



Antifertility Effect of Ethanol Extracts of *Feronia elephantum* Correa Leaf and Bark on Male Albino Rats

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ABSTRACT

The present study was carried out to evaluate the effective concentration of ethanol extract of *Feronia elephantum* leaf and bark on male reproductive system of albino rats. The study was divided into four groups of five animals each. The first group (I) received distilled water for a period of 14 days, served as control. The groups II, III and IV of animals were administered the ethanol leaf extract daily at 400mg/kg body weight, bark extract daily at 400 mg/kg body weight and 1:1 ratio of leaf (200mg/kg b.wt) and bark (200mg/kg b.wt) extract of *Feronia elephantum* respectively for a period of 14 days. Significant decreases in the weight of testis ($p < 0.05$), epididymis ($p < 0.01$) and seminal vesicle ($p < 0.05$) were observed. The results of the hormonal assay showed that, increased serum levels of FSH and estrogen but decreases in the serum levels of LH and testosterone compared to control. The results showed that, *Feronia elephantum* has effects on male rat reproduction, affecting the sexual behavior and epididymal sperm concentration.

Keywords: *Feronia elephantum*, epididymis, testis, seminal vesicle.

INTRODUCTION

Population explosion is a leading cause of poverty and pollution in developing countries. Several potential approaches for infertility have been investigated over a long period, including chemical, hormonal and immunological approaches. However, no suitable method has emerged that is effective and free from side effects. The World Health Organization (WHO) has constituted a population control programme, which includes studies having traditional medical practices. Medicinal plants [1-2] products have a long history of indigenous use in India as well other countries. Phytotherapy has a very long tradition, although proper scientific explanation is relatively new. In our country as well as in the world, there are several medicinal plants associated with antifertility properties. [3-4] A large number of plant species with antifertility effects have been screened in China and India beginning about 50 years ago and were subsequently fortified by national and international agencies. [5] However, the search for an orally active, safe and effective plant preparation or its compound is yet to be needed for fertility regulation due to incomplete inhibition of fertility or

side effects.

Feronia elephantum is one of the medicinally important plants belonging to Rutaceae, commonly known as wood apple. The leaves are used traditionally in Ayurveda as antiemetic, aromatic, expectorant, purgative, useful in anorexia, bronchitis, calculus, cardiac debility, cough, gastropathy. [6] Fruit pulp is sour, sweet edible stomachic. The pulp is applied externally as a remedy for the bites of venous insects. The bark is occasionally prescribed for biliousness and useful in liver diseases. [7]

An extensive survey of literature available from all scientific sources revealed no information about the pharmacological validation of the antifertility activity of *Feronia elephantum*. Therefore the present work has been undertaken to evaluate the antifertility activity of *F. elephantum* leaf and bark using animal models.

MATERIALS AND METHODS

Plant material

The leaf and bark of *Feronia elephantum* Correa were collected in the month of Feb and March-2012 from the Agasthiarmalai Biosphere Reserve, Western Ghats, Tirunelveli. The plant was identified with the help of Local flora and voucher specimen, preserved in Ethnopharmacology unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin-628008, Tamil Nadu, India.

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Preparation of plant extract

Leaves and Bark of *F. elephantum* were 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 (95%). The ethanol extract were concentrated in a rotary evaporator. The concentrated ethanol leaf and bark extracts were used for antifertility activity.

Animals

Normal healthy male Wistar albino rats (180-240 g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) and light and Dark (12:12h). Rats were fed with standard pellet diet (Gold Mohur brand, MS Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method); albino rats of either sex selected by random sampling were used for acute toxicity study. [8] The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as 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 higher doses such as 50, 100 and 2000 mg/kg body weight.

Experimental Design

The male rats were divided into four groups consisting of 5 animals each.

Group I: Rats received normal saline daily for 14 days, orally (Normal control).

Group II: Rats received ethanol leaf extract of *F. elephantum* at the dose of 400mg/kg body weight daily for 14 days.

Group III: Rats received ethanol bark extract of *F. elephantum*, at the dose of 400mg/kg body weight daily for 14 days.

Group IV: Rats received the 1: 1 ratio of ethanol leaf (200mg/kg b.wt) and bark (200mg/kg) extract of *F. elephantum* daily for 14 days.

After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by decapitation. Blood was collected; Sera were separated by centrifugation at 3000 rpm for 10 minutes and stored at 20°C until used for various biochemical assays. Then testes, epididymis, vas deferens, seminal vesicle and ventral prostate were dissected out, trimmed off extraneous and weighed accurately on torsion balance. The organ weights were expressed in terms of mg/100g body weight.

Sperm count

Epididymal fluid (for sperm count) was collected from caput and cauda segments separately and diluted with Sorenson's buffer (pH 7.2). The separated fluid was taken for sperm count. Sperm count was carried out by using Neubauer's haemocytometer as described by Zaneveld and Pelakoski. [9]

Sperm motility and abnormality

After anaesthetizing the rats, the caudal epididymis was then dissected. An incision (about 1mm) was made in the caudal epididymis and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was

then assessed by calculating motile spermatozoa per unit area.

Morphology (abnormality) was evaluated on sperm from the cauda epididymis. The total morphological abnormalities were observed as described by Linde *et al.* [10]

Serum biochemical analysis

Serum protein [11] and serum albumins were determined by quantitative colorimetric method by using bromocresol green. The total protein minus albumin gives the globulin, urea [12], creatinine [13], serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel. [14] Serum alkaline phosphatase (ALP) was measured by the method of king and Armstrong. [15]

Hormonal Assay

Blood removed from the rats by intracardiac method. Blood was centrifuged at 3000 rpm to separate the serum for the measurement of testosterone, Luteinizing hormone (LH), estrogen and follicle stimulating hormone (FSH). The quantitative determination of hormones was done by using Enzyme Immunoassay Method (EIA). The EIA kit was obtained from Immunometrics (London, UK).

Statistical Analysis

Data were expressed as Mean ± SEM. Student's t test was used for statistical comparison.

RESULTS**Body and Reproductive organ weight**

Acute toxicity study revealed that the non toxic nature of ethanol extract of *F. elephantum* leaf and bark. In the period, the treatment with *F. elephantum* leaf and bark extracts, no significant clinical and behavioral changes were observed in Group II, Group III and Group IV animals. The treatment of rats with plant extract caused no effect on the body weight of the animals; weight gain was normal in all the experimental groups. The treatment with *F. elephantum* ethanol leaf and bark extracts treated rats caused a highly significant ($p<0.05$) decrease in the accessory sex organ weights, namely testis, epididymis and seminal vesicle in all treated groups (Table 1). In the Group II animals, the sex organ weights were highly reduced when compared to the Group-III and Group-IV as well as Group-I (Normal) animals.

Sperm count and Sperm motility

Sperm motility and sperm density in caudal epididymis, significantly ($p<0.05$) decreased and the reduction was severe in *F. elephantum* ethanol leaf extract treated group (Group II) followed by 1:1 ratio of *F. elephantum* ethanol leaf and bark extract treated group (Group IV) and *F. elephantum* ethanol bark extract treated group (Group III) (Table 2) and the same trend was seen in the caput epididymal sperm density when compared to control (Group I).

Sperm abnormality

Sperm abnormality in caput and caudal region was drastically affected by ethanol leaf and bark extract of *F. elephantum* ($p<0.05$). Among the three extract treated groups, bark extract of *F. elephantum* treated group has shown significant and drastic abnormality in the sperm morphology, further tail region of the sperm in all the treated groups much affected than the head region.

Serum biochemical profile

Serum protein, albumin, globulin, urea and creatinine and the activity of liver marker enzymes (SGOT, SGPT AND ALP)

Table 1: Effect of ethanol leaf and bark extract of *Feronia elephantum* on the reproductive organ weight of adult male albino rats

Treatment	Body wt (g)		Testis (g)	Epididymis (g)		VD (mg)	SV (mg)	Prostrate (mg)
	Before	After		Caput	Cauda			
Group-I (Control)	214.33±08.45	226.42±09.34	1.678±0.34	113.57±2.89	263.12±08.14	94.41±6.32	289.56±9.44	158.78±5.97
Group-II (<i>F. elephantum</i> leaf 400mg/Kg)	205.56±10.22	231.32±07.23	1.321±0.45*	108.56±2.76	185.34±5.45**	82.55±3.98	219.34±6.45*	127.34±2.12*
Group-III (<i>F. elephantum</i> bark 400mg/Kg)	198.35±7.45	214.67±8.53	1.412±0.12	128.34±2.31	229.33±6.11	81.22±2.67	269.53±10.34	148.67±6.23
Group-IV (<i>F. elephantum</i> leaf and bark 200:200mg/Kg)	223.89±10.34	228.32±10.32	1.321±0.24*	103.34±1.34	198.12±3.34*	96.24±2.67	229.45±11.9*	129.54±5.78*

Each Value is SEM of 5 animals * $p < 0.05$, ** $p < 0.01$. Compared to vehicle control.

Table 2: Effect of ethanol leaf and bark extract of *Feronia elephantum* on the sperm concentration and motility in the epididymis of adult male albino rats

Treatment Groups	Sperm Concentration (Counts * 10 ⁶ mil)		Sperm Motility (FMI) @ (cauda)	Sperm Abnormality (%)	
	Caput	Cauda		Head (%)	Tail (%)
Group -I (control)	389.24±16.51	408.34±9.56	169.26±12.41	2.35±0.29	5.78±0.23
Group-II (<i>F. elephantum</i> leaf 400mg/Kg)	326.58±12.3*	331.23±8.74*	129.38±10.19*	19.23±2.36*	26.58±3.21*
Group-III (<i>F. elephantum</i> leaf 400mg/Kg)	374.67±13.67	381.88±10.12	143.65±8.09	61.34±1.98*	63.26±2.12*
Group-IV (<i>F. elephantum</i> leaf and bark 200:200mg/Kg)	331.65±10.34*	329.34±9.39*	135.45±8.43*	13.29±1.75**	12.44±1.34*

Each Value is SEM of 5 animals * $p < 0.05$, ** $p < 0.01$. Compared to vehicle control.

@: Motility is movement recorded after 5 min in suspension of caudal epididymal spermatozoa in phosphate buffered solution.

Table 3: Effect of ethanol leaf and bark extract of *Feronia elephantum* on few serum biochemical profile of adult male albino rats

Parameters	Serum biochemical profile			
	Group - I (Control)	Group - II (<i>F. elephantum</i> leaf 400mg/Kg)	Group - III (<i>F. elephantum</i> bark 400mg/Kg)	Group - IV (<i>F. elephantum</i> leaf and bark 200:200mg/Kg)
Protein (g/dl)	7.23±0.32	8.96±0.45*	7.12±0.51	7.85±0.18
Albumin(g/dl)	4.01±0.38	4.96±0.13*	4.05±0.88	4.31±0.29
Globulin(g/dl)	3.22±0.13	3.60±0.21	3.07±0.63	3.54±0.18
Urea(mg/dl)	11.29±1.98	22.45±2.78*	18.53±1.22*	15.58±1.23
Creatinine(mg/dl)	0.79±0.45	0.97±0.02	0.81±0.05	0.85±0.04
SGOT (U/L)	16.77±0.56	25.22±1.78*	26.78±1.23*	21.56±2.04
SGPT(U/L)	17.39±0.48	20.31±1.22	23.56±1.67	24.98±0.58
ALP (U/L)	131.86±4.33	153.48±3.56*	169.45±3.76	179.33±4.98

Each value is SEM of 5 animals * $p < 0.05$

Table 4: Effect of ethanol leaf and bark extract of *Feronia elephantum* on sex hormone levels and pituitary gonadotropins in male albino rats

Treatment	Hormone levels			
	Testosterone (ng/ml)	LH/ICSH (μIU/ml)	Estrogen(pg/ml)	FSH (μIU/ml)
Group - I (Control)	3.56±0.93	1.89±0.01	19.59±0.13	0.84±0.03
Group - II (<i>F. elephantum</i> leaf 400mg/Kg)	1.98±0.31**	0.95±0.02*	29.66±2.13*	4.53±0.68**
Group - III (<i>F. elephantum</i> bark 400mg/Kg)	2.34±0.53	1.67±0.74	25.39±0.24	1.98±0.12*
Group - IV (<i>F. elephantum</i> leaf and bark 200:200mg/Kg)	1.84±0.56*	1.16±0.77	27.58±0.34*	3.98±0.63**

Each Value is SEM of 5 animals * $p < 0.05$, ** $p < 0.01$. Compared to vehicle control

levels of control and treated rats were depicted in Table 3. No significant changes were noted in the serum biochemical and liver marker enzymes in the entire drug treated groups when compared to control group.

Reproductive hormone file

Serum Testosterone level

The ethanol extract of *F. elephantum* leaf and bark (400 mg/kg) body weight) caused a significant ($p < 0.01$) decrease in the serum level of testosterone in male rats (Table 4).

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the ethanol extract of *F. elephantum* leaf for 14 days caused a significant decrease in the serum level of LH (Table 4). The level of decrease was statically significant ($p < 0.05$).

Serum Estrogen Level

The ethanol extract of *F. elephantum* leaf caused an increase in the serum level of estrogen in male rats (Group II) when

compared to the Group III and Group IV as well as Group I (Normal) animals.

Serum follicle stimulating hormone (FSH) level

Pretreatment with ethanol leaf extract of *F. elephantum* for 14 days caused an increase in the serum level of FSH in male rats (Group II) when compared to the Group III and Group IV as well as Group I (Normal) animals. The increase in the serum level of FSH in male rats statistically significant ($p < 0.05$).

DISCUSSION

Studies on the effects of plant products on male reproductive system and fertility are comparatively few and far fetched. From a public health perspective, the head for contraception has never been greater.

The administration of ethanol extracts of *F. elephantum* leaf and bark to rats did not cause any significant change in the

body weight and on the libido of treated rats, whereas, weights of testes and other accessory sex organs were decreased significantly during the experiment. Among the accessory sex organs, a significant weight reduction was seen in the testes, caudal epididymal segments. Reduction in the weight of testis and other accessory sex organs might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories.^[16] It is known that the accessory sex organs viz., epididymis and vas deferens are androgen dependent target organs and manifest differential sensibility to androgens for maintenance of their structure and function. It is also known that, any change in circulating androgens would affect the internal microenvironment of epididymis and thereby lead to alteration in sperm motility and metabolism.^[17]

The development of normal mature sperm is the key to optimum male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and Luteinizing hormone (LH), which are released from the anterior pituitary.^[18] FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in leydig cells of the testis.^[19] Many studies on the testis of rat treated with plant extracts have also demonstrated that the inhibitory activity on the proliferation of spermatogonia in mammals.^[20-22] Spermatogenesis is therefore, a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatids during their preformation.^[13-14] The result of the present study suggests that ethanol extract of *F. elephantum* may affect the normal function of the sertoli and leydig cells on continuous oral administration for fourteen days.^[23]

Among the ethanol extract treated rats, Group II produced a significant reduction in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis.^[24-25] The presence of immature sperms was also observed in the experimental rats treated with 400mg/kg body weight of ethanol leaf extract and 1:1 ratio of leaf and bark ethanol extract of *F. elephantum* could affect the maturation of the spermatozoan in the male rats, which might also be a contributory factor to the decrease in the mean total sperm count. The data generated in the present study, by and large, confirm to those already reported and studied with various plant extract.^[26-28]

The decrease in the caudal epididymal sperm counts are clear indications that, *F. elephantum* extract can affect one or more aspects of spermatogenesis as well as spermigenesis. Though a direct effect of *F. elephantum* extract on the cellular mechanisms of spermatogenesis cannot be concluded, it is likely that the impairment of the hormonal mechanisms concerned with the regulation of spermatogenesis may be underlying cause.

The various other sperm abnormalities like sluggish motility, coiled tail and sperm immaturation are also due to *F. elephantum* toxicity. The hitherto unreported abnormal sperm morphology, coiled tail and malformed head could be attributed to both testicular and epididymal effects of *F. elephantum* extract. Coiling of the sperm tail is usually the

product of abnormal axoneme and or outer dense fibril. The outcome of the present study affirms the male reproductive toxic effects of *F. elephantum* when applied as therapeutic agent. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequence of *F. elephantum* on the sperm may be taken as the advantage for further study. By the treatment employed in the study, no toxic effect was produced in the liver and kidney, neither was it directly involved on the development of functioning of the male reproductive system nor in the reproductive organs.

The present study revealed a decrease in the serum level of testosterone. This observation was similar to the earlier findings.^[29-31] Testosterone is produced by Leydig cell in the testes and decreased number of Leydig cells and their nuclear area in the treated rats diminished the production of testosterone^[23] which might have affected the fertility in treated rats. The reduction in the serum level of testosterone could probably be due to the decrease of serum levels of LH/ICSH observed in this investigation. Leydig cells secrete testosterone by the stimulatory effect of LH.^[31-33] In males, reduction of testosterone level may impair spermatogenesis and cause male infertility. This study further observed a significant increase in the serum estrogen level of ethanol leaf extract of *F. elephantum* treated rats. This increase might probably be due to the conversion of testosterone to estrogen. In the present investigation, there is an increase in the serum estrogen level of ethanol leaf extract of *F. elephantum* treated rats. This is in agreement with earlier reports.^[34-35] This shows that the plant possessed antifertility activity.

Treatment with the ethanol extracts of *F. elephantum* leaf and bark (400mg/kg b.wt) was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicated structural and functional alteration in testis, epididymis and seminal vesicle. Depletion of sperm count and sperm motility in the drug treated rats suggests alteration in sperm production in the testes and maturation in the epididymis. Changes in both sperm count and motility resulted in partial infertility within fourteen days. This resulted in abnormal sperm functions which ultimately gave rise to complete male sterility. Among the plant based contraceptives, inhibition of male fertility after administration of natural substances has been related to decreased spermatozoa density.^[36] For male contraception, it is not necessary to stop spermatogenesis, but it is enough to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of sperm.^[37]

Antifertility activity of *F. elephantum* has been attributed due to the presence of certain phytochemical compound such as stigmasterol, psoralon, bergapten, orientin, vitedin, sapononis, tannins and an essential oil found to be observed earlier in *F. elephantum* leaf.^[38] Saponins are important mainly because of their steroid structure. They are precursors for the hemisynthesis of birth control pills (with progesterone and esotrogens) as well as similar hormones and corticosteroids.^[39] Recently many laboratories are engaged in developing male contraceptives from plants.^[40] Plant products as contraceptives will be more acceptable for economic reasons in terms of self reliance and the possible practicability for a male pill approach in counties where population pressure is high. Recently extensive efforts have been made to study the antifertility drugs from plants.^[41-43]

In the present study, the treatment of ethanol extracts of *F. elephantum* leaf and bark in male albino rats and duration suggests marked alterations in the male reproductive organs. Further studies are needed to prove whether the alterations are reversible or permanent after cessation of treatment and for understanding the exact mechanism.

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