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EFFECT OF *ATRIPLEX NUMMULARIA, FICUS INGENS* AND *SCORZONERA ALEXANDRINA* EXTRACTS ON REPRODUCTIVE ORGANS AND FERTILITY OF MALE RATS

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ABSTRACT

We have investigated the effect of *Atriplex nummularia, Ficus ingens* and *Scorzonera alexandrina* extracts on the fertility of male rats. Serum reproductive hormone levels, weight of testes and accessory sex organs, sperm characters and histopathology of testes were used to evaluate the reproductive efficiency of extracts-exposed rats. None of the sexual activity parameters was significantly different between *A. nummularia* and *F. ingens*-treated groups and the control rats. The ethanolic extract of *S. alexandrina* at doses of 200 and 400 mg/kg for 7 weeks did not cause body weight loss but significantly decreased the weight of testes and accessory sex organs of male rats in a significant manner. Serum testosterone level, sperm count and sperm motility were reduced significantly in *S. alexandrina*-medicated rats. Moreover, fertility success of males treated with *S. alexandrina* at 200 or 400 mg/kg was markedly reduced by 58.33% and 41.66%, respectively after mating with normal females. Histologically,

seminiferous tubules of *S. alexandrina*-treated rats showed marked arrests of spermatogenesis. *S. alexandrina* extract at doses of 200 and 400 mg/kg demonstrated potential antifertility effects without altering general body metabolism. The antifertility effect of this extract could be attributed to the reduction in testosterone level.

Keywords: Sperm Count, Sperm Motility, Accessory Sex Organs, Testosterone

INTRODUCTION

Infertility is a major clinical concern, affecting 15% of all reproductive age couples. Male factors, including decreased semen quality, are responsible for 25% of these cases. A large number of plant species affecting fertility have been screened and were subsequently fortified by national and international agencies **[1]**.

The species of the genus *Atriplex* belonging to family Chenopodiaceae are partly spontaneous in the WANA (West Asia and North Africa) area and have partly been introduced to determine their adaptability for use as fodder species **[2]**. *A. nummularia* Lindl. is a species of saltbush known by the common names old man saltbush, bluegreen saltbush, and giant saltbush. The plant is generally palatable to grazing animals, but the palatability can be limited by the concentration of salt in the plant tissues as the plant takes in water from saline soils **[3]**. It is widely used as a forage crop in Tunisia. Australian Aborigines used the seeds as a traditional food source.

Ficus is a genus belongs to the large Moraceae family that occurs in the temperate regions of South Africa, the Northern provinces and further northwards into tropical Africa as far north as Ethiopia and Saudi Arabia **[4]**. All parts of *F. ingens* have milky latex when broken. The latex is used as a substitute disinfectant for iodine. Extracts of the bark are administered to cows with a low milk production. Mammals and birds eat the fruit of *F.ingens* which are not always as palatable as *F.carica*.

The genus *Scrozenera* is a member of the family Asteraceae which is a known source of numerous classes of bioactive natural products **[5]**. *Scrozenera* encompasses about 160 species that are widely spread in arid regions of Europe and Africa. In Egypt, the genus *Scrozenera* is represented by five species, namely; *S. pseudolanata* Grossheim, *S. mollis* Bieb, *S. schweinfurthii* Boiss, *S. drarii* Tackh and *S. alexandrina* Boiss. The first four species are very rare in the Egyptian deserts while *S. alexanderina* is very common.

The use of plant extracts as antifertility or as fertility enhancer is now in the increase because of the shifting of attention from synthetic drugs to natural plant products **[6]**. The effect of *A. nummularia, F. ingens* and *S. alexandrina* on fertility of males has not been explored and, hence, this study was undertaken.

METHODOLOGY

Plant Material

A. nummularia and S. alexandrina plants were collected from the North Western coast of Egypt during spring 2010, while *F. ingens* was collected from Tabouk area, Kingdom of Saudi Arabia during summer 2010. The collected plants were identified by Prof. Dr. Abd El Naser Al Gefri, Pharmacognosy Department, Faculty of Pharmacy, Salman bin Abdulaziz University, Al-Kharj, KSA. A specimen from each plant has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Salman bin Abdulaziz University, Al-Kharj, KSA.

Extract Preparation

The collected plants were dried under shade and then grinded to fine powders. The dried powders of *A. nummularia, F. ingens* and *S. alexandrina* (750, 750 and 500 g, respectively) were extracted by percolation in 70% aqueous ethanol with occasional shaking for 48 h. This process was repeated three times, and then the ethanolic extracts of each plant were combined and concentrated under reduced pressure to give total extracts; 64, 68 and 51g for *A. nummularia*, *F. ingens* and *S. alexandrina*, respectively.

Preparation of the Extract for Biological Studies

The total ethanolic extracts of *A. nummularia*, *F. ingens* and *S. alexandrina* were separately suspended in 3% v/v Tween 80 in distilled water (vehicle) to obtain concentration of 200 mg/ml. Suspensions were administered to animals at the dose levels of 100, 200 and 400 mg/kg in the dose volume of 10 mL/kg. Extract suspensions were freshly prepared every two days. The control animals were administered vehicle only.

Animals

Healthy adult male (200-220 g) and female (180-200 g) Wistar rats were used in the fertility study. Both sexes of adult albino mice (25–30 g body weight) were used in the acute toxicity test. Animals were obtained from Lab Animal Care Unit, Pharmacy College, Salman bin Abdulaziz University, Al-Kharj, KSA. All animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, fed with standard rodent diet and water *ad libitum*. Commercially obtained sawdust was used as bedding material. The cages were washed

once a week. The animals were allowed to acclimatize to the laboratory condition for one week before commencement of the experiment. The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval.

Acute Toxicity Study

Acute toxicity of *A. nummularia*, *F. ingens* and *S. alexandrina* was conducted in albino mice using the procedure of **[7]**. The animals were fasted overnight and each extract was administered orally at three dose levels (1000, 2000 and 4000 mg/kg body weight). The vehicle control group received 3% v/v Tween 80 in distilled water. Animals were observed individually after dosing for a total of 14 days for any clinical sign of mortalities. Animals were also observed for the presence of toxic symptoms such as weakness, aggressiveness, refusal of food, diarrhea, noisy breathing, and fluid discharge from eyes and ears. Surviving animals were sacrificed by sodium pentobarbital overdose and given a complete gross pathology examination.

Justification for Dose Selection

Results of acute toxicity studies in albino mice indicated that *A. nummularia*, *F. ingens* and *S. alexandrina* were nontoxic up to the maximum dose level of 4000 mg/kg. On the basis of these results, the doses selected for the study was 100, 200 and 400 mg/kg. The oral route was selected for use because oral route is considered to be a proposed therapeutic route **[8]**.

Male Rat Fertility Study

The daily doses of the vehicle or the test extracts were administered to each rat every morning as follows:

a) Control group: Received the vehicle in a dose of 10 mL/ kg.

b) *A. nummularia* group: Received *A. nummularia* at doses of 100, 200 and 400 mg/kg.

c) *F. ingens* group: Received *F. ingens* at doses of 100, 200 and 400 mg/kg.

d) *S. alexandrina* group: Received *S. alexandrina* at doses of 100, 200 and 400 mg/kg.

Both vehicle and the test extracts were given by gavage for seven weeks. This administration period is necessary to determine the effect of the extracts on sperm production because rats need a period of 48–52 days for the exact spermatogenic cycle **[9]**.

Mating Studies

Five days before the termination of the experiment, each male rat in all groups was cohabited individually with two proestrous females. Successful mating in each case was confirmed by the presence of spermatozoa in the vaginal smear the following morning **[10]**.

All mated females were observed for the incidence of pregnancy. The following reproductive parameters were then computed: mating success % = ([number mated/number paired] × 100); fertility success % = ([number pregnant/number paired] × 100); Fertility index = ([number pregnant/number mated] × 100).

Sample Collection

Blood samples were collected from rats, 24 h after the last dose of treatment under light ether anesthesia by cardiac puncture into centrifuge tubes and left to clot for 10 min at room temperature. The tubes were centrifuged at 3000 rpm for 5 min and the sera separated, stored frozen and used within 12 h of preparation for the estimation of testosterone **[11]**, prolactin **[12]** follicle-stimulating hormone (FSH) and luteinizing hormone (LH) **[13]**. Moreover, liver functions were evaluated by measuring the serum activity of alanine amino transferase (ALT) and aspartate aminotransferase (AST) **[14]**. Serum concentrations of urea **[15]** and creatinine **[16]** were determined colorimetrically as measures of kidney functions.

Body and Organ Weight Measurements

Initial and final body weights of the animals were recorded. The animals were dissected and the reproductive organs, namely, testes, epididymis, seminal vesicle and ventral prostate, were excised, cleared of adhering fat and connective tissue and weighed. Organ weights were reported as relative weights (organ weight/body weight×100).

Sperm Characteristic Analysis

Assessment of Sperm Count

The cauda epididymidis on one side was exposed and incised. The semen that oozed was quickly sucked into a red blood pipette to the 0.5 mark, and then diluted with warm normal saline which was sucked up to the 101 mark. The normal saline at the stem of the pipette was discarded and then the contents of the bulb of the pipette were mixed thoroughly. A drop of the semen mixture was placed on the Neubauer counting chamber which then spread under the cover- slip by capillary action. The counting chamber was then mounted on the slide stage of the microscope and viewed under the magnification of x40. A grid system divides the counting chamber into 5 major squares using the top and right or left and bottom system of counting **[17]**. The total numbers of sperm cells were counted and expressed as million/mL.

Assessment of Sperm Motility

The percentage of forward progressive sperm motility was evaluated **[17]**. A slide was placed on a light microscope with a heated stage warmed up to 37 °C, and then 2-3

droplets of Tris buffer solution [0.3 M Tris(hydroxymethyl) aminomethane, 0.027 M glucose, 0.1 M citric acid] were dropped on the slide.A small droplet of fluid obtained from left cauda epididymis with a pipette was added to the Tris buffer solution and mixed by a cover-slip. The percentage of forward progressive sperm motility was evaluated visually at 400× magnification. Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score.

Assessment of Sperm Viability and Morphology

Eosin–nigrosin stain (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) was prepared to evaluate sperm viability according to WHO protocol **[18]**. One drop of semen was mixed with two drops of eosin- nigrosin stain. Thin smears were then prepared and observed under a light microscope at ×100 magnification. Viable sperms remained colorless while nonviable sperms stained red **[19]**.

To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin–nigrosin viewed under a light microscope at 400× magnification. A total of 300 sperm cells was examined on each slide (1800 cells in each group), and the head, tail and total abnormality rates of spermatozoa were expressed as a percent **[20]**.

Histological Examination

For the histologic examination, testis of each rat was fixed in Bouin's solution immediately after removal. The tissues were then placed in ethanol 70% for 24 h, dehydrated, embedded in paraffin, sectioned at 5 μm, subsequently stained with haematoxylin/eosin and examined by light microscopy **[20]**.

Statistical Analysis

Data are expressed as the mean ± SEM. One-way ANOVA complemented with Student's *t*-tests were used to evaluate significant differences between control and the extract-treated groups. Differences within values at $P < 0.05$ were considered statistically significant. All statistical analyses were performed using the SPSS 13.0 software.

RESULTS

Acute Toxicity Study

No mortality or any signs of behavioral changes or toxicity were observed throughout the 14-day period after single oral administration of *A. nummularia, F. ingens and S. alexandrina* extracts up to the dose levels of 4000 mg/kg. Morphological characteristics (fur, skin, eyes, and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual

behaviors such as self-mutilation, walking backward and so forth were observed; gait and posture, reactivity to handling or sensory stimuli, grip strength were all normal. Further, there were no gross pathological abnormalities in mice of all groups. The non-toxic nature of the ethanol extract of *A. nummularia*, *F. ingens* and *S. alexandrina* in acute toxicity study is well supported by the normal levels of ALT, AST, urea and creatinine following 7-weeks treatment period in rats (data not shown). In addition, there were no deaths during the course of the fertility study (7 weeks) in any of the control and treated groups.

Effect on Male Fertility

Oral administration of *A. nummularia* and *F. ingens* extracts did not affect any of male fertility parameters **(Tables 1-4)**.

Hormonal Assay

Levels of testosterone, prolactin, FSH and LH in the serum of males treated with *A. nummularia* and *F. ingens* at different dose levels were comparable to control values. *S. alexandrina* extract at doses of 200 and 400 mg/kg body weight significantly reduced the serum concentration of testosterone in male rats, whereas the values of FSH and LH were not significantly altered **(Table 1)**. On the contrary, significant elevation of serum prolactin level was observed.

Body and Reproductive Organs Weights

No alterations were recorded in the final body weight of rats following exposure to all extracts in doses of 100, 200 and 400 mg/kg for 7 weeks **(Table 2)**. No statistically significant differences in relative weights of testes and accessory sex organs were noted in any of *A. nummularia* and *F. ingens*-treated groups compared with the control one. Oral administration of ethanolic extract of *S. alexandrina* in doses of 200 and 400 mg/kg to male rats for 7 weeks decreased the weight of testes, epididymis, seminal vesicle and ventral prostate in a significant manner **(Table 2)**.

Epididymal Sperm Characteristics

The effects of different doses of the test extracts on epididymal sperm characters are presented in **Table 3**. Sperm count and percentages of sperm motility, sperm viability and sperm abnormalities did not show any significant alteration after exposure to all doses of *A. nummularia* and *F. ingens* extracts and were comparable to the control group. The count, motility and viability of epididymal spermatozoa were reduced significantly in *S. alexandrina*-treated group at doses of 200 and 400 mg/kg as compared to control rats. Significant increase in sperm abnormality was observed in spermatozoa of rats treated with the same doses of *S.*

alexandrina extract. Majority of spermatozoa showed coiled mid piece and bent tail.

Fertility Test

There were no effects of *A. nummularia* and *F. ingens* treatment on the fertility of male rats. The fertility of *S. alexandrina*-treated male rats declined in a dose-dependent pattern, and their ability to mate was also reduced as evidenced by the number of mated females in 200 and 400 mg/kg groups (8/12 and 7/12, respectively) as compared to 12/12 in control animals **(Table 4)**. Seven and five female rats that mated with *S. alexandrina*-males at doses of 200 and 400 mg/kg conceived as compared to 12 pregnant females in the control group. While the fertility success was 100% for the control male rats, it dropped to 58.33 and 41.66% in rats exposed to *S. alexandrina*-extract in doses of 200 and 400 mg/kg, respectively.

Histological Examination

Histopathology of testes of the control rats showed normal round or oval seminiferous tubules with normal germ cells at various stages covering complete spermatogenic cycle **(Figure 1-A)**. Cross section of testes obtained from rats exposed to various doses of *A. nummularia* and *F. ingens* extracts revealed well-preserved seminiferous tubules with normal amount of stroma **(Figure 1-B)**. Testes of rats treated with *S. alexandrina* extract showed degeneration of spermatogoneal cells linning seminiferous tubules with incomplete spermatogenesis **(Figure 1-C)**.

DISCUSSION

In the current study, the acute toxicological evaluation revealed that oral administration of the ethanolic extract of *A. nummularia, F. ingens* and *S. alexandrina* did not produce any demonstrable acute toxic effect or death in all groups of mice. Since no remarkable changes in animal behavior were observed at doses up to 4000 mg/kg as compared to control group, it can be inferred that *A. nummularia, F. ingens* and *S. alexandrina* extracts are nontoxic. Accordingly, it suggested that oral LD50 of the extracts was higher than 4000 mg/kg b.wt. Therefore, the tested extracts can be categorized as highly safe since substances possessing LD50 higher than 50 mg/kg are non-toxic **[21]**.

The non-toxic nature of the ethanolic extracts of *A. nummularia, F. ingens* and *S. alexandrina* in acute toxicity study is well supported by the biochemical data following 7-week treatment period in rats. Since the activity of ALT and AST are specific assayable liver enzymes, their normal levels in serum of experimental groups of rats treated for 7 weeks means that the three tested plants are not hepatotoxic. Urea and

creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. In kidney damage, there will be retention of urea and creatinine in the blood **[22]**, therefore marked increase in serum urea and creatinine are indications of functional damage to the kidney **[23]**. By these indicators, ethanolic extracts of *A. nummularia, F. ingens* and *S. alexandrina* are therefore, not nephrotoxic in rats.

Testosterone is the main male gonadal hormone secreted from the testicular Leydig cells **[24]**. It promotes differentiation of spermatozoa during the process of spermatogenesis. So, a lack of testosterone level would have direct effects on the process of spermatogenesis **[25]**. Accordingly, decreased level of testosterone is one of the indicators of the chemical toxicity on reproductive system **[26]**. The results of this work show that rats treated with *S. alexandrina* extract (200 and 400 mg/kg) exhibited a significant decrease in testosterone level compared to animals in the control group. The decrease in testosterone level by *S. alexandrina* extract may be adduced to reduction of the hormone synthesis by the Leydig cells, as the cells are the main source of testosterone **[27]**.

It is well established that FSH and testosterone are both required by Sertoli cells/germ cells to support the process of spermatogenesis. Depletion in the biosynthesis of any one of these hormones, therefore, could block formation of spermatozoa. In males, LH stimulates the testicular Leydig cells to secrete testosterone. The fact that serum LH and FSH levels were unaltered in the treated animals suggested that *S. alexandrina* extract was acting directly on the testis **[28]**.

It is known that monitoring body weight provides information on the general health level of animals, which can be important to the interpretation of reproductive effects **[29]**. In this study, the body weight of rats treated with *A. nummularia, F. ingens* and *S. alexandrina* extracts were not altered indicating that the general metabolic condition of the animals was within normal range. According to **[30]**, weights of testes and accessory organs are sensitive end points that can be used in evaluation of deleterious effect on male reproduction. The weight of the testes and epididymis is basically dependent on the mass of the differentiated spermatogenic cells and spermatozoa in the tissue **[31]**. Differentiation of spermatozoa during the process of spermatogenesis was promoted under the effect of testosterone **[32]**. In the present study, testes, epididymis, and accessory organs obtained from rats

exposed to *A. nummularia* and *F. ingens* in doses of 100, 200 and 400 mg/kg were not atrophic and their weights did not decrease markedly following 7 weeks-medication. The non-significant effect of *A. nummularia* and *F. ingens* on the relative weights of the epididymis, seminal vesicles and ventral prostate may be an indication that the general metabolic conditions of these sex organs were within the normal range **[33]**. Conversely, *S. alexandrina* extract in doses of 200 and 400 mg/kg caused a significant reduction in the testes and accessory sex organ weights which might be due to the decrease in serum testosterone level and inhibition of spermatogenesis **[31]**. The decreasing weight of testes, epididymis, seminal vesicle and ventral prostate clearly indicated that *S. alexandrina* extract caused structural and functional alteration in the reproductive organs of male rats **[34]**.

Reduced epididymal sperm count could be a direct impact of suppressed concentration of testosterone in the circulation **[35]**. In this investigation, it was found that treatment of male rats with the ethanolic extract of *S. alexandrina* at doses of 200 and 400 mg/kg result in reducing count, motility and viability of sperms. Lowering of epididymal sperm count suggested an undersupply of testosterone to the epididymis, thereby possibly causing impaired epididymal function. Further, decrease in sperm count is correlated with decrease in the testicular weight indicating that the germ cell death or cell loss from the epithelium may be due to tubular atrophy which is a main reason for decreased testis **[36]**.

Alterations in the sperm motility, viability and morphology are indications of a disturbed epididymal microenvironment **[37]**. Therefore, the absence of significant effect of *A. nummularia* and *F. ingens* at all the tested doses on the epididymal sperm motility, viability and morphology could possibly imply that the extracts did not produce disturbance in the microenvironment of the epididymis. This may rule out impairment/hindrance in the normal functioning of the epididymal sperm cells during the experimental period. It is also possible that the extracts were unable to cross the blood-testes barrier and thus could not interfere with the normal functioning of the testicular epithelium **[38]**. The results of this study have shown that exposure of rats to *S. alexandrina* extract at doses of 200 and 400mg/kg caused a decrease in sperm motility and viability. The significant reduction of sperm viability, sperm motility and sperm counts shows that the extract has the potential to penetrate the blood-testis barriers.

Moreover, the reduction in sperm motility could be ascribed to the reduction in plasma testosterone level **[39]**.

Testosterone is required for the maintenance of normal sexual desire and penile erection in males. In the present study, the fertilizing ability of male rats was significantly reduced by the administration of *S. alexandrina* extract at doses of 200 and 400 mg/kg. The reduction in mating and fertility success of male rats by doses of 200 and 400 mg/kg of *S. alexandrina* extract could be due to decreased testosterone level that reduces androgen-dependent parameters like mating behavior, libido and penile erection **[40]**. In addition, sperm motility is an important functional measurement to predict sperm fertilizing capacity, so the negative impact of *S. alexandrina* extract at doses of 200 and 400 mg/kg on motility would seriously affect the fertilizing ability **[41]**.

Sections taken through the testes of control and *A. nummularia* and *F. ingens* treated-animals demonstrated normal histology. In contrast, *S. alexandria* in doses of 200 and 400mg/kg provoked some histopathological changes in the testes such as disorganized seminiferous tubules with incomplete spermatogenesis and sloughing of degenerated germ cells. One possible explanation for the incomplete spermatogenesis is the reduction in testosterone level **[25]**. Moreover, sloughing of germ cells was observed in the lumen of some seminiferous tubules indicating testicular dysfunction **[41]**.

In conclusion, our results suggest the absence of male reproductive toxicity of *A. nummularia* and *F. ingens* extracts at doses tested. Conversely, the ethanolic extract of *S. alexandrina* in doses of 200 and 400mg/kg could significantly alter the fertility potential of male rats. This is demonstrated by the decrease in the fertility parameters (sperm count, motility, testosterone level, and fertility rate) in treated rats.

DECLARATIONS

Conflict of Interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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Table 1: Effect of *A. nummularia, F. ingens* and *S. alexandrina* Extracts for 7 Weeks on Serum Levels of Reproductive Hormones of Male Rats, (n = 6)

Groups	Doses (mg/kg)	Testosterone (ng/mL)	Prolactin (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
Control	00	4.92±0.29	0.73±0.05	7.50±0.21	0.58±0.03
A. nummularia	100	4.08±0.27	0.69±0.04	6.98±0.20	0.55±0.03
	200	5.15±0.20	0.78±0.04	7.00±0.32	0.61±0.04
	400	4.73±0.22	0.69±0.04	7.11±0.28	0.57±0.04
F. ingens	100	5.21±0.10	0.70±0.05	6.91±0.31	0.56±0.02
	200	4.52±0.21	0.73±0.04	7.28±0.45	0.58±0.03
	400	4.46±0.27	0.71±0.04	7.26±0.26	0.60±0.04
S. alexandrina	100	4.11±0.28	0.91±0.08	7.62±0.35	0.61±0.04
	200	3.52±0.25*	1.43±0.04*	7.00±0.46	0.59±0.03
	400	3.13±0.20*	1.69±0.03*	7.98±0.22	0.57±0.02

NOTE: Data are Expressed as Mean±S.E.M. of 6 Animals; * Indicate Significance Compared to Control Group (p< 0.05)

Table 2: Effect of *A. nummularia, F. ingens* and *S. alexandrina* Extracts for 7 Weeks on Body and Sexual Organs Weights of Male Rats, (n=6)

Groups	Doses (mg/kg)	Initial b.wt (g)	Final b.wt (g)	Relative weight of reproductive organs (g/100 g b.wt)			
				Testes (Pair)	Cauda epididymis	Seminal vesicles	Ventral prostate
Control	00	198.58±8.48	210.16±8.34	1.72±0.12	0.57±0.03	0.61±0.03	0.44±0.02
A. nummularia	100	196.42±9.35	206.85±8.85	1.60±0.11	0.55±0.02	0.59±0.04	0.44±0.02
	200	189.53±9.10	202.52±9.16	1.67±0.15	0.58±0.03	0.59±0.03	0.43±0.01
	400	182.11±7.96	201.85±9.55	1.65±0.13	0.54±0.04	0.57±0.05	0.43±0.03
F. ingens	100	183.52±8.53	210.56±7.84	1.64±0.14	0.60±0.04	0.56±0.04	0.42±0.03
	200	196.62±9.77	205.42±9.43	1.65±0.15	0.58±0.03	0.54±0.04	0.42±0.02
	400	183.10±9.28	206.60±8.84	1.68±0.14	0.59±0.02	0.54±0.03	0.40±0.03
S. alexandrina	100	192.30±9.37	201.94±7.87	1.51±0.13	0.52±0.03	0.55±0.03	0.40±0.03
	200	184.17±8.80	206.25±9.50	1.36±0.10*	0.46±0.02*	0.51±0.02*	0.37±0.02*
	400	185.30±8.64	207.50±9.38	1.34±0.11*	0.40±0.02*	0.49±0.03*	0.35±0.02*

NOTE: Data are Expressed as Mean±S.E.M. of 6 Animals; * Indicate Significance Compared to Control Group (p< 0.05)

Table 3: Effect of *A. nummularia, F. ingens* and *S. alexandrina* Extracts for 7 Weeks on Semen Characteristics of Male Rats, (n=6)

Groups	Doses (mg/kg)	Sperm count (X 10^6/mL)	Sperm motility (%)	Sperm viability (%)	Total sperm abnormalities (%)
Control	00	67.97±2.80	93.3±2.84	89.8±2.55	3.62±0.19
A. nummularia	100	69.29±1.53	92.6±2.67	88.5±2.81	3.24±0.18
	200	66.24±2.11	90.7±2.55	86.5±2.62	3.90±0.23
	400	68.62±2.26	92.8±2.17	84.8±2.50	4.11±0.27
F. ingens	100	63.85±2.65	90.2±3.70	90.2±3.11	3.10±0.25
	200	61.75±2.85	89.0±2.47	87.7±3.27	3.43±0.28
	400	60.35±2.82	90.2±3.14	89.0±3.53	4.25±0.22
S. alexandrina	100	59.74±2.85	86.4±2.10	82.6±2.19	4.10±0.24
	200	52.50±1.68*	83.5±2.62*	80.8±2.16*	5.82±0.28*
	400	50.63±1.96*	80.2±2.25*	77.5±2.14*	6.50±0.26*

NOTE: Data are Expressed as Mean±S.E.M. and % of 6 Animals; * Indicate Significance Compared to Control Group (p< 0.05)

Table 4: Effect of *A. nummularia, F. ingens* and *S. alexandrina* Extracts for 7 Weeks on the Fertility of Male Rats (Mating Ratio= 1 Male: 2 Females)

Groups	Doses (mg/kg)	No of mated females	Mating success %	No of pregnant females	Fertility success %	Fertility index %
Control	00	12/12	100	12/12	100	100
A. nummularia	100	12/12	100	11/12	91.66	91.66
	200	11/12	91.66	11/12	91.66	100
	400	10/12	83.33	10/12	83.33	100
F. ingens	100	11/12	91.66	11/12	91.66	100
	200	10/12	83.33	10/12	83.33	100
	400	10/12	83.33	10/12	83.33	100
S. alexandrina	100	10/12	83.33	9/12	75	90
	200	8/12	66.66	7/12	58.33	87.5
	400	7/12	58.33	5/12	41.66	71.4

NOTE: Data are Expressed as Numbers And % of 6 Males and 12 Females

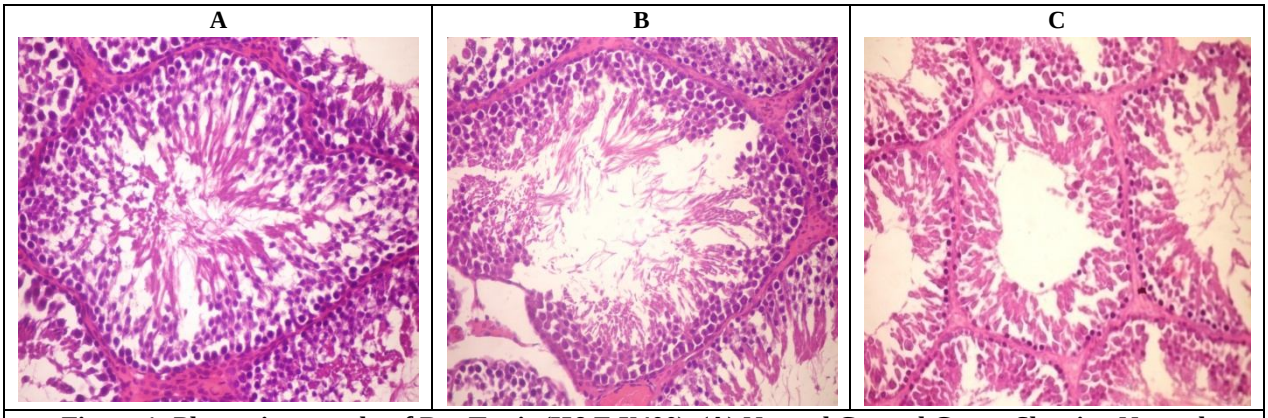

Figure 1: Photomicrographs of Rat Testis (H&E X400), (A) Normal Control Group Showing Normal Seminiferous Tubules with Complete Spermatogenesis; (B) *A. nummularia*(400 mg/kg) Showing the Normal Histological Structure of Seminiferous Tubule; (C)*S. alexandrina* (400 mg/kg) Showing Degeneration of Spermatogoneal Cells Lining Seminiferous Tubules with Incomplete Spermatogenesis