastic cups	Shangai blopak	China
Surgical set	Hebson	India
Syringe	Citioglas	China

3-1-6 The Drug Employed

Hydroxychloroquine sulfate (HCS) was used in this experiment, (HCS; quinoric tablets with an estimated dose of 200 mg/kg (manufactured by Bristol Laboratories, United Kingdom) .

3-2 Preparing Doses

To prepare the doses hydroxychloroquine tablets were ground into powder by a grinding machine. The dose was weighed daily by a sensitive balance and it was resolved using distilled water Tanenbaum and Tuffanelli (1980), and the process was repeated every day during the experiment period the treatment was administered orally by gavage tube at a dose equivalent to the human dose ,two concentrations were used in the experiment, the dose with a concentration of 200 mg /km was prepared by dissolving 0.06 mg in 0.2 ml of distilled water, and the double dose was 400 mg/kg that was used on the first day of the experiment, which was prepared by dissolving 0.12 mg in 0.2 ml distilled water.

3-3 Plant Collection :

The aerial parts of the wormwood plant were obtained from local shop selling medicinal herbs in one of the local markets in Maysan Governorate , the plant was classified by a classification specialist in the College of Science/University of Basrah (Dr.Sahar Abdulabbas Malik Al-Saadi) .

3-4 Preparation of the aqueous extract of *Artemisia herba alba*

The dry aerial parts of the wormwood were crushed in an electric grinder until a fine powder was obtained. 25 gm of dry plant powder was used with 250 ml of heated distilled water at a temperature of 40 °C and placed over the magnetic stirrer at a temperature of 40 °C for 24 hours.The mixture was filtered using several layers of medical gauze, and then the solution was separated using a centrifuge at 3000 rpm for 10 minutes. The filtrate was has been separated and

placed in clean, sterile glass dishes and dried using an incubator at a temperature of 40 °C. For a period ranging from (24-48) hours,to dry the extract and keep the solid substance only, then take the weight of the solid substance and from this solid substance, the required concentrations were prepared to used in experience (Mukinda and Syce,2007).

3-5 GC-Mass analysis :

The dry sample obtained from drying the filtrate of Artemisia extract was sent for the purpose of GC-Mass analysis, GC-MS analysis was carried out at the Basra oil company, laboratory quality control department, by using an Agilent Technologies , 7890B GC system coupled to an Agilent Technologies 5977A MSD with EI Signal detector , using HP-5ms 5% phenyl , 95% methyl siloxane (30m*250um*0.25) , the oven temperature was set at 40 C hold for 5 min then raised to 10 C/min to 300 C for 20 min , Helium carrier gas flow rate was 1 ml/min and purge flow 0f 3 ml/min .

The injection mode was pulsed splitless with injection temperature 290º C and the injection sample volume was 1 micro letter. The mass spectrometer used Ion Source temperature 230º C , With scan speed 1562 (N2) , and the mass range 44-750 m/z , Data was run through the NIST 2014 ,and Wiley 9 Library databaseas an additional tool to confirm the identity of compounds .

3-6 Determine the lethal dose (LD50) of artemisia extract:

24 laboratory mice of BALB/C strain, males with weights 20-25 g were used. The median lethal dose was determined ,LD50 (the dose that kills 50% of laboratory animals) and animals were divided into 6 groups (four mice in each group), the first group was considered as a control group, administrated orally with 2ml of normal saline solution.While, the other five groups were dosed with 2 ml Artemisia aqueous extract at concentrations (6000, 8000, 10000, 12000,

14000) mg/kg body weight of the mice and the animals were monitored for 72 hours (Doull *et al*,1980) .

3-7 The Experimental Design

The total number of mice is (80 males weighing 20-25 gm) which divided into four groups(20 mice) for each group,6 mice were killed after 6 days from the start of the experiment, 6 other mice were killed after 12 days, and the last 6 mice were killed after 18 days after administration, while 2 were left to mate and were placed each male with 4 females for fertility test (figer.3-2).

The drug and extract were administered to mice for a maximum of 18 days as below :

1-The first group (the control group) was given an oral dose of 0.2 ml of normal sline daily, two doses each day, one in the morning and one in the evening.

2-The second group , which was received hydroxychloroquine, where the dose was calculated in proportion to the amount of body weight of the mice and dissolved in distilled water and administration to mice by two doses, morning and evening, with a volume of 0.2 ml contains a concentration of 400 mg/kg body weight of mice for the first day,and a concentration of 200 mg/kg for the rest of the days

3- The third group , was given two doses, one dose in the morning and it was 0.2 ml of hydroxychloroquine with the same concentrations mentioned above and an evening dose of aqueous extract of *Artemisia herba alba* ,0.2 ml contains a concentration of 8000 mg/kg.

4 -The fourth group , was dosed with aqueous extract of Artemisia plant at an amount of 0.2 ml twice daily(one morning and other evening) with a concentration of 8000 mg/kg,was prepared by dissolving 0.16 g of dry extract of the plant in 0.2 ml of distilled water.

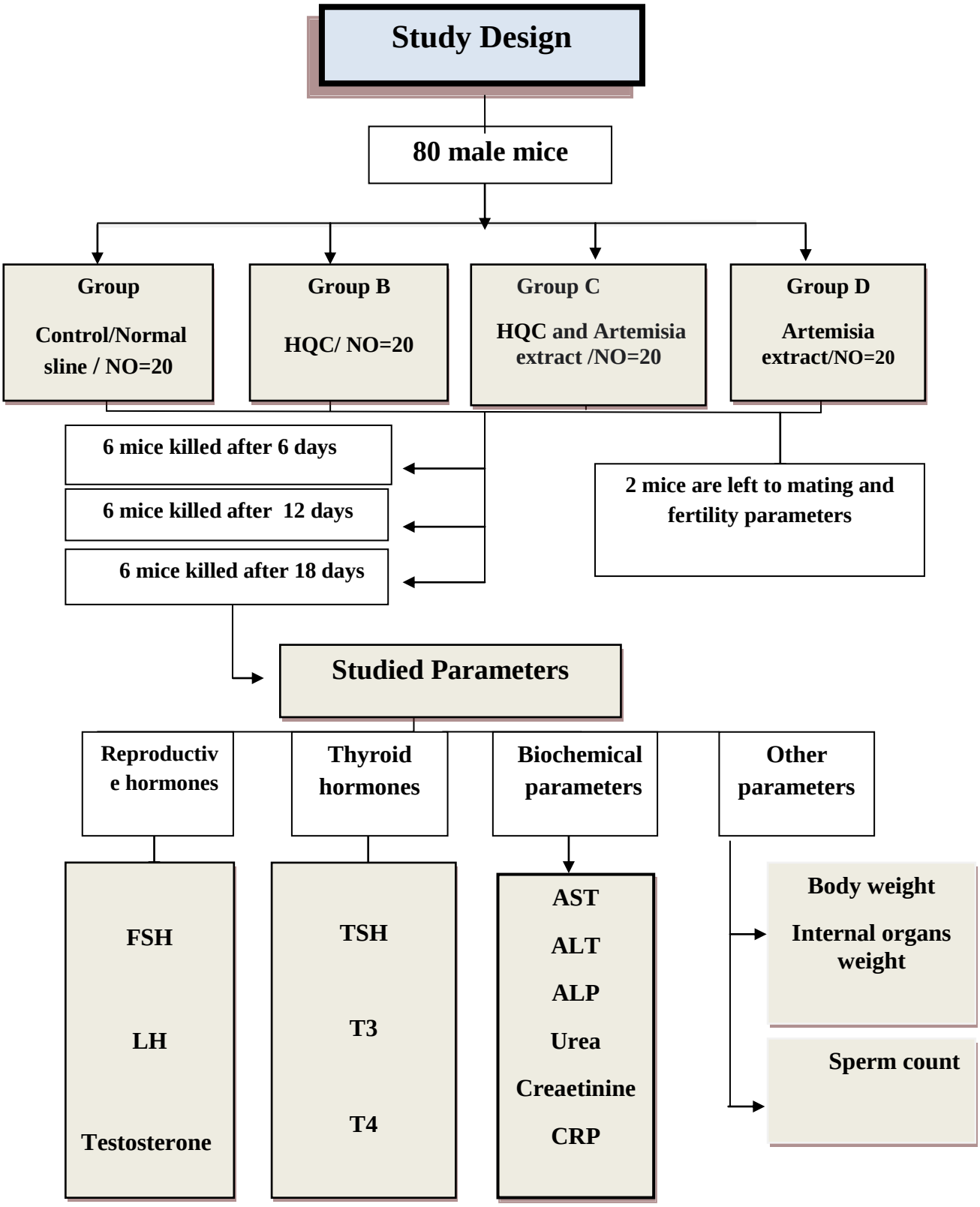


Figure (3-2) :The Experimental Design

3-8 Blood Samples Collection

Mice were euthanized by asphyxiation with chloroform,blood was collected from the heart of mice using a 5 ml syringe ,after collection, the blood was kept in a gel tube and then centrifuged at 3000 rpm for 15 min ,serum was collected from the clear top layer after centrifugation and kept in Eppendorf tubes and stored in freezeruntil parameters were measured (Iriadam*et al*.,2006).

3-9 Studied Parameters

3-9-1 Measurement of Reproductive Hormones

3-9-1-1 Determination of Serum Follicle-Stimulating Hormone(FSH)

Principle of the test :Sandwich principle (The Elecsys FSH assay employs two differentmonoclonal antibodies specifically directed against human FSH) (Wu , 2006).

1-lst incubation: 40 µL of the sample, a biotinylated monoclonal FSH‑specific antibody, and a monoclonal FSH‑The specific antibody labeled with a ruthenium complex (a)*form a sandwich complex.

- Complex (a) :Tris(2,2'-bipyridyl) ruthenium(II)-complex (Ru(bpy)

2- nd incubation: After addition of streptavidin-coated microparticles, the complex become linked to the solid phase by interference of biotin and streptavidin.

3- The reaction mixture is aspirated into the measuring cell were the microparticles are magnetically captured onto the surface of the electrode.

Unbound substances are then removed with ProCell/ProCell M, application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

4-The result can be obtained from the screen showing the data of eachsample and the number of tests.

3-9-1-2 Determination of Serum Luteinizing Hormone (LH)

Sandwich principle (The Elecsys LH assay employs two monoclonal antibodies specifically directed against human LH) (Tietz and Ash,1995).

1- st incubation: 20 µL of the sample, a biotinylated monoclonal LH specific antibody, and a monoclonal LH specific antibody labeled with a ruthenium complexes form a sandwich complex.

2- nd incubation: After addition of streptavidin-coated microparticl ,the complex becomes bound to the solid-state by interference of biotin and streptavidin.

3- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode.

Unbound substances are then removed with ProCell/ProCell M. Applicationof a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier .

4-The result can be obtained from the screen showing the data of each sample and the number of tests.

3-9-1-3 Determination of Total Testosterone (TT)

Principle of the Test Competition principle (The Elecsys Testosterone II assay is based ona competitive test principle using a high-affinity monoclonal antibody specifically directed against testosterone) (Rosner *et al.*,2006) .

1-st incubation: 20 µL of sample is incubated with a biotinylated monoclonal testosterone‑specific antibody. The binding sites of thelabeled antibody become occupied by the sample analyte (depending on its concentration).

2- nd incubation: After addition of streptavidin-coated microparticlesand a testosterone derivate labeles with a ruthenium complex, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

3- The reaction mixture is aspirated into the measuring cell were themicroparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCellM.Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

4-The result can be obtains from the screen showing the data of each sample and the number of tests.

3-9-2 Measurement of Thyroid Hormones

3-9-2-1 Determination of Thyroid Stimulating Hormone (TSH)

Test principle: Sandwich principle (The Elecsys TSH test uses a monoclonal antibody specifically directed against human TSH) (Wu, 2006).

1-First incubation: 50 µl of the sample, monoclonal TSH-monoclonal antibody ,and TSH-monoclonal solution react labeled specific antibodywith ruthenium compound to form a sandwich complex.

2- Second incubation: After adding the streptavidin-coated microparticles, the compound becomes bound to the solid phase by biotin-streptavidin reaction ,the unbound material is then removed with a ProCell/ProCell M. The application of a voltage to the electrode causes a chemical luminous emission that is measured by a photomultiplier.

3-A result can be obtained from the screen showing the data of each sample and the number of tests.

3-9-2-2 Determination of Triiodothyronine (T3)

Principle of the test competition principle (The Elecsys T3 test uses a principle of competitive testing with polyclonal antibodies (Klee, 1996)

1- First incubation: 30 µl of sample and specific anti-T3 antibody, labeled with ruthenium complex; T3 bound is released from the binding proteins in the sample by ANS.

2- Second incubation: After incorporation of streptavidin-coated microparticles and biotinylated T3, the T3 is filled. The binding sites are still free from the labeled antibody, with the formation of the antibody active complex The formed complex binds to the reaction between biotin and 3-streptavidin .

3- The reaction mixture is suctioned into the reading cell, where the microparticles are magnetically attached to the electrode surface.Then the unconnected elements are removed Using a ProCell.Applying an electric current to the electrode causes a chemical flash that is measured by a photomultiplier.

4-The result can be obtained from the screen showing the data of each sample and the number of tests

3-9-2-3 Determination of Thyroxine (T4)

the principle of a competition test (The Elecsys T4 test uses a competitive test principle with an antibody specifically directed against T4) (Nelson and Wilcox, 1996).

1-First incubation: 9 µl of sample and a ruthenium complex-labeled T4- specific antibody; The bound T4 is released from the binding proteins in the sample by the ANS.

2-Second incubation:After the addition of streptavidin- and T4-coated microparticles, the labeled antibody-free binding sites become occupied,

with the antibody-complex formed. The entire complex becomes bound to the solid phase by biotin-streptavidin .

3- Reaction ,the reaction mixture is drawn into the measuring cell where the microparticles are magnetically captured on the electrode surface ,the unbound material is then removed with a ProCell II M ,the application of a voltage to the electrode causes a chemical luminous emission that is measured by a photomultiplier .

4-The result can be obtained from the screen showing the data of each sample and the number of tests

3-9-3 Measurement of Biochemical Parameters

3-9-3-1 Estimation of Aspartate Aminotransferase(AST)

Test Principle :This test follows IFCC recommendations, but is optimized for performance and stability (Bergmeyer *et al* .,1986).

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate ,the oxaloacetate then reacts with NADH, in the presence of malate e4dehydrogenase (MDH), to form NAD.

Pyridoxal phosphate acts as a coenzyme in the amine transfer reaction. Ensures complete enzyme activation.

L-Aspartate + 2-oxoglutarate ⟶ oxaloacetate + L-glutamate

Oxaloacetate + NADH + H ⟶ L-malate + NAD

The rate of oxidation of NADH is directly proportional to the catalytic activity of AST. It is determined by measuring the decrease in absorbance.

Working - Reagents

R1	TRIS buffer	264 mmol/L
	L-aspartate	792 mmol/L
	MDH	≥ 24 µkat/L
	LDH	≥ 48 µ kat / L
	Albumin	0.25%
PYP	Pyridoxal Phosphate	730 µmol/ L
SR	NADH	≥ 1.7 mmol/L
	2-oxoglutarate	94 mmol/L

3-9-3-2 Estimation of Alanine Aminotransferase (ALT)

Test principle: This essay follows the recommendations of the IFCC (Bergmeyer *et al* .,1986).

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. pyruvate formed reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+ ,pyridoxal phosphate serves as a coenzyme in the amino transfer reaction ,it ensures full enzyme activation.

L-alanine + 2-oxoglutarate ⟶ pyruvate + L-glutamate

Pyruvate + NADH + H+ ⟶ L-lactate + NAD+

The rate of NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Working – Reagents

R1	TRIS buffer	224 mmol/L
	L-alanine	1120 mmol/L
	Albumin	0.25 %
	LDH	≥2 45 ukat/L
PYP	Pyridoxal phosphate	730 µmolVL
SR	preservative NADH (yeast):	> 1.7 mmol/L
	2-oxoglutarate	94 mmol/L

3-9-3-3 Estimation ofAlkaline Phosphatase (ALP)

Test principle (Schumann *et al*,2011)

Colorimetric assay According to a standard method in the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into p-phosphate and p-nitrophenol.

p-nitrophenyl phosphate + H2O ⟶ phosphate + p-nitrophenol

The release of p-nitrophenol is directly proportional to the catalytic activity of ALP. It is determined by measuring the increase in absorbance.

Working - Reagents

R1	2-amino-2-methyl-1-propanol	1.724 mol / L
	Magnesium acetate	3.83 mmol/L
	Zinc sulfate	0.766 mmol
	N-(2-hydroxyethyl)-triethylenediamine triacetic acid	3.83 mmol / L
SR	nitrophenyl phosphate	132.8 mmol / L

3-9-3-4 Estimation of Urea

Test principle Kinetic test with urease and glutamate dehydrogenation (Richterich and Colombo ,1978; Sampson *et al*.,1980)

1-First reaction:Urea is hydrolyzed by urease to yield ammonium and carbonate.

Urea + 2 H2O ⟶ 2 NH4 + CO3

2- In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the enzyme NADH to produce L-glutamate, and 2 moles of NADH are oxidized to NAD per mole of hydrolyzed urea.

NH4 +2-oxoglutarate + NADH ⟶ L-glutamate + NAD + H2O

The rate of decrease in the concentration of NADH is directly proportional to the urea concentration in the specimen and measured photometrically.

Working reagents

TRIS buffer solutions	220 mmol/L
2-oxoglutarate	73 mmol/L
NADH	2.5 mmol/L
ADP	6.5 mmol / L
Urease	2300 uF/L
GLDH	80 µkat/L

3-9-3-5 Estimation of Creatinine

Test principle (Fabiny and Ertinghausen ,1971; Jaffé ,1986) This colorimetric kinetic test is based on the Jaffé method.

In an alkaline solution, creatinine forms a yellow-red complex with picrate. The rate of pigment formation is proportional to the creatinine concentration in the sample. The test uses 'rate-blanking' to reduce bilirubin interference.

To correct for a nonspecific reaction caused by pseudo-creatinine staining in serum/plasma, including proteins and ketones, the serum or plasma results are corrected by -18 μmol/L (-0.2 mg/dL).

Creatinine + picric acid ⟶ yellow - red-complex

Working - Reagents

R1	potassium hydroxide	900 mmol / L
	Phosphate	135 mmol / L
SR	Picric acid	38.2 mmol/L
	non reactive buffer	

3-9-3-6 Estimation of C-reactive protein

Test principle (Price *et al.*,1987;Eda *et al.*,1998) :Particle-enhanced immunoturbidimetric assay human C-reactive protein (CRP) agglutinates with latex particles coated with monoclonal anti-CRP antibodies ,the aggregates are determined turbidimetrically.

Working – Reagents

R1	TRIS* buffer with bovine serum albumin
SR	Latex particles coated with anti-CRP (mouse) in glycine buffer
	immunoglobulins (mouse)

*TRIS=Tris(hydroxymethyl)-aminomethane

3-9-4 Body Weight and Weight of the Internal organ

The animals were weighed daily from the beginning to the end of the experience, the weights were recorded to be entered in the statistical analysis and to know the difference in the weight of the animals between the beginning and end of the experiment,The internal organs of the animals are also measured after the end of each period of dosing, where the organs were cut using the surgical set and were measured using a sensitive balance ,the organs that were weighed included heart, stomach,liver, intestines, spleen and kidneys.

3-9-5 Epididymal Sperm Analysis

sperm concentration in the epididymis was calculated according to the method of (Robb *et al*., 1978; Soto.,1983)

To perform the sperm examination, the right epididymis of each animal is cut and placed in a tube containing 5 ml of normal saline, then it was placed in a centrifuge at a speed of 3000 cycles for 5 minutes, after which 5 ml of normal saline was withdrawn and disposed of, using a pipette for red blood cells, draw 5 microns of the solution containing the sperm heads and fill the volume using dilution solution (eosin tincture solution) to mark 101. One drop of the solution is dropped on the Neubauer chamber which was previously covered with a sliding cap. Sperm in the five squares used for counting red blood cells using the objective lens (40x). Sperm count was calculated in cubic millimeters as follows:

Sperm count/cm = n x 10000

n = Number of sperm in 5 squares

3-9-6 Fertility Tests

To perform fertility tests, two male mice were left from each group, and each male was placed with four females in a plastic cage. After 16 days, pregnant females were isolated and left until birth. After birth, the number of births for each female was calculated and the weights of the newborns in each group were measured , the fertility rate was calculated based on the equations shown by (Maertens and Coudert ,1980) as follwes:

Fertility rate(%) = number of pregnant females / number of mated females * 100

3-10 Statistical Analysis of Data

Statistical analysis was performed by a one-way ANOVA (Analyses Variation) followed by the LSD test and Chi square test. Data were expressed as Mean+SE. Statistical significance was set at $P<0.05$ (SPSS, 2001).

Chapter Four

Results

4-1 According to the Groups

4-1-1 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of FSH ,LH and Testosterone in Male Mice

The results in table (4-1) showed a significant (P<0.05) decrease in serum FSH concentration in the treatment group B compared to the control group, and there was nosignificant (P>0.05) difference among the treatment groups B,C and D , as well as there is no significant (P>0.05) difference between the two groups D, C and the control group .The results in the same table also showed that there was a significant (P<0.05) increase in the concentration of LH in group B compared to the control group and the other groups, and there was a significant(P<0.05) decrease in the concentration of LH in the two groups C ,D compared to the control group and group B.The results showed that there was a significant (P<0.05)decrease in the concentration of testosterone in B group compared to control and other groups, also there was a significant (P<0.05)increase in its concentration in group C compared to control and B group, while there was no significant difference(P>0.05) in the concentration of testosterone between control and group D as shown in table (4-1) .

Table (4-1):The values of FSH,LH and Testosterone in Serum of Different Groups (M± SE)

Hormones / Groups	FSH (mlu/ml)	LH (mlu/ml)	Testosterone (ng/ml)
A (Control)	a 0.18 ±0.03	b 1.13±0.22	b 0.19±0.05
B (HCQ)	b 0.10±0.00	a 1.58±0.15	c 0.03±0.01
C (HCQ +Artemisia extract)	ab 0.14±0.03	c 0.10±0.00	a 0.30±0.010
D (Artemisia extract)	ab 0.11±0.01	c 0.10±0.00	b 0.20±0.02

Different letters refer to a significant differences among groups at the level of (P<0.05).

Similar letters refer to non-significant differences among groups

Letter a = highest value , letter c = Lowest value

4-1-2 Effect of Hydroxychloroquine and Artemisia Extract on TSH ,T3 and T4 Serum Concentration in Male Mice

Table (4-2) showed that there was no significant (P>0.05) difference in the concentration of serum TSH between the treatment groups and the control group, and there were no statistically significant(P>0.05) differences in the concentration of TSH among all the treated groups. On the other hand, there was a significant(P<0.05) increase in the concentration of T3 in group B compared to the control group and the other groups, while there was a significant(P<0.05) decrease in the concentration of T3 in C and D groups compared to the control group and group B. As for T4, a significant(P<0.05) decrease was observed in T4 serum concentration for each of the three treatment groups compared to the control group, while there was a significant (P<0.05) difference in T4 concentration between C , D groups and B group, while there was no significant (P>0.05) difference between the two groups C and D in the serum concentration of T4 as shown in table (4-2).

Table (4-2) :The values of TSH,T3 and T4 in serum of different groups (M± SE)

Hormones / Groups	TSH (uIU/ml)	T3 (nmol/L)	T4 (nmol/L)
A (Control)	a 0.007±0.002	b 2.13±0.25	a 66.84±3.38
B (HCQ)	a 0.007±0.002	a 2.95±0.20	b 49.69±3.21
C (HCQ +Artemisia extract)	a 0.005±0.00	c 0.96± 0.02	c 39.92±1.29
D (Artemisia extract)	a 0.005±0.00	c 0.86±0.03	c 39.85±2.76

Different letters refer to a significant differences among groups at the level of (P<0.05).

Similar letters refer to non-significant differences among groups.

Letter a = highest value , letter c = Lowest value

4-1-3 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of AST,ALT and ALP in Male Mice

The results in the table (4-3) showed a significant (P<0.05) increase in (P<0.05) the serum AST concentration in group B compared to the control group. There was also a significant(P<0.05) increase in AST concentration in C and D groups compared to the control group, but at a lower rate, and there was a significant(P<0.05) difference between B and D groups ,while there was no significant(P>0.05) difference between C and D groups, also there was nonsignificant (P>0.05) difference between C and B groups.The results in the same table showed a significant (P<0.05) increase in the serum ALT concentration in the treatment group B compared to the control group and the other groups, while there was no significant (P>0.05) difference among C, D and control groups. The results also showed a significant(P<0.05) increase in the concentration of ALP in all treated groups B,C and D compared to the control group and there was no significant(P>0.05) difference between the B and C groups, while there was a significant(P<0.05) difference between D group and other treated groups B and C as shown in table (4-3).

Table (4-3): The values of AST, ALT and ALP in Serum of Different Groups (mean ± SE):

Paramters / Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
A (Control)	c 153.26±4.20	b 39.33±2.75	c 51.14±2.11
B (HCQ)	a 257.07±5.80	a 72.96±10.23	a 85.77±2.59
C (HCQ +Artemisia extract)	ab 238.86±9.92	b 50.46±3.18	a 87.00±1.84
D (Artemisia extract)	b 229.33±11.36	b 47.16±2.12	b 69.31±2.38

Different letters refer to a significant differences among groups at the level of (P<0.05).

Similar letters refer to non-significant differences among groups

Letter a = highest value , letter c = Lowest value

4-1-4 Effect of Hydroxychloroquine and Artemisia Extract on Serum concentration of Urea,Creatinine and C-Reactive Protein in Male Mice

The results in the table (4-4) showed a significant($P<0.05$) increase in urea concentration in group B compared to the control and C groups, also there was a significant($P<0.05$) decrease in urea concentration in group C compared to control and other treated groups, while there was no significant($P>0.05$) difference between D and control groups and there was no significant($P>0.05$) difference in urea concentration between B and D groups. In the same table, the results showed that there was no significant($P>0.05$) difference in creatinine concentration between the control group and all other treated groups. Also, the group D did not differ significantly($P>0.05$)in comparison with B and C groups, while there was a significant ($P>0.05$)difference in creatinine value between B and C groups. The results also showed that there was no significant($P>0.05$) difference in the concentration of CRP between the control group and the treatment groups B,C and D while there was a significant ($P<0.05$)difference in concentration of CRP between D and B groups , as shown in the table(4-4).

Table (4-4): The values of urea, creatinine and C reactive protein in serum of different groups (mean ± SE):

Parameters / Groups	Urea (mg/dL)	Creatinine (mg/dL)	CRP (mg/L)
A (Control)	**b 38.02±1.40**	**ab 0.17±0.02**	**ab 0.12±0.005**
B (HCQ)	**a 52.16±4.33**	**b 0.12±0.01**	**b 0.11±0.008**
C (HCQ +Artemisia extract)	**c 29.50±1.67**	**a 0.27±0.06**	**ab 0.12±0.009**
D (Artemisia extract)	**ab 44.83±2.70**	**ab 0.21±0.01**	**a 0.14±0.005**

Different letters refer to a significant differences among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups
Letter a = highest value , letter c = Lowest value

4-2 According to the Periods

4-2-1 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of FSH ,LH and Testosterone in Male Mice

The results in the table (4-5) showed that there was no significant($P>0.05$) difference in FSH concentration among B, C and D groups after 6 days of administration , also there was no significant ($P>0.05$) difference between these groups and the control group as well as there was no significant difference($P>0.05$) among groups B, C, D and control after 12 days .

While there was a significant($P<0.05$) decrease in FSH concentration in treatment groups B, C and D compared to the control group after 18 days of dosing.The results in the same table also showed that there was asignificant($P<0.05$) decrease in the concentration of LH in the two treated groups C and D compared to the control group and group B after 6 days of treatment, while there was no significant($P>0.05$)difference in the concentration between B group and the control group in the same period.

After 12 days of treatment, the results also showed a significant($P<0.05$) decrease in LH concentration in groups C and D compared to the control group and group B, while there was no significant($P>0.05$) difference between the two groups C and D, and there was also no significant($P>0.05$) difference between group B and the control group.

After 18 days of treatment, the results showed a significant ($P<0.05$) increase in LH concentration in group B compared to the control group and the other treated groups, while there was no significant($p>0.05$) difference in the other groups among themselves, as well as no significant($P>0.05$) difference between them and the control group, as shown in table (4-5)

As for testosterone, the results showed a significant($P<0.05$) increase in the concentration of testosterone in the D group after 6 days of administration compared to the control group, while there is no significant ($P>0.05$)difference among the B,C and control groups .

After 12 days of admistration, the results showed a significant ($P<0.05$) decrease in testosterone concentration in group B compared to the control and other groups ,while there was no significant($P>0.05$) difference between the control and the other groups (C and D) while, there was a significant($P<0.05$) difference among groups B, C and D,also there was a significant($P<0.05$)decrease in the hormone concentration in B and D groups compared to group C.

After 18 days of dosing, the results also showed a significant ($P<0.05$) decrease in testosterone concentration in group B compared to the control group, and there was no significant($P>0.05$) difference between group B and group C, as well as there was no significant($P>0.05$) difference between group C and control group, while the results showed a significant($P<0.05$) increase in group D compared to control group and other treated groups as shown in table (4-5).

Table (4-5) : The values of FSH ,LH and Testosterone in Serum of Different Periods (M± SE)

Hormones	Groups	After 6 days	After 12 days	After 18 days
FSH (mlu/ml)	A (Control)	a 0.19±0.06	a 0.13±0.03	a 0.21±0.06
	B (HCQ)	a 0.10±0.00	a 0.10±0.00	b 0.10±0.00
	C(HCQ+Artemisia extract)	a 0.16±0.06	a 0.17±0.07	b 0.10±0.00
	D Artemisia extract	a 0.10±0.00	a 0.14±0.03	b 0.10±0.00
LH (mlu/ml)	A (Control)	a 1.60±0.37	a 1.70±0.20	b 0.10±0.00
	B (HCQ)	a 1.64 ±0.34	a 1.77±0.20	a 1.34±0.22

	C(HCQ+Artemisia extract)	b 0.10±0.00	b 0.10±0.00	b 0.10±0.00
	D(artemisia extract)	b 0.10±0.00	b 0.10±0.00	b 0.10±0.00
Testosterone (ng/ml)	A (Control)	b 0.02±0.00	ab 0.43±0.13	b 0.13±0.03
	B (HCQ)	b 0.05±0.03	c 0.02±0.00	c 0.02±0.00
	C(HCQ+Artemisia extract)	ab 0.12±0.04	a 0.71±0.25	bc 0.08±0.00
	D(artemisia extract)	a 0.20±0.07	b 0.20±0.05	a 0.22±0.02

Different letters refer to a significant differences among groups at the level of ($P<0.05$)

Similar letters refer to non-significant differences among groups

Letter a = highest value , letter c = Lowest value.

4-2-2 Effect of Hydroxychloroquine and Artemisia Extract on TSH,T3 and T4 Serum Concentration in Male Mice

The results in the table (4-6) showed that there was no significant ($P>0.05$) difference in the TSH concentration among all groups A, B,C and D after 6,12 and 18 days of administration,

In the same table, the results showed a significant($P<0.05$) decrease in T3 concentration in groups D and C after 6 days of administration compared to the control group and group B, also there was no significant ($P>0.05$) difference between the group of C and D group and there was no significant ($P>0.05$) difference between group B and the control group.

After 12 days of dosing, the results showed a significant($P<0.05$) increase in T3 concentration in group B compared to the control group and other treated groups, there was a significant ($P<0.05$) decrease in T3 concentration in the D and C groups compared to the control group.

After 18 days of dosing, the results also showed a significant($P<0.05$) increase in T3 concentration in group B compared to the control group and the other groups, while there was no significant($P>0.05$) difference among the

control group and the two groups D and C, and there was nosignificant (P>0.05) difference between group C and group D (Table,4-6).

As for the T4 hormone, the results showed a significant(P<0.05) decrease in the concentration of T4 in the treatment groups B, C and D compared to the control group after 6 days of dosing, while there was no significant (P>0.05) difference in its concentration among treated groups B, Cand D.

After 12 days of administration, the results showed a significant (P<0.05) decrease in the concentration of T4 in group D compared to the control group, and there was a decrease in groups C and B, so there is no significant(P>0.05) difference between groups B,C and the control group, and there was no significant(P<0.05) difference among all treated groups (B,C and D).

After 18 days of administration, there was a significant(P<0.05) decrease in the concentration of T4 in the treatment groups D, C, and B compared to the control group, while there was no significant(P>0.05) difference among groups B, C and D as shown in the table (4-6)

Table (4-6) : The Value of TSH ,T3 and T4 in Serum of Different Period (M±SE)

Hormones	Groups	After 6 days	After 12 days	After 18 days
TSH (uIU/ml)	A (Control)	a 0.012±0.00	a 0.005±0.00	a 0.005±0.00
	B (HCQ)	a 0.005±0.00	a 0.012±0.00	a 0.005±0.00
	C(HCQ +Artemisia extract)	a 0.005±0.00	a 0.005±0.00	a 0.005±0.00
	D(artemisia extract)	a 0.005±0.00	a 0.005±0.00	a 0.005±0.00
T3 (nmol/L)	A (Control)	a 2.61±0.39	b 2.50±0.52	b 1.27±0.05
	B (HCQ)	a 2.82±0.31	a 3.53±0.28	a 2.51±0.36
	C(HCQ +Artemisia extract)	b 0.94±0.04	c 1.07±0.05	b 0.88±0.01
	D(artemisia extract)	b 0.89±0.08	c 0.84±0.05	b 0.87±0.03
T4 (nmol/L)	A (Control)	a 70.08±4.94	a 58.26±6.76	a 72.16±4.93
	B (HCQ)	b 48.16±4.84	ab 43.84±5.31	b 57.08±5.95
	C(HCQ +Artemisia extract)	b 40.69±2.06	ab 42.10±2.27	b 36.96±1.33
	D(artemisia extract)	b 43.25±3.59	b 35.52±6.44	b 40.79±4.13

Different letters refer to a significant differences among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups

Letter a = highest value , letter c = Lowest value

4-2-3 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of AST,ALT and ALP Male Mice

The results in the table (4-7) showed a significant (P<0.05)increase in AST concentration in B and C groups compared to the control group and group D after 6 days of administration while the results showed a significant(P<0.05) increase in its concentration in all groups B, C and D compared to the control after 12 days of administration.

Also the results showed a significant(P<0.05) increase in the AST concentration in B, C and D groups , while there was no significant(P>0.05) difference between groups B and D,at the same time, there was no significant (P>0.05)difference between D and C groups after 12 days of administration.

The results in the same table showed a significant(P<0.05) increase in ALT concentration in B group after 6 days of administration compared to the control and the other groups, while there was no significant (P>0.05) difference between C,D and the control group.

The results also showed a significant (P<0.05) increase in the enzyme concentration in group B after 12 days of administration compared to the control group, and there was no significant(P>0.05) difference between groups D, C and the control group.There was also a significant (P<0.05)increase in ALT concentration in groups B, C and D after 18 days of administration compared to the control group, while there is no significant(P>0.05) difference among the treatment groups B, C and D, table (4-7).

In the same table, the results showed a significant (P<0.05)increase in ALP concentration in groups B, C and D after 6 days of administration compared to the control group, while there was no significant(P>0.05) difference in ALP concentration in C and B groups. The results also showed a significant(P<0.05) increase in ALP concentration in the two groups C, B after 12 days of

administration compared to the control group, while there was no significant($P>0.05$) difference between the D group and the control group in the same period, and there was a significant ($P<0.05$) increase in the level of ALP in the three groups B, C and D after 18 days of administration compared to the control group. While, there was no significant($P>0.05$) difference in ALP concentration between C and B groups. Also, there was a significant ($P<0.05$) difference between them and D group as shown in the table (4-7).

Table (4-7) :The Values of AST,ALT and ALP in Serum of Different Periods(M±SE)

Parameters	Groups	After 6 days	After 12 days	After 18 days
AST (IU/L)	A (Control)	b 145.48±8.90	b 161.40±6.48	c 152.90±5.82
	B (HCQ)	a 250.46±7.00	a 249.53±12.97	a 271.21±8.16
	C(HCQ+Artemisia extract)	a 228.56±16.98	a 262.00±12.47	b 226.01±19.99
	D(artemisia extract)	b 182.10±23.87	a 250.08±4.28	ab 255.83±7.47
ALT (IU/L)	A (Control)	b 38.90±3.89	b 44.60±3.95	b 34.51±6.01
	B (HCQ)	a 102.06±26.79	a 65.08±8.18	a 51.73±3.29
	C(HCQ+Artemisia extract)	b 45.21±5.02	ab 59.65±6.88	a 46.53±2.32
	D(artemisia extract)	b 38.60±3.17	ab 51.60±3.34	a 51.30±1.71
ALP (IU/L)	A (Control)	c 50.05±3.62	b 52.20±4.48	c 51.18±3.4
	B (HCQ)	a 84.72±4.51	a 83.90±6.35	a 88.69±2.32
	C(HCQ+Artemisia extract)	a 89.11±2.17	a 86.06±4.55	a 85.81±2.79
	D(artemisia extract)	b 71.95±4.18	b 63.28±3.74	b 72.71±3.94

4-2-4 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of Urea,Creatinine and C-Reactive Protein in Male Mice

The results in the table (4-8) showed a significant (P<0.05) decrease in the urea concentration in group C after 6 days of administration compared to the control group and the other groups, while there was no significant(P>0.05) difference between the other groups for the same period and after 12 days of administration the results showed a significant(P<0.05) increase in the urea concentration in group B compared to the control group and the other groups, while there was a significant(P<0.05) decrease in the urea concentration in the C group compared to the control group and the other groups.

After 18 days of administration, the results showed a significant (P<0.05)increase in urea concentration in groups B and D compared to the control group and group C, while there was no significant(P>0.05) difference between the control group and group C, and there was no significant(P>0.05) difference in the concentration of urea in both groups B and D.

The results showed that there was no significant(P>0.05) difference in creatinine concentration in all treated groups(B,C and D), after 6 ,12 and 18 days of administration compared to the control group (Table ,4-8). Also showed that there was no significant(P>0.05)difference in the CRP value after 6 days of administration. Whereas, after 12 days the results showed a significant(P<0.05) decrease in the CRP value in B group compared to group D(which had the highest value) while there was no significant(P>0.05) difference among control ,B and C groups as well as there was no significant(P>0.05) difference among control, C and D groups, but after 18 days of treatment, the results also showed no significan (p>0.05) difference in CRP value in all groupsas shown in table(4-8).

Table (4-8):The Value of Urea , Creaitinin and CRP in Serum of Different Periods (M±SE).

Parameters	Groups	After 6 days	After12 days	After 18 days
Urea (mg/dL)	A (Control)	a 34.57±2.10	b 43.15±1.34	b 36.34±2.38
	B (HCQ)	a 34.90±3.66	a 59.32±4.90	a 62.25±7.99
	C(HCQ+Artemisia extract)	b 22.02±1.18	c 29.56±1.98	b 36.91±1.10
	D(artemisia extract)	a 34.44±1.60	b 44.47±2.36	a 55.58±4.68
Creatinine (mg/dL)	A (Control)	a 0.16±0.03	a 0.25±0.07	a 0.11±0.01
	B (HCQ)	a 0.11±0.01	a 0.13±0.02	a 0.11±0.01
	C(HCQ+Artemisia extract)	a 0.18±0.04	a 0.23±0.02	a 0.30±0.12
	D(artemisia extract)	a 0.16±0.02	a 0.23±0.02	a 0.23±0.04
CRP (mg/L)	A (Control)	a 0.13±0.008	a 0.12±0.009	a 0.13±0.011
	B (HCQ)	a 0.12±0.012	b 0.09±0.020	a 0.12±0.008
	C(HCQ+Artemisia extract)	a 0.13±0.014	ab 0.11±0.022	a 0.13±0.011
	D(artemisia extract)	a 0.12±0.015	a 0.15±0.006	a 0.14±0.002

Different letters refer to a significant differences among groups at level of ($P<0.05$).

Similar letters refer to non-significant differences among groups

Letter a = highest value , letter c = Lowest value

4-3 Effect of Hydroxychloroquine and Artemisia Extract on Body Weight Gain in Male Mice:

The results of the study after 6 days showed there were a significant ($P<0.05$) increase in the body weight of the mice in C group compared to control and other groups.

After 12 days the results also showed there were a significant ($P<0.05$) increase in the body weight of mice in C group compared to control and other groups, and there was no significant($P<0.05$) difference among B, D and control groups after 6 and 12 days .

While after 18 days the results showed there were a significant ($P<0.05$) decreased in the body weight of mice in B group compared to control and other groups and there was no significant($P<0.05$) difference among C , D and control groups as shown in table (4-9).

Table (4-9) : Body Weight Gain after Different Period in Male Mice (M±S.E)

Body weight of the mice (g)			
Periods / Groups	**After 6 days**	**After 12 days**	**After 18 days**
A (Control)	b 22.18±0.28	b 21.57 ±0.36	a 25.16±0.54
B (HCQ)	b 22.40 ±0.61	b 20.75 ±0.45	b 20.50±0.29
C (HCQ +artemisia extract)	a 24.06±0.13	a 23.49±0.55	a 24.29±0.70
D (Artemisia extract)	b 22.23±0.66	b 21.08±0.39	a 24.01±0.30

Different letters refer to a significant differences among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups

Letter a = highest value , letter b = Lowest value.

4-4 Effect of Hydroxychloroquine and Artemisia Extract on the Relative Weight of Internal Organs

The results showed that there was no significant ($P>0.05$) difference in heart weight among the four groups after 6 days of treatment.

The results also showed a significant($P<0.05$) decrease in heart weight in treatment groups B, C and D after 12 days of administration compared to the control group, and there was no significant($P>0.05$) difference between the two

groups B and C. As for group D, it showes a significant (P<0.05) decrease compared to the control, B and C groups.

After 18 days of administration, the results showed a significant(P<0.05) decrease in heart weight in the three treatmentgroups(B, C and D) compared to the control group, while there was no significant(P>0.05) difference among the three treated groups B,C and D table (4-10).

The results showed a significant (P<0.05) decrease in liver weight in group B compared to the control group and C ,D groups after 6 days of administration, while there was no significant(P>0.05) difference between C and D groups As well as, there were no significant(P>0.05) differences between C, D groups and control group after 6 days of administration .

The results also showed no significant (P>0.05) difference among the four groups A ,B,C and D after 12 days of adminstration .

The results showes a significant (P<0.05) decrease in liver weight of the treatment groups B, Cand D compared to the control group after 18 days of administration , and there was no significant (P>0.05) difference between groups B and C, and there was no significant(P>0.05) difference in liver weight between groups C and D

In the same table,the results showed a significant(P<0.05) decrease in intestines weight in group B after 6 days of treatment compared to the control group and the other groups, while there was no significant(P>0.05) difference between the B, C, also between C and D groups.

After 12 days of administration the results showed no significant (P>0.05) difference in intestine weight among all groups A, B, C and D.

While ,the results showed a significant(P<0.05) decrease in weight in the three groups B, C, and D after 18 days of administration compared to the control group, while there was no significant(P>0.05) difference among the treated

groups. The results showed that there was no significant(P>0.05) difference in stomach weight after 6, 12 and 18 days of administration (Table ,4-10).

Also ,the results showed that there was no significant (P>0.05) difference in spleen weight between all groups after 6 days of administration. While ,there was a significant(P<0.05) decrease in spleen weight in group B after 12 days of administration compared to the control and C groups, while there was no significant (P>0.05) difference between B and Dgroups, and there was no significant(P>0.05) difference among D, C and control groups.

Whereas,after 18 days of administration , the results showed a significant(P<0.05) decreased in spleen weight in group C compared to control group and other treatment groups B and D, while there was no significant(P>0.05) difference between groups B, D, and control group.

The results also showed a significant (P<0.05) increase in kidney weight in B group compared to the control ,B and D groups after 6 days of administration . While ,the results showed a significant (p<0.05) decreased in kidney weight in group D compared to group C, while there was no significant(P>0.05) difference between group C and (A, B groups) ,after 12 days of administration . The results also showed a significant(P<0.05) decreased in all treatment groups D, C, B compared to the control groupafter 18 days of administration . While but, there was no significant (P>0.05) difference between treatment groups (B,C and D),as shown in table (4-10)**.**

table (4-10):Effect of Hydroxychloroquine and Artemisia Extract on Relative Weight of Internal Organs:

Organs weight (g)				
Organs	**Periods / Groups**	**after 6 days**	**after12 days**	**after18 days**
heart	A (Control)	a 0.130±0.012	a 0.148±0.007	a 0.156±0.010
	B (HCQ)	a 0.140±0.015	b 0.116±0.004	b 0.110±0.006
	C (HCQ +Artemisia extract)	a 0.265±0.163	b 0.116±0.008	b 0.1200±0.006
	D (Artemisia extract)	a 0.121±0.006	c 0.095± 0.005	b 0.124± 0.006
Liver	A (Control)	a 1.220±0.066	a 1.158±0.035	a 1.586± 0.033
	B (HCQ)	b 0.763±0.158	a 0.964±0.096	c 0.870± 0.056
	C (HCQ +Artemisia extract)	a 0.910±0.163	a 1.111±0.087	bc 0.940± 0.03
	D (Artemisia extract)	a 1.091± 0.052	a 0.995±0.065	b 1.084± 0.096
intestines	A (Control)	a 1.892± 0.171	a 1.940±0.034	a 2.128±0.062
	B (HCQ)	b 1.548±0.096	a 1.260±0.100	b 1.693± 0.099
	C (HCQ +Artemisia extract)	ab 1.84±0.084	a 1.851±0.128	b 1.725± 0.078
	D (Artemisia extract)	a 2.013±0.070	a 1.816± 0.188	b 1.618±0.099

Organs weight (g)				
Organs	**Periods / Groups**	**after 6 days**	**after12 days**	**after18 days**
Stomach	A (Control)	a 0.410±0.087	a 0.424± 0.039	a 0.452± 0.070
	B (HCQ)	a 0.498± 0.187	a 0.556±0.085	a 0.393±0.027
	C (HCQ +Artemisia extract)	a 0.288± 0.032	a 0.401± 0.069	a 0.316± 0.045
	D (Artemisia extract)	a 0.443± 0.062	a 0.460± 0.044	a 0.444± 0.070
Spleen	A (Control)	a 0.130± 0.020	a 0.124± 0.008	a 0.120± 0.008
	B (HCQ)	a 0.101± 0.016	b 0.087± 0.003	ab 0.095± 0.013
	C (HCQ +Artemisia extract)	a 0.101± 0.013	a 0.125± 0.015	b 0.078±0.006
	D (Artemisia extract)	a 0.108± 0.013	ab 0.096± 0.012	a 0.114± 0.007
Kidney	A (Control)	b 0.158±0.016	ab 0.150± 0.005	a 0.200± 0.007
	B (HCQ)	a 0.183± 0.012	ab 0.160±0.003	b 0.158± 0.016
	C (HCQ +Artemisia extract)	b 0.1500±0.004	a 0.168±0.016	b 0.155± 0.009
	D (Artemisia extract)	b 0.160± 0.068	b 0.125± 0.016	b 0.154± 0.002

Different letters refer to a significant differences among groups at the level of ($P<0.05$).

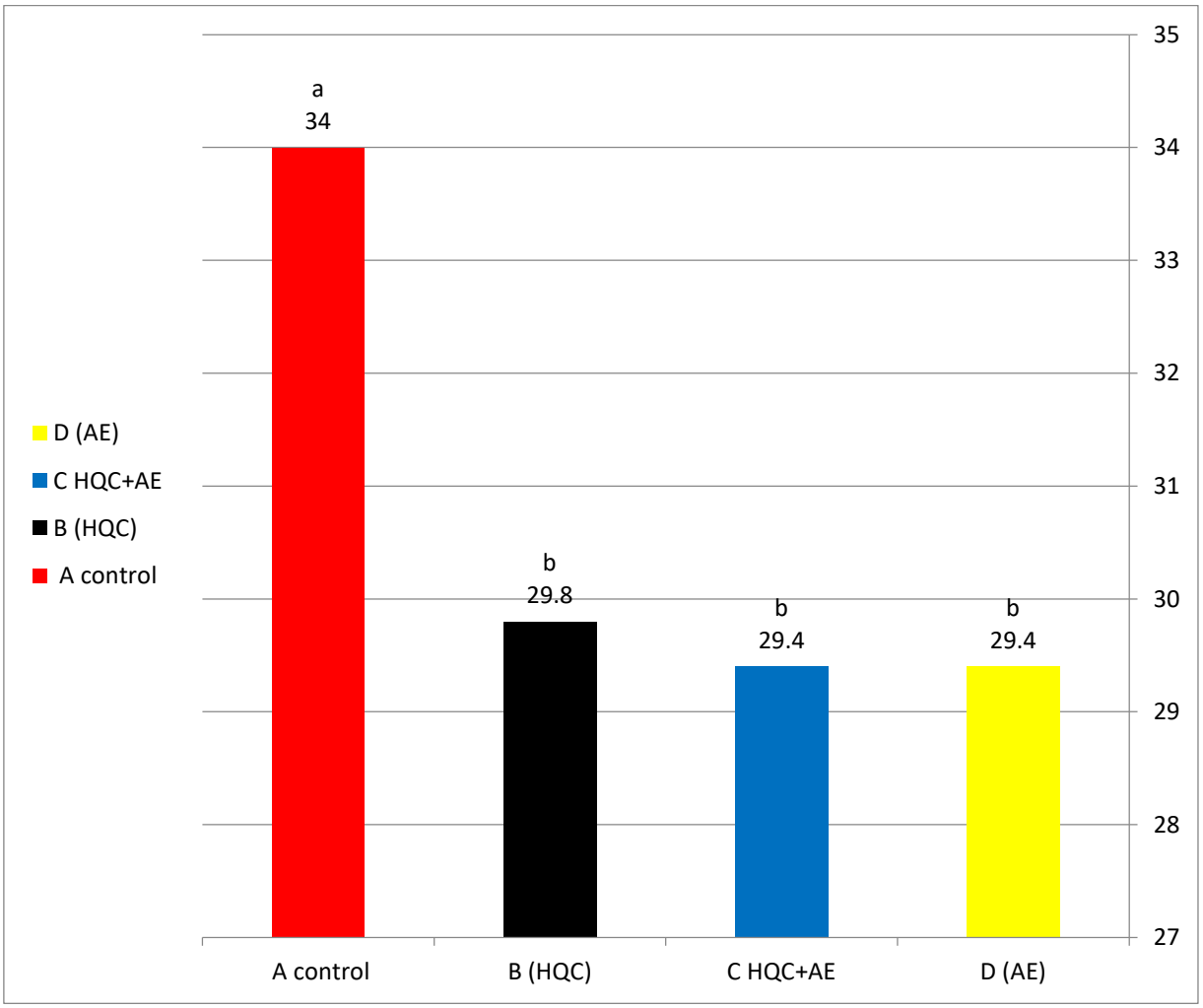
Similar letters refer to non-significant differences among groups.

Letter a = highest value , letter c = Lowest value

4-5 Effect of Hydroxychloroquine and Artemisia Extract on Fertility

4-5-1 The Sperm Count in Adult Male Mice

The results showed a significant (P < 0.05) decrease in the number of the sperm in all treated groups (B , C and D) (29.8 , 29.4 and 29.4 million / ml of Seminal fluid) respectively , in Comparison with the control group (34 million / ml of Seminal fluid). Also , the results showed a non significant (P>0.05) differences in the number of sperm among all the treated group as described in figure (4-1).



AE=Artemisia extract*

Figure (4-1) :Effect of Hydroxychloroquine and Artemisia Extract on Sperm Count in Male Mice

4-5-2 The Fertility and Pregnancy Rates in Females Mice

The results showed a significant ($p<0.05$) decrease in the fertility rate in group B compared to the control group and the other groups. There was also a significant($p<0.05$) decrease in the pregnancy rate in group B compared to the control group and the other groups. significant($p<0.05$) decrease in the pregnancy rate was also observed in group D compared to the control group and C group as shown in table (4-11).

Table(4-11):Effect of Hydroxychloroquine and Artemisia Extract on Fertility and Pregnancy Rates.

Groups	fertility rate %	Pregnancy rate %	X^2	P.value
A Control)	75	75	6.49	0.05
B (HCQ)	50	50		
C(HCQ +Artemisia extract)	75	75		
D (Artemisia extract)	63	62.5		

4-5-3 Reproductive Efficiency in Females After Mated with Males Treated (HQC, HCQ+Artemisia Extract and Artemisia Extract)

The effect of hydroxychloroquine, Artemisia extract and combined treatment fertility efficiency in female mice was described in the table (4-12).The results showes a non significant ($P>0.05$) decrease in the number of the offspring in all treated groups (B, C and D),in compared to the control group. The results in the same table, showed a significant ($p<0.05$) decreased in the weight of the offspring. in the HCQ treated group(1.32 ± 0.05),compared to the control and other groups. But, there was non significant ($p>0.05$) differences among C, D and control groups(1.59 ± 0.13,1.58 ± 0.13 and 1.54 ± 0.11) respectively.

Table (4-12): The Number of Females, Number of Offspring and Weight of Offspring in Different Groups(Mean±SE)

Groups / Parameters	NO of female	NO of offspring	Weightof offspring
A (Control)	8	a 27.50±0.95	a 1.54±0.11
B (HCQ)	8	a 24.25±0.75	b 1.32±0.05
C (HCQ +Artwmisia extract)	8	a 25.16±0.65	a 1.59±0.13
D (Artemisia extract)	8	a 24.80±0.66	a 1.58±0.13

Different letters refer to a significant differences among groups at the level of ($P<0.05$).

Similar letters refer to non-significant differences among groups.

Letter a = highest value , letter b = Lowest value

4-6 Gas Chromatography-Mass Spectrometry Analysis

The chemical name of each compound, Peak, Retention time, and Area % were obtained, 153 compounds were detected, the main chemical components as shown in Table(4-13), Figure (4-2), where the area of piperidine was (10.515) and Peak 84, 2,3-Butanediol(1.567) and Peak 45, ethylamine,(8.669) and Peak 78,2(5H)-Furanone(5.228) and Peak 84, I-pyrrolid-2-one,N-carbamoyl(1.309) and Peak 84, ethyl pipecolinate (1.990) and Peak 84, 1-methyl-2-phenoxyethylamine(7.043) and Peak 44, n-Hexadecanoic acid(1.623) and Peak 212, 4-methyl-2,4-bis(hydroxyphenyl)(7.104) and Peak 207, Olean-12-en-28-oic acid(1.315) and Peak 203.

Table (4-13) : The Main Chemical Composition of Artimisia Extract

NO	Composite name	Peak	RT	Area%	Chemical formula
1	Piperidine	84	3.623	10.515	C5H11N
2	2,3-Butanediol, [S-(R*,R*)]-	45	3.829	1.567	C4H10O2
3	Glycine, N,N-dimethyl-, methyl ester	58	4.277	1.905	C5H11NO2
4	Ethylamine, 2-((p-bromo-.alpha.-methyl-.alpha.-phenylbenzyl)oxy)-N,N-dimethyl-	78	7.073	8.669	C18H22BrNO
5	2(5H)-Furanone, 5-(1-methylethyl)-	84	9.979	5.228	C7H10O2
6	l-Pyrrolid-2-one, N-carbamoyl-	84	10.12	1.309	C5H8N2O2
7	Ethyl pipecolinate	84	10.318	1.990	C8H15NO2
8	1-Methyl-2-phenoxyethylamine	44	10.692	7.043	C9H13NO
9	n-Hexadecanoic acid	212	12.914	1.623	C16H32O2
10	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	207	14.882	7.104	C24H36O2Si2
11	Olean-12-en-28-oic acid, 3-oxo-, methyl ester	203	16.462	1.315	C31H48O3

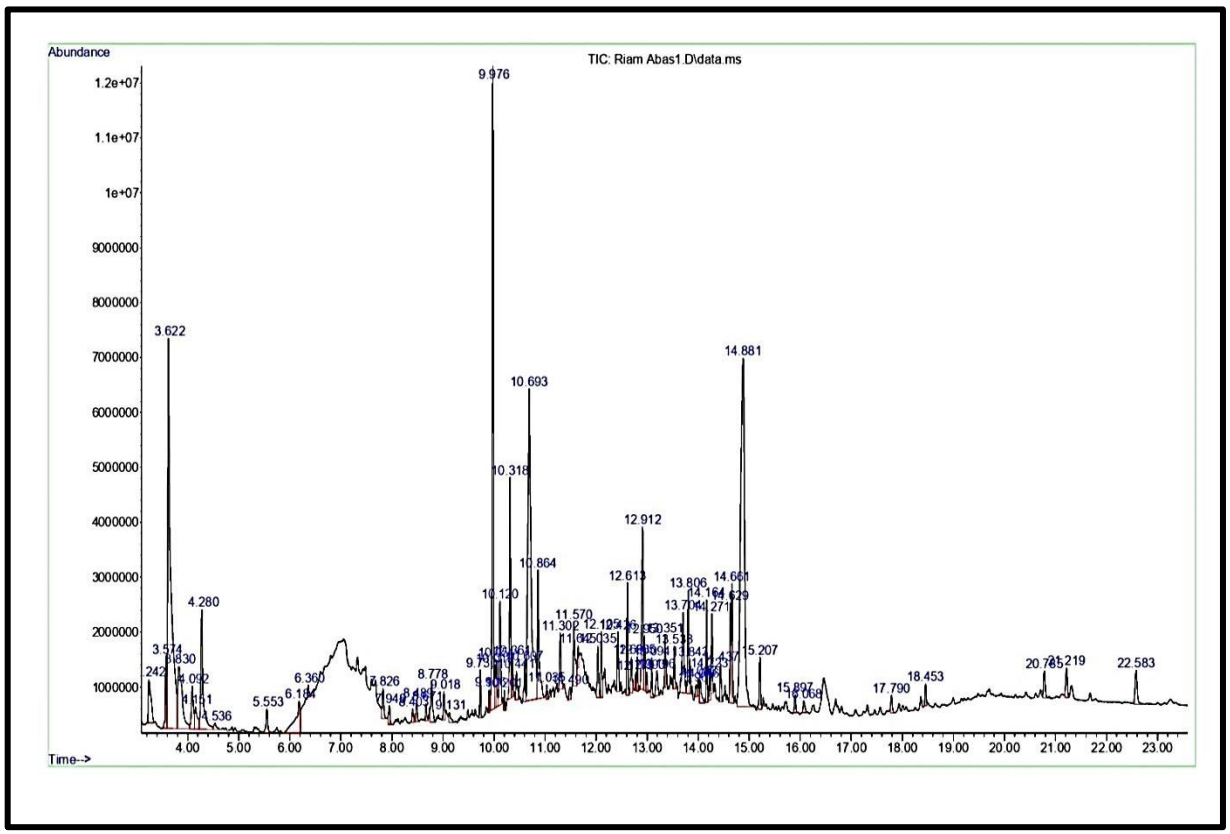


Figure (4- 2) : Chromatogram Showes the Identity of the Main Compounds of Artemisia Extract

Chapter Five

Discussion

5-1 According to groups:

5-1-1 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of FSH ,LH and Testosteroneof Male Mice:

The results of the current study showed a significant($P<0.05$) decrease in FSH and testosterone concentrations in B group compared to the control group table (4-1).

These results of study done by Ejebe *et al*.,(2008) indicated those adult men who took quinine at a dose of 600 mg every 8 hours for five days, as well as other group which took 4 tablets of CQ (250 mg each) for two days, then 2 tablets for one day there was a significant decrease in testosterone concentration , while there was no changes in FSH and LH in adult men who took CQ. As well as there was no changes in FSH and testosterone and significant increase of LH in quinine group.

In studies contradict our study done by Korte *et al*., (1979) showed that giving mefloquine (4-aminoquinelon related to hydroxychloroquine) to dogs and monkeys for 90 days does not cause a significant change in testosterone level.

The decrease may be attributed to changes in the enzymatic activity of cytochrom p450 (CYP450)operating in the liver, which leads to the stimulation of metabolism process in liver and thus leads to changes in the enzymatic activity associated with hormonal metabolism, as hormones are mostly synthesized by members of the CYP450 enzyme family(Stupans *et al* .,1995).

In study by Long *et al*.,(2009) showed that the decrease in FSH and LH has a direct effect on the decrease in the level of testosterone .

The toxic effects of hydroxychloroquine on the male reproductive system can be explained by the fact that it is 2-3 times less toxic than chloroquine.(McChesney,1983)

The results of the current study showed that there was no difference in FSH and testosterone, and a decrease in LH in C and D groups compared to control group (table,4-1).

The results of this study were similar to study done by Trinh *et al.*, (2017) which indicated that orally administration of *Artemisia capillaris* extract to rats at dose 200 mg/kg for 3 weeks leads to lowering the level of LH and maintaining the level of FSH in female rats.

In another study condradict to our by Boareto *et al*.,(2008) they indicated that the administration of *Artemisia annua* extract to pregnant female laboratory rats at dose 7,35 and 70 for 13,14 and 20 days led to a decreased in the level of testosterone.

Also, in the other study that done by Zerrougui *et at*.,(2018) which showed that the administration of artemisia extract to male rabbits at dose(10 and 20 mg /kg) for 33 days led to an increased in the level of testosterone,that is due to contained of this extract various antioxidants that eliminate free radicals resulting from various cellular metabolic processes, thus maintaining the cellular membranes of various organs. Therefore, artemisia extracts have a positive effect on the reproductive system. Despite the great benefits of antioxidants, it may in some cases lead to defect in reproductive system .

On the effect of antioxidants ,the study conducted by Akour *et al.*, (2016) proved that the use of the wormwood plant of the species *Artemisia herba alba*, (which is the species used in our experiment) has fertility-reducing effects that may lead to sterility in women.

The excessive intake of artemisia leads at first to the activity of reproductive system cells, especially leydig cells, and thus increased the weight of reproductive organs and increased the testosterone as shown by(Zerrougui *et al*.,2018).

In turn, this excessive activity convert into a deficit and deficiency in the function of the testicles, thus affecting the production of testosterone (Ajah and Eteng,2010).

5-1-2 Effect of Hydroxychloroquine and Artemisia Extract on TSH ,T3 and T4 Serum Concentration of Male Mice

The results in the table (4-2) showed no significant difference in TSH values, a significant increase in T3 and a significant decreased in T4 in the group treated with hydroxychloroquine compared to the control group.

Shiroky *et al*.,(1993) they mentioned that hydroxychloroquine plays a role in lowering thyroid hormones in rheumatoid arthritis patients with hypothyroidism.

Chen and Liou (2005) noted that the hydroxychloroquine may helps control on the thyroid inflammation in patients with lupus erythematosus, and suggested that hydroxychloroquine may alter abnormal thyroid function and reduce levels of thyroid hormones.

The results in current study were agreement with Amichai *et al*.,(2007) which suggested that HCQ does not cause changes in thyroid hormones, as the results showed normal thyroid functions and reduction the levels of thyroid hormones in patients with rheumatoid arthritis .

While the results showed no difference in TSH with a decrease in T3 and T4 hormones in the C and D groups

This may be due to the presence of flavonoids in the plant , which inhibit the activity of thyroid hormones , affecting the availability of these hormones by inhibiting the activity of the deiodineation (Dos Santos *et al*.,2011) .

Both natural and synthetic flavonoids are potent inhibitors for thyroid hormones (Kohrle *et al* ., 1989) This may be explains the decreased in thyroid hormones in the D group in our study.

Many conditions can affect thyroid function and the secretion and availability of thyroid hormones in tissues ,among these nutritional and environmental factors that have a role in interfering with thyroid hormones are flavonoids (Panda and Kar,1998).

It has been shown that some flavonoids are able to increase the uptake of iodide in the thyroid gland and thus help in iodine uptake in thyroid cancer patients. Of the most important flavonoids tested, rutin was the only one capable of increasing the uptake of thyroid iodide (Gonçalves et al.,2013).

5-1-3 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of AST,ALT and ALP of Male Mice

The results of the current study showed a significant increased in the level of liver enzymes ALT, AST, and ALP in B group , table(4-3).

These results are similar to a study by El-Shishtawy *et al.,* (2015) they showed that liver enzymes ALT, AST, and ALP were increased after daily administration of HCQ at a dose of 124 mg/kg to albino rats for 6 weeks.

The results were also in agreement with another study by Abdel Galil (2015), which observed an increase in liver enzymes ASTand ALT in patient with systemic lupus erythematosus whom took 400 mg of HCQ per day for one year , after stopping the drug the enzymes returned to normal level.

The enzymes ALT, AST, and ALP are important enzymes in the liver, and the rise in these enzymes is an indication of liver damage, as these enzymes rise to weaken the normal level in the case of viral diseases and other liver diseases, in addition to taking some different medications. The main indicator for

assessing liver function is the measurement of these enzymes(Thapa and Walia,2007;Corwin,2008).

Lin *et al*.,(1997)indicated that the largest percentage of ALT enzyme is found in the liver, while AST is most found in the heart, liver and kidneys. Therefore, AST may increase when kidney and pancreatic tissue are damaged and liver cells necrosis, which supports an increase in AST, ALT, and ALP.AST ,also it rises as a result of impaired liver function resulting from the use of the drugs.

The reason of rise in enzymes maybe due to the accumulation of drug doses in the liver, as the liver is primarily responsible for metabolizing these drugs, as the drug accumulates in Kupffer cells in the liver .Thus, overloading the liver lysosomes with indigestible substances and increasing their size and number(Schneider *et al*.,1997) but the accumulation is less when using hydroxychloroquine compared to chloroquine,because, it is less toxic (Smith and Klein_Schwartz,2005) .

Also,the rise may be attributed to free radicals, as elevated liver enzymes are an indicator of cellular damage, loss of plasma membrane function, and liberation of enzymes into the interfluid and then into the blood (Dakshayani *et al*.,2005;Pannerselvam *et al*.,2009) and oxidative stress which causes an elevation in liver enzyme values (Eze *et al*.,2012).

The results of the current study showed that there was no significant change in the value of ALT and an increased in the values of AST and ALP group D compared to the control group but the increasing was less compared to the group B.

Our results agreed with Adam *et al*.,(2000) they noted when rats fed a diet containing 10% leaf extract of *Artemisia abyssinica* shown rising in levels of AST compared to control.

In another study, no significant changes were observed in the level of ALT enzyme ,and increased in value AST after dosing 1 g/kg of aqueous extract of *Artemisia afra* to rodents for three months (Mukinda and Syce,2007).

The results contradicted with our results by Iriadam *et al.*, (2006) they shows that giving the aerial parts extract of Artemisia herba alba at a dose of 85 mg/kg to diabetic rabbits reduced ALT and AST levels

Also, results of study by Al-Sogeer, (2011) was opposite to the results of the current study, as it indicated a significant($P<0.05$) decreased in the AST and ALT when *Artemisia amonosperma* extract (5%) is given to rats that were suffering from high levels of AST, ALT enzymes due to being dosed with lead acetate,

In the C and D groups, the results showed that the levels of AST and ALT decreased compared to the group B. In similar study by Sekiou *et al* .,(2019) showed that the administration of *Artemisia herba alba* extract to alloxan-induced diabetic rats which received 400 mg/kg of the extract for 30 days leds to reduction of liver enzymes concentration of AST and ALT.

The decline in the values of AST and ALT is an indication of the restoration of hepatocytes,treatment of rats with plant extract enhanced the fight against free radicals which is widely used to inactivate reactive oxygen species (ROS) Coskun *et al*.,(2005), where free radicals caused damage to cell membranes, including liver cells, which leads to the activity of liver enzymes inside the cytosol and leads to their entry into the blood circulation.Therefore, the increased in these enzymes indicates damage hepatocytes (Shariati and Zarei ,2006;Taheri *et al*.,2012).

A study by Cordova *et al*.,(2002) showed that polyphenols, especially flavonoids, have an inhibitory effect on the cytochrome p Cyp450 system,

preventing the metabolism of drug compounds, thus reducing free radicals (Jafari *et al*,2007).

As shown by Rezaei *et al*.,(2013) which indicated that the injection thioacetamide to male rats led to an increase in liver enzymes while when they used Artemisia extract(at dose100,200 and 300 mg/kg) with 50 mg/kg thiostamide led to a decrease in liver enzymes AST, ALT and ALP compared to other group that received thioacetamide only .

Artemisia contains many compound as (flavonoids, alkaloids, phenols, glycosides, terpenes) that have protective effects for the liver, as it led to a reduction in liver enzymes levels such as AST, ALT that rise as due to diabetes (Patil *et al*.,2011;Chang *et al*.,2015; Abdullah *et al*.,2015).

5-1-4 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of Urea,Creatinine and C Reactive Protein in Male Mice

The results of the current study showed an increased in the value of urea in group B compared to the control group table(4-4).

The results of this study were similar to the results of El-Shishtawy *et al*.,(2015) which showed that hydroxychloroquine caused a relative increase in the urea value in experimental animals compared to the control group.

Lee (2005) and Ling Nagan wong(2008), also explained that the side effects of using HCQ were represented in the disturbance of liver and kidney functions. One of the important indicators in evaluating kidney functions is the measurement of the concentration of urea and creatine in the blood serum, as the rise in urea is caused by a decrease in the glomerular filtration rate as a result of the disturbance of the renal tubules (Guyton and Hall,2006).

The results of the current study also showed that there was no difference in the level of creatinine in B group, creatinine is a useless product that is excreted from the blood into the urine by the kidneys. It is easily filtered through the glomeruli and is not recycled or metabolized, but it is only slightly excreted through the tubules (Preminger and Curhan,2003).

But the results of the study conducted by El-Shishtawy *et al*.,(2015) were contradictory with ours, as they showed a significant increased in creatinine level in rats treated with hydroxychloroquine compared to the control group.

As for the group D the results showed that there is no significant difference in the values of urea and creatinine in the mice treated with Artemisia extract compared to the control group table(4-4).

These results were similar to the results of the study done by Noori *et al*.,(2014) which showed no significant differences in the levels of urea and creatinine in rats treated interaperitoneally with *Artemisia deserti* extract 100 and 200 mg/kg respectively for 6 day. Also,the results of Mongi *et al*.,(2021) which in agreement with our study, where the levels of urea and creatinine did not change in rats treated interaperitoneally with 200 mg/kg of *Artemisia campestris* essential oil(ACEO) by for two weeks,there were no significant differences between treated group and the control group.

While the results of Jayasimha *et al*.,(2011) was shown that *Artemisia absinthum* extract reduces high levels of urea and creatinine in diabetic rats, and this is due to its anti-diabetic effect. This explains the difference between these results and our results, as the extract was administrated to infected rats suffering from high urea and creatinine, so the extract led to reducing high levels, and this confirms the protective role played by the artemisia extract, as it preserved the kidneys by reducing the high level of urea.

This was showed in a study by Al-Sogeer(2011), which showed that high levels of urea in rats due to lead acetate were reduced after using *Artemisia monospermous* extract.

C-reactive protein is a protein that is produced by liver cells, and the rise in this protein is one of the important indicators of the presence of various infections, as it helps to detect the infection and its severity and tracks the effect of treatment. In our study,CRP was included, and the results showed no significant differences in all treatment groups B , C and D groups compared to the control.

In one of the similar study by Olsen *et al*.,(2016) where they reported that taking HCQ did not cause any change in the CRP value in patients with incomple lupus erythematosus.

In a study by El-Shishtawy *et al*.,(2015) the results showed an increased in CRP value in rats treated with hydroxychloroquine 124 mg/kg for 6 weeks and these results were contradictory with ours.

Scott *et al*.,(1989) explained that hydroxychloroquine causes a decrease in the value of CRP in patients with rheumatoid arthritis, because hydroxychloroquine plays a role in reducing inflammatory cytokines, and that reducing the severity of inflammation leads to a gradual reduction of C-reactive protein.

As for the D group also, there were no changes in the values of C-reactive protein, and these results were contradictory with Yang *et al*.,(2017) which showed that Artemisia can cause a decrease in CRP in rheumatoid arthritis patients who suffer from high levels of CRP and ESR , and the effective role of Artemisia in arthritis can be explained, as this extract contains antioxidant compounds the most important of which are flavonoids (Mohammed *et al*.,2011).

5-2 According to Periods

5-2-1 Effect of Hydroxychloroquine and Artemisia Extract on Serum Concentration of FSH ,LH and Testosterone of Male Mice

The results of this study table (4-5) shows that there was no significant change in FSH and LH values compared to control after 6 and 12 days of administration in group B.

This result is in agreement with Khezri *et al*.,(2007) which observed that the administration of HCQ to adult male mice at a dose of 15,30,60 mg/kg for 14 days did not lead to significant changes in the values of FSH and LH.

Also, they noted that the administration of HCQ,there is a significant decrease in the value of testosterone, and this decrease was observed in our study, where the values of testosterone in HCQ group were significantly lower compared to the control group, especially in the last periods after 12 and 18 days.

In a study, the results of which were opposite to ours by Elgndy *et al*., (2017) when rats that received 124 mg/kg for six weeks shows a significant decrease in the serum concentration of the FSH and LH.

These differences maybe explained by the difference in the dose of the drug, the duration of the treatment , or the difference in the animals used in the experiment. As it noted in our study that the effect increases in the last period of adminstration. Through this, it can confirm that the prolongation of the treatment duration has a significant effect.

As for C and D groups the result showed no significant difference in FSH , a significant decrease in LH during all treatment periods and significant

increase in testosterone after 6 and 18 in comparesion to control in artemisia extract group.

It was similar to the study by Martin and Touaibia (2020) which showed that the flavonoids contained in Artemisia extract help improve testosterone production and contribute prevention of age-related degenerative diseases associated with testosterone deficiency.

While the results contradicts with study done by (Khataibeh and Dardka , 2007) which they indicated that administration of *Artemisia herba alba* extract at a dose of 100 mg / kg to rats for 60 days leads to a significant decrease in testosterone , FSH ,this may be due to the activity effects of *Artemisia herba alba* on the enzymes involved in the oxidative phosphorylation proces. In conclusion, *Artemisia herba alba* ingestion diet possesses strong compound or principles that decreased fertility mainly by affecting pituitary gland cells.

5-2-2 Effect of Hydroxychloroquine and Artemisia Extract on TSH ,T3 and T4 Serum Concentration of Male Mice

The results of the current study indicated that there was no significant change in TSH values in all treatment groups ,and increase in T3 after 12 and 18 days and decrease in T4 after 6 and 18 days in group B (Table 4-6).

In a study agreement with ours done by Strtoer *et al*.,(2000)they showed that injection of chloroquine at dose 50 mg / kg for 7,14 and 21 days to rats caused an increase in T3 and non significant changes in T4.

Also , in a study done by moattar *et al*.,(2013) included Graves ' disease patients they was suffer from elevated thyroid hormones T3 and T4 and law level of TSH, chloroquine was used at a dose of 250 mg daily for 6 months the result showed a significant decrease in T3 and T4 concentration and an increase in the level of TSH during 4 to 6 months of starting treatment .

The result done by Barbosa *et al* (2001) on patient with homozygous arthritis who was treated with 400 mg/kg of HCQ For four weeks the researchers reported that there was a significant increase in T3 values that agreement with our results but also there was asignifican increase in T4 values that conflicted with our reslts .

While in C and D groups T3 values decreased in groups after 6, 18 days. T4 decreased in this group after 6,12,18 days compared to the control group.

In a study involving a group of male albino rats in which oxidative stress was induced by CCl4 in thyroid tissue, which were dosed in addition to CCl4 with extract of *Sonchus asper* (asteraceae family) at a dose of 100,200 mg/kg for 3 days led to the restoration of the normal levels of T3 and T4, which decreased as a result of exposure to CCl4. Also, the level of TSH returned to the level close to the control group after it increased in the group that was dosed with CCl4 ,this can be explained by the fact that the herbs contain antioxidants, which play a key role in removing free radicals and reactive oxygen species that are produced when exposed to toxic chemicals(Khan,2012).

One of this antioxidants is polyphenolic compouned such as flavonoids which are the most powerful natural compouned that affect the thyroid hormones by antioxidant activity , but a study by Van Der Heide *et al*.,(2003) showed that the use of natural and laboratory flavonoids compounds in the body and laboratory leads to disorders of thyroid gland. While in another study by Dos Santos *et al*.,(2011), they proved that many flavonoids can inhibit thyroid activity and lower T3andT4 concrntration .This may agree with our results.

5-2-3 Effect of Hydroxychloroquine and Artemisia Extract on AST,ALT and ALP Serum Concentration of Male Mice

The results of the current study in table (4-7) showed an increased in the values of AST, ALT and ALP in all treatment periods, after 6,12 and18 days in group B.

Galvan *et al.,* (2007)which indicate increase in AST and ALT in woman with mixed connective tissue disease after treatment with 200 mg of HCQ with prednisone 40 mg for eight to ten hours led to increase in ALT and AST ,normal liver function returned after discontinuation of HCQ this result agreed with our results.

The results of our study also agreed with El-Shishtawy *et al*.,(2015), which noticed an increase in ALT, AST enzymes after administering 124 mg/kg of HCQ to albino rats daily for 6 weeks, while there was no increased in ALP.

Also, Chen *et al*.,(2020) noted after conducting a marker on COVID-19 patients who received HCQ at 200 mg twice daily for 10 days, the researchers observed a significant increase in ALP and AST concentration and after 3 days of stopping taking HCQ these enzymes was decreased.

The values of ALP and ALT in this study contradicted with Rajeshkumar *et al*.,(2020) which they did not observe a significant change in ALP and ALT values after rats were dosed with 60 mg HCQ once daily for 4 weeks.

These changes may be related to the dose, as the values of these enzymes were high when using higher doses, but they did not change when using a lower dose (Sontheiner,2000).

As for, the C and D groups ,ALP and AST values were significantly elevated in all treatment periods 6,12 and 18 days, while the ALT value was high after 18 days.

These results agreement with Li *et al* .,(2022) who observed that administration of a combination treatment of artemisinin-hydroxychloroquine sulfat to male Sprager Dawley rats at different doses 146,219,328 and 492 mg/kg resulted in an increased in AST and ALT at the highest dose of treatment (492 mg/kg).

As for the group that was treated with the extract only, there was an increase in AST After 12 and 18 days and increase in ALT and ALP After 6 and 18 days compared to control group.

The results agreement with study done by (Khataibeh and Dardka , 2007) which they indicated that administration of *Artemisia herba alba* extract at a dose of 100 mg / kg to rats for 60 days leads to an increase in AST and ALT .

Ekam *et al*.,(2019) noted that the dose of 300 mg of *Artemisia annua* extract to a group of malaria induced Wistar rats twice daily for three days led to a significant increase in AST compared to control group, while there was no significant change in ALT value.

The increase in the liver enzymes in the serum may be related to tissue injury with the enzymes being released later into the circulation from the damaged liver tissue(Kumar Mishra *et al*.,2013).Or due to an increase in reactive oxygen species that are responsible for oxidative damage(Ozer *et al*.,2008).

It also noted that the increased in serum levels of AST, ALT, and ALP may be seen in liver diseases such as cirrhosis, hepatitis, and sedation from certain antimalarial drugs or drinking (Vasudevan and Skrecumari ,2007).

While in another study, included a group of rats exposed to CCl4 which suffering from elevated in AST level ,when it was injected with *Artemisia campestris* extract it reduced the level of AST ,this explains the protective role of Artemisia extract.. The same result was also obtained by oral administration, ,

plant extracts play an important role by reducing the disturbances caused by oxidative stress caused by drugs and toxic substances, due to the fact that these herbs contain phenolic derivatives such as flavonoids, which are known for their antioxidant activity(Aniya *et al*.,2000).

5-2-4 Effect of Hydroxychloroquine and Artemisia Extract on Urea and Creatinine Serum Concentration and C Reactive Protein in Male Mice

The results of the current study, in the group treated with HCQ showed no change in urea concentration after 6 days of dosing, while the results showed a significant increase in urea values after 12 and 18 days compared to the control group (Table,4-8).

These results are conflicted with Singh *et al*.,(2018) which explained that giving HCQ for a group of liver patients with a certain treatment for 6 months does not result in a significant change in its urea values

And our results also contradicted other results by El-Shishtawy *et al*.,(2015) which did not notice a change in the urea value after the albino rats were fed 124 mg/kg of HCQ for 6 weeks.

Also, Rajeshkumar *et al*.,(2020) did not observe a change in urea after administration of 60 mg/kg HCQ to mice by intraperitoneal injection.

While the results of the group C showed a significant decrease in the urea concentration after 6 and 12 days of administration, and there was no significant change in the urea concentration after 18 days.This result agreed with Sekiou *et al*.,(2021) which indicated that administration 400 mg/kg of *Artemisia herba alba* extract to group of mice(which exposure to alloxan) for 30 days led to a decrease in the urea level that was raised by alloxan, while the urea level did not change after administration of the Artemisia extract to (healthy mice) in the same study.

This result was also consistent with Li *et al*.,(2022). They indicated that administration male Sprager Dawley rats with a combination treatment of artemisinin-hydroxychloroquine for 14 days did not lead to a change in urea values.

Creatinine did not show any significant change in all treated groups and this agreed with the above studies(Rajeshkumar *et al* .,2020;Sekiou *et al* .,2021;Li *et al*,2022) which indicated that the creatinine level does not change using both hydroxychloroquine or Artemisia extract

Also, there was no significant difference among all period in all treated group that was contradict to study by El-Shishtawy *et al*.,(2015) the showed an increased in CRP value in rats treated with hydroxychloroquine 124 mg/kg for 6 weeks and these.

5-3 Effect of Hydroxychloroquine and Artemisia Extract on Body Weight Gain in Male Mice

The results of the current study showed a significant($P<0.05$) decrease in total body weight in group B compared to the control group after 18 days of administration (Table,4-9).

The results of the current study were in agreement with Rangwala *et al* .,(2014)which indicated that HCQ caused the emergence of symptoms including loss of appetite and weight loss and the symptoms was more severe in the 1200 mg/kg dose (doses used in the study 200, 400, 800 and 1200 mg/kg) and this may be due to the side effect of hydroxychloroquine on the stomach.

Where Lofgren *et al*.,(2020) indicated in their study that the most common intestinal symptoms when taking HCQ. Included, stomach upset and nausea, followed by diarrhea, vomiting and abdominal pain.

Srinivasa *et al*.,(2017),also showed that a large number of patients were suffering from very harmful effects after taking HCQ, and most of these effects were represented by gastrointestinal symptoms (indigestion, abdominal cramps), and this was attributed to the use of the (Quinoric) brand after the production of the brand (Plaquenil) was stopped in 2015.

Or maybe the cause is the occurrence of damage in the membranes of the gastric and intestinal cells of the animal, thus reducing the digested and absorbed nutrients, such as proteins and sugars, which are necessary to build body mass,or due to the stress that the animal exhibits during the dosing, as the mice showed strong resistance and unwillingness to the treatment during the dosing.

Our results also contradicted the study done by Pareek *et al*.,(2009) which indicated that oral hydroxychloroquine prevents weight loss in diabetic male rats, as HCQ raised the insulin level and thus reduced glucose in diabetic rats,they also suggested in the same study that HCQ doses to these rats do not affect food and water intake.

As for C and D groups, the values in all periods were similar to those of the control group.

In a study similar to our results by Muto *et al*.,(2003), they noted that *Artemisia absinthium* extract did not affect body weight, as the weights of the groups that were dosed with the extract were equal to the weights of the control group, and also Mongi *et al*.,(2021) they showed that treated with Artemisia 200mg/kg for 2 weeks could prevent weight loss which induced by other drugs. It leads to the restoration of body weight to normal, which was reduced due to treatment of rats with chlorpyrifos (CPF)

5-4 Effect of Hydroxychloroquine and Artemisia Extract on Relative Weight of Internal Organs

The results in the table (4-10) showed a decrease in the weight of the heart in group B after 12,18 days, as well as a decrease in the weight of the liver and intestines after 6 and 18 days. In a study by Rajeshkumar *et al* .,(2020) when gave the rats 60 mg of HCQ once a day for four weeks, there was no observed change in the weight of the internal organs (liver, kidneys, spleen and heart), and the body weight also did not change, and a decreased in the weight of the spleen after 12 days .This is the opposite of our study, and this can be explained by the fact that they used a low dose of drug.

Also, ElShishtawy *et al*.,(2015) they noticed that the dose of 124 mg/kg of HCQ for male albino rats for 6 weeks decreased the weight of the heart and an increase in the weight of the liver and spleen, while the weight of the kidneys did not change .

While there was a decrease in the weight of the heart after 12 and 18 days as well as a significant decrease in the weight of the liver, intestines ,spleen and kidneys after 18 days and no change in the weight of the stomach in all periods in group C.

In a study by Li *et al*.,(2022) including male Sprago Dawley rats, they were given a combination treatment consisting of HCQ and artemisinin in different doses, the smallest of which dose was 146 mg/kg and the largest dose was 492 mg/kg for 14 days. The researchers noted that the weights of the organs did not show a significant change in all doses after 14 days of administration .

As for the weights of the members in the group D, there was a decrease in the heart, liver, intestines and kidneys after 18 days of administration compared to the control group.

In a study done by Muto *et al*.,(2003) they indicated that the administration of Artemisia extract to a group of rats for 13 weeks at different concentrations (0.125.0%, 0.5% and 2%) led to an increase in the weight of the liver at concentration 2%, and these effects were not interpreted as toxic effects because there is no change in body weights.

In our study can be noted that the weights were low in all treatment groups after 18 days, mostly compared to the control group, with no significant differences between groups , and thus it can conclude that this decrease occurred as a result of prolonging the treatment period or effects maybe occasional, as noted in the previous study, or due to the use of high doses **.**

5-5 Effect of Hydroxychloroquine and Artemisia Extract on Sperm Count in Adult Male Mice

The results of the study showed that the number of sperms decreased in B group compared to control

The results were similar to study by Olumid and Raji,(2011) which indicated that antimalarials lead to impaired spermatogenesis.Also, Abayomi *et al*.,(1992) indicate that administration chloroquine to rats leads to infertility by causing the growth of germ cells and stop depletion of sperm cells. While ,Ashiru *et al*.,(1991) indicated that the elimination of Leydig cells by injecting mice with chloroquine is expected to significantly affect spermatogenesis by lowering the level of testosterone in the blood.

The results of the study contradicted to study by Silva *et al*.,(2010) which pointed out that Hydroxychloroquine is not causing a decrease or weakness in sperm or fertility in patient with rheumatic diseases.

It could be that the treatment may act directly or indirectly on the pituitary gland secretory function leading to an increase in the main hormones controlling spermatogenesis process. It has ben demonstrated that the process of

spermatogenesis and the accessory reproductive organs functions are androgen dependent. Therefore, a change in the androgen production would reflect and explain the decrease in number of mature Leydig cells and their functional status(Agrawal *et al*.,1986).

The results also showed that the number of sperms decreased in C and D groups compared to control

In a study by (Khataibeh and Daradka,2007) which coclude that oral administration of *Artemisia herba alba* extract with a dose of 100 mg/kg for 60 days to a group of male laboratory rats showed a decrease in sperm preparation and this is consistent with our study ,this may be due to the activity effects of *Artemisia herbaalba* on the enzymes involved in the oxidative phosphorylation proces. *Artemisia herba alba* ingestion diet possesses strong compound or principles that decreased fertility mainly by affecting pituitary gland cells.

It is well known that the weight, size and the secretory function of tests, epididymes, seminal vesicles, ventral prostate, and vasa differential are closely regulated by androgens hormones (Chowdhury and Steinberger, 1975).

5-6 Fertility Rate in Male Mice which Trated with (HCQ, HCQ+Artemisia Extract and Artemisia Extract) and Reproductive Efficiency in Mice that Mated with Trated Male

The results of the study showed a lower fertility rate in the HCQ and Artemisia extract group but did not reach to the significant level. The study was consistent with Osinabi *et al* .,(2006), which showed that administration of antimalarials to laboratory animals may be associated with failure of reproductive functions in males.

The reduced fertility rate in the HCQ group can be attributed to the decrease in the level of testosterone, as testosterone plays an essential role in spermatogenesis, as testosterone within the Sertoli cells binds selectively to the androgen receptor,and thus activation of the receptor initiates the process of spermatogenesis and in preservation and thus the deficiency, in the production of testosterone leads to a decrease in spermatogenesis (Dohle *et al* .,2003). In contradictory study of our study by (Ostensen *et al*., 2006) they indicated that hydroxychloroquine does not cause impairment in fertility. The results showed a non significant decrease in the number of newborns in all treatment groups, As for the birth weights, the decrease was observed in the hydroxychloroquine group,this is consistent with Diav-Citrin *et al*.,(2013)where 114 cases of pregnant women with rheumatic diseases which taken of the HCQ were followed and the result was a decrease in birth weights.

While, the results are inconsistent with Seo *et al*.,(2019) the study included 151 pregnancies with lupus erythematosus 80 female of them received hydroxychloroquine, as it was noted that the group that was treated with HCQ showed an increased in birth weight compared to the HCQ untreated group. hydroxychloroquine or with Artemisia extract or both, and this is consistent with the results of our study related to reproductive hormones and the number of sperms. As for the birth weights, the decrease was observed in the hydroxychloroquine group.

5-7 Gas Chromatography-Mass Spectrometry Analysis

The genus of Artemisia contain many chemical components this shows that it is a source of diverse bioactive compounds such as essential oils, sesquiterpenes lactones, sesquiterpenes ,alkaloids, diterpenes, triterpenes, alkamides, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes(Bourgou *et al*.,2017).

The results of current study indicated that the highest percentage in the components of *Artemisia herba alba* extract were Piperidine 10.5% , Embramine 8.6%,4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative 7.10%,1-Methyl-2-phenoxyethylamine 7%, 2(5H)-Furanone, 5-(1-methylethyl)- 5.2%

Whereas Mohsen and Ali,(2009) indicated that the highest percentage in the components of the oil of *Artemisia herba alba* were cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone.

In the current studies, there were differences from those that indicated that cineole is the major component, it is a high percentage ,where in another studies indicated that cineole has been reported as the main component of Artemisia essential oil in Morocco (Lamiri *et al*.,1997) ,Spain (Salido *et al*.,2004) and Egypt(Soliman,2007).As for our study ,it showed that the active compound ,which occupied a high percentage ,is the Piperidine compound .

This difference in compounds can be explained by several factors, including soil, environmental conditions, climate change, plant age, plant parts, harvest time, geographical location and extraction method.

Conclusion

and

Recommendation

Conclusions

The study concluded:

1- Hydroxychloroquine had a significant effect on some hormones and other parameters which led to reduced reproductive hormones, a decrease in the number of sperm and weight of births. Also it was led to a decrease in T3 , an increase in urea concentrations ,and loss of body weight and appetite.
2- In the groups treated with the extract and combined group showed an opposite effect to the group treatrd with HCQ, which indicates that the extract had a protective effect on some parameters.
3- The liver enzymes were increased in all treatment groups.
4- The values of TSH, creatinine and CRP were not affected by either the drug or the extract.

Recommendations

1. The use of other types of Artemisia and the use of the essential oil of Artemisia ,Also use other extraction methods such as alcoholic extraction or cold aqueous extraction.
2. Use of different concentrations of HCQ and plant extracts.
3. Measure other biochemical and hormonal parameter,also antioxidant markers.
4. Studying other parameters and conduct a histological study of different organs .

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Appendixes

Appendix(1):Chemical components of Artemisia herba alba

Compound Label	RT	Area%
N,N-Dimethylaminoethanol	3.242	1.11
L-Lactic acid	3.284	0.11
Piperidine	3.623	10.51
2,3-Butanediol, [S-(R*,R*)]-	3.829	1.56
Propane, 2-isocyanato-2-methyl-	3.874	0.5
Pyridine, 2,3,4,5-tetrahydro-	4.093	0.83
1,2-Ethanediamine, N,N,N',N'-tetramethyl-	4.153	0.23
Glycine, N,N-dimethyl-, methyl ester	4.277	1.9
1,2-Cyclopentanedione	5.554	0.32
(1-Oxa-2-aza-spiro[2.5]oct-2-yl)-phenylmethanone	6.184	0.14
Phenol	6.361	0.23
2H-Pyran-2,6(3H)-dione	6.601	0.17
Ethylamine, 2-((p-bromo-.alpha.-methyl-.alpha.-phenylbenzyl)oxy)-N,N-dimethyl-	6.681	5.15
Propiolactone	6.802	0.5
Ethylamine, 2-((p-bromo-.alpha.-methyl-.alpha.-phenylbenzyl)oxy)-N,N-dimethyl-	7.073	8.66
Bromomethyl methyl ether	7.185	0.27
1,3-Dioxol-2-one,4,5-dimethyl-	7.228	0.12
1-Butanamine, 3-methyl-N-(3-methylbutylidene)-	7.321	0.18
Ethylamine, 2-((p-bromo-.alpha.-methyl-.alpha.-phenylbenzyl)oxy)-N,N-dimethyl-	7.336	2.62
Furaneol	7.445	0.1
1-Piperidineacetonitrile	7.487	0.15
2-Pyrrolidinone	7.596	0.1
Glycerin	7.65	0.62
Cyclopentane, 1-ethenyl-3-ethyl-2-methyl-	7.684	0.13
Phenol, 2-methoxy-	7.827	0.26
Nonane, 5-(1-methylpropyl)-	7.952	0.21
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.408	0.14
1-Butanol, 2-methyl-, acetate	8.482	0.25
Amphetamine-3-methyl	8.492	0.13
Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl-	8.671	0.26
2-Piperidinone	8.735	0.12
Benzoic acid	8.773	0.47
Benzofuran, 2,3-dihydro-	9.018	0.4
2-Propenal, 2-methyl-, diethylhydrazone	9.492	0.11
Valeramide, 5-chloro-N-methyl-	9.57	0.13
2H-Quinolizin-1-ol, octahydro-, cis-	9.633	0.11
Ethanone, 1-(2-hydroxy-5-methylphenyl)-	9.732	0.4
Hygrine	9.905	0.24
Cathine	9.928	0.18

Compound Label	RT	Area%
Phenol, 2,6-dimethoxy-, acetate	9.956	0.11
2(5H)-Furanone, 5-(1-methylethyl)-	9.979	5.22
Cathine	10.02	0.35
3,4-dimethyl-5-phenyloxazolidine	10.053	0.64
l-Pyrrolid-2-one, N-carbamoyl-	10.12	1.3
Ethyl 3,5-dimethylphenylacetate	10.145	0.13
Benzene, (1,1,4,6,6-pentamethylheptyl)-	10.201	0.17
9H-Purine-6(1H)-thione, 9-(piperidinomethyl)-	10.29	0.12
Ethyl pipecolinate	10.318	1.99
2-Ethylpiperidine	10.361	0.52
(2S,6R)-1-Methyl-2,6-dipentylpiperidin-4-one	10.44	0.46
Benzeneethanol, 4-hydroxy-	10.462	0.16
Fumaric acid, di(2-fluorophenyl) ester	10.489	0.13
Acetophenone, 4'-hydroxy-	10.52	0.11
Pyrazine, 2,6-diethyl-	10.576	0.14
Methylparaben	10.596	0.14
1,1'-Bicyclohexyl, 2-methyl-, cis-	10.61	0.38
1-Methyl-2-phenoxyethylamine	10.692	7.04
Phosphorus pentafluoride	10.76	0.16
2(3H)-Naphthalenone, 4,4a,5,6-tetrahydro-7-methyl-	10.793	0.3
Cyclohexanol, 2-methyl-3-(1-methylethenyl)-, (1.alpha.,2.alpha.,3.alpha.)-	10.819	0.18
Anabasine	10.863	1.04
3,4-methylenedioxypyrovalerone	10.88	0.23
.beta.-D-Glucopyranose, 1,6-anhydro-	10.889	0.22
Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	10.937	0.11
Homovanillyl alcohol	11.036	0.13
3',5'-Dimethoxyacetophenone	11.16	0.23
Isoborneol, pentamethyldisilanyl ether	11.237	0.17
1-Amino-2-methylnaphthalene	11.29	0.12
a-Benzyl-N-formyl-N-methylphenethylamine	11.306	0.82
1-Cyclopentene-1-carboxylic acid, 2-methyl-3-vinyl-	11.361	0.18
Methyl(methyl 4-O-methyl-.alpha.-d-mannopyranoside)urinate	11.479	0.16
N,N-Dimethyloctylamine	11.566	0.71
Myo-Inositol, 4-C-methyl-	11.604	0.7
1-Piperidinecarboxaldehyde, 2-(3,4-dihydro-2H-pyrrol-5-yl)-	11.645	0.26
Myo-Inositol, 4-C-methyl-	11.683	1.11
.beta.-d-Fructofuranose, 1,3,4-tri-O-acetyl-2,6-anhydro-	11.703	0.16
Phenylephrine	11.745	0.75
Borane, chlorodimethoxy-	11.803	0.32
(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	11.837	0.13
p-Hydroxynorephedrine	11.882	0.42
4-(1-Hydroxyallyl)-2-methoxyphenol	12.038	0.46

Compound Label	RT	Area%
N-Ethyl-2-isopropoxycarbonylazetidine	12.049	0.12
p-Anisic acid, 2-hydroxy-, monoanhydride with 1-butaneboronic acid, cyclic ester	12.107	0.62
Methanone, (4-methoxyphenyl)phenyl-	12.136	0.19
2-Pyridinamine, 5-methyl-	12.167	0.19
4H-1-Benzopyran-2-carboxylic acid, 6-amino-4-oxo-, ethyl ester	12.185	0.13
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	12.237	0.11
Piperidine, 1-(4-aminobenzoyl)-	12.318	0.11
Butanamide, N-(3-methylphenyl)-	12.353	0.15
s-Triazine, 2-amino-4-(piperidinomethyl)-4-piperidino-	12.424	0.59
1,2-Dimethyl-3-formylindole	12.44	0.18
Tyrosol, acetate	12.489	0.14
2-Pyrrolidin-1-yl-bicyclo[3.2.1]octan-8-one	12.612	0.71
Iron, (1,3-butadiene)tricarbonyl-	12.671	0.16
Matridine, (6.beta.)-	12.694	0.38
1H-Pyrazole, 3,5-bis(1,1-dimethylethyl)-4-ethyl-	12.723	0.14
Hexadecanoic acid, methyl ester	12.779	0.17
Spiro[4H-1,3,2-benzodioxaborin-4,1'-cyclohexane], 2-ethyl-5,6,7,8-tetrahydro-	12.805	0.38
l-Leucine, N-cyclopropylcarbonyl-, butyl ester	12.875	0.11
Butanamide, N-(3-methylphenyl)-	12.893	0.6
n-Hexadecanoic acid	12.914	1.62
2-Pyrrolidin-1-yl-bicyclo[3.2.1]octan-8-one	12.95	0.36
Matridine, (5.beta.,6.beta.)-	12.996	0.18
trans-Sinapyl alcohol	13.092	0.38
3,4-Dihydrocoumarin, 6-amino-4,4-dimethyl-	13.171	0.13
2,7(1H,8H)-Pteridinedione	13.188	0.13
2H-pyrrol-2-one, 4-amino-1-cyclohexyl-1,5-dihydro-3-(1-piperidinyl)-	13.196	0.26
5-Hydroxy-4-octanone	13.267	0.11
Di(cyclopentanonyl-2)methane	13.351	0.7
Benzamide, N-[2-(2-benzylphenoxy)ethyl]-2-methoxy-4-methylthio-	13.379	0.16
Tyramine, N-aminoacetyl-	13.453	0.26
2(1H)-Pyridone, 4-hydroxy-6-methyl-3-valeryl-	13.484	0.17
Di(cyclopentanonyl-2)methane	13.535	0.46
2-Cyclopenten-1-one, 2,3-dimethyl-	13.658	0.17
1,4-Cyclohexanedimethanol	13.689	0.31
Dodecahydro-1H,6H,11H-tripyrido[1,2-a:1',2'-c:1",2"-e][1,3,5]triazine	13.706	1.15
4-Epipallensin	13.804	1.02
4-Epipallensin	13.843	0.34
4'-Hydroxy-3'-methoxyacetophenone, propyl ether	13.953	0.14
3,6-Dimethylpiperazine-2,5-dione	14.002	0.2

Compound Label	RT	Area%
4,8-Propanoborepino[1,2-b][1,2,5]oxadiborole, 2,3,3-triethyloctahydro-3a-methyl-	14.045	0.21
Tributyl acetylcitrate	14.167	0.81
.alpha.-Isolupanine	14.225	0.29
Tricyclo[3.3.1.1(3,7)]decane, 5,7-dimethyl-2-(thiophen-2-yl)-1,3-diaza-	14.275	1.05
2-O-Benzyl-d-arabinose	14.327	0.23
1,3-Dimethyl vinbarbital	14.432	0.31
Oxiraneoctanoic acid, 3-octyl-, methyl ester	14.45	0.16
1,3-Dimethyl vinbarbital	14.528	0.14
Piperidine, 2-(tetrahydro-2-furanyl)-	14.661	0.89
4H-Indazol-4-one, 1-(4,6-dimethyl-2-pyrimidinyl)-1,5,6,7-tetrahydro-	14.781	0.12
2-Methyl-2-hexyl-1,3-dithiane	14.82	0.12
Tetradecanamide	14.835	0.44
4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	14.882	7.1
2-(1H-Indol-3-yl)-N,N-dimethyl-acetamide	14.894	0.48
9-Acridinecarboxylic acid	15.209	0.47
Ethyl stearate, 9,12-diepoxy	15.275	0.12
2,4-Diamino-7-n-butyl-5,6,7,8-tetrahydropyrido[4',3',4,5]thieno[2,3-d]pyrimidine	15.899	0.2
Benzaldehyde, 4-[[(4-methyl)phenylamino]carbonylmethoxy]-	16.07	0.17
Olean-12-en-28-oic acid, 3-oxo-, methyl ester	16.462	1.31
4H-Benzo[def]carbazole, 4-methyl-	16.696	0.19
Quinazolin-4(1H)-one, 2,3-dihydro-2-ethyl-3-(4-methoxyphenyl)-2-methyl-	16.738	0.12
Salicylaldehyde hydrazone	17.317	0.15
1-cyclohexene-1-carboxylic acid, 3,4-dihydro-2,2-dimethyl-4-(1-methylethyl)-2H-1-benzopyran-6-yl ester	17.791	0.22
Terephthalic acid, piperidide, pentyl ester	18.365	0.13
1-Azacyclotridecane, N-(9-borabicyclo[3.3.1]non-9-yl)-	18.455	0.3
Silane, triethoxypentyl-	19.655	0.33
2-Phenazinecarbonitrile, 7-amino-	19.703	0.17
1-methylpentacyclo[6.6.6.0(2,7).0(9,14).0(15,20)]icosane	20.71	0.1
Stigmasterol	20.783	0.36
.gamma.-Sitosterol	21.22	0.46
3-Fluoro-5-trifluoromethylbenzoic acid, 2-nitro-5-fluorophenyl ester	21.299	0.13
Stigmastanol	21.324	0.23
Olean-12-en-28-oic acid, 3-oxo-, methyl ester	22.584	0.48

الخلاصة

أجريت هذه الدراسة في البيت الحيواني التابع لقسم علوم الحياة في كلية العلوم / جامعة ميسان باختيار 104 فار من ذكور الفئران البيضاء في الفترة من 10/2/2021 إلى 14/8/2021 , 24 فار استخدمت لقياس نصف الجرعة المميتة للمستخلص النباتي بالاضافة لذلك 80 منها قسمت عشوائيا إلى ٤ مجموعات، كل مجموعة تتكون من 20 فأر (تم قتل 18 منهم وترك 2 للتزاوج). كان الهدف تقييم تأثير التجريع الفموي لعقار الهيدروكسي كلوروكوين والمستخلص المائي لنبات الشيح على الهرمونات التناسلية وهرمونات الغدة الدرقية بالاضافة الى تاثيرهما على بعض المعايير الكيموحيوية ومنها اختبار وظائف الكبد و الكلى وقياس قيمة بروتين C التفاعلي ، وكذلك تقييم تأثيرهما على وزن الجسم وأوزان الأعضاء ومعايير الخصوبة.

قسمت الدراسة الى مجاميع حسب نوع المادة التي تم تجريعها:

١. المجموعة الضابطة(مجموعة A) التي جرعت 0.2مل من المحلول الملحي العادي فمويا
٢. مجموعة هيدروكسي كلوروكوين(مجموعة B) والتي جرعت فمويا 0.2 مل من هيدروكسي كلوروكوين مرتين يومياً بتركيز400 مجم / كجم لليوم الأول و200 مجم / كجم لبقية فترة العلاج.
٣. مجموعة HCQ + مستخلص الشيح(مجموعة C) والتي جرعت فمويا 0.2 مل من هيدروكسي كلوروكوين في الصباح و 0.2 مل من مستخلص الشيح في المساء.
٤. مجموعة مستخلص الشيح(مجموعة D) والتي جرعت فمويا 0.2 مل من المستخلص بتركيز 8000 مجم / كجم جرعتين يومياً.

اظهرت نتائج الدراسة الحالية مايلي :

بحسب المجموعات:

١. انخفاض معنوي (P <0.05) في تركيز الهرمون المنبه للجريب (FSH) والتستوستيرون في مجموعة B وزيادة معنوية في تركيزه في مجموعة C . لوحظ أيضًا زيادة معنوية (P <0.05) في تركيز الهرمون اللوتيني (LH) في مجموعة B وانخفاض معنوي (P <0.05) في تركيزه في مجموعة C .
٢. لم يكن هناك تغير معنوي (P> 0.05) في تركيز TSH في جميع المجموعات المعالجة ، وزيادة معنوية (P <0.05) في T3 وانخفاض معنوي في T4 في مجموعة B ، وانخفاض معنوي (P <0.05) في تركيز T3 و T4 في مجموعتي C و D .

٣. زيادة معنوية (P <0.05) في AST و ALT و ALP في المجموعة المعالجة B ، كما كانت هناك زيادة معنوية (P <0.05) في AST و ALP في المجموعة المعالجة C و D

٤. زيادة معنوية (P <0.05) في تركيز اليوريا في مجموعة B وانخفاض معنوي (P <0.05) في اليوريا لمجموعة C ، ولم يكن هناك تغير معنوي (P> 0.05) في تركيز الكرياتين في المجموعات المعالجة مقارنة لمجموعة السيطرة

٥. لم يكن هناك تغير معنوي (P> 0.05) في قيمة بروتين C التفاعلي في جميع المجموعات العلاجية.

حسب الفترات:

١- لم يكن هناك تغير معنوي (P> 0.05) في FSH في جميع المجموعات العلاجية بعد 6 ،12 يوم ، بينما كان هناك انخفاض معنوي (P <0.05) في قيمته في جميع المجموعات العلاجية بعد 18 يومًا مقارنة بمجموعة التحكم. انخفاض معنوي (P <0.05) في LH في مجموعتي C و D بعد 6 و 12 و18 يومًا، بينما لم يكن هناك تغير معنوي (P> 0.05) في قيمة LH في مجموعة B في كل الفترات. انخفاض معنوي (P <0.05) في تركيز هرمون التستوستيرون في مجموعة B بعد 6 و12 و18 يوم . بينما لم يكن هناك فرق معنوي (P> 0.05) في قيمته في المجموعات الأخرى في جميع الفترات مقارنة مع مجموعة التحكم

٢- لم يكن هناك تغير معنوي (P> 0.05) في هرمون TSH بعد 6 و 12 و 18 يوم في جميع المجموعات العلاجية. انخفاض معنوي (P <0.05) في تركيز T3 في مجموعة C و D بعد 6 و 12 يوم ، بينما كان هناك انخفاض معنوي (P <0.05) في تركيز T3 في مجموعة B بعد 12 و 18 يوم، وانخفاض معنوي (P <0.05) في تركيز T4 في مجموعات B وC بعد 6 و18 يوم وكذلك انخفاض معنوي (P> 0.05) في تركيز T4 في مجموعة D في جميع الفترات.

٣- زيادة معنوية (P <0.05) في قيم AST في كل مجاميع المعالجة في جميع الفترات مقارنة بمجموعة التحكم ، وزيادة معنوية (P <0.05) في تركيز ALT في مجموعة B في جميع الفترات وكان هناك زيادة معنوية (P <0.05) في ALT في مجموعتي B وC بعد 18 يوم مقارنة بمجموعة السيطرة . زيادة معنوية (P <0.05) في ALP في مجموعات B وC في جميع الفترات مقارنة بمجموعة السيطرة ، وزادت قيمها في مجموعة D مقارنة بمجموعة السيطرة بعد 6 و18 يوم من التجريع .

٤- زيادة معنوية (P <0.05) في تركيز اليوريا في مجموعة B وانخفاض معنوي (P <0.05) في اليوريا في مجموعة C في جميع الفترات وزيادة معنوية (P <0.05) في تركيزها في مجموعة D بعد 18 يوم بينما لم يكن هناك تغير معنوي (P> 0.05) في قيمة الكرياتينين في الدم في جميع مجموعات العلاج مقارنة بمجموعة السيطرة ولكل الفترات.

٥- لم يكن هناك تغير معنوي (P> 0.05) في بروتين سي التفاعلي في جميع المجموعات المعالجة ولكل الفترات مقارنة بمجموعة السيطرة.

تاثير التجريع على وزن الجسم، اوزان الاعضاء والخصوبة تضمن التالي :

١- انخفاض معنوي (P <0.05) في وزن الجسم في مجموعة B وزيادة معنوية (P <0.05) في وزن الجسم في مجموعة C .

٢- في المجموعة B لوحظ انخفاض معنوي (P <0.05) في وزن القلب بعد 12و 18 يوم من التجريع ونقص في وزن الكبد والامعاء بعد 6 و12 يوم من المعالجة ولم يكن هناك تغيرمعنوي (P> 0.05) في وزن المعدة مقارنة بمجموعة السيطرة ، انخفاض معنوي (P <0.05) في وزن الطحال بعد 12 يوم وزيادة معنوية (P <0.05) في وزن الكلى بعد 6 أيام وانخفض معنويا (P <0.05) في وزنها بعد 18 يوم . في المجموعة C لم يكن هناك تغير معنوي (P> 0.05) في أوزان القلب والكبد والأمعاء والمعدة والطحال والكلى. أما المجموعة D فقد أظهرت انخفاضاً معنوياً (P <0.05) في وزن القلب بعد 12 يوم وانخفاض في وزن الكبد والامعاء بعد 18 يوم ، ولم تظهر هذه المجموعة تغير(P> 0.05)معنوي في وزن المعدة ، الطحال و الكلى.

٣- انخفاض معنوي (P <0.05) في عدد الحيوانات المنوية في المجموعات المعالجة مقارنة بمجموعة السيطرة ، وكذلك انخفاض معنوي (P <0.05) في معدلات الخصوبة والحمل في مجموعة B ، عدم وجود اختلاف معنوي (P >0.05) في عدد الولادات في جميع المجموعات المعالجة وانخفاض في أوزان المواليد في مجموعة B .

وزارة التعليم العالي والبحث العلمي

جامعة ميسان/ كلية العلوم

قسم علوم الحياة

دراسة تاثيرعقار هيدروكسي كلوروكوين ومستخلص الشيح *Artemisia herba alba* في الهرمونات الجنسية وهرمونات الدرقية وبعض المعايير الكيموحيوية في ذكور الفئران المختبرية

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

ريام عباس عبد الدراجي

بكالوريوس علوم /علوم الحياة (٢٠١٨)

بأشراف

أ.د زينب عبد الجبار رضا

آذار٢٠٢٢ م

شعبان١٤٤٣هـ