


Original article

Effect of Sub Toxic Dose of *Ephedra Altissima* Methanolic Extract on Reproductive System of Male Albino Mice

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ABSTRACT

Background and objectives: *Ephedra* is nonflowering plants belonging to Ephedraceae family. There is little information available concerning toxicity. The aim of this work is to carry out phytochemical screening, sperm parameters, and histological study. **Materials and Methods:** Fresh aerial parts of *Ephedra* collected, identified, then air-dried. Phytochemical screening of methanolic extract of *Ephedra* powder using standard procedures were carried out. Twelve male albino mice (25-30 g), were divided into two groups. Mice were treated (i.p.) for 14 days with 1% T80 (5mL/kg), and extract (3000mg/kg) as treated group. At the end of experiment, mice were killed by cervical dislocation, dissected and testis, cauda epididymis and vasa deferentia collected. Seminal fluid collected for sperm count, motility and morphology. Testis were kept in formalin for histological examination. **Results:** Phytochemical screening showed the presence of carbohydrates, phenols, sterols, saponins, tannins, terpenoids, flavonoids and alkaloids in *Ephedra Altissima* stem extracts. *Ephedra* extract caused decrease in sperm count; but it did not show any change in sperms motility compared to the control mice. There is increase in the number of abnormal sperms morphology in mice treated with extract; the sperms tail treated group were much affected than the head. Histological examination showed no structural alteration in the seminiferous epithelium of the treated mice testis compared to control mice. **Conclusions:** It is concluded that methanolic extract of *Ephedra altissima* stems have the potential to affect male mice reproductive functions but not affect the structure at the given high dose and short period of treatment.

INTRODUCTION

In recent years, there is increasing interest for studying of medicinal plants [1]. Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. However, there is little information available concerning the possible toxicity that

medicinal plants may cause to the consumers [2].

Ephedra is a genus of nonflowering plants belonging to Ephedraceae family [3]. The family Ephedraceae has the only genus *Ephedra*, which includes more than 60 species in the world. *Ephedra* generally are xerophytic plant that grows wild in arid and semiarid climates mainly in the desert areas of Asia, America, Europe and North Africa.

Ephedra consists of a group of perennial, evergreen, and dioecious, sub-shrubs, shrubs, or climbers [4].

Among these species, *Ephedra altissima*, which was recorded in Nafusa Mountain in Libya; also, it can be found in Algeria, Chad, Mauritania, Morocco, Spain, Tunisia, and Western Sahara [5]. This species can grow up to two meters in height, has long narrow and jointed stems, and small leaves that are reduced to scales at the nodes. Stamens and pistils are on separate flowers and separate plants (Dioecious), and seeds are enclosed in cones. This plant has a distinct pine odor and is reported to have a very astringent taste [6].

Ephedra genus commonly used in the folk medicine for treatment of bronchial asthma, chills, colds, fever, coughs, flu, circular system, and digestive system disorders, as well as cancer; also, it has antibacterial and antifungal actions [7]. Phytochemical studies revealed that more than 145 compounds have been isolated and identified from the genus Ephedra, including alkaloids, flavonoids, tannins, and polysaccharides [4]. The major active ingredients of Ephedra are alkaloids that constitute 0.5 to 2.5 percent of the total mass, and are referred to as ephedrine-type alkaloids; it is the biologically active constituents of pharmacological and toxicological relevance of Ephedra herb mainly ephedrine and pseudoephedrine [8]. In addition to the ephedrine-type alkaloids, other alkaloids and amino compounds have been isolated from different species of Ephedra. The macrocyclic spermine alkaloids, Ephedradines A-D, kynurenic acid derivatives, cyclopolylglycine, methanoproline amino acids, flavones, flavanols, tannins, carboxylic acids and volatile terpenes [9]. Ephedrine and related alkaloids produce sympathomimetic effects, including vasoconstriction, increased heart rate; and stimulation of central nervous system [4].

Therefore, the current study aimed to investigate the effect of *Ephedra altissima* methanolic extract on reproductive system in male adult albino mice using sub toxic dose determined by previous work for assessment of acute toxicity of methanolic extract. Assessment of sperm parameters are used as an indicator for identifying and characterizing male reproductive toxic agents. Sperm analyses such as analyses of sperm count, motility, and morphology are the most reliable endpoint tests for male reproductive status [10]. Histopathology, sperm count, sperm motility and sperm morphology were examined for the control and ephedra methanolic extract treated groups.

METHODS

Plant material

Fresh aerial parts of *Ephedra altissima* were collected from naturally growing populations located in Nafusa Mountain, in Libya during January 2018. The plant collected during the flowering stage of growth, sample were identified, and the plant species is properly authenticated by Dr. Mohammed Abu-Hadra at herbarium section, department of botany, faculty of science, University of Tripoli. The plant was air dried in shadow at room temperature, the dried stems milled into coarse powder using electric grinder, and then stored in tightly closed container in fridge until needed.

Chemicals

Hexane, Ethyl acetate, Methanol, Ethanol, α -Naphthol, Dragendroff's reagent, Mayer's reagent, Wagner's reagent, Hager's reagent, Tween 80 and Paraffin wax were obtained from (Sigma-Aldrich, Steinheim, Germany); Formaldehyde solution, Glacial acetic acid, Fehling solution B, Haematoxylin and Ethanol absolute were obtained from (PARC Scientific limited, Northampton, United Kingdom); Chloroform, Ammonia solution, Ninhydrin and Eosin were obtained from (Riedel-Dehaen AG, Hanover, Germany); Ferric chloride, Sodium hydroxide and Anhydrous calcium chloride were obtained from (WINLAB, Leicestershire, United Kingdom); Fehling solution A was obtained from (GFS Chemicals, Ohio, USA); Benzene, Xylene, Hydrochloric acid and Sulphuric acid were obtained from (BDH Chemicals Ltd, Poole, England).

Experimental animals

Albino mice were bred in the animal house of the pharmacology department, Faculty of pharmacy, University of Tripoli. Mice were housed in plastic cages; food pellet diet and water were free available. The animals were kept at room temperature, and on 12-hour dark/light cycle.

Preparation of Ephedra extracts

The powdered plant sample (1kg) was extracted with different solvents respectively (Hexane, Ethyl acetate then Methanol) by cold maceration for 7 days for each solvent, the extracts then filtered through Whatman filter paper No. 1, subsequently, by using rotary evaporator at 40 - 60 °C and reduced pressure the solvents was evaporated. The resulting crude extracts were stored at 4 °C until used for analysis.

Phytochemical screening

Chemical investigations were carried out on the Methanolic, Ethyl acetate, and Hexane extracts using standard procedures as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973) [11-13].

1. Tests for alkaloids

Few ml of extracts mixed with 2ml of HCl and heated gently. Few drops of Dragendroff's, Mayer's, Wagner's and hager's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2. Test for anthraquinones (Borntragers test)

One milliliter of the plant extracts was shaken with 10 ml of benzene, the mixture then allowed to stand for 10 min then filtered. 10 ml of 10% ammonia solution added to the filtrate and shaken for 30 sec. pink, red or violet colour in the ammonia phase indicated the presence of anthraquinones in the extract.

3. Test for tannins

One milliliter of the filtrate was mixed with 2 ml of FeCl₃ solution; a dark green color indicated a positive test for tannins.

4. Test for saponins

One milliliter of the extract filtrate was diluted with 2 ml of distilled water; the mixture was vigorously shaken and left to stand for 10 min. The development of foam on the surface of the mixture lasting for more than 10 min indicates the presence of saponins.

5. Test for flavonoids (Alkaline reagent test)

Few ml of each extract separately was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

6. Test for terpenoids (Salkowski test)

Few ml of each extract separately was mixed in 4 ml of chloroform, and concentrated H₂SO₄ carefully added to form a layer, a reddish-brown coloration of the interface was formed and become yellow after 2 min indicate the presence of terpenoids.

7. Test for cardiac glycosides (Keller-Killiani test)

Extract (2 ml) treated with 2ml of glacial acetic acid with one drop of FeCl₃ solution and 1 ml of H₂SO₄ concentrated. A brown ring formed between the layers indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form.

8. Test for carbohydrates

(a) Molish test – to about 2 ml of each extract few drops of α-naphthol (20% in ethyl alcohol) was added. Then about

1 ml of Conc. H₂SO₄ was added along the sides of the test tube. Reddish violet ring at the junction of two layers separated in the presence of carbohydrates

(b) Fehlings solution test – the extract was heated with dilute HCl to hydrolyze polysaccharides. The reaction mixture is neutralized by adding NaOH solution and then Fehlings solution 1 and 2 were added. A red precipitate formed in cases of reducing sugars /carbohydrates.

9. Test for phenolic compounds

Ferric Chloride Test: 1 ml of solvent extracts, 3 ml of distilled water was added. To this, a few drops of neutral 5% FeCl₃ solution was added. Formation of a dark green color indicated the presence of phenolics.

10. Test for proteins and amino acids

(a) Ninhydrin test: Boil 2 ml of 0.2% Ninhydrin solution with the plant extracts, appeared violet color indicate the presence of proteins and amino acids.

(b) Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added to the extracts. Appearance of pink or purple colour indicates the presence of proteins and free amino acids

11. Test for steroids

(a) Salkowski's test: 2 ml of extract was mixed with 2 ml of chloroform and 2 drops concentrated H₂SO₄ was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

(b) Libermann Burchard's test: mixing 2 ml extract with 2ml of chloroform. Then 2 ml of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

12. Test for Fixed oils and Fats (Stain Test)

Small quantities of the extracts were pressed between 2 filter papers. Formation of an oily stain on the filter paper indicates the presence of fixed oils and fats.

13. Test for coumarins

A few drops of ammonia were added on a filter paper. To this, a drop of the extract was added and the paper was observed for fluorescence under UV light.

Sperm analysis

Twelve male albino mice, weighing (25-30 g), were divided into two groups (n=6). Mice were treated intraperitoneally (I.P) for 14 days with: 1% Tween 80 (5mL/kg) Group I (control), methanolic extract (3000mg/kg) group II (treated). Mice were weighed at the beginning of the experiment, before injection and before killing. At the end of experiment, mice were killed by cervical dislocation. The mice were dissected and testis, cauda epididymis and vasa deferentia

of male mice were dissected out carefully processed and prepared accordingly and evaluated for sperm count, sperm motility and sperm morphology. Testes were collected for histopathological examination.

Seminal fluid collection

Sperm of each mouse were obtained by squeezing the vasa deferentia gently into 1ml normal saline in small dish. The specimen was mixed gently by a special dropper to distribute the seminal fluid. Sperm suspension was incubated for 15 minutes at 32 °C to allow sperm separation [14].

Assessment of sperm count

Sperm count in the vasa deferentia of control and methanolic extract treated mice was determined using the Neubauer chamber of hemocytometer. Spermatozoa were counted by charging both chambers of improved neubauer hemocytometer with pre-prepared sperm suspension. The number of spermatozoa in the squares of the hemocytometer was counted under the microscope (ZEISS) at 400X magnification. Sperm count was expressed in millions per milliliter ($10^6/\text{mL}$) [15].

Assessment of sperm motility

Sperms from control and methanolic extract treated mice were examined using the improved neubauer hemocytometer (American optical Co., Buffalo. N.Y.). A drop of diluted sperm suspension was taken up by fine pipette; the mouth of the pipette was emptied near the edge of the cover slip. The number of motile and non-motile sperms of control and extract treated mice were counted from five diagonal squares on the part of the hemocytometer containing graticules (ruling) under the 400x light microscope (ZEISS) [16].

Assessment of sperm morphology

To examine the presence of any sperm morphological abnormalities, two smears were made from each mouse, and allowed to dry in air. Smears were stained with 1% eosin Y in water for 10 minutes. Sperm smear slides were randomly read with regard to slides from control or treated groups to eliminate any bias. From each mouse, 250 sperms were examined at 400x magnification of light microscope (ZEISS) for morphological abnormalities. The result was expressed as percentage of abnormal sperms [17].

Histopathological examination of testis

Testis of control and methanolic extract treated mice were removed and then were fixed in formalin for 24 hours. The

specimens were washed twice with 70% alcohol. The fixed tissues were dehydrated in an ascending series of alcohol ranging from 70% to 100% (absolute). The dehydrated tissues were cleared in xylene (twice), infiltrated and then were embedded in paraffin wax. Testis sectioned by rotary microtome, sections were 5µm in thickness. The prepared sections were stained by routine methods using Hematoxylin-eosin method. The stained sections were examined under the microscope and the different cell types were carefully studied and photographed. Testis sections from each study group were evaluated for structural changes, blind by a histologist. Light microscopy (Leica, Germany) was used for the evaluations [18].

Statistical analysis

The data was analyzed by independent samples t-test using SPSS version 20 software. The statistical significance of difference between the control and treated group for sperm parameters: sperm count, sperm motility, and sperm morphology were determined, the difference was considered significant at the P value ≤ 0.05 . Values are expressed as the mean \pm standard error.

RESULTS

Phytochemical screening

Preliminary Phytochemical screening of hexane, ethyl acetate and methanolic extracts of *Ephedra altissima* stems identify the presence of different medically active compounds (Table 1). Hexane crude extract showed the presence of terpenoids, carbohydrates, fixed oils and lipids, with very little traces of alkaloids; while glycosides, saponins, tannins, flavonoids, coumarins, and proteins were absent. On the other hand, ethyl acetate crude extract showed the presence of terpenoids, carbohydrates, and traces of alkaloids; while glycosides, saponins, tannins, flavonoids, coumarins, proteins, and fixed oils were absent. Phenols, carbohydrates, flavonoids, tannins, cardiac glycosides, saponins, terpenoids, coumarins, and alkaloids were identified in methanolic extract; while proteins, anthraquinones and fixed oils and lipids were not detected.

Table 1. Preliminary phytochemical screening of *Ephedra altissima* stems extracts

Serial NO.	Phytochemical compounds	Methanol extract	Ethyl-acetate extract	Hexane extract
1	Alkaloids	+	+	+
2	Anthraquinones glycosides	-	-	-
3	Saponins	+	-	-
4	Tannins	+	-	-
5	Flavonoids	+	-	-
6	Terpenoids	+	+	+
7	Carbohydrates	+	+	+
8	Cardiac glycosides	+	-	-
9	Coumarins	+	-	-
10	Fixed oils and fats	-	-	+
11	Proteins and amino acids	-	-	-

Sperm parameters

A significant ($P \leq 0.05$) decline in sperm count of extract treated mice (13.5 ± 2.78) was observed as compared to control group (170.5 ± 8.61). The percentage of motile sperm of control and extract treated groups were insignificantly different ($P > 0.05$). The percentage of abnormal sperm morphology in extract treated group (79 ± 8.59) was significantly increased compared to the control (47.6 ± 10.82) at ($P \leq 0.05$) (Table 2).

The types of abnormal sperm shapes observed in this study included amorphous head, banana head, pointed head, hookless head, sperm with cytoplasmic droplet, bent neck, bent tail, and sperm with two tails. The majority of abnormality observed are sperms with bent tail (Figure 1).

Table 2. Mean value and percentage of sperm parameters of control and extract treated mice.

Treatment	Sperm parameters			
	Sperm count $\times 10^6/\text{ml}$	Motility %	Normal sperm morphology %	Abnormal sperm morphology %
5ml/kg 1% T80	170.5 ± 8.61	42.91 ± 4.44	52.4 ± 10.82	47.6 ± 10.82
3000mg/kg Extract	$13.5 \pm 2.78^*$	50.95 ± 10.99	$21 \pm 8.59^*$	$79 \pm 8.59^*$

*, Significantly different from the control at $p \leq 0.05$.

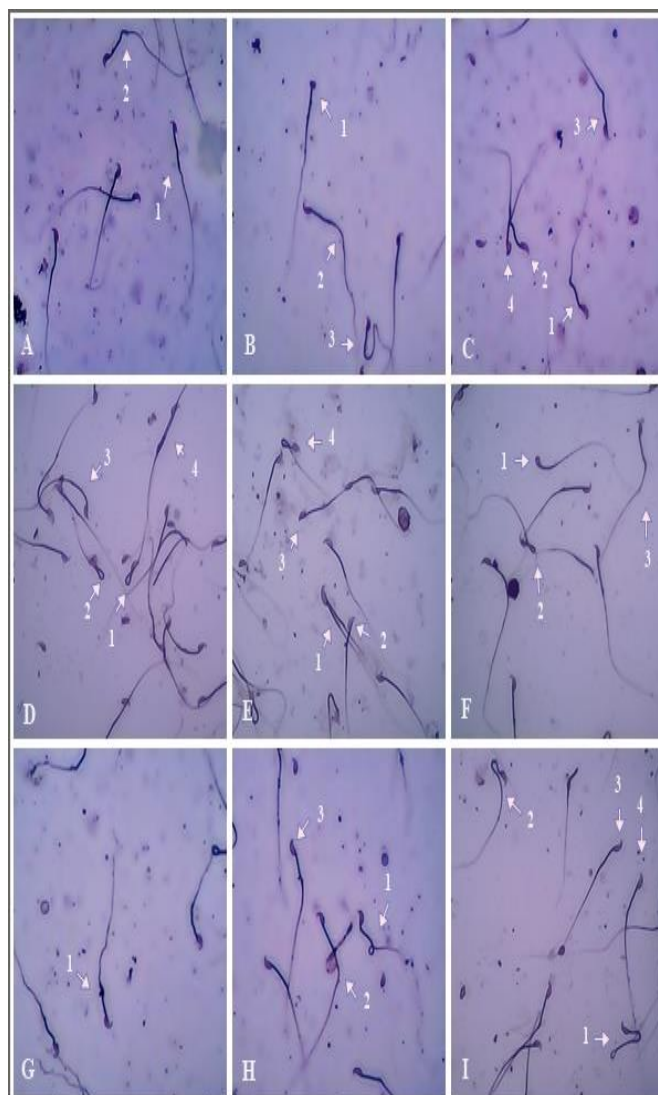


Figure 1 Photomicrograph (400x) of mice spermatozoa stained with 1% eosin
 A: 1. Normal sperm; 2. hookless head and curved tail; B: 1. Amorphous head; 2. 3. Curved tail; C: 1. 2. 3. Banana head and curved tail; 4. Hookless head; D: 1. 2. Bent neck; 3. Curved tail; 4. Bent tail; E: 1. Two tails; 2. 3. Hookless head; 4. Bent neck; F: 1. Pointed head; 2. Bent neck; 3. Curved tail; G: 1. Cytoplasmic droplet; H: 1. Coiled tail; 2. Curved tail; 3. Hookless head; I: 1. Bent tail; 2. Bent neck; 3. 4. Hookless head.

Histopathological examination of testis

Group I (control mice)

Sections of the testis of the control group showed the normal histological features of seminiferous tubules; tightly packed seminiferous tubules, separated from each other by narrow interstitial spaces containing interstitial cell of Leydig (Figure 2). These seminiferous tubules containing spermatogenic cells and Sertoli cells. Sertoli cells have pale cytoplasm, which were formed of spermatogonia, primary spermatocytes and spermatides. The spermatogonia appeared as small cell under basement membrane. Meyoid cells in the connective tissue under basement membrane are present and appeared as a spindle shape (fig. 3).

Group II (Ephedra altissima methanolic extract treated mice):

Sections of testis of *Ephedra altissima* methanolic extract treated group showed tightly packed seminiferous tubules, separated from each other by narrow interstitial spaces contain normal spermatogenic cells. The spermatogenic cells are spermatogonia, primary spermatocytes and elongated spermatids. The interstitial spaces are clearly visualized and contain Leydig cells (fig 4, 5). Tunica albuginea are surrounding seminiferous tubules (fig. 6).

There was no obvious great variation between group I & group II, where the histological structures of the seminiferous tubules in the *Ephedra altissima* extract- treated group was similar to that of the control group.

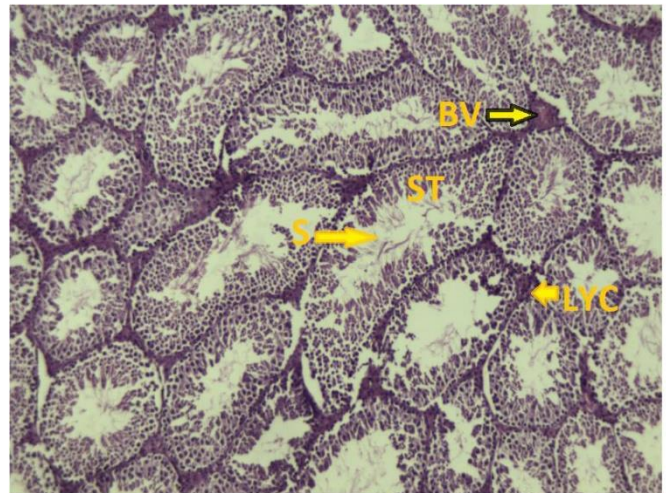


Fig.2, A photomicrograph of (low power) section of the testis of the control group I, showed the normal histological features of seminiferous tubules (ST) that contain normal spermatogenic cells (SGC) at the wall of seminiferous tubules. Normal formation of sperms (S) in the lumen of seminiferous tubules. Normal interstitial space between seminiferous tubules contains leydig cells (LYC) and normal blood vessels. (H&E, 20x).

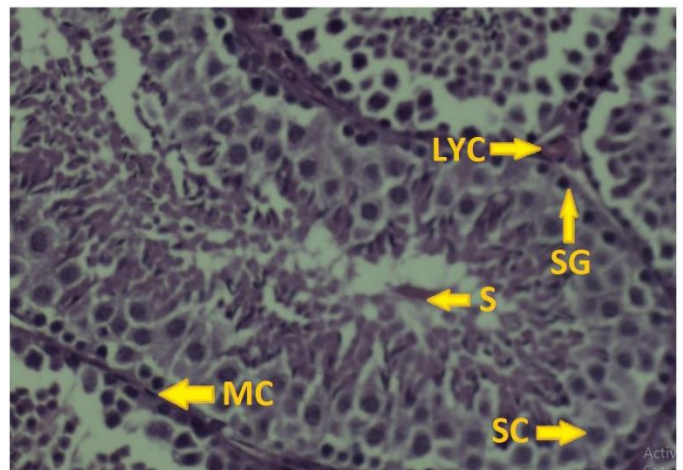


Fig.3, A photomicrograph (high power) of section of the testis of the control group I, showed the normal histological features of: Seminiferous tubules contain normal spermatogonia (SG) and sertoli cells (SC). Meyoid cells (MC) with flat nuclei under a basement membrane. Normal formation of sperms (S) in the lumen of seminiferous tubules. Normal interstitial space contains leydig cells (LYC). (H&E, 40x)

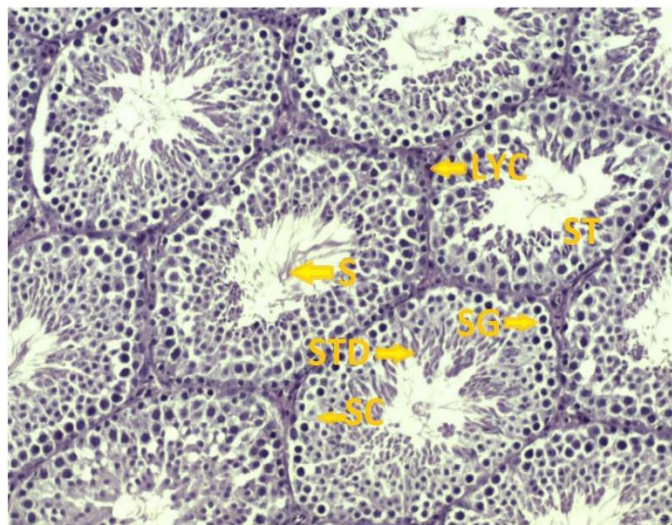


Fig.4, A photomicrograph of (low power) sections of testis of *Ephedra altissima* extract treated group II, showed; tightly packed seminiferous tubules, (ST) separated from each other by narrow interstitial spaces contain leydig cells (LYC) and blood vessels (BV). Normal spermatogenic cells including normal spermatogonia (SG) and sertoli cells (SC). Normal formation of spermatids (STD). (H&E, 20x)

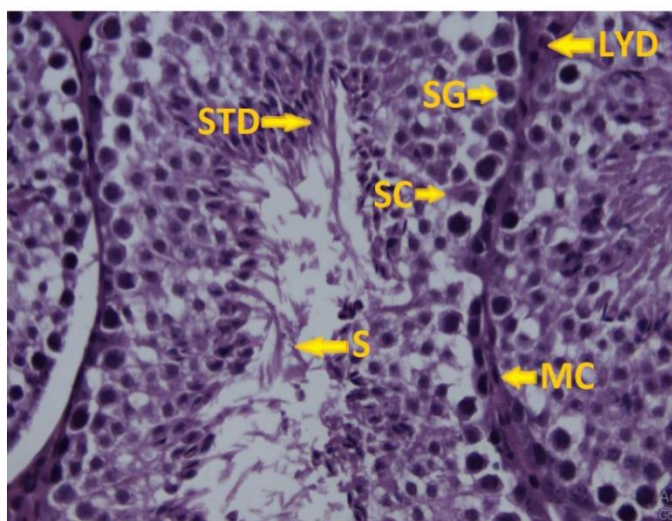


Fig.5, A photomicrograph of (high power) sections of testis of *Ephedra altissima* extract treated group II, showed; normal seminiferous tubules contain normal spermatogonia (SG), sertoli cells (SC) and spermatids. Normal meioid cells (MC) with flat nuclei under a basement membrane. Normal formation of sperms (S) in the lumen of seminiferous tubules. Normal interstitial space contains leydig cells (LYC). (H&E, 40x)

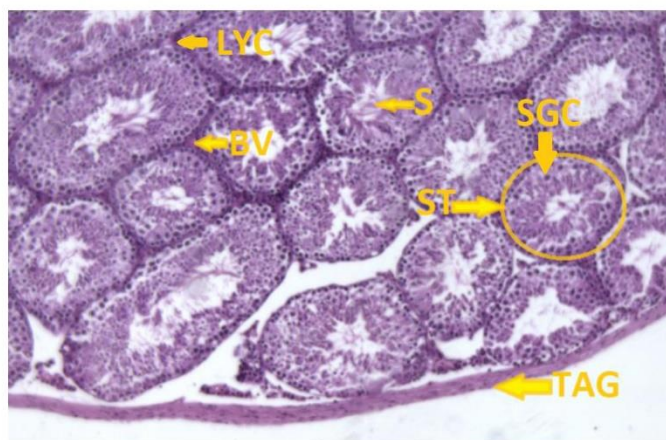


Fig.6, A photomicrograph of (low power) sections of testis of *Ephedra altissima* extract treated group II, showed; normal seminiferous tubules (ST) contain normal spermatogenic cells (SGC). Normal formation of sperms (S) in the lumen of seminiferous tubules. Normal testicular wall with normal tunica albuginea (TAG). Normal interstitial space contains leydig cells (LYC) and blood vessels (BV). (H&E, 20x)

DISSCUSION

Male reproductive toxicology is the study of the potential adverse effects of an agent on the structure and function of organ systems involved in male reproductive system [19].

Antifertility effects of medicinal plants included: spermicidal, anti-zygotic, inhibition of spermatogenesis and sperm motility, inhibition of androgen synthesis either by inhibiting Leydig cell function or by disrupting the hypothalamic - pituitary axis, and disruption of seminiferous tubules, erosion of germinal epithelium and disorganized histo-architecture of the testis [20].

The species ephedra is widely used in folk medicine for their various health effects; it is one of the best-known medicinal plants with nearly 5000 years history of application in traditional medicine for treatment of asthma, nasal congestion, and central nervous system disorders. The plant is widely distributed in the world and its chemical composition is mainly dependent on species, parts used, harvesting date, geographic location, and extraction techniques [21]. However, there are few studies on its toxicological activity especially the reproductive toxicity. This species contains different biologically active macromolecules; the preliminary phytochemical screening showed the presence of carbohydrates, phenols, sterols, saponins, tannins, terpenoids, flavonoids and alkaloids in *Ephedra*

altissima stem extracts. The observations indicate that methanolic extract was the richest with bioactive compounds, in comparison to ethyl acetate and hexane extracts. Which appear to be responsible for the therapeutic properties of the plant but are also may be capable of exerting adverse actions in the body including the reproductive system.

It is fundamental to evaluate the concentration and quality of sperm to assess the effects of chemicals on male reproductive function. In the present study, effects of methanolic extract of *Ephedra Altissima* stems on the reproductive parameters were evaluated in adult albino male mice. The results demonstrate that, *Ephedra altissima* methanolic extract caused significant decrease in sperm count. This may be as a result of the ability of the extract at the given high dose; either interfere with spermatogenic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins, which may result in alteration of spermatogenesis [22].

The quality of sperms evaluated, by assessing the motility and morphology of sperms. It was found that there is no significant difference in the motility of sperms of treated mice compared to the control mice, but there is increase in the number of sperms with abnormal morphology in mice treated with extract. The tail region of the sperm in the treated group were much affected than the head region. Morphological alterations of spermatozoa along with reduction in total sperm count may affect male mice fertility and can cause significant reduction in fertility rate.

It has been found that some phytochemicals possess antifertility side effects, some of these compounds include Cathinone alkaloid the major component of *Catha edulis* has been shown to produce decrease in sperm count and motility and increase in the number of abnormal sperms in rats treated with toxic dose. Histopathological examination of testes revealed degeneration of interstitial tissue, cellular infiltration and atrophy of Sertoli and Leydig's cells [23]. After six weeks treatment, alkaloids of *Tripterygium wilfordii* may produce damage in epithelial cells of

seminiferous tubules in rats' testes; the number of spermatogenic cells in seminiferous tubules decreases and the wall of seminiferous tubules becomes thinner; as the dosage increase the damage aggravates [24]. Another research has been shown that alkaloids of tobacco induced significant decrease of sperm-count and motility; the histological study revealed a pronounced atrophy of the interstitial tissue and a deterioration of somniferous tubules within testis. It has been observed that alkaloids effects are associated to high levels of lipid peroxidation [25].

Treatment with terpenoids isolated from roots of *Echinops echinatus* showed a decrease in the relative weight of the reproductive organs of Wister albino rats, and significant decrease in serum testosterone levels and cauda epididymal sperm concentration. Histology of testis showed a significant reduction in the seminiferous tubular diameter and germinal epithelial cell thickness [26]. Andrographolide diterpenoid present in *Andrographis paniculate*, (20 mg daily for 60 days) has been shown to inhibit spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells, and regressive or degenerative changes in the epididymis, seminal vesicle, ventral prostate, and coagulating glands in male albino rats. Andrographolide also produced similar results when orally administered to male Wistar albino rats for 48 days [27].

Treatment with Gossypol, a phenolic compound, found in seeds of *Gossypium herbaceum* resulted in reduction in sperm count and motility, increased abnormal sperm count, and reduced serum levels of testosterone, LH, and FSH; histopathological examination showed Sertoli cell and seminiferous tubules damage [28].

Cyanogenic glycosides of *Carica papaya* linked with suppression of cauda epididymal, sperm motility, and reduced sperm count. It also caused degeneration of germinal epithelium and germ cells, reduction in the number of Leydig cells and vacuoles in the tubules of treated rats [29].

It was indicated that saponins isolated from seeds of *Camellia oleifera* decreased percent of live sperm and sperm count; also, the number of abnormal

spermatozoa increased in mice treated with isolated saponins. Testicular weight and seminiferous tubular area gradually decreased as dosage increased [30].

In this research work, the histological examination revealed that there was no structural alteration in the seminiferous epithelium of the treated mice testis compared to control mice after treated period for fourteen days. The damage of the histological structure may need longer duration of treatment to be observed. The dose of Ephedra methanolic extract and the duration of treatment, in this study, produce damage in the function of the reproductive male system of albino mice.

CONCLUSION

The net observation from this study allowed to conclude that the components of the methanolic extract of *Ephedra altissima* stems have the potential to affect male mice reproductive functions but not affect the structure at the given high dose and short period of treatment. The development of abnormal sperm and reduction in sperm count may affect male fertility, and consequently impaired fertility, which might be a consequence.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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