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RESEARCH ARTICLE

Effect of Hydroethanolic Extract of Nasturtium on Parameters of Reproductive and Sex Hormones of Male Rats

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Abstract

Nasturtium is a plant in the family *Trapaeolaceae* cultivated in Europe, Asia, Africa, and America, which has numerous healing, antiseptic, and expectorant activities. It is the most important herbal medicine used for curing some diseases like diabetes, bronchitis, cardiovascular disorders, and urinary tract infections. There are many chemical constituents isolated from Nasturtium including polyphenols, glucosilonates and fatty acids. Many systems of the body like the reproductive system are affected by some herbal plants, which contain essential chemical active substances with biological properties. The present study was conducted to investigate the effect of hydroethanolic extract of Nasturtium on parameters of reproductive and sex hormones on male rats. Sixty mature male albino rats weighing 180-200 g were divided into three equal groups at random. The first group was kept as a monitor and consisted of 20 rats that were given distilled water daily for 30 days. The second group consisted of 20 rats, which were given hydroethanolic extract of Nasturtium flowers orally via a stomach tube at a dose of 250 mg/kg body weight (BW.), and the third group consisted of 20 rats, those were given hydro-alcoholic extract of Nasturtium leaves every day for four weeks. Blood samples were collected from all rats to estimate serum levels of luteinizing hormone (LH) and follicular stimulating hormone (FSH), testosterone and prolactin. The testicles, seminal vesicles and prostate glands were weighted and the sperms were collected from the epididymis to calculate the sperm count. The results showed decreased testosterone level in flower extract (1.10 ± 0.238) and leaves extract (0.89 ± 0.08) compared to the control group (3.75 ± 0.35). Also, LH decreased in flower extract (0.225 ± 0.025) and leave extract (0.103 ± 0.003) compared to the control group (0.105 ± 0.003). Meanwhile, serum FSH level in flower extract (0.60 ± 0.07) and leaves extract (0.45 ± 0.05) showed non-significant change compared with the control group (0.35 ± 0.029). The fast mobility of sperm indicated non-significant change compared with the control group. In conclusion, hydroethanolic extract of Nasturtium make attributed levels on sex hormones, which give negative effect on fertility despite of its chemical compounds.

Introduction

The infertility is a major problem in the world and has effects on people's life. It is evaluated that the rate of infertility between 50 to 80 million couples all over the world. Several factors affecting sperm releasing and are related to incidence of infertility. These factors include specific medication system, cancers, antibiotics, toxic compounds, radiation, stress, environmental pollutants, and inadequate intake of vitamins [1]. Infertility and following complications are known as one of the most important issues affecting sperm production and spermatogenesis [2-4]. Nasturtiums contain vitamin E, which is a strong anti-oxidant and has protective function in spermatogenesis. Vitamin E improves the living viability of semen. Deficiency of vitamin E causes testicular tissues damages and reduces testosterone synthesis [5, 6]. Utilizations of Nasturtiums have positive

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effects on the increase of fertilization and improve or control male fertility. They increased libido (sexual desire), stimulatory activity gonadotrophic hormones, luteinizing hormones (LH) and follicular stimulating hormones (FSH), and testosterone. Nasturtiums were used as a male contraceptive [7]. Some effects of medicinal plants are spermicidal, anti-zygotic, inhibition of spermatogenesis and sperm motility, and inhibition of androgen production either by pituitary axis, destruction of seminiferous tubules or erosion of germinal epithelium. Leydig cell activity is inhibited or disrupted by the histoarchitecture of the testes. The complicated mechanism of hormonal stimulation and complex interactions between the seminiferous epithelium's sertoli cells and germ cells in the testes creates spermatozoa [7]. Sertoli cells secrete hormonal and nutrient factors into the adluminal compartment, providing a specialized microenvironment for the growth and viability of resident germ cells. Furthermore, these cells are proliferating and have a reliable paracrine signaling pathway as well as a physical support [8]. The belief that medicinal plants are low in toxicity, because they are "natural herbs", allowed them to be used in the treatment of diseases. Treatments with medicinal plants, as well as traditional medicine can have side effects of drug reactions [9, 10]. Herbal toxicity is a significant problem that poses a substantial risk to human health [11]. Nasturtium contains a number of bioactive compounds, including flavonoids, carotenoids, and other polyphenols that have been shown to have anti-inflammatory properties [12]. Defendant Nasturtium is a plant of family *Trapaeolaceae* that is grown in Europe, Asia, Africa, and America; it has many different species. Nasturtium is a herbaceous annual plant with trailing stems. The leaves are nearly circular and wide. Nasturtium has been used for centuries in herbal medicine to purify the blood, prevent hemorrhage, cleanse the liver, expel excess bile, and stimulate the immunity. Herbal therapies, which present on traditional remedies, are generally safer than modern medicine. Herbs may aid in fertilization and the elimination of issues such as sexual dysfunction, sperm count, and mobility. It can be used as an ornamental or a medicinal plant. The plant's flowers contain vitamin C and lutein [13]. Nasturtium is an edible plant with antibacterial properties that aid in the healing of skin conditions such as eczema and dermatitis and helpful in healing problems because of its vitamins and chlorine content so, it is helpful in rejuvenating skin tissues and in healing problems [14]. Tropaeolum majus has effect on uterine contractility as it has carotenoids.2 phenethyle secondary metabolites of glucosinolates and hydrolysis product in isothiocyanate [15].This study was designed to evaluate the effect of Nasturtium on fertilization indexes, the rate of sex hormones and the effect on sex organs weight index (seminal vesicles, prostate gland, and testicles) as well as histopathological changes.

Material and methods

Sixty rats were collected from Animal Breeding Unit, Faculty of Veterinary Medicine Zagazig University. They were acclimatized for two weeks in clean hygienic plastic cages, and fed a healthy pelleted diet and water. Animals were housed in stainless –steel cages, maintained in a 12 h light – dark cycle at a controlled temperature (21-24 °C) and humidity (50-60%). They were kept for 15 days without medication for acclimation before beginning the study. The care and welfare of animals conformed to the guidelines of the Animal Use Research Ethics Committee of Zagazig University, Egypt.

Preparation of a hydroethanolic extract of Nasturtium flowers and leaves

Nasturtium:

The hydroethanolic extract of Nasturtium was made by macerating 100 g of powdered Nasturtium leaves and flowers in 2 L of solvent (70 ethanol: 30 distilled water

ratios) for 3 h at 37 °C. After 4 days, the extract was filtered and concentrated under reduced pressure using a rotatory apparatus [16]. The residual content was transferred into a Petri dish and was kept in an oven at 40 °C for drying up, and then it was kept at -20°C until use**.** The residual material was transferred to a Petri dish and dried in a 40°C oven before being stored at -20°C until use [17].

Experimental animals

Sixty mature male albino rats weighing between 180 and 200 g were used in this study. The animals were divided into three equal groups, each consisting of twenty rats. Group 1: consists of 20 rats that were given distilled water every day for 30 days and kept in a cage. Group 2: includes 20 rats, those were administered the hydroethanolic extract of Nasturtium flowers orally via a stomach tube at a dose of 250 mg/kg B. wt. /day, orally [17] once a day for four weeks. Group 3: comprises of 20 rats, which were given the hydroethanolic extract of Nasturtium leaves orally via a stomach tube at a dose of 250 mg/kg B. wt. /day, orally [17] once a day for four weeks.

Blood samples

Blood samples were obtained from five humanly euthanized rats of each group on the 10^{th}, 20^{th} and 30^{th} days after the end of therapy. The blood was collected in centrifuge tubes for serum preparation by centrifugation for 15 minutes at 3000 round per minute (rpm) for measuring serum FSH, LH, testosterone, and prolactin. After the blood was collected, the rats were dissected and their sex organs were weighted. Index weight (I.W.) measure (organ weight/ body weight) x100 was calculated. They were then put in jars containing 10% formol saline for histopathological analysis.

Weight index of sex organs

The rats were individually weighed and blood was collected, during which rats were dissected and seminal vesicles, prostate glands, and testicles were extracted and weighed. Measurement of the weight index (I.W) for each organ was calculated as described previously [18].

Weight index (W.I) =

$$\frac{organ\ weight}{body\ weight} x100 \frac{organ\ weight}{body\ weight} x100$$

After that, they were put in jars containing 10% formol. For histopathological research, a saline solution will be used.

Collection of sperm samples and spermo gram analysis

The epididymis and testis were separated in a Petri dish and then evacuated into another Petri dish containing normal saline [19]. Immediately after selection, sperm samples were tested for the following:

Mass motility:

A tiny drop of undiluted semen was taken with a capillary pipette and put over a warm clean glass slide immediately after the semen sample was collected. The semen droplet was microscopically examined at 10 X magnification power. The sperm wave motion was measured using the previously published method [20].

To assess mass motility, the following scores were used:

- Very good sample: strong active rapid wave motions ++++
- Good sample: good and satisfactory +++
- Fair sample: appropriate and fair (++)
- Bad sample: slow, with varying levels of individual motility (+)
- Low quality sample with no wave motion and immotile sperm cells.

Individual progressive sperm motility

A small volume of semen was diluted in a reasonable dilution rate of 1:10 with 2.9 % sodium citrate solution. A small drop of this mixture was mounted on a warm glass slide, covered with a cover slip, and examined by 40X magnification lens microscopically. According to a described protocol [18], several fields were investigated to estimate the percent of motile spermatozoa.

Assessment of total sperm abnormalities

One drop of diluted sperm was put on a dry and clean glass slide to assess the total sperm abnormalities .A number of films were produced and then exposed to warm air. After drying, the films were placed in an alkaline methyl violet solution-filled container. The stained smears were washed with continuous distilled water for five minutes before being plotted with a filter paper. A total of 100 sperms were used and sperm cell concentration was calculated by counting sperm cells using a haemocytometer as defined previously [21].

Sperm cell concentration:

Sperm cells were counted using a haemocytometer to determine the sperm concentration using an aqueous solution of eosin 2% as a diluent, to destroy the sperm [18]. Eosin discolors sperm heads, making it easier to be counted. The pipette was filled with the diluent after the semen was pulled to the mark 1.0. A small drop of this mixture was put in the haemocytometer counting chamber, covered with a cover slip for five minutes to allow all sperm cells to settle. Spermatozoa were counted in five non-adjacent squares at a magnification of 40x (80 small squares).The amount of sperms per mL was calculated by multiplying the number of sperms in five squares by 10.000.

Calculation*:*

Sperm count/mm3 = N x5x200x10

When and where:

- Nx5 = the number of spermatozoa in each of the 80 small squares.
- The dilution factor = 200
- Depth = 10

Assessment of sex hormones

An Enzyme Linked Immunosorbent Assay (ELISA) was used to assess the total testosterone [22] and serum LH, and FSH levels [23] in the blood.

Serum prolactin levels were measured in the following way:

The quantitative prolactin test is based on the solid phase ELISA theory [24, 25]. For solid phase (microtiter well) immobilization, in this assay we used mouse monoclonal anti-prolactin Another mouse monoclonal anti-prolactin was used in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was required to react with the control sample at the same time. The prolactin molecules were sandwiched between the solid phase and enzyme-linked antibodies after the test sample reacts simultaneously with the antibodies. The wells were washed with water after 45 minutes incubation at room temperature to extract the unbound labeled antibodies. Tetra methyl benzidine (TMB) solution was added then incubated for 20 minutes until the formation of a blue pigment. With the addition of IN HCL, the color production was halted, and the resulting yellow color was measured spectrophotometrically at 450 nm. The color strength of the test sample was directly proportional to the concentration of prolactin.

Reagents and materials provided:

All reagents were allowed to reach room temperature (18-25ºC) before use. All reagents were mixed by gentle inversion or swirling prior to use. Each lyophilized standard was reconstituted with 1.0 mL distilled water. The reconstitute material was allowed to stand for at least 20 minutes. Reconstitute standards were stored sealed at 2-8 ºC and are stable at 2-8 ºC for at least 30 days.

Histopathological examination

Seminal vesicles, prostates, and testicles from all groups were collected after the end of the plant administration, then at 10, 20 and 30 days later and fixed in 10% formalin for 24 h. Prior to routine processing in paraffin wax, samples were cut into sections of around five microns thickness stained with Mayer's Haematoxylin and

eosin stain (H&E) then they were examined microscopically as previously described [26].

Statistical Analysis:

The data were analyzed using the One Way ANOVA F-test**.** Means at the same column followed by different letters were significantly different and the highest value was represented with the letter a [27].

RESULTS

Effect on weight index of sex organs

The weight index of testicles was clearly evident from the oral administration of hydroethanolic extract of Nasturtium of either leaves or flower extract daily for 30 successive days in a dose of 250 mg /kg BW The rats afforded a significant decrease in I.W of testicles at 20 and 30 days post end of treatment and non-significant changes on the 10th day compared with the control group along the entire period of the experiment. Whereas, the results showed a significant increase in I.W of testicles of the leaves and flower groups on the 30th day post treatment compared with the control group together with the non-significant changes at the 10th and the 20th day post end of treatment. Meanwhile, the IW of the seminal vesicles showed a significant decrease only in the group given leaves extract after the 10th day post-treatment. The weight index of the prostate gland of male rats treated with therapeutic doses of hydroethanolic extract of flowers and leaves of Nasturtium with doses of 250mg /kg b. wt. showed a significant increase ($P \leq 0.05$) relative to the normal control group at the third time post treatment along the entire period of the experiment Table (1).

Table 1: The Effect of Flower and Leaves Treatment on Weight Index of Testes, Prostate, Seminal Vesicle (SV), and Semen Analysis (Sperm Motility, Abnormalities and Count) (Mean Values± SE). N=5

Parameters Groups	Weight index of testes	Weight index of prostate	Weight index of SV	Sperm motility %	Sperm abnormalities	Sperm count
Control	0.988 ^{a} ±0.052	0.016 ^{b} ±0.002	0.493 ^{a} ±0.056	87.5 ±1.443	12.64^{cd} ±0.691	30.00 ^{c} ±1.826
Flower I after 10 days	0.850 ^{ab} ±0.075	0.028 ^{b} ±0.005	0.413 ^{ab} ±0.019	85 ±2.041	16.61 ^{b} ±1.089	43.5 ^{bc} ±2.723
Flower II after20 days	1.003 ^{a} ±0.056	0.056 ^{b} ±0.028	0.420 ^{ab} ±0.027	78.75 ±5.154	13.25 ^{c} ±0.750	52.75 ^{b} ±3.568
Flower III after 30 days	0.655 ^{b} ±0.108	0.114 ^{a} ±0.008	0.398 ^{ab} ±0.020	87.5 ±1.443	8.76 ^{e} ±0.562	68.5 ^{a} ±5.331
Leaves I after10 days	0.928 ^{ab} ±0.061	0.014 ^{b} ±0.001	0.348 ^{b} ±0.018	76.25 ±2.394	19.68 ^{a} ±0.684	29.75 ^{c} ±3.425
Leaves II after 20 days	1.028 ^{a} ±0.075	0.020 ^{b} ±0.002	0.475 ^{ab} ±0.025	80 ±4.082	14.99 ^{bc} ±0.965	47.5 ^{b} ±2.901
Leaves IIIafter30 days	0.648 ^{b} ±0.059	0.132 ^{a} ±0.015	0.393 ^{ab} ±0.028	82.5 ±1.443	10.15^{de} ±0.219	53.25 ^{b} ±4.423

Mean values with different superscripts in the same column are significantly different at $P \leq 0.05$

A) Effect on sperm image

a- Sperm motility

The sperm motility in both treated groups showed a very good mass motility along the entire period of the study except on the 10th day post end of treatment in response to both extracts compared with the normal control group ($P \leq 0.05$). In contrast to the standard control group, the sperm motility in both treated groups showed no substantial improvements over the course of the study, except on the 10th day after the end of treatment Table (1).

b- Effect on sperm count

The sperm count of the control group was 30.0 ± 1.83, in flowers extract group was 68.5 ± 5.33 and in leaves extract one was 53.25 ± 4.42, which increased significantly in all groups treated with both extracts along the entire course of the study. In Table (1), sperm count showed significant increase in all groups compared with the control one ($P \leq 0.05$).

c- Effect on sperm abnormalities

Bent back, curved tail, broken head, coiled tail, protoplasmic droplet, and detached tail were among the sperm anomalies found in both groups treated with flower and leaves extracts.

Effects on sex hormones

Follicle Stimulating Hormone (FSH)

Serum FSH levels showed significant ($P \leq 0.05$) increased levels in flower extract at 20 days (0.575 ± 0.085) and in 30 days (0.60 ± 0.07) after treatment ended relative to control group (0.35 ± 0.029), flower extract (0.60 ± 0.07) and leaves extract (0.45 ± 0.05) groups along the entire period of the experiment but were non-significant at 10 days the control group (0.35 ± 0.0290, flower group (0.45 ± 0.096) and the leaves group (0.45 ±0. 065) during the study period Table(2)..

Serum levels of LH hormone

Serum LH levels in the control (0.105± 0.003), flower extract (0.225 ± 0.025) and leave extract (0.103 ± 0.003) groups were estimated**.** These values were significantly elevated in response to both extracts during the study period as compared to the control group along the entire period of the experiment ($P \leq 0.05$), with the exception of the control group (0.105±0.003), flowers group (0.35±0.065) and leaves group (0.125 ± 0.025).whereas at 30 days, the control (0.105 ± 0.003), flowers extract (0.225 ± 0.025) and leaves extract (0.103 ± 0.003) groups showed lower levels (Table 2).

Table 2: The Effect of Flower and Leaves Treatment on Serum Hormones. (Mean ±S.E.), N =5

Parameters Groups	FSH	LH	Prolactin	Testosterone
Control	0.35 ±0.029	0.105 ^{c} ±0.003	1.1625 ^{a} ±0.094	3.75 ^{a} ±0.352
Flower I after 10 days	0.45 ±0.096	0.375 ^{ab} ±0.063	0.8525 ^{ab} ±0.064	0.935 ^{b} ±0.333
Flower II after20 days	0.575 ±0.085	0.35 ^{ab} ±0.065	0.875 ^{ab} ±0.063	1.04 ^{b} ±0.101
Flower III after30 days	0.60 ±0.071	0.225 ^{bc} ±0.025	0.935 ^{ab} ±0.096	1.10^{b} ±0.238
Leaves I after 10 days	0.45 ±0.065	0.4675 ^{a} ±0.062	1.185 ^{a} ±0.212	0.86 ^{b} ±0.257
Leaves II after20 days	0.475 ±0.075	0.125 ^{c} ±0.025	0.945 ^{ab} ±0.032	1.65 ^{b} ±0.250
Leaves III after 30 days	0.45 ±0.050	0.1025 ^{c} ±0.003	0.6175 ^{b} ±0.044	1.8925 ^{b} ±0.082

FSH, follicular stimulating hormone; LH, luteinizing hormone

Mean values with different superscripts in the same column are significantly different at $P \leq 0.05$

Serum prolactin

Serum prolactin levels were evaluated at 10 [control (1.153± 0.094), flowers extract (0.853 ± 0.064) and leaves extract (1.185 ± 0.212) groups], 20 [control (1.163 ± 0.094), flowers extract (0.815 ± 0.063), and leaves extract (0.945 ± 0.032) groups] and at 30 days [control (1.163 ± 0.094), flowers extract (0.935 ± 0.096) and leaves extract (0.618 ±0.044) groups] of the experiment. These values did not alter significantly over the entire period of the experiment of the hydroethanolic extract of Nasturtium flowers and leaves Table (2).

Serum total testosterone

Serum testosterone levels showed significant ($P \leq 0.05$) decreased levels in flower extract (1.10±0238) and leaves extract (1.89±0.08) than the control group (3.75±.35) along the entire period of the experiment (Table 2).

Histopathological findings

Effect of hydroethanolic extract of Nasturtium flowers

Testicles:

As shown in Figure (1), the male rat testis (control), one day post end of administrated extract of Nasturtium flower, showing normal semineferous tubules and active spermatogenesis. After 10 days, the rats administrated flower extract of Nasturtium showing moderate spermatogenesis and congested blood vessels in tunica albugenia**.** After 20 days, they showed mild spermatogenesis and degenerate spermatocytes with congested blood vessels (Figure 2). After 30 days, the testicles showed intense degenerated necrotic spermatocytes with spermatid giant cells**,** the majority of tubules had vacuolated and degenerated or necrosed spermatogonial and spermatocytes beside spermatid giant cells, pronounced interstitial edema and congested blood vessels (Figure 2).

Prostate gland:

The male rat prostate gland (control) showed normal prostatic acini with secretion, while the glandular tissue and stroma were normal. After 10 days, the prostate gland showed apparently normal acini containing secretion. All the glandular tissue contains secretion and was normal (Figure 1). After 20 days, prostate gland showed mild hyperplastic acini containing little secretion, all glandular tissue appeared normal and a few acini lined by hyperplastic epithelium and contain a little secretion (Figure 2). After 30 days, the prostate gland showed cystic dilated prostatic acini devoid from secretion; some acini appeared cystic and lined by flattened epithelium and devoid from secretion (Figure 2).

Seminal vesicle:

The seminal vesicle of mal rat (control) showed normal tubulo alveolar glands and secretion; trabeculae and covering Tunica were normal. After 10 days, the seminal vesicle of male rat showed distended glands with secretion and atrophy of their lining epithelium (Figure 1). After 20 days, the seminal vesicle of male rats showed hyperplastic tubulo alveolar glands containing little secretion (Figure 2). After 30 day, the seminal vesicle of male rats showed atrophied tubular glands with retained secretion (Figure 2).

Effect of hydroethanolic extract of Nasturtium leaves

Testicles: The male rat testis (control) showed normal seminiferous tubules and active spermatogenesis. After 10 days, it demonstrated necrotic seminiferous tubules containing edema and necrotic debris. After 20 days, it showed intense edema and necrosis of the seminiferous tubules and arrest of spermatogenesis. After 30 days, it showed spindle cell invasion for necrotic seminiferous tubules (arrows) and interstitial edema.

Prostate gland:

The male rat prostate gland (control) showed normal prostatic acini with secretion. After 10 days, it showed normal prostatic acini and interstitial edema. After 20 days, it showed slightly cystic acini with little secretion. After 30 days, it showed cystic acini without secretion and interstitial edema**.**

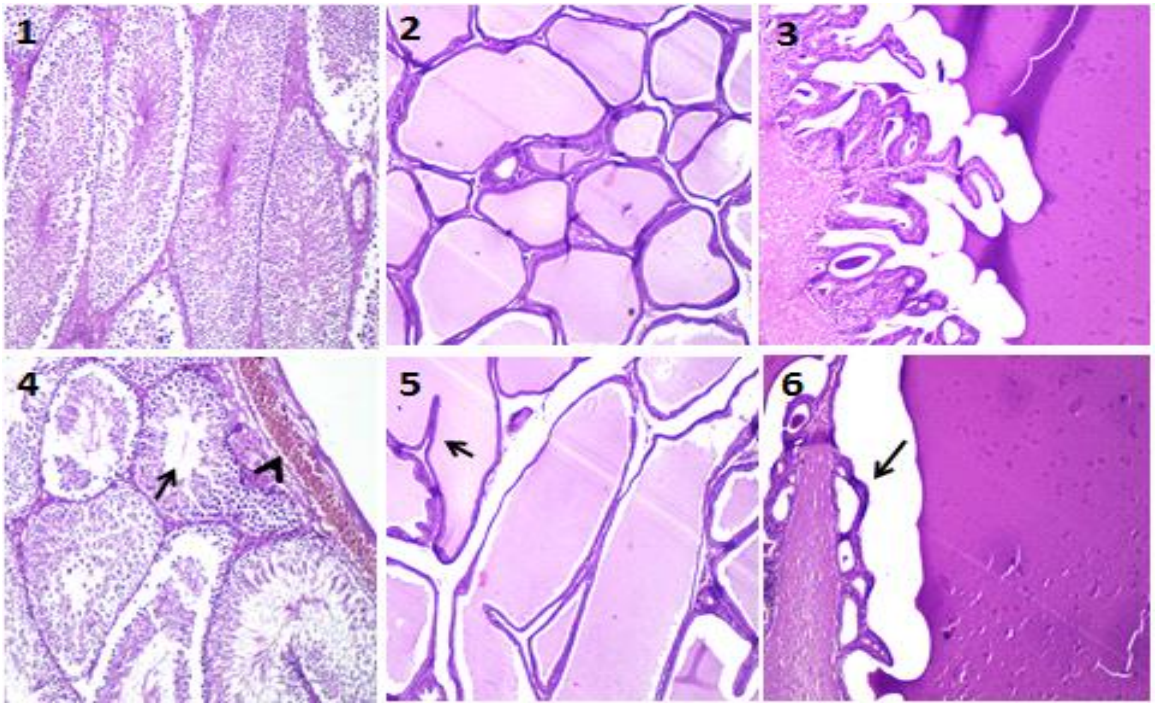

Figure 1: Histological finding of the control group of albino rats without treatment with hydroethanolic extract of Nasturtium. 1, 2, 3: a male rat testis (control) one day post end of administrated extract of Nasturatium flower showing normal semineferous tubules and active spermatogenesis. 4: A photo micrograph of a male rat testis (flower I) administrated flower extract of Nasturtium showing moderate spermatogenesis (arrowhead) of congested blood vessels in tunica albugenia. 5: A photo micrograph of a male rat prostate gland (Flower I) showing apparently normal acini containing secretion (arrow). 6: A photo micrograph of a male rat seminal vesicle (Flower I) showing distended glands with secretion and atrophy of their lining epithelium (arrow).

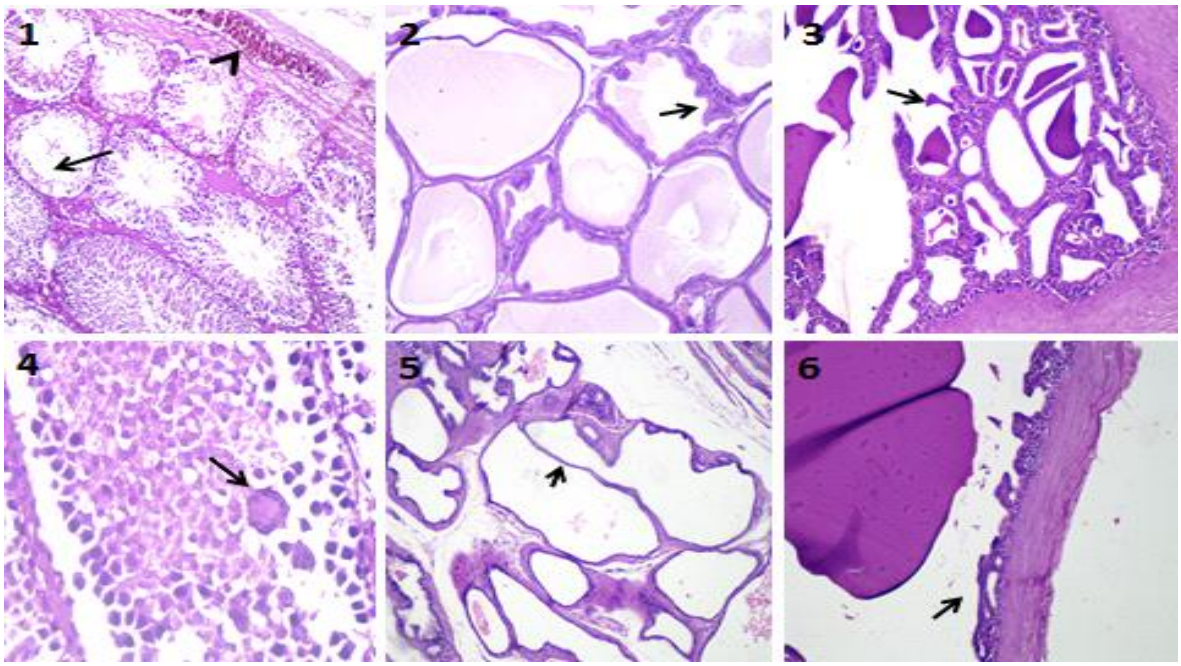

Figure 2: Histological findings in albino rats treated with hydroethanolic extract of Nasturtium flowers. 1: Flower I: A photo micrograph of male rat testis showing mild spermatogenesis and degenerated spermatocytes (arrows) with congested blood vessels (arrowhead). 2: Flower II: A photo micrograph of male rat prostate gland showing mild hyperplastic acini containing little secretion (arrows). 3: Flower II: A photo micrograph of male rat seminal vesicles showing hyperplastic tubuloalveolar glands (arrows) containing little secretion. 4: Flower III: A photo micrograph of male rat testis showing intense degenerated necrotic spermatocytes with spermatid giant cells (arrows). 5: Flower III: A photo micrograph of male rat prostate showing cystic dilated prostatic acini devoid from secretion (arrow). 6: Flower III: A photo micrograph of male rat seminal vesicle showing atrophied tubular glands with retained secretion (arrows).

Seminal vesicle:

The seminal vesicle of mal rat (control) showed normal tubulo alveolar glands and secretion. After 10 days, it showed normal tubule alveolar glands and edema. After 20 days, it showed partial desquamation of some glandular epithelium and foamy secretion**,** some glands had partially desquamate epithelium and filled with foamy esinophilic colloidal secretion. After 30 days, it showed hyperplastic glands without secretion, tubuloalveolar glands were hype plastic without secretion and tunica muscularis had partial edema or necrosis.

Discussion

The results showed that the oral administration of Nasturtium hydroethanolic extract for 30 days in male rats affected on their fertility. The two basic functions of male reproductive tract are production of spermatozoa and testosterone to cause spermatogenesis and release sperm to transport to epididymis where sperm maturation and storage occur [28]. Semen is a mixture of spermatozoa, produced by testicles, seminal plasma secreted at different sites by accessories glands and by the epididymis, which are combined at the time of ejaculation [29]. The average duration of spermatogenesis in rats is about 43.6 - 51.8 days [30], so that this study lasted for 2 months to cover the complete cycle of spermatogenesis in rat. Spermatogenesis is dependent on hormonal stimulation and on dynamic interactions between the sertoli cells and the germ cells of the seminiferous epithelium. This complex process produces spermatozoa in the testes [31, 32]. Some toxic agents disrupt spermatogenesis through cellular reactions that decrease testicular tissue

damage [32]. A significant decrease in testicles weight along the entire period of the experiment was recorded in this study. Mechanism of Nasturtium effects on rat testes was not clarified in this study, but this plant might affect sertoli and leydig cells' functions. The size of testicles depends on the amount of sertoli cells and sperm making in a way that testicles size reflects the amount of germ cells in its [33]. It was reported that spermatogenic function during puberty depends on the number of sertoli cells in testicular tissues [34]. The proliferation of sertoli cell in pre-puberty ages is limited. Ramaswamy *et al.* [35] found that index weight of male sex organs was significantly increased compared with the normal control group. The difference in our results might be possibly attributed to the species of the experimental animal, dose of the plant extract and duration of the study. The histopathological changes in the testes represented by the testicular morphology showed severe edema, congestion of testicular blood vessels in capsule and intersitium, epithelial vacuolization, sloughing and atrophy and intense necrosis inside their lumina. After 10 days post treatment, Nasturtium flowers administered group showed moderate spermatogenesis and congested blood vessels and intersitium and tunica albuginea. After 20 days post treatment, edema in seminiferous tubules, hyperplastic epithelium with little secretions and distended ducts and glands were observed with mild atrophy of some epithelial lining of a few glands. These results were confirmed by WHO [36] and Guneli *et al.* [37]. After 30 days post treatment, vacuolation and degeneration or necrosed spermatogonial and spermatocytes beside spermatid giant cells and interstitial edema with congested blood vessels and atrophied epithelium of tubule alveolar glands were also recorded [38]. The obtained result revealed increase in prostate gland index weight. The decrease in testis and seminal vesicles may be due to the vacuolar degeneration of epididymal epithelium present in histopathological changes in flower group at 30 days post end of treatment. The majority of tubules had vacuolated and degenerated or necrosis spermatogonial stem cells and spermatocytes beside spermatid giant cells. Pronounced interstitial edema and congested blood vessels could be also seen [33, 34].Our results coincides with Cardenas-Valenica *et al.* [39] who concluded that hydroethanolic extract of nasturtium induce testicular function in rat following treatment with extracts prepared from leaves and flowers. The obtained results revealed changes in testes, prostate and vesicular glands with hydro ethanolic extract of nasturtium flowers and leaves 10 days post treatment as well as mild atrophy of some epithelial lining of few glands. At 20 days post treatment, edema was noticed in seminiferous tubules and hyperplastic epithelium with little secretions. At 30 days post end of treatment, vacuolation and degeneration or necrosed spermatogonial and spermatocytes beside spermatid giant cells and interstitial edema with congested blood vessels and atrophied epithelium of tubuloalveolar glands were observed, which was similar to the results of Moffit et al. [40]. Khaki *et al.* [34] reported that nasturtium contains flavonoids, anti-oxidant compounds, vitamins and quercetin, which cause improvement of semen pictures. In the present study, sperm motility was non-significant changed along the study. Concerning sperm motility, our findings showed a decrease in the mass motility of sperms after administration of hydro ethanolic extract of nasturtium flowers and leaves. Moreover, there was change in the sperm count, which displayed a progressive forward motility in treated groups. The decrease in both mass and individual progressive sperm motility may be due to the significant increase in sperm abnormalities due to presence of flavonoids in nasturtium. The significant increase in the sperm abnormalities in this study were in accordance with the previous results of Chen *et al.* [41]. This result could be due to Nasturtium contains vitamin E that

confirmed by WHO [36], Aitken and Roman [42], and Glade and Smith [43]. The sperm cell count was significantly increased 10 days and 30 days post administration of hydro-ethanolic extract of flowers and leaves. The increase in sperm count indicates positive effect on spermatogenesis in rats administrated the extract of Nasturtium flowers and leaves. The effect on sperm abnormality showed a significant increase flower group after 30 days. Sperm abnormalities includes bent tail, curved tail, broken head and detached tail were very high significant compared with the control group [41]. Spermatogenesis in mammals requires the actions of a complex assortment of peptide and steroid hormones, each of which plays an important role in the normal functioning of the seminiferous epithelium. These hormones include GnRH, LH, FSH, and testosterone [44]. The results demonstrated that the hydroethanolic extract of Nasturtium leaves and flowers showed a significant decrease in the serum total **t**estosterone, which might be due to Nasturtium contents of flavonoids, phenolic compounds, and glycosides, which are responsible for hypo cholesterolemia and hypolipidemic activities as the results recorded by Verma and Kanwar [45] and Gruel *et al*. [46], which showed also a decrease in lipid peroxidation. Testosterone is a steroid hormone, synthesized from cholesterol in the testicular leydig cell under control of pituitary gonadotropin LH. Cholesterol is the essential precursor for all steroidal hormones. Hadjzadeh *et al.* [47] demonstrated that hydro alcoholic extract of Nasturtium demonstrated a significant decrease in serum total cholesterol and low-density lipoprotein. Nasturtium flavonoids, phenolic compounds, and glycosides were responsible for hypo cholesterolemia and hypolipidemic activities. Nasturtiums which decrease cholesterol may be another reason for decrease of testosterone. LH showed a significant increase in flower and leaves after 20 days post treatment. Prolactin in control group (1.163±0.094), in flower extract (0.935±0.096) and in leaves extract (0.618±0.044) showed a significant decrease in leaves after 30 days. Serum FSH showed non-significant change compared with control group. These hormones have been reported to play a major role in the improvement of reproductive function in male reproductive system [48, 49]. These results are in accordance with the previous results of Lei *et al.* [50] who concluded that in males, the regulation of testosterone synthesis seems to be the only indispensable function of LH within the adult testes. Sharpe [51] and McLachlan *et al.* [52] reported that androgens are essential for male fertility and the maintenance of spermatogenesis. These results are in full agreement with Singh *et al.* [53]. A significant increase in histological criteria and a significant decrease in testes weight were confirmed by an earlier study [54]. This conclusion might be supported by hypothesis that an agent acting directly on the brain, hypothalamus or anti pituitary gland will indirectly affect the testes and will possibly affect sexual activity. Boekelheide [55] concluded that mechanism of Nasturtium effects on rat testes not clarified, but this plant might affect Sertoli and Leydig cells 'functions. There are negative feedbacks of testosterone and LH hormones. It is well accepted that secretion of the gonadotropins, LH and FSH, in males depends on release of gonadotrophic hormone from the hypothalamus into the hyperphysical portal blood reported by Tilbrook and Clarke [56]. The synergistic action of LH/testosterone and FSH is necessary for the initiation, maintenance and also for reinitiating normal spermatogenesis [57]. In the testes, LH binds to receptors on the surface of Leydig cells and stimulates the production of testosterone [58]. FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors and is critical for the initiation of spermatogenesis [58]. These hormones are controlled by the negative feedback regulation mediated by testicular factors, where level of testosterone increase,

it act on the hypothalamus and pituitary through a negative feedback loop and inhibit the release of GnRH and gonadotropins and consequently inhibits the secretion of LH and FSH [58]. We suggested that the existence of flavonoids and some other antioxidants in Nasturtium able to make attributed on sex hormones.

Conclusion

The study showed that hydroethanolic extract of Nasturtium make attributed levels on sex hormones, which gave negative effect on fertility despite of its chemical compounds, that had positive effect on fertility. So, more research is required to fully evaluate the plant extracts such as toxicity and fertility index after mating males with females, according to the findings.

Conflict of interest:

The authors declare that they have no conflict or interest.

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الملخص العربى

تأثير المستخلص الهيدروكحولى من نبات أبو خنجر على التكاثر والهرمونات التناسلية لذكور الجرذان

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ينتمي نبات أبو خنجر إلى عائله النباتات الكثيفة المتسلقة حقيقية الأوراق يزرع في أوروبا وآسيا وأفريقيا وأمريكا وله خصائص طبية كثيرة تستخدم لعلاج الكثير من الأمراض مثل مرض السكر والالتهاب الرئوي والقلب وأمراض الجهاز البولي ويستخدم كمطهر وطارد للحشرات وترجع أهميته لأنه يحتوى على مواد كيميائية وبيولوجية هامه مثل جلوكوزات, كاروتينات ,ومركبات فلوبيبدية. بعض الأجهزة فى الجسم مثل الجهاز التناسلي والذي يتأثر بالنباتات الطبية لأنه يحتوى على مواد كيميائيه وبيولوجيه وتم إجراء هذه الدراسة باستخدام 60 جرذا من الذكور البالغة وزنها بين 180-200 جرام للذكر الواحد تم تقسيمها عشوائيا إلى ثلاثة مجموعات كل مجموعه تحتوى على عشرون جرذا. المجموعة الأولى تم إعطائها ماء مقطر يوميا لمده ثلاثون يوما واستخدمت كمجموعه ضابطه. المجموعة الثانية تم إعطائها 250 مجم لكل كجم من وزن الجسم من مستخلص الزهور النبات في الفم بأنبوبة إلى المعدة يوميا لمده ثلاثون يوما. المجموعة الثالثة تم إعطائها 250 مجم لكل كجم من وزن الجسم من مستخلص أوراق النبات في الفم بانبوبة إلى المعدة يوميا لمده ثلاثون يوما وتم جمع عينات الدم في أنابيب جهاز الطرد المركزي بعد 10 و20 و30 يوم من انتهاء تناول الخلاصة وذلك لتحضير السيرم لاستخدامه في قياس هرمونات التستوستيرون الكلى و LH هرمون التبويض و FSH الهرمون المحفز لنمو حويصلات جراف وكذلك هرمون اللبن prolactin وتم حفظ السيرم عند درجه حرارة -20 مئوية حتى إجراء القياسات المختلفة .وبعد ذلك تم تشريح الجرذان واستخلاص الخصى والبروستاتا والحويصلات المنوية ووزنها بميزان حساس وذلك لحساب معامل الوزن للأعضاء التناسلية وأظهرت النتائج أن هرمون التيستوستسرون نقص في مجموعه الزهور والأوراق مقارنة بالمجموعة ألضابطه وكذلك هرمون التبويض والهرمون المحفز لحويصلات جراف وكذلك هرمون اللبن. وأظهرت زيادة معنوية في حركه الحيوانات المنوية مقارنه بالمجموعة الضابطة. الخلاصة أن المستخلص الهيدرو كحولى من نبات أبو خنجر أحدث تغيرات سلبية في الهرمونات الجنسية وأعطتنا نتيجة سلبية على الخصوبة على الرغم من احتوائه على مواد كيميائية جيدة.

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