Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Science
Department of Biotechnology

Phytochemical investigation and Evaluation of the biological effect of *Costus* and *Cyperus* secondary metabolites on fertility in male mice

A Thesis

Submitted to the College Board of Science / AL-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology.

By

Wid Ibraheem Kadm

B.Sc. Biotechnology/ College of Science/ Al-Nahrain University, 2014

Supervised by

Prof. Dr. Khulood W. Abood

July 2017 Shawal 1438

Acknowledgements

First of all praise to Allah the lord of the universe, peace be upon Mohammed the messenger of Allah and upon his relatives and companions.

Great thanks to my supervisor professor Dr. Khulood Waheeb Abood for her support and important guidance throughout my study.

I would like to extend my thankful, respect and appreciation to Dr. Jasem Alithawi who had given me a hand, support, and important guidance throughout this study.

I am very thankful for the staff of the Department of Biotechnology at Al-Nahrain University for their appreciable help.

I would gratefully like to express my gratitude and feelings of love to my mother, father, brothers, sisters, friends and my fiancé for their encouragement, patience and the many sacrifices they presented during all stages of my study.

For all of them, I said 'thank you all"

Wid.

Summary

The present study was designed to detect the effect of *Saussurea lappa* and *Cyperus esculentus* extracts on male albino mice fertility in addition to the histopathological changes in liver, kidney and testes. *Saussurea lappa* extract was prepared by maceration of 50 gm of *Saussurea lappa* roots with 90% methanol, then chemical detection of flavonoids, alkaloids, tannins, saponins, terpenes and steroids was carried out. The use of high performance liquid chromatography technique helped in detection of querciten, rutin, kaempferol, Myricetin and Luteolin. The same procedure was also used for the extraction and chemical detection of *C. esculentus* tubers.

The effect of *S. lappa* and *C. esculentus* methanolic extracts on the sperm including sperms concentration, percentage of viable sperms, percentage of morphologically abnormal sperms and an assay of serum testosterone were studied. Then the histopathological sections of liver, kidney and testis were examined.

Forty mice were divided equally into eight groups, Group1 (negative control): The mice were treated with water, Group2 (positive control): mice were treated with 0.36 mg/kg Mesterolone (Proviron), Group3: mice were treated with 8.3 mg/ ml of *S.lappa* extract (200mg/kg), Group4: mice were treated with 12 mg/ ml of *S.lappa* extract (400mg/kg), Group5: mice were treated with 13.56 mg/ ml of *S.lappa* extract (600mg/kg), Group6: mice were treated with 7 mg/ ml of *C.esculents* extract (200mg/kg), Group7: mice were treated with 14.8 mg/ ml of *C.esculents* extract (400mg/kg),Group8: mice were treated with 23.4 mg/ ml of *C.esculents* extract (600mg/kg). The extracts were administered orally for 3 weeks.

The results showed a significant increase ($p \leq 0.01$) in sperms concentration after three weeks of treatment with the *S. lappa* extract at doses 200 and 400 mg/kg when compared with controls and also when compared with other groups treated with extract at doses 600 mg/kg and significant increase ($p \leq 0.01$) in sperms concentration after 3 weeks of treatment with the *C. esculentus* extract at doses 200, 400 and 600 mg/kg when compared with controls.

A significant increase ($p \leq 0.01$) in dead sperms and percentage of morphologically abnormal sperms was observed after treatment with *S. lappa* extract at doses 600 mg/kg when compared with controls and with other treatments, (extract doses of 200 and 400mg/kg) and a significant decrease ($p \leq 0.01$) in dead sperms and percentage of morphologically abnormal sperms was observed after treatment with with *C. esculentus* extract at doses (200, 400 and 600) mg/kg when compared with controls.

A significant increase ($p \leq 0.01$) in Serum testosterone was observed in mice treated with 200 and 400 mg/kg when compared with controls and with another group treated with *S. lappa* extract at dose of 600 mg/kg and the same result was observed for the groups treated with *C. esculentus* extract.

The histopathological study of testis, liver and kidney in controls showed no significant changes and there is no variation in liver and kidney of mice treated with *S. lappa* extract at dose 200 mg/kg and mice treated with *C. esculentus* extract at doses 200, 400 and 600 mg/kg . The testis sections of mice treated with *S. lappa* extract at dose 200 mg/kg and testis sections of mice treated with *C. esculentus* at doses 200, 400 and 600 mg/kg clarify the normal maturation of spermatogonia and the presence of numerous sperms inside the lumen.

The dose 400 mg/kg of *S. lappa* extract led to the presence of irregular dilatation of sinusoids, fragmentation of nuclear chromatin of hepatocyte cells with rare apoptotic cells and mild degenerative changes of renal epithelial cells with congestion. Mice treated with *S. lappa* extract at dose 600 mg/kg showed sever congestion with irregular dilatation of sinusoids in addition to few inflammatory cells inside the sinusoids with dispersed necrotic cells in liver section and rare epithelial cells of renal tubules, mild degenerative changes in kidney section, were observed..

The testis section of mice treated with *S. lappa* extract at dose 600 mg/kg showed that some of seminiferous tubules showing immaturity of spermatogonia cells and no sperm inside the lumen, while few seminiferous tubules showing maturation of spermatogonia cells with sperms inside the lumen, and the testis section of mice treated with *C. esculentus* extract at dose 600 mg/kg showed normal maturation of spermatogonia cells with presence of few sperms inside the lumen and shrinkage of lyedig cell which are present in between seminiferous tubules.

These results may suggest that high doses of *S.lappa* may have negative effect on liver, kidney and testis and the high doses of *C. esculentus* have negative effect on testis.

List of contents

No.	*Title*	*Page No.*
	Chapter One Introduction and Literature review	
1.1	**Introduction**	1
1.2	**Literature Review**	4
1.2.1	***Saussurea lappa***	4
1.2.1.1	**Common Names and Taxonomy**	4
1.2.1.2	**Plant Distribution**	5
1.2.1.3	**Plant Description**	5
1.2.1.4	**Chemical Constituent**	6
1.2.1.5	**Folkloric Medicinal Uses**	6
1.2.1.6	**Biological Potential and Pharmaceutical Application *S. lappa*.**	8
1.2.1.6. A	**Anti-inflammatory Activity**	8
1.2.1.6. B	**Anti-Spasmolytic Activity**	9
1.2.2	***Cyperus esculentus***	9
1.2.2.1	**Common Names and Taxonomy**	11
1.2.2.2	**Plant Distribution**	11
1.2.2.3	**Plant Description**	12

1.2.2.4	**Chemical Constituent of *Cyperus esculentus***	13
1.2.2.5	**Folkloric Medicinal Uses**	13
1.2.2.6	**Biological Potentials and Pharmaceutical Applications**	14
1.3	**The Reproductive System of male Mouse**	16
1.3.1	**Spermatogenesis**	18
1.3.2	**Testosterone**	20
1.3.3	**How is Testosterone Controlled**	21
1.3.4	**Semen Analysis**	23
1.3.5	**Male Infertility**	24
1.4	**Antioxidants**	25
1.4.1	**Antioxidant Activity of *saussurea lappa***	27
1.4.2	**Antioxidant Activity of *Cyperus esculentus***	28
Chapter Two Materials and Methods		
2	**Materials**	29
2.1	**Equipments and Instruments**	29
2.2	**Chemicals and Reagents**	30
2.3	**Kit**	31

2.4	**Solutions Preparation**	31
2.4.1	**Phosphate Buffer Saline (PBS)**	31
2.4.2	**Ferric Chloride Solution (1%)**	31
2.4.3	**Potassium Hydroxide Solution**	32
2.4.4	**Haematoxylin Stain**	32
2.4.5	**Eosin Stain**	32
2.4.6	**Mayer's Reagent**	32
2.4.7	**Mesterolone (Proviron) dose preparation**	32
2.5	**Methods**	32
2.5.1	**Plants Collection**	33
2.5.2	**Plants Extraction**	33
2.5.3	**Measurement of the Extract Acidity**	33
2.6	**Detection of Some Active Compounds in Plants Extracts**	35
2.6.1	**Chemical Detection of Plants Extracts**	35
2.6.1.1	**Detection of Terpenes and Steroid**	35
2.6.1.2	**Detection of Flavonoids**	35
2.6.1.3	**Detection of Resins**	35
2.6.1.4	**Detection of Tannins**	35
2.6.1.5	**Detection of Alkaloids**	35

2.6.1.6	**Detection of Saponins**	36
2.6.2	**Detection of Flavonoids Compound by HPLC**	36
2.7	**Laboratory Animals**	37
2.8	**Experimental Design**	37
2.9	**Collection of Blood Samples and Determination of Testosterone levels**	38
2.10	**Semen Preparation**	38
2.10.1	**Sperm viability and morphology**	38
2.10.2	**Sperm Concentration**	39
2.11	**Histopathological Study**	40
2.12	**Statistical Analysis**	41
Chapter Three **Results and Discussion**		
3.1	**Plants Extracts**	42
3.1.1	**Methanolic Extract of *S. lappa***	42
3.1.2	**Methanolic Extract of *C.esculentus***	42
3.1.3	**Chemical Detection of *S. lappa* Active Compound**	42
3.1.4	**Chemical Detection of *C. esculentus* Active Compound**	43
3.1.5	**HPLC Analysis for *S. lappa***	44
3.1.6	**HPLC Analysis of *C. esculentus***	46
3.2	**Testosterone assay**	47

3.3	**Sperms Concentration**	50
3.4	**Morphologically Abnormal Sperms**	53
3.5	**Sperms Viability**	56
3.6	**Histopathological Evaluation of Liver, Kidney and Testes**	60
Conclusions		79
Recommendations		80
References		81

List of Tables

No. of table	***Title of table***	***Page No.***
Table (1-1)	**Hormones released by Testis**	**22**
Table (2-1)	**The equipments and instruments**	**29**
Table (2-2)	**The chemicals and reagents**	**30**
Table (2-3)	**Testosterone kit**	**31**
Table (3-1)	**Chemical detection of some active compounds in *S.lappa* methanolic extract.**	**43**
Table (3-2)	**Chemical detection of some active compounds in *C.esculentus* methanolic extract.**	**44**
Table (3-3)	**Effect of S. lappa methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on serum testosterone concentration in mice .**	**47**
Table (3-4)	**Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on serum testosterone concentration in mice .**	**49**
Table (3_5)	**Effect of *S.lappa* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on sperms concentration in mice .**	**51**
Table (3_6)	**Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with C esculentus on sperms concentration in mice .**	**52**
Table (3-7)	**Effect of *S.lappa* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on percentage of morphologically abnormal sperms in mice .**	**53**
Table (3_8)	**Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on percentage of morphologically abnormal sperms in mice.**	**56**
Table (3_9)	**Effect of *S.lappa* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on percentage of sperms viability in mice .**	**57**
Table (3_10)	**Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on percentage of sperms viability in mice.**	**58**

List of Figures

No. of figure	*Title of figure*	*Page No.*
Figure (1-1)	**Dried roots of *Saussurea lappa***	**6**
Figure (1-2)	**Dried tubers of *Cyperus esculentus***	**13**
Figure (1-3)	**Reproductive system of male mouse**	**17**
Figure(1-4)	**Schematic representation of the process of spermatogenesis**	**19**
Figure (1-5)	**Steroid hormones derived from cholesterol**	**21**
Figure (1-6)	**Regulation of male androgens (Sex hormones)**	**23**
Figure (1-7)	**Factors contributing to oxidative stress-induced male infertility**	**25**
figure (2-1)	**Main steps of the research plan**	**34**
Figure (3-1)	**HPLC analysis of the *S.lappa* dried roots methanolic extract**	**45**
Figure (3-2)	**HPLC analysis for flavonoid standard.**	**45**
Figure (3-3)	**HPLC analysis of the *C. esculentus* dried tubers methanolic extract.**	**46**
Figure (3-4)	**Level of testosterone in different group of mice treated with *S. lappa* extract**	**48**
Figure (3-5)	**Level of testosterone in different group of mice treated with *C. esculentus* extract**	**49**
Figure (3-6)	**Sperm count in male mice treated with methanolic extract of S. lappa for 3 weeks**	**51**
Figure (3-7)	**Sperm count in male mice treated with methanolic extract of *C. esculentus* for 3 weeks**	**52**
Figure (3-8)	**Morphologically abnormal sperms in male mice treated with methanolic extract of *S. lappa* for 3weeks**	**54**
Figure (3-9)	**Effect of *S. lappa* extract at dose 600mg/kg on sperm morphology in mice**	**54**
Figure (3-10)	**Morphologically abnormal sperms in male mice treated**	**55**

	with methanolic extract of *C. esculentus* for 3weeks	
Figure (3-11)	**Sperm viability in male mice treated with methanolic extract of S. lappa for 3 weeks**	**58**
Figure (3-12)	**Sperm viability in male mice treated with methanolic extract of *C. esculentus* for 3 weeks**	**59**
Figure (3-13)	**Liver Section of Mice Treated with (water)**	**61**
Figure (3-14)	**Kidney Section of Mice Treated with (water)**	**61**
Figure (3-15)	**Testis Section of Mice Treated with (water)**	**62**
Figure (3-16)	**Liver Section of Mice Treated with (proviron)**	**63**
Figure (3-17)	**Kidney Section of Mice Treated with (proviron)**	**63**
Figure (3-18)	**Testis Section of Mice Treated with (proviron)**	**64**
Figure (3-19)	**Liver section of mice treated with *S. lappa* extract at dose 200 mg/kg**	**65**
Figure (3-20)	**Kidney section of mice treated with *S. lappa* extract at dose 200 mg/kg**	**65**
Figure (3-21)	**Testis section of mice treated S. *lappa* extract at dose 200 mg/kg**	**66**
Figure (3-22)	**Liver section of mice treated with S. lappa extract at dose 400 mg/kg**	**67**
Figure (3-23)	**Kidney section of mice treated with S. lappa extract at dose 400 mg/kg**	**67**
Figure (3-24)	**Testis section of mice treated with S. lappa extract at dose 400 mg/kg**	**68**
Figure (3-25)	**Liver section of mice treated with S. lappa extract at dose 600 mg/kg**	**69**
Figure (3-26)	**Kidney section of mice treated with S. lappa extract at dose 600 mg/kg**	**69**
Figure(3_27)	**Testis section of mice treated *S. lappa* extract at dose 600 mg/kg**	**70**
Figure (3-28)	**Liver section of mice treated with *C. esculentus* extract at dose 200 mg/kg**	**71**
Figure (3-29)	**Kidney section of mice treated with *C. esculentus* extract at dose 200 mg/kg**	**71**
Figure (3-30)	**Testis section of mice treated with *C. esculentus* extract at dose 200 mg/kg**	**72**
Figure (3-31)	**Liver section of mice treated with *C. esculentus* extract at dose 400 mg/kg**	**73**

Figure (3-32)	**Kidney section of mice treated with *C. esculentus* extract at dose 400 mg/kg**	**73**
Figure (3-33)	**Testis section of mice treated with *C. esculentus* extract at dose 400 mg/kg**	**74**
Figure (3-34)	**Liver section of mice treated with *C. esculentus* extract at dose 600 mg/kg**	**75**
Figure (3-35)	**Kidney section of mice treated with *C. esculentus* extract at dose 600 mg/kg look**	**75**
Figure (3-36)	**Testis section of mice treated with *C. esculentus* extract at dose 600 mg/kg**	**76**

List of abbreviations

Abbreviations	Full name
ElISA	**Enzyme-linked immunosorbent assay**
FSH	**Follicle Stimulating Hormone**
GnRH	**Gonadotropin Releasing Hormone**
HPLC	**High performance liquid chematography**
kg	**kilogram**
LH	**Luteinizing Hormone**
mg	**milligram**
S. lappa	***Saussaria lappa***
C. esculentus	***Cyperus esculentus***
PBS	**Phosphate buffered saline**
ROS	**Reactive oxygen species**
rpm	**Rotation per minute**
WHO	**World Health Organization**
PH	**Power of Hydrogen**
RBC	**Red blood cells**
FAO	**Food and Agriculture Organization**

Chapter one

Introduction and Literature Review

Chapter one

Introduction and literature review

1.1. Introduction

Herbal medicine is a growing area of health care that demands attention. Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species (Srivastav *et al*., 2011). According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care (Vijayan *et al*., 2007).

Chemical components of the medicinal plant are the most important for pharmaceutical companies. People are interested in medicines prepared from plants due to their little side effects, cheap and almost available compared with synthetic drugs. This may be because of the low concentrations of the active compounds found in plants which the human body would need (Mackin, 1993).

Herbal medicine is sometimes referred to as herbalism or botanical medicine for the use of herbs in therapy or medicinal uses (Barens, 2002). Arokiyaraj *et al*. (2007) mentioned that a large number of these plants, plant extracts, plant derivatives and/or their isolated constituents have shown beneficial biological effects: including immunomodulatory, anti-oxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic and anti-microbial effects.

Plant secondary metabolites have different structures that correlate with different chemical terminologies (flavonoids, alkaloids, tannins, glycosides and others), and therefore they may have different functions with regard to their biological potentials (XD *et al*., 2008).

Medicinal plants and herbal medicine are one of the current areas of investigation in which various drugs have been identified which affect fertility, both in a positive and a negative sense but some of which have side effects that are undesirable (Delaszlo and Henshaw, 1954; Ahmad *et al*., 1998).

Saussurea lappa is one of the most commercially viable species among all the species of genus *Saussurea*. It is widely utilized in various indigenous system of medicine all around the world for treating variety of disorders such as tenesmus, diarrhea, vomiting, dyspepsia, inflammation (Xiao *et al*., 2006; Irshad *et al*., 2012). Chauhan *et al*. (2014) reported that *Saussurea lappa* is one of the aphrodisiac plants which purify and improve the quality of semen.

Cyperus esculentus is a perennial plant species that belongs to the Cyperaceae family and grows abundantly in the Mediterranean region (Pascual *et al*., 2000; Ezeh *et al*., 2014).*C. esculentus* has been reported to be high in dietary fiber content (Alegrıa-Toran and Farre-Rovira, 2003).which could be effective in the treatment and prevention of many diseases including colon cancer (Adejuyitan *et al*., 2009),coronary heart disease (Chukwuma *et al*., 2010) ,obesity, diabetes, gastrointestinal disorders (Anderson *et al*., 2009),and in loosing weight (Borges *et al*., 2008). *C. esculentus* tubers can be used for their aphrodisiac properties(Caius, 1998).

Al-Shaikh *et al*. (2013) reported protective effects of *C. esculentus* on testicular weight and spermatogenesis process in mice treated with lead acetate. They speculated that these effects could be due to either the antioxidant ability of *C. esculentus* or its positive influence on sex hormones.

The Aims of This Study

1.Extraction and identification of classes of secondary metabolites in *saussurea lappa* and *cyperus esculentus.*

2. Evaluate the effect of extracted secondary metabolites of *saussurea lappa and cyperus esculentus* on fertility using sexual hormones.

3.Histopathological study on the effect of *saussurea lappa* and *cyperus esculentus* secondary metabolites on liver, kidney and testis of treated mice.

1.2. Literature Review

1.2.1. *Saussurea lappa*

Saussurea lappa has been used as medicines for thousands of years (Suffredini *et al*., 1999). Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of disease. Compounds such as carbohydrates, proteins, enzymes, fats, oils, terpenoids, flavonoids, sterols simple phenolic compounds are responsible for various pharmacological activites of plants. (Lewis and Elvin, 1997; Prabakaran *et al*., 2011).

1.2.1.1. Common Names and Taxonomy

The Asteraceae family comprises approximately 1000 genera and 30,000 species, distributed more or less throughout the globe, of which approximately 177 genera and 1052 species are found in India (Rao *et al*., 1988). The genus *Saussurea* of the same family comprises about 300 species in the world (Bremer, 1994). *Saussurea lappa*, one of the best-known species within this genus, is commonly known as costus in English and has different vernacular names in India like, Kut (Gujrati), Kur (Bengali), Koshta (Kannada) and Kuth (Hindi) (Kirtikar and Basu, 2001).

Scientific Classification

- Kingdom: Plantae
- Sub-group: Phanerogamae
- Division: Angiosperms
- Class: Dicotyledon
- Sub-Class: Gamopetalae
- Order: Asterales
- Family: Asteraceae (Compositae)

• Tribe: Cynareae

• Genus: *Saussurea*

• Species: *lappa* (Chadha, 1972).

1.2.1.2. Plant Distribution

Saussurea lappa distributed between 2500- 3000 m altitude in the Himalaya, and native to the Himalayan region. Its natural populations are reported from the higher elevations of Jammu and Kashmir and Himachal Pradesh and now cultivated in Kashmir, Himachal Pradesh and in some part of Uttarakhand (Aswal and Mehrotra, 1994).

1.2.1.3. Plant Description

S. lappa commonly known as Costus which is a tall, perennial herb that grows to a height of 1-2 m; stem is upright, stout and fibrous while root is a long stout of approximately 60 cm with a characteristic odour; leaves are lobate, stalked, membranous, irregularly toothed; upper leaves are small while basal leaves are large with long lobately winged stalks. Flowers are stalkless, dark purple to black in colour and are arranged in terminal and axillary heads. Pappas is approximately 1.7 cm long, fluffy, feathery giving an inquisitive appearance to the fruiting flower heads. Fruit of *S. lappa* is cupped, curved, compressed and hairy (Pandey *et al.*, 2007).

Figure (1-1): Dried roots of *Saussurea lappa.*

1.2.1.4. Chemical Constituent

Studies on the chemical ingredients of *S. lappa* could be traced back to 1950s, and many compounds have been found until now. Its active ingredients are mainly terpenes, but it also contains alkaloids, anthraquinones, and flavonoids. *S. lappa* has many diverse terpenes that mainly have anti-inflammatory and antitumor properties, such as costunolide, dihydrocostunolide, dihydrocostus lactone, dehydrocostus lactone, it is also contained resins and tannins (Yang *et al*., 1998).

1.2.1.5. Folkloric Medicinal Uses

S. lappa is one of the most commercially viable species among all the species of genus Saussurea. It is widely utilized in various indigenous system of medicine all around the world for treating variety of disorders such as tenesmus, diarrhea, vomiting, dyspepsia, inflammation (Irshad *et al*., 2012; Xiao *et al*., 2006). It is also prescribed in irregular menstruation and abdominal pain (Ko *et al*., 2005; Sarin *et al.,* 1967).

Medicinal properties of this plant are well documented in traditional Chinese medicine, ayurvedic medicine and the Tibetan system of medicine (Singh, 1999). In the Handbook of Traditional Tibetan Drugs, out of the 175 formulations it was reported that *S. lappa* was one of the main ingredients in 71 formulations (Tsarong, 1986).

The roots of *S. lappa* have a strong and sweet aromatic odour with a bitter taste, and are used in controlling bronchial asthma and as an antiseptic. Preparations made from this species are also reported to cure various diseases and conditions including ophthalmic conditions, fever, cough, paralysis, deaf, hysteria and headache (Jain, 1984; Basu and Kirtikar, 1987).

In Indian traditional systems of medicine, *S. lappa* is used either in combination with other drugs or as a single drug. The essential oil obtained from roots is mostly used in medicine (Indian Drug Manufactures' Association, 2002). Different preparations of roots are used by Ayurvedic physicians for the treatment of a range of ailments like flatulence, pruritus, gout, epilepsy, itching and leukoderma (Koul, 1941). *S. lappa* is used as an important medicine for promotes spermatogenesis.

The roots possess carminative, anthelmintic, analgesic and emmenagogic properties. It stimulates the brain and cures blood, liver and kidney disorders. Different preparations of *S. lappa* are prescribed in helminthic infestations, convulsions, gas, leprosy, persistant hiccups, tuberculosis, quartan malaria, rheumatism, intestinal carcinogenesis and edema (Lee *et al.*, 2001; Malik *et al.*, 2011). It is also used traditionally in treatment of chronic skin diseases such as scabies, ringworm, bruises and cuts (Council of Scientific and Industrial Research, 1972; Shah, 1982).

1.2.1.6. Biological Potential and Pharmaceutical Application *S. lappa*.

S. lappa is a medicinally important plant. Various active compounds isolated from plant are reported to have medicinal properties e.g. the major components are sesquiterpene lactones such as costunolide and dehydrocostus lactone. *S. lappa* possesses various bioactivities such as antifungal, antidiabetic, anthelmintic, antitumor (Ko *et al*., 2005), antiulcer, antimicrobial, immune stimulant (Hamilton, 2004). anti-inflammatory and antihepatotoxic (Yashvanth *et al*., 2010).

A. Anti-inflammatory Activity

In Korean traditional prescriptions, *S. lappa* is frequently used for inflammatory diseases. It was observed that at 0.1 mg/ mL concentration of methanol extract of *S. lappa* exhibited more than 50% of inhibition on the cytokine induced neutrophil chemotactic factor induction (Lee *et al*., 1995).

Ethanolic extract of *S. lappa* was studied at a dose range of 50-200 mg/kg, for the acute and chronic inflammation induced in both mice and rats. The result of this study revealed that the extract showed considerable values for anti-inflammatory activity through carrageenan induced paw edema and peritonitis animal models (Gokhale *et al*., 2002). Costunolide, which is on of *S. lappa* component was investigated for anti-inflammatory activity and it was observed that costunolide hindered the protein and mRNA expression of interleukin-1b. By means of an electrophoretic mobility shift assay, it was confirmed that it also concealed the AP-1 transcription activity. So, all these activities proved the anti-inflammatory activity of costunolide (Kang *et al*., 2004).

The potential of dehydrocostus lactone for the oxidative osteoblast damage was investigated and showed considerable increase in the osteoblast growth and hydrogen peroxide. At 0.4-2 µg/mL of dose, the factors for example calcium deposition, collagen and alkaline phosphatase were improved. These results confirmed that dehydrocostus lactone compound had potential to be used against oxidative osteoblast damage (Choi *et al*., 2009). At the doses of 50, 100 and 200 mg/kg, the ethanolic extracts of *S. lappa* were assessed for their action on acute and chronic inflammation and at 50-200 mg/kg it showed considerable inhibition on carrageenan induced paw edema (Gokhale *et al*., 2002).

B. Anti-Spasmolytic Activity

It was proved that *S. lappa* was significantly able to relax the contraction induced by carbachol (30 µmol/ L). *S. lappa* has been reported to have antiperoxidative effects possibly due to the presence of sesquiterpene lactones. It is known to suppress contractions in guniea-pig aorta. Sesquiterpenes are recognized to stimulate the sGC which stimulates extrusion of K^+ ions and thereby reduces intrinsic Ca^{++} ions through activation of cGMP and PKG pathway, leading to relaxation of smooth muscles (Hsu *et al*., 2009).

1.2.2. *Cyperus esculentus*

Is a perennial plant species that belongs to the Cyperaceae family and grows abundantly in the Mediterranean region (Pascual *et al*., 2000; Ezeh *et al*., 2014). Tubers of this plant are considered one of the earliest food sources known to humanity, where they have been documented to be cultivated by ancient Egyptians since 5000 BC (Defelice, 2002; Ezeh *et al*., 2014). These tubers are commonly known by several names such as chufa, earth almond, and tiger nut.

C. esculentus is a potentially valuable food source for humans and animals due to its rich nutritional contents of fat, carbohydrates, and minerals (Tunde-Akintunde and Oke, 2012).

In addition to being a food source, *C. esculentus* tubers have several other purposes. For example, in Spain, they are used in the preparation of a milk-like beverage named “horchata” (Pascual *et al*., 2000). The milk concentrate of the tubers is also used in the manufacturing of some cosmetic products (Forner and Conde, 2010). According to Ayurvedic medicine, *C. esculentus* tubers can be used for their aphrodisiac properties (Caius, 1998). In the Middle East, they are known to the public as “Hab Al-zulom” (Arabic), which translates to “the seeds of men”, owing to their apparent ability to improve male sexual activity; thus, they are frequently given to grooms during their honeymoons as a sexual invigorator. However, there has been no scientific evidence to date on the influence of *C. esculentus* tubers on male sexual behavior.

In a previous study, Al-Shaikh *et al*. (2013) reported protective effects of *C. esculentus* on testicular weight and spermatogenesis process in mice treated with lead acetate. They speculated that these effects could be due to either the antioxidant ability of *C. esculentus* or its positive influence on sex hormones. In addition, it has been claimed that treatment with *C. esculentus* methanolic extract improves sperm count and motility in male rats, which is associated with increased gonadotropins and testosterone serum levels (Agbai and Nwanegwo, 2013).

1.2.2.1. Common Names and Taxonomy

This plant is classified as follows :

• Kingdom: Plantae

• Subkingdom: Viridiplantae.

• Division: Tracheophyta.

• Class: Magnoliopsida.

• Sub-Class: Monocots.

• Order: Poales.

• Family: Cyperaceae.

• Genus: *Cyperus.*

• Species: *Esculentus.* (Gleason, 1963).

1.2.2.2. Plant Distribution

It is a tuber that grow freely and is consumed widely in Nigeria, other parts of west Africa, east Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula (Abaejoh *et al.*, 2006). In many thousand years, ago tiger nut, in Spanish called chufa, was cultivated in region of chufa between Sudan and Egypt on the borders of the Nile River.
There are documents that certify this product over 400 years ago. Proof of this is that on many occasion archeologists found earthen jars containing tiger nut in graves of pharaohs. (Obadina *et al.*, 2008). previously, it was cultivated in the ancient Mesopotamia between the rivers Tigris and Euphrates.

At the same time, historical Persian and Arab documents mentioned the nutritive, digestive and disinfected value of tiger nut. During the era, the tiger nut milk was classified as medicinal drink due to its been highly energetic and diuretic, rich in mineral, predominantly phosphorus and potassium and also vitamins C and E. It was in the 8th century that Arab traders introduced the cultivation of tiger nut in the Mediterranean region of Valencia (Spain), for elaboration of tiger nut milk (leche de chufa), to know the tiger nut cultivation as it arrived to our days. It has been reported that grainy sandy group and mild temperatures are special for the cultivation growth of earth tuber (Abaejoh *et al.*,2006).

1.2.2.3. Plant Description

Erect perennial herb; culms simple with triangular section, growing from perennial, tuber-bearing rhizomes; leaves in three ranks, mostly basal; inflorescence in terminal umbels; umbel subtended by unequal leaf-like brackets varying from 5 to 25 cm long; spikelets yellowish-brown or straw-coloured, 1-3 cm long, of several flowers, flattened, two-ranked; stamens three; style three-cleft; achenes (fruit) three-angled, narrowing gradually from a square-shouldered apex towards the base, about 1.5 mm long, covered with very fine granulation (Holm *et al.*, 1977).It propagates by rhizomes, basal bulbs and tubers.

This light-bright green perennial sedge grows to about 0.8 m in height. A basal bulb is formed by a swelling of the culm below the soil surface and rhizomes grow out from this basal bulb to terminate in new shoots (under long days over 14 hours long) or underground tubers (under shorter days, less than 14 hours).

Figure (1-2): Dried tubers of *Cyperus esculentus* .

1.2.2.4. Chemical Constituent of *Cyperus esculentus*

Chukwuma *et al*. (2010) analysed tigernut tuber for the presence of phytochemicals, it was observed that alkaloids, cyanogenic glycosides, resins, tannins, sterols and saponins were present in the raw tuber, however only alkaloids, sterols and resins were present in the roasted sample. The nut is rich in energy content (starch, fat, sugars and protein), mineral (phosphorus, potassium and vitamins E and C (Belewu and Belewu, 2007). The oil of the tuber was found to contain 18% saturated (palmitic acid and stearic acid) and 82% unsaturated (oleic acid and linoleic acid) fatty acid (Eteshola and Oraed, 1996).

1.2.2.5. Folkloric Medicinal Uses

Since ancient times the chufa tuber has been considered a foodstuff; it was an important food product in ancient Egypt according the references of Teophrastus and Pliny (Serrallach, 1927; Negbi, 1992). According to Zohary and Hopf (1993) there are almost no contemporary records of this plant in other parts of the Old World. In Egypt, the tubers of *Cyperus esculentus* were roasted and used as sweetmeat.

Tubers were also recorded as having medicinal properties (Negbi, 1992). In southern Europe chufa, has been cultivated for several centuries. It seems to have been introduced in Europe during the Middle Ages by the Arabs after their expansion across the north of Africa. There are written records from the 13th century which mention the consumption of a drink made from chufa in the Mediterranean areas of the present-day Valencian community (Spain). This beverage could be considered an ancestor of the horchata drunk nowadays. In northern Nigeria and Ghana, it is made into a sweetmeat, and Togo, where it is used principally uncooked as a side dish (Omode *et al.,* 1995).

1.2.2.6. Biological Potentials and Pharmaceutical Applications

The globoid rhizomes of *C. esculentus* are used by the rural population of Ceara in the northeastern of Brazil as an aphrodisiac and antivenin. They are also used in Brazil for the treatment of measles and fever, but generally as a dessert for its sweet flavor (De Abreu Matos *et al.,* 2008). Tiger nut has been reported to be high in dietary fiber content (Alegrıa-Toran and Farre-Rovira 2003). which could be effective in the treatment and prevention of many diseases including colon cancer (Adejuyitan *et al*., 2009), coronary heart disease (Chukwuma *et al*., 2010). obesity, diabetes, gastrointestinal disorders (Anderson e*t al*., 2009). and in loosing weight (Borges *et al*., 2008).

It has been reported to be used in the treatment of flatulence, indigestion, diarrhea, and dysentery (Bixquert-Jimenez, 2003). and its starch content presumably provides prebiotic properties for colon bacteria (Alegrıa-Toran and Farre-Rovira, 2003).

In addition, tiger nut has been demonstrated to contain higher essential amino acids than those proposed in a protein standard by the FAO/WHO for satisfying adult needs (Bosch *et al*., 2005).
Tiger nut milk has been found to be good for preventing arteriosclerosis, since its consumption can help to prevent heart problems and thrombosis and activate blood circulation (Chukwuma *et a*l., 2010). mainly because its unsaturated fatty acid content is similar to that of olive oil (Linssen *et al.,* 1988). and its arginine is a precursor of nitric oxide which helps the veins to expand (Martınez-Valls, 2003).

Tiger nut milk or “horchata” can be drunk by diabetics for its content in low-glycemic carbohydrates (mainly starch) and due to its arginine, which liberates hormones that produce insulin (Alegrıa-Toran and Farre-Rovira, 2003). It is also a suitable drink for celiac patients, who are not able to tolerate gluten and also for the lactose-intolerant who stay away from cow milk and many dairy foods. It could also be recommended for those who have problems with digestion, flatulence, and diarrhea because it provides some digestive enzymes like catalase, lipase, and amylase (Bixquert-Jimenez, 2003; Adejuyitan, 2011).

Alkaloids, saponins and tannins found in this plant are known to have antimicrobial activity, as well as other physiological activities (Sofowora, 1993; Evans, 2005). Alkaloids are known for their toxicity, they inhibit certain mammalian enzymic activities such as those of phophodiesterase, prolonging the action of cAMP, they also affect glucagons and thyroid stimulating hormones, while some forms have been reported to be carcinogenic (Okaka *et a*l., 1992). Some have been used either as an analgesic, antispasmodic, bactericidal agents (Frantisek, 1991).
Saponins have been reported to be useful in reducing inflammation of upper respiratory passage and also chiefly as foaming and emulsifying agents and detergents (Frantisek, 1991).

Tannins have astringent properties that hasten the healing of wounds and prevention of decay. Tannin compounds have antimicrobial activities and are responsible for preventing and treating urinary tract infections and other bacterial infections.

The result of the determination of phytochemical test indicated that the tuber possess some biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine.

These phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals (Amadi *et al*., 2006).

1.3. The Reproductive System of male Mouse

The Reproductive System of male mouse consists of the primary reproductive organs, the testes and the secondary reproductive organs, which include the scrotum, epididymis, ductus deferens, seminal vesicles, coagulating glands, urethra, prostate glands and bublourethral glands (Seeley *et al*., 1996). As shown in figure (1-3). The testis is a paired oval tubular gland in which the male sex cells, the spermatozoa, develop. They are located in the scrotal sacs which lies just anterior to the anus, on either side of the urethra (Foster *et al*., 1982). This organ is covered by a fibrous connective tissue capsule, the tunica albuginea, from which at the hilus, thin septa projects into the gland and divide it into lobules.

These lobules contain convoluted seminiferous tubules (Bearden and Faquay, 1992; Saladin and Porth, 1998). The walls of these tubules are lined with two types of cells: spermatogenic cells, which give rise to sperm and Sertoli cells. Between the seminiferous tubules are clusters of endocrine cells, called interstitial endocrinocytes or leydig cells, which secrete the male sex hormone, testosterone (Mader, 1993; Miller and Harley, 1996). The seminiferous tubules empty into tubular network called the rete testis. The rete testis empties into three to five tubules

called efferent ductules, these efferent ductules exit the testis into the epididymis. The epididymis is a tightly coiled series of thread like tubule located at the posterior side of the testis and it consists of three parts, the caput epididymis (head), corpus epididymis (body) and the cauda epididymis (tail) (Foster *et al* 1982).

The epididymal functions include transport, as a duct system leading from the testis to the ductus deferens, concentration, storage of spermatozoa until they mature and ready to be ejaculated, maturation in which they acquire motility and fertilizing capacity during passage through the epididymis and loss of cytoplasmic droplet which formed during spermatogenesis (Austin and Short, 1982; Knobit and Neill, 1998; Martini and Mark, 1998).

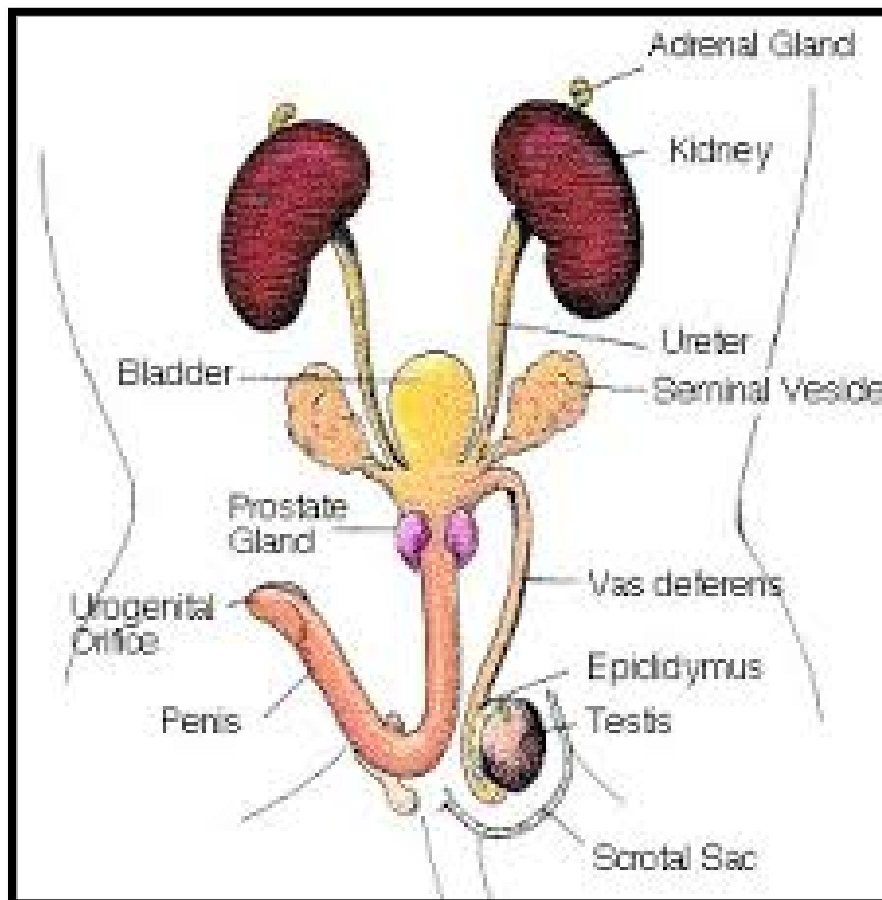


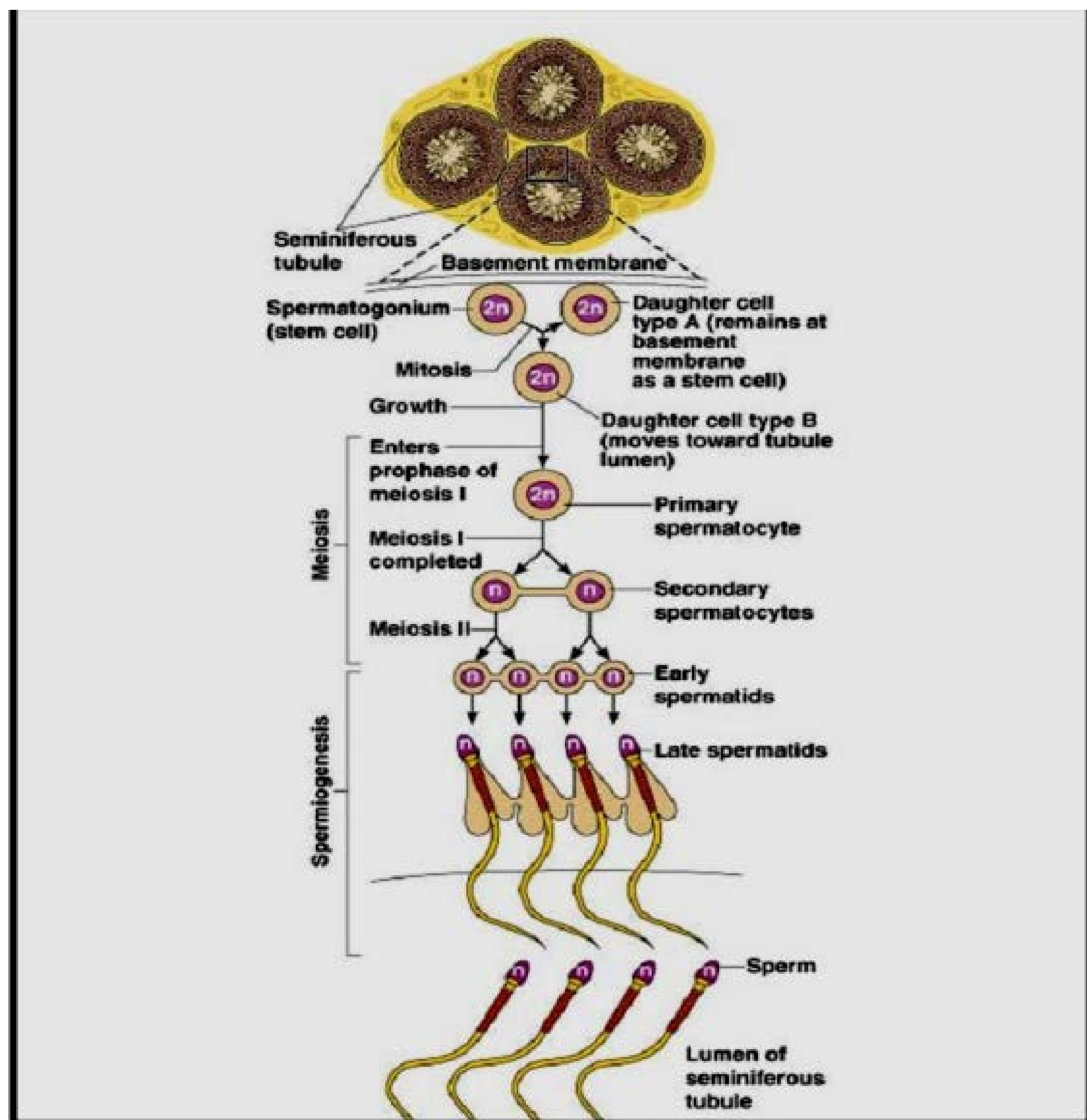
Figure (1-3): Reproductive system of male mouse (John R. 2009)

The ductus deferens emerges from the cauda epididymis and widens into an ampulla before entering the dorsal wall of the urethra near the neck of the bladder. The paired seminal vesicles are white, curved, elongated structures notched on the convex surface and hooked at the lateral tips. Each gland has a wide duct which enters the urethra with the ampulla of the ductus deferens. The paired coagulating glands are less conspicuous than seminal vesicles and are translucent in appearance. The paired ventral prostate glands are pinkish in color having several ducts which empty into the urethra on the ventral wall. A third pair of prostate glands which lie dorsal to the urethra open laterally into the urethra. The paired bulbouretral (Cowpers) gland lie lateral to the junction of the membranous urethra and penis (Foster *et al.*, 1982).

1.3.1. Spermatogenesis

The entire process of sperm formation, beginning with spermatogonia and resulting in mature spermatozoa is referred to as spermatogenesis, this process occurs in the seminiferous tubules of the testis. The seminiferous epithelium lining the seminiferous tubule is composed of two basic cell types: the developing germ cells and the Sertoli cells (Guyton, 1989; Kalthoff, 2001). The germ cells undergo a continuous series of cellular division and developmental changes, beginning at the periphery and progressing toward the lumen of the tubule in which each stage is little closer to the lumen of the tubule than the earlier stage (Saladin and Porth, 1998; Hafez and Hafez, 2000). The stem cells, called spermatogonia, divide through mitosis in which some daughter cells produced from these mitotic divisions remain as spermatogonia and continue to divide by mitosis. Other daughter cells come from primary spermatocytes. These primary spermatocytes undergo meiosis1giving rise to haploid secondary spermatocytes and each of these undergoes meiosis2 to produce spermatids; each stage is a little closer to the lumen of the

tubule than the earlier stage (Dorit *et al*., 1991; Seeley *et al*., 1996; Kalthoff, 2001). The spermatids are then transformed into spermatozoa by a series of morphologic changes collectively known as spermiogenesis as shown in figure (1-4). These changes include condensation of the nuclear chromatin, formation of the sperm tail, and development of the acrosomal cap (Arab *et al*., 1989; Austin and Short, 1982; Hafez and Hafez, 2000). The fully formed spermatozoa are extruded into the lumen of the seminiferous tubules by a process called spermiation (Berne and Levy, 1988).



Figure(1-4):Schematic representation of the process of spermatogenesis (Honaramooz *et al*., 2002).

1.3.2. Testosterone

Testosterone is a steroid hormone that derived from cholesterol molecules (figure 1-5), which is responsible for many of the physical characteristics specific to adult males. It plays a key role in reproduction and the maintenance of bones and muscles strength (Ricki *et al.*, 2007). Testosterone is mainly produced by the gonads (by the Leydig cells in testes in men and by the ovaries in women), although small quantities are also produced by the adrenal glands in both sexes. It is an androgen, meaning that it stimulates the development of male characteristics (Te-Chi Liu *et al.*, 2009). Testosterone is present in much greater levels in men than women, it initiates the development of the male internal and external reproductive organs during fetal development and is essential for the production of sperm in adult life. This hormone also signals the body to make new blood cells, ensures that muscles and bones stay strong during and after puberty and enhances libido in men (Richard *et al.*, 2004). Testosterone is linked to many of the changes seen in boys during puberty (including an increase in height, body and pubic hair growth, enlargement of the penis, testes and prostate gland and changes in sexual and aggressive behavior). It also regulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). For all these changes, testosterone is often converted into another androgen called dihydrotestosterone (Roger, 2004).

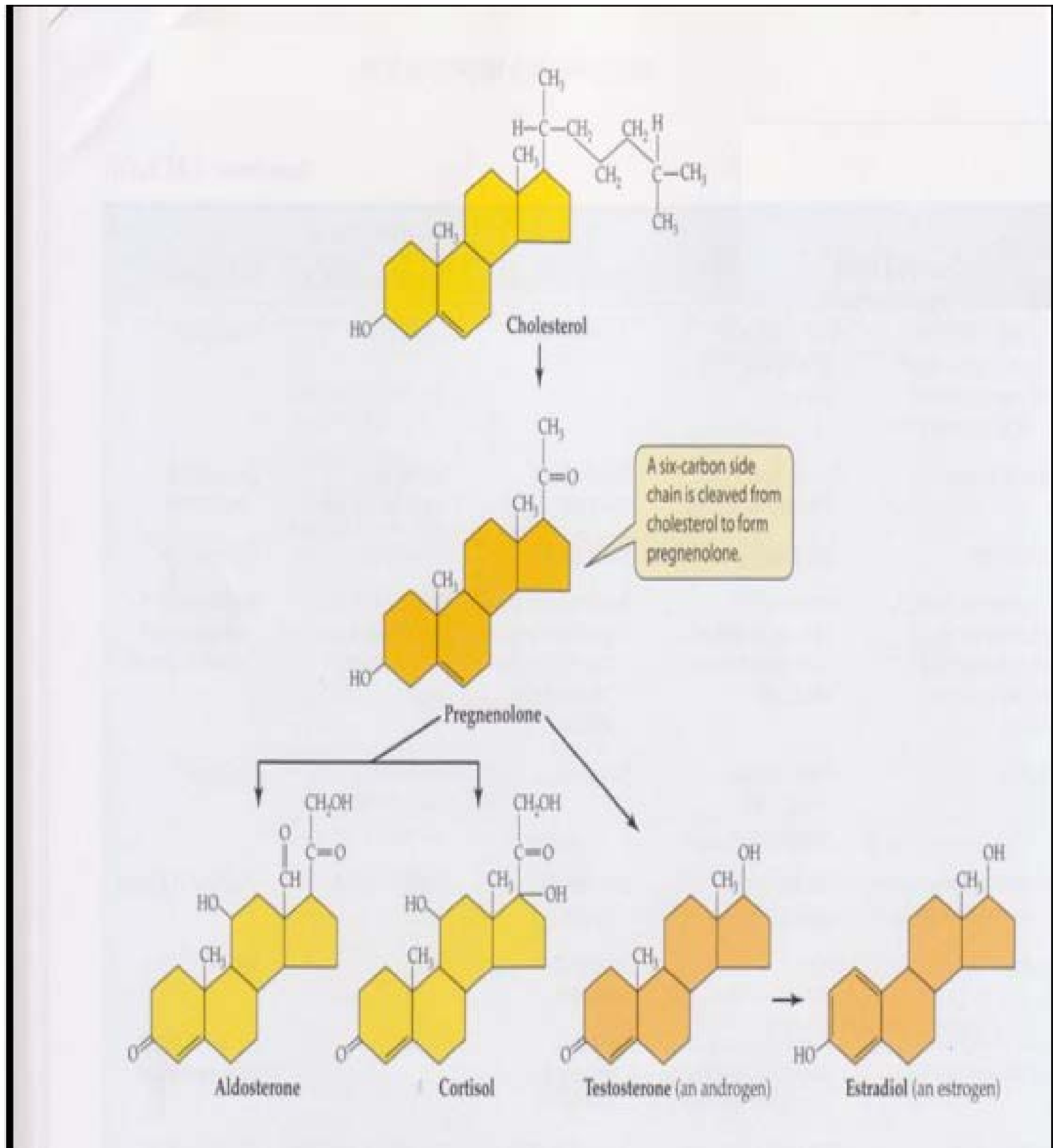


Figure (1-5): Steroid hormones derived from cholesterol (Richard *et al.*, 2004).

1.3.3. How is Testosterone Controlled

The regulation of testosterone production is tightly controlled to maintain normal levels in blood, although levels are usually highest in the morning and fall after that. The hypothalamus and the pituitary gland are important in controlling the amount of testosterone produced and other hormones released by testes as shown in table (1-1). In response to gonadotrophin-releasing hormone (GnRH) from the hypothalamus, the

pituitary gland produces LH which travels in the blood stream to the gonads and stimulates the production and release of testosterone (Ricki *et al*., 2007). As blood levels of testosterone increase, this feeds back to suppress the production of GnRH from the hypothalamus which in turn, suppresses the production of LH by the pituitary gland. Levels of testosterone begin to fall as a result, so negative feedback decreases and the hypothalamus resumes secretion of GnRH (Jeffrey and Gingrich, 2010). (figure 6).

Table (1-1): Hormones released by Testes (Pentikäinen *et al*., 2006).

Secreted hormone	**From cells**	**Role**
Androgens (chiefly testosterone)	Leydig cells	Anabolic: growth of muscle mass and strength, increases bone density, growth and strength, Virilizing: maturation of sex organs, formation of scrotum, deepening of voice, growth of beard and axillary hair.
Estradiol	Sertoli cells	Prevents apoptosis of germ cells
Inhibin	Sertoli cells	Inhibit production of FSH

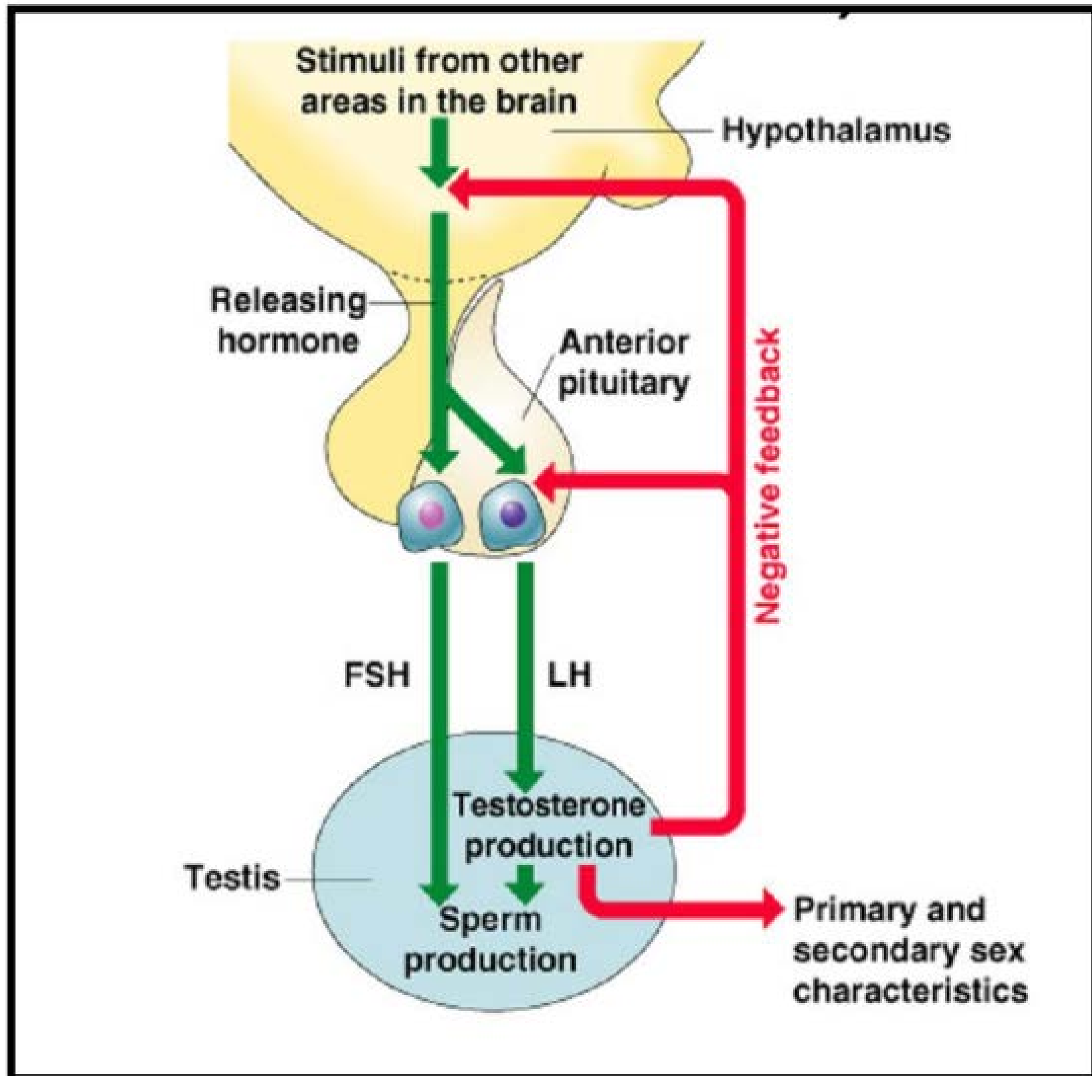


Figure (1-6): Regulation of male androgens (Sex hormones) (Honaramooz *et al*., 2002).

1.3.4. Semen Analysis

Semen analysis is one of the methods used in the fertility laboratories for the study of possible male infertility. The analysis evaluates certain characteristics of sperms in order to diagnose the percentage of patients' fertility potential. The characteristics measured by semen analysis are factors concerning semen quality and quantity which means considering microscopical and macroscopical analysis of the sample. The inicial macroscopic examination considers: liquefaction, appearance (color, odour, consistency), volume, viscosity, pH, prostatic crystals and presence of bacteria (Purvis and Christiansen ,1992). One source states

that 30% of men with a normal semen analysis actually have abnormal sperm function. The microscopical parameters considered in a semen analysis are sperm concentration, motility, morphology and vitality (Agostini and Lucas, 2005).

1.3.5. Male Infertility

More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. The remaining cases of male infertility can be caused by a range of conditions including anatomical problems, hormonal imbalances, and genetic defects (Zhu *et al*., 2006). infertility is seen as an alteration in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyses (World Health Organization, 1999).

Free radicals contribute to the pathogenesis of male infertility as shown in figure (1-7). There are a group of highly reactive chemical molecules with one or more unpaired electrons that can oxidatively modify biomolecules they encounter. Superoxide anion hydroxyl radical and hydrogen peroxide are major reactive oxygen species (ROS) present in seminal plasma. Cells living under aerobic conditions require oxygen to support life; however, metabolites, such as ROS, can modify cell functions and endanger cell survival (Agarwal *et al*., 2003). Male germ cells at various stages of differentiation have the potential to generate ROS and low physiologic levels are needed to regulate sperm cap citation, acrosome reaction and sperm–oocyte fusion (Agarwal and Saleh, 2002; Agarwal *et al*., 2004).

To maintain normal cell function, excess ROS must be continuously inactivated by seminal plasma antioxidants. These block the formation of new ROS or act as scavengers and remove ROS already generated (Agarwal and Sekhon, 2010).

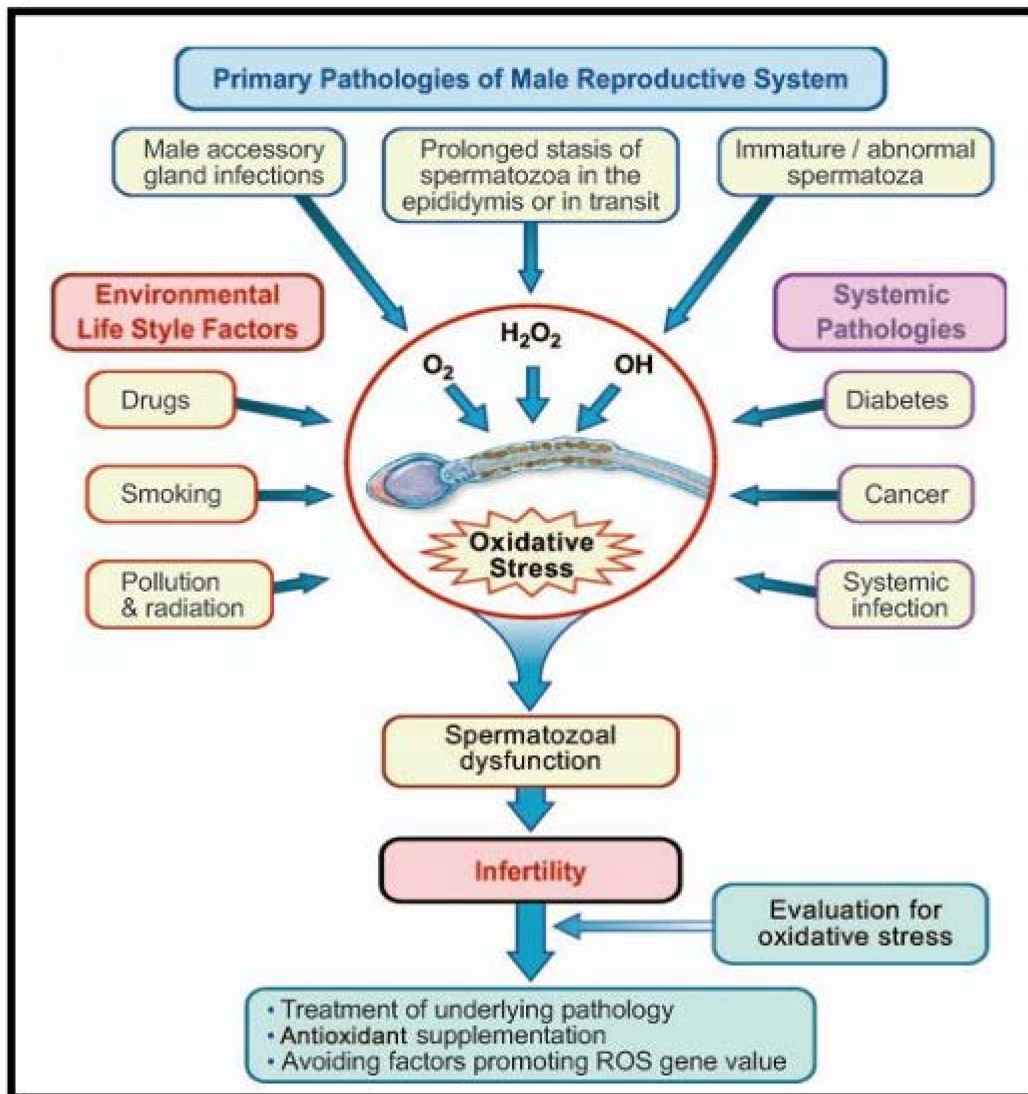


Figure (1-7): Factors contributing to oxidative stress-induced male infertility (Agarwal and Sekhon, 2010).

1.4. Antioxidants

Anti-oxidants are molecules capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reaction can produce free radicals, which start chain reactions that damage cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions (Bjelakovic *et al*., 2007). Antioxidants are particularly important in biology, and all organisms maintain a reducing environment inside their

cells and contain complex systems of antioxidants to prevent damage by oxidation. These antioxidants include glutathione and ascorbic acid and these chemicals are substrates for enzymes such as peroxidases and oxidoreductases. Low levels of antioxidants or inhibition of the antioxidant enzymes causes oxidative stress and may damage or kill cells (Manna *et al*., 2006). Many investigations suggest that antioxidant-rich foods as well as some medicinal plants and their derivatives can reduce damages to cells and biochemical from free radicals. This may slow down, prevent, or even reverse certain diseases that result from cellular damage (Shetty, 1997). In this regard, dietary phenolic antioxidants have been shown to play important roles in delaying the development of chronic diseases such as cardiovascular diseases, cancer, inflammatory bowel syndrome and Alzheimer's disease (Akyon, 2002). Phenolic antioxidants are products of secondary metabolism in plants and are good sources of natural antioxidants in human diets (Botsoglou *et al*., 2002). Aromatic containing plant such as herbs and spices are rich in their phenolic content, and have been widely used to extend the shelf life of foods (Adam *et al*., 1998), and in traditional medicine as treatment for many diseases (Shetty, 1997). Knowledge on the protective mechanisms against toxin- and druginduced organ toxicities leads scientists to look for biologically active relevant compound from herbal plants, which can possess intrinsic antioxidant activity and protect those organs from unwanted oxidative stress. The widespread use of traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. The roles of herbs in disease prevention and cure have been attributed, in part, to antioxidant properties of their constituents; liposoluble and water soluble vitamins, and a wide range of amphipathic molecules (Morel *et al*., 1994; Rice-Evans *et al*., 1997). One reason for the continued interest in examining the antioxidant effects of

medicinal plants is the desire to find natural antioxidants that have a minimal impact on the characteristics of food (Brown *et al*., 2006).

1.4.1. Antioxidant Activity of *Saussurea lappa*

several sesquiterpenes, such as costunolide and dehydrocostus lactone isolated from the methanolic extract of *saussurea lappa* were found to show a potent inhibitory effect on nitrite (NO2-) accumulation in LPS (lipopolysaccharide)-activated mouse macrophages (IC50=3.5 mg/ml). there have been many pharmacological studies on the activities of extracts or principal constituents, costunolide and dehydrocostus lactone from the root of *S.lappa* such as the anti-ulcer, anti-carcinogenesis in rats, the vasorelaxant effect and the inhibitory effects on killing activity of cytotoxic T lymphocyte. In addition, previous studies of this species and other herbal medicines demonstrated that sesquiterpene lactones including costunolide and dehydrocostus lactone inhibited nuclear factor –KB activation there by preventing iNOS (inducible nitric oxide synthase) and TNF- α (tumour necrosis factor – α) expression. Matsuda and other from Japan studied the methanolic extract of the roots and found that it inhibits nitric oxide (NO) production in lipopolysaccharide – activated mouse peritoneal macrophage.

Among the constituents from the methanolic extract, two sesquiterpene lactones (costunolide and dehydrocostus lactone) and two amino acid sesquiterpene conjucates (saussureamines A and B) potently inhibited LPS-induced NO production (IC50=1.2-2.8mm).

Saussureamines A and B in addition to costunolide and dehydrocostus lactone did not inhibited iNOS Enzyme activity, but they inhibited both induction of inducible NO synthase and activation of nuclear factor –KB

in accordance with induction of heat shock protein 72 (Matsuda *et al*., 2003).

1.4.2. Antioxidant Activity of *Cyperus esculentus*

It's important to have a diet rich in antioxidants for protection from oxidative damage over time, and tiger nuts are a great source of antioxidants, *Cyperus esculentus* oil is also known as the new health care oil. it's contained oleic acid, linoleic acid and palmitic acid. In vitro results showed that the total antioxidant activity and the scavenging capacity of hydroxyl radicals and diphenyl picryl hydrazyl radicals increased with increasing concentration of the plant oil (Chen *et a*l., 2008).

Because it contains some natural antioxidants, *C.esculentus* oil shows very good resistance to oxidation. *Cyperus* oil also contains phytosterol, vitamin E and β-carotene Such substances, together with the unsaturated fatty acids of the plant oil, are responsible for the overall antioxidant activity of *C. esculentus* oil. A scavenging capacity of *Cyperus* oil was equal to the clearance capacity of a similar concentration of vitamin C (Ozcan *et al*., 2010).

Certain preparation styles change the antioxidant properties of the plant, although they're all be beneficial to us. For example, when preparing *horchata* (tiger nut milk), using germinated tiger nuts helps retain higher antioxidant content than using fresh tiger nuts because the extract of germinated tigernut has a significantly higher phenolic content than the drinks prepared with with fresh tigernut extract.

Phenolics have the ability to scavenge free radicals, which would otherwise build up in the body due to an imbalance between the antioxidant system of the body and the formation of reactive oxygen species (Savikin *et al*., 2009).

Chapter Two
Materials and Methods

Chapter Two

Materials and Methods

2. Materials

2.1. Equipments and Instruments

The following laboratory equipments and instruments were employed in the present study:

Table (2-1): The equipments and instruments used in this study

Equipment	Company	Origin
Compound light microscope	Motic	Japan
Disposable micropipette tips (Different sizes)	Jippo	Japan
Disposable Petri -dishes	Sterilin	England
Disposable syringes	CMP	Turkey
Electric Balance	Sartorius	Germany
Electric blender	Sartorius	Germany
Filter papers	Halzfeld	Germany
Gas burner	Grade	England
Gauzes	Halzfeld	Germany
Glass flasks	Terumo	Japan
Glass slides and cover slips	Sail Bran	China
Hemocytometer	Neubauer	Germany
HPLC Apparatus	Shimadzu	Japan
Incubator	Memmert	Germany
Laminar flow hood	Heraeus	Germany
Lyophilizer	Fisher	U.K.

Magnetic stirrer	Gallenkamp	England
Microcenteifuge	Beckman	England
Micropipette(Different sizes)	Gilson	France
Oven	Osaw	India
PH_meter	Metter –Tolledo	U.K.
Refrigerator	Ariston	Japan
Rotary evaporator	Buchi	Switzerland
Shaking water bath	Gallenkamp	England
Water distiller	GLF	Germany

2.2. Chemicals and Reagents

The chemicals and reagents used in this study are the following classified according to the manufactured company.

Table (2-2): The chemicals and reagents used in the present study

Chemical Material	Company	Origin
Acetic anhydride	BDH	England
Chloroform	BDH	England
Eosins stain	BDH	England
Ethanol	BDH	England
Formalin	Analar	England
Ferric chloride (FeCl2)	Fluka	Switzerland
Haematoxylin stain	BDH	England

Hydrochloric acid (HCl)	BDH	England
Methanol absolute	BDH	England
mercuric chloride (HgCl2)	Fluka	Switzerland
Potassium iodide(KI)	Fluka	Switzerland
Potassium hydroxide(KOH)	BDH	England
Proviron	Bayer	Germany
Sulphric acid	Analar	England

2.3. Kit

The following kit was used in this study:

Table (2-3): Testosterone kit

Kit	Company	Origin
Testosterone Enzyme Immunoassay test kit	ICN	USA

2.4. Solutions Preparation.

2.4.1. Phosphate Buffer Saline (PBS)

One tablet of PBS was dissolved in 200 ml of distilled water sterilized by autoclave then used.

2.4.2. Ferric Chloride Solution (1%)

The solution was prepared by dissolving 1g of ferric chloride in 100 ml of distilled water (Stahl, 1969).

2.4.3. Potassium Hydroxide Solution

Prepared by dissolving (50g) of potassium hydroxide in (100ml) of distilled water (Jaffer *et al*., 1983).

2.4.4. Haematoxylin Stain

The stain solution was ready supplied by the Laboratories of Baghdad University\college of medicine. It was used to stain the histological sections of mice.

2.4.5. Eosin Stain

The stain solution was ready supplied by the Laboratories of Baghdad University\college of medicine. It was used to stain the histological sections of mice.

2.4.6. Mayer's Reagent

Two solutions were prepared; the first one was prepared by dissolving 1.58 grams of mercuric chloride ($HgCl_2$) in 60 ml of distilled water, while the second solution was prepared by dissolving 5 grams of potassium iodide (KI) in 10 ml of distilled water. Both solutions were mixed and the volume was made – up to 100 ml with distilled water (Smolensk *et al*., 1972).

2.4.7. Mesterolone (Proviron) dose preparation

A daily therapeutic dose of 25 mg of oral proviron tablet was used in this study. However, we calculated the human dose based on the physiological calculation for a 70kg man, such that Proviron tablet was dissolved in distilled water to prepare a dose of 0.36 mg/kg administered to the animal (Shittu, 2009).

2.5. Methods

The Main steps of the research plan was summarized in figure (2_1).

2.5.1. Plants Collection

S. lappa and *C.esculentus* were obtained from a local markets (Herbs shope) in Baghdad and identified by prof. Dr. Khulood Al-sammarrai.

2.5.2. Plants Extraction

The dried roots of the *S. lappa* was powdered using a blender for 10 minutes, and then extracted with methanol (90%), 50 grams of the processed plant were extracted in 250 ml of the solvent and left in shaking water bath (40ºC) for 24hrs. Extract was then filtered with gauze and then with filter paper. The obtained extract was then evaporated at (55ºC) using a rotary evaporator, and the resultant crude extract was dried using lyophilizer. Dried extract was collected, weighed and stored in frozen at (-20ºC) until used to prepare the required doses and concentrations. The dried tubers of *C.esculentus* were extracted by the same procedure (Arokiyaraj *et al*., 2007). The weight of residue of *S.lappa* root extracts was 4.9g which represents 9.8% of the original roots sample weight and the appearance of the residue was light brown in color. The weight of residue of *C.esculentus* tuber extracts was 5.5g which represents 11% of the original tubers sample weight and the appearance of the residue was yellow in color.

2.5.3. Measurement of the Extract Acidity

An aliquot of 10g of *S.lappa* root powder was mixed with 50 ml of absolute methanol for 10 minutes using a magnetic stirrer. The suspension was filtered and the acidity of the filtrate was measured using a pH meter and An aliquot of 10g of *C.esculentus* powder was mixed with 50 ml of absolute methanol for 10 minutes using a magnetic stirrer. The suspension was filtered and the acidity of the filtrate was measured using a pH meter (Shihata, 1951).

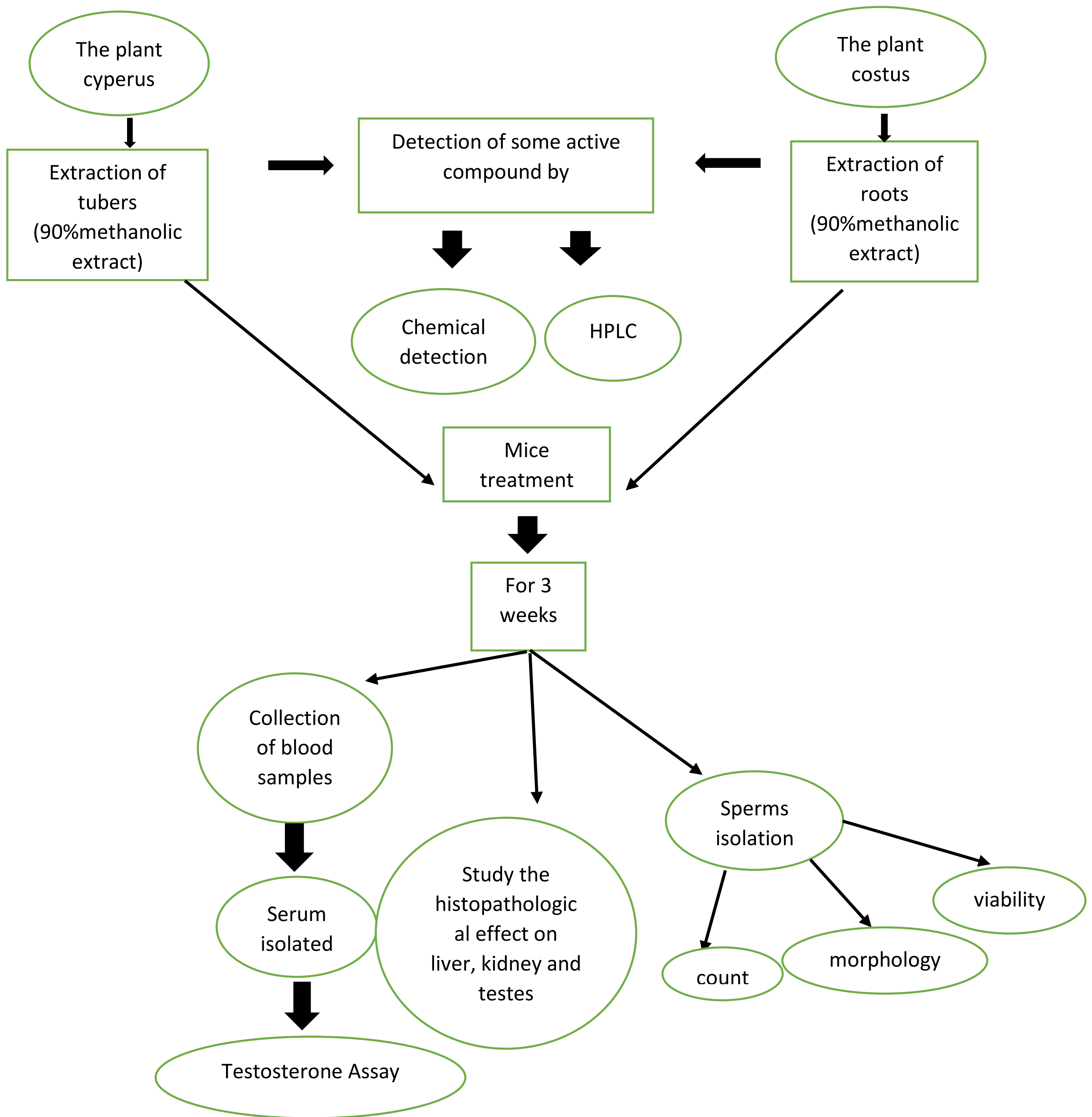


Figure (2-1): Main steps of the research plan

2.6. Detection of Some Active Compounds in Plants Extracts

2 .6.1. Chemical Detection of Plants Extracts

1. Detection of Terpenes and Steroid

An aliquot of 1 ml of methanol extract was mixed with few drops of chloroform, then a drop of acetic anhydride and drop of concentrated sulphuric acid were added, brown precipitate appeared which representing the presence of terpenes, the appearance of dark blue color after few minutes would represent the presence of steroids (Al-Abid, 1985).

2. Detection of Flavonoids

The detecting solution was prepared by mixing 10 ml of ethanol (50%) with 10 ml of potassium hydroxide (50%), and then 5 ml of this solution was added to 5 ml of the plant extract. The appearance of yellow color was an indicator of the presence of flavonoids (Jaffer *et al*., 1983).

3. Detection of Resins

An aliquot of 10 ml of distilled water acidified with 4% hydrochloric acid were added to 5 ml of the plant extract, the appearance of turbidity indicated the presence of resins (Shihata, 1951).

4. Detection of Tannins

An aliquot of (25ml) of methanolic extract was mixed with ferric chloride solution ($FeCl2$) (1%; w/v), the appearance of greenish-blue color was an evidence for the presence of tannins (Harbone, 1984).

5. Detection of Alkaloids

An aliquot of 10 ml of the plant extract was acidified by adding HCL, test it by Mayer’s reagent and appearance of white precipitate indicates the presence of alkaloids (Trease and Evans,1987).

6. Detection of Saponins

This method was done according to the method described by (Stahle, 1969).

Saponins were detected by two methods:

A. A solution of plant powder was shaken vigorously in a test tube. The formation of foam standing for a time indicates a positive result.

B. An aliquot of 5 ml of the plant extract was added to 1-3 ml of 3% ferric chloride solution, a white precipitate was developed indicating the presence of saponins.

2.6.2. Detection of Flavonoids Compound by HPLC

At December in Ministry of Science and Technology, HPLC application for flavonoids standards rutin, quercetin ,Myricetin , Luteolin and for kaempferol of the *S.lappa* roots extract which was used for qualitative detection of the flavonoids. The condition for detection of qurciten, rutin, kaempferol, Myricetin and Luteolin as follow:

Mobile phase: were 0.1% phosphoric acid: acetonitrile (20:80, V/V).

Column: C_18 (25cm).

Flow rate: 1.5ml/min.

Injected volume: 20µl.

Temperature: 25ºC

Wave length: 285nm.

Instrument: refractive index detector RF Shimadzu.

The same procedure were used for the detection of flavonoids compound of the *C.esculantus* tubers extract.

2.7. Laboratory Animals

Albino male mice were the laboratory animals that were employed in carrying out the experiments of the study. They were supplied by the Drug Control Center (Ministry of Health), and their age at the start of the experiment was 8-10 weeks, and their weight was 21-44 grams. They were divided into groups; each group was kept in a separate plastic cage. The cages were put in a room with optimal temperature (25 ºC). The animals were given water and fed throughout the experimental work.

2.8. Experimental Design

The experiment was designed to assess fertility, histopathological effects of three doses (200, 400 and 600 mg/kg) of *S.lappa extract* (methanol extract) and three doses (200, 400 and 600 mg/kg) of *C.esculentus* extract (methanolic extract), as well as, proviron (positive controls) and water (negative controls) Therefore, the animals were divided into eight groups (each group contains 5 mice). Just before treatment, the extracts were dissolved in distilled water to facilitate oral administration to the mice. Then the mice were sacrificed after 3 weeks and showed the results.

Group1 (negative control): mice were treated with water.

Group2 (positive control): mice were treated with 0.36 mg/kg

Mesterolone (Proviron).

Group3: mice were treated with 8.3 mg/ ml of *S.lappa* extract (200mg/kg).

Group4: mice were treated with 12 mg/ ml of *S.lappa* extract (400mg/kg).

Group5: mice were treated with 13.56 mg/ ml of *S.lappa* extract (600mg/kg).

Group6: mice were treated with 7 mg/ ml of *C.esculents* extract (200mg/kg).

Group7: mice were treated with 14.8 mg/ ml of *C.esculents* extract (400mg/kg).

Group8: mice were treated with 23.4 mg/ ml of *C.esculents* extract (600mg/kg).

2.9. Collection of Blood Samples and Determination of Testosterone levels

At the end of the experiment, blood was drawn from the heart directly by stab the heart (using syringe) to get the largest amount of blood and collected into micro centrifuge tubes. Blood samples were centrifuged at 3000 rpm for15min to get serum, then the serum had frozen (-20°C) in refrigerator until the testosterone assay. The testosterone concentration was determined using the Testosterone Enzyme Immunoassay kit.

2.10. Semen Preparation

Soon after killing mice and dissection, the epididymes and testes were removed for study the sperm concentration, morphological and viability.

2.10.1. Sperm viability and morphology

The epididymes minced with small scissors in Petri dish containing phosphate buffer saline (PBS) 1ml. A drop of semen suspension was mixed with a drop of eosin stain (1%) a thin smear of semen -eosin was put on the slide and then mixed by others slide which used to make a thin smear in a third slide and the third slide left to dry at room temperature, the slides were examined under light microscope at (40x). The dead sperms stain pink color while the live one is bright without color. Also the morphology of abnormal sperm was determined by this stain. The sperm viability was estimated according to the following equation (Hafez, 1987).

Percentage of dead sperm % = (NO. of dead sperm /total NO. of sperm) X 100

The percentage of sperm abnormality was estimated according to the following equation (Dale and Edler, 1997).

Abnormality % = (No. of abnormal sperms/ total NO. Of sperm) X 100

2.10.2. Sperm Concentration

Sperm concentration was calculated according to the following steps:

i. The sperm suspension prepared was pulled by RBC pipette till "0.5" mark, and then diluted with the diluting solution till "101" mark, so the dilution rate is 1:200.

ii. The content of the pipette was mixed; the coverslip was placed over the counting chamber of hemocytometer.

iii. A small amount of the diluting solution containing sperms was placed at the edge of the coverslip and drawn by the capillary action under the coverslip.

iv. The slide was placed under the microscope and the number of sperms was counted in five large squares.

The number of sperm / ml was calculated using the following formula (Salisbury and VanDemark,1961).

$$No. = n \times D \times 400 \times 1000 / N \times 1/10$$

n: number of sperms in the five squares

D: inversion of the dilution rate (200).

400: inversion of the small square size.

N: number of small squares in the hemocytometer (80).

1/10: depth of the hemocytometer.

2.11. Histopathological Study

Mice were sacrificed by cervical dislocation, then dissected to obtain the liver, kidney and testes which were washed with D.W., before fixed in 10% formalin for 48 hours, and the procedure of Bancroft and Stevens (1982) was followed to prepare sections for histopathological examinations. The procedure is outlined at the following:

Washing: Samples were placed in 70% ethanol overnight.

Dehydration: They were dehydrated with ascending concentrations (70, 80, 90 and 99%) of ethanol. With two hours for each concentration.

Clearing: Samples were placed in xylene for two hours.

Infiltration: The samples were first placed in paraffin- xylene (1:1) for 30 min at 57-58 °C, and then in paraffin alone for 2 hrs at 60-70°C inside the oven. **Embedding:** The samples were embedded in pure paraffin wax (melting temperature 60-70 °C) and left to be solidified at room temperature.

Sectioning: The paraffin block was sectioned by a rotary microtome at a thickness of 5 microns, and then the sections were transferred to a slide covered with Mayer's albumin. The sections of tissues were placed in a water bath (35-40°C) for a few seconds.

Staining: The slides were first placed in xylene for 15-20 min, then it was put in descending concentrations (100, 90, 80 and 70%) of ethanol (2 min for each concentration) and finally put in tap water. After that, the slides were stained with haematoxylin for 5-15 min and then washed with tap water for 5 min. Then, the slide was placed in acidic alcohol for a few seconds and washed with distilled water. After washing, the slides were placed in eosin stain for 10-15 seconds, and then in ascending concentrations (70, 80, 90 and 99%) of ethanol

(two minutes for each concentration). Finally, the slides were cleared with xylene for 10 min.

Mounting: The slides were mounted with Canada balsam and covered with a cover slip. Then, each slide was examined microscopically to inspect the histopathological changes.

2.12. Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means in this study.

Chapter Three
Results and Discussion

Chapter Three

Results and Discussion

3.1 Plants Extracts

3.1.1 Methanolic Extract of *S. lappa*

Fifty grams of costus powdered roots was used for the preparation of the extract, the pH value of the methanolic extract was 7.11. The weight of the residue obtained after lyophilized was 4.9g, which represents 9.8% of the original roots sample weight. The appearance of the extract was light brown in color.

3.1.2 Methanolic Extract of *C.esculentus*

Fifty grams of *C.esculentus* powdered tubers was used for the preparation of the extract, the pH value of the methanolic extract was 7. The weight of the residue obtained after lyophilized was 5.5g, which represents 11% of the original roots sample weight. The appearance of the extract was light yellow in color.

3.1.3 Chemical Detection of *S. lappa* Active Compound

In this study the active constituent of methanolic extract of *S.lappa* were determined using different specific reagents. The results indicated the presence of different active compound which are (flavonoids, alkaloids, steroids, tannins, saponins, terpenes and resin) (Table 3-1). This result was in accordance with previous studies on the same plant indicated the presence of (flavonoids, steroids, terpenes and alkaloids) (Yua *et al*., 2007).

Another study indicated that *S. laapa* roots contained (tannins, saponins and resin) (Bose *et al.,* 1961; Uma Chandur *et al*., 2011).

Table (3-1): Chemical detection of some active compounds in *S.lappa* methanolic extract.

Secondary Metabolites	Reagent	Indication	Result of detection
Alkaloids	Mayer's reagent	White ppt.	+
flavonoids	Ethanol with KOH	Yellow color	+
Tannins	Ferric chloride	Greenish-blue color	+
Resins	Hydrochloric acid	Turbidity	+
Terpenes and Steroids	Chloroform, acetic anhydride and sulphuric acid	Brown ppt. Blue color	+ +
Saponins	Extract shaking Ferric chloride solution	Foam White ppt	+ +

Note: + indicate the presence of the active compound

3.1.4 Chemical Detection of *C. esculentus* Active Compound

The active constituent of methanolic extract of *C. esculentus* were determined using different specific reagents. The results indicated the presence of different active compound which are (flavonoids, alkaloids, steroids, tannins, saponins, terpenes and resin) (Table 3-2).

Previous pharmacological and chemical studies indicated the presence of several active compounds, including (alkaloids, flavonoids, steroids, tannins, resins and saponins) (Chukwuma *et al*., 2010; Jing *et al*., 2016).

Another study indicated that *C. esculentus* contained terpenes (Klein *et al*., 2014).

Table (3-2): Chemical detection of some active compounds in *C.esculentus* methanolic extract.

Secondary Metabolites	Reagent	Indication	Result of detection
Alkaloids	Mayer's reagent	White ppt.	+
flavonoids	Ethanol with KOH	Yellow color	+
Tannins	Ferric chloride	Greenish-blue color	+
Resins	Hydrochloric acid	Turbidity	+
Terpenes and Steroids	Chloroform, acetic anhydride and sulphuric acid	Brown ppt. Blue color	+ +
Saponins	Extract shaking Ferric chloride solution	Foam White ppt	+ +

Note: + indicates the presence of the active compound.

3.1.5 HPLC Analysis for *S. lappa*

Detection of flavonoids in *S. lappa* methanolic extract obtained from dried roots by HPLC analysis indicated the presence of:

A. Myricetin, with retention time (1.885) minutes, figure (3-1) in comparison with myricetin standard (1.793) figure (3-2).
B. Quercetin, with retention time (3.175) minutes, figure (3-1) in comparison with quercetin standard (3.122) figure (3-2).
C. Kaempferol, with retention time (4.157) minutes, figure (3-1) in comparison with kaempferol standard (4.022) figure (3-2).
D. Rutin, with retention time (5.037) minutes, figure (3-1) in comparison with rutin standard (4.977) figure (3-2).
E. Luteolin, with retention time (5.923) minutes, figure (3-1) in comparison with luteolin standard (5.875) figure (3-2).

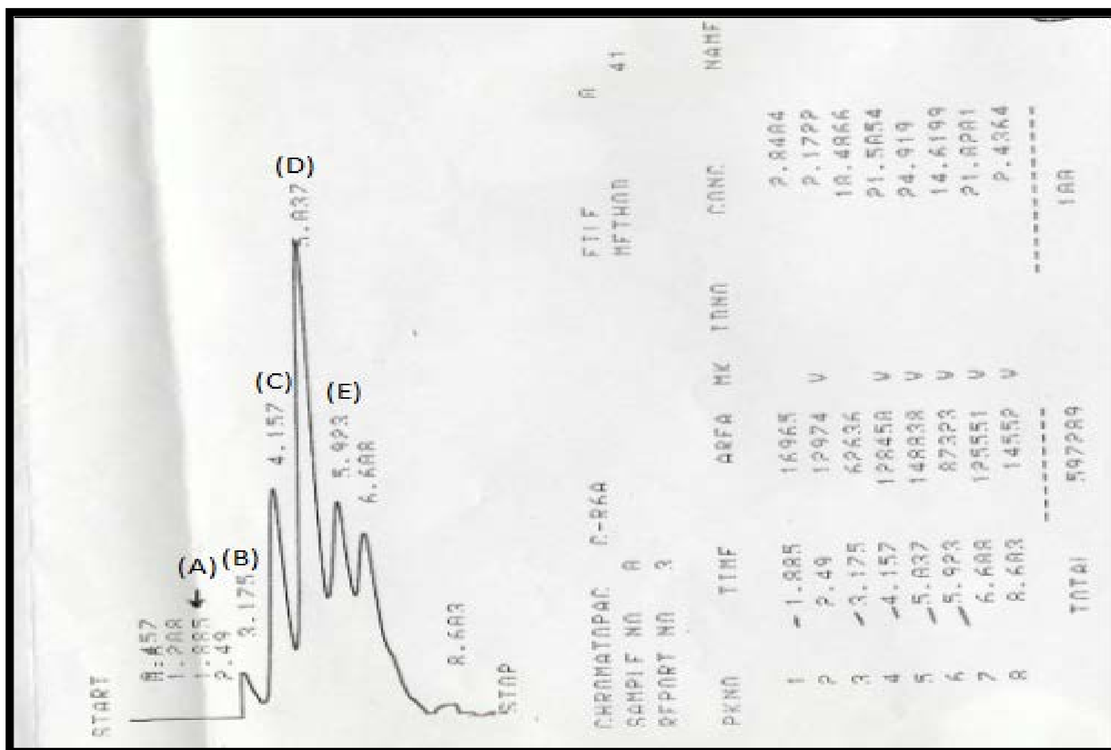


Figure (3-1): HPLC analysis of the *S.lappa* dried roots methanolic extract.
A: myricetin **B:** quercetin **C:** kaempferol **D:** rutin **E:** luteolin

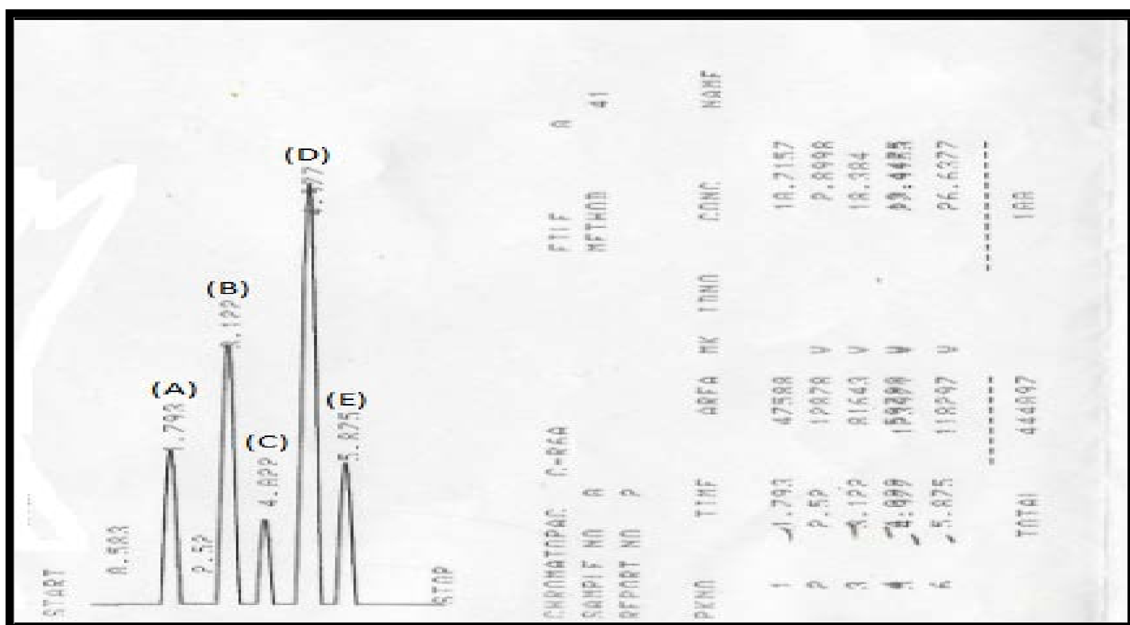


Figure (3-2): HPLC analysis for flavonoid standard.
A: myricetin **B:** quercetin **C:** kaempferol **D:** rutin **E:** luteolin

3.1.6 HPLC Analysis of *C. esculentus*

Detection of flavonoids in *C.esculentus* methanolic extract obtained from dried tubers by HPLC analysis indicated the presence of:

A. Myricetin, with retention time (1.892) minutes, figure (3-3) in comparison with myricetin standard (1.793) figure (3-2).
B. Quercetin, with retention time (3.233) minutes, figure (3-3) in comparison with quercetin standard (3.122) figure (3-2).
C. Kaempferol, with retention time (4.163) minutes, figure (3-3) in comparison with kaempferol standard (4.022) figure (3-2).
D. Rutin, with retention time (5.05) minutes, figure (3-3) in comparison with rutin standard (4.977) figure (3-2).
E. Luteolin, with retention time (5.988) minutes, figure (3-3) in comparison with luteolin standard (5.875) figure (3-2).

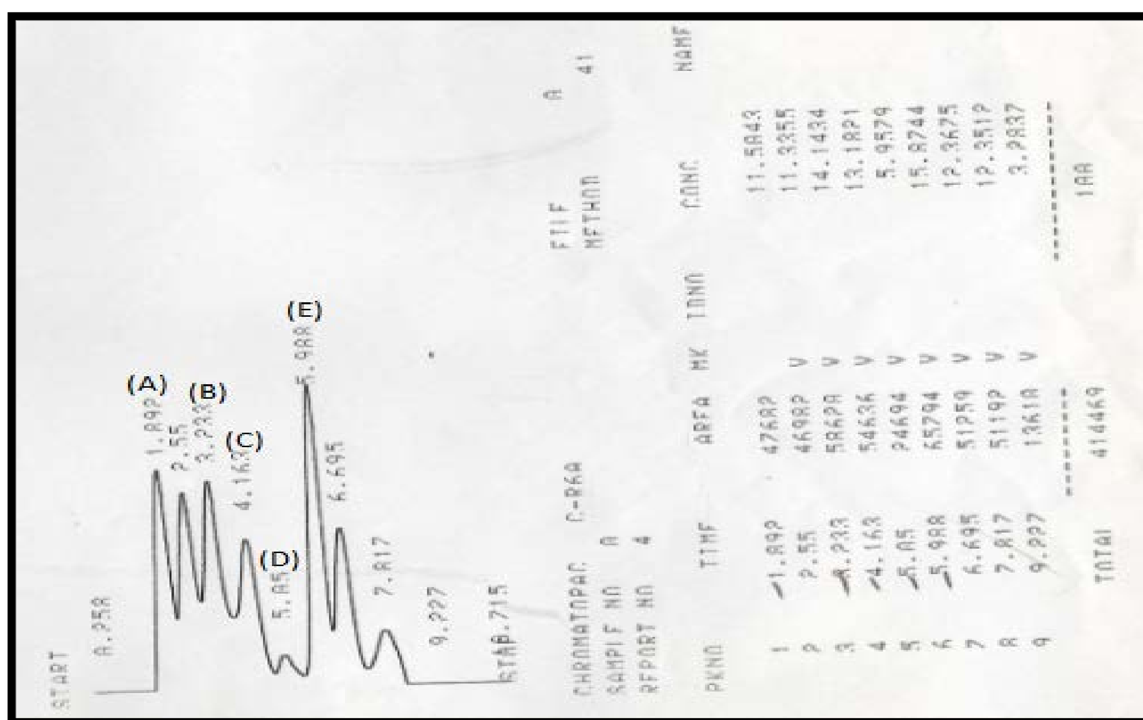


Figure (3-3): HPLC analysis of the *C. esculentus* dried tubers methanolic extract.
A: myricetin **B:** quercetin **C:** kaempferol **D:** rutin **E:** luteolin

3.2 Testosterone assay

Testosterone serum concentration after 3 weeks of treatment

Result in table (3-3) indicated a significant increase (p<0.01) in serum testosterone concentration after 3 weeks in mice treated with *S. lappa* extract compared with negative and positive controls-treated mice. serum testosterone in *S. lappa* treated mice with concentrations 200 and 400 mg/kg were elevated to (1.244± 0.09 and 0.964±0.05 ng/ml) while in the negative control, water treated mice was (0.878±0.006 ng/ml) and in positive control group, mesterolone drug treated mice was (0.906±0.005 ng/ml). The levels of testosterone in all treated groups were measured (figure 3-4). Testosterone concentration were elevated in doses (200 and 400) mg/kg compared with the dose (600) mg/kg. So, there is a significant increase (p<0.01) in serum testosterone concentration as compared with the dose 600 mg/kg.

Table (3.3): Effect of *S. lappa* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on serum testosterone concentration in mice (mean ± SD)

Mice groups	Mean ± SD of Testosterone concentration (ng/ml)
200 (mg/kg)	1.244 ± 0.09 a
400 (mg/kg)	0.964 ± 0.05 b
600(mg/kg)	0.814 ± 0.01 c
Negative Control (water)	0.878 ± 0.006 bc
Positive Control (mesterolone)	0.906 ± 0.005 bc
LSD value	0.1235 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	

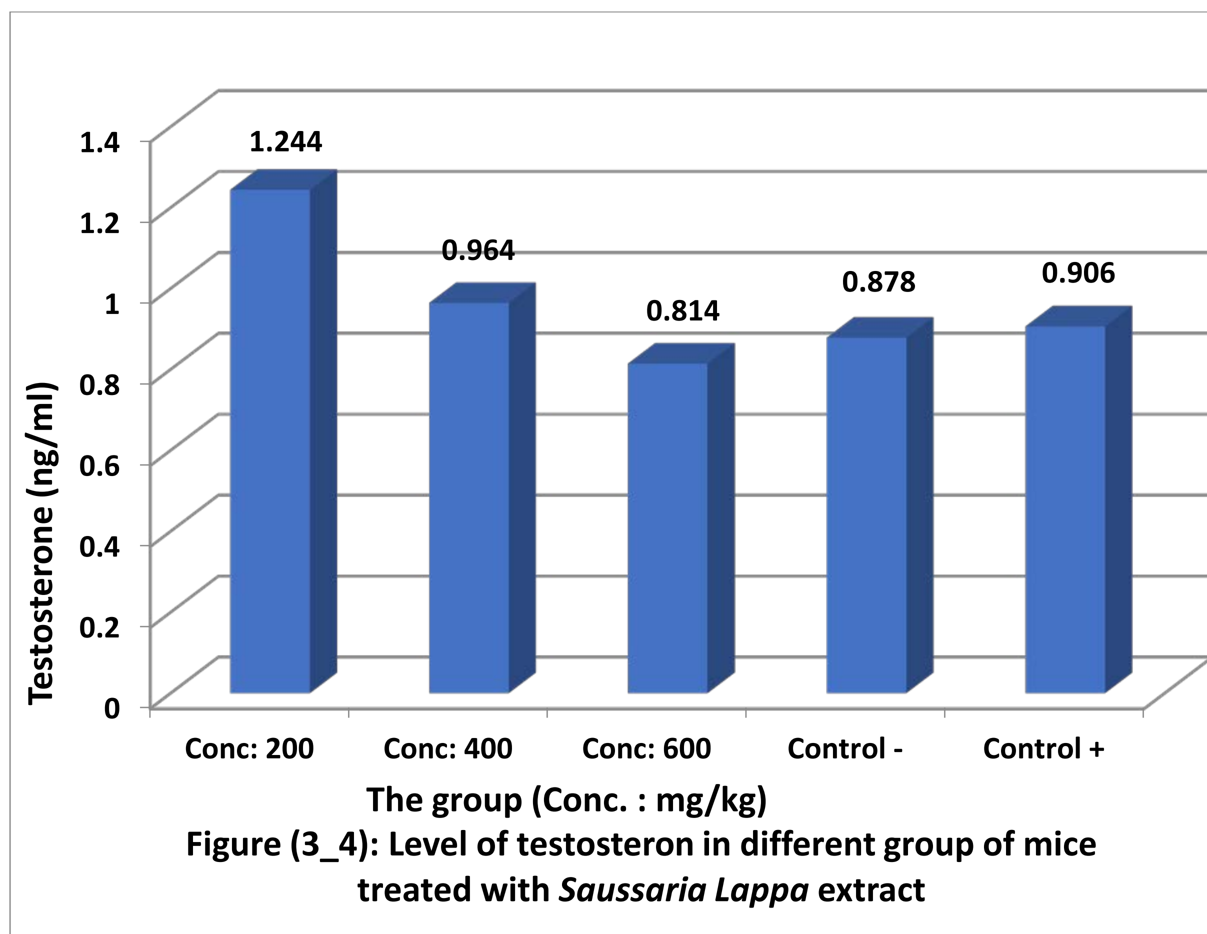
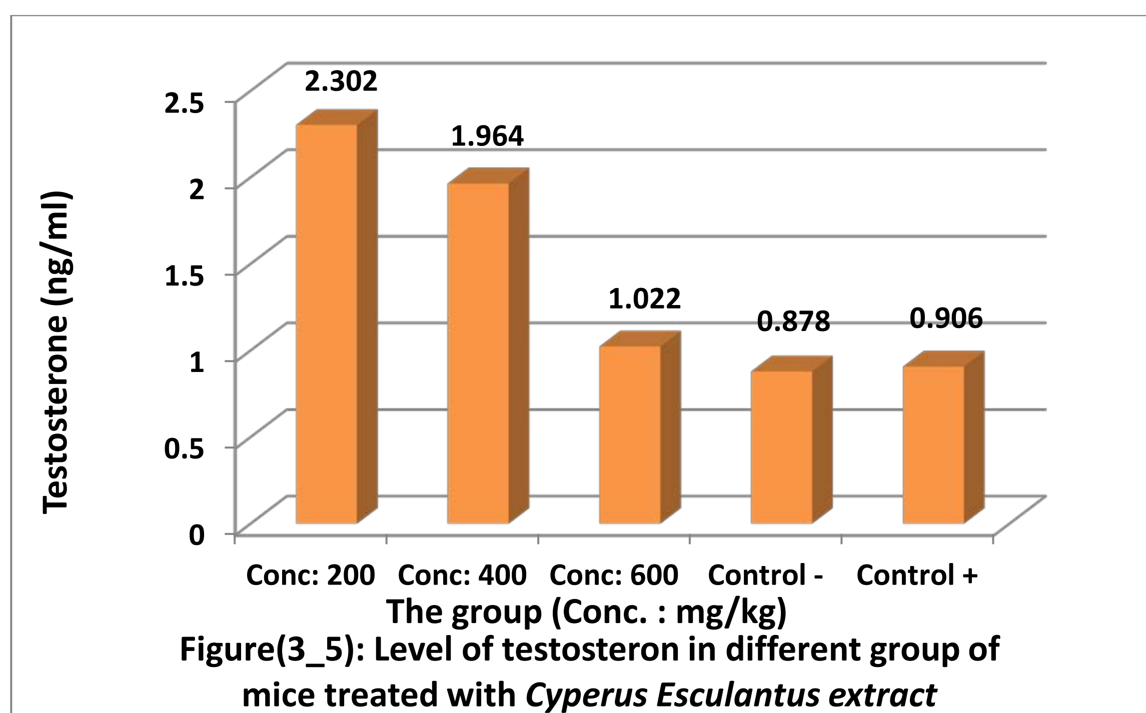


Figure (3_4): Level of testosteron in different group of mice treated with *Saussaria Lappa* extract

Results in table (3-4) indicated a significant increase ($p<0.01$) in serum testosterone concentration after 3 weeks in mice treated with *C. esculantus* extract compared with negative and positive controls-treated mice. serum testosterone in *C. esculantus* treated mice with concentrations 200, 400 and 600 mg/kg were elevated to (2.302$\pm$ 0.008, 1.964$\pm$ 0.046 and 1.022$\pm$ 0.011 ng/ml) while in the negative control, water treated mice was (0.878$\pm$0.006 ng/ml) and in positive control group, mesterolone drug treated mice was (0.906$\pm$0.005 ng/ml). The levels of testosterone in all treated groups were measured (figure 3-5). Testosterone concentration were elevated in doses (200 and 400) mg/kg compared with the dose (600) mg/kg. So, there is a significant increase ($p<0.01$) in serum testosterone concentration as compared with the dose 600 mg/kg.

Table (3-4): Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on serum testosterone concentration in mice (mean ± SD)

Mice groups	Mean ± SD of Testosterone concentration (ng/ml)
200 (mg/kg)	2.302 ± 0.008 a
400 (mg/kg)	1.964 ± 0.046 b
600(mg/kg)	1.022 ± 0.011 c
Negative Control (water)	0.878 ± 0.006 d
Positive Control (mesterolone)	0.906 ± 0.005 d
LSD value	0.0646 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	



Figure(3_5): Level of testosteron in different group of mice treated with *Cyperus Esculantus extract*

Both plants contained flavonoids compounds such as qureceine and rutin. Qurecetine could increase serum testosterone levels in male and found to

improve the action of sex hormone (LH). This hormone stimulates male testicles to produce greater levels of testosterone, which in turn helps increased sexual drive (Maz, 2004). Androgenic effect is attributable to testosterone levels in blood; *S. lappa* and *C. esculentus* extracts have a role in testosterone secretion confer best availability of hormone to gonads. The testes, epididymis and other reproductive organs are structurally and physiologically dependent upon the testosterone (Amini, 2005).

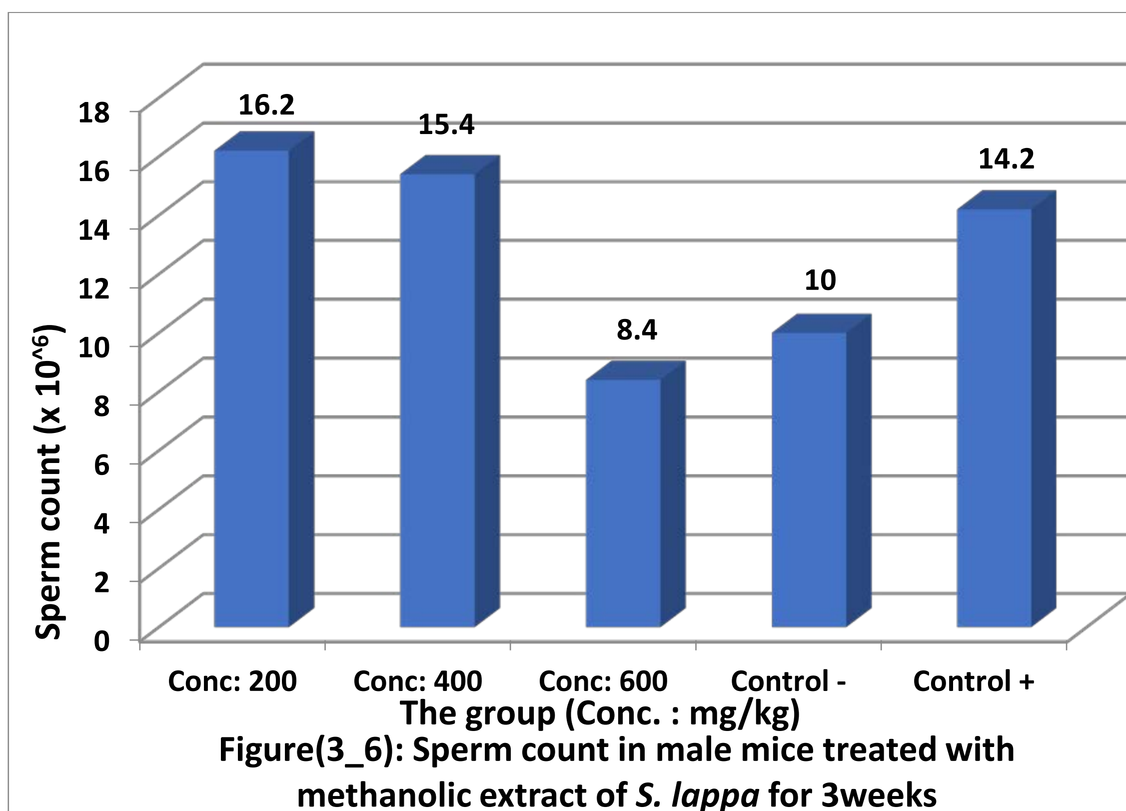
3.3 Sperms concentration

The results of sperms concentration in table (3-5) and figure (3-6) showing a significant increase (p<0.01) in sperms concentration after treatment with the *S. lappa* extract at doses 200 and 400 mg/kg (16.20±0.37 and 15.40±0.51 sperm/ml) when compared with negative control, water (10.00± 0.71 sperm/ml), positive control, mesterolone drug (14.20± 0.58 sperm/ml) and also when compared with another group treated with *S. lappa* extract at dose 600 mg/kg (8.40± 0.51 sperm/ml). and the results of sperms concentration in table (3-6) and figure (3-7) showing a significant increase (p>0.01) in sperms concentration after treatment with the *C. esculentus* extract at doses 200, 400 and 600 mg/kg (20.40±0.51, 19.00± 0.54 and 17.60± 0.51 sperm/ml) when compared with negative control, water (10.00+ 0.71 sperm/ml), positive control, mesterolone drug (14.20+ 0.58 sperm/ml).

The *S.lappa* and *C. esculantus* extracts contained many active compounds especially flavonoids that contributed in an increasing sperms concentration. The mechanism for increased sperms concentration may be due to the presence of quercetin. It was known that quercetin can increase the numbers of spermatogonial cells by reduceing the oxidative damage in the testes (Mi and Zhang, 2005). Other study found that quercetin increases the testosterone level so that quercetin led to boost sperm quality and fertility (Taepongsorat, 2008).

Table (3_5): Effect of *S.lappa* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on sperms concentration in mice (mean ± SD)

Mice groups	Mean ± SD
	Sperm count (x 10^6)
200 (mg/kg)	16.20 ± 0.37 a
400 (mg/kg)	15.40 ± 0.51 ab
600(mg/kg)	8.40 ± 0.51 c
Negative Control (water)	10.00 ± 0.71 c
Positive Control (mesterolone)	14.20 ± 0.58 b
LSD value	1.615 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	

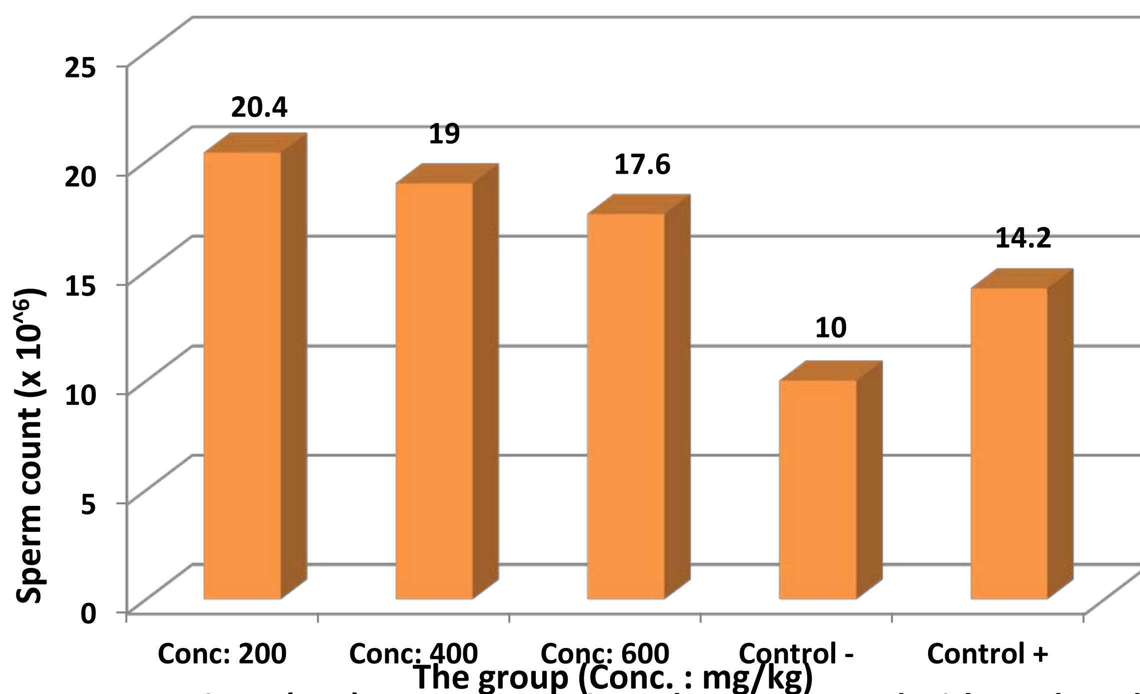


Figure(3_6): Sperm count in male mice treated with methanolic extract of *S. lappa* for 3weeks

Table (3_6): Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C esculentus* on sperms concentration in mice (mean ± SD)

Mice groups	Mean ± SD
	Sperm count (x 10^6)
200 (mg/kg)	20.40 ± 0.51 a
400 (mg/kg)	19.00 ± 0.54 ab
600(mg/kg)	17.60 ± 0.51 b
Negative Control (water)	10.00 ± 0.71 d
Positive Control (mesterolone)	14.20 ± 0.58 c
LSD value	1.699 **
P-value	0.0001

** (P<0.01).
Means having with the different letters in same column differed significantly.



Figure(3_7): Sperm count in male mice treated with methanolic extract of *Cyperus Esculantus* for 3weeks

3.4 Morphologically Abnormal Sperms

Morphological study of sperms is an important aspect in the assessment of sperm functions (Katz *et al.*,1982). Results in table (3-7) and Figure (3-8) revealed a significant decrease ($p \leq 0.01$) in percentage of morphologically abnormal sperms after treatment with *S. lappa* extract at doses 200 and 400 mg/kg (13.20± 0.80 and 13.80± 1.02) when compared with negative control (water treatment) (20.40 ± 0.51) and positive control (mesetorlone drug) (17.60 ± 0.67) and also when compared with other group treated with *S. lappa* extract at dose 600mg/kg (33.00 ± 1.22), morphological abnormalities of sperms were observed in figure (3-9), Results in table (3-8) and Figure (3-10) revealed a significant decrease ($p \leq 0.01$) in percentage of morphologically abnormal sperms after treatment with *C. esculentus* extract at doses 200, 400 and 600 mg/kg (11.00 ± 0.71, 12.60 ± 0.40 and 13.60 ± 0.08) when compared with negative control (water treatment) (20.40 ± 0.51) and positive control (mesetorlone drug) (17.60 ± 0.67).

Table (3-7): Effect of *S.lappa* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on percentage of morphologically abnormal sperms in mice (mean ± SD)

Mice groups	Mean ± SD
	Abnormal sperm (%)
200 (mg/kg)	13.20 ± 0.80 d
400 (mg/kg)	13.80 ± 1.02 d
600(mg/kg)	33.00 ± 1.22 a
Negative Control (water)	20.40 ± 0.51 b
Positive Control (mesterolone)	17.60 ± 0.67 c
LSD value	2.605 **
P-value	0.0001

** (P<0.01).
Means having with the different letters in same column differed significantly.

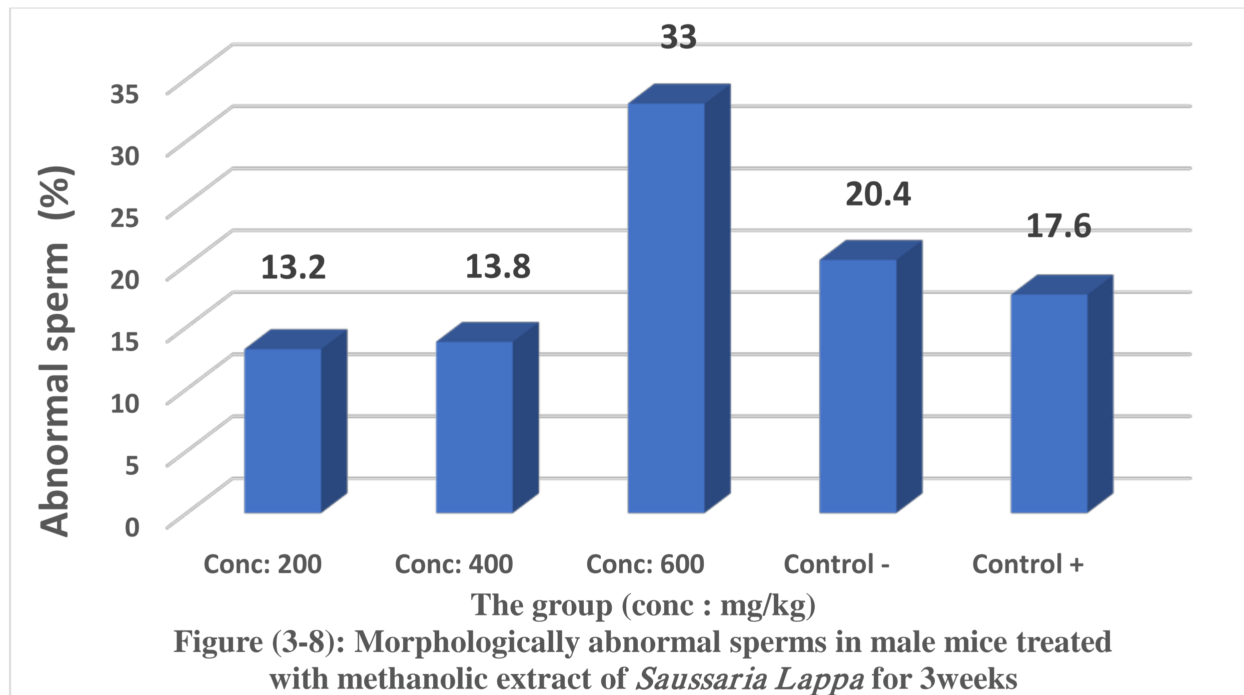
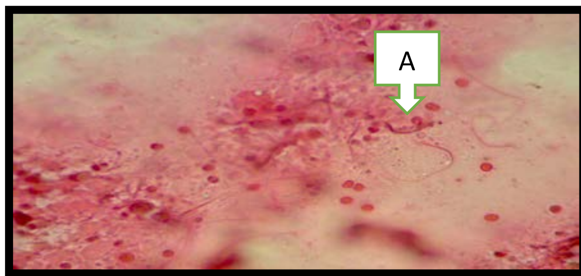
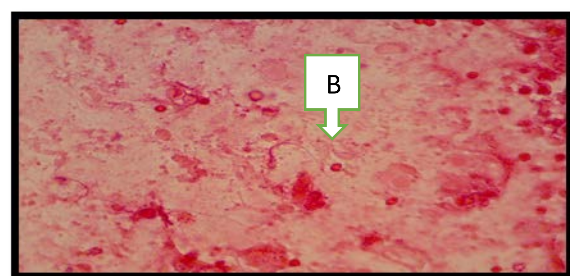


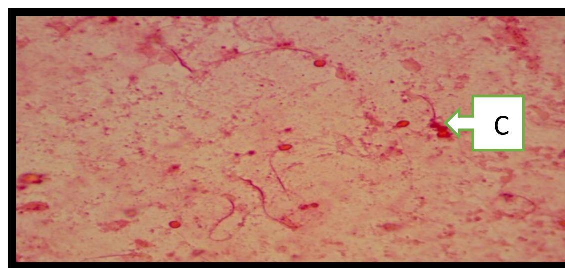
Figure (3-8): Morphologically abnormal sperms in male mice treated with methanolic extract of *Saussaria Lappa* for 3weeks



A: Tapering head



B: Fragmented head



C: Double head

Figure (3_9): Effect of *S. lappa* extract at dose 600 mg/kg on sperm morphology in mice.

Table (3_8): Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on percentage of morphologically abnormal sperms in mice (mean ± SD)

Mice groups	Mean ± SD
	Abnormal sperm (%)
200 (mg/kg)	11.00 ± 0.71 d
400 (mg/kg)	12.60 ± 0.40 dc
600(mg/kg)	13.60 ± 0.08 c
Negative Control (water)	20.40 ± 0.51 a
Positive Control (mesterolone)	17.60 ± 0.67 b
LSD value	2.102 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	

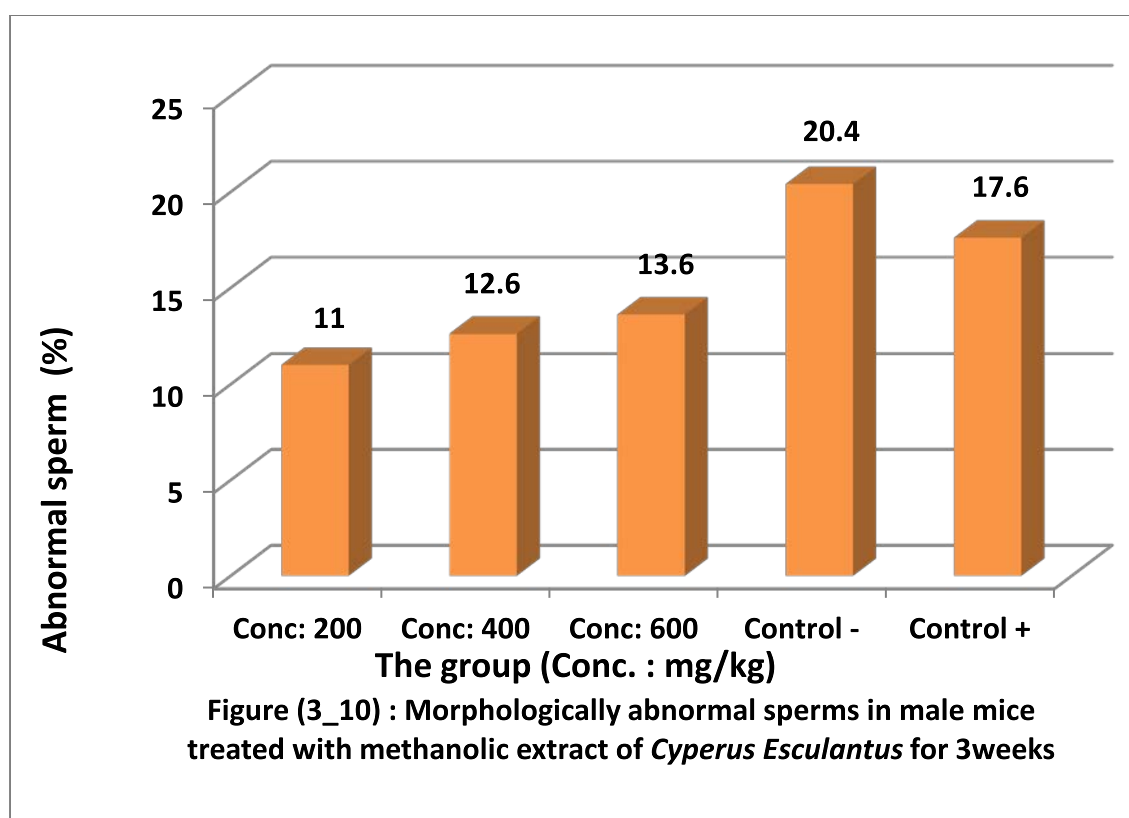


Figure (3_10) : Morphologically abnormal sperms in male mice treated with methanolic extract of *Cyperus Esculantus* for 3weeks

The results in table (3-7) and figure (3_8) showed that after 3 weeks treatment with *S. lappa* extract at dose (600 mg/kg). The plant extract affects on

morphological abnormalities of sperms on the basis of a dose-dependent. The activity of plant extract can be referred to many active compound including flavonoids and also other compounds that act as antioxidant (Yua *et al*., 2007). These compounds protected the plasma membrane of the sperm against the influence of oxidative stress. Depending on our results, the dose 600mg/kg of *S. lappa* extract caused a significant increase ($p \leq 0.01$) in the percentage of morphologically abnormal sperm in comparison with the doses 200 and 400mg/kg. Which means that low doses were less effective on sperm morphology than high doses; this could be explained that high dose (600 mg/kg) was toxic and caused reduction in testosterone levels. Results in table (3-8) and Figure (3-10) revealed a significant decrease ($p \leq 0.01$) in percentage of morphologically abnormal sperms after 3 weeks treatment with C. esculentus extract at doses 200, 400 and 600 mg/kg when compared with negative control (water treatment) and positive control (mesetorlone drug). The activity of plant extract can be referred to the presence of flavonoids and oleic acid, linoleic acid and palmitic acid that act as antioxidant (Chukwuma *et al*., 2010; Jing *etal*., 2016). These compounds protected the plasma membrane of the sperm against the influence of oxidative stress.

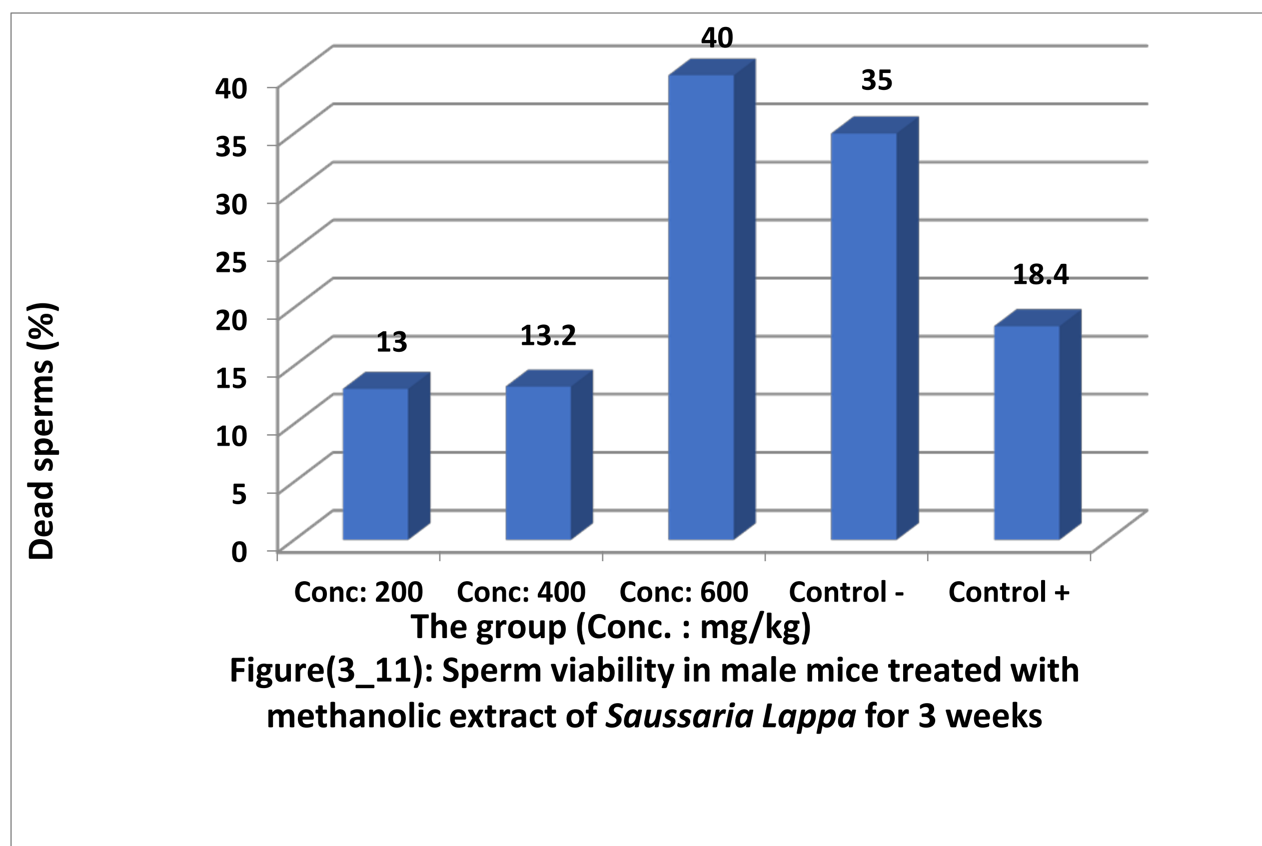
3.5 Sperms viability

Results in table (3-9) and figure (3-11) clarified a significant decrease ($p<0.01$) in percentage of dead sperms after treatment with *S. lappa* extract at doses (200 and 400) mg/kg, with a percentage of dead sperms are (13.00 $\pm$ 0.71 and 13.20 $\pm$ 0.58) respectively when compared with negative control (water treatment) (35.00 $\pm$ 0.71) and positive control (mesterolone drug) (18.40 $\pm$ 0.51) while the group treated with dose (600) mg/kg a significant increase ($p<0.01$) in percentage of dead sperms was observed when compared with other groups treated with (200 and 400) mg/kg. In table (3-10) and figure (3-12) there is a significant decrease ($p>0.01$) in percentage of dead sperms after treatment with

C. esculentus extract at doses (200, 400 and 600) mg/kg, with a percentage of dead sperms (12.40 ± 0.51, 13.00 ± 0.71 and 13.40 ± 0.67) respectively when compared with negative control (water treatment) (35.00 ± 0.71) and positive control (mesterolone drug) (18.40 ± 0.51).

Table (3_9): Effect of *S.lappa* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *S. lappa* on percentage of sperms viability in mice (mean ± SD)

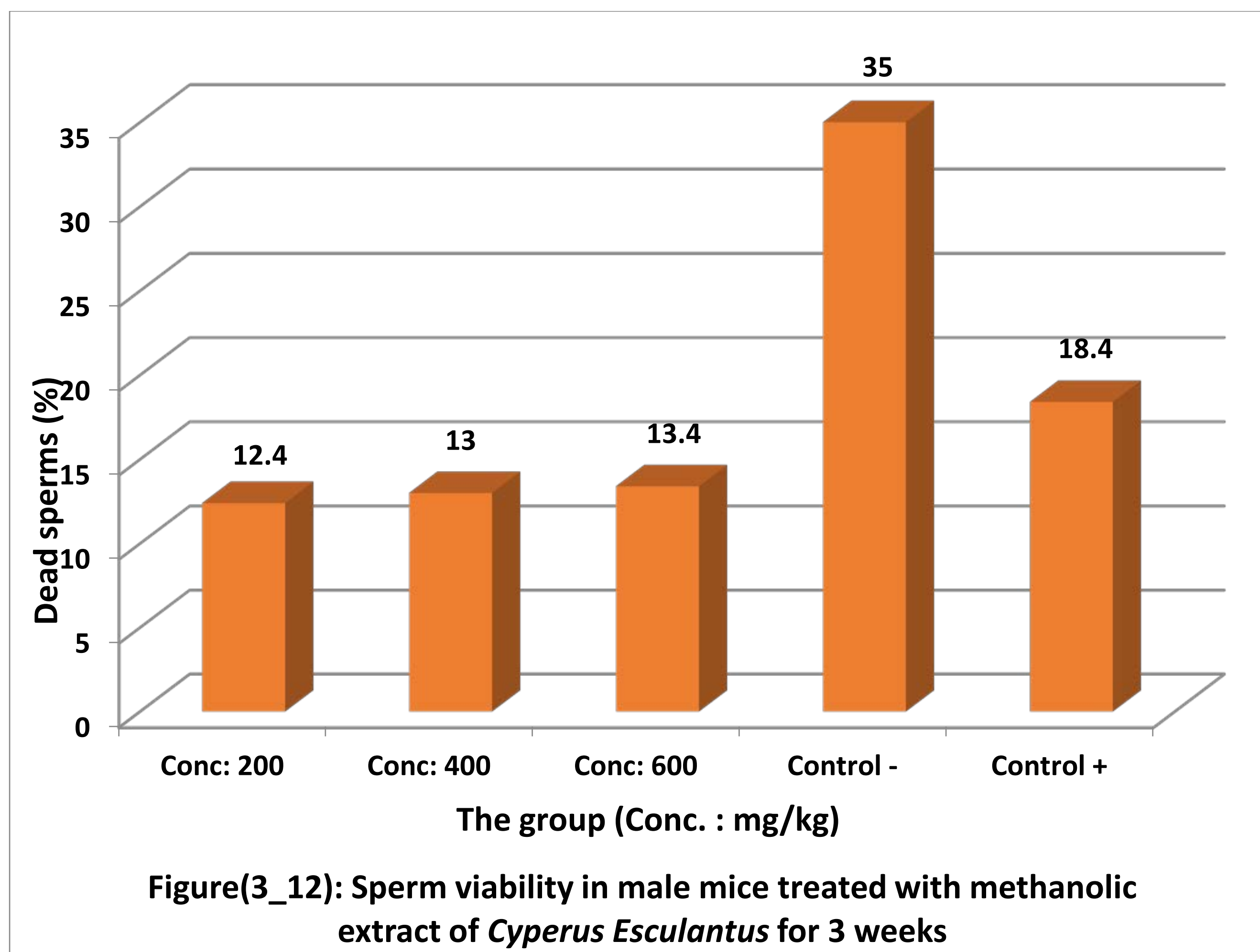
Mice groups	Mean ± SD
	Dead sperms (%)
200 (mg/kg)	13.00 ± 0.71 d
400 (mg/kg)	13.20 ± 0.58 d
600(mg/kg)	40.00 ± 0.71 a
Negative Control (water)	35.00 ± 0.71 b
Positive Control (mesterolone)	18.40 ± 0.51 c
LSD value	1.912 **
P-value	0.0001
** ($P<0.01$). Means having with the different letters in same column differed significantly.	



Figure(3_11): Sperm viability in male mice treated with methanolic extract of *Saussaria Lappa* for 3 weeks

Table (3_10): Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on percentage of sperms viability in mice (mean ± SD)

Mice groups	Mean ± SD
	Dead sperms (%)
200 (mg/kg)	12.40 ± 0.51 c
400 (mg/kg)	13.00 ± 0.71 c
600(mg/kg)	13.40 ± 0.67 c
Negative Control (water)	35.00 ± 0.71 a
Positive Control (mesterolone)	18.40 ± 0.51 b
LSD value	1.856 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	



Figure(3_12): Sperm viability in male mice treated with methanolic extract of *Cyperus Esculantus* for 3 weeks

The low doses of *S. lappa* were more effective on sperms viability than high doses; this could be explained that high dose (600 mg/kg) may be toxic. While in the case of *C. esculentus* methanolic extract the results showed significant decrease ($p>0.01$) in percentage of dead sperms after treatment with *C. esculentus* extract at doses (200, 400 and 600) mg/kg, when compared with negative control (water treatment) and positive control (mesterolone drug). Flavonoids, like Rutin has shown a significant stimulating effect on sperm parameters like sperm count, sperm morphology and sperm viability, these results were in agreement with other studies on flavonoids effect on male reproductive system, flavonoids including (quercetin) showed positive effect on the function of prostate (Shuk-mei ho, 1993). In this study, it has found that oral administration of *C. esculentus* extract significantly enhanced certain sperm

function parameters such as sperm concentration, sperms viability and percent of abnormal sperms. *C. esculentus* locally known as Hhabb el aziz which used to treat male infertility and increase sperm count (Bedevian, 1994).

3.6 Histopathological Evaluation of Liver, Kidney and Testes

Different histopathological changes were observed in liver, kidney and testes of groups of mice. For the ease of presentation, under each picture, the histopathological profile is given.

- Liver section of mice treated with water as negative control showing normal looking appearance of hepatocyte cells around central vein figure (3-13).
- Kidney section of mice treated with water as negative control showing normal histological changes which consist of glomerulus, proximal convoluted tubules and distal convoluted tubules figure (3-14).
- Testis section of mice treated with water as negative control showing seminiferous tubules with normal maturation of spermatogonia and the presence of spermatid and sperms inside the lumen figure (3-15).

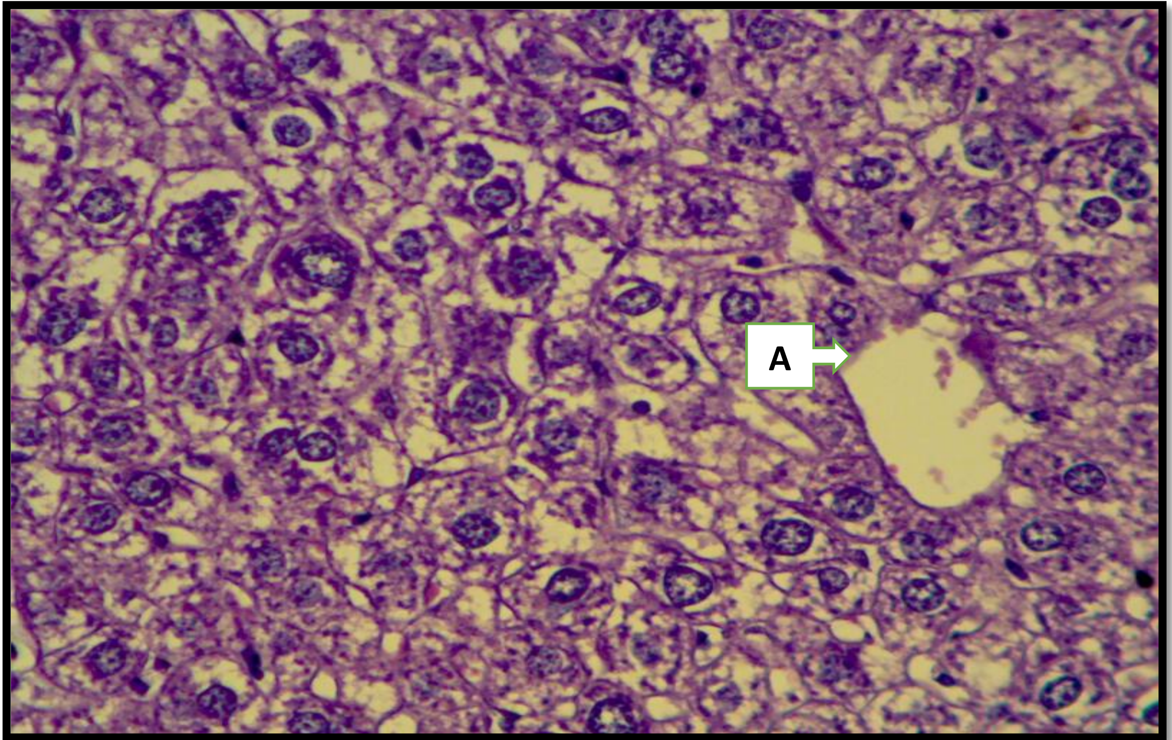


Figure (3-13): liver section of mice treated with (water) showing normal appearance of hepatocyte cells **(A)** central vein (400X; H&E).

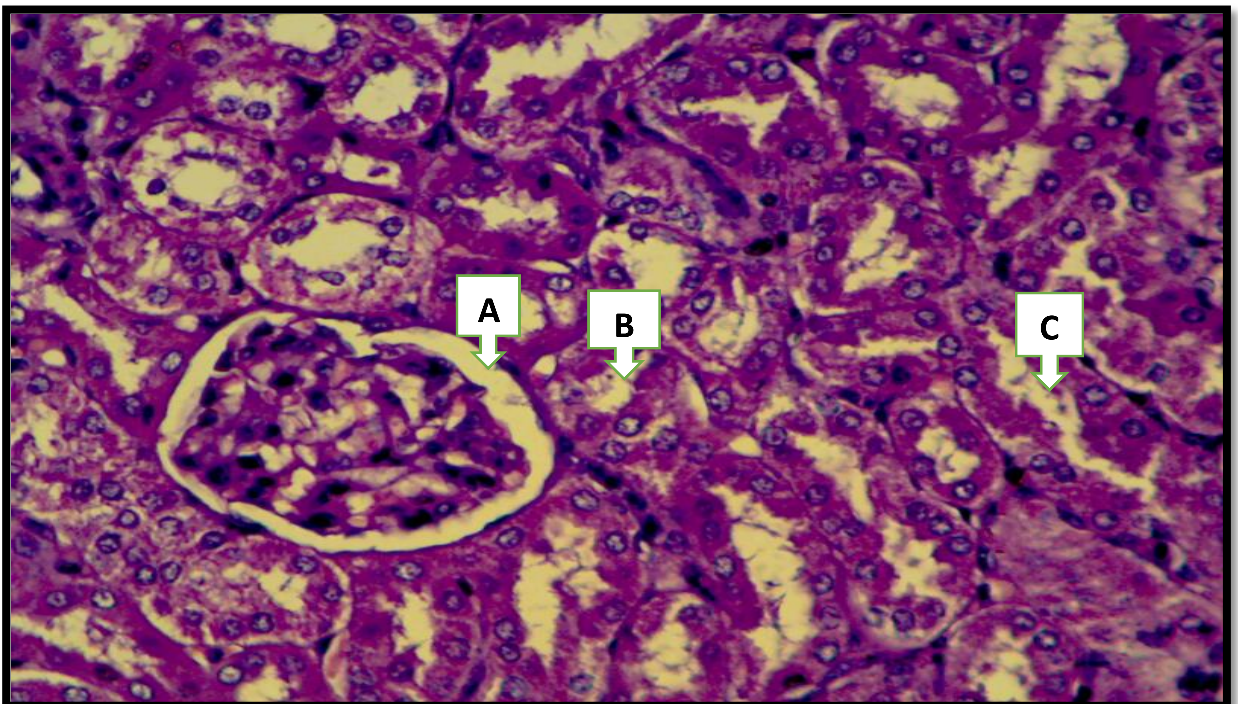


Figure (3-14): kidney section of mice treated with (water) showing normal appearance (A) represent the glomerulus; (B) represent the proximal convoluted tubule and (C) distal convoluted tubules (400X; H&E).

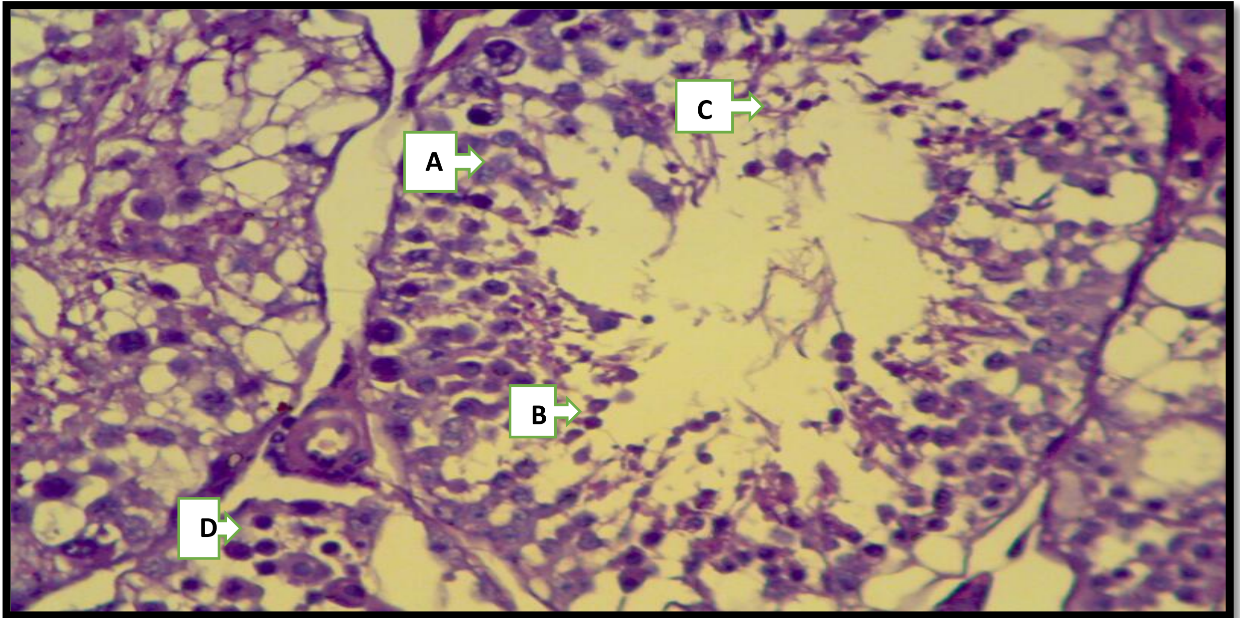


Figure (3-15): testis section of mice treated with (water) showing normal maturation of spermatogonia cells(A) with the appearance of spermatid(B) and sperm(C) and leydig cell (D) (400X; H&E).

- Liver section of mice treated with Proviron as positive control showing normal looking appearance of hepatocyte cells around central vein; with very mild dilatation of sinusoids figure (3-16).
- Kidney section of mice treated with Proviron as positive control showing normal histological changes which consist of glomerulus, proximal convoluted tubules and distal convoluted tubules with only congestion of the blood vessels figure (3-17).
- Testis section of mice treated with Proviron as positive control showing normal maturation of spermatogonia with the presence of spermatid and sperms inside the lumen. The section also showing hyperplasia of leydig cells figure (3-18).

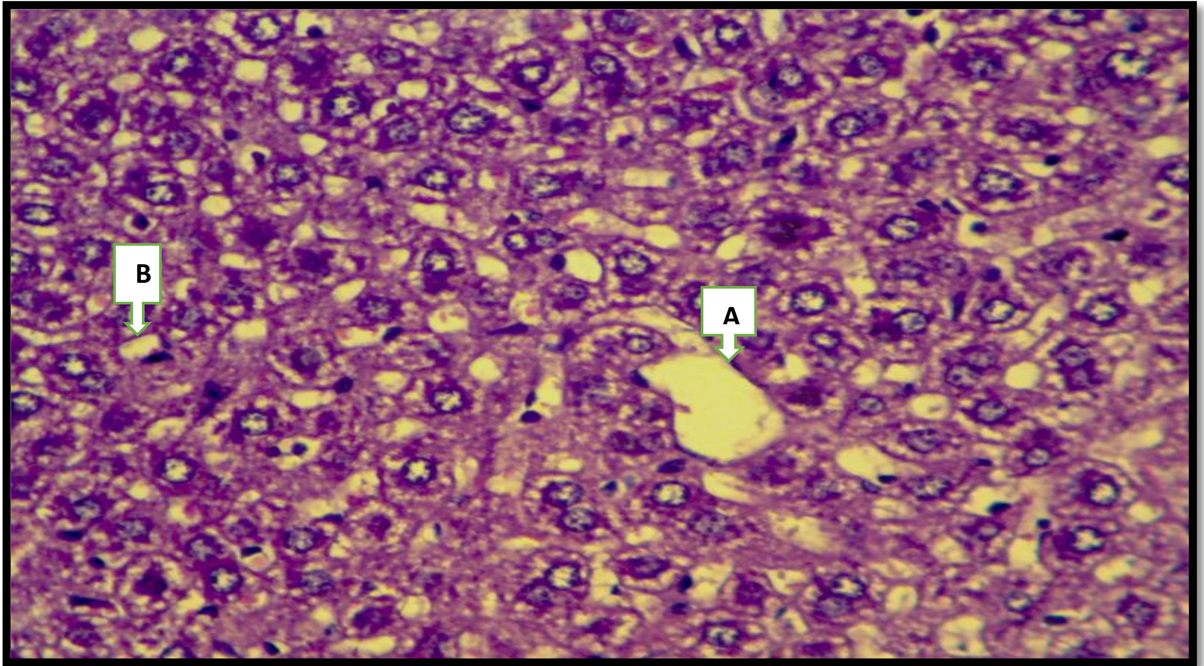


Figure (3-16): liver section of mice treated with (proviron) showing normal appearance of hepatocyte cells (A) central vein with mild dilatation of sinusoids (B) (400X; H&E).

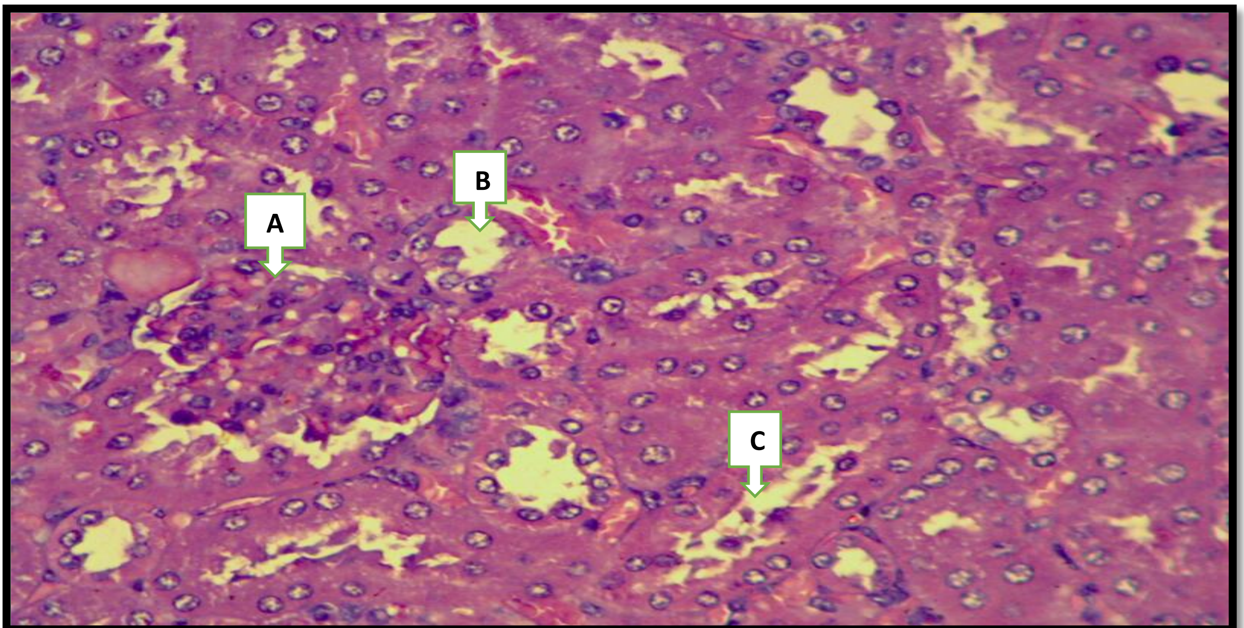


Figure (3-17): kidney section of mice treated with (proviron) showing normal appearance (A) represent the glomerulus; (B) represent the proximal convoluted tubule and (C) distal convoluted tubules (400X; H&E).

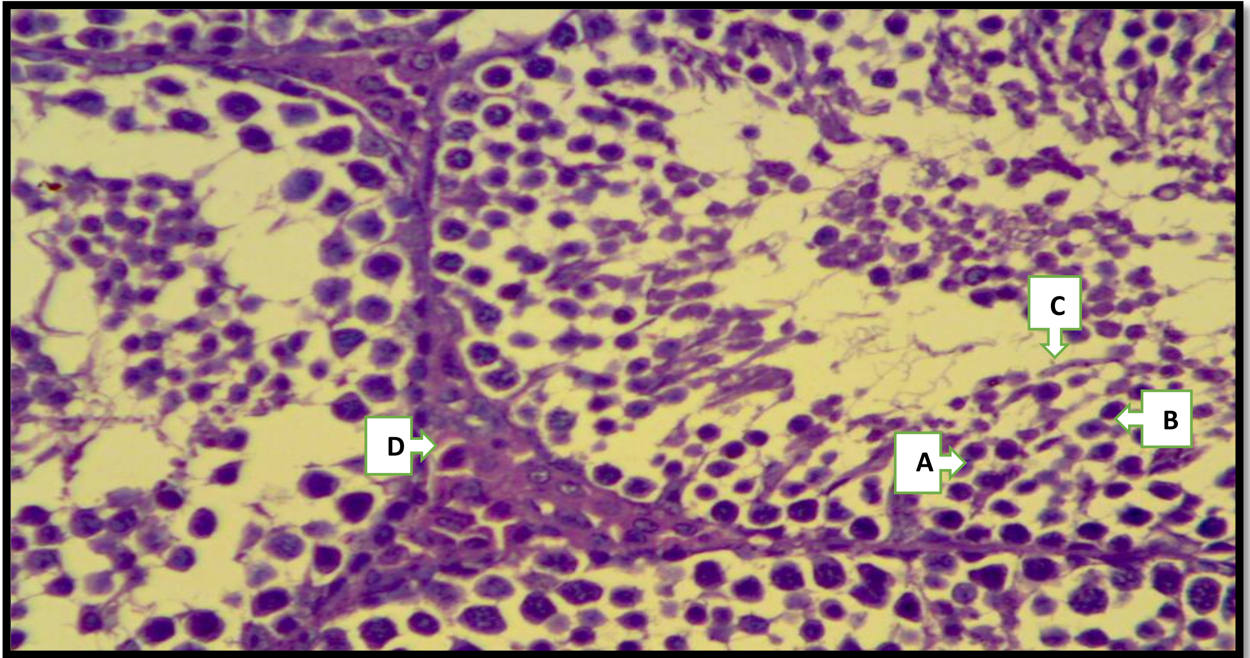


Figure (3-18): testis section of mice treated with (proviron) showing normal maturation of spermatogonia cells(A) with the appearance of spermatid(B) and sperm(C) The section also showing hyperplasia of leydig (D) (400X; H&E).

- Liver section of mice treated with *S. lappa* extract at dose 200 mg/kg showing normal appearance of hepatocyte cells around the central vein, with congestion (3_19).
- Kidney section of mice treated with *S. lappa* extract at dose 200 mg/kg showing normal appearance of renal tissue which consist of glomerulus, distal convoluted tubules and proximal convoluted tubules figure (3-20).
- Testis section of mice treated *S. lappa* extract at dose 200 mg/kg showing normal maturation of spermatogonia cells with presence of numerous sperms inside the lumen figure (3-21).

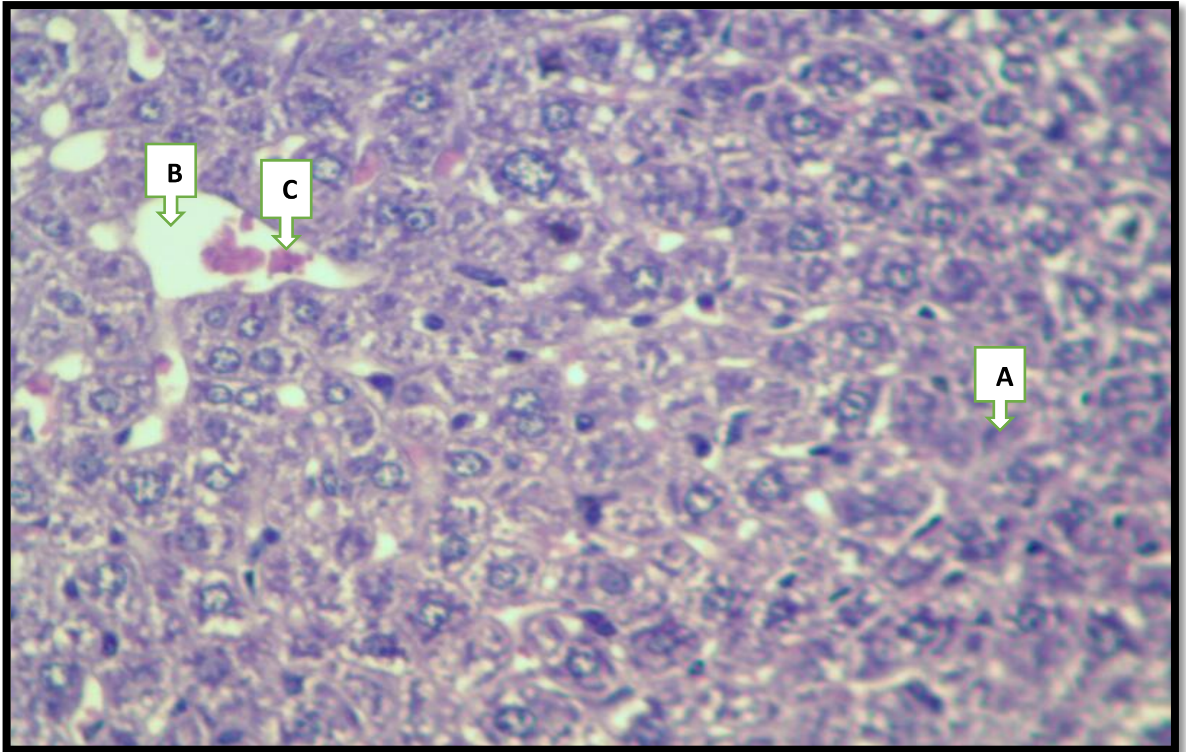


Figure (3-19): Liver section of mice treated with *S. lappa* extract at dose 200 mg/kg showing normal appearance of hepatocyte cells(A) around the central vein(B) with congestion(C) (400X; H&E).

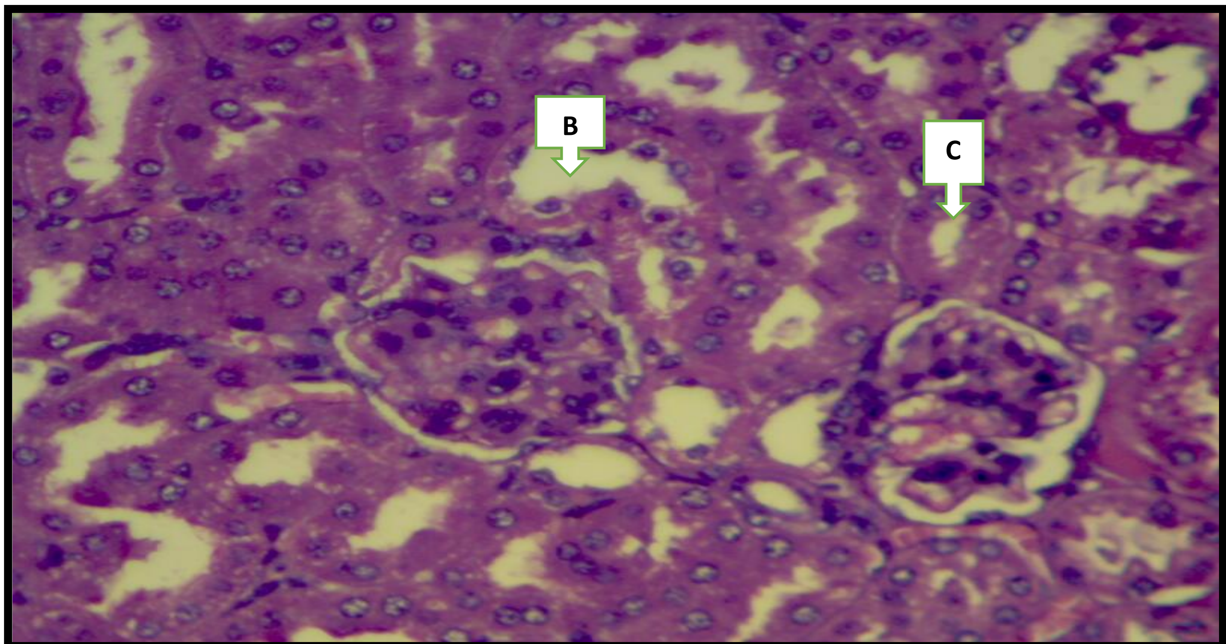


Figure (3-20): Kidney section of mice treated with *S. lappa* extract at dose 200 mg/kg showing normal appearance of renal tissue which consist of glomerulus(A) distal convoluted tubules(B) and proximal convoluted tubules(C) (400X; H&E).

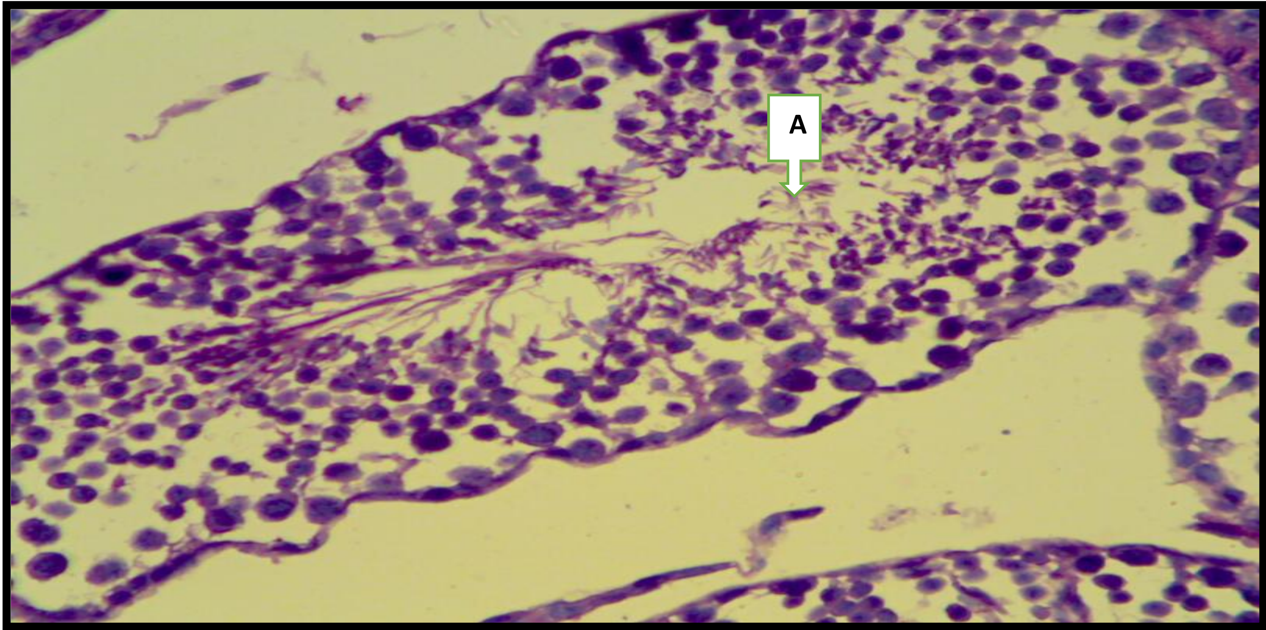


Figure (3-21): Testis section of mice treated *S. lappa* extract at dose 200 mg/kg showing normal maturation of spermatogonia cells with presence of numerous sperms(A) inside the lumen (400X; H&E).

- Liver section of mice treated with *S. lappa* extract at dose 400 mg/kg showing irregular dilation of sinusoids with fragmentation of nuclear chromatin of hepatocyte cells, with rare apoptotic cells figure (3-22)
- Kidney section of mice treated with *S. lappa* extract at dose 400 mg/kg showing mild degenerative changes of renal epithelial cells with congestion figure (3-23).
- Testis section of mice treated *S. lappa* extract at dose 400 mg/kg showing normal maturation of spermatogonia cells with presence of numerous sperms inside the lumen figure (3-24).

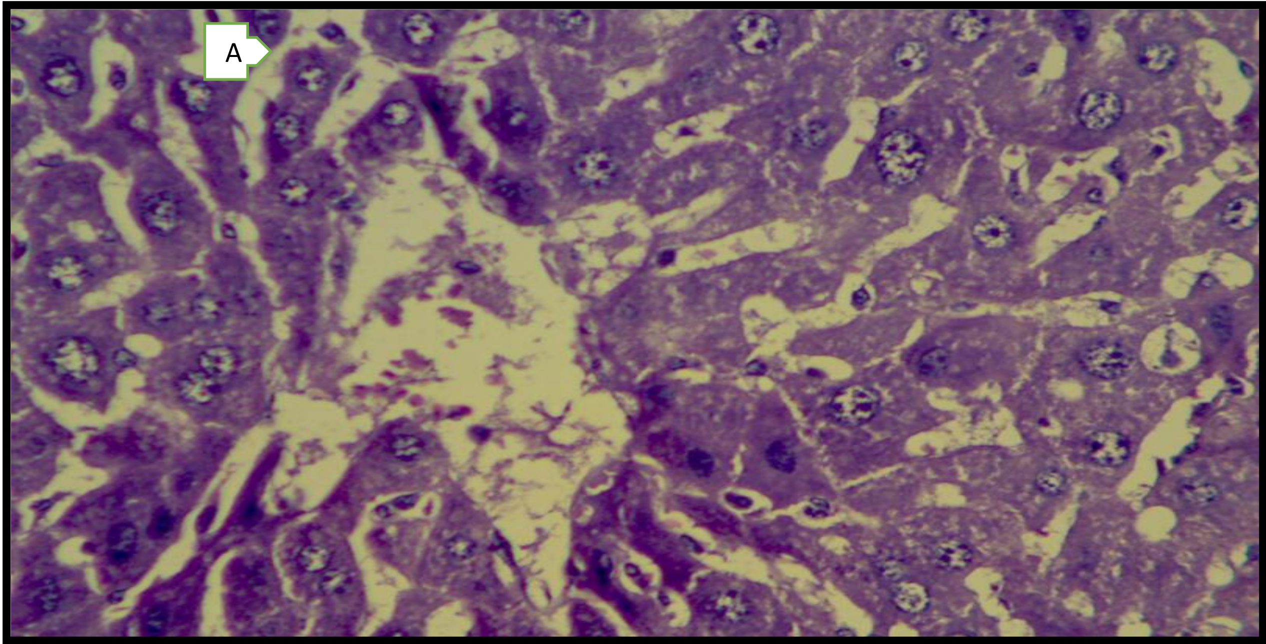


Figure (3-22): Liver section of mice treated with *S. lappa* extract at dose 400 mg/kg showing irregular dilation of sinusoids(A) with fragmentation of nuclear chromatin of hepatocyte cells, with rare apoptotic cells (400X; H&E).

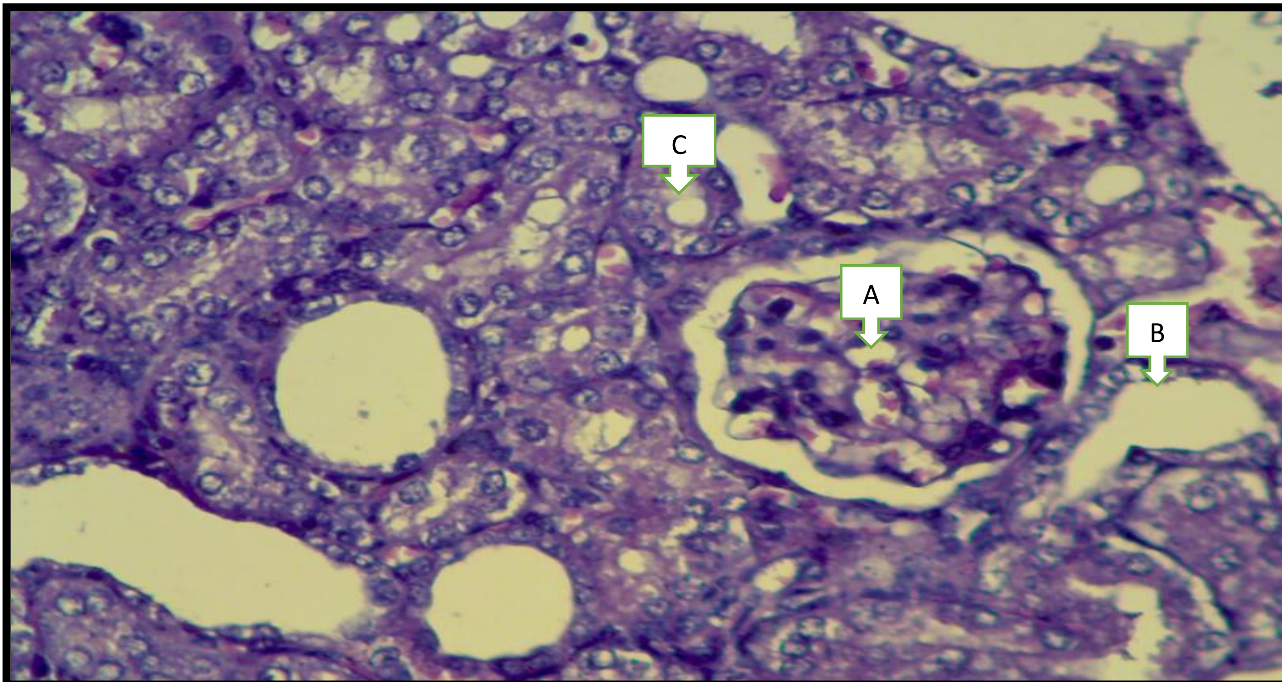


Figure (3-23): Kidney section of mice treated with *S. lappa* extract at dose 400 mg/kg showing mild degenerative changes of renal epithelial cells which consist of glomerulus(A) distal convoluted tubules(B) and proximal convoluted tubules(C) with congestion (400X; H&E).

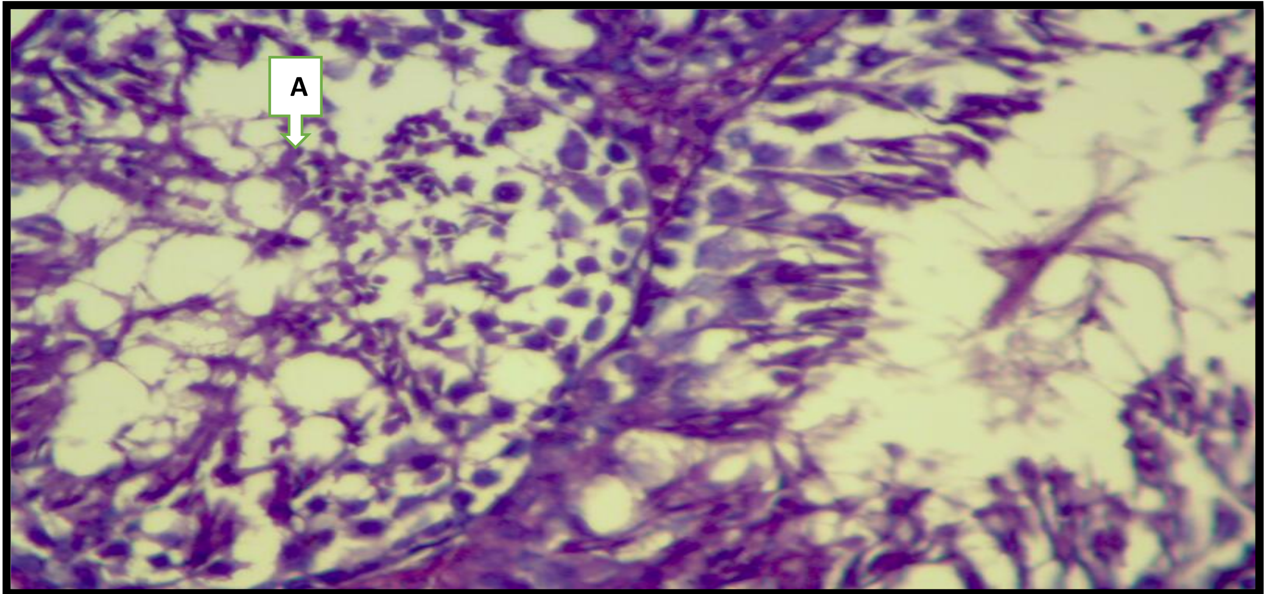


Figure (3-24): Testis section of mice treated with *S. lappa* extract at dose 400 mg/kg showing normal maturation of spermatogonia cells with presence of numerous sperms(A) inside the lumen (400X; H&E).

- Liver section of mice treated with *S. lappa* extract at dose 600 mg/kg showing sever congestion with irregular dilatation of sinusoids with present of few inflammatory cells inside the sinusoids with dispersed necrotic cells figure (3-25).
- Kidney section of mice treated with *S. lappa* extract at dose 600 mg/kg showing rare epithelial cells of renal tubules with mild degenerative changes figure (3-26)
- Testis section of mice treated *S. lappa* extract at dose 600 mg/kg showing that some of seminiferous tubules showing immaturity of spermatogonia cells and no sperm inside the lumen, while few seminiferous tubules showing maturation of spermatogonia cells with sperms inside the lumen figure (3-27).

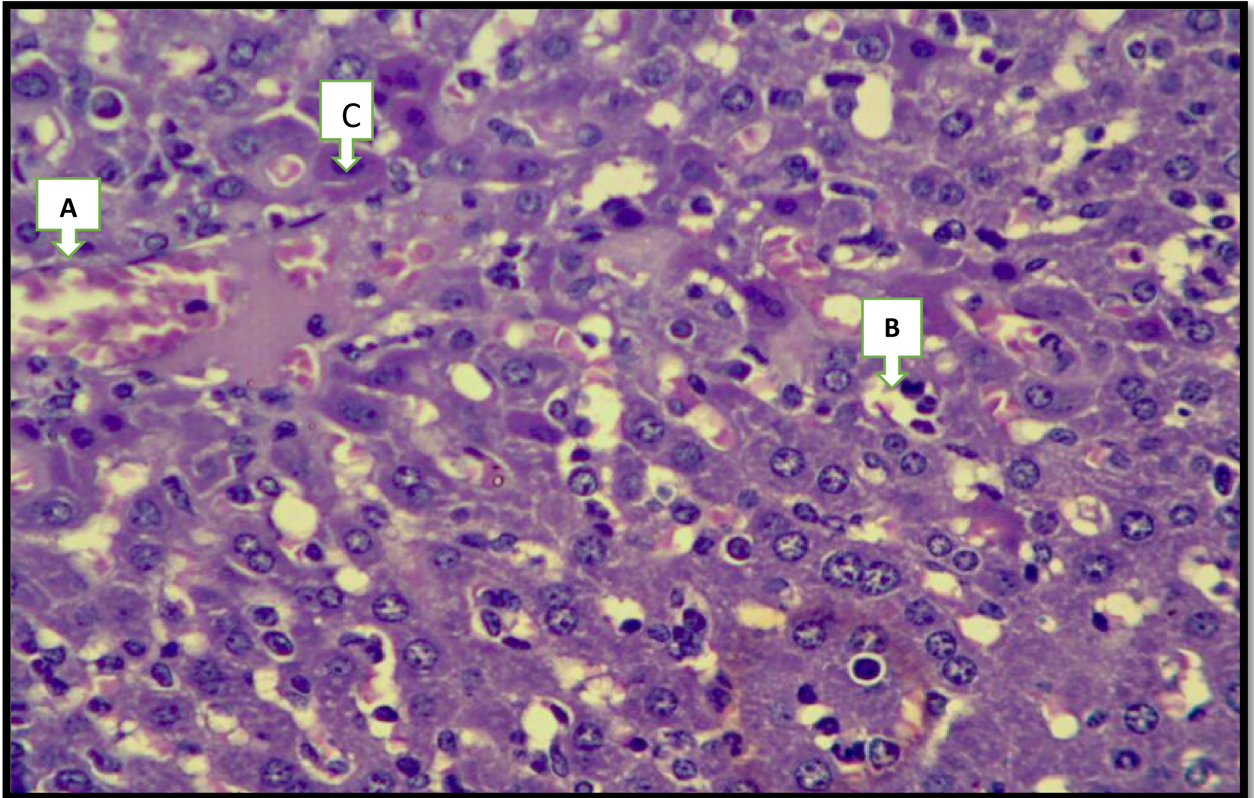


Figure (3-25): Liver section of mice treated with *S. lappa* extract at dose 600 mg/kg showing sever congestion(A) with irregular dilatation of sinusoids with present of few inflammatory cells(B) inside the sinusoids with dispersed necrotic cells(C) (400X; H&E).

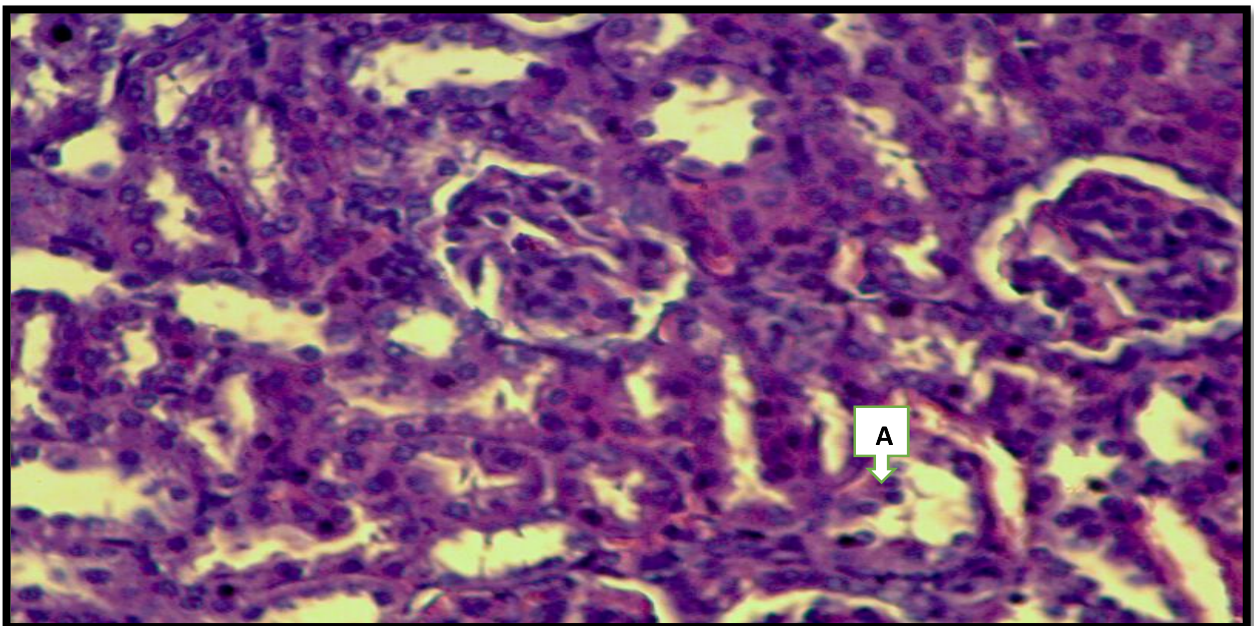


Figure (3-26): Kidney section of mice treated with *S. lappa* extract at dose 600 mg/kg showing rare epithelial cells of renal tubules with mild degenerative changes(A) (400X; H&E).

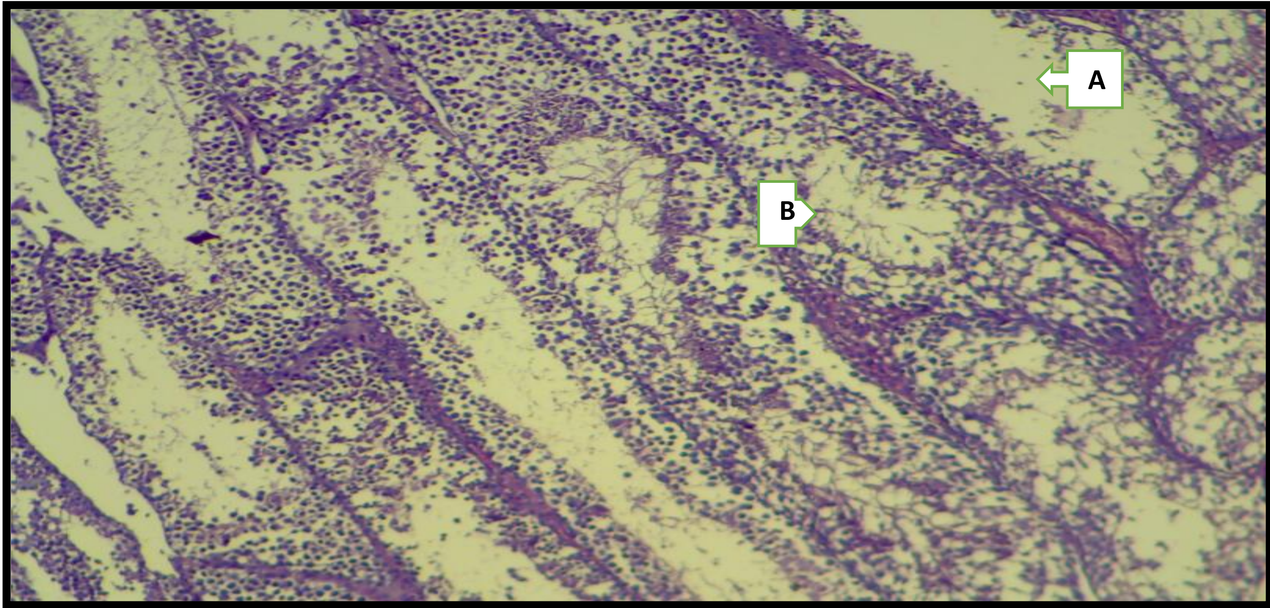


Figure (3-27): Testis section of mice treated *S. lappa* extract at dose 600 mg/kg showing that some of seminiferous tubules showing immaturity of spermatogonia cells and no sperm(A) inside the lumen, while few seminiferous tubules showing maturation of spermatogonia cells with sperms inside the lumen(B) (100X; H&E).

- Liver section of mice treated with *C. esculentus* extract at dose 200 mg/kg showing excessive accumulation of glycoprotein granules with very mild dilation of sinusoids figure (3-28).
- Kidney section of mice treated with *C. esculentus* extract at dose 200 mg/kg showing normal histological changes which consist of glomerulus, proximal convoluted tubules and distal convoluted tubules figure (3-29).
- Testis section of mice treated with *C. esculentus* extract at dose 200 mg/kg with showing normal maturation of spermatogonia and the presence of numerous sperms inside the lumen figure (3-30).

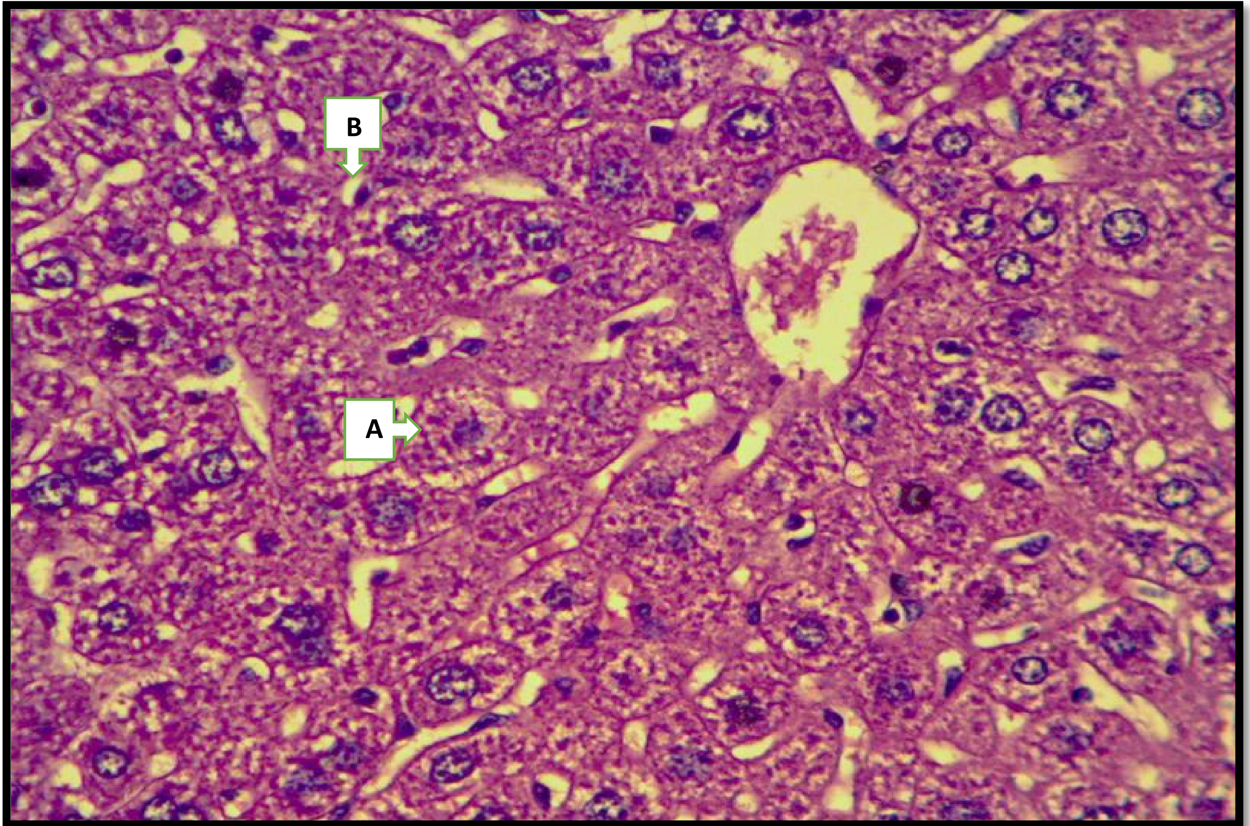


Figure (3-28): Liver section of mice treated with *C. esculentus* extract at dose 200 mg/kg showing excessive accumulation of glycoprotein granules(A) with very mild dilation of sinusoids(B) (400X; H&E).

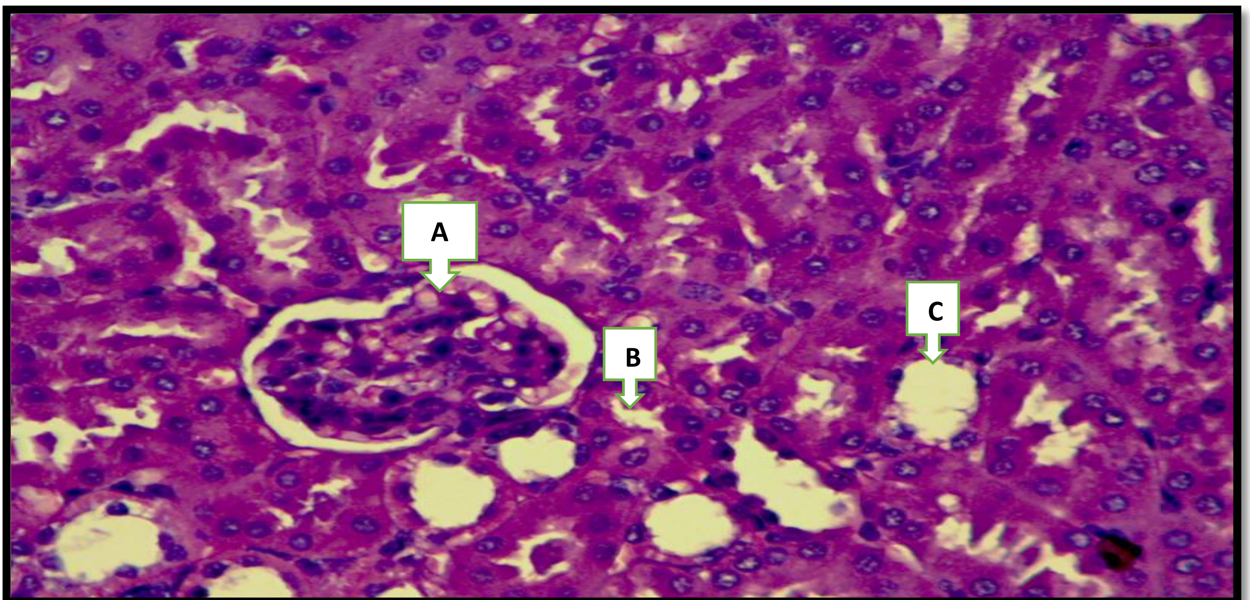


Figure (3-29): Kidney section of mice treated with *C. esculentus* extract at dose 200 mg/kg showing normal histological changes which consist of glomerulus(A) proximal convoluted tubules(B) and distal convoluted tubules(C) (400X; H&E).

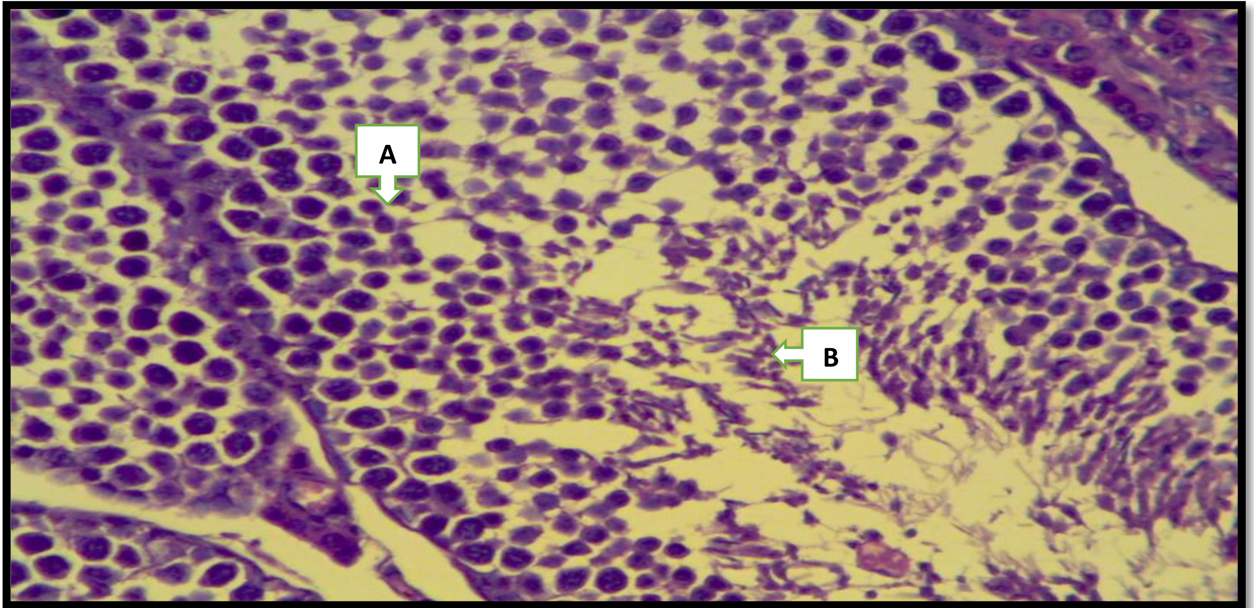


Figure (3-30): Testis section of mice treated with *C. esculentus* extract at dose 200 mg/kg with showing normal maturation of spermatogonia (A) and the presence of numerous sperms(B) inside the lumen (400X; H&E).

- Liver section of mice treated with *C. esculentus* extract at dose 400 mg/kg look-like normal histological appearance of hepatocyte cells arrange as a sheet around the central vein (3-31).
- Kidney section of mice treated with *C. esculentus* extract at dose 400 mg/kg look- like normal appearance of renal tissue which consist of glomerulus, distal convoluted tubules and proximal convoluted tubules (3-32).
- Testis section of mice treated with *C. esculentus* extract at dose 400 mg/kg showing normal maturation of spermatogonia cells with presence of sperms inside the lumen, lyedig cell look- like normal present in between seminiferous tubules figure (3-33).

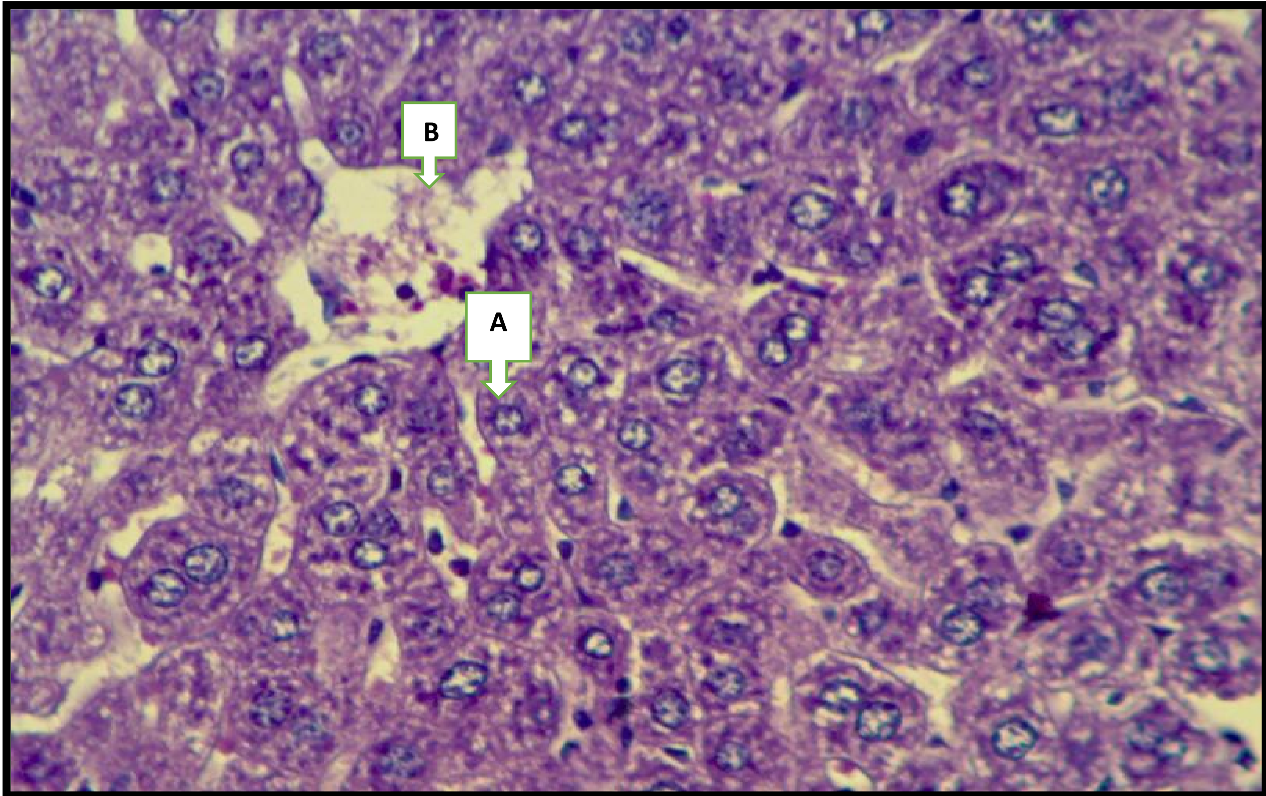


Figure (3-31): Liver section of mice treated with *C. esculentus* extract at dose 400 mg/kg look-like normal histological appearance of hepatocyte cells(A) arrange as a sheet around the central vein(B) (400X; H&E).

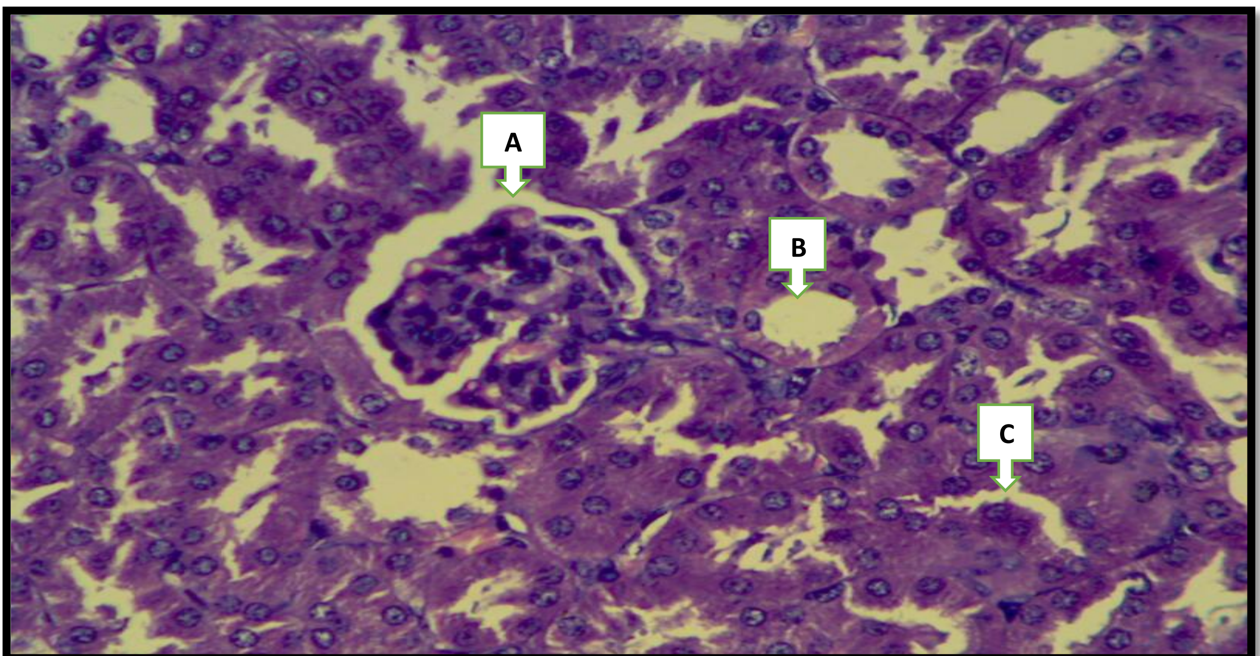


Figure (3-32): Kidney section of mice treated with *C. esculentus* extract at dose 400 mg/kg look- like normal appearance of renal tissue which consist of glomerulus(A) distal convoluted tubules(B) and proximal convoluted tubules(C) (400X; H&E).

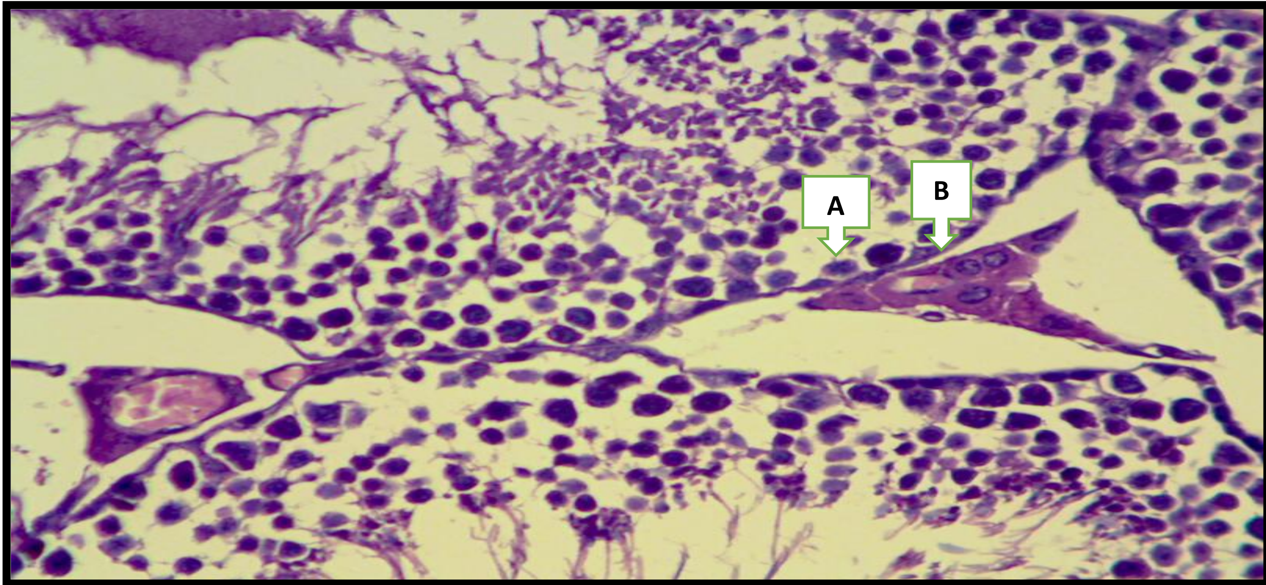


Figure (3-33): Testis section of mice treated with *C. esculentus* extract at dose 400 mg/kg showing normal maturation of spermatogonia cells (A)with presence of sperms inside the lumen, lyedig cell(B) look-like normal present in between seminiferous tubules (400X; H&E).

- Liver section of mice treated with *C. esculentus* extract at dose 600 mg/kg showing normal looking appearance of hepatocyte cells around central vein figure (3-34).
- Kidney section of mice treated with *C. esculentus* extract at dose 600 mg/kg look- like normal appearance of renal tissue which consist of glomerulus, distal convoluted tubules and proximal convoluted tubules (3-35).
- Testis section of mice treated with *C. esculentus* extract at dose 600 mg/kg showing normal maturation of spermatogonia cells with presence of few sperms inside the lumen and shrinkage of lyedig cell which are present in between seminiferous tubules figure (3-36).

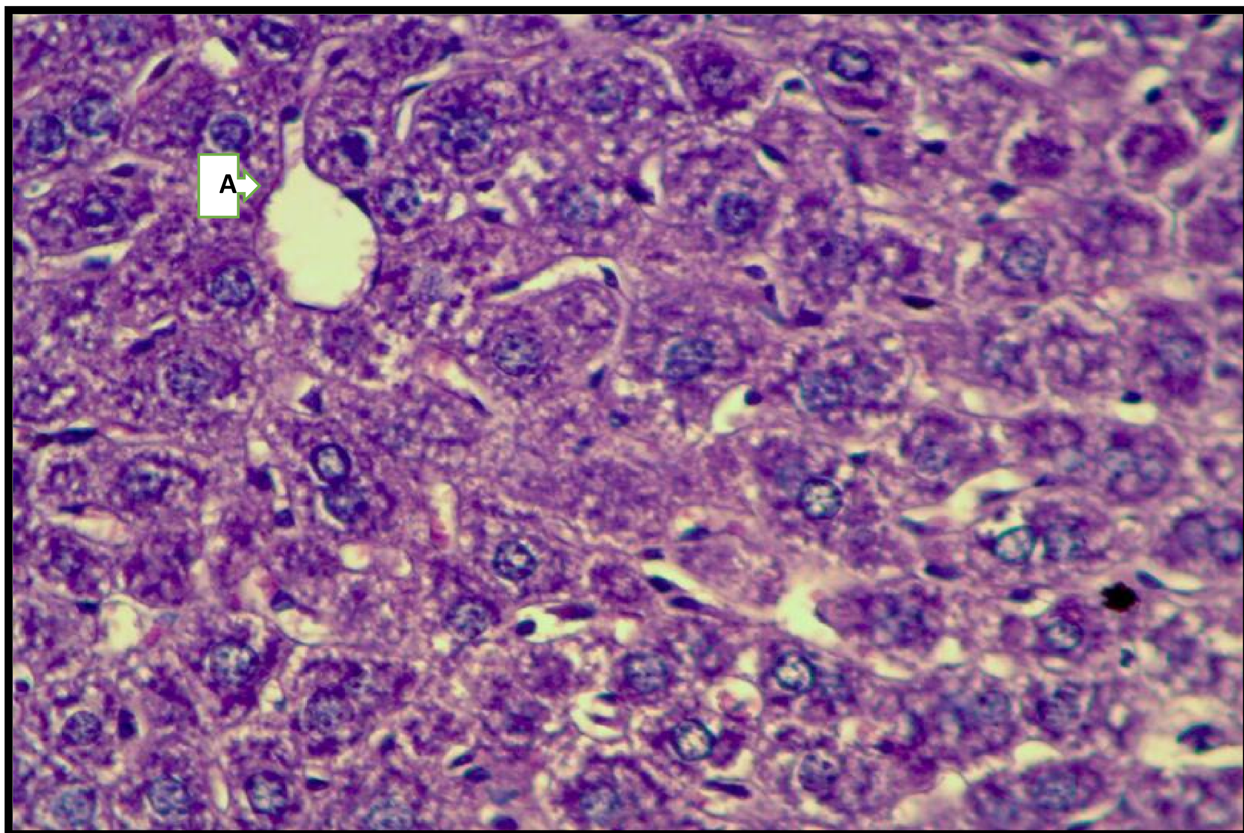


Figure (3-34): Liver section of mice treated with *C. esculentus* extract at dose 600 mg/kg showing normal looking appearance of hepatocyte cells around central vein(A) (400X; H&E).

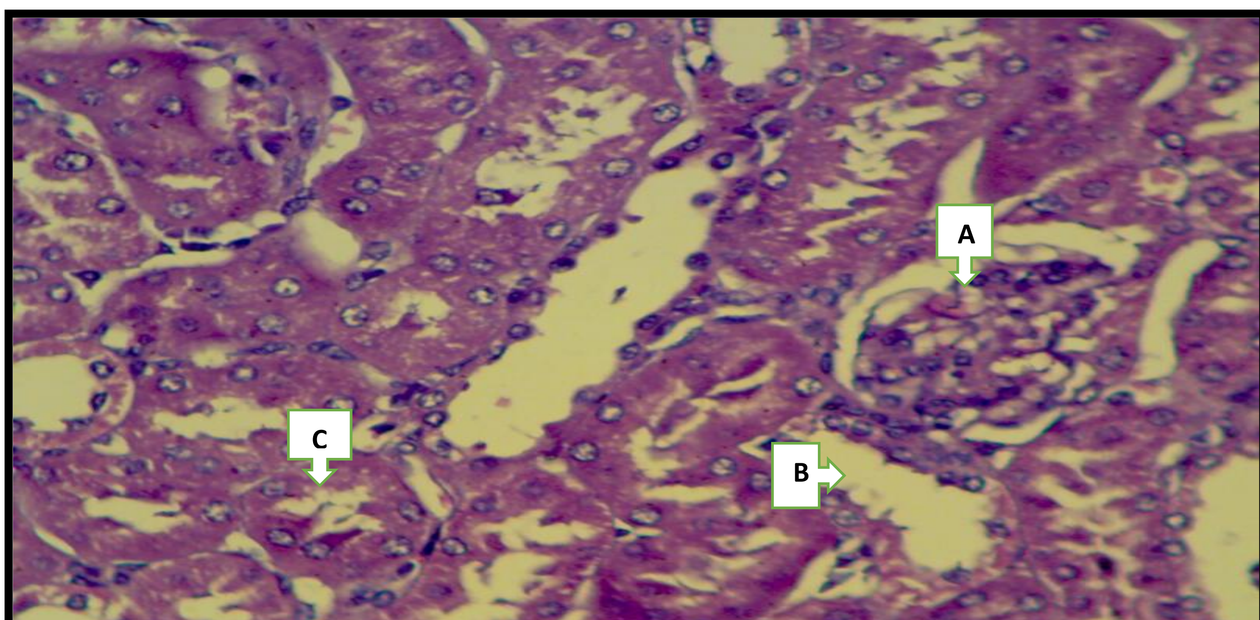


Figure (3-35): Kidney section of mice treated with *C. esculentus* extract at dose 600 mg/kg look- like normal appearance of renal tissue which consist of glomerulus(A) distal convoluted tubules(B) and proximal convoluted tubules(C) (400X; H&E).

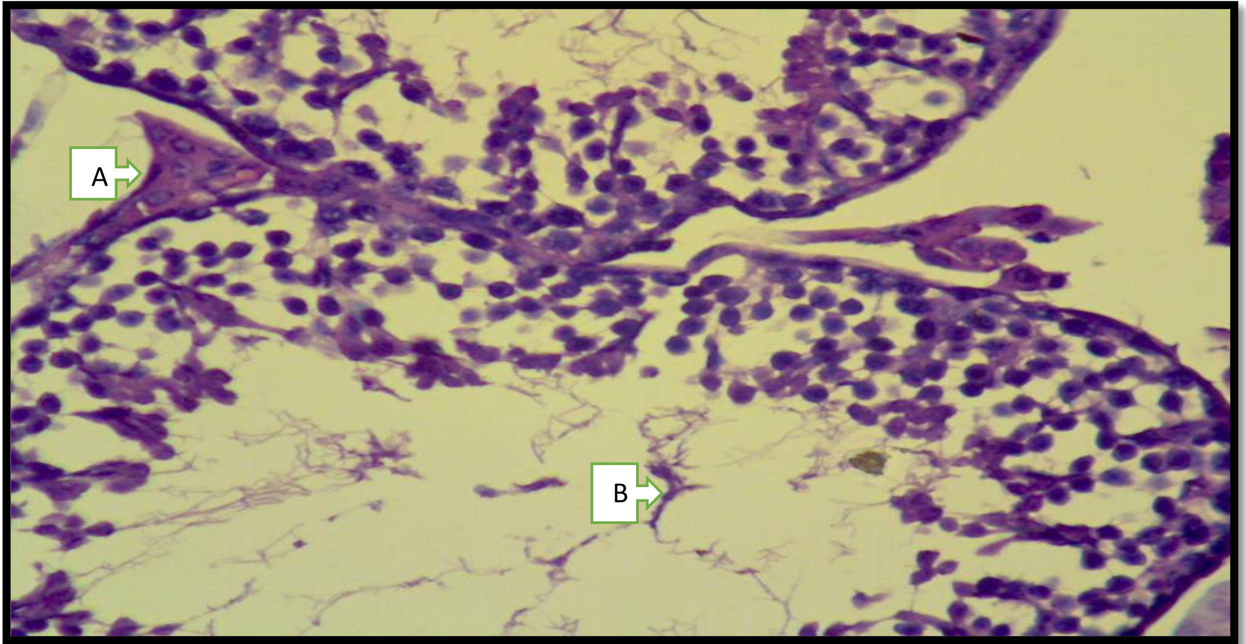


Figure (3-36): Testis section of mice treated with *C. esculentus* extract at dose 600 mg/kg showing normal maturation of spermatogonia cells with presence of few sperms(A) inside the lumen, shrinking of lyedig cell(B) were present in between seminiferous tubules (400X; H&E).

Mice representing negative control (treatment with water) showed the presence of the normal appearance of liver, kidney and testis. Mice treated with positive control (proviron) also appeared with the normal appearance of liver, kidney and testis. There is no variation in liver and kidney of mice treated with *S. lappa* extract at dose 200 mg/kg and mice treated with *C. esculentus* extract at doses 200, 400 and 600 mg/kg while the testis sections of mice treated with *S. lappa* extract at dose 200 mg/kg and testis sections of mice treated with *C. esculentus* at doses 200, 400 and 600 mg/kg clarify the normal maturation of spermatogonia and the presence of numerous sperms inside the lumen. Theses doses were very active and causing an increase in serum testosterone level and increase in viability, morphology and count of sperms, this is because the *S. lappa* and *C. esculentus* extracts contained active compounds such as flavonoids (Yua *et al*., 2007) (Jing *et al*., 2016) detected in the extracts. The flavonoids are

a group of benzopyran derivatives which occur widely in plants, they are effective antioxidants because of their free radical scavenging properties and because they are chelators of metal ions (Trivedi *et al*., 2001); thus, they may protect tissue against free oxygen radicals and lipid peroxidation.

Many studies showed that treatment with antioxidants improves steroidogenesis by enhancing the primary effect of on leydig cell endocrine function along with increase circulatory testosterone production and stimulation of spermatogenesis (Prasad and Rajalakshmi, 1989).

The dose 400 mg/kg of *S. lappa* extract showed the presence of irregular dilatation of sinusoids, fragmentation of nuclear chromatin of hepatocyte cells with rare apoptotic cells with mild degenerative changes of renal epithelial cells with congestion. Mice treated with *S. lappa* extract at dose 600 mg/kg showed sever congestion with irregular dilatation of sinusoids in addition to few inflammatory cells inside the sinusoids with dispersed necrotic cells in liver section and rare epithelial cells of renal tubules, mild degenerative changes in kidney section, were observed.

The testis section of mice treated with *S. lappa* extract at dose 600 mg/kg showed that some of seminiferous tubules showing immaturity of spermatogonia cells and no sperm inside the lumen, while few seminiferous tubules showing maturation of spermatogonia cells with sperms inside the lumen, and the testis section of mice treated with *C. esculentus* extract at dose 600 mg/kg showed normal maturation of spermatogonia cells with presence of few sperms inside the lumen and shrinkage of lyedig cell which are present in between seminiferous tubules. A significant number of studies provide evidence that the biologic activities of flavonoids may play a dual role in mutagenesis and carcinogenesis. They can act as antimutagens/ promutagens and antioxidants/pro-oxidants, which is largely dependent upon the levels consumed

as well as the physiological conditions in the body. These results may be because exposure to increased levels of flavonoids, whether through the diet or by supplementation, may potentially overwhelm the system, leading to the formation of reactive oxygen species, and ultimately DNA damage. Furthermore، these effects may be enhanced in fetal development where there is rapid cell growth, which may increase sensitivity to phytochemical exposure.(Christine and Martyn , 2000), or these results can be attributed to alkaloids, according to the chemical detection both plants contain alkaloids which was demonstrated to reduce sperm counts and motility in male rats (Er *et al*., 2006). Alkaloids can penetrate the blood–testis barrier and affect the process of spermatogenesis possible mechanism may be that the administration of alkaloids results in a reactive oxygen species (ROS)-induced oxidative stress in the sperm (Wu *et al*., 2010),the effect was strongly dose dependent.

Alkaloids also have hepatotoxic and nephrotoxic effect at high doses, the mechanism by which the alkaloids exert their hepatotoxic effect could be considered that the essential action is an alkylation, brought about by an alkyl_oxygen fission.(Chugh, 2003)(Manske, 1970).

Chapter Four
Conclusions and Recommendations

4.1. Conclusions

1. Different classes of active compounds detected in *S. lappa* and *C. esculentus* methanolic extracts including alkaloids, flavonoids, saponins terpenes, steroids, resins and tannins are considered to be responsible for the activity of these two plant.
2. Methanol extracts of *S. lappa* and *C. esculentus* increase testosterone level in serum after 3 weeks of oral ingestion.
3. *S. lappa* extract and *C. esculentus* extract caused increase in sperms count, viability under two doses 200 and 400 mg/kg when compared with controls.
4. *S. lappa* and *C. esculentus* enhance fertility in male mice, high doses of *S. lappa* showed a significant histological changes in liver, kidney and testis while high doses of *C. esculentus* showed a significant histological changes in testis.

4.2. Recommendations

1. Isolation and Purification of different active compounds of *S. lappa* and *C. esculentus*.
2. Qualitative and quantitative study of different active compounds presents in *S. lappa* and *C. esculentus*.
3. Further pharmacological studies on the effects of *S. lappa* and *C. esculentus* on different organs and different hormones in male and female.
4. Establish a pharmacological controls in the use of these two plants as herbal medication by the people due to their toxicity especially with high doses.

References

References

(A)

- **Abaejoh, R.;** Djomdi, I. and Ndojouenkeu, R. (2006). Characteristics of tigernut (*Cyperus esculentus*) tubers and their performance in the production of a milky drink. Journal of Food Process and Preserve, 30: 145-163.
- **Adam, K.;** Sivropoulou, A.; Lanalas, T. and Aresnakis, M. (1998). Antifungal activity of Origanum vulgare subsp. *Hirtum, Mentha spicata, Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. Journal of Agricultural and Food Chemistry, 46: 1739-1745.
- **Adejuyitan ,J. A. (2011).** Tiger nut processing: its food uses and health benefits. American Journal of Food Technology, 6(3):197–201.
- **Adejuyitan, J. A.,** Otunola , E. T., Akande , E. A., Bolarinwa , I. F., &Oladokun , F. M. (2009). Some physicochemical properties of flour obtained from fermentation of tigernut (*Cyperus esculentus*) sourced from a market in Ogbomoso, Nigeria. African Journal Food Science , 3:51–5.
- **Agarwal, A.** and Saleh, R. A. (2002). Role of oxidants in male infertility: rationale, significance, and treatment. Urologic Clinics of North America, 29:817–827.
- **Agarwal, A.** and Said, T.M. (2004). Carnitines and male infertility Reproductive Biomedicine Online, 8: 376-384.
- **Agarwal, A.** and Sekhon, L. H. (2010). The role of antioxidant therapy in the treatment of male infertility. Human Fertility, 13(4): 217–225.
- **Agarwal, A.;** Saleh, R. A., and Bedaiwy, M. A. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction. Fertility and Sterility, 79: 829–843.

- **Agbai ,E. O.** and Nwanegwo, C. O. (2013).Effect of methanolic extract of *Cyperus esculentus*L. (Tigernut) on luteinizing hormone, follicle stimulating hormone, testosterone, sperm count and motility in male albino wistar rats. Journal of Medicine and Biosciences ,5:52–61.
- **Agostini, A.** and Lucas, H. (2005). Semen analysis. Postgraduate Course for Training in Reproductive Medicine and Reproductive Biology. 31:36.
- **Ahmad, F.;** Khan, R. A. and Rashid, S. (1998). Pharmacological evaluation of medical plants for their analgesic activity in mice. Medical Journal of the Islamic Republic of Iran ,10 (2): 149-152.
- **Akyon, Y. (2002).** Effect of antioxidants on the immune response of Helicobacter pylori. Clinical Microbiology, 8: 438-441.
- **Al-Abid, M. R. (1985).** Zur *zusammen* setrung der Absschlu B memtrame in phoenix dactily Frawuzburg University, Wurzburg F. R. of Germany.
- **Alegrıa-Toran, A.** and Farre-Rovira, R. (2003). Horchata y salud: Aspectos nutricionales y dieteticos. In: Fundaci ´ on Valenciana de Estudios Avanzados, editor. Jornada Chufa y Horchata: Tradicion y Salud. Valencia, Spain: ´ Conseller´ıa de Agricultura, Pesca y Alimentacion, 55–70.
- **Al-Shaikh, M. N.;** Abdul Wahab , T. A. ; Abdul Kareem, S. H. ; Hamoudi, S. R. (2013). Protective effect of chufa tubers (*Cyperus esculentus*) on induction of sperm abnormalities in mice treated with lead acetate. International Journal of Drug Development and Research , 5:387–392.
- **Amadi, B.A.;** C.O. Ibegbulem ,and A.C. Egbebu, (2006). Assessment of the effect of aqueous extract of (*Asimina triloba*) root on organ weights and liver function of albino rats. International Journal of Natural and Applied Sciences ,2: 79-81.

- **Amini, A. (2005).** The effects of gossypol on spermatogenesis in NMRI mice. Iranian Journal of Science and Technology (Sciences) , 29(1), 123-133.
- **Anderson , J.** W. ;Baird, P. ; Davis , R. H. ; Ferreri, S. ; Knudtson, M. ; Koraym, A. (2009). Health benefits of dietary fibre. Nutr Rev, 67:188–205.
- **Arab, Y. M.;** Al-Flaugy, S. N.; Karmasha, F. N. and Yas, M. A. (1989). Anatomy and Physiology, Ministry of High Education and Scientific Research, Baghdad University, Al-Hekma house.
- **Arokiyaraj, S.;** Perinbam, K.; Agastian, P. and Balaraju, K. (2007). Immunosuppressive effect of medicinal plants of kolli hills on mitogen-stimulated proliferation of the human peripheral blood mononuclear. Indian Journal of Pharmacology ,39:180-183.
- **Aswal, B. S.** and Mehrotra , B. N. (1994). Flora of Lahaul-Spiti (A cold desert in North Western Himalaya), Published by Bishen Singh Mahendra pal Singh, Dehra Dun, Uttarakhand, India, 591.
- **Austin, C. R.** and Short, R. V. (1982). Reproduction in mammals (book 1): Germ cells and Fertilization. 2nd ed. Cambridge University press.
- **Awasthi, P.;** Sood, I. and Syal, S. (2008). Isolating diesel-degrading bacteria from air. Current Science, 94:168_180.

(B)

- **Bancroft, J. D.** and Stevens, A. (1982). Theory and Practice of Histological Technique. Churchill living stone. Edinburgh, London. 662.16.
- **Barens, J. (2002).** An introduction to herbal medicine products. The pharmaceutical Journal 268: 304-306.
- **Basu BD,** Kirtikar KR.(1987). Indian medicinal plants. Derhadun: International Book Distributors; p. 915.

- **Bearden, H. J.** and Faquay, J. W. (1992). Applied Animal Reproduction. 3rd ed. Simen and Schuster company, Englewood and Cliffs, New Jersey.
- **Bedevian, A. k. (1994).** Illustrated polyglottic dictionary of plant. Pp 222.
- **Belewu, M.A.** and K.Y. Belewu.(2007). Strain physicochemical evaluation of tigernut, soybean and coconut milk sources. International Journal of Agriculture and Biology ,5: 785- 78.
- **Berne, R. M.** and Levy, M. M. (1988). The reproductive glands, in physiology. Berne, R. M. and Levy, M. M. (eds.), The C.V. Mosly Company. St. Louis.
- **Bixquert-Jimenez , M. (2003).** Horchatay Salud: Propiedades saludablesy de prevencion de enfermedades digestivas in: Fundaci on Valenciana de Estudios Avanzados, editor. Jornada Chufa y Horchata: Tradicion y Salud. Valencia, ´ Spain: Conseller´ıa de Agricultura, Pesca y Alimentacion, 71–85.
- **Bjelakovic, G.;** Valko, M. and Wolf, W. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA, 297: 842-857.
- **Borges, O.;** Gonc¸alves ,B. ; Sgeoeiro, L; Correia , P. ; Silva, A. (2008). Nutritional quality of chestnut cultivars from Portugal. Food Chemistry, 106:976–84.
- **Bosch, L. ;** Alegrıa-Toran, A. and Farr e-Rovira, R. (2005). RP-HPLC determination of tiger nut and orgeat amino acid contents. Food Science Technology International ,11:33–40.
- **Bose, B. C.;** Saifi, A. Q.; Vijayvargiya, R. and Sharma, S. K. (1961), Some aspects of phytochemical and pharmacological study of *Saussurea lappa clarke*. Journal of Pharmaceutical Sciences ,50: 679–681.

- **Botsoglou, N. A.;** Christaki, E.; Fletouris, D. J.; Florou-Paneri, p. and Spais, A.B. (2002). The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. Meat Science 62:259-265.
- **Brown, M. J.;** Henderson, D. E. and Hunt, C. H. (2006). Composition of antioxidant properties of supercritical fluid extracts of herbs and the confirmation of pinocembrin as a principle antioxidant in Mexican Oregano (Lippa gravolens). Electronic Journal of Environmental Agriculture and Food Chemistry, 5:1265-1277.
- **Bermer, K. (1994).** Asteraceae_Ladistic and Classification. Timber Press, Portland, Oregon, U.S.A.

(C)

- **Caius, J. F. (1998).** The medicinal and poisonous plants of India. Jodhpur: Scientific Publishers, 167–168.
- **Chandel, A.,** Dhindsa, S., Topiwala, S., Chaudhuri, A., & Dandona, P. (2008). Testosterone concentration in young patients with diabetes. Diabetes care, 31(10), 2013-2017.
- **Chauhan , N. S. ;** Sharma, V. ; Dixit, V. K. and Tahkur, M. (2014). A review on plants used for improvement of sexual performance and virility. BioMed Res, 1-19.
- **Chadha, Y. R. (1972).** The wealth of India, Vol. IX : Rh-So, CSIR, New Delhi, 196.
- **Chen , X. ;** Chen , D. and Liu, L. (2008). *Cyperus esculentus* whole component analysis. Food and Science Technology, 34:165–168 .
- **Choi , E. M. ;** Kim, G. H. and Lee, Y. S. (2009). Protective effects of dehydrocostus lactone against hydrogen peroxide-induced dysfunction and oxidative stress in osteoblastic MC3T3-E1 cells. Toxicol In Vitro, 23: 862-867.

- **Christine, F.;** Skibola and Martyn T. Smith.(2000). Potential Health Impacts Of Excessive Flavonoid Intake, Free Radical Biology and Medicine, Vol. 29 pp. 375–383
- **Chugh, K. S. (2003).** Nephropathy associated with animal, plant, and chemical toxins in the tropics. Seminars in Nephrology, 23:49–65.
- **Chukwuma , E. R. ;** Obioma, N. and Cristopher, O. I. (2010). The phytochemical composition and some biochemical effects of Nigerian tiger nut (*Cyperus esculentus* L.) tuber. Pakistan Journal of Nutrition , **9**(7):709–15.
- **Council of Scientific and Industrial Research.(1972).** The wealth of India: a dictionary of Indian raw materials and industrial products. New Delhi: Council of Scientific and Industrial Research.

(D)

- **Dale, B.** and Elder, K. (1997). Sperm oocyte interaction, sperm parameters and sperm preparation techniques. in vitro, 20-120.
- **De Abreu Matos , F. J. ;** Cavalcanti , F. S. and Parente, J. P. (2008). Qualitative and quantitative agronomic study of *Cyperus esculentus L.* (earth almond) – an unexplored source of energetic food. Revista Ciencia Agronomica ,39(1):124–9.
- **Defelice, M. S. (2002).**Yellow nut sedge *Cyperus esculentus* L.─Snack food of the gods. Weed Technol ,16:901–907.
- **Delaszlo, H.** and Henshaw, P. S. (1954). Plant materials used by primitive people to affect fertility. Science, 199: 626-631.
- **Dorit, R. L.;** Walker, W. F. and Barnes, R. D. (1991). Zoology. Saunders college. USA.

(E)

- **Er TK,** Tsai EM, Tsai LY, Ko YC, Lee JN.(2006). In vitro effects of arecoline on sperm motility and cyclooxygenase-2 expression. Journal of Toxicology Science 31:75–82.
- **Eteshola, E.** and Oraedy, A. C. I. (1996). Fatty acid composition of tiger nut tubers (*Cyperus esculantus L.)* Baobab seeds, and their mixture. JAOCS ,(73):255-257.
- **Evans, N.S. (2005).** Trease and Evans. Pharmocognosy, 15th Edn., Elsevier, India, pp: 1-24.
- **Ezeh, O.** and Gordon , M. H. (2014). Niranjan K. Tiger nut oil (*Cyperus esculentus L.*): a review of its composition and physic-chemical properties. European Journal of Lipid Science and Technology ,116:783–794.

(F)

- **Forner, D. T.** and Conde, J. A. N. (2010). Tiger nut milk base concentrate and method for obtaining a cosmetic product from said base concentrate. U.S. Patent 20100183526 A1.
- **Foster, L. H.;** Small, J. D. and Fox, G. F. (1982). The mouse in biomedical research. Public press, INC. (London).
- **Frantisek, S.S. (1991).** The natural guide to medicinal herbs and plants. Tiger Barks Cast, Twinkemhan, United Kingdom, pp: 1-5.

(G)

- **Gleason, H. A. (1963).** Illustrated Flora of the Northeastern United States and Adjacent Canada. Vol. 1. Hafner Publishing Co., Inc., New York. 482 pp.
- **Gokhale , A. B. ;** Damre , A. S. ; Kulkarni , K. R. and Saraf, M. N. (2002). Preliminary evaluation of anti-infammatory and anti-arthritic

activity of *S. lappa*, A. speciosa and A. asoara. Phytomedicine, 9: 433-437.

- **Guyton, A. C. (1989).** Reproduction and hormonal function of the male and female. In textbook of Medical Physiology. Guyton, A. C (Eds.) W. B. Saunder, Company, Philadelphia, USA.

(H)

- **Hafez, E. S. E. (1987)** .Artificial insemination. In Reproduction in Farm Animals (Ed. Hafez E. S. E), 5th edn, pp. 481-497. Lea & Fediger, Philadelphia, PA.
- **Hafez, B.** and Hafez, E. S. (2000). Reproduction in farm animals. Awolters Kluwer Company.
- **Hamilton, A. C. (2004).** Medicinal plants, conservation and livelihoods. Biodivers Conserv, 13: 1477-1517.
- **Harborne, J. B. (1984).** Phytochemical Methods (A guide to Modern Technique of Plant Analysis), Chapman Hall, London.
- **Holm , L. G. ;** Plucknett, D. L. ; Pancho, J. V. and Herberger, J. P. (1977). The World's Worst Weeds. Distribution and Biology. Honolulu, Hawaii, USA: University Press of Hawaii.
- **Honaramooz, A.;** Snedaker, A.; Boiani, M.; Scholer, H.; Dobrinski, I. and Schlatt, S. (2002). Sperm from neonatal mammalian testes grafted in mice. Nature 418: 778-781.
- **Hsu, Y.L.;** Wu, L.Y. and Kuo, P. L. (2009). Dehydrocostuslactone, a medical plant derived sesquiterpene lactone induces apoptosis coupled to endoplamic reticulum stress in liver cancer cells. Journal of Pharmacology and experimental therapeutics 329:809_819.
- **Hyeon-Hee Yua;** Jun-Sup Leeb; Ki-Hyun Leeb; Ki-Young Kimc; Yong-Ouk Youb. (2007), *Saussurea lappa* inhibits the growth, acid

production, adhesion, and water-insoluble glucan synthesis of Streptococcus mutans. Journal of Ethnopharmacology ,111, 2, 413–417.

(I)

- **Irshad, S. ;** Mahmood , M. and Perveen, F. (2012).In-vitro anti-bacterial activities of three medicinal plants using agar well diffusion method. Research Journal of Biological , 2(1): 1-8.

(J)

- **Jaffer, H. ;** Mahmoud, M.; Jawad, A.; Naji, A. and Al Naib, A. (1983). Phytochemical and biological screening of some Iraqi plants. Fitoterapia, 59, 229-238.
- **Jain, S. P.(1984).** Ethnobotany of Morni and Kalesar (Ambala, Haryana). Journal of Economic and Taxonomic Botany , 5: 809-813.
- **Jeffrey, R.** and Gingrich, M. D. (2010). Pathophysiology Course Endocrine Module Male Gonadal Disorders (Testicular Disorders & Clinical Conferences).

(K)

- **Kalthoff, K. (2001).** Analysis of Biological Development. 2nd ed., McGraw Company New York.
- **Kang, J. S. ;** Yoon, Y. D. ; Lee, K. H. ; Park, S. K. ; Kim, H. M. (2004). Costunolide inhibits interleukin-1beta expression by down-regulation of AP-1 and MAPK activity in LPS-stimulated RAW 264.7 cells. Biochemical and Biophysical Research Communications , 313: 171-177.
- **Kirtikar, K.R.** and Basu, B.D. (2001). Indian Medicinal Plants, vol. 6., second ed. Oriental Enterprises, pp. 1961–1965.
- **Klein, B.;** Gallardo-Chacón, J.-J.; Codina-Torrella, I.; Trujillo, A.-J. and Juan, B. (2014) Evaluation of Volatile Compounds of “Tiger Nut Beverage” (Orxata de Xufla) Headspace by Optimized Solid-Phase Micro-Extraction. Open Access Library Journal, 1: e1015.

- **Knobit,** E. and Neill, J. D. (1998). Encyclopedia of Reproduction, Vol. I. Academic press. USA.
- **Ko, S. G. ;** Kim, H. P. ; Jin, D. H. ; Bae, H. S. ; Kim ,S. H. ; Park, G. H. *etal.* (2005). *Saussurea lappa* induces G2-growth arrest and apoptosis in AGS gastric cancer cells. Cancer Letters ,220: 11-19.
- **Koul , S. C. (1941).** Some wild flowers of Kashmir and their indigenous use. Journal of Bombay Natural History Society , 42: 452-454.

(L)

- **Lee, G. I. ;** Ha, J. Y.; Min, K. R. ; Nakagawa , H.; Tsurufuji, S. ; Chang, I. M. *etal.*(1995).Inhibitory effects of oriental herbal medicines on IL-8 induced in lipopolysacharide activated rat macrophages. Planta Med ,61: 26-30.
- **Lee, M. G.;** Lee, K. T. ; Chi, S. G. and Park, J. H. (2001). Costunolide induces apoptosis by ROS-mediated mitochondrial permeability and cytochrome C release. Biol Pharm Bull, 24: 303-306.
- **Lewis, W. H.** and Elvin-Lewes, M. P. F. (1997) Medicinal Botany; Plants affecting Man's health. John Wiley and Sons, New York, 515.
- **Linssen, J. P. H. ;** Kielman , G. M. ; Cozijnsen, J. L. and Pilnik, W. (1988). Comparison of chufa and olive oils. Food Chemistry , 28:279–85.

(M)

- **Mackin, J. (1993).** Cornell Book of Herbs and Edible Flowers. Cornell Cooperative Extension Ihaca, New York, U.S.A., Pp: 78- 99.
- **Mader, S. S. (1993).** Biology. 14th ed., McGraw-Hall Company. USA.
- **Malik , A. H. ;** Khuroo, A. A. ; Dar , G. H. and Khan, Z. S. (2011).Ethnomedicinal uses of some plants in the Kashmir Himalaya. Indian Journal of Traditional Knowledge, 10(2): 362-366.

- **Manna, P.;** Sinha, M. and Sil, C.P. (2006). Aqueous extract of Terminalia arjuna prevents carbon tetrachloride induced hepatic and renal disorders. BMC Complementary and Alternative Medicine, 6:33.
- **Manske, R.H. (1970).**The alkaloids chemistry and physiology. Academic press. New York. London. Pp520.
- **Martınez-Valls, J. F. (2003).** Horchata y Salud: Posibles beneficios de la horchata de chufa en la prevencion de la arteriosclerosis. In: Jornada Chufay Horchata: Tradicion y Salud, editor. Fundaci on Valenciana de Estudios Avanzados. Valencia, Spain: Conselleria de Agricultura, Pescay Alimentacion, 87–94.
- **Martini, F. H.** and Mark, D. (1998). Fundamentals of Anatomy and Physiology. Prentice-Hall, New Jersey.
- **Ma Z, H. N. T.;** Hoa H. T.; Tien, D. P.; Huynh, H. (2004). Reproduction of rat prostate weight by combined quercetin finasteride treatment is associated with cell cycle deregulation, Journal of Endocrinology,181,493-495.
- **matsuda , *etal*., (2003).** Bioorganic and Medicine Chemistry , 11, 709-715.
- **Mi Y, Z. C. (2005).** Protective effect of quercetin on Aroclor 1254 – induced oxidative damage in cultured chicken spermatogonial cells. Toxicological Sciences, 88(2), 545-550.
- **Miller, S. A.** and Harley, J. P. (1996). Zoology, 3rd ed., McGraw-Hall Company. USA.
- **Morel, I.;** Lescoat, G.; Cillard, P. and Cillard, J. (1994). Role of flavonoids and iron chelation in antioxidant action. Methods of Enzymoogy, 234:437-443.

(N)

- **Negbi, M. (1992).** A sweetmeat plant, a perfume plant and their weedy relatives: a chapter in the history of *Cyperus esculentus L.* and *C. rotundus L.* Economic Botany 46:64-71.

(O)

- **Obadina, A.O.;** Oyawole, O.B. and Ayoola, A.A. (2008). Quality assessment of Gari produced using rotary drier: In Food Processing, Methods, Techniques and Trends. Edited by Valerie C Bellinghouse. Nova Science Publishers.
- **Okaka, J.C.;** N.J. Enoch, and N.C. Okaka, (1992). Human nutrition: an integrated approach. Enugu, ESUT Publications, pp: 57-58.
- **Omode, A. A.;** O. S. Fatoki, and K. A. Olaogun, (1995). Physicochemical properties of some underexploited and nonconventional oilseeds. Journal of Agricultural Food Chemistry 43:2850-2853.
- **Ozcan, M. M. ;** Gumuscu , A; Er F; Arslan, D. and Ozkalp, B.(2010). Chemical and fatty acid composition of *Cyperus esculentus*. Chemistry of Natural Compounds , 46:276–277 .

(P)

- **Pandey , M. M. ;** Rastogi , S. and Rawat, A. K. (2007). *Saussurea costus*: Botanical, chemical and pharmacological review of an ayurvedic medicinal plant. Journal of Ethnopharmacology, 110(3): 379-390.
- **Pascual, B.** ; Maroto, J. V. ; Lopez-Galarza, S. ; Sanbautista , A. ; Alagarda , J. (2000). Chufa (*Cyperus esculentus* L. var*sativus*Boeck.): an unconventional crop. Studies related to applications and cultivation. Econ Bot. 54:439–448.
- **Pentikäinen, V.;** Erkkilä, K.; Suomalainen, L.; Parvinen, M.; and Dunkel, L. (2006). Estradiol Acts as a Germ Cell Survival Factor in the Human Testis in vitro. The Journal of Clinical Endocrinology and Metabolism, 85:2057-2067.

- **Prabakaran, M. ;** Chandrakala, N. and Panneerselvam, A. (2011) Antimicrobial activity of *Indigofera glandulosa* (wild). Asian Journal of Plant Science and Research, 1(2): 18-25.
- **Prasad, M.R.N** and M. rajalakshmi, (1989). Spermatogenesis and Accessory Gland Secretions. In Textbook of Biochemistry and human Biology, Talwar, G.P., L.M. Srivastava and L.M. Moudgil (Eds). 2nd Edn. Prentice-Hall of india Pvt. Ltd., New Delhi, India, pp:883.
- **Purvis, K.** and Christiansen, E. (1992). Male infertility current concepts. Ann Med., 24:258-272.

(R)

- **Rao, R.R.;** Chawdhery, H.J.; Hajra, P.K.; Kumar, S.; Pant, P.C.; Naithani, B.D.; Uniyal, B.P.; Mathur, R.; Mamgain, S.K. (1988). Florai Indicae Enumaratio-Asteraceae. BSI, Calcutta.
- **Rice-Evans, C. A.;** Miller, N. J. and Pagang, G. (1997). Antioxidant properties of phenolic compounds. Trends of Plant Science, 2:152-159.
- **Richard, W.;** Gordon, A. and Margaret, A. (2004). Animal Physiology. Endocrine and Neuroendocrine Physiology.1st edition. Chapter 14.
- **Ricki, L.;** Bruce, P.; Douglas, G. and Marielle, H. (2007). Life. The Endocrine System. 6th edition. Chapter 34.
- **Roger, M. (2004).** Testosterone Is Your Friend. Understanding & Controlling One of Nature's Most Potent Hormones. 5th edition. Chapter 1.

(S)

- **Saladin, K. S.** and Porth, C. M. (1998). Anatomy and Physiology, the unity of form and function. McGraw-Hall, USA.

- **Salisbury, G. W.** and Van Demark, M. L. (1961). Physiology of Reproduction and Artificial Insemination of Cattle. W. H. Freeman and Co., San Francisco and London.
- **Sarin, Y. K. (1967).** A survey of vegetable raw material resources of Lahul. Indian Forester 93: 459-462.
- **SAS. (2012).** Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- **Savikin , K. ;** Zdunić, G. ; Janković, T. ; Tasić, S. ; Menković, N. ; Stević, T. ; Dordević, B. (2009). Phenolic content and radical scavenging capacity of berries and related jams from certificated area in Serbia. Plant Foods Human Nutrition , 64:212–217.
- **Seeley, R. R.;** Stephens, T. D. and Tate, P. (1996). Essentials of anatomy and physiology. Second edition.
- **Serrallaeh, J. (1927).** Die wurzelknolte von *Cyperus esculentus L.* Ph.D. thesis, University Frankfurt am Main.
- **Sesquiterpene lactone induces apoptosis coupled to endoplasmic reticulum stress in liver cancer cells.(2009).** Journal of Pharmacology and Experimental Therapeutics, 329: 809-819.
- **Shah, N. C.(1982).** Herbal folk medicines in northern India. Journal of Ethnopharmacology, 6: 293-301.
- **Shetty, K. (1997).** Biotechnology to Harness the benefits of dietary phenolics: focus on Lamiaceae. Asia Pacific Journal of Clinical Nutrition, 6:162-171.
- **Shihata, I. M. (1951).** A pharmacological study of anagalis arvensis. M.D. Vet. Thesis, Cairo University, P.43.
- **Shittu Lukeman, A.;** Shittu Remilekun, K. and Osinubi Abraham, A. (2009). Mesterolone (Proviron) induces low sperm quality with reduction in sex hormone profile in adult male Sprague Dawley rat's testis.

- **Shuk-mei ho;** Viccione, T.; Yu, M. (1993). In-vitro and in-vivo inhibition of nuclear type II estrogen binding sites in the dorsolateral prostate of noble rats. Journal of steroid biochemistry and molecular biology, 46: 489–495.
- **Singh, S. K. (1999).**Ethnobotanical study of useful plants of Kullu districts in north western Himalayas, India. Journal of Economic and Taxonomic Botany , 23: 185-198.
- **Siqun Jing;** Saisai wang; Qian Li; Lian Zheng; Li Yue; Shaoli Fan; Guanjun Tao. (2016). Dynamic high pressure microfluidization-assisted extraction and bioactivities of *Cyperus esculentus (C. esculentus L.)* leaves flavonoids. Food Chemistry ,192 : 319–327.
- **Smolensk, S. J.;** Silinis, H. and Fransworth, N. R. (1972). Alkaloid screening. Annals of Biochemistry, 35: 31-34.
- **Sofowora, E.A. (1993).** Medicinal plants and Traditional Medicine in Africa. Ibadan-Owerri-Kaduna-Lagos Spectrum Books Limited, pp: 159-238.
- **Srivastav ,S. ;** Singh, P. ; Mishra, G. ; Jha, K. K. ; Khosa, R. L .(2011) .Achyranthes aspera-Animportant medicinal plant: A review. Journal of Natural Product and Plant Resources ,1(1): 1-14.
- **Stahle, E. (1969).** Thin layer chromatography a laboratory hand book, 2nd ed., translated by Ashworth, M. Spring-verag, Berlin, heidelberty, New York.
- **Suffredini, I. ;** Bacchia, E. ; Sertie, J. (1999) Antiulcer action of *Microgramma squamulosa* (Kaulf.) Sota. Journal of Ethnopharmacology, 65: 217.

(T)

- **Taepongsorat, L.;** Tangpraprutgul, P.; Kitana, N. & Malaivijitnond, S. (2008). Stimulating effects of quercetin on sperm quality and

reproductive organs in adult male rats. Asian journal of andrology ,10(2):249-258.

- **Te-Chi Liu;** Chia-Hua Kuo and Wang, P. S. (2009). Exercise and Testosterone. Adaptive Medicine, 1(1): 26-31.
- **Trease, J. E.** and Evans, W. C. (1987). Pharmacognocy. 13th ed., Balliere. Tindall, pp. 62-68.
- **Trivedi, P. P. ;** Tripathi, D. N. and Jena, G. B. (2011). Hesperetin protects testicular toxicity of doxorubicin in rat: role of NFkB, p38 and caspase-3. Food and Chemistry Toxicology ,49(4): 838-847.
- **Tsarong , T. J.(1986).** Handbook of traditional Tibetan drugs, their nomenclature, composition, use and dosage. India: Tibetan Medical Publications, p. 101.
- **Tunde-Akintunde , T. Y.** and Oke, M. O.(2012). Thin-layer drying characteristics of tiger nut (*Cyperus esculentus*) seeds. Journal of Food Process and Preserve , 36:457–464.

(U)

- **Uma Chandur, S. ;** Shashidhar, S.B.; Chandrasekar, M. Bhanumathy and T. Midhun. (2011). Phytochemical Evaluation and Anti-Arthritic Activity of Root of *Saussurea lappa*. Pharmacologia, 2: 265-267.

(V)

- **Vijayan, A. ;** Liju, V. B. ; John, J. V. ; Reena; Parthipan, B. ; Renuka, C. (2007) Indian Journal of Traditional Knowledge, 6(4): 589-594.

(W)

- **Wu, P. F. ;** Chiang, T. A. ; Chen, M. T. ; Lee, C. P. ; Chen , P. H. ; Ko, A. M. ; Yang, K. J. ; Chang, P. Y. ; Ke , D. S. ; Ko , Y. C. (2010). A characterization of the antioxidant enzyme activity and reproductive

toxicity in male rats following sub-chronic exposure to areca nut extracts. Journal of Hazardus Materials ,178: 541–6.

(X)

- **XD, X.;** XR, H. and JS, Y. (2008). Review of studies on active components of antitumor medicinal plants. Zhongguo Zhong Yao Za Zhi. 33: 2073-2081.
- **Xiao, Q. N. ;** Kuo, Y. H. ; Zhang , Y. ; Barker , D. M. ; Won, D. J. (2002). A tropical cyclone bogus data assimilation scheme in the MM5 3D-Var system and numerical experiments with typhoon Rusa near landfall. Journal of Meteorological Society of Japan ,84: 671-689.

(Y)

- **Yang , H. ;** Xie, J. L. and Sun, H. D. (1998). Resarch progress on the medicinal plant-*Saussurea lappa.* Tian Ran Chan Wu Yan Jiu Yu Kai Fa 10(2):90-8.
- **Yashvanth , S. ;** Robinson, A. ; Babu, K. S. ; Naidu,V. G. M. ; Vishnuvardhan , M. V. P. S. ; Ramakrishna, S. *etal*., (2010).Anti-inflammatory and cytotoxic activity of chloroform extract of roots of *Saussurea lappa* Clarke. Journal of Pharmacy Research , 3(8): 1775-1778.

(Z)

- **Zhu, J. L.;** Basso, O.; Obel, C.; Bille, C. and Olsen, J. (2006). Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. BMJ. (7570):679.
- **Zohary , D.** and Hopf, M. (1993). Domestication of plants in the Old World. 2nd ed. Oxford: Clarendon Press. 279 p.

الخلاصة

تم تصميم هذه الدراسة للكشف عن تأثير مستخلص القسط الهندي ومستخلص حب العزيز على خصوبة الفئران البيضاء الذكور اضافة الى التغايرات النسيجية في خلايا الكبد والكلى والخصي نتيجة المعاملة . أعد مستخلص القسط الهندي بعملية النقع في محلول ٩٠% الميثانول وأجري الكشف عن المواد الكيميائية من الفلافونويد والقلويدات و الصابونيات و التربينات والدهون. ساعد استخدام تقنية الكروموتوكرافي السائل عالي الجودة في الكشف عن وجود الكوارستين، الروتين، اليوتيولين، الكامفيرول والميريستين.وقد تم استخدام نفس الطريقة اعلاه لاستخلاص والكشف الكيميائي لنبات حب العزيز.

تضمنت الدراسة تأثير مستخلص القسط الهندي ومستخلص حب العزيز على تركيز الحيوانات المنوية و نسبة حيوية الحيوانات المنوية ونسبة الحيوانات المنوية المشوهة بألاضافة الى فحص تركيز هرمون التستوستيرون في الدم، كما اجريت دراسة للتأثيرات المرضية النسيجية للمركبات الفعالة للنباتين في الكبد والكلى والخصي.

تم توزيع ٤٠ من الفئران البيضاء بألتساوي الى ثماني مجموعات، المجموعة الاولى: (السيطرة السلبية) وهي الفئران التي عوملت بالماء، المجموعة الثانية: (السيطرة الايجابية) الفئران التي عوملت مع ٠.٣٦ملغم/كلغم من عقار الميسترلون، المجموعة الثالثة: الفئران التي عوملت مع ٨.٣ملغم/مل من مستخلص القسط الهندي المثانولي (٢٠٠ملغم/كغم)، المجموعة الرابعة: الفئران التي عوملت مع ١٢ملغم/مل من مستخلص القسط الهندي الميثانولي (٤٠٠ملغم/كغم)، المجموعة الخامسة: الفئران التي عولجت مع ١٣.٥٦ملغم/مل من مستخلص القسط الهندي الميثانولي (٦٠٠ملغم/كغم)، المجموعة السادسة: الفئران التي عوملت مع ٧ملغم/مل من مستخلص حب العزيز الميثانولي (٢٠٠ملغم/كغم)، المجموعة السابعة: الفئران التي عوملت مع ١٤.٨ملغم/مل من مستخلص حب العزيز الميثانولي(٤٠٠ملغم/كغم)، المجموعة الثامنة: الفئران التي تمت معاملتها مع٢٣.٤ملغم/مل من مستخلص حب العزيز الميثانولي (٦٠٠ملغم/كغم)،كانت تعطى هذه المستخلصات عن طريق الفم لمدة ٣اسابيع.

اظهرت النتائج زيادة معنوية ($p \leq 0.01$) في تركيز الحيوانات المنوية التي عوملت بمستخلص القسط الهندي وبجرعات ٢٠٠و٤٠٠ملغم/كغم مقارنة بألسيطرة السلبية والايجابية وايضا بالمقارنة مع المجموعة الاخرى التي عوملت مع المستخلص وبجرعة ٦٠٠ملغم/كغم وزيادة معنوية ($p \leq 0.01$) في تركيز الحيوانات المنوية بعد٣اسابيع من المعالجة بمستخلص حب العزيز وبجرعات ٢٠٠،٤٠٠و٦٠٠ملغم/كغم بألمقارنة مع السيطرات السلبية والايجابية.

اظهرت النتائج زيادة معنوية ($p \leq 0.01$) في نسبة الحيوانات المنوية الميتة و ذات الشكل الغير طبيعي بعد المعاملة بمستخلص القسط الهندي بجرعة ٦٠٠ملغم/كغم مقارنة بالسيطرة السلبية والايجابية وايضا بالمقارنة مع المجموعات الاخرى التي عوملت بالمستخلص النباتي بجرعات ٢٠٠و٤٠٠ملغم/كغم، وانخفاض معنوي في الحيوانات المنوية الميتة وغير طبيعية شكليا بعد المعاملة بمستخلص حب العزيز بجرعات ٢٠٠،٤٠٠و٦٠٠ملغم/كغم بالمقارنة مع السيطرات السلبية والايجابية.

وجدت زيادة معنوية ($p \leq 0.01$) في هرمون التستوستيرون في مصل الفئران التي عوملت بمستخلص القسط الهندي وبجرعات ٢٠٠و ٤٠٠ ملغم/كغم بالمقارنة مع السيطرة السلبية والايجابية وايضا بالمقارنة مع المجموعة التي عوملت بالمستخلص بجرعة ٦٠٠ملغم/كغم، وكانت النتائج في المجاميع التي جرعت بمستخلص حب العزيز مماثلة لتلك في المعاملة بمستخلص القسط الهندي.

وقد لوحظت تغييرات نسيجية مرضية مختلفة في الكبد والكلى والخصيتين لمجموعات الفئران التي عوملت بالجرعات العالية لمستخلص القسط الهندي تضمنت تغيرات انتكاسية ونخر واحتقان وكما لوحظت تغييرات نسيجية مرضية في الخصيتين لمجموعات الفئران التي عوملت بالجرعات العالية لمستخلص حب العزيز تضمنت تقلص خلايا لأيدك .ولم تظهر تغييرات نسيجية مرضية في الكبد والكلى والخصيتين لمجموعات الفئران التي عوملت بمستخلصات القسط الهندي وحب العزيز بجرعة ٢٠٠ملغم/كغم اضافة الى السيطرة السلبية والايجابية لجميع الانسجة.

تشير هذه النتائج الى ان الجرعات العالية لمستخلص القسط الهندي تمتلك تأثير سلبي على الكبد والكلى والخصى، والجرعات العالية لمستخلص حب العزيز تمتلك تأثير سلبي على الخصى.

بسم الله الرحمن الرحيم

ومن يتق الله يجعل له مخرجا ويرزقه

من حيث لا يحتسب ومن يتوكل على الله

فهو حسبه إن الله بالغ أمره قد جعل الله

لكل شيء قدرا

صدق الله العظيم

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعه النهرين
كلية العلوم
قسم التقانة الاحيائية

تشخيص المركبات الفعالة لنبات القسط الهندي ونبات حب العزيز ودراسة تأثيراتها البيولوجية على خصوبة ذكور الفئران

رسالة

مقدمة الى مجلس كلية العلوم\جامعة النهرين

كجزء من متطلبات نيل درجة الماجستير في علوم التقانة الاحيائية

من قبل

ود ابراهيم كاظم

بكلوريوس تقانة احيائية-كلية العلوم-جامعة النهرين-٢٠١٤

بأشراف

أ. د. خلود السامرائي

شوال ١٤٣٨ تموز ٢٠١٧