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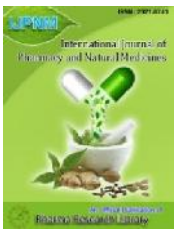

Research Article **Open Access**

Investigation of *Leonotis Nepetifolia* for its Antioxidant and Anti Steroidogenic Properties in Male Rats

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A B S T R A C T

The objective of the present study is to explore anti-steroidogenic and antioxidant properties of ethanolic extract of whole plant of *Leonotis nepetifolia* (EELN). The different dose of ethanolic extract were given by gavage to rats in the In Vivo test at a dose of 100, 200, 300mg/kg of body weight to rats in group II, II & III respectively, along with control dose. At the end of study various parameter such as lipid profile and anti-oxidant level in the testicular tissue were analyzed. Finding of this study explored a significantly increase ($p<0.05$) the total cholesterol and triglycerides levels, LDL and VLDL cholesterol in EELN treated rats when compared to control. But HDL cholesterol and anti-oxidant levels were significantly decreased in EELN treated rats in dose dependent manner and also we observed the role of *Leonotis nepetifolia* on antioxidant defense mechanism in rat testis. The present study, concluded that ethanolic extract of *Leonotis nepetifolia* capable to suppress the fertility in male rats without altering general metabolism.

Keywords: Anti steroidogenic; antioxidant; *Leonotis nepetifolia*

A R T I C L E I N F O

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1. Introduction

The rapid increase of population has got an adverse effect on the international economy and as the increase is only limited to the developing countries, the problem becomes an acute on the fruits of improvement in the different sectors, which are being eroded by the growing population. Moreover, increasing number of births has got a deleterious effect on the health of mother and child and hinders social and economic progress. The regulation of human fertility has global consequences in terms of resources depletion, population and poverty [1]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources [2].

Male pills containing a combination of testosterone, estrogens and progesterone have also been tried to prevent spermatogenesis. Biomedical research supported by WHO has investigated the potential of developing new male contraceptive methods that would function at any step in male reproductive process, from sperm production in the testes through to sperm-egg interactions in female genital tract [3].

Leonotis nepetifolia is an erect, branched herb belongs to family lamiaceae. It is commonly referred to as Lion's ear and Christmas candlestick [4]. *Leonotis nepetifolia* is found in the all over the world especially tropical Africa and southern India. The whole plant is used for the menstrual pain and unspecified female complaints. The plant is being used by the local peoples and tribal of Maharashtra as ethno medicine on various ailments. The infusion of leaves is traditionally being used to cure the stomach pain of the children and also to cure cough and cold by tribals of Melghat (MS) India [5]. The earlier phytochemical studies carried out have reported the Presence of Labdanic acid, Laballenic acid nepetaefolinol and leonotinin and a coumarin, characterized as 4, 6, 7-trimethoxy-5-methyl chromen-2-one [5].

2. Materials and Methods

Plant material

The whole plant of *Leonotis nepetifolia* was collected from Kalakadu, Thirunelveli district. Plant material was dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction in a Soxhlet apparatus using ethanol. 50g of powered plant was extracted with 500mL of ethanol at 60^0C.Then extract was poured in petri plates and allowed to air dried [6].

Animals

Adult male Wistar rats were used for the study. Animals were housed under 12 h light/12 h dark cycle with controlled conditions and were fed by standard food and allowed water *ad libitum.* Food and water are given by animals are noted. Body weights of animals were also recorded on day 0 of the experiments and at end of the experiments.

Design of the experiment

Experimental animals

Male Wister albino rats were divided into 4 groups and their body weight ranging from 160g to 250g. Each groups containing 5 animals. Three groups are considered as the Treatment groups and one group is considered as the control group. Animals were administrated orally to the rat for 55 days by Plant extract of *L. nepetifolia* using oral gavage tube. The animals are divided into 4 groups based on their body weight and given by different doses, Group 1- Control, Group 2 - 100mg/kg (b/w), Group 3 - 150mg/kg (b/w), Group 4 - 200mg/kg (b/w). At the end of the treatment, animals were sacrificed by cervical dislocation and testis were collected and stored at -20°C for lipid profile and antioxidant estimation.

Determination of lipid profiles

Estimation of Cholesterol

Principle

The cholesterol esters are hydrolysed by enzyme cholesterol esterase to give free cholesterol and fatty acid molecules. This free cholesterol gets oxidized in presence of cholesterol oxidase to liberate cholesterol 4 en -3 one and H_2O_2. Liberated H_2O_2 by this reaction combines with phenol and 4-amino antipyrine in presence of peroxidase to from red colored quinonimine complex, the intensity of which is measured at 505nm. (490-530nm).It is directly proportional to cholesterol concentration present in sample.

$$\text{Cholesterol} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesterol 4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4 aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinonimine} + 4H_2O$$

Procedure

All the reagents were brought to the room temperature (37^0C). The Assay was performed with the following working assay table using un-hemolysed serum.

Table 1

Addition sequence	Procedure for 1mL		
Enzyme Reagents	1mL	1mL	1mL
Standard	-	10µL	-
Sample	-	-	10µL

The Sample and reagent mixture were mixed well and incubated for 5 minutes at 37^0C, measured the absorbance of standard and the test sample against the reagent blank at 480nm (490nm) [7].

Estimation of Triglycerides by Enzymatic Method [8]

Principle

Triglyceride in the samples originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry.

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty acid.}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol3-phosphate} + \text{ADP}$$

$$\text{Glycerol} \xrightarrow{\text{Glycerol 3- phosphate oxidase}} O_2\text{Dihydroxyacetonephosphate} + \text{3-phosphate} + H_2O_2$$

$$H_2O_2 + \text{Amino antipyrine} + \text{ADPS} \xrightarrow{\text{Peroxidase}} \text{Red quinonimine dye} + H_2O$$

The intensity of purple colored complex formed during the reaction is directly proportional to the triglyceride concentration in the sample and is measured at 546nm.

Reagents:

Enzyme chromogen, Buffer, Standard (Triglyceride 200mg/dl)

Procedure:

Table 2

Addition sequence	Blank (ml)	Standard (ml)	Test (ml)
Enzyme chromogen	1	1	1
Cholesterol standard	-	10 µl	-
Serum sample	-	-	10 µl

The Reaction mixture was incubated for 5 minutes at 37^0C and mixed well. The color formed was read at 546 nm (520-570nm). The final color is stable for 30 minutes.

Calculation:

HDL Cholesterol in mg % =

$$\frac{\text{Absorbance of HDL Test (TH)} \times 100 \times 1.1}{\text{Absorbance of standard}}$$

From the above estimated values of TC and HDL, the values of LDL and VLDL can be obtained using the following arithmetic calculations.

LDL Cholesterol in mg %

= Concentration of Triglycerides/5

VLDL Cholesterol in mg % =

Concentration of Total cholesterol – Concentration of HDL cholesterol + Concentration of LDL cholesterol

Determination of Anti-oxidant enzymes in Testicular tissues

The testis was removed, cleared of excess fat and minced with anatomical scissors. The testicular tissue homogenate (10% w/v) was prepared in 0.1 M phosphate buffer (pH 7.4), centrifuged for 15 minutes at 500 x *g*. The supernatant obtained thereafter was used for various biochemical assays.

Determination of catalase

The catalase activity was assayed by the method of Sinha (1972) [9]. In brief, the incubation mixture contained in a final volume of 2.0 ml. 0.1 ml of diluted homogenate, 1.5 ml of phosphate buffer and 0.4 ml of distilled water to which 0.5 ml of H_2O_2 solution was added to initiate the reaction, while the H_2O_2 solution was left out in control tubes. After incubating for 1 minute at 37ºC, the reaction was stopped by addition by 2 ml of potassium dichromate acetic acid reagent. The samples were kept in boiling water bath for 10 minutes, finally cooled and the absorbance measured at 570 nm against control.

$$\text{Activity} = \frac{\Delta \text{ OD x Std conc. (µ mol)}}{\text{Enzyme (ml) x Std. OD x Protein (mg/ml)}}$$

Unit = µ moles/min/mg/protein.

Determination of Superoxide Dismutase (SOD)

SOD activity was determined according to the method of Marklund and Marklund (1974)[10]. In this test, the degree of inhibition of pyrogallol autoxidation by supernatant of the lenticular homogenate was measured. The change in absorbance was read at 470 nm against blank every minute for 3 min on a spectrophotometer. The enzyme activity was expressed as units per milligram protein.

Determination of Glutathione Peroxidase (Gpx)

The activity of Gpx was determined essentially as described by Rotruck et al. 1973[11]. The principle of this method is that the rate of glutathione oxidation by H_20, as catalyzed by the Gpx present in the supernatant, is determined; the color that develops is read against a reagent blank at 412 nm on a spectrophotometer. In the test, the enzyme activity was expressed as units per milligram protein (one unit was the amount of enzyme that converted 1 µmol of reduced glutathione to the oxidized form of glutathione in the presence of H_20_2/min)

Determination of Reduced Glutathione

The GSH content was estimated by the method of Moron et al. 1979[12]. Each lens was homogenized in 1 ml of 0.1 M phosphate buffer and was centrifuged at 5,000 rpm for 15 min at 4°C. To the supernatant of the lenticular homogenate, 0.5 ml of 10% trichloroacetic acid was added and recentrifuged. The protein-free supernatant thus obtained was reacted with 4 ml of 0.3 M Na_2HPO (pH 8.0) and 0.5 ml of 0.04% (w/v) 5, 5dithiobis-2-nitrobenzoicacid. The intensity of the resulting yellow color was read spectrophotometrically at 412 nm. A parallel standard was also maintained. The results were expressed in micromoles per gram of tissue.

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 $p<0.05$. All data were recorded systematically in preformed data collection sheet. Statistical analysis was performed by using SPSS for windows version 16.0. 95% confidence limit was taken.

3. Results and Discussion

Effect of EELF on lipid profile

Exposure to *Leonotis nepetifolia* resulted in an increase of absolute and relative Lipid profile compared to the control in a dose-dependent manner. Total Cholesterol, VLDL,

LDL cholesterol, and triglyceride levels were significantly higher in EELF 200mg group. Whereas the total: HDL cholesterol ratio was significantly decreased in EELF 150 & 200mg treated groups (Table 1). The concentration of lipid profile on 100 and 150mg EELF treated groups is less compared to 200mg treated group.

Effect of EELF on antioxidant enzyme in testicular tissues

Catalase

The mean activity of catalase in testicular tissues of ethanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of catalase was noted in group IV ($p<0.05$) than in group I control rats (Table.2).

Superoxide Dismutase (SOD)

The mean activity of SOD in testicular tissues of ethanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of SOD was noted in group IV ($p<0.05$) than in group I control rats (Table.2).

Glutathione reductase (GSH)

The mean activity of GSH in testicular tissues of ethanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of GSH was noted in group IV ($p<0.05$) than in group I control rats (Table.2).

Glutathione peroxidase (GP_X)

The mean activity of GP_X in testicular tissues of ethanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of GP_X was noted in group IV ($p<0.05$) than in group I control rats (Table.2).

Discussion

Evaluation of herbs for anti-fertility effects has been in progress worldwide for several decades to identify effective and safe substances for control of population explosion. The anti-fertility effect of ethanolic extract of *Leonotis nepetifolia* was confirmed by following measures. Cholesterol is involved in steroidogenesis in testes. It is the most important precursor in synthesis of steroid hormones and its level is related to fertility of individuals [13]. Increased level of cholesterol may be due to decreased androgen production, which results in accumulation of cholesterol in testes and impaired spermatogenesis [14].

For example, *Pleurotus florida*, possessed significant antioxidant enzymes activity [15]. *Indigofera tinctoria* had strong antioxidant effect [16] and *Coriandrum sativum* increased the antioxidant enzyme activity [17]. *Leonotis nepetifolia* is widely used for various ailments. But the available literature on *Leonotis nepetifolia* does not reveal the effect of its antioxidant enzyme activities in testis. Hence, we studied the role of *Leonotis nepetifolia* on lipid peroxidation and antioxidant defense mechanism in rat testis.

Reactive Oxygen Species (ROS) such as superoxide anions (O^{2-}) hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and nitric oxide (NO) are directly or indirectly involved in DNA damage leading to mutations. Some antioxidant defenses are present in the plants and their byproducts mainly edible vegetables and spices, have a key role in chemo preventers in human diet.

Table 3: Effect of EELF on total cholesterol and triglycerides

Group	Treatments	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group 1	Saline	147.34±0.02	0.24±0.10
Group 2	EELF-100mg/kg	166.05±0.03	0.67±0.09
Group 3	EELF - 150mg/kg	171.95±0.02	0.87±0.78
Group 4	EELF - 200mg/kg	209.34±0.02	1.23±0.06

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

Table 2: Effect of EELF on antioxidant enzymes in testicular tissues

Group	Treatment	SOD U/mg of protein	Catalase U/mg of protein	Gpx U/mg of protein	GSH U/mg of protein
Group 1	Saline	0.04± 0.013	61.51± 0.04	134.78± 0.18	1.35± 0.02
Group 2	EELF 100mg /kg	0.04± 0.008	41.75± 0.02	120.35± 0.26	1.23± 0.01
Group 3	EELF 150mg /kg	0.03± 0.006	35.94± 0.03	113.47± 0.26	1.15± 0.02
Group 4	EELF 200mg /kg	0.02± 0.007	34.68±0. 03	83.52±0. 04	1.08±0.0 4

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

4. Conclusion

In conclusion, from the overall results, it could be inferred that whole plant of *Leonotis nepetifolia* showed potent male antifertility effect. Further long term studies are in progress for the evaluation of complete and reversible fertility with this extract and also other effects of this important plant.

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