RESEARCH ARTICLE

Evaluating the antifertility potential of the ethanol extracts of *Heliotropium europaeum* and *Taraxacum serotinum* in male rats

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SUMMARY

The aim of the present investigation was to evaluate the effect of Taraxacum serotinum and Heliotropium europaeum extracts on reproductive organs and fertility of male rats. The study was divided into three groups of rats. The first group received vehicle alone to serve as control. The second and third groups of animals were administered the ethanol extracts of T. serotinum and H. europaeum, respectively. Each extract was given orally at 3 dose levels; 100, 200 and 400 mg/kg for a period of 7 weeks. Sperm motility, count, viability and morphology and serum levels of testosterone, follicle stimulating hormone (FSH), leutinizing hormone (LH) and prolactin were assessed. Percentage of mating and fertility success and fertility index were also calculated. The testes, liver and kidney were processed for histological examination. The effect on biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine were estimated. Hematological profiles such as red blood cell (RBC) count, total leucocyte count (TLC), hemoglobin (Hb) concentration and packed cell volume (PCV) were quantified. The results of this study suggest that the ethanol extract of H. europaeum possesses potential fertility lowering effects without altering general body metabolism.

Key Words: *Heliotropium europaeum, Taraxacum serotinum, male fertility, serum sex hormones, testosterone, mating trial.*

Heliotropium europaeum ve Taraxacum serotinum etanol ekstrelerinin erkek sıçanlarda antifertilite potansiyellerinin değerlendirilmesi

ÖZET

Bu araştırmanın amacı, Taraxacum serotinum ve Heliotropium europaeum ekstrelerinin erkek sıçanlarda üreme organları ve doğurganlık üzerine etkilerini değerlendirmektir. Çalışmada sıçanlar üç gruba ayrılmıştır. İlk grup olan kontrole sadece çözücü uygulanmıştır. İkinci ve üçüncü gruplardaki hayvanlara, sırasıyla, T. serotinum ve H. europaeum'un etanol ekstreleri uygulanmıştır. Her bir ekstre, 7 hafta süreyle 3 farklı dozda oral olarak verilmiştir; 100, 200 ve 400 mg/kg. Sperm hareketliliği, sayısı, canlılık ve morfolojisi ile serum testosteron, folikül uyarıcı hormon (FSH), luteinleştirici hormon (LH) ve prolaktin seviyeleri değerlendirilmiştir. Çiftleşme ve doğurganlık başarı yüzdesi ve doğurganlık indeksi de hesaplanmıştır. Testis, karaciğer ve böbreklerde histolojik incelemeler yapılmıştır. Aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), üre ve kreatinin gibi biyokimyasal parametreler üzerindeki etki incelenmiştir. Hematolojik profil; eritrosit sayımı, toplam lökosit sayımı, hemoglobin konsantrasyonu ve sıkıştırılmış eritrosit hacmi belirlenmiştir. Bu çalışmanın sonuçları, H. europaeum'un etanol ekstresinin genel vücut metabolizmasını değiştirmeden potansiyel fertiliteyi azaltıcı etkiye sahip olduğunu göstermektedir.

Anahtar kelimeler: *Heliotropium europaeum, Taraxacum serotinum, erkek fertilitesi, serum seks hormonları, testosteron, çiftleşme denemesi.*

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INTRODUCTION

Several plants are reported to enhance reproductive processes but, on the other hand, may also hinder testicular functions. Population growth is a great concern world-wide and most of the developing countries are characterized by rapid population growth (1). Therefore, numerous methods are being used to reduce the total fertility rate in both men and women. Plants have been attracting medical attention for their effective and amazing cures for thousands of years and are the most widely used medicines in the world today (2). A large number of plant species with anti-fertility effects have been screened and were subsequently fortified by the international agencies (3). However, the search for an orally active, safe and effective plant preparation is yet to be needed for fertility regulation due to incomplete inhibition of fertility or side effects.

Asteraceae is the largest family of flowering plants, comprising about 1,100 genera and more than 20,000 species. Plants of the genus *Taraxacum*; also known as dandelions have long been used as medicinal herbs. These plants have been employed as diuretics for over 2000 years in both traditional Chinese medicine and in Ayurvedic medicine (4). *T. serotinum* (Waldst. & Kit.) Poiret plant is a member of the Asteraceae family. It is known in Turkey as "sütlük" and is considered as one of the vitamin-rich plants (5). The leaves of *T. serotinum* are used in the Turkish folk medicine as cardiotonic (6). The plant is also used as appetizer and digestant (5).

Heliotropium is one of the important genera of the Boraginaceae family. Boraginaceae has hundred genera and eighteen hundreds species which are distributed through temperate regions but more abundantly in the Mediterranean region (7). The genus *Heliotropium* is represented by 14 species in the Turkish flora and two of which are endemic. *H. europaeum* is an annual herb distributed in European Turkey and North, South, East and Inner Anatolia (8) and is known in Turkey as "temarotu". *Heliotropium* species are known to contain pyrrolizidine alkaloids which have a wide variety of biological activities such as antitumor, antibacterial, antifungal, insecticide, antispasmodic, mydriatic, mutagenic, teratogenic and hepatotoxic activities (9). Juice of the crushed *H. europaeum* is used topically to treat dermatophytosis of hair, nails and skin in domestic animals (10), while boiled leaves are applied on skin to treat pimples and eruption (11).

The aim of this study was to investigate the effect of *T. serotinum* and *H. europaeum* plants on various male fertility parameters.

MATERIALS AND METHODS

Animal model

Both sexes of adult albino mice (27–30 g b. wt) were used in the acute toxicity test. Adult male (200–220 g) and female (180–190 g) Wistar rats were used in male fertility 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*. 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 Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

Plant material

Fresh flowering aerial parts of *T. serotinum* L. and *H. europaeum* (Waldst. & Kit.) Poiret were collected in summer 2011, from Ankara: Kızılcahamam, Çeltikçi way and Hasanoğlan, surrounding Yeşildere Village, respectively. Taxonomic identification was determined by Prof. Dr. Galip Akaydin and a voucher specimen from each plant (Akaydın 13430 & Akaydın 13498, respectively) was deposited at the Herbarium of Faculty of Education (HEF), Hacettepe University, Ankara, Turkey.

Extraction of plant materials

The collected plants were shade dried and then grinded to fine powder. An amount of 100 g dried powder from each plant was extracted by percolation in 70% aqueous ethanol with occasional shaking for 48 h. Percolation was repeated three times, and then each ethanol extract was combined and concentrated under vacuum to give the total extracts of *T. serotinum* and *H. europaeum* (25.5 g/12.75% and 25.0 g/12.5%, respectively).

Acute toxicity study

Acute toxicity study was performed according to Health Effect Test Guidelines (12). Two groups of mice (6 animals/ group) were fasted overnight then treated orally with *T. serotinum* and *H. europaeum* extracts, respectively at a dose of 2000 mg/kg using intragastric tube. An equal volume of the vehicle (3% v/v Tween 80 in distilled water) was given to control group animals which were kept under the same conditions without any treatment. The animals were observed for 48 hours and the number of dead mice was recorded and used in the calculation of the acute toxicity value (LD_{50}). The mice were also observed for clinical signs of toxicity, such as, excitation, tremors, twitches, motor coordination, righting reflex and respiratory changes. Since, there was no mortality at this level; the dose of both extracts was increased to 4000 mg/kg and animals were observed for another 48 h.

Justification for dose selection

T. serotinum and *H. europaeum* extracts were nontoxic at the dose of 4000 mg/kg so, 1/40th, 1/20th and 1/10th of this dose (100, 200 and 400 mg/kg, respectively) were selected for the study (13).

Effect on male fertility

Fourty two sexually mature male Wistar rats were randomly divided into three groups. The 1st group (6 animals) received the vehicle (5 mL/kg) and was kept as control. The 2nd group (18 animals) was divided into 3 equal sub-groups that received *T. serotinum* extract at doses of 100, 200 and 400 mg/kg, respectively. Rats of the 3rd group (18 animals divided into 3 equal sub-groups) received *H. europaeum* extract at doses of 100, 200 and 400 mg/kg, respectively. Both extracts and vehicle were administered to animals by oral intubation for 7 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 (14).

Sacrification schedule

Twenty-four hours after their last dose, the rats were weighed and sacrificed under anesthesia. The following steps were taken to minimize the suffering of the rats. First, the rats were handled gently to reduce their discomfort and distress. Second, anesthesia was adminis-tered prior to blood sample collection, body weight measurements (40 mg/kg sodium pentobarbital intraperitoneally) and before animal sacrifice (70 mg/kg sodium pentobarbital intraperitoneally). Additionally, anesthesia, examinations and animal sacrifice were undertaken in separate rooms to avoid instilling fear in other rats.

Parameters

Estimation of sex hormones

Blood samples were withdrawn from experimental rats of all groups by retro orbital puncture into sampling tubes. Sera were separated by centrifugation of blood samples at 3500 rpm for 15 min. the separated sera were stored frozen and used within 12 h of preparation for estimation of testosterone (15), FSH and LH (16) and prolactin (17).

Assessment of sperm motility and count

Progressive motility was tested immediately. The right cauda epididymitis was incised and semen was squeezed on a pre-warmed slide. Two drops of warm 2.9% sodium citrate was added to semen and mixed by a cover-slip. The percentage of progressive sperm motility was evaluated visually at 400× magnification (18). 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.

For sperm count, the left cauda epididymitis was incised and semen that oozed was quickly sucked into a red blood pipette to the 0.5 mark, and then diluted with warm normal saline up to the 101 mark. A drop of the semen mixture was placed on the Neubauer counting chamber and viewed under the magnification of ×40 (19). The total numbers of sperm cells were counted and expressed as 10^6/mL.

Assessment of sperm viability and morphology

A viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. A drop of semen was squeezed onto a microscope slide and two drops of the stain were added. Thin smears were then prepared and observed under a light microscope at ×400 magnification. Viable sperm remained colorless while non-viable sperm stained red (20). The stained and the unstained sperm cells were counted using x40 microscope objectives and an average value for each was recorded from which percentage viability was calculated.

To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin-nigrosin (5 slides/rat) viewed under a light microscope at 400× magnifications. A total of 300 sperm cells was examined on each slide (1500 cells for each rat), and the head, tail and total abnormality rates of spermatozoa were expressed as a percent (14).

Mating trial test

Mating trial test of male rats was conducted 5 days before the termination of the experiment. Each male rat was cohabitated overnight with proestrous females in a ratio of 1:2 and housed in a single cage. Positive mating was confirmed by presence of sperm and vaginal plug in the vaginal smear the following morning (21). Each sperm positive female was kept under observation and the resultant pregnancies were noted, when dam gave birth. 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).

Body and sex organ weights

The initial and final body weights of the animals were recorded. The testes, epididymitis, seminal vesicle and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed to the nearest milligram. Organ weights were reported as relative weights (organ weight/body weight ×100).

Quantification of fructose in seminal vesicle

For fructose quantification, seminal vesicular homogenate was prepared at a tissue concentration of 50 mg/mL. The supernatant (seminal plasma) was deproteinized by adding 50 μL of zinc sulphate and sodium hydroxide to make a total dilution of seminal plasma 1:16, followed by centrifugation at 2500 rpm for 15 min. For fructose measurement, 200 μL of clear seminal plasma was used and the optical density of standard and samples were measured against blank at 470 nm. The concentration of fructose was obtained by plotting the value in standard curve and the value expressed in the unit of μM/ mL of seminal plasma (22).

Histological analysis

Testes were carefully dissected out following abdominal incision and fixed in 10% formol-saline and processed routinely for paraffin embedding. Sections of 5 μm were obtained with rotary microtome, stained with Hematoxylin and Eosin Stain (H/E) and observed under a light microscope.

Measurement of some biochemical and blood parameters

The biological activities of AST and ALT were measured by enzyme kits method. Serum concentrations of creatinine and urea were determined colorimetrically as measures of kidney function according to Kroll et al. (23) and Wills & Savory (24), respectively. The blood samples collected directly from the heart of each rat at the time of scarification into heparinized tubes were immediately used for determination of some hematological parameters. Total red blood cell and white blood cell counts were estimated according to the visual method of Dacie & Lewis (25). Hb concentration was determined as described by Schalm et al. (26) using the cyanomethemoglobin method, while PCV was done using the macrohaematocrit method (25).

Statistical analysis

The values are expressed as mean ± SEM of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Bonferroni post hoc test to determine the intergroup variability by using SPSS ver. 14.0. A comparison was made with the experimental control group. Differences were regarded statistically significant at the $P \leq 0.05$ and 0.001 levels.

RESULTS AND DISCUSSION

In the current study, the acute oral LD_{50} of ethanol extracts of *T. serotinum* and *H. europaeum* was determined in mice and their effects on fertility of male rats were examined.

Acute toxicity experiment

In toxicity experiment, oral administration of *T. serotinum* and *H. europaeum* extracts at doses up to 4000 mg/kg failed to kill any mouse within 48 h of observation. These findings prove that both extracts have high safety as no toxic symptoms and mortalities were reported when mice were given the extracts at doses up to 4000 mg/kg. Accordingly, it suggested that oral LD_{50} of both extracts was higher than 4000 mg/kg. Therefore, *T. serotinum* and *H. europaeum* can be categorized as

non-toxic plants since substances possessing LD_{50} higher than 50 mg/kg are considered safe (27). Contrary to our results, pigs and poultry (28) have become poisoned due to consumption of feeds contaminated with *H. europaeum*. This toxic effect may be attributed to the presence of pyrrolizidine alkaloids among the plant constituents (29). The lack of toxicity in the current study may be attributed to the low level of pyrrolizidine alkaloids in our crude extract and/or to the difference in the metabolic pathway within pigs, poultry and rats.

Effect on male fertility

Oral administration of *T. serotinum* (100, 200 and 400 mg/kg) and *H. europaeum* (100 mg/kg) extracts for 7 weeks did not affect any of male fertility parameters (Tables 1-4).

Table 1. Effect of oral administration of *Taraxacum serotinum* and *Heliotropium europaeum* extracts for 7 weeks on serum levels of reproductive hormones of male rats, (n = 6).

Groups	Doses (mg/kg)	Testosterone (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)	Prolactin (ng/mL)
Control	-	4.25±0.30	6.45±0.33	0.54±0.04	0.74±0.06
T. serotinum	100	4.10±0.31	6.24±0.28	0.59±0.03	0.70±0.06
	200	3.47±0.26	5.95±0.31	0.52±0.04	0.75±0.07
	400	3.35±0.31	5.63±0.36	0.58±0.03	0.73±0.05
H. europaeum	100	4.11±0.30	6.18±0.38	0.55±0.03	0.77±0.05
	200	3.28±0.22 [a]	5.92±0.30 [a]	0.50±0.03	0.69±0.04
	400	2.55±0.15 [b]	4.65±0.21 [b]	0.52±0.02	0.68±0.05

Values represent the mean ± S.E. of six rats for each group.
[a] P<0.05: Statistically significant from control (Dunnett's test).
[b] P<0.001: Statistically significant from control (Dunnett's test).

Table 2. Effect of oral administration of *Taraxacum serotinum* and *Heliotropium europaeum* extracts for 7 weeks on semen characteristics and fructose content in seminal vesicle of male rats, (n=6).

Groups	Doses (mg/kg)	Sperm motility (%)	Sperm count (X 10⁶/mL)	Sperm viability (%)	Total sperm abnormalities (%)	Fructose level (µm/mL)
Control	-	86.5±2.5	47.3±1.53	89.7±3.5	4.2±0.23	5.10±0.25
T. serotinum	100	87.3±2.8	50.2±1.79	84.5±3.2	4.4±0.26	4.96±0.26
	200	84.7±2.4	48.5±1.47	85.1±3.0	4.5±0.24	5.12±0.31
	400	85.5±2.5	49.3±1.30	85.8±2.7	4.7±0.28	4.92±0.28
H. europaeum	100	81.2±2.3	43.8±1.47	85.2±3.2	4.7±0.26	4.45±0.22
	200	73.1±2.6[a]	40.5±1.49 [b]	72.1±3.6 [a]	5.6±0.36[a]	4.11±0.25 [a]
	400	69.2±2.7[b]	34.6±1.50 [b]	68.0±3.3 [b]	6.0±0.31[b]	3.25±0.21 [b]

Values represent the mean ± S.E. of six rats for each group.
[a] P<0.05: Statistically significant from control (Dunnett's test).
[b] P<0.001: Statistically significant from control (Dunnett's test).

Table 3. Effect of oral administration of *Taraxacum serotinum* and *Heliotropium europaeum* extracts for 44 days on the mating trial of male rats with normal untreated females (mating ratio = 1 male: 2 females).

Groups	Doses (mg/kg)	No. of females mated[a]	Mating success %	No. of females pregnant	Fertility success %	Male fertility index (%)[b]
Control	-	12/12	100.00	12/12	100.00	100.00
T. serotinum	100	12/12	100.00	12/12	100.00	100.00
	200	12/12	100.00	12/12	100.00	100.00
	400	12/12	100.00	12/12	100.00	100.00
H. europaeum	100	11/12	91.66	10/12	83.33	90.90
	200	9/12	75.00	8/12	66.66	88.88
	400	9/12	75.00	6/12	50.00	66.66

Data are expressed as numbers and % of 6 males and 12 females.
[a] Evidenced by vaginal plug and sperms in a vaginal smear.
[b] Male fertility index = ([number pregnant/number mated] × 100).

Table 4. Effect of oral administration of *Taraxacum serotinum* and *Heliotropium europaeum* extracts for 7 weeks on body and sexual organs weights of male rats, (n=6).

Groups	Doses (mg/kg)	Final body weight (g)	Relative weight of reproductive organs (g/100 g b.wt)			
			Testes (Pair)	Cauda epididymis	Seminal vesicles	Ventral prostate
Control	00	259.99±7.34	1.88±0.11	0.63±0.04	0.65±0.03	0.50±0.03
T. serotinum	100	246.87±7.38	1.84±0.16	0.65±0.02	0.65±0.03	0.52±0.01
	200	253.94±8.43	1.92±0.12	0.65±0.05	0.62±0.04	0.48±0.02
	400	249.40±6.80	1.85±0.17	0.66±0.03	0.64±0.03	0.49±0.02
H. europaeum	100	245.72±8.53	1.73±0.12	0.60±0.03	0.61±0.02	0.46±0.01
	200	248.79±7.65	1.25±0.15 [a]	0.51±0.02 [a]	0.53±0.03 [a]	0.37±0.02 [a]
	400	252.71±7.55	1.10±0.11 [b]	0.40±0.03 [b]	0.45±0.02 [b]	0.32±0.01 [b]

Values represent the mean ± S.E. of six rats for each group.
[a] P<0.05: Statistically significant from control (Dunnett's test).
[b] P<0.001: Statistically significant from control (Dunnett's test).

Serum level of sex hormones

Testosterone and gonadotropins are the prime regulators of germ cell development. Testosterone was reported to act on the seminiferous tubules to initiate and maintain spermatogenesis (30). The quantitative production of spermatozoa generally requires the presence of FSH and LH. FSH acts synergistically with testosterone to increase spermatogenesis efficiency and fertility in the male, whereas LH stimulates the production of testosterone in Leydig cells (31).

The means of serum testosterone and FSH levels of rats treated with *H. europaeum* (200 and 400 mg/kg) for 7 weeks significantly reduced as compared to the controls (Table 1). The reducing effect was dose-dependent. The decrease in testosterone level by *H. europaeum* extract may be adduced to reduction of the hormone synthesis by the Leydig cells, as the cells are the main source of testosterone (32). In addition, the deficiency of FSH prevents the gonads from producing sperms (33). Serum levels of LH and prolactin did not reveal any significant change in all treated groups when compared with their control counterparts.

Sperm motility and count

Sperm motility and their count are considered as the important factors that affect the process of spermatogenesis and fertility (34). Testosterone promotes differentiation of spermatozoa during the process of spermatogenesis so; a lack of testosterone level would have direct effects on the process of spermatogenesis (35). In this investigation, epididymal sperm motility was sig-

nificantly reduced in a dose-dependent manner (Table 2) following administration of *H. europaeum* extract at doses of 200 and 400 mg/kg (73.1% and 69.2%, respectively) as compared to control rats (86.5%). The epididymal spermatozoa of *H. europaeum*-treated rats exhibited gradual decline in sperm count in a dose-dependent manner showing 14.4% and 26.8% decline at doses of 200 and 400 mg/kg, respectively. The decrease in epididymal sperm motility and count could be connected to the reduction in the serum testosterone level reported in our study. Gong & Han (36) confirmed this explanation as they stated that lowering of epididymal sperm motility and count suggested an undersupply of testosterone to the epididymis.

It has also been demonstrated that the deficiency of FSH prevents the gonads from producing sperms (33). Further, it is well established that FSH and testosterone are both required by Sertoli cells/germ cells to support the process of spermatogenesis (37). Depletion in the biosynthesis of any one of these hormones, therefore, could block formation of spermatozoa. In addition, low fructose concentration in the seminal fluid of *H. europaeum*-medicated rats may be another cause of low sperm motility as fructose supplies energy for sperm motility. The impaired sperm motility and viability may also be due to the reduced activity of the testes, which affects the normal passage of testicular fluid into the epididymis (38). Moreover, reduced 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 weight (39).

Sperm viability and morphology

Alterations in the sperm viability are indications of a disturbed epididymal microenvironment (40). The results of this study have shown that exposure of rats to *H. europaeum* at doses of 200 and 400 mg/kg caused a decrease in sperm viability (72.1 & 68.0%, respectively). The reduction in sperm viability agreed with reduction in the progressive sperm motility because immobile sperms were considered dead as they took up the Eosin/Nigrosin stain when the smear was examined. The significant reduction of sperm viability, motility and count shows that *H. europaeum* extract has the potential to penetrate the blood-testis barriers and suggests alteration of sperm maturation in the epididymitis (41).

In the literature, tail abnormalities have been reported to start either during spermatogenesis or during epididymal maturation (42). Accordingly, coiling of end tail of spermatozoa of treated rats could possibly be due to either a direct effect of *H. europaeum* on maturing germ cells or interference with sperm maturation process in epididymis.

Seminal fructose level

Fructose concentration has been noted to be essential for spermatozoal metabolism and motility as an energy source. In this connection, Montagnon et al., (43) reported that fructose concentration in seminal plasma is one of the most important markers of seminal vesicular function and that when seminal vesicular function is decreased, sperm motility and viability are affected. In Table 2, the fructose concentration of the seminal fluid of the animals treated with *H. europaeum* extract at doses of 200 and 400 mg/kg showed significant decrease (4.1 & 3.3 μm/mL, respectively) when compared with that of the control (5.1 μm/mL). This depletion of seminal fructose invariably affects the sperm motility and viability since fructose is androgen-dependent and serves as the driving energy of the sperm which may indicate reduction in circulating androgen levels.

Mating trial test

Drugs may impair male fertility by interfering with spermatogenesis, sperm motility or the fertilizing capacity of spermatozoa (44). In the current study, mating trial during 44–49 days of treatment schedule culminated in normal pregnancy outcome in control animals. After mating of estrous female rats with *H. europaeum*-med-

icated males (200 and 400 mg/kg), the mating success, fertility success and fertility index were declined in a dose-dependent pattern as compared to the untreated group (Table 3). These results might be due to the low serum testosterone and reduction of sperm motility and quantity that were evident in the present study. Gauthaman et al., (45) concluded that decreased testosterone level may reduce androgen-dependent parameters like mating behavior, libido and penile erection.

Pregnancy rates of the untreated female rats were reduced following mating with *H. europaeum*-medicated males (200 and 400 mg/kg). The decrease in the pregnancy rate might be due to the effect of *H. europaeum* extract on the progressive epididymal sperm motility as sperm motility is positively correlated with fertilization of oocytes and pregnancy rates (46)., The decline in either fertilization of oocytes or pregnancy rates is one of the markers of male reproductive toxicity and also an indicator for the onset of infertility (47). Moreover, sperm motility is an important functional measurement to predict the fertilizing capacity of sperms, so the negative impact of *H. europaeum* extract on sperm motility would seriously affect the fertilizing ability (48).

H. europaeum is known to contain pyrrolizidine alkaloids which have a wide variety of biological effects including cytotoxic activity (9). The mechanism of action of most cytotoxic agents is interference with obligatory cell processes, such as DNA synthesis, in the rapidly dividing cancer cells. However, all cells that undergo rapid division including spermatogonial stem cells are susceptible to the toxic effect of cytotoxic agents. Accordingly, the antifertility effect of *H. europaeum* extract in male rats could be attributed to its pyrrolizidine alkaloids content as the cytotoxic agents do not discriminate between cancer cells and normal cells undergoing rapid division. In this connection, Meistrich (49) stated that cytotoxic agents are most toxic to the rapidly proliferating spermatogonia, which can be reproduced from the germinal stem cell layer. Additionally, the results obtained from an experiment in men (50) suggest that after some cytotoxic therapy regimens, there is a potentially reversible block to spermatogonial differentiation.

Effect on body and sex organs weights

In this study, there were no significant changes in the final body weight of all medicated rats compared with the controls after 7 weeks of medication indicating that the

general metabolic condition of the animals was within normal range. The alteration of the testicular weight suggests injury of the gonad. Creasy (51) has reported that weights of testes and accessory organs are sensitive end points that can be used in evaluation of deleterious effect on male reproduction. A significant decline in the relative weights of testes, epididymides, seminal vesicle and ventral prostate were observed in *H. europaeum*-treated rats (200 and 400 mg/kg) when compared with the control (Table 4). The decreasing weight of testes and the accessory reproductive glands clearly indicated that *H. europaeum* extract caused structural alteration in the reproductive organs of male rats (52). It has also been demonstrated that maintenance of the weights of the accessory reproductive glands depends on testosterone level; accordingly, the significant reduction in the reproductive organ weights of male rats in this study may be attributed to the decrease in testosterone levels and inhibition of spermatogenesis (53). In addition, Zitzmann (54) have demonstrated that physiologic concentrations of testosterone and FSH play an important role in spermatogenesis, so a significant decrease of these hormones in our study could decrease the number and function of somatic and germinal cells of testis followed by testis weight reduction.

Histologic evaluation

Histopathological examination of the testes of normal control rats showed normal histological picture of the seminiferous tubules as demonstrated in Fig. 1-A. Histological studies of control rat testis showing all successive stages of spermatogenesis, where the lumen was filled with sperm. Similarly, histopathology of the seminiferous epithelium of *T. serotinum*-treated rats showed preserved seminiferous epithelium, with normal concentric distribution of germ cells (Fig.1-B). The deleterious effects of *H. europaeum* extract at doses of 200 and 400 mg/kg on male fertility were supported by the histopathological findings in the testes of treated rats. Different degrees of damage were noted in the seminiferous tubules of *H. europaeum*-treated rats according to the dose. Testicular sections of *H. Europaeum* medicated rats at a dose of 400 mg/kg showed disorganized seminiferous epithelium, associated with incomplete spermatogenesis and sloughing of degenerated germ cells (Fig. 1-C). Sloughing of germ cells was observed in the lumen of some seminiferous tubules of *H. Europaeum*-medicated rats indicating testicular dysfunction (39). Another possible explanation for the incomplete spermatogenesis is the reduction in testosterone level (35).

Histopathology of liver and kidney of *T. serotinum* and *H. europaeum*-medicated rats did not reveal any treatment related changes (Figures 2 & 3). The observation allows conclusion that both plants are non-hepatotoxic and non-nephrotoxic. In a previous histopathological study by Christie & Le Page (55), although extensive liver-cell necrosis was noted in the purified pyrrolizidine alkaloid treated rats, we did not notice any adverse effects with *H. europaeum* extract-treated rats in our study. This difference may be due to the low level of phytochemicals present in the crude extract of *H. europaeum* compared to the purified alkaloid.
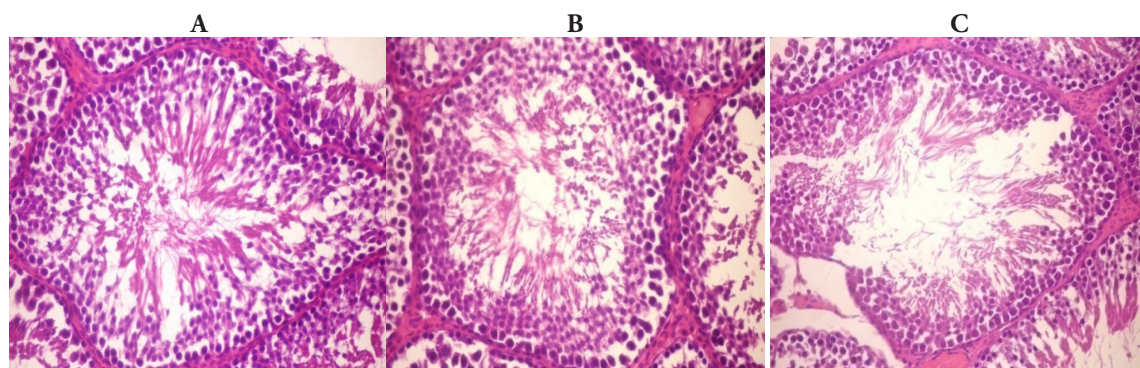

Figure 1. Photomicrographs of rat testis (H&E X400), **(A)** Normal control group showing normal seminiferous tubules with all successive stages of spermatogenesis; **(B)** *T. serotinum* (400 mg/kg) showing preserved seminiferous epithelium, with normal concentric distribution of germ cells; **(C)** *H. europaeum* (400 mg/kg) showing disorganized seminiferous epithelium, associated with incomplete spermatogenesis and sloughing of degenerated germ.
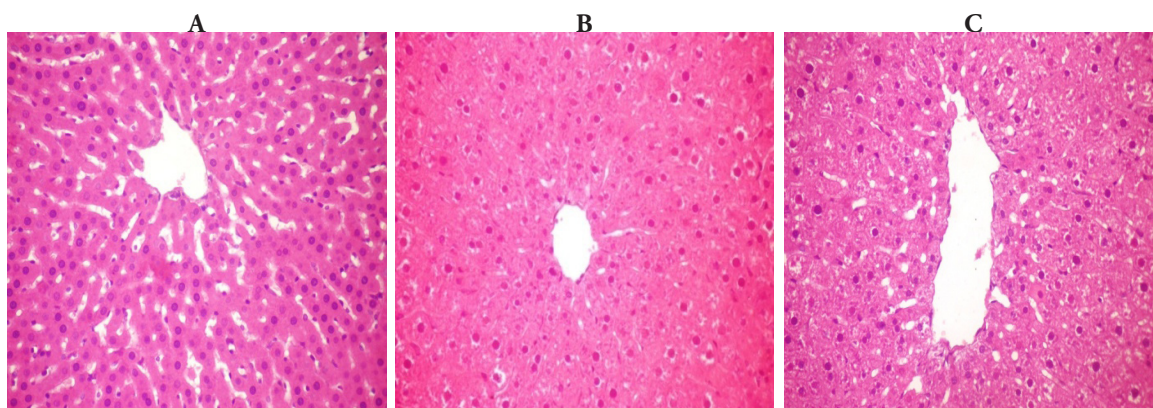
Figure 2. Photomicrographs of a section of rat liver (H&E X400), **(A)** Normal control group; **(B)** *T. serotinum* (400 mg/kg) and **(C)** *H. europaeum* (400 mg/kg) showing normal structure.
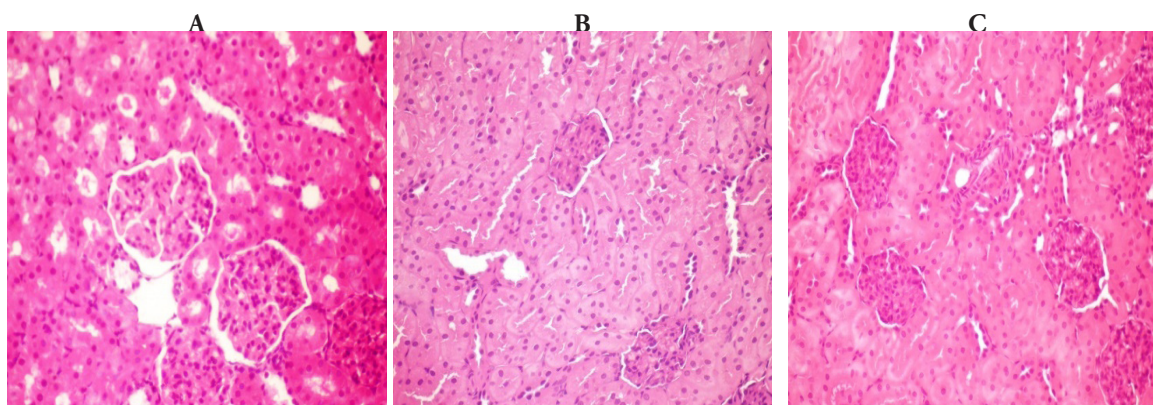

Figure 3. Photomicrographs of a section of rat kidney (H&E X400), **(A)** Normal control group; **(B)** *T. serotinum* (400 mg/kg) and **(C)** *H. europaeum* (400 mg/kg) showing normal structure.

Measurement of some biochemical and blood parameters

The non-toxic nature of *T. serotinum* and *H. europaeum* extracts in acute toxicity study is well supported by the biochemical data following 7-weeks treatment period in rats. Doses of 100, 200 and 400 mg/ kg of both extracts were well tolerated by all the animals, as there were no toxic effects observed by direct visual observation of the animals throughout the experiment. There were no deaths and apparent behavioral changes recorded during the course of the experiment in all treatment groups as compared to the control animals. These observations might suggest the non-toxic effect of *T. serotinum* and *H. europaeum* plants (56).

The changes in the liver enzyme system have been used clinically in evaluating the toxicity of any extraneous substance to the living system. Fortunate enough, oral administration of both extracts to male rats at doses up to 400 mg/kg for 7 weeks had not any significant effect on their serum activities of AST and ALT (Table 5). Since the activity of ALT and AST are specific assayable liver enzymes, their normal levels in the serum of rats indicated that *T. serotinum* and *H. europaeum* extracts are not hepatotoxic.

Serum creatinine and urea levels are sensitive and reliable biochemical indices for evaluation of renal function in animal models (57). In the present study, the mean values of urea and creatinine in serum of rats were not affected by treatment with *T. serotinum* and *H. europaeum* extracts for up to 7 weeks. In kidney damage, there will be retention of urea and creatinine in the blood (58); therefore marked increases in serum urea and creatinine are indications of functional damage to the kidney (59). By these indicators, ethanol extracts of *T. serotinum* and *H. europaeum* are therefore, not nephrotoxic in rats.

It is well established that hematological tests form the

Table 5. Effect of oral administration of *Taraxacum serotinum* and *Heliotropium europaeum* extracts for 7 weeks on serum levels of AST, ALT, urea and creatinine of male rats, (n = 6).

Groups	Doses (mg/kg)	AST (U/L)	ALT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	00	66.56±4.54	153.26±7.77	44.36±2.14	0.42±0.03
T. serotinum	100	62.63±4.78	155.58±8.74	47.47±1.94	0.46±0.03
	200	71.58±4.20	163.17±7.89	43.73±1.90	0.41±0.04
	400	69.15±4.63	165.73±7.32	47.43±2.43	0.45±0.04
H. europaeum	100	60.27±4.22	162.55±8.42	46.74±2.11	0.44±0.02
	200	68.29±3.62	155.27±8.79	40.55±1.85	0.45±0.02
	400	69.22±3.74	161.28±7.25	45.62±2.10	0.39±0.03

Values represent the mean ± S.E. of six rats for each group.
No statistically significant from control (LSD test).

Table 6. Hematological analysis of male rats after treatment with *Taraxacum serotinum* and *Heliotropium europaeum* extracts for 7 weeks (n= 6).

Groups	Doses (mg/kg)	RBCs (x 10^6/ mL)	Hb (g%)	PCV (%)	TLC (x 10^3/ mL)
Control	00	9.6±0.36	12.23±0.55	33.38±1.67	13.2±0.64
T. serotinum	100	9.5±0.52	11.59±0.68	34.73±1.79	14.2±0.65
	200	8.8±0.49	11.84±0.74	35.50±1.54	14.0±0.69
	400	9.4±0.55	12.10±0.85	35.32±1.82	13.7±0.62
H. europaeum	100	9.0±0.44	12.53±0.63	34.24±1.84	13.4±0.68
	200	8.9±0.35	11.88±0.58	34.55±1.66	12.8±0.66
	400	9.2±0.48	12.35±0.53	31.65±1.85	13.5±0.63

Values represent the mean ± S.E. of six rats for each group.
No statistically significant from control (LSD test).

very front-line investigations on which diagnosis of various diseases is based. RBC count, Hb concentration, PCV and TLC in *T. serotinum* and *H. europaeum* groups were found to be within the normal range when compared with the control animals (Table 6). Unaltered hematological parameters in all of the treatment groups in the present investigation in rats suggest that *T. serotinum* and *H. europaeum* extracts did not cause any adverse effects on the general health of the animals. The biochemical and hematological data were supported by the normal histopathological findings in the livers and kidneys of treated rats.

CONCLUSION

The results of the present study indicate absence of male reproductive toxicity of *T. serotinum* at the dose levels tested. It is concluded that the ethanol extract of *H. europaeum is* capable to suppress male fertility without altering general body metabolism at 200 and 400 mg/kg doses. This is demonstrated by the decrease in the fertility parameters (motility, count and viability of sperms, serum level of sex hormones, and fertility rate) in treated rats. However, further studies are required for better understanding of the mechanism of reproductive toxicity induced by *H. europaeum*.

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