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Aphrodisiac activity and curative effects of *Pedalium murex* (L.) against ethanol-induced infertility in male rats

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Abstract: It has been suggested that chronic ethanol exposure may result in testicular damage and infertility in males. Petroleum ether extract of *Pedalium murex*, family Pedaliaceae (PEPM), is evaluated in this study for its ability to increase aphrodisiac activity and to cure ethanol induced germ cell damage and infertility in male rat models. Doses of 200 and 400 mg/kg of PEPM showed a significant increase ($P < 0.01$, $P < 0.001$) in mating and mounting behaviour. The effect on fertility factors such as total body weight, percentage of pregnancy, litter size were also significantly increased ($P < 0.01$) in comparison with the ethanol-treated group. Significant increases in sperm motility and count were observed in PEPM-treated groups in a dose-dependent manner ($P < 0.01$; $P < 0.001$) as compared with the ethanol-treated group. Similarly, reductions in the percentage of abnormal sperm were noted in animals treated with PEPM 400 mg/kg. The effects of PEPM on total protein, total cholesterol and testosterone were satisfactory, the levels being increased significantly for protein ($P < 0.05$), cholesterol ($P < 0.01$) and testosterone ($P < 0.05$) by 400 mg/kg PEPM. Microtome sections of the testes of animals treated with 400 mg/kg PEPM exhibited restoration and recovery of germinal cells and the luminal spermatozoa and were comparable with the control group animals. These effects of PEPM make this natural herb ideal as an aphrodisiac and a potent fertility enhancing drug.

Key words: Aphrodisiac, ethanol, fertility, *Pedalium murex*, sperm count

Erkek ratlarda etonolün neden olduğu kısırlığa karşı *Pedalium murex* (L.)'ın ilaç etkisi ve afrodizyak aktivitesi

Özet: Kronik etanole maruz kalmanın erkeklerde testislerde hasara ve kısırlığa neden olduğu öne sürüldü. Pedaliaceae familyasına ait olan *Pedalium murex*'in petrol eter özütü (PEPM) bu çalışmada erkek rat modellerinde afrodizyak aktivitesindeki artış yeteneği ve etanolün neden olduğu germ hücre zararı ve kısırlığı tedavi etmek için değerlendirildi. PEPM' in 200 ve 400 mg/kg dozlarında çiftleşme ve binme davranışında önemli bir artış ($P < 0,01$, $P < 0,001$) görüldü. Toplam vücut ağırlığı, hamilelik yüzdesi ve çöp boyutu gibi doğurganlık üzerindeki etkisi de etanol uygulanmış grup ile karşılaştırıldığında önemli ölçüde ($P < 0,01$) artmıştır. Sperm hareketliliği ve sayısındaki önemli bir artış da doza bağlı şekilde PEPM uygulanmış gruplarda gözlendi ($P < 0,01$; $P < 0,001$). Benzer şekilde, anormal spermlerin yüzdesindeki azalmalar 400 mg/kg PEPM ile muamele edilmiş hayvanlarda dikkati çekti. PEPM' nin toplam protein, toplam kolesterol ve testosteron üzerine etkisi tatmin ediciydi, seviyeleri protein için ($P < 0,05$), kolesterol için ($P < 0,01$) ve testosteron için ($P < 0,05$) 400 mg/kg PEPM konsantrasyonunda önemli derecede arttı. 400 mg/kg PEPM ile muamele edilmiş hayvanların testislerinin mikrotom kesitlerinde onarım gözlendi ve tohum hücrelerinin ve luminal spermatozoaların yeniden kazanımları kontrol grubu hayvanlar ile karşılaştırıldı. TEPM'nin bu etkisi bu bitkiyi ideal bir afrodisiak yapar ve bu bitkide fertiliteyi artırma potansiyeli olan bir ilaç olabilir.

Anahtar sözcükler: Afrodizyak, etanol, doğurganlık, *Pedalium murex*, sperm sayısı

Introduction

Pedalium murex L. (Pedaliaceae) is a diffuse, more or less succulent herb found near the sea coast of south India, Mexico, and tropical Africa (1). The fruits as well as the leaves and stems produced milk mucilage when agitated, and it is recommended as a treatment for gonorrhoea (2). An infusion or extract prepared from leaves is diuretic and demulcent, useful in treating disorders of the urinary system such as ardor urine, dysuria, spermatorrhoea, and incontinence of urine. As an emmenagogue, the juice is used in puerperal diseases and also to promote lochial discharge (3). The mucilage from leaves and young shoots is used as an aphrodisiac in seminal debility (4). The petroleum ether extract of *P. murex* is effective against Japanese encephalitis vector culex (5). The aqueous extract of the whole plant has been found to possess analgesic and anti-inflammatory properties (6). Extensive phytochemical investigations on the plant have revealed the presence of Pedalitin and Pedalin (major flavanoids) along with Diosmetin, Dinatin, Dinatin-7-glucoronide, Quercetin, Quercimeritin, and Quercetin-7-glucorhamnoside (7). Triterpenoids such as α-amyrin acetate, Rubusic acid, ursolic acid, and lupeol acetate are reported (8). Steroids such as β-sitosterol (9), Sapogenins (10) and Diosgenin (11) have also been reported. Lipids (12), phenolic acids such as caffeic acid, ferulic acid, protocathechic acid, and vanillic acid (9), and amino acids such as aspartic acid, glutamic acid, and histidine are other phytoconstituents present in *P. murex* (13). Although the plant contains several phytoconstituents, they have not been evaluated for their pharmacological activities in detail. Since no scientific data are available on the aphrodisiac and fertility-enhancing properties of this medicinal plant, the present study was carried out, which aims to evaluate aphrodisiac activity along with the potential of the plant (leaves and fruits) to promote sperm count, motility, restoration of normal architecture of testis and testosterone in testicular injury male rat models.

Materials and methods

Animals

In-bred male albino rats (*Rattus norvegicus*) weighing 150-200 g procured from the animal house of the C.L.Baid Metha College of Pharmacy were used for the study. The animals were housed in polypropylene cages and fed with standard rodent pellet obtained from Amruth Laboratory feeds, Sangli, and water ad libitum. The animals were subjected to a 12:12 h light:dark cycle under standard laboratory conditions at a temperature of 24-28 °C with a relative humidity of 60%-70%. The study was carried out after approval by the Institutional Animal Ethical Committee (Ref No. IAEC/05/XIV/CLBMCP/2005-06, dated 22.12.2005).

Plant material

The plant specimen used for the study was collected from a crude drug market (Chennai, India). The identity of the specimen was confirmed as *Pedalium murex* by Dr. Sasikala Ethirajulu, Research officer, Pharmacognosy, Central Research Institute for Siddha, Govt. of India (Chennai, Tamil Nadu, India). A voucher specimen was deposited in the Department of Pharmacology and Toxicology, C.L.Baid Metha College of Pharmacy, Chennai.

Preparation of the extract

The plant was deprived of the roots, shade dried, and made into a coarse powder. The powder was then passed through sieve no: 40 to obtain uniform particle size and used for the purpose of extraction (14). A weighed quantity of the powder was packed in Whatman filter paper and subjected to Soxhlet extraction using petroleum ether as solvent. The extract was evaporated to dryness using a rotary vacuum evaporator. The percentage yield of the extract (PEPM) was 19.2% w/w from the crude material. For the study PEPM was administered to the animals by dissolving it in 5 % v/v of Tween 80.

Phytochemical analysis

PEPM was subjected to phytochemical screening through qualitative chemical analysis for confirmation of the phytoconstituents (15).

Acute toxicity study

Albino rats weighing 150-200 g selected by random sampling were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines (16). The animals were fasted overnight, provided only with water, and afterwards PEPM was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation, and the groups were observed for 14 days. If mortality was observed in 2 or 3 animals, then the dose administered was identified as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsions, and mortality for 72 h.

Mounting behaviour

A mount is operationally defined as the male assuming the copulatory position but failing to achieve intromission. To quantify mounting behaviour, non-oestrous female rats were paired with males treated with a single dose of the drugs (200 and 400 mg/kg, p.o.) as well as with male rats in the control group and male rats treated with ethanol (20% v/v). Animals were observed for 3 h and their behaviours were scored (17). Males were placed individually in a glass cage. After 15 minutes of acclimatisation, a non-oestrous female was introduced into the arena. The numbers of mounts were recorded during a 15-min observation period at the start of the 1st h. Then the female was separated for 105 min. Again the female was introduced and the number of mounts was observed for 15 min as before at the 3rd h. All the experiments were performed between 0900 to 1200 h during day time at room temperature, 26-27 °C (18).

Assessment of mating

Healthy and sexually experienced male rats were selected for the study and they were divided into 4 groups (6 animals in each group). Group I served as the control and received 1 mL of 5% Tween 80. Group II received ethanol 20 % v/v. Groups III and IV received PEPM doses of 200 and 400 mg/kg body weight respectively once a day for 7 days at 1700-1800 h. Since male animals should be tested in familiar circumstances, they were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment.

The female rats allow mating only during the oestrus phase. Thus, they were artificially brought into oestrus (heat). They were administered suspension of ethinyl oestradiol orally at a dose of 100 μg/animal 48 h prior to the pairing plus progesterone injected subcutaneously, at a dose of 1 mg/animal 6 h before the experiment (19,20). The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test, and standard animals. The most receptive females were selected for the study. The experiment was carried out on the 7th day after commencement of the treatment of the male animals. The experiment was conducted at 2000 h in the same laboratory and under the light of same intensity. The receptive female animals were introduced into the cages of male animals with 3 females to 1 male in each cage and they were cohabitated overnight (18). The test was terminated if the male failed to evince sexual interest. If the female did not show receptivity it was replaced by another artificially warmed female. The stage of oestrous cycle was determined (21). The vaginal smear of each female rat was examined under a microscope for the presence of sperm. The number of sperm-positive females was recorded in each group (18).

Effect on fertility

In these experiments, each male was cohabitated with one female with proven fertility. All the female rats were sperm positive. These females were watched for pregnancy and birth of offspring. The litter size and ratio of male and female pups were recorded in each group (22).

Evaluation of sperm motility, count, viability, and abnormalities

Experimental groups

The male rats were divided into 4 groups, each consisting of 6 rats. All the groups except group I were fed with 20% v/v ethanol for 5 weeks to induce testicular injury

Group I: This group served as the control and received 5% v/v Tween 80 for 60 days.

Group II: This group served as the negative control and received ethanol 20% v/v as an aqueous solution for 5 weeks to induce testicular damage. (23).

Group III: Animals received 200 mg/kg p.o. PEPM in 5% v/v Tween 80, as a single dose daily for 60 days.

Group IV: Animals received a single daily dose of 400 mg/kg p.o. PEPM in 5% v/v Tween 80 for 60 days.

The samples for these studies were obtained by making small cuts in the caudae epididymis and vas deferens, and placed in 1 mL of modified Krebs Ringer-bicarbonate buffer (pH 7.4). The sperm suspension was evaluated for sperm count and percent motility. The percent motility was determined by the progressive and nonprogressive movements of sperms observed under a compound microscope (24). The sperm content was carried out under a Neubauer haemocytometer (25). To evaluate abnormal sperm, the sperm suspension was stained with eosin, and smears were made on slides, air dried, and made permanent. The slides were examined by bright field microscope with an oil immersion lens. The percentages of normal and abnormal sperm were calculated (26).

Biochemical estimations

All the groups of male rats were sacrificed at the end of treatment on day 61 under ether anaesthesia in lethal chamber and the blood samples were collected by carotid bleeding. The samples were centrifuged and serum was separated and used for the estimation of total protein (27), total cholesterol (28), and testosterone (29) using respective standard biochemical kits.

Histopathological examination

Testes were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Tissues were removed and dehydrated through upgraded ethanol, then cleared with xylene, and finally embedded in paraffin. Sectioning was done by using microtome (7 μm thickness). Tissues were counterstained with haematoxylin in eosin, then examined and photographed under a Leica microscope (30,31).

Statistical analysis

The data represent mean ± S.E.M. Results were analysed statistically using one way ANOVA followed by Dunnet's t-test. $P < 0.05$ was considered significant.

Results

Phytochemical screening

The petroleum ether extract of *Pedalium murex* subjected to preliminary phytochemical screening revealed the presence of steroids, sterols, flavanoids, carbohydrates, proteins, gums and mucilage, saponins and terpenes.

Acute oral toxicity study

PEPM produced no toxic symptoms or mortality up to a dose level of 2000 mg/kg body weight orally in rats, and hence the drug was considered safe for further pharmacological screening. According to OECD-423 guidelines for acute oral toxicity, an LD_{50} dose of 2000 mg/kg and above is categorised as unclassified.

Effect of PEPM on mounting behaviour of rats

Male rats treated with PEPM (200 and 400 mg/kg) displayed excessive mounting behavior 1 h after treatment as well as 3 h after treatment when compared to controls. Mounting behaviour was diminished or decreased in Group II animals. Between the 2 dose levels, 400 mg/kg ($P < 0.001$) was more effective than 200 mg/kg ($P < 0.01$) (Table 1).

Table 1. Effect of PEPM on mounting behaviour.

Group and Treatment	mounting behaviour	
	1st h	3rd h
I Normal control	4.167 ± 0.3073	2.833 ± 0.3073
II Ethanol 20% v/v	2.500 ± 0.2236^{a*}	1.667 ± 0.2108^{a*}
III PEPM 200 mg/kg	6.000 ± 0.3651^{b*}	5.000 ± 0.2582^{b*}
IV PEPM 400 mg/kg	10.67 ± 0.3333^{b**}	10.00 ± 0.3651^{b**}

Values are mean ± S.E.M. of 6 observations, a- Group I vs. Group II; b-Group II vs. Group III and IV, *P < 0.01, **P < 0.001.

Effect of PEPM on assessment of mating

The administration of PEPM resulted in a dose-dependent increase in the mating performance of the rats. Out of 6 animals in Group I, only 1 mated with 2 females and the remaining 5 males mated with 1 female each during the overnight experimental period. Animals treated with PEPM (200 mg/kg) mated with 1 female each except 1 male which mated with 2 females. In Group IV animals, treated with the 400 mg/kg, 4 males mated with 2 females each and 2 males mated with 3 females each ($P < 0.01$) (Table 2).

Table 2. Effect of PEPM on mating performance.

Group and Treatment	mating performance
I Normal control	1.167 ± 0.1667
II Ethanol 20% v/v	$0.500 \pm 0.2236^{a*}$
III PEPM 200 mg/kg	$1.670 \pm 0.1084^{b*}$
IV PEPM 400 mg/kg	$2.500 \pm 0.2108^{b*}$

Values are mean ± S.E.M. of 6 observations, a- Group I vs. Group II; b-Group II vs. Group III and IV, *P < 0.01.

Effect of PEPM on fertility

Total body weight

The total body weight was significantly increased ($P < 0.01$) in Group III and IV animals in comparison with Group II animals treated with ethanol 6% v/v at day 61. There was a significant reduction in the body weight of Group II animals ($P < 0.01$) compared with Group I animals (Table 3).

Percentage of pregnancy

The percentage of pregnancy was 33.33 in the female group mated with Group II animals treated with ethanol 6% v/v. The pregnancy rates of female groups mated with Group III and Group IV animals were calculated to be 83.33% and 100%, respectively (Table 3).

Litter size

Litter size was significantly lower in females mated with Group II males receiving ethanol 6% v/v ($P < 0.01$). The litter size of female animals mated with Group IV male animals (400 mg/kg) was significantly increased ($P < 0.01$). The difference in litter size was statistically nonsignificant for female animals mated with Group III male animals (200 mg/kg) when compared with controls (Table 3).

Pups sex ratio

There was an increased ratio of males to females in pups fathered by PEPM treated groups, but this was statistically non significant.

Effects of PEPM on sperm motility, count, viability, and abnormalities

The percentage of caudae epididymal sperm motility exhibited a significant ($P < 0.01$) decrease in Group II animals, which received ethanol 6% v/v, indicating germinal cell damage, while Group IV animals treated with 400 mg/kg of PEPM exhibited a significant increase ($P < 0.001$), and Group III animals treated with 200 mg/kg of PEPM showed a moderate increase ($P < 0.01$) in sperm motility in comparison with Group II, indicating significant and moderate restoration of germ cell damage (Table 4).

Table 3. Effect of PEPM on fertility.

Group and Treatment	Total body weight		% pregnancy	Litter size
	1st day	60th day		
I Normal control	176.6 ± 3.300	193.0 ± 1.607	100	8.667 ± 0.2108
II Ethanol 20% v/v	173.0 ± 2.817	$132.8 \pm 3.084^{a*}$	33.33	$3.167 \pm 0.3073^{a*}$
III PEPM 200 mg/kg	171.8 ± 2.648	$193.3 \pm 2.109^{b*}$	83.33	8.000 ± 0.2582^{NS}
IV PEPM 400 mg/kg	172.0 ± 2.446	$207.9 \pm 3.218^{b*}$	100	$11.67 \pm 0.3333^{b*}$

Values are mean ± S.E.M. of 6 observations, a- Group I vs. Group II; b-Group II vs. Group III and IV, *P < 0.01, NS- Non-Significant.

Table 4. Effect of PEPM on sperm motility, count, viability, and abnormality.

Group	Motility (%)	Count (%) ($mL \times 10^6$)	Viability (%)	Abnormality (%)
I	70.67 ± 0.8819	55.17 ± 1.515	81.67	5.80
II	20.50 ± 0.8851	15.67 ± 1.116	17.50	30.66
III	56.17 ± 2.535	50.17 ± 0.9458	54.00	11.83
IV	67.67 ± 1.476	60.83 ± 1.167	80.17	3.33

Group IV animals (400 mg/kg PEPM) showed a significant increase ($P < 0.001$) and Group III animals (200 mg/kg) exhibited a moderate increase ($P < 0.01$) in sperm content against Group II animals with testicular injury (Table 4).

The percentage of viable sperm was 81.67% in Group I rats, which was drastically reduced to 17.50% in the testicular injury groups. Moreover, 80.17% of viable sperm was found in Group IV rats treated with PEPM 400 mg/kg, indicating a significant level of restoration.

The percentages of abnormal sperm were 3.33, 11.83, and 88.17 in Group IV, III, and II, respectively. Group I animals had 5.80% of abnormal sperms (Table 4) (Figure 1).

Effects of PEPM on total protein, total cholesterol, and testosterone levels

Ethanol treated Group II male rats exhibited decreased levels of total proteins, total cholesterol, and testosterone due to induction of damage to germ cells in comparison with Group I control animals. The levels of protein ($P < 0.01$), cholesterol ($P < 0.01$), and testosterone ($P < 0.01$) were restored significantly in Group IV animals treated with PEPM 400 mg/kg. Group III animals treated with PEPM 200 mg/kg exhibited moderate restoration when compared with Group II animals (Table 5).

Effects of PEPM on testes morphology

Sections of testes from Group I control rats showed normal architecture of the testicular tubules with different types of germinal cells and lumen filled with spermatozoa, whereas the Group II animals treated with ethanol 20% v/v exhibited detachment, loss of germinal cells, and presence of vacuoles in the tubular lumen. Sections of testes from Group III animals treated with 200 mg/kg of PEPM for 60 days showed partial loss of germinal cells and decreased number of vacuoles in the tubular lumen in comparison with Group II animals. Microtome sections of the testes of Group IV animals which received 400 mg/kg of PEPM for 60 days showed recovery of germinal cell and the luminal spermatozoa. The histogram was comparable with Group I control animals (Figure 2).

Discussion and conclusion

Herbal medicine and products derived from plants are still being used in medical practice, though the mechanisms of action of many herbal drugs are unknown, and the active principles in these drugs are seldom satisfied. Qualitative phytochemical investigation on PEPM has found it to contain higher concentrations of steroids and sterols, and moderate concentrations of flavanoids, phenols, glycosides, alkaloids, proteins, terpenes, carbohydrates, and gums and mucilage. It has been reported that steroidal constituents found in the plants possess fertility potentiating properties, and they have been found to be useful in the treatment of impotence (4).

Generally elevated testosterone levels enhance sexual behaviour in humans. Moreover, drugs inducing changes in neurotransmitter levels or their action at the cellular level could also change sexual behaviour (22). In this connection it should be noted that the action of this drug as a sexual invigorator may be due to its nervine stimulating property. Increased blood circulation in the body also increases sexual stimulation. This property may be attributed to the nervous stimulating effect for increased sexual behaviour in animals. Both of the tested doses

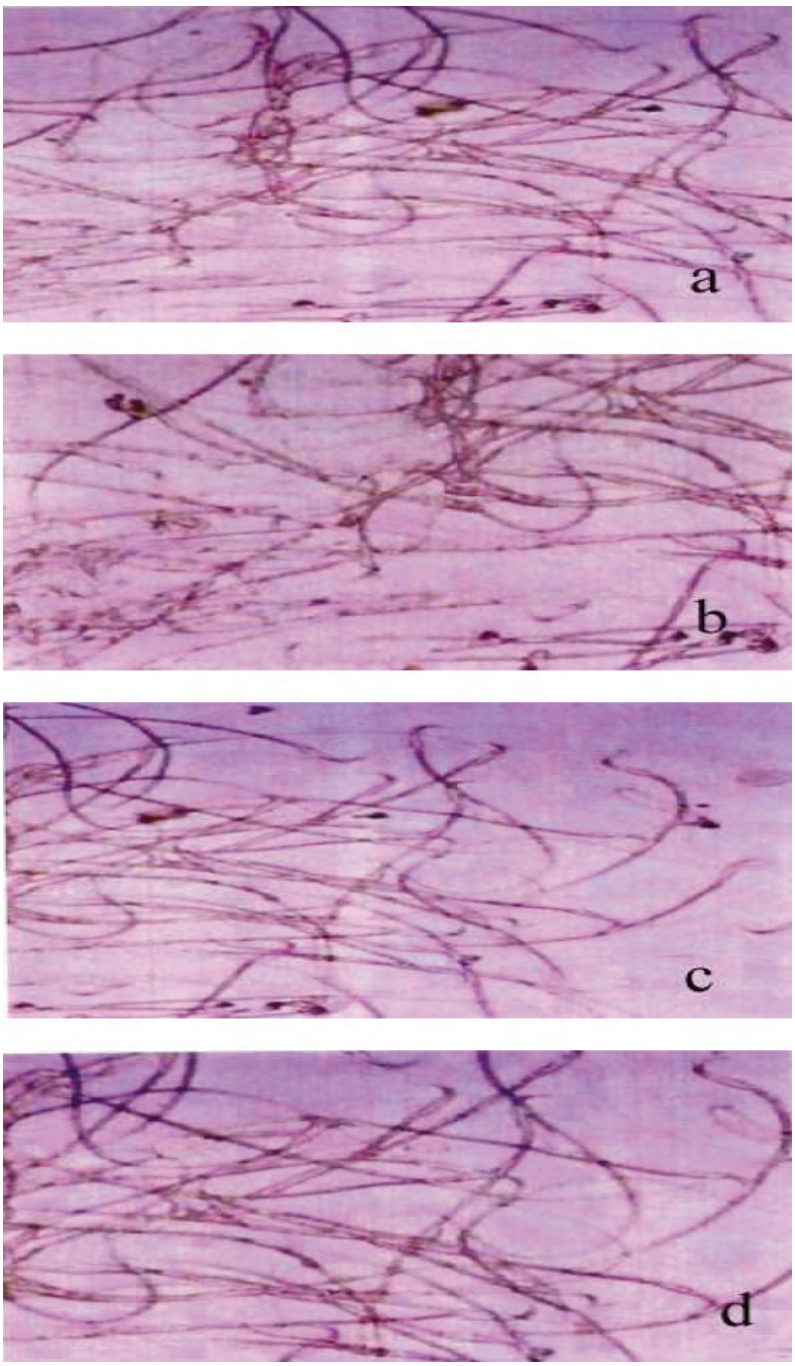

Figure 1. Spermiogram of rats (×100).
a: Control group; b: Ethanol-induced testicular injury group;
c: PEPM 200 mg/kg treated group; d: PEPM 400 mg/kg treated group.

exhibited a higher increment of mating performance. As ethanol is a known aphrodisiac, it was used for quantitative comparison and not for mechanistic purposes (24).

The decrease in body weight in ethanol treated rats is essentially due to fat mass reduction, reduced adipose tissue, and inadequate nutritional intake. Although ethanol can supply >50% of dietary energy,

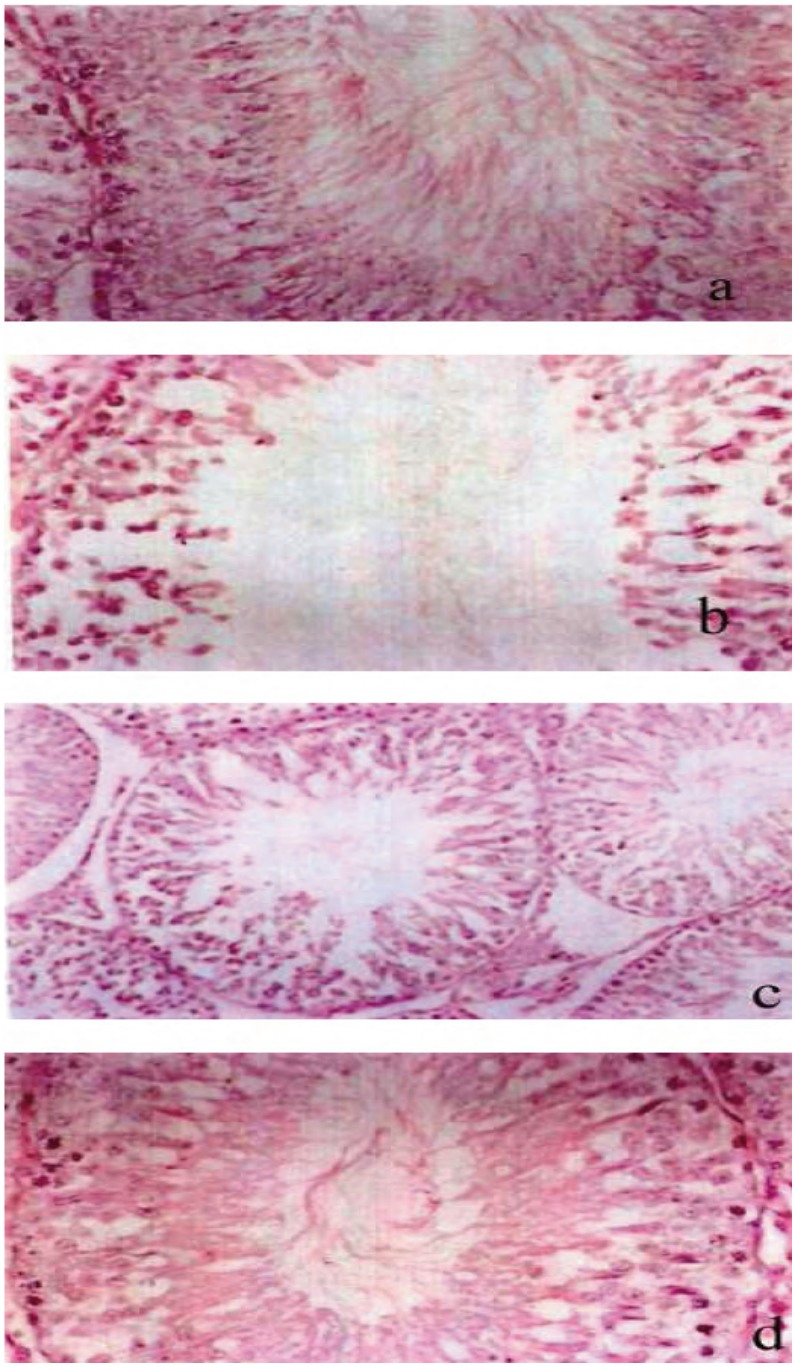

Figure 2. Histology of testes (×100).
a: Control group; b: Ethanol-induced testicular injury group;
c: PEPM 200 mg/kg treated group; d: PEPM 400 mg/kg treated group.

the calories provided by ethanol cannot be stored, and energy from ethanol may not be utilised to maintain body weight. Ethanol slows down the rate of hepatic protein metabolism and albumin synthesis. Hypoalbuminaemia, a common feature of alcohol abuse, is attributed to the nutritional status of the subject. Albumin forms adducts with acetaldehyde, which can stimulate the formation in

Table 5. Effect of PEPM on biochemical parameters.

Group and Treatment	Serum protein (mg/dL)	Serum cholesterol (mg/dL)	Serum testosterone (ng/dL)
I Normal Control	8.870 ± 0.5878	245.2 ± 0.7286	4.950 ± 0.0428
II Ethanol 20% v/v	6.757 ± 0.172 [a*]	138.7 ± 0.9667 [a*]	2.083 ± 0.0477 [a*]
III PEPM 200 mg/kg	7.720 ± 0.3894 [NS]	194.2 ± 1.114 [b*]	3.933 ± 0.0333 [b*]
IV PEPM 400 mg/kg	8.604 ± 1.5878 [b*]	197.0 ± 1.620 [b*]	4.883 ± 0.0307 [b*]

Values are mean ± S.E.M. of 6 observations, a- Group I vs. Group II; b-Group II vs. Group III and IV, *P < 0.01.

immunoglobulin. The increased protein level in the group treated with PEPM might be due to the presence of steroids and other antioxidants, which favours protein metabolism by depressing oxidative damage. The weight gain in the drug treated group is mainly due to protein mass.

The increased pregnancy rate in the drug treated group may be due to the healthy and viable sperm of the male rats. There was a lower rate of pregnancy in the female group mated by the ethanol treated group, indicating germinal cell loss, which in turn results in diminish viable sperm. All the pups fathered by the drug treated group were normal and healthier, indicating the safety of the drug and the lack of any teratogenic potential. An increased tendency of birth of male pups was observed, but this was not statistically significant.

The decrease in percentage of motility in ethanol treated animals is due to free radical injury to the spermatozoa. The chronic consumption of ethanol decreases the motility of spermatozoa.

The ethanol treated group showed abnormal sperm morphology due to the injury and destruction of germ cells. The spermatozoa head was flexed in such a way that the pointed tip of the head was facing away from the flagellum. Several spermatozoa had flagellum curved at various points resulting in a wavy appearance, and several other spermatozoa had the tail coiled. A large percentage of the spermatozoa remained agglutinated or entangled in loose epithelial cells or in a large epithelial cell mass. Another abnormality of spermatozoa was sticking or fusion of the spermatozoa at various points and over short and long distances. (32). The probable explanation for the curved and or curled nature of the flagellum may be sought in the microtubule support of the axoneme of the sperm. The microtubules are undoubtedly the established targets for ethanol actions; any disruption caused in the microtubules of the sperm axoneme would result in weakening of the axoneme and curvature/coiling of the flagellum (33).

The agglutination and attachment of sperm may be explained in the light of imminent changes in the surface protein. It is known with certainty that the spermatozoa, during their epididymal maturation, are altered with respect to the principle cells of the initial segment and caput; they secrete several proteins, some of which get translocated on the spermatozoa. It is already known that changes in the sperm surface protein and the pH of the medium can cause sperm agglutination.

The cytoplasmic droplet is a small portion of cytoplasm, which the spermatozoa carry while leaving the semniferous tubules. The droplet is shed when the spermatozoa leave the corpus epididymides; the spermatozoa in storage at the caudae are devoid of the droplet. Spermatozoa which retain extra cytoplasm inhibit motility.

Alcohol abuse is well known to impair reproductive performance in experimental animals and also in human beings. Alcoholics are found to have fertility abnormalities, with low sperm count, impaired testosterone production, and testicular atrophy (31). Ethanol significantly augments lipid peroxidation in the testis and inhibits the conversion of both dehydroepiandrosterone and androstendione to testosterone by decreasing the activities of 3b hydroxyl steroid dehydrogenase (33).

The testes produce spermatozoa and testosterone, the most important sex hormone in the male. The slightly increased levels of testosterone in Group III and Group IV when compared with Group II animals were due to the protection of germ and sertoli cells by antioxidants, which prevents the spermatozoa and maintains the quality and quantity of sperm and its functions (34).

The overall observation of the results suggests a highly significant effect in Group IV animals, which were treated with 400 mg/kg of PEPM. Histopathology of the testes of Group IV animals showed protection from testicular injury induced by ethanol when compared with other Groups II and III. The present pharmacological investigation reveals that PEPM can remarkably enhance male sexual activity in rats with testicular injury. The mechanistic approach and the effects of the drug on female sexual behaviour remain to be studied in our laboratory.

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