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ORIGINAL ARTICLE

Effect of *Emex spinosa*, *Leptadenia pyrotechnica*, *Haloxylon salicornicum* and *Ochradenus baccatus* extracts on the reproductive organs of adult male rats

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Abstract

Context: *Emex spinosa* (L.) Campd. (Polygonaceae), *Leptadenia pyrotechnica* (Forsk.) Decne (Asclepiadaceae), *Haloxylon salicornicum* (Moq.) Bunge ex Bioss. (Chenopodiaceae) and *Ochradenus baccatus* Delile (Resedaceae) are used in folk medicine for treatment of male sexual disorders.

Objective: To investigate the effects of *E. spinosa*, *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* extracts on the reproductive system of male rats after prolonged period of treatment.

Methods: Seventy-eight healthy adult male Wistar rats were divided into 13 groups (6 animals, each). The plant extracts (100, 200 and 400 mg/kg) were given daily by gavage to different groups of rats for 65 days. The thirteenth group (control) received the vehicle only. Test and control rats were mated with estrus female rats on days 30, 45 and 60 of treatment. Body and relative reproductive organ weights, and sperm parameters were recorded.

Results: Animals treated with the ethanol extracts of *E. spinosa* and *L. pyrotechnica* showed significant improvement of the relative weight of reproductive organs, sperm count, sperm motility and total sperm abnormality. The mean sperm count for *E. spinosa* group (400 mg/kg) was $233.7 \pm 4.50 \times 10^6$/mL, for *L. pyrotechnica* (200 and 400 mg/kg) groups were $237.0 \pm 5.22 \times 10^6$/mL and $240.3 \pm 4.64 \times 10^6$/mL, respectively and that of the control group was $218.1 \pm 4.28 \times 10^6$/mL. The sperm motility of the control group was 77.5 ± 2.12, those of *E. spinosa* (400 mg/kg) group was $87.3 \pm 3.50\%$ and those of *L. pyrotechnica* (200 and 400 mg/kg) groups were 86.0 ± 3.11 and $89.7 \pm 2.90\%$, respectively. Ethanol extracts of *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg) significantly elevate the serum levels of testosterone (5.30 ± 0.15, 5.32 ± 0.20 and 5.66 ± 0.19 ng/mL, respectively *vs* 4.64 ± 0.16 ng/mL) and luteinizing hormone (0.69 ± 0.03, 0.70 ± 0.03 and 0.74 ± 0.03 mIU/mL, respectively *vs* 0.59 ± 0.02 mIU/mL). On the other hand, no alterations were observed in body and relative organ weights, sperm numbers as well as sperm morphology of the male rats after the exposure to the *H. salicornicum* and *O. baccatus* extracts for 65 days.

Conclusions: *E. spinosa* and *L. pyrotechnica* extracts appear to possess fertility improvement activity in male rats due to their testosterone increasing property. Moreover, the results suggest the absence of male reproductive toxicity of the *H. salicornicum* and *O. baccatus* extracts at tested doses.

Keywords: Male fertility, testosterone, follicle-stimulating hormone, luteinizing hormone, prolactin, sperm parameters

Introduction

Fertility regulation with plants or plant preparations, in the indigenous systems of medicine, has been reported. A large number of plant species affecting fertility have been screened in China and India more than 50 years ago and were subsequently fortified by national and

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international agencies (Lohiya, 2000; World Health Organization (WHO), 2000).

Emex spinosa (L.) Campd. (Polygonaceae) is an annual stout herb with spreading stems and sweet roots. It is one of the important medicinal plants used as purgative, diuretic and a remedy for stomach disorders (Watt & Breyer-Brandwijk, 1962). The boiled leaves are used by African tribes to stimulate appetite and for the cure of dyspepsia and biliousness (Mossael al., 1987). The aqueous ethanol extracts (70%) of *E. spinosus* leaves exhibited free radical scavenging activity against 2,2-diphenyl-2-picrylhydrazyl (Emam et al., 2010). Three anthraquinone pigments were detected in *Emex spinosus*; chrysophenol, physcion, emodin in addition to four sterols: stigmasterol, campesterol, β-sitosterol and β-sitosterol-glucoside (Abdel-Fattah et al., 1990).

Leptadenia pyrotechnica (Forsk.) Decne (Asclepiadaceae) is a typical desert shrub growing in different parts of Africa, Asia, and the Mediterranean region (McLaughlin, 2006). The whole plant of *L. pyrotechnica* afforded 18 new pregnane glycosides with sarcostin, 11-hydroxysarcostin, and deacetyl metaplexigenin as a glycone moieties and acetyl, benzoyl, cinnamoyl, *p*-coumaroyl, and nicotinoyl ester moieties (Cioffi et al., 2006).

Haloxylon salicornicum (Moq.) Bunge ex Bioss. (Chenopodiaceae) is a desert plant. The plant is reported to be used as anti-diabetic (Ajabnoor et al., 1984), antibacterial (Al-Saeed, 2002) and anti-inflammatory (Al-Shanawani, 1996).

Ochradenus baccatus Delile (Resedaceae) is a yellowish green shrub, distributed nearly in all the deserts of Egypt (Tackholm, 1974). The flavonoids quercetin 3-*O*-β-glucosyl (1→2)-α-rhaminoside-7-*O*-α-rhaminoside and quercetin 3-*O*-*p*-coumaryl (1→6)-β-glucosyl (1→6)-β-glucoside-7-*O*-α-rhaminoside were isolated from the aerial part of *O. baccatus* (Barakat et al., 1991).

Infertility is one of the major health problems and approximately 30% of this problem is due to male factors (Isidori et al., 2006). Several factors can interfere with the process of spermatogenesis and reduce sperm quality and quantity. The present study was carried out to evaluate the effect of *E. spinosa, L. pyrotechnica, H. salicornicum* and *O. baccatus* extracts on the reproductive organs and fertility of male rats.

Materials and methods

Plant material

E. spinosa was collected from desert around Tabuk area, Saudi Arabia during spring 2009, while *L. pyrotechnica, H. salicornicum* and *O. baccatus* were collected from Eastern Desert of Egypt during spring 2009. The collected plant samples were kindly identified by Dr. Ahmed Morsy Ahmed, botanist (Desert Research Center, Egypt) and by comparison with the published plant description (Migahid, 1974). Voucher specimens are deposited in the herbarium of Chemistry Department, Faculty of Sciences, King Saud University. Plant material was air-dried in shade, reduced to fine powder, packed in tightly closed containers and stored at room temperature for phytochemical and biological studies.

Extraction

The air-dried powders of *E. spinosa*, *H. salicornicum*, *L. pyrotechnica* and *O. baccatus* and their aerial parts (1 kg, each) were separately extracted by percolation in 90% ethanol at room temperature for two days. The ethanol extract was filtered and the residues were re-percolated for four times. The total ethanol extract was concentrated under reduced pressure at a temperature not exceeding 35°C to yield a dry extract of 195, 200, 155, and 178 g for *E. spinosa*, *H. salicornicum*, *L. pyrotechnica* and *O. baccatus*, respectively.

Phytochemical screening

Powdered samples from the aerial parts of *E. spinosa*, *H. salicornicum*, *L. pyrotechnica,* and *O. baccatus* were subjected to preliminary phytochemical screening (Sofowora, 1993, Trease & Evance, 1989).

Animals

Healthy adult male Wistar rats with initial weight of 200–220 g were used in this study. The animals were housed in standard polypropylene cages with wire mesh top. Feeding pens and water bottles were mounted outside the cages. The cages were washed once a week. Animals were maintained under standard laboratory conditions on a 12 h light/dark cycle in a temperature-controlled room at 21 ± 3°C and fed with standard pellet diet with water *ad libitum*. Adult female Wistar rats (180–200 g) of proven fertility were used for the fertility test. All animals were acclimatized to the laboratory conditions for 10 days before the beginning of the experiments. The care and handling of the animals were in accordance with the internationally accepted standard guidelines and was approved by an institutional review board.

Preparation of the extracts for biological studies

The total ethanol extracts of *E. spinosa*, *L. pyrotechnica, H. salicornicum* and *O. baccatus* were suspended separately in 3% v/v Tween 80 in distilled water (vehicle).

Acute toxicity and determination of median lethal dose (LD_{50})

LD_{50} of the ethanol extracts of *E. spinosa*, *L. pyrotechnica, H. salicornicum* and *O. baccatus* were determined according to the method of Lorke (1983). Male Wistar rats in groups of six, received one of 1000, 2000, or 4000 mg/kg of the tested extracts. Control animals received the vehicle and kept under the same conditions. Signs of acute toxicity and number of deaths per dose within 24 h were recorded. LD_{50} was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no lethality at all.

Doses

The dose selection for the ethanol extracts of *E. spinosa*, *L. pyrotechnica, H. salicornicum* and *O. baccatus* was based on the acute toxicity study, which did not show any toxicity with the oral administration of doses up to 4000 mg/kg. Accordingly, experimental oral doses of 100, 200 and 400 mg/kg that equal to one-fortieth, one-twentieth and one-tenth of the maximum possible dose of the extracts that did not cause mortalities in rats were selected.

Experimental protocol

A total number of 78 male Wistar rats were divided into 13 groups of 6 animals each. The tested extracts were given to the rats by gavage daily for 65 consecutive days (the period needed for spermatogenic cycle) (Amann, 1982).

Group I: Control rats received 0.5 mL/day of the vehicle, i.e., Tween 80 in equivalent amount of normal saline. Groups II, III and IV: Rats were treated with *E. spinosa* at 100, 200 and 400 mg/kg, respectively. Groups V, VI and VII: Rats were treated with *L. pyrotechnica* at 100, 200 and 400 mg/kg, respectively. Groups VIII, IX and X: Rats were treated with *H. salicornicum* at 100, 200 and 400 mg/kg, respectively. Groups XI, XII and XIII: Rats were treated with *O. baccatus* at 100, 200 and 400 mg/kg, respectively.

Fertility index

The effects of the tested extracts on fertility index were estimated by serial mating technique of the treated males with normal (untreated) females of regular estrous cycle. Prior to the mating, the females were isolated for one month to rule out pre-existing pregnancy. Each male was cohabited with two coeval females in the evening for 5 days. Presence of sperms in the vaginal smears, examined on the next day morning, indicated positive mating (Dehghan et al., 2005) and the day of mating was taken to be day one of pregnancy. The process was repeated for three successive times at 30, 45 and 60 days during the experimental period with new females in estrous cycle. A single time point fertility index for each rat was carried out using the following formula WHO, 1983.

$$\text{Fertility index} = \frac{\text{Number of pregnant females} \times 100}{\text{Number of mated females}}$$

The litter size of the pregnant rats was also determined at the end of the gestation period.

Sample collection

The animals were weighed and sacrificed under light ether anesthesia, 24 h after the last dose of treatment. Blood samples were collected by cardiac puncture into centrifuge tubes and left to clot for 10 min at room temperature. The tubes were centrifuged at 3000 *g* for 5 min and the sera were separated, stored frozen and used within 12 h of preparation for the estimation of circulatory levels of hormones, namely, testosterone (Chen et al., 1991), prolactin (Tietz, 1995), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Uotila et al., 1981). Moreover, liver functions were evaluated by measuring the serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following the method of Reitman & Frankel (1957). Serum concentrations of urea (Wills & Savory, 1981) and creatinine (Kroll et al., 1987) were determined colorimetrically as measures of kidney functions.

Body and relative organ weight measurements

Initial and final body weights of the animals were recorded. The animals were dissected and testes, epididymis, seminal vesicle and ventral prostate were excised, cleared of adhering fat and connective tissue. Testes, seminal vesicle and ventral prostate were weighed to the nearest milligram on a digital electric balance. Organ weights were reported as relative weights (organ weight/body weight×100).

Sperm characteristic analysis

Right caudal epididymis was finely minced by anatomical scissors in 1 mL of isotonic saline in a Petri dish. It was completely squashed by a tweezers for 2 min, and then allowed to incubate at room temperature for 4 h to provide the migration of all spermatozoa from epididymal tissue to the fluid. The epididymal sperm concentration was determined with a hemocytometer using a modified method described by Sönmez et al. (2007) and Türk et al. (2007).

The percentage of forward progressive sperm motility was evaluated using a light microscope with heated stage as described by Sönmez et al. (2005). The left cauda epididymis from each animal was incised and a very small droplet of epididymal fluid obtained with a pipette was dropped on the slide. Several drops of Tris buffer solution [0.3 M Tris (hydroxymethyl) aminomethane, 0.027 M glucose, 0.1 M citric acid] were added to the epididymal fluid 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 means of the three successive estimations were used as the final motility score.

A sperm viability test was done by the method described by WHO, 1999. To evaluate sperm morphology, the ducts deferens from each animal was rinsed with 5 mL 0.9% NaCl to obtain a sperm suspension. Smears prepared from each sperm suspension were stained with eosin–nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate). The slides were then viewed under a light microscope at 400× magnification. A total of 200 sperm cells was examined on each slide (1200 cells in each group), and the head, tail and total abnormality rates of spermatozoa were expressed as a percentage (Türk et al., 2007).

Statistical analysis

Results were expressed as the mean ± SD. One-way ANOVA complemented with Student's *t*-tests were used to evaluate significant differences between the extract-treated groups and control one. Differences within values at $p<0.05$ and $p<0.01$ were considered statistically significant (Mahajan, 1997).

Results and discussion

Acute toxicity and determination of LD_{50}

The obtained results indicated that different doses of *E. spinosa*, *L. pyrotechnica, H. salicornicum* and *O. baccatus* extracts (1000, 2000 and 4000 mg/kg) did not produce any symptoms of acute toxicity and no mortality during 24 h of observation. No diarrhea, haematuria, restlessness, uncoordinated muscle movements, and respiratory distress were appeared. Accordingly, it suggested that oral LD_{50} of the tested extracts were higher than 4000 mg/kg. Therefore, the tested plants can be categorized as highly safe since substances possessing LD_{50} higher than 50 mg/kg are nontoxic (Buck et al., 1976).

The nontoxic nature of the ethanol extracts of *E. spinosa*, *L. pyrotechnica, H. salicornicum* and *O. baccatus* in acute toxicity study is well supported by the biochemical data following 65-day treatment period in rats (Table 1). The serum transaminases level is the most widely used measure of hepatic injury. Since the activity of ALT and AST are specific assayable liver enzymes, their normal levels in the serum of experimental groups of rats treated for 65 days indicated that the tested extracts are not hepatotoxic. Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. No significant change in the mean values of urea and creatinine was found in serum of rats following 65 days of extract administration at all dose levels when compared with control rats. By these indicators, the ethanolic extracts of *E. spinosa*, *L. pyrotechnica, H. salicornicum* and *O. baccatus* are therefore, not nephrotoxic in rats.

Effect on body and relative reproductive organ weights

General observations showed that all the rats in the study looked healthy. Their body weights did not change, indicating that the general metabolic conditions of the animals were within the normal range. However, administration of the ethanol extracts of *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg) to the rats for 65 days caused a significant increase in the relative weights of the testes, seminal vesicles and ventral prostate compared with the controls (Table 2). The weights of the accessory sex organs require continuous androgenic stimulation for their normal growth and functions. Therefore, the increased weight of the sex organs could be attributed to the increased levels of serum LH and testosterone by *E. spinosa* and *L. pyrotechnica*. There were no significant changes in the relative weight of the testes, seminal vesicles and ventral prostate in *H. salicornicum* and *O. baccatus*-treated groups (Table 1).

Serum hormone levels

FSH, LH and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH, LH and testosterone. FSH acts directly on the seminiferous tubules, whereas LH stimulates spermatogenesis indirectly *via* testosterone (Anderson et al., 1997).

The effect of *E. spinosa*, *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* on serum hormone profile in male rats is shown in Table 3. The means of serum testosterone and LH levels of rats treated with *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg) for 65 days significantly increased as compared to the controls. The stimulatory effects were dose-dependent. In fact, LH binds to Leydig cells and increases cAMP which increases protein secretion and the side-chain cleavage of cholesterol, as well as other likely steps, to increase steroidogenesis and the production of testosterone and other androgens. In addition, the deficiency of LH and FSH prevents the gonads from either

Table 1. Effect of oral administration of the tested ethanol extracts for 65 days on the serum activity of ALT and AST and serum levels of urea and creatinine in rats ($n=6$).

Groups	Dose (mg/kg)	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	0.00	65.11 ± 2.62	144.62 ± 5.39	32.16 ± 1.83	0.36 ± 0.03
E. spinosa	100	62.37 ± 3.47	145.20 ± 5.51	33.50 ± 1.85	0.37 ± 0.03
	200	64.50 ± 3.56	140.25 ± 4.22	35.17 ± 1.78	0.39 ± 0.02
	400	66.14 ± 3.22	146.71 ± 5.63	32.55 ± 1.55	0.35 ± 0.03
L. pyrotechnica	100	67.20 ± 4.72	142.47 ± 4.83	35.36 ± 1.85	0.33 ± 0.04
	200	63.78 ± 4.17	148.11 ± 4.16	35.08 ± 1.50	0.39 ± 0.03
	400	64.50 ± 3.64	141.47 ± 5.20	34.82 ± 1.92	0.40 ± 0.04
H. salicornicum	100	67.39 ± 4.26	146.50 ± 4.27	33.40 ± 1.96	0.37 ± 0.03
	200	63.15 ± 3.24	141.62 ± 4.83	31.72 ± 1.85	0.39 ± 0.03
	400	66.28 ± 4.50	145.95 ± 5.27	33.15 ± 1.55	0.36 ± 0.03
O. baccatus	100	64.82 ± 3.58	143.13 ± 4.85	34.58 ± 1.91	0.40 ± 0.04
	200	62.58 ± 4.14	144.52 ± 5.16	34.17 ± 1.77	0.37 ± 0.03
	400	65.72 ± 3.82	146.30 ± 4.30	30.85 ± 1.83	0.38 ± 0.02

Table 2. Effect of oral administration of the tested ethanol extracts for 65 days on the body weight and relative reproductive organ weights of male rats ($n=6$).

		Body weight (g)		Relative reproductive organs weight (g/100 g b.wt)		
Groups	Dose (mg/kg)	Initial	Final	Testes (pair)	Seminal vesicles	Ventral prostate
Control	0.00	212.5 ± 6.47	231.6 ± 6.83	1.19 ± 0.08	0.25 ± 0.03	0.17 ± 0.03
E. spinosa	100	216.7 ± 7.50	235.4 ± 7.59	1.27 ± 0.09	0.27 ± 0.04	0.19 ± 0.03
	200	220.7 ± 8.26	236.4 ± 8.22	1.35 ± 0.09	0.33 ± 0.04	0.22 ± 0.04
	400	207.7 ± 7.47	225.4 ± 7.85	1.45 ± 0.08*	0.38 ± 0.03*	0.26 ± 0.02*
L. pyrotechnica	100	215.3 ± 6.24	232.8 ± 7.77	1.36 ± 0.08	0.30 ± 0.04	0.21 ± 0.04
	200	204.3 ± 7.62	222.8 ± 7.36	1.44 ± 0.07*	0.39 ± 0.05*	0.27 ± 0.03*
	400	211.3 ± 6.80	228.8 ± 6.25	1.52 ± 0.08*	0.42 ± 0.06*	0.31 ± 0.03**
H. salicornicum	100	213.8 ± 8.33	234.5 ± 8.62	1.21 ± 0.10	0.23 ± 0.03	0.18 ± 0.02
	200	210.8 ± 6.18	225.5 ± 6.15	1.21 ± 0.08	0.27 ± 0.04	0.17 ± 0.03
	400	205.8 ± 7.57	223.5 ± 7.29	1.17 ± 0.11	0.28 ± 0.03	0.15 ± 0.04
O. baccatus	100	218.2 ± 6.75	234.2 ± 7.15	1.22 ± 0.10	0.25 ± 0.03	0.18 ± 0.02
	200	207.2 ± 7.29	226.2 ± 8.50	1.20 ± 0.09	0.29 ± 0.05	0.20 ± 0.03
	400	208.2 ± 8.08	249.2 ± 7.36	1.17 ± 0.08	0.26 ± 0.04	0.19 ± 0.02

*$p<0.05$ **$p<0.001$, significantly different from vehicle control group.

Table 3. Effect of oral administration of the tested ethanol extracts for 65 days on the serum hormone profile in male rats ($n=6$).

Groups	Dose (mg/kg)	Testosterone (ng/mL)	Prolactin (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
Control	0.00	4.64 ± 0.16	0.60 ± 0.03	7.34 ± 0.15	0.59 ± 0.02
E. spinosa	100	4.83 ± 0.15	0.63 ±.03	7.17 ± 0.28	0.61 ± 0.03
	200	5.04 ± 0.19	0.62 ± 0.02	7.22 ± 0.21	0.66 ± 0.04
	400	5.30 ± 0.15*	0.65 ± 0.02	7.35 ± 0.20	0.69 ± 0.03*
L. pyrotechnica	100	4.91 ± 0.21	0.62 ± 0.03	7.53 ± 0.14	0.64 ± 0.04
	200	5.32 ± 0.20*	0.60 ± 0.02	7.63 ± 0.17	0.70 ± 0.03*
	400	5.66 ± 0.19**	0.64 ± 0.02	7.86 ± 0.19	0.74 ± 0.03**
H. salicornicum	100	4.50 ± 0.18	0.62 ± 0.03	7.04 ± 0.16	0.58 ± 0.02
	200	4.46 ± 0.12	0.60 ± 0.04	7.12 ± 0.19	0.60 ± 0.03
	400	4.52 ± 0.15	0.64 ± 0.03	7.27 ± 0.20	0.61 ± 0.02
O. baccatus	100	4.70 ± 0.18	0.65 ± 0.04	7.49 ± 0.17	0.57 ± 0.02
	200	4.63 ± 0.20	0.64 ± 0.03	7.63 ± 0.16	0.59 ± 0.04
	400	4.65 ± 0.16	0.64 ± 0.04	7.30 ± 0.18	0.58 ± 0.03

*$p<0.05$ **$p<0.001$, significantly different from vehicle control group.

producing sperms or sufficient quality of testosterone (Nieschlag, 1997).

The serum levels of prolactin and FSH did not reveal any significant change in all treated groups when compared with their control counterparts.

Epididymal sperm characteristics

The effects of different doses of *E. spinosa, L. pyrotechnica, H. salicornicum* and *O. baccatus* extracts on sperm counts, motility, viability and abnormalities were shown in Table 4.

Epididymal sperm count

In Table 4, daily administration of *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg) extracts to rats for 65 days significantly increased the means of epididymal sperm counts (233.7 ± 4.50, 237.0 ± 5.22 and 240.3 ± 4.64 × 10^6 sperm/mL, respectively) compared with their control group (218.1 ± 4.28 × 10^6 sperm/mL). Testosterone in humans or androstenedione in animals are synthesized in the Leydig cells under the influence of LH (Vasudevan & Sreekumari, 2005). Thus, increased testosterone level is responsible for the increased sperm counts noted in *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg)-treated groups when compared with the control.

Sperm motility

Oral administration of the total ethanol extract of *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg) for 65 days, progressively and significantly increased sperm progressive motility compared with the control groups. There was insignificant effect in the percentage of sperm motility in *H. salicornicum* and *O. baccatus* groups compared with the control one (Table 4). Ganong (1999) mentioned that seminal vesicle secretes fructose, phosphorylcholine, ergothioneine and prostaglandins. These chemical components of seminal fluid are responsible for enhancing motility of sperm; hence its increased secretion by the organ will lead to increased motility.

Table 4. Effect of oral administration of the tested ethanol extracts for 65 days on semen characteristics of male rats ($n = 6$).

Groups	Dose (mg/kg)	Sperm count ($\times 10^6$/mL)	Sperm motility (%)	Viable sperms (%)	Total sperm abnormalities (%)
Control	0.00	218.1 ± 4.28	77.5 ± 2.12	90.7 ± 2.16	3.28 ± 0.11
E. spinosa	100	226.5 ± 5.84	80.8 ± 2.27	91.3 ± 3.25	3.15 ± 0.13
	200	227.3 ± 4.24	83.0 ± 2.22	93.0 ± 2.14	2.90 ± 0.10
	400	233.7 ± 4.50*	87.3 ± 3.50*	94.5 ± 3.37	2.85 ± 0.12*
L. pyrotechnica	100	229.8 ± 4.17	83.3 ± 2.47	93.0 ± 3.65	2.98 ± 0.12
	200	237.0 ± 5.22*	86.0 ± 3.11*	94.4 ± 2.53	2.88 ± 0.10*
	400	240.3 ± 4.64**	89.7 ± 2.90**	96.2 ± 3.91	2.80 ± 0.11*
H. salicornicum	100	220.3 ± 4.55	77.6 ± 2.55	90.7 ± 2.44	3.25 ± 0.14
	200	222.5 ± 4.82	78.6 ± 2.50	90.2 ± 3.25	3.19 ± 0.16
	400	223.1 ± 5.14	78.6 ± 3.72	91.0 ± 3.60	3.27 ± 0.18
O. baccatus	100	221.5 ± 4.26	78.5 ± 2.65	90.6 ± 3.21	3.22 ± 0.15
	200	220.7 ± 4.80	79.5 ± 2.87	90.3 ± 2.86	3.00 ± 0.19
	400	220.2 ± 5.27	79.5 ± 3.36	91.8 ± 3.54	3.31 ± 0.15

*$p < 0.05$, **$p < 0.001$, significantly different from vehicle control group.

Table 5. Effect of oral administration of the tested ethanol extracts for 65 days on the fertility index of male rats ($n = 6$) after mating with normally cycling female rats ($n = 12$).

		Number of females showed positive mating			Fertility index (%)			Litter size of the mated females (M ± SE)		
Groups	Dose (mg/kg)	30	45	60	30	45	60	30	45	60
Control	0.00	9/12	10/12	10/12	75.00	83.33	83.33	6.42 ± 0.49	7.06 ± 0.47	7.14 ± 0.48
E. spinosa	100	9/12	9/12	10/12	75.00	75.00	83.33	6.54 ± 0.44	6.73 ± 0.43	7.39 ± 0.42
	200	10/12	10/12	10/12	83.33	83.33	83.33	7.35 ± 0.47	7.66 ± 0.46	7.84 ± 0.46
	400	11/12	11/12	11/12	91.67	91.67	91.67	8.18 ± 0.46*	8.62 ± 0.42*	8.75 ± 0.43*
L. pyrotechnica	100	9/12	10/12	10/12	75.00	83.33	83.33	6.83 ± 0.45	7.46 ± 0.43	7.85 ± 0.49
	200	11/12	12/12	12/12	91.67	100.0	100.0	8.24 ± 0.45*	8.70 ± 0.42*	8.64 ± 0.42*
	400	12/12	12/12	12/12	100.0	100.0	100.0	9.00 ± 0.46**	9.10 ± 0.42**	9.17 ± 0.41**
H. salicornicum	100	9/12	9/12	10/12	75.00	75.00	83.33	6.45 ± 0.42	6.74 ± 0.43	7.32 ± 0.42
	200	9/12	10/12	10/12	75.00	83.33	83.33	6.62 ± 0.47	7.50 ± 0.45	7.41 ± 0.43
	400	8/12	9/12	9/12	66.67	75.00	75.00	6.20 ± 0.43	6.78 ± 0.47	6.58 ± 0.47
O. baccatus	100	9/12	9/12	9/12	75.00	75.00	75.00	6.44 ± 0.39	6.50 ± 0.42	6.53 ± 0.43
	200	9/12	9/12	10/12	75.00	75.00	83.33	6.83 ± 0.47	6.50 ± 0.43	7.47 ± 0.41
	400	8/12	8/12	9/12	66.67	66.67	75.00	6.25 ± 0.42	6.77 ± 0.39	6.45 ± 0.42

*$p < 0.05$, **$p < 0.001$, significantly different from vehicle control group.

Sperm viability

The sperm viability did not reveal any significant change in all treated groups when compared with their control counterparts.

Total sperm abnormalities

The commonest morphological abnormality of sperm in control rats that received the vehicle for 65 days was the "curved tails" and "detached heads" which accounted for over 60% and 30% of the abnormalities observed. The percentages of these abnormalities were significantly reduced (Table 4) in groups medicated with *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg). Both *H. salicornicum* and *O. baccatus* extracts provided non-significant effect on the sperm abnormalities when compared to the control group.

Fertility tests

Mating of male rats treated with *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg) for 30, 45 and 60 days with normal cycling females revealed enhancement effects on their fertilizing capability and a remarkable increase in pregnancy rate (Table 5). As a huge number of spermatozoa were seen in vaginal smears from the females taken after mating, the improved fertility of treated males was in part a consequence of mating success. In addition, the fertility of these rats was significantly improved by *E. spinosa* (400 mg/kg) and *L. pyrotechnica* (200 and 400 mg/kg) treatments in terms of the number of litters born by the cohabited female rats after 30, 45 and 60 days. Female rats cohabited with those treated male rats bore significantly increased number of litters: 8.75 ± 0.43, 8.64 ± 0.42 and 9.17 ± 0.41 for 60 days, respectively (control: 7.14 ± 0.48). The improvement of fertilizing efficiency of the treated animals was attributed to their high sexual desire as a result of elevated testosterone levels. The fertility of *H. salicornicum* and *O. baccatus*-treated rats in terms of percentage mated female rats or the number of litters born by the cohabited female rats was unaffected. Testosterone is known to regulate the reproductive behavior and

fertilizing ability of the male. The observed tendency of the increase in potency may therefore be the consequence of increased testosterone levels.

Conclusions

Results of this investigation indicated that oral administration of *E. spinosa* and *L. pyrotechnica,* in particular with high doses, induce a significant improvement in fertility parameters as a result of increased testosterone levels. In addition, our preliminary phytochemical study indicates the presence of flavonoids and anthraquinone in the ethanol extract of *E. spinosa* and *L. pyrotechnica*. Hence, the sexual function improving effect of both extracts might be due to the presence of such compounds. Moreover, further research is needed for the identification of their active constituent(s) responsible for sexual function improvement activities and the mechanism by which they augment sexual function.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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