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Evaluation of antifertility potential of *ethanolic* extract of *Bacolepis* nervosa in male albino rats

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ABSTRACT

The present investigation was carried out to assess the antifertility activity of ethanol extract of *Bacolepis nervosa* stem and leaf in male albino rats. Acute toxicity study of the extract was determined in adult male albino rats. The antifertility activity of ethanol extract at the dose levels 150 and 300 mg/kg orally was evaluated in experimental animal models and effect of extract on reproductive organs, sperm count, motility and sperm abnormality and fertility test were investigated. The treatment caused decrease in weight of testis, sperm count and revealed a reduction in the size of seminiferous vesicles. The epididymas sperm count, motility and sperm abnormality were reduced significantly in treated rats. When compared with control rats, *B. nervosa* extracts led to significant testosterone and lutinizing hormone suppression associated with consequent significant rise in estrogen. There was an increase in serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of control and treated rats. The activities of serum antioxidants (CAT, SOD, GPx, GST and GRD) in plant extract treated rats were decreased. The results of fertility test indicated that the treated adult male rats reduced the number of female impregnation. The results conclude the disruption of spermatogenic as well as androgenic compartment. The present study suggests that *B. nervosa* extracts suppress male fertility without altering the general metabolism.

INTRODUCTION

The future of life on the planet is under the pressure of the population explosion. The world's population for mid-year 2011, is estimated to be 6,928,198,253 [[1]] and continues to grow by 83 million people per year. Population growth is a great concern world-wide and most of the developing countries are characterized by rapid population growth[2]. Hence, various methods are being used to reduce the total fertility rate in both men and women. 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[3]. Phytotherapy has a very long tradition, although a proper scientific explanation is relatively new. In our county as well as in the world, there are several medicinal plants associated with antifertility properties[4].

Many studies have been done on male contraception. The traditional use of medicinal plants to treat different sorts of diseases, including fertility related problems is widespread throughout the world as many plant substances are known for their interferences with the male reproductive system[5]. Some of the plants had spermicidal effects; others caused reduction in sperm counts and alter the mobility of the sperms. Some of the plants caused testicular change and altered hormone levels[6]. Fertility regulation using plants or plant products has been reported in the ancient literature of indigenous systems of medicines. 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[7,8].

Bacolepis nervosa (Wight & Arn.) Decne. ex Moq. (Periplocaceae) is an endemic plant to Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu, India. This plant contains rich source of bioactive compounds such as phenolic compounds,

flavonoids, steroids and alkaloids. The impact of this plant in various disease treatments should be considered to discover new drug molecule or its derived compounds. But no such literatures are revealed for its antifertility activity. Hence the present study focuses on evaluating the antifertility activity of stem and leaf extracts of *Bacolepis nervosa.*

MATERIALS AND METHODS

Plant material

The whole plant of *Bacolepis nervosa* (Wight & Arn) Decne. ex. Moq. was collected from Kothagiri, Nilagiri Biosphere Reserve, Western Ghats, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen (specimen number VOCB6413) was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Preparation of plant extract

The stem and leaves of the plant were dried under shade and then powdered separately with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a Soxhlet apparatus using ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for antifertility activity.

Experimental animal

Normal healthy adult male Wistar albino rats weighing about 180 - 240 g body weight were selected for this work. They were housed under standard environmental conditions at room temperature (25 ± 2°C) in a well-ventilated animal house with constant 12 hrs of darkness and 12 hrs of light schedule. The rats were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The study was carried out as per IAEC approval no. 1012/CO6/CPSEA-Corres-2008-2009.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD- 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after that the extracts were administered orally at 5mg/kg body weight by gastric intubations 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 upto 2000 mg/kg body weight.

Experimental design

The animals were divided into five groups and each group was consisting of 5 animals.

Group I : Rats given normal saline daily for 14 days, orally by using an intragastric catheter tube (IGC).

Group II : Rats given stem extract of *B. nervosa* at the dose of 150 mg/kg body weight daily, orally for 14 days by using an IGC.

Group III : Rats given stem extract of *B. nervosa* at the dose of 300 mg/kg body weight daily, orally for 14 days by using an ICG.

Group IV : Rats given leaf extract of *B. nervosa* at the dose of 150 mg/kg body weight daily, orally for 14 days by using an ICG.

Group V : Rats given leaf extract of *B. nervosa* at the dose of 300 mg/kg body weight daily, orally for 14 days by using an ICG.

Suitable controls were maintained for each duration of treatment. However, as there was no obvious difference on any parameter among control groups, a common control was employed in the present study. All the treatments were given between 8.00 and 11.00 hrs in the morning. After 24 hrs of last treatment, the final body weight was recorded and the animals were sacrificed by decapitation. Blood was collected, sera separated by centrifugation at 3000×g for 10 minutes and stored at -20°C until used for various biochemical assays. Then testis, epididymis, vasdeferens, seminal vesicle and ventral prostate were dissected out, trimmed off extraneous tissues and weighed accurately on a torsion balance. The weight of the organs was expressed in terms of mg/100 g body weight.

Sperm count determination

Epididymal fluid was collected from caput and cauda segments separately for sperm count, minced in 2 ml Sorenson's

Table 1 : Effect of ethanol extracts of stem (BNS) and leaf (BNL) of *Bacolepis* nervosa on the body and reproductive organ weight of adult male albino rats

Group	Dose (mg/kg body weight)	Body wiight (gm)		Testis (g)	Epididymis (mg)		VD (mg)	SV (mg)	Prostrate (mg)
		Before	After		Caput	Cauda			
I	Control (Saline)	204.16±11.46	226.54±10.84	2.646±0.32	216.84±6.54	312.13±9.16	146.13±3.16	316.42±6.84	193.62±3.16
II	BNS(150)	219.62±10.85	211.37±8.64^{ns}	1.934±0.91*	189.16±4.34^{ns}	282.13±7.84*	118.29±4.54**	293.19±8.16^{ns}	179.28±3.94
III	BNS(300)	196.16±7.36	193.19±6.18*	1.741±0.54**	172.84±6.56**	264.19±5.84**	101.16±3.84**	281.93±3.94*	156.16±4.24*
IV	BNL (150)	219.46±8.54	206.16±8.56*	1.962±0.36*	186.16±3.45^{ns}	283.16±5.54*	121.17±4.62*	284.16±7.56^{ns}	181.13±2.16
V	BNL (300)	212.16±11.96	186.54±7.54**	1.816±0.84**	176.18±5.14**	251.93±10.22**	113.24±4.35**	273.18±5.94*	163.59±5.46*

Values are expressed as mean ± SEM, n= 5 in each group.
* $p < 0.05$. ** $p < 0.01$. Control *Vs* Treated. ns - not significant.

Table 2 : Effect of ethanol extracts of stem (BNS) and leaf (BNL) of *Bacolepis nervosa* on the sperm concentration and motility in the epididymis of adult male albino rats.

Group	Dose(mg/kg body weight	Sperm Concentration (Counts x 10^6 mil)		Sperm Motility (FMI)@ (cauda)	Sperm Abnormality#	
		caput	cauda		Head (%)	Tail (%)
I	Control (Saline)	326.13±10.84	352.18±18.13	178.19±5.83	5.91±0.28	7.16±0.19
II	BNS(150)	308.05±11.26	308.16±11.92**	151.27±4.84**	20.17±1.06	20.48±0.78*
III	BNS(300)	274.16±10.33**	256.28±9.38***	112.84±3.84***	24.13±0.96*	26.15±0.84**
IV	BNL (150)	304.18±9.54	316.65±11.46*	143.94±4.55**	16.32±0.67	19.31±0.54*
V	BNL (300)	284.65±8.38*	269.13±12.65**	118.54±4.67***	26.16±0.92*	24.84±0.86**

Values are expressed as mean ± SEM, n= 5 in each group.
* $p < 0.05$. ** $p < 0.01$; *** $p<0.001$- Control *Vs* Treated. ns - not significant.
@ : Motility is movement recorded after 5 min in the suspension of caudal epididymal spermatozoa in phosphate buffer solution.
# : Expressed in percentage; ns - not significant.

buffer (pH 7.2) and passed through nylon mesh of 75 µ size. The separated fluid was taken for sperm count. Sperm count was carried out by using Neubauer haemocytometer[9].

Sperm motility and abnormality

The rats were anesthetized with 25% urethane at a dose of 0.6 ml/100 g intraperitoneally. The caudal epididymis was then dissected. An incision (about 1 mm) was made in the caudal epididymis, drops of sperm fluid were squeezed on to 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 caudal epididymis. The total morphological abnormalities were observed as described by Linde *et al*. [10].

Serum biochemical analysis

Serum proteins [11] and serum albumins were determined by quantitative colorimeric method by using bromocresol green. The total protein minus albumin give the globulin, Urea,[12] Creatinine,[13] Serum glutamate pyruvate transaminase (SGPT) and Serum glutamate oxaloacetate transaminase (SGOT) was measured specrophotomerically by using the method of,[14] Serum alkaline phosphatase (ALP) was measured[15].

Serum antioxidants

Serum antioxidant Catalase[16], Superoxide dismutase[17], Glutathione peroxidase[18], Glutathione s- transferase [19], and glutathione reductase [20] were analyzed.

Hormonal assay

The blood removed from the animals by intra-cardiac method was centrifuged at 2000 rpm (Revolutions per minute) to separate the serum for the measurement of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone and Estrogen. The quantitative determination of hormones was done by using Enzyme Immuno Assay method (EIA). The EIA kit was obtained from immunometrics (London, UK).

Fertility test

Fertility was estimated in adult male rats treated with stem and leaf ethanol extracts of *B. nervosa* in the control males' counterparts. Each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for 10 days during which two estrons cycles had elapsed[21]. One week after the removal of the exposed males, pregnant females were killed by cervical dislocation under light ether anesthesia and the number of implantation sites, the number of foetuses and the number of resorption sites were recorded.

Statistical analysis

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

RESULTS

Body and reproductive organ weight

The treatment of rats with stem and leaf extracts of *B. nervosa* showed decrease in body weight compared to control. The treatment with stem and leaf extracts treated rats caused a highly significant ($p<0.05$; $p<0.01$) decrease in the accessory sex organ weights namely testis, epididymis and seminal vesicle in all treated groups. In Group III and V (300 mg/kg body weight), the sex organ weights were significantly ($p<0.01$) reduced when compared to that of Group II and IV (150 mg/kg body weight) (Table 1).

Sperm count and sperm motility

Table 2 shows that the sperm motility and sperm density in epididymis were decreased to a significant ($p<0.01$; $p<0.001$) level in treated animals with the ethanol extracts of stem and leaf of *B. nervosa* compared to control group. The reduction was very severe in rats treated with stem and leaf extracts of *B. nervosa* at

Table 3 : Effect of ethanol extracts of stem (BNS) and leaf (BNL) of *Bacolepis nervosa* on the activity of serum Catalase, Glutathione peroxidase, Glutathione-S-transferase, Superoxide dismutase and Glutathione reductase in adult albino rats

Group	Dose (mg/kg body weight)	Catalase (µ moles of H_2O_2 decomposed/min /mg protein)	Glutathione peroxidase (µ moles of NADPH oxidized/min/mg protein)	Glutathione-S-transferase (µ moles of conjugate formed/mg protein)	Superoxide dismutase (Units/mg protein)	Glutathione reductase (µ moles of NADPH/mg protein)
I	Control (Saline)	6.516±0.84	0.394±0.05	14.81±1.13	21.93±1.84	28.16±1.93
II	BNS (150)	5.39±0.24^{ns}	0.311±0.04^{ns}	7.28±0.65**	14.92±0.46*	18.26±0.37*
III	BNS (300)	3.55±0.18**	0.296±0.05***	4.81±0.45***	8.22±0.59***	12.86±0.91***
IV	BNL (150)	5.16±0.91^{ns}	0.354±0.03^{ns}	8.93±0.84*	15.42±1.52	21.56±1.84*
V	BNL (300)	4.82±0.45*	0.306±0.05*	5.66±0.93***	10.82±0.94**	13.27±0.94**

Values are expressed as mean ± SEM, n= 5 in each group.
$^{*}p < 0.05$, $^{**}p<0.01$; $^{***}p<0.001$. Control *Vs* Treated. ns - not significant.

Table 4 : Effect of ethanol extracts of stem (BNS) and leaf (BNL) of *Bacolepis* nervosa on the serum biochemical profile of adult male albino rats

Group	Dose (mg/kg body weight)	Parameters							
		Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	SGOT (U/L)	SGPT(U/L)	ALP(U/L)
I	Control (Saline)	7.84±0.14	4.36±0.24	3.48±0.21	14.93±0.84	0.73±0.05	13.67±0.93	16.89±1.05	196.13±4.81
II	BNS(150)	7.44±0.31	4.52±0.16	2.93±0.21	36.93±1.22*	1.13±0.06	34.13±1.93*	61.36±2.51**	228.63±2.16
III	BNS(300)	7.16±0.53	4.06±0.23	3.10±0.26	39.68±1.65**	1.84±0.56*	50.32±1.36**	76.96±1.93**	238.15±5.86**
IV	BNL (150)	7.68±0.23	4.08±0.17	3.60±0.13	21.63±1.22	0.98±0.05	32.56±1.31	38.66±1.84	213.19±4.81
V	BNL (300)	7.48±0.13	4.65±0.13	2.83±0.11	42.16±2.17**	1.39±0.16*	39.65±3.13**	52.83±3.96**	236.51±8.34*

Values are expressed as mean ± SEM, n= 5 in each group.
$^{*}p < 0.05$; $^{**}p<0.01$ Control *Vs* Treated.

Table 5 : Effect of ethanol extracts of stem (BNS) and leaf (BNL) of *Bacolepis nervosa* on the sex hormones level and pituitary gonadotrophins in male albino rats

Group	Dose (mg/kg body weight)	Parameters			
		Testosterone (ng/ml)	LH (µIu/ml)	Estrogen (pg/ml)	FSH (µIu/ml)
I	Control (Saline)	3.96±0.21	2.54±0.36	16.13±1.83	1.16±0.07
II	BNS (150)	2.56±0.18*	1.96±0.05**	17.94±1.36^{ns}	0.73±0.07*
III	BNS (300)	1.94±0.12**	1.63±0.08**	23.22±1.10*	0.56±0.04**
IV	BNL (150)	3.41±0.16^{ns}	2.06±0.15	18.96±1.13^{ns}	1.08±0.05
V	BNL (300)	1.96±0.15**	1.76±0.08*	21.56±1.15*	0.97±0.03*

Values are expressed as mean ± SEM, n= 5 in each group.
$^{*}p < 0.05$; $^{**}p<0.01$ - Control *Vs* Treated
ns - not significant

Table 6 : Effect of ethanol extracts of stem (BNS) and leaf (BNL) of *Bacolepis nervosa* on the fertility of adult male albino rats

Groups	Dose (mg/kg body weight)	No. of males	No. of females	No. of pregnant females	No. of implantation	No. of viable fetuses	Total No. of resorption sites
I	Control (Saline)	2	7	7/7	9.84±0.54	6.81±0.94	6
II	BNS (150)	2	7	4/7	5.26±0.26*	3.58±0.62*	4
III	BNS (300)	2	7	3/7	3.92±0.13**	3.16±0.53*	3
IV	BNL (150)	2	7	4/7	4.93±0.83**	5.38±0.16ns	4
V	BNL (300)	2	7	2/7	3.16±0.12**	3.96±0.21**	2

Values are expressed as mean ± SEM, n= 5 in each group.
* $P < 0.05$,** $P<0.01$- Control *Vs* Treated

the dose of 300 mg/kg body weight (Group III & V) followed by Group II and Group IV rats treated with plant extracts at the dose of 150 mg/kg body weight respectively. The same trend was seen in the caput epididymal sperm density when compared to control rats (Group I).

Sperm abnormality

Sperm nature, in caput and caudal regions, was affected by the ethanol extracts of *B. nervosa* (Table - 2). The results obtained from all the treated groups showed that the tail region of the sperms was much affected than the head.

Serum antioxidants

The activities of CAT, SOD, GPx, GST and GRD in the serum of control and plant extracts treated rats were presented in Table - 3. In the present study, plant extracts treated rats showed decreased activities of all the studied antioxidants when compared to control rat.

Serum biochemical profile

Serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) of the control and plant extracts treated rats are depicted in Table 4. The results showed no significant changes in the serum protein, albumin and globulin. The level of creatinine slightly increased in rats treated with the plant extracts at 150 mg/kg body weight and 300 mg/kg body weight dose. The level of urea and liver marker enzymes like SGOT, SGPT and ALP increased in a dose dependent manner, in both the stem and leaf treated groups, when compared to the control group.

Reproductive hormone profile

Serum testosterone level

The ethanol extracts of stem and leaf of *B. nervosa* (150 and 300 mg/kg body weight) repeated treatment for 14 days caused a significant ($p<0.05$; $p<0.01$) decrease in serum level of testosterone in male rats. The level of testosterone decrease was dose related (Table - 5).

Serum Luteinizing Hormone (LH) level

Repeated treatment of male rats with the ethanol extracts of *B. nervosa* for 14 days caused a dose related decrease in the serum level of LH (Table 5).

Serum estrogen level

The ethanol extracts stem and leaf of *B. nervosa* (150 and 300 mg/kg) caused an increase in the level of serum estrogen in male rats. Doses of 150 and 300 mg/kg body weight, administered daily for 14 days, caused rise in the serum level of estrogen.

Serum Follicle Stimulating Hormone (FSH) level

Pretreatment with ethanol extracts of stem and leaf of *B. nervosa caused* a decrease in the serum level of FSH in male rats compared to control (Table 5).

Fertility test

The results presented in Table 6 showed that intragastric administration of stem and leaf extracts of *B. nervosa* (150 mg/kg and 300 mg/kg body weight), for 14 days to male rats, caused a significant ($p<0.01$) decrease in the number of females impregnated by plant extracts treated male rats. When compared to females impregnated with untreated male rats, the number of viable foetuses formed decreased significantly ($p<0.05$; $p<0.01$) in female rats impregnated by treated males. Similarly, the number of resorption sites was found to be reduced in female rats impregnated by treated male rats when compared to control.

DISCUSSION

Fertility control is an issue of global and national public health concern. Current methods of contraception result in an unacceptable rate of unintended pregnancies. Contraceptive vaccines and inhibition of spermatogenesis and sperm motility provide a potential for non hormonal male contraceptive. Use of antifertility agent is one of the methods in controlling human population. In recent years, there has been a concern about the use of plant products in affecting fertility of humans. India has vast resources of natural products. People have been using many of the medicinal plants for inducing abortion and permanent sterility[22]. A large number of herbal drugs are used to control fertilization with considerable success.

The results of the present study revealed a little change in the body weight of rats treated with the stem and leaf ethanol extracts of *B. nervosa* at doses of 150 and 300 mg/kg body weight for fourteen days. The weight of testis and other accessory sex organs

was decreased significantly during the experiment. Among the accessory sex organs, a significant weight reduction was noticed in the caput and caudal epididymal segment and the weight reduction was dose dependent. Significant reduction was observed in the vas deferens (VD) ($p<0.01$), seminal vesicle (SV) ($p<0.05$) and prostate ($p<0.05$). 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[23]. 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 micro environment of epididymis and thereby lead to the alteration in sperm motility and metabolism[24].

In the present study, the rats treated with stem and leaf ethanol extracts of *B. nervosa* showed significant decreased sperm motility ($p<0.001$) and sperm density in caudal and caput epididymal segments. Drastic effect on the nature of the normal sperms, in the caput and cauda region was observed in treated rats with the stem and leaf ethanol extracts of *B. nervosa.* Further head and tail regions of the sperm were affected in all the treated groups (Group II, III, IV and V). The development of normal and mature sperm is the key to optimum male fertility. Decline in sperm motility in males might have affected fertilization and implantation. Inadequate concentration, sluggish or non-motile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova[25, 26]. 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[27]. FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in the Leydig cells of the testis[28]. Many studies on the testis of rat treated with plant extracts have also revealed the inhibitory activity on the proliferation of spermatogonia in mammals[29,30]. Spermatogenesis is a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatids during their preformation [27, 28]. The results of the present study suggest that oral administration with stem and leaf ethanol extracts of *B. nervosa* may affect the normal function of the sertoli cells.

Sex cells can occur during the reproductive phase, mitotic division of the spermatogonia or during the maturation of the spermatozoa, thereby affecting the number and quality of the sperm cells produced in the testis. Among the treated groups with the ethanol extracts of stem and leaf of *B. nervosa,* Group III and V (300 mg/kg body weight) produced a significant ($p<0.01$; $p<0.001$) decrease in sperm density and sperm motility. This may be due to the ability of the extract at the given dose, 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[31,32]. The presence of immature sperms was also observed in the experimental rats treated with ethanol extracts of stem and leaf of *B. nervosa* at the dose of 300 mg/kg body weight. This suggested that 300 mg/kg body weight dose level could affect the maturation of the spermatozoa 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, coincide to those already reported and studied with various plant extracts[33, 34, 35]. The decrease in the caudal epididymal sperm count is a clear indication that the stem and leaf extracts of *B. nervosa* can affect one or more aspects of spermatogenesis as well as spermiogenesis. Though a direct effect of stem and leaf ethanol extracts of *B. nervosa* 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 the underlying cause.

No toxic effect was observed in the liver and kidney of the rats treated with extracts of the experimental plant. It is because the liver and kidney are neither directly involved on the development nor functioning of the male reproductive system/ reproductive organs. The present study clearly exposed a decrease in the sperm density and sperm motility in the caudal epididymis of all the treated groups which led to the proven impairment of fertility in all the treated groups. The results also indicated that the treatment of male rats with the stem and leaf ethanol extracts of *B. nervosa* reduced the number of impregnation of females with the treated males. In addition, the number of implantations and the number of viable foetuses were also decreased. This decrease in viable foetuses observed in this study, may be due to the decrease in sperm motility and sperm density. This may be due to the effect of the plant extracts on the enzymes involved in the oxidative phosphorylation process.

The present study revealed a decrease in the serum level of testosterone. This observation was similar to the earlier findings[36,37,38]. The reduction in the level of testosterone, observed in this investigation, could be probably due to the decrease in the levels of LH/FSH. Leydig cells secrete testosterone by the stimulatory effect of LH[39]. In males, the reduction of testosterone level may impair spermatogenesis and causes male infertility. Physiologic concentrations of testosterone, LH and FSH play an important role in spermatogenesis[40]. The study also revealed a dose dependent increase in the serum estrogen level. This increase might probably be due to the conversion of testosterone to estrogen[41].

Treatment with the ethanol extracts of stem and leaf of *B. nervosa* 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 suggested the alteration in sperm production in the testis and maturation in the epididymis. Changes in both sperm count and sperm motility resulted in a partial infertility. This resulted in abnormal sperm function which ultimately gave rise to complete male sterility. Among the plant based contraceptives, inhibition of male fertility after administration with normal substances has been related to decreased sperm density[42]. 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 the sperm[43].

The antifertility activity of *B. nervosa* has been attributed to the action of various steroidal saponin. Saponins are important mainly because of their steroid structure. They are precursors for the hemi-synthesis of birth control pills (with progesterone and estrogens) as well as similar hormones and corticosteroid[44]. From the present study, it can be concluded that *B. nervosa* is capable to suppress male fertility without altering general metabolism. Hence, the possible male contraceptive efficacy of *B. nervosa* stem and leaf extracts cannot be ignored paving way to the smooth

development for the clinicians' interests in clinical trials towards emergence of a potent herbal male contraceptive.

CONCLUSION

Recently many laboratories are engaged in developing male contraceptives from plants[45]. 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 countries where population pressure is high. Recently extensive effects have been made to study the antifertility drugs from plants[24, 46]. In the present study, dose dependent treatment of *B. nervosa* stem and leaf extracts 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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