ORIGINAL ARTICLE

Antifertility and Antioxidant Activities of Ethanolic Extract of *Leonotis nepetifolia* in Male Albino Rats

B Kadalmani[1], R Niranjana[2], Uma A Natarajan[3], I Sarath Chandiran[4]

Abstract

Background and objective: This study based on the antifertility action of *Leonotis nepetifolia* ethanol extract (whole plant) in the animal model male Wistar Albino rats. *Leonotis nepetifolia* is reported to have antioxidant activity, antiproliferative potential, hypotensive potential, anti-inflammatory, antiplasmodial, antibacterial, antifungal, analgesic, anticancer, laxative and narcotic activities. The plant aids in the recovery of malaria, diarrhea, bronchial asthma, common cold, cough, and fever, particularly used during menstrual pain.

Materials and methods: The animals were grouped into four with five rats each. Control group (group I) received normal saline. The other three groups of rats were treated with the specified dose (group II-100 mg/kg, group III-150 mg/kg, and group III-200 mg/kg) of *Leonotis nepetifolia* ethanolic extract for a period of 55 days.

Results: A marked reduction in the weight of accessory sex organs/testis, sperm count, and sperm motility were observed in the treated groups. Serum hormonal levels and few biochemical parameters also showed variation compared to the control group. The reports revealed that the treated adult male rats had decreased the counts of female's impregnation.

Conclusion: Hence, this current research concluded that *L. nepetifolia* ethanolic extract (whole plant) repressed sperm motility, concentration, and the testosterone proving dose-dependent antifertility effect.

Keywords: Antifertility, Impregnation, *Leonotis nepetifolia*, Motility, Sperm count.

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Introduction

India being a developing country is still not able to cope up with the demerits of having high population. There are a large number of devices, mechanisms, and techniques adopted for antifertility treatment.[1] Moreover, increasing number of births has got a deleterious effect on social and economic progress.[2] And on the other hand growth and control of population is an important issue as it is directly proportional to country's life-supporting systems.[3] The World Health Organization recommended traditional medicine for the antifertility effect as an alternate to synthetic drugs due to its cost-effectiveness in the control of birth.[4] *Leonotis nepetifolia* is a short-lived perennial plant belonging to Lamiaceae family found all over the world, especially tropical Africa and southern India. The whole plant is used for the menstrual pain and unspecified female complaints. It is reported to have antioxidant, antiproliferative potential, hypotensive potential, anti-inflammatory, antiplasmodial, antibacterial, antifungal, analgesic, anti-cancer, hypotensive, laxative, and narcotic activities. The plant is also used in traditional medicine for bronchial asthma, diarrhea, fever, malaria, and as an analgesic agent in menstrual pain. It also used to treat common cold and alleviate cough.[5] The aim of this current research work was to determine the antifertility effect of *L. nepetifolia* ethanolic extract in Wistar albino rats.

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Conflict of interest: None

Materials and Methods

Collection/Identification of Plant

The well-grown whole plant of *L. nepetifolia* was collected from Kalakadu, Tirunelveli District, Tamil Nadu. The collected plants were identified using Botanical Survey of India, Coimbatore. The whole plant of *L. nepetifolia* was shade dried and powdered using grinder. The fresh finely ground powder was subjected to extraction in a Soxhlet apparatus using ethanol. The quantitative analytical tests were done on recently prepared extract to identify the phytochemical constituents using standard procedures[6] and then concentrated using rotatory evaporator for further studies.

Animal Studies

Male Wistar Albino rats weighing 160–250 g were maintained under controlled conditions of temperature, humidity, and 12-hour light–dark cycles. All the animals were acclimatized for 7 days before the study. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum.

The initial body weight of the animals was recorded. The acute toxicity test was carried out per OECD guidelines 423. The animals were observed for gross behavior changes as well as for motility for 14 days.

Experimental Design

The animals were equally divided into four groups (5/group). Group I animals were given normal saline for 55 days and served as control group. Groups II, III, and IV animals received ethanolic extract at oral dose of 100, 150, and 200 mg/kg body weight, respectively, for 55 days. Final weight of the animals was measured/recorded, and blood samples were also collected after 24 hours of last treatment. Then, the animals were sacrificed. The serum were collected separately from blood by centrifugation (3,000*g*/10 minutes) and stored at −20°C. Then, the ventral prostrate, testes, vas deferens, epididymis, and seminal vesicle were dissected, trimmed, weighed, and fixed in 10% formalin for further analysis.

Determination of Sperm Count

Sperm count was carried out by using Neubauer's hemocytometer after collecting the epididymal fluid. The procedure was carried out per Lawerence et al. The sperms' viability was determined by AO/EB staining method.[7,8]

Determination of Hematological Parameters

The blood sample collected was tested for red blood cell count (RBC), white blood cell count (WBC), platelets, and hemoglobin using Neubauer counting chamber.

Assay Procedure for Hormone Detection

The stored serum was used to measure the level of follicle-stimulating hormone (FSH), testosterone, and luteinizing hormone (LH) by enzyme immunoassay method.

Estimation of Biochemical Profile

The biochemical assays of alanine aminotransferase (ALT/GPT), alkaline phosphatase (ALP), and aspartate aminotransferase(AST/GOT) were analyzed.[9–11]

Estimation of Oxidative Parameters

The oxidative parameters such as catalases (CAT), superoxide dismutase (SOD), glutathione peroxidase, and reduced glutathione were analyzed in homogenates of liver, kidney, and testis.[12–15] Cholesterol and triglycerides levels of the samples were measured using standard procedures.[16]

Histopathology of Testis

The testis fixed in 10% formalin were entrenched in paraffin, sectioned using microtome, and subjected to hematoxylin and eosin staining per procedure of Kadalmani et al.[17]

Fertility Test

Male rat and virgin untreated females (same strain) were positioned in a separate cage in the ratio of 1:2 (male:female) and kept together for 16 days. At the end of 16 days, the male rats were detached and the pregnant female rats were sacrificed. The parameters, namely, number of pregnant rats, implantation sites, and fetus numbers, were recorded.[18]

Statistical Analysis

All data for control and experimental groups were subjected to statistical evaluation using analysis of variance (ANOVA) for significant differences between the controls and experimental groups at p value < 0.05. All data were recorded systematically in preformed data collection sheet. Statistical analysis was performed by using SPSS for windows version 16.0, and 95% confidence limit was obtained.

Table 1: Preliminary phytochemical screening

S. no	*Secondary metabolites*	*Result*
1	Steroids	Present
2	Triterpenoids	Absent
3	Alkaloids	Present
4	Phenolic compounds	Absent
5	Flavonoids	Present
6	Reducing sugars	Present
7	Sugars	Absent
8	Catechins	Absent
9	Saponins	Absent
10	Tannins	Present
11	Anthroquinone	Absent
12	Amino acids	Absent

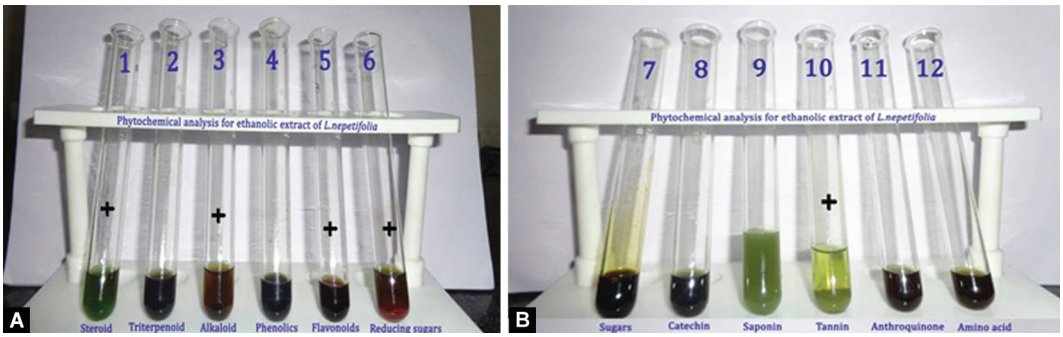
Results

The phytochemical screening of ethanolic extract of *L. nepetifolia* whole plant revealed the presence of steroids, alkaloids, flavonoids, reducing sugars, and tannins as shown in Table 1 and Figure 1. The nontoxic nature of the *L. nepetifolia* ethanol extract was proved by the acute toxicity study. The reduction in the final body weight and sex organs, namely, seminal vesicle, vas deferens testis, prostate, and epididymis, were observed in all three treatment groups when compared to the control group (Table 2).

A substantial decrease in the sperm count and presence of abnormal sperm in caudal and caput was noticed in all three treated groups when compared to control group as tabulated in Table 3. Nearly more than half the total percentage of the sperm exhibited abnormal morphologies of different kinds, which encompassed DNA-damaged sperm, broken head, coiled tail area, etc. along with reduction in epididymal sperm concentration and motility (Figs 2 and 3).

The hematological parameters such as platelets, RBC, PCV, and hemoglobin of *L. nepetifolia*–extract treated rats showed significant changes when compared to control and are presented in Table 4. Reduction in serum testosterone, LH, and FSH levels were witnessed in all the three treated groups when compared to group I. The respective levels in groups II, III, and IV were reduced when compared to the group I. Most significance reduction of serum testosterone, LH, and FSH levels were observed at the dose of 200 mg/kg/bw (Table 5). The serum biochemical parameters such as AST, ALT, and ALP of *L. nepetifolia* extract treated rats was significantly decreased when compared to control as shown in Figures 4 to 6, respectively.

The activities of antioxidants CAT, SOD, GPx, and GSH in the tissue homogenates of liver, kidney, and testis of group I and all other treated groups are shown in Figures 7 to 10. In this current research work, ethanolic extract–treated rat indicated suppressed effects of all the antioxidants when compared to group I. Administration of *L. nepetifolia* extract at the dose of 100, 150, and 200 mg/kg showed significant increase in triglyceride



Figs 1A and B: Preliminary phytochemical screening

Table 2: Effect of ethanolic extract of *Leonotis nepetifolia* on body weight and reproductive organ weight

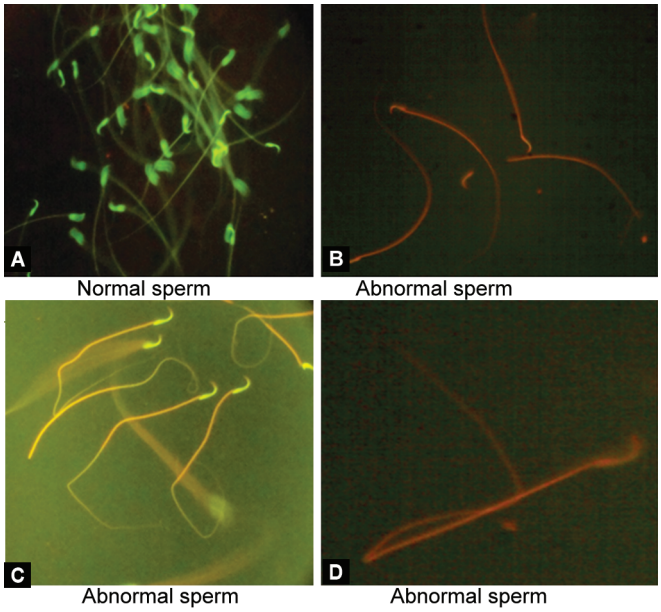
		Body weights (g)		*Reproductive organ weights (g)*			
Groups	*Treatments*	*Initial*	*Final*	*Testis*	*Epididymis*	*Seminal vesicles*	*Prostate gland*
Group I	Saline	200 ± 15.81	192 ± 8.36	2.2 ± 0.03	0.67 ± 0.02	0.45 ± 0.02	0.27 ± 0.01
Group II	*L. nepetifolia* (100 mg/kg)	184 ± 8.94	160 ± 15.8	1.45 ± 0.02	0.45 ± 0.01	0.41 ± 0.01	0.27 ± 0.01
Group III	*L. nepetifolia* (150 mg/kg)	206 ± 8.94	184 ± 20.7	1.37 ± 0.04	0.33 ± 0.02	0.37 ± 0.01	0.23 ± 0.01
Group IV	*L. nepetifolia* (200 mg/kg)	250 ± 10.0	234 ± 27.0	1.15 ± 0.02	0.24 ± 0.03	0.32 ± 0.02	0.22 ± 0.02

Values represent the mean ± SD of the observation made on five rats in each group. Statistical analysis one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

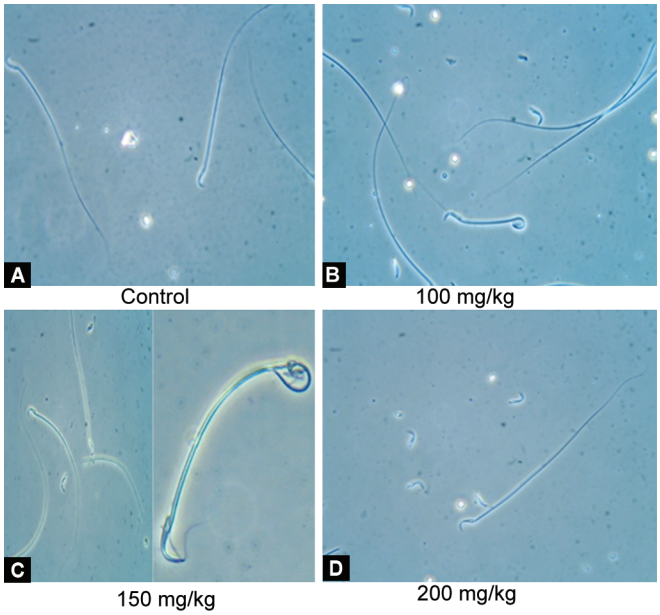
Table 3: Effect of ethanolic extract of *Leonotis nepetifolia* on sperm concentration, motility and abnormality

	Total sperm count			
Treatment	*Head*	*Tail*	*Sperm motility*	*Abnormal sperm morphology*
Control	97.34 ± 3.78	93.24 ± 3.09	98.76 ± 6.32	3.98 ± 0.23
100 mg/kg	74.61 ± 10.31	71.57 ± 9.76	74.43 ± 9.78	32.65 ± 7.65
150 mg/kg	51.23 ± 12.79	55.12 ± 11.47	56.51 ± 14.78	39.07 ± 6.54
200 mg/kg	35.17 ± 7.15	33.78 ± 7.09	34.23 ± 7.64	55.34 ± 7.02

Values represent the mean ± SD of the observation made on five rats in each group. Statistical analysis one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference



Figs 2A to D: Effect of ethanolic extract of *Leonotis nepetifolia* on AO/EtBr staining of sperm morphology



Figs 3A to D: Effect of ethanolic extract of *Leonotis nepetifolia* on light microscopic observation of sperm morphology

Table 4: Effect of ethanolic extract of *Leonotis nepetifolia* on hematological parameters

S. no.	Groups	Differential count		Platelet (lakhs/ cu.mm)	RBC (million/ cu.mm)	PCV (%)	Hemoglobin
		L (%)	E (%)				
1	Group I	57.06 ± 0.07	3.12 ± 0.07	4.03 ± 0.04	1.50 ± 0.34	61.34 ± 0.21	20.37 ± 0.19
2	Group II	46.04 ± 0.06	3.06 ± 0.02	3.04 ± 0.04	6.46 ± 0.34	59.32 ± 0.19	19.56 ± 0.20
3	Group III	43.16 ± 0.10	3.024 0.03	2.4 ± 0.22	6.28 ± 0.24	56.21 ± 0.11	18.42 ± 0.28
4	Group IV	37.08 ± 0.06	2.038 0.04	1.58 ± 0.24	5.48 ± 0.36	53.30 ± 0.25	17.48 ± 0.35

Values represent the mean ± SD of the observation made on five rats in each group. Statistical analysis one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

Table 5: Effect of ethanolic extract of *Leonotis nepetifolia* on LH, FSH and testosterone

Groups	Treatments	FSH	LH	Testosterone
Group I	Saline	8.16 ± 0.27	1.69 ± 0.15	4.52 ± 0.17
Group II	*L. nepetifolia* 100 mg/kg	8.13 ± 0.43	1.29 ± 0.13	4.02 ± 0.19
Group III	*L. nepetifolia* 150 mg/kg	8.10 ± 0.39	1.09 ± 0.14	3.36 ± 0.20
Group IV	*L. nepetifolia* 200 mg/kg	7.95 ± 0.35	0.97 ± 0.09	1.84 ± 0.24

Values represent the mean ± SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

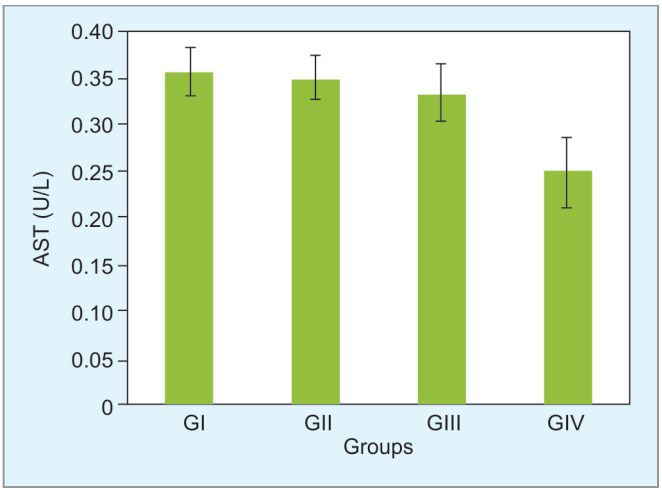


Fig. 4: Effect of ethanolic extract of *Leonotis nepetifolia* on AST values represents the mean ± SD of the observation made on five rats in each group. Statistical analysis by one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

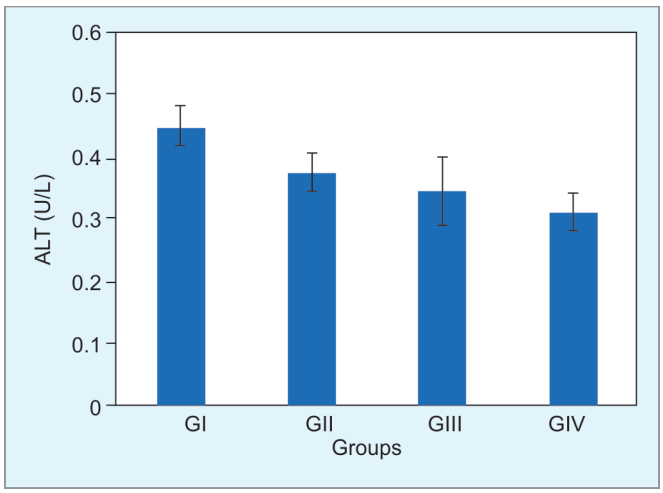


Fig. 5: Effect of ethanolic extract of *Leonotis nepetifolia* on ALT values represents the mean ± SD of the observation made on five rats in each group. Statistical analysis by one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

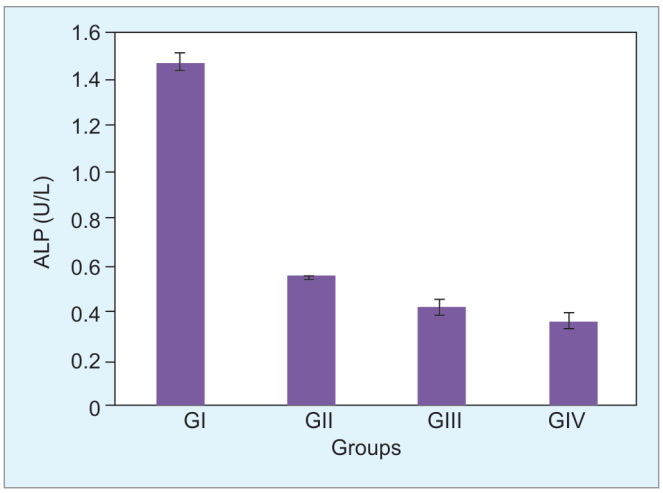


Fig. 6: Effect of ethanolic extract of *Leonotis nepetifolia* on ALP values represents the mean ± SD of the observation made on five rats in each group. Statistical analysis by one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

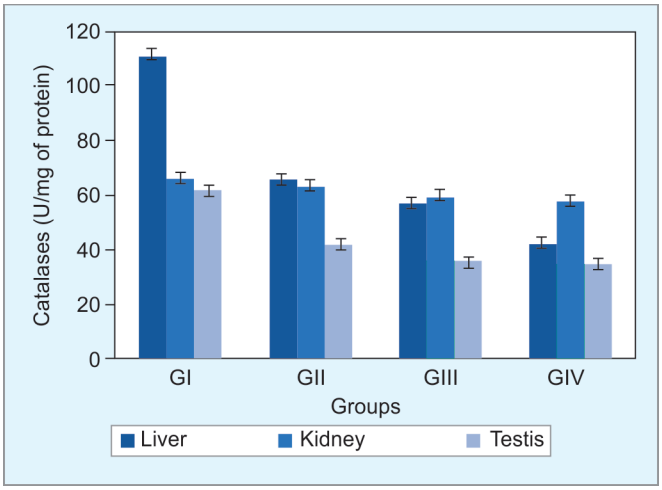


Fig. 7: Effect of ethanolic extract of *Leonotis nepetifolia* on catalase values represents the mean ± SD of the observation made on five rats in each group. Statistical analysis by one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

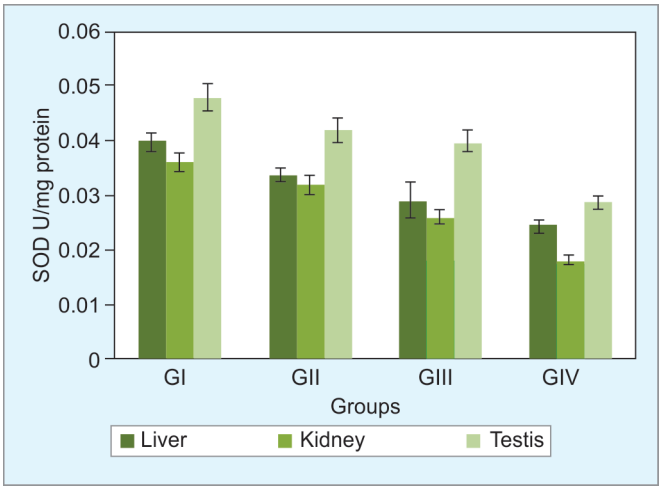


Fig. 8: Effect of ethanolic extract of *Leonotis nepetifolia* on SOD values represents the mean ± SD of the observation made on five rats in each group. Statistical analysis by one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

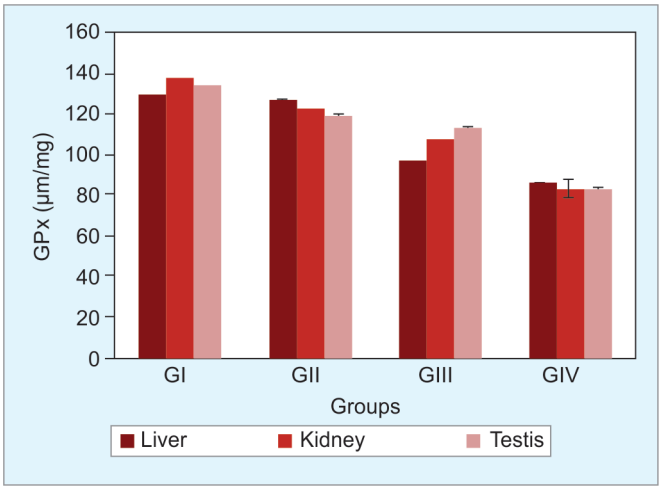


Fig. 9: Effect of ethanolic extract of *Leonotis nepetifolia* on GPx values represents the mean ± SD of the observation made on five rats in each group. Statistical analysis by one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

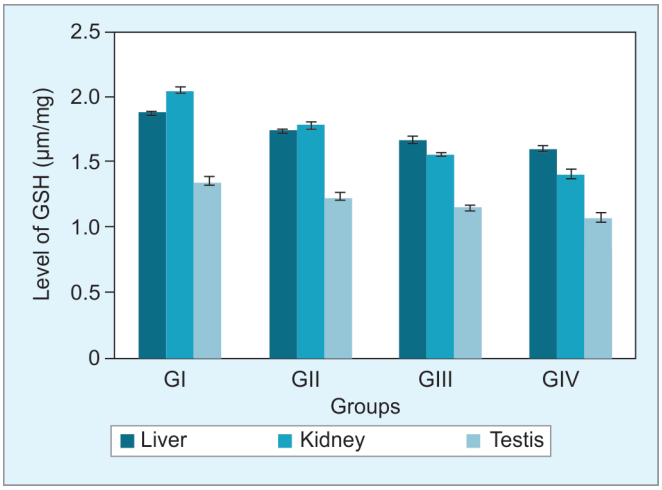


Fig. 10: Effect of ethanolic extract of *Leonotis nepetifolia* on GSH values represents the Mean ± SD of the observation made on five rats in each group. Statistical analysis by one-way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

Table 6: Effect of ethanolic extract of *Leonotis nepetifolia* on cholesterol and triglycerides

Groups	*Treatments*	*Cholesterol (mg/dL)*	*Triglycerides (mg/dL)*
Group I	Saline	147.34 ± 0.02	0.24 ± 0.10
Group II	*L. nepetifolia* 100 mg/kg	166.05 ± 0.03	0.67 ± 0.09
Group III	*L. nepetifolia* 150 mg/kg	171.95 ± 0.02	0.87 ± 0.78
Group IV	*L. nepetifolia* 200 mg/kg	209.34 ± 0.02	1.23 ± 0.06

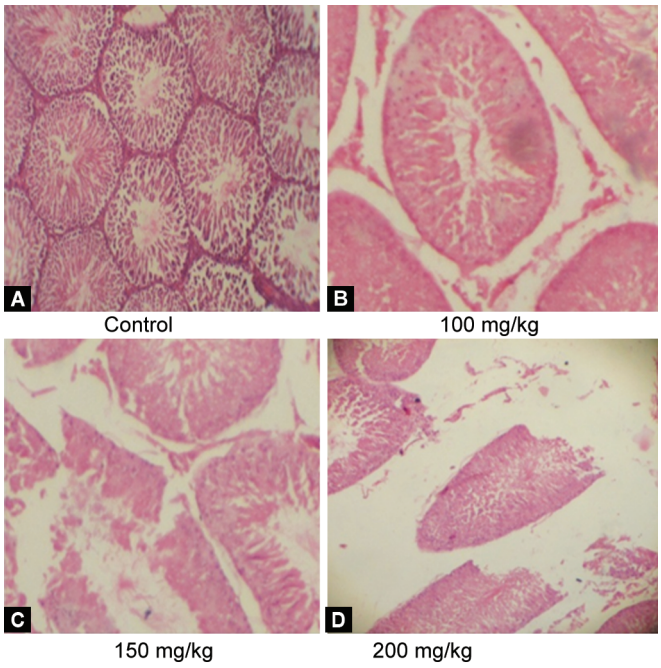
Values represents the mean ± SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

and total cholesterol of all groups, compared to the control in a dose-dependent manner. Total cholesterol and triglyceride levels were significantly higher in group treated with 200 mg/kg/bw of *L. nepetifolia*, whereas the total cholesterol ratio was significantly decreased compared to group I (Table 6).

Leonotis nepetifolia ethanolic extract at 100 mg/kg presented minor changes such as degeneration of seminiferous tubules along with interstitial edema. On the other hand, group I (control) animal's testicular section represented undamaged Sertoli cells/basement membrane, complete lumen of seminiferous tubule, and unharmed leydig, interstitial tissue, and peritubular venules/capillaries. Necrosis of the Sertoli cell was also well observed. Moderate to severe degeneration of the seminiferous tubules and shrinkage for rats treated with 150 and 200 mg/kg/bw *L. nepetifolia* ethanolic extract are also indicated in Figure 11. The results shown in Table 7 revealed a dose-dependent reduction in the number of pregnant females and number of implantation.

Discussion

Based on the extensive use of herbal medicines, many research works have been assumed to determine the welfare and efficiency of traditional therapies. The current study was undertaken to



Figs 11A to D: Effect of ethanolic extract of *Leonotis nepetifolia* extract on testis

Table 7: Effect of ethanolic extract of *Leonotis nepetifolia* on fertility of male albino rats

Groups	*No. of male*	*No. of females*	*No. of pregnant females*	*No. of implantation*
Group I	5	10	9/10	9.350 ± 0.231
Group II	5	10	7/10	6.864 ± 0.576
Group III	5	10	4/10	4.548 ± 1.623
Group IV	5	10	2/10	3.000 ± 0.685

Values represent the mean ± SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with *ad hoc* testing least significant difference

evaluate the male reproductive toxicity of the 50% ethanolic extract of *L. nepetifolia,* which is an herbal medicine. In the male reproductive system, weight loss of the gonads, seminal vesicles, prostate gland, vas deferens as well as histology of gonads, biochemistry of testis, and epididymis are considered as the characterization of toxic agents that may cause fertility problems in the treated animals.[19]

The treatment of *L. nepetifolia* extracts (100, 150, 200 mg/kg/bw for 55 days) in rats suppresses the testicular and epididymal sperm counts and causes lesions in the seminiferous tubules. There is no effect on body weight and on general behavior of extract-treated rats. The reproductive organ weight decreased in all the treated groups when compared to the control groups. The treatment showed significant reduction in the serum concentration of LH, FSH, and testosterone level after the 55 days of treatment when compared to the control group. The primary step in the mechanism of the effects on testis induced by the *L. nepetifolia* extract was the suppression of LH. Absence of stimulation by LH causes Leydig cell dysfunction which results in the decline of testosterone secretion that is most responsible for the diminished spermatogenesis, and finally the total sperm count was reduced. The reduction in sperm motility in cauda epididymides is observed all treated animals when compared to the control group. Sperm morphology and viability are also decreased.[20] The male antifertility activity of *L. nepetifolia* ethanolic extract at the dose level 100, 150, and 200 mg/kg/bw has been proved by all said parameters.

The rise in cholesterol concentration of testis in ethanolic extract–treated male animals may represent the reduced conversion of cholesterol to testosterone. Glucose is the major substrate for metabolism in spermatids and spermatocyte in that testes. Glycogen levels were increased suggesting an inhibitory action of glycolysis in testis and epididymides. Cholesterol is the precursor for testosterone biosynthesis. Accumulation of cholesterol in the testis is the main evidence of antiandrogenic activity. Triglyceride is said to be energy source for spermatozoa, and its increase or decrease is suggestive of imbalanced synthesis. This will increase all treated groups when compared to the control group.[21] A possible explanation for these observations could be attributed to the flavonoids contained in the plant extract. Flavonoids containing *L. nepetifolia* has been documented to possess antifertility and cytotoxic activity.[22] Reactive oxygen species also involved DNA damage leading to mutations. Some antioxidant defenses are present in plants and their byproducts, mainly edible vegetables and spices, play a key role in human diet. The serum levels of ALT, AST, and ALP suggest that the treatment with the plant extract has decreased toxic effects when compared to the control rats. The antioxidant enzymes of catalases, SOD, GSH, glutathione peroxidase, and lipid peroxidation are also decreased in all treated groups when compared to the control groups.[23] The result shows that the all doses (100, 150, 200 mg/kg/bw) of *L. nepetifolia* ethanolic extract decreased the antioxidant activity. The treatment of crude extract of *L. nepetifolia* causes the alterations in male reproductive organ and suppression of fertility in males.

Conclusion

The present study concludes that the ethanolic extract of *L. nepetifolia* plant inhibited the fertility potential of male rats which is mediated by altered oxidative stress and decreased testosterone concentration. The daily administration of oral dose of *L. nepetifolia* ethanolic extract of 100, 150, and 200 mg/kg for a period of 55 days of in male animal (prior to contraceptive effects) did not elicit reproduction. The results achieved so far revealed that *L. nepetifolia* plant is orally effective safe and reversible, and thus meets out all the essential criteria of an ideal male contraceptive. The *L. nepetifolia* contain several efficacious subcomponents, and more studies have to emerge to explore the antifertility activity of other traditional herbs for the product management and mass application.

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