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Astudy of Histopathological Effects of Methanolic Buds of Flowers Extract of *Lavandula officinalis L.* on Selected Organs of Male Mice

Raghad Akram Aziz **Asmma E.Al-Niaame**

Department of Science- College of Basic Education/ University of Al-Mustansiriyah

Abstract

***Lavandula officinalis*(Lavendar) flowers is widely used in traditional medicine to treat various types of ailments. The evaluation of toxic properties of *Lavandula officinalis* flowers is crucial when considering public health protection because exposure to plant extracts can result in undesirable effects on consumers. Hence, in this study the oral toxicity of methanolic buds of flowers extract of *Lavandula officinalis* was investigated in mice. The Qualitative Phytochemical analysis of methanolic extract of of *Lavandula officinalis* contained many bioactive chemical constituents including tanninis, alkaloids, saponins, flavonoids, coumarins, terpenoids, glycosides, phenol, and absence of steroids. Twelve male albino mice were randomly selected and divided into four groups A, B, C and D of three animals each. Groups A, B, C and study groups were treated orally 500,1000and1500 mg/kg body weight (bd. wt). with of methanolic buds of flowers extract of *Lavandula officinalis* respectively, while group D control received equivalent quantity normal saline for the same period. Animals received their doses once a day daily orally, via polythene cannula for a period of 14 days. The mice were observed daily for clinical/pharmacological signs of toxicity and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for 14 days. On the 15th day, the mice were humanely sacrificed by cervical dislocation for necropsy examination. The Internal organs including liver, kidney, heart, testis were surgically removed. The gross pathological observations of the tissues were performed by histopathological examination. No toxic symptoms or mortality**

were observed in any animals, which lived up to 14 days after the administration of methanolic buds of flowers extract at single dose level of 500and1000 mg/kg bd. wt. The behavioral patterns of animals were observed after the administration at the first, second, fourth and sixth hour and once daily for 14 days. And the animals in both vehicle treated and extract-treated groups were normal and did not display significant changes in behavior. but in the treated group1500mg/kg bd. wt. In the one hr of 14 days rapid heartbeat and Tremors were observed after the receiving the oral administration of the extract. Histological sections of the liver, kidney, heart and testis derived from experimental mice treated with methanolic buds of flowers extract of *Lavandula officinalis* 500,1000,1500 mg/kg bd.wt./day for 14-days appeared abnormalities were observed in sections when compared with the control group. Histological sections of the liver derived from mice treated with the dose of 500,1000,1500 mg/kg bd.wt. /day, showed infiltration of lymphocyte and hemorrhage, infiltration of lymphocyte respectively compared to the controls.. Histological sections of the kidney derived from mice treated with the dose of 500,1000,1500 mg/kg bd. /day, showed infiltration of lymphocyte and hemorrhage, necrosis respectively compared to the control. And distruction of basement membrane of the seminiferous tubules. was observed in histological sections of testis derived from mice treated with 500,1000,1500mg dose compared to the control. There were no observable microscopic lesions in the heart the mice in the all groups. This study has shown the toxicity characteristics of the methanol extract of the buds of flowers extract of *Lavandula officinalis* in short time treatment with the extract. Despite the reported potentially beneficial effects *lavandula officinalis*, its use as a medicinal plant should be with great caution and Additional future pharmacokinetics studies are suggested.

Key words: *Lavandula officinalis*, Qualitative phytochemical screening, histopathological effects.

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دراسة التأثيرات المرضية النسجية لمستخلص الميثانولي لبراعم ازهار *Lavandula officinalis* L. على بعض الاعضاء الحيوية لذكور الفئران

رغد أكرم عزيز أسماء عزت النعيمي
قسم العلوم– كلية التربية الأساسية/ الجامعة المستنصرية

الخلاصة

تعد ازهار نبات *Lavandula officinalis*(Lavendar) flowers والمعروف بالافندر واسعة الاستخدام في الطب التقليدي لمعالجة امراض عديدة. ان تقييم الخصائص السمية لنبات لافندر تعد ضرورية لحماية صحة الناس لأن التعرض لمستخلصات النبات ممكن ان تسبب نتائج غير مرغوب بها للمستهلك. لذا هدفت الدراسة الحالية معرفة سمية المستخلص الميثانولي لبراعم ازهار *Lavandula officinalis* على الفئران المختبرية وبعد تجريعها فمويا وبجرع مختلفة. اما بخصوص الكشف النوعي ولبعض المركبات الفعالة لمستخلص الميثانولي لبراعم ازهار نبات اللافندر، فقد تبين احتواءه على كل من التانينات والقلويدات والصابونينات والفلافونيدات والكومارينات والتربينات والكلايكوسيدات والفينولات فضلا عن غياب تواجد الستيرودات. وعند دراسة التأثيرات الأمراضية للمستخلص الميثانولي على ذكور الفئران وبعد تجريعها فمويا وبجرع مختلفة 500، 1000، 1500 ملغرام لكل كيلو غرام من وزن الجسم يوميا ولمدة 14 يوم مقارنة بمجموعة السيطرة مع ملاحظة العلامات السريرية وتسجيل حالات الوفاة خلال فترة البحث. اظهرت نتائج الدراسة الحالية بأن مجموعتي الفئران المجرعة فمويا وبجرع500، 1000 ملغرام لكل كغم من وزن الجسم لم تظهر عليها اي علامات سريرية واضحة وذات العلاقة بسمية المستخلص، في حين ان المجموعة المجرعة بـ 1500 ملغرام لكل كغم من وزن الجسم قد اظهرت وفي الساعة الاولى من التجريع وفي اليوم الرابع عشر علامات سريرية والمتمثلة بسرعة ضربات القلب مع رجفة. في حين لم تظهر اي حالة وفاة وفي المجاميع الثلاثة. وعند دراسة التأثيرات المرضية النسجية للمستخلص، أظهرت نتائج الدراسة الحالية تغيرات مرضية نسجية على الاعضاء الحيوية لاسيما الكبد والكلى والخصى وفي التراكيز الثلاثة

وعلى التوالي مقارنة بمجموعة السيطرة. من خلال ارتشاح الخلايا اللمفية ونزف دموي في الكبد مع ارتشاح الخلايا اللمفية ونزف دموي فضلا عن نخر في النبيبات البولية في الكلى فضلا عن تحطم الغشاء القاعدي للنبيبات المنوية في الخصى في حين لم يظهر المستخلص اي تأثير واضح على المقاطع النسجية المأخوذة من القلب. وبهذا قد تبين بأن المستخلص الميثانولي لنبات اللافندر ذات تأثير سمي واضح وفي مدة زمنية قصيرة .وعلى الرغم من الاثار المفيدة والمحتملة للنبات فمن الضروري ان يستخدم بحذر شديد وكما نقترح بأجراء دراسات أضافية لتحديد الجرع المميتة للانسان.

الكلمات المفتاحية: *Lavandula officinalis*، الكشف النوعي للمركبات الفعالة، التأثيرات الأمراضية النسجية.

Introduction

All over the world, especially in developing countries herbal drugs are playing an important role in health care programmes.This is because they are being cheap and locally available. There is a general belief amongst the consumers globally that herbal drugs are always safe because they are “natural"(4). Till now no much is known about the dose-related toxicity of medicinal plants, particularly at the histological side. Bioactive compounds derived from medicinal plants can be useful but might have serious dose-related side effects (13).

Therefore, in view of their wide spread use of medicinal plants in alternative medicine, toxicological assessment becomes imperative in order to arrive at potencies that can be considered as safe formulations for clinically efficient remedies. *L. officinalis* L. (Lavander) belongs to Labiatae (Lamiaceae),It is cultivated throughout Europe as well as in different parts of Iran (16). *L. officinalis*, is a common dense, evergreen, aromatic shrub grown in any parts of the world. The fresh and dried flowers are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods. A tisane can also be made from them. They are extensively used in cooking, and a distinct mustard smell gives off while they are burned, therefore, they often are used to flavor foods while barbecuing (8). In recent years, *L. officinalis* flowers exhibit such various biological and pharmacological activities as anti- tumour, anti- inflammatory, antihistaminic, antidiabetic, and antimicrobial activity and modulating the central nervous system (6). The study therefore is aimed at investigating histopathological effects of intake of the crude methanolic buds of flowers extract of *L. officinalis* on selected organs of male mice.

Materials and methods

Plant sample collection:

Buds of flower *Lavandula officinalis* were obtained from the local herbal store, Baghdad, Iraq in April 2012, and the identity of these buds of flower were confirmed by an expert a plant taxonomist Dr. Ali Al-mosawi, Department of Biology, Baghdad University, Baghdad.

Preparation of buds of flower powder:

The healthy buds of flower were dried in shade condition and to avoid decomposition of chemical constituents, pulverized using a

mechanical grinder and the obtained powder was stored in clean and dry airtight containers for further studies.

Preparation of plant extract:

Extract of of the buds of flower were obtained according to following methodology Methanol extraction 10g of air-dried powder was added to 150 ml of methanol in a conical flask,pluged with cotton wool and kept for 24 h. After 24h the extract was filtered using Whatman filter paper NO.1 and centrifuged at×10000g for15 min the supernatant was collected. The procedure was repeated twice and the supernatant was evaporated to dryness using oven at 40°C and was later stored at 4°C until used (1).

Phytochemical screening:

Qualitative phytochemical analysis of methanol extract were used preliminary screening of secondary metabolites , were done as follows:

Tannins:

20 mg powder was dissolved in 2 ml distilled water and filtered. 2 ml $FeCl_3$ was added to the filtrate, blue-black precipitate indicated the presence of tannins(7).

Alkaloids:

20 mg extract was dissolved in 2 ml distilled water and filtered. To the filtrate, 2–4 drops of 1% HCl was added and steam was passed through it. To the 1 ml of this solution 6 drops of Wagner's reagent was added. Brownish-red precipitate indicated the presence of alkaloids(7).

Saponins:

To 0.5 ml of the filtrate obtained in alkaloids test 5 ml distilled water was added. Frothing persistence indicated the presence of saponins(7).

Flavonoids:

A 2 g of powdered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample. The mixture was filtered while hot. The filtrate was cooled and 5 mL of 20% sodium hydroxide was added to equal volume of the filtrate. Ayellow solution indicates the presence of flavonoids(15).

Coumarins:

0.5 g of the moistened methanolic plant extract was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined

under the UV light for yellow fluorescence indicated the presence of coumarins(12).

Terpenoids:

Salkovski test was performed using a small amount of extract solution. To this solution 5 drops of conc. H_2SO4 and 1 ml Chloroform were added. Change of yellow colour into red indicated the presence of terpenoids(3).

Glycosides:

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer(12).

Phenols:

A small amount of material was extracted in Methanol and evaporated to dryness. Residue was dissolved in distilled water and 0.5 ml Folin-ciocalteau reagent was added followed by 2 ml 20% Na_2CO3 solution. Development of bluish colour indicated the presence of phenols(3).

Steroids (Steroidal ring):

About 0.1 g of the extract was dissolved in 2 mL of chloroform; sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interphase is indicative of the presence of steroidal ring (11).

Experimental animals:

Twelve male Albino mice were used for the experiment. They were obtained from the animal house of National Center For Drug Control and Research, weighing between 23-25 g, The animals were housed in separate cages, and kept under controlled environmental conditions of temperature (25 ± 5^oC), relative humidity (50 ± 5)% and 12 hour light/dark cycle. And food with commercial feed pellets and water *ad-libitum.* All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (9).

Experimental Design:

Twelve male albino mice were randomly selected and divided into four groups A, B, C and D of three animals each. Groups A, B, C

and study groups were treated orally 500,1000and1500 mg/kg body weight. with methanolic buds of flowers extract of *L. officinalis* respectively, while group D control received equivalent quantity normal saline for the same period. Animals received their doses once a day at 5:00 pm daily orally, via polythene cannula for a period of 14 days. The mice were observed daily for clinical/pharmacological signs of toxicity included (behavior, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss were observed)and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for 14 days. On the 15th day, the mice were humanely sacrificed by cervical dislocation for necropsy examination. The Internal organs including liver, kidney, heart, testis were surgically removed. The gross pathological observations of the tissues were performed by histopathological examination. The specimens for histopathology were fixed in 10% neutral, buffered formalin for 18 h at 4°C.

Histological examination of vital organs:

The fixed tissues of mice were dehydrated with progressively increasing concentrations of ethanol. The tissues were passed through xylene solution to clear the ethanol and facilitate molten paraffin wax infiltration 55°C. After that, they were embedded in a wax block. Paraffin sections of 3-4 μm thickness were cut with the rotary microtome and placed on cleaned glass slides. Finally, the sections were stained with hematoxylin and eosin. Photomicrographs of the slides 10x were taken for histological examination and the slides in tests were compared to that of the control for histological variations(2).

Results and discussion:

Phytochemical screening is usually carried out to screen and characterized the constituents available in a given plant sample. Generally, in the phytochemical screening of any plant one normally identifies secondary metabolites that have accumulated to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological activity when tested on animals.The Qualitative Phytochemical analysis of methanolic extract of *L. officinalis* buds of flower gave positive reactions for tanninis, alkaloids, saponins, flavonoids, coumarins, terpenoids, glycosides, phenol, and negative reactions for steroids as shown on (Table, 1).

(Table, 1): Qualitative phytochemical analysis of methanolic extract of *L. officinalis* buds of flowers**.**

Constituents	Result
Tannins	+
Alkaloids	+
Saponins	+
Flavonoids	+
Coumarins	+
Terpenoids	+
Glycosides	+
Phenol	+
Steroids	_

General sign and behavioral analysis:

The toxic effect of methanolic extract of buds of flowers of *L. officinalis* on the appearance and the general behavioural pattern of mice are shown in (Table 2 and 3). No toxic symptoms or mortality were observed in any animals, which lived up to 14 days after the administration of methanolic buds of flowers extract at single dose level of 500and1000 mg/kg bd. wt. The behavioral patterns of animals were observed after the administration at the first, second, fourth and sixth hour and once daily for 14 days. And the animals in both vehicle treated and extract-treated groups were normal and did not display significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss. but in the treated group1500mg/kg. In the one hr of 14 days rapid heartbeat and tremors were observed after the receiving the oral administration of the extract**.**

(Table, 2): Potential toxic effects of the methanolic buds of flowers extract of *L. officinalis***.**

Control [a]	**Test groups[b] (mg/ kg bd.wt.)**		
	500	1000	1500
0/3[c]	0/3	0/3	0/3

[a] Control groups (treatment without crude extract); [b] test groups (treatment with 500,1000,1500 mg/kg bd.wt.); [c] Number of dead mice/ number of mice used.

(Table, 3): General appearance and behavioral observations for control and treated group with(1500mg/kg bd. wt.).

Observation	**Control group**				**Test group(1500mg/kgbd.wt.)**			
	4 hrs	**6hrs**	**24hrs**	**14 days**	**4 hrs**	**6hrs**	**24hrs**	**14 days**
Skin and fur	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
Eyes	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
Mucous membrane	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
Behavioural patterns	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Rapid heart beat**
Salivation	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
Lethargy	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
Sleep	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
Diarrhea	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**	**Normal**
Coma	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**
Tremors	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**N.o.[a]**	**Observed**

[a] Not Observed.

Histopathology analysis:

Gross lesions:

Macroscopic examination of the organs of the animals treated with extract showed no changes in color compared to control. Autopsy at the end of the experiment period revealed no apparent changes in the liver, kidney, heart and testis organs from both control and treated mice in the histopathology analysis.

Microscopic lesions:

In control group no structural changes were identified by histopathology in the liver, kidneys, heart and testis suggesting that these animals were healthy and the conditions under which the experiment was conducted were proper. Histological sections of the liver, kidney, heart and testis derived from experimental mice treated with methanolic buds of flowers extract of *L. officinalis* 500, 1000, 1500 mg/kg/day for 14 -days appeared abnormalities were observed in sections when compared with the control group.

Histological sections of the liver derived from mice treated with the dose of 500,1000,1500 mg/kg/day, showed infiltration of lymphocyte and hemorrhage, infiltration of lymphocyte respectively compared to the controls (Figure 1). Liver is the major site of detoxification in the body for all drugs/toxins. Therefore it is an important organ in any toxicological study (14).

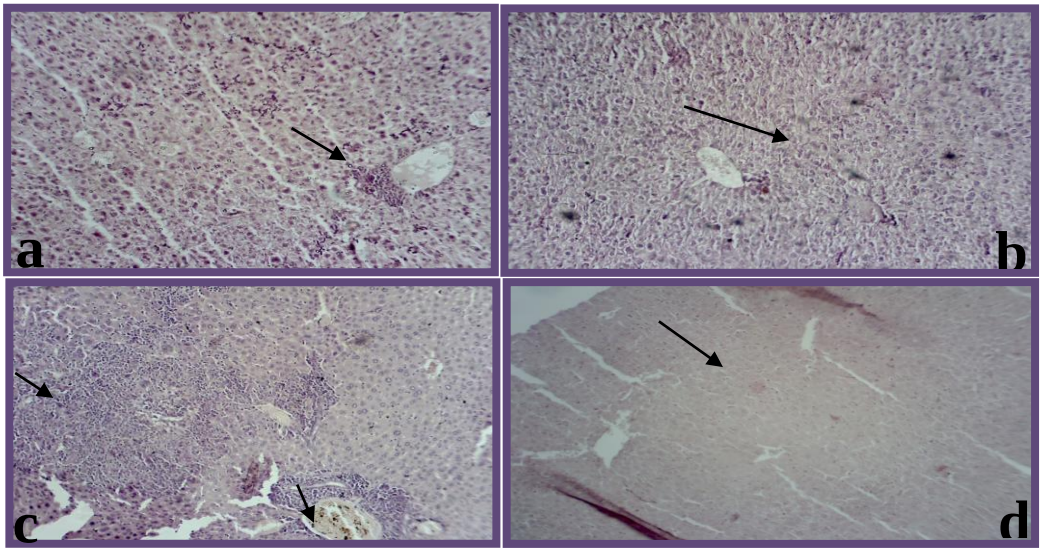

Fig.(1): Stained sections of Liver at 100X magnification, H&E.

1a & b) Liver sections of treated animals (with methanolic buds of flowers extract of *L. officinalis*) of dose 500&1000mg/kg/day for 14 - days, showing Infiltration of lymphocyte (black arrow).

1c) Liver sections of treated animals (with methanolic buds of flowers extract of *L. officinalis*) of dose 1500 mg/kg/day for 14 - days, showing 1- hemorrhage.
2-Infiltration of lymphocyte (black arrow).
1d) Liver sections of control animal showing normal structure (black arrow).

Histological sections of the kidney derived from mice treated with the dose of 500,1000,1500 mg/kg/day, showed infiltration of lymphocyte and hemorrhage , necrosis respectively compared to the control (Figure 2).

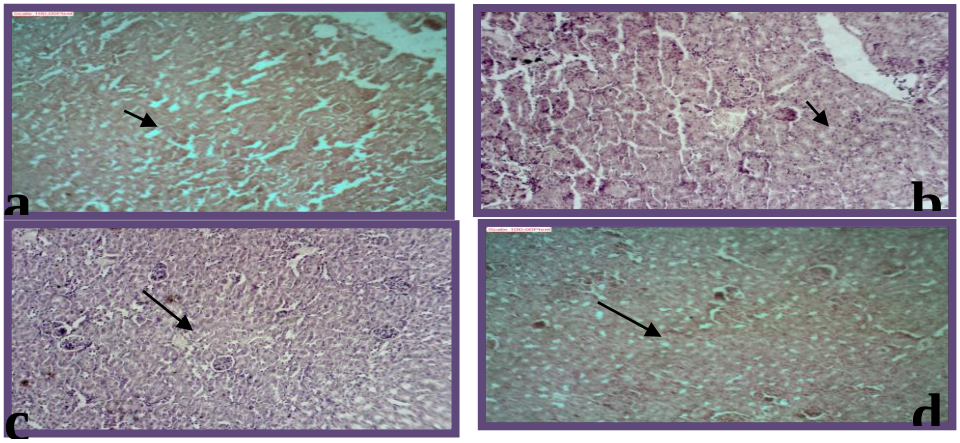

Fig.(2): Stained sections of Kidney at 100X magnification, H&E.

2a & b) Kidney sections of treated animals (with methanolic buds of flowers extract of *L. officinalis*) of dose 500&1000 mg/ kg/ day for 14- days, showing Infiltration of lymphocyte (black arrow).

2c) Kidney sections of treated animals (with methanolic buds of flowers extract of *L. officinalis*) of dose 1500 mg/kg/day for 14- days, showing, showing 1- hemorrhage 2- necrosis (black arrow).

2d)Kidney sections of control animal showing normal structure (black arrow).

And distruction of basement membrane of the seminiferous tubules. Was observed in histological sections of testis derived from mice treated with 500, 1000, 1500 mg dose compared to the control

(Figure 3) .There were no observable microscopic lesions in the heart the mice in the all groups.

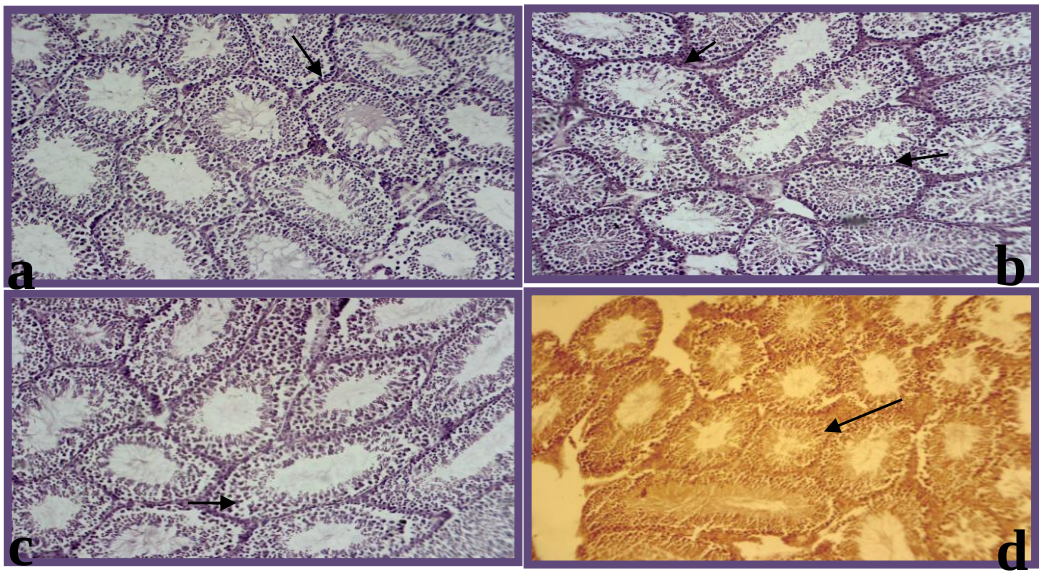

Fig.(3): Stained sections of Testis at 100X magnification, H&E.

3a & b) Testis sections of treated animals (with methanolic buds of flowers extract of *L. officinalis*) of dose 500&1000mg/ kg/ day for 14 - days, showing destruction of basement membrane of the of seminiferous tubules (black arrow).

3c) Testis sections of treated animals (with methanolic buds of flowers extract of *L. officinalis*) of dose 1500 mg/ kg/day for 14- days, showing, destruction of basement membrane of the of seminiferous tubules (black arrow).

3d) Testis sections of control animal showing normal histology (black arrow).

The herbal and natural products of folk medicine have been used by men since the beginning of the human race. However, the general acceptability of herbal medicines has been limited by a lack of defined chemical characterization, dose regimen and adequate toxicity data to evaluate their safety. The indiscriminate increase in the use of plant extract is further aggravated by the belief that herbs are safe simply because they are natural in origin (5). Plants produce bioactive compounds which act as defense mechanisms against predators and at the same time, may be toxic in nature (10). Therefore, it has become

imperative to assess the safety of plants used for medicinal purposes for possible toxicity. Phytochemical screening is usually carried out to screen for and to characterized the constituents available in a given plant sample. Generally, in the phytochemical screening of any plant one normally identifies secondary metabolites that have accumulated to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological activity when tested on animals. Result of phytochemical screening of the methanolic extract of *L. officinalis* buds of flowers of the various extracts showed the

presence of tannins, alkaloids, saponins, flavonoids, coumarins, terpenoids, glycosides and phenol. The presence of pathological lesions in the liver and kidney may not be surprising since the kidney is the primary organ of the excretion while the liver is the main organ of biotransformation in the body. So, it is possible that the two organs may have been exposed to the toxic substances present in the extract. It is recommended that caution should therefore be advocated in the intake of this product and further work is required to investigate the mechanisms by which methanolic extract of *L. officinalis* buds of flowers its effect on the organs studied**.**

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