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EVALUATION OF ANTI-FERTILITY ACTIVITY OF ETHANOL EXTRACT OF *CYNOGLOSSUM ZEYLANICUM* (VEHL EX HORNEM) THUMB. EX LEHM (BORAGINACEAE) WHOLE PLANT ON MALE ALBINO RATS

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ABSTRACT

Anti-fertility effect of ethanol extract of whole plant extract of *Cynoglossum zeylanicum* was observed in male albino rats. The relative weight of the testes and epididymis were decreased. The epididymal sperm count, motility and sperm abnormality were reduced significantly in treated rats. There was an increase in serum urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of plant extract treated rats. The activities of serum antioxidants (CAT, SOD, GP_X, GST and GRD) in plant extract treated rats were decreased. The results of the hormonal assay showed that increased serum levels of FSH and estrogen but decreased in the serum levels of LH and testosterone compared to control. The results of fertility test indicated that the treated adult male rats reduced the number of female's impregnation. In addition, the number of implantations and the number of viable fetuses were also decreased. The results of the present study concluded that, ethanol extract of whole plant of *Cynoglossum zeylanicum* inhibited sperm concentration, motility and testosterone which might result in a male sterility.

Key words: *Cynoglossum zeylanicum*, Testosterone, Antioxidant, Sterility.

INTRODUCTION

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 inhibitors of spermatogenesis and sperm motility, provide a potential for non hormonal male contraceptive. Use of anti-fertility 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[1]. A large number of herbal drugs are used to control fertilization with considerable success.

There has been a steady accumulation of information regarding the screening of plants having anti-fertility efficacy. The folklore information and the ancient literature about the plants and herbs can help the anti-fertility program. In the recent past, a number of plants have been identified and evaluation of extracts

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and active principles from different parts of plants like seeds, root, leaves, flowers, stem or stem barks have been done by various researchers[2-4].

Cynoglossum zeylanicum belongs to Boraginaceae family. It is commonly known as "Jathakkai". Decoction prepared from the whole plant is used to arrest vomiting by Badaga community in Nilgiri Biosphere Reserve, Tamil Nadu. The biological activities such as hepatoprotective, *in vitro* antioxidant, antihyperglycemic and antitumor were reported[5-8].

However, no systematic attempts have been made to establish scientific basis of beneficial effects of *Cynoglossum zeylanicum* whole plant extracts. To our knowledge, no reports on the anti-fertility activity of *Cynoglossum zeylanicum* whole plant. This study was therefore undertaken to evaluate the effect of ethanol extract of whole plant of *Cynoglossum zeylanicum* on anti-fertility activity.

EXPERIMENTAL

Materials and methods

Plant material

The well grown whole plant of *Cynoglossum zeylanicum* (Vehl ex Hornem) Thumb. ex Lehm was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further references.

Preparation of plant extract

The whole plants of *Cynoglossum zeylanicum* (CZW) were dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected for extraction in a Soxhlet apparatus using ethanol. The ethanol extracts were concentrated in a rotatory evaporator. The concentrated ethanol extracts of whole plant of *Cynoglossum zeylanicum* were used for anti-fertility 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 ± 2^0C) and light and Dark (12 : 12 h). Rats were fed with standard pellet diet (Goldmohur 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[9]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5 mg/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 and 2000 mg/Kg body weight.

Experimental design

The male rats were divided into 4 groups consisting of 5 animals.

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

Group II : Rats received ethanol extract of whole plant of *Cynoglossum zeylanicum* at the dose of 50 mg/Kg body weight daily for 14 days.

Group III : Rats received ethanol extract of whole plant of *Cynoglossum zeylanicum*, at the dose of 100 mg/Kg body weight daily for 14 days.

Group IV : Rats received ethanol extract of whole plant of *Cynoglossum zeylanicum*, at the dose of 150 mg/Kg body weight 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 20^0C 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[10].

Sperm motility and abnormality

After anaesthetizing the rats, the caudal epididymis was then dissected. An incision (about 1 mm) 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 caudal epididymis. The total morphological abnormalities were observed as described by Linde *et al.*[11]

Serum biochemical analysis

Serum proteins[12] and serum albumins were determined by quantitative colorimetic method by using bromocresol green. The total protein minus albumin gives the globulin, urea[13], creatinine[14], serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel[15]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong[16].

Serum antioxidants

Serum antioxidants Catalase (CAT)[17], Superoxidedismutase (SOD)[18], Glutathione peroxidase (GP_X)[19], Glutathione s-transferase (GST)[20] and Glutathione reductase (GRD)[21] were analyzed.

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).

Fertility test

Fertility was estimated in adult male rats treated with ethanol extracts of whole plant of *Cynoglossum zeylanicum* and in the control male's 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

estron cycles had elapsed. One week after the removal of the exposed males, pregnant females were killed by cervical dislocation under light ether anesthesis and the number of implantation sites, the number of fetuses and the number of resorption sites were recorded[22].

Statistical analysis

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

RESULTS AND DISCUSSION

Body and reproductive organ weight

The ethanol extract of whole plant of *Cynoglossum zeylanicum* (CZW) at different concentration were treated on male Wistar albino rats for anti-fertility activity.

The administration of ethanol extract of whole plant of *C. zeylanicum* to rats, slightly decrease the body weight (Table 1) and on the libido of treated rats whereas weight of testes and other accessory organs were decreased ($p < 0.001$) (Table 1). Among the accessory sex organs, a significant weight reduction was seen in the caput and caudal epididymal segment. The weight reduction was dose-dependent i.e. high dose (150 mg/Kg body weight) treated groups (Group-IV) drastically reduced followed by less in low dose group (50 mg/Kg body weight) (Group-II). Slight changes were observed in vas deferens, seminal vesicle and prostate.

Table 1: Effect of CZW extract on the body and reproductive organ weight of adult male albino rats

Treatment Groups	Body wt. (g)		Testes (g)	Epididymis (mg)		VD (mg)	SV (mg)	Prostate (mg)
	Before	After		Caput	Cauda			
Group-I	214.93 ± 5.61	219.16 ± 3.92	2.214 ± 0.27	268.11 ± 2.65	318.51 ± 3.68	134.17 ± 2.94	243.81 ± 4.39	203.62 ± 3.93
Group-II	198.56 ± 3.16	204.16 ± 3.14	2.014 ± 0.19*	211.53 ± 2.19*	256.55 ± 5.29*	119.62 ± 2.94ns	204.33 ± 3.54	183.54 ± 4.81
Group-III	206.58 ± 5.11	193.62 ± 3.64	1.843 ± 0.17**	191.36 ± 2.16**	241.38 ± 3.84***	121.22 ± 4.39*	208.56 ± 2.91	181.04 ± 2.68
Group-IV	198.91 ± 1.82	193.14 ± 2.63	1.821 ± 0.11***	183.18 ± 2.84***	224.84 ± 4.93***	113.84 ± 1.66**	214.56 ± 1.42	205.24 ± 3.47

Each Value is SEM of 5 animals * P < 0.05; ** P < 0.01; *** P < 0.001. Control vs Treated: Not significant

Sperm count and sperm motility

Sperm motility and sperm density in caudal epididymis, significantly decreased and the reduction was severe in higher dose treated group (Group-IV) followed by low dose group (Group-II) (Table 2) and the same trend was seen in the caput epididymal sperm density when compared to control (Group-I).

Serum biochemical profile

Serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of control and treated rats were depicted in the Table 3. Except protein and albumin, all the other parameters were slightly increased.

Table 2: Effect of CZW on the sperm concentration and motility in the epididymis of adult male albino rats

Treatment groups	Sperm concentration (Counts x 10^6 mil)		Sperm motility (FMI) @ (cauda)	Sperm abnormality #	
	caput	cauda		Head (%)	Tail(%)
Group-I	284.54 ± 5.11	334.63 ± 6.54	153.22 ± 5.51	6.11 ± 0.08	11.22 ± 0.14
Group-II	262.17 ± 3.16ns	306.53 ± 1.17* ↓-	136.34 ± 3.94ns	7.27 ± 0.29	12.17 ± 0.16
Group-III	251.24 ± 4.10**	293.19 ± 2.27** ↓-	124.11 ± 2.63* ↓-	8.16 ± 0.11ns	17.39 ± 0.14* ↓-
Group-IV	214.16 ± 3.19***	271.68 ± 2.60***↓-	113.16 ± 1.38** ↓-	8.63 ± 0.21ns	21.17 ± 0.84* ↓-

Each Value is SEM of 5 animals * P < 0.05,** P < 0.01; *** P < 0.001; Control vs Treated : ↓-Significantly reduced ;↑ Significantly increased; @ : Motility is movement recorded after 5 min in suspension of caudal epididymal spermatozoa in phosphate buffered solution; # : Expressed in percentage

Table 3: Effect of CZW on the serum biochemical profile of adult male albino rats

Parameter	Treatment groups				
	Group I	Group II	Group III	Group IV	Units
Protein	8.38 ± 0.23	8.54 ± 0.13	8.01 ± 0.24	7.65 ± 0.19	gm/dl
Albumin	4.81 ± 0.28	4.89 ± 0.11	4.14 ± 0.17	3.91 ± 0.27	gm/dl
Globulin	3.57 ± 0.12	3.65 ± 0.07	3.87 ± 0.04	3.74 ± 0.12	gm/dl
Urea	13.54 ± 0.17	16.22 ± 0.73	42.54 ± 2.17	49.54 ± 0.16	mg/dl
Creatinine	0.83 ± 0.05	1.21 ± 0.15	1.98 ± 0.55	0.81 ± 0.24	mg/dl
SGOT (U/L)	12.56 ± 0.12	17.54 ± 0.34	34.84 ± 0.94	58.66 ± 1.21	(U/L)
SGPT(U/L)	14.39 ± 0.32	16.33 ± 0.16	41.63 ± 0.15	63.51 ± 1.65	(U/L)
ALP(U/L)	174.39 ± 4.51	182.65 ± 4.64	198.66 ± 5.11	189.16 ± 5.21	(U/L)

Each Value is SEM of 5 animals Control vs Treated

Serum antioxidants

The activities of CAT, SOD. GPx, GST and GRD in the serum of control and plant extract treated rats were presented in Table 4. In the present study, plant extract treated rats had shown decreased activities of all the studied antioxidants when compared to control rat.

Reproductive hormone level

Serum testosterone level

The ethanol extract of whole plant of *C. zeylanicum* (50, 100 and 150 mg/Kg body weight) repeated treatment for 14 days caused a significant decrease in serum level of testosterone in male rats. The level of testosterone decrease was dose related (Table 5).

Table 4: Effect CZW on the activity of serum catalase, glutathione peroxidase, glutathione-stransferase, superoxide dismutase and glutathione reductase in adult albino rats

Parameter	Treatment groups				
	Group I	Group II	Group III	Group IV	Units
Catalase	9.84 ± 0.26	9.64 ± 0.21	8.61 ± 0.14	8.14 ± 0.26*	μ moles of H_2O_2 decomposed/min/mg protein
Glutathione peroxidase	0.482 ± 0.07	0.434 ± 0.16ns	0.458 ± 0.04ns	0.407±0.06*	μ moles of NADPH oxidized/min/mg protein
Glutathione-Stransferase,	10.59 ± 0.17	8.63 ± 0.24ns	8.17 ± 0.32*	7.94 ± 0.11**	μ moles of conjugate formed
Superoxide dismutase	24.81 ± 0.67	20.11 ± 0.14ns	20.94 ± 0.34ns	17.58 ± 0.33*	Units
Glutathione reductase	16.88 ± 0.34	16.64 ± 0.11	14.56 ± 0.38*	11.39 ± 0.54**	μ moles of NADPH

Each value is SEM of 5 animals * P < 0.05, ** P<0.01 Control vs Treated: ↑ significantly increased

Table 5: Effect of CZW extract on the Sex hormone levels in male albino rats

Treatment groups	Parameters			
	Testosterone (ng/mL)	LH (μIu/mL)	Estrogen (pg/mL)	FSH (μIu/mL)
Group I	4.38 ± 0.74	3.84 ± 0.11	15.32 ± 0.77	1.32 ± 0.17
Group II	3.84 ± 0.93ns	3.71 ± 0.23	14.34 ± 0.91ns	1.45 ± 0.14
Group III	2.13 ± 0.61**	3.12 ± 0.94ns	16.27 ± 1.08ns	1.68 ± 0.26
Group IV	1.24 ± 0.34**	2.64 ± 0.27*	17.98 ± 0.94*	1.93 ± 0.17

Each value is SEM of 5 animals * P < 0.05 ,** P<0.01 Control vs treated

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the ethanol extract of whole plant of *C. zeylanicum* for 14 days caused a dose related decrease in the serum level of LH. The level of decrease is statistically significant.

Serum estrogen level

The ethanol extract of whole plant of *C. zeylanicum* (50, 100 and 150 mg/Kg body weight) caused an increase in the serum level of estrogen in male rats. Doses of 50, 100 and 150 mg/Kg body weight administered daily for 14 days cause raise in the serum level of estrogen.

Serum Follicle Stimulating Hormone (FSH) level

Pre-treatment with ethanol extract of whole plant of *C. zeylanicum* caused an increase in the serum level of FSH male rats compared to control. An increase in the serum level of FSH was statistically significant.

Fertility test

The results represented in Table 6 shows that intragastric administration of ethanol extract of whole plant of *C. zeylanicum* (50, 100 and 150 mg/Kg body weight) for 14 days to male rats causes a significant decrease ($p < 0.05$) in the number of females impregnated by male treated rats. The number of viable fetuses calculated after cesarean sections were significantly decreased ($p < 0.05$) in female rats impregnated with untreated male rats. On the other hand, the number of resorption sites was found to be increased in female impregnated by extract treated male rats when compared to controls.

Table 6: Effect of CZW extract on the Fertility of adult male albino rats

Groups	No. of male	No. of females	No. of pregnant females	No. of implantation	No. of viable fetuses	Total No. of resorption sites
Group-I	4	8	8/8 (100%)	9.57 ± 0.14	7.20 ± 0.65	**3**
Group-II	4	8	6/8 (75%)	7.84 ± 0.11	5.15 ± 0.16	**2**
Group-III	3	8	4/8 (50%)	6.84 ± 0.26*	3.19 ± 0.72**	**2**
Group-IV	3	8	2/8 (25%)	4.81 ± 0.17**	3.01 ± 0.13**	**2**

Each value is SEM of 5 animals * P < 0.05 ,** P<0.01 Control vs Treated

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 results revealed slight changes in the body weight of rats treated with ethanol extract of whole plant of *C. zeylanicum* (50, 100 and 150 mg/Kg body weight) for 14 days. The testes and other accessory sex organs, a significant weight reduction were seen in the testes, caput and caudal epididymal segments and the weight reduction was dose dependent. Reduction in the weight of testes 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 microenvironment of epididymis and thereby lead to alteration in sperm motility and metabolism[24].

In the present study, ethanol extract of whole plant of *Cynoglossum zeylanicum* treated rats decreased the sperm motility and sperm density in caudal and caput epididymal segments (Table 2). Drastic effect on the nature of the normal sperms in the caput and caudal region was observed in ethanol extract of whole plant of *C. zeylanicum* treated rats. Further tail region of the sperm in all the treated groups (Group-II, III & IV) were much affected than the head regions (Table 2). The development of normal and mature sperm is the key to optimum male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testes are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary[25]. FSH stimulates spermatogenesis in the steroli cells, while LH stimulates the production of testosterone in the leydig cells of the testes[26]. Many studies on the testes of rat treated with plant extracts has also demonstrated that the inhibitory activity on the proliferation of spermatogonia in mammals[27-29]. 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 pre-formation. The result of the present study suggest that ethanol extract of whole plant of *C. zeylanicum* for 14 days may affect the normal function of the steroli and leydig cells.

Sexual 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 testes. Among the ethanol extract treated groups II, III & IV (50, 100 and 150mg/Kg body weight) produced a significant reduction in the 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 somniferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis[30,31]. The presence of immature sperms was also observed in the experimental rats treated with ethanol extract of whole plant of *C. zeylanicum* (50, 100 and 150 mg/Kg body weight). This suggests that the 50, 100 and 150 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, confirm to those already reported and studied with various plant extracts[32-34]. The decrease in the caudal epididymal sperm counts are clear indications that, the ethanol extract of whole plant of *C. zeylanicum* can affect one or more aspects of spermatogenesis as well as spermigenesis. Though a direct effect of the ethanol extract of whole plant of *C. zeylanicum* 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.

The various other sperm abnormalities like sluggish motility, coiled tail and sperm maturation are also due to *C. zeylanicum* toxicity. The hitherto unreported abnormal sperm methodology, coiled tail and malformed head could be attributed to both testicular and epididymal effects of the ethanol extract of whole plant of *C. zeylanicum*. Coiling of the sperm tail is usually the product of abnormal axoneme and/or the other dense fibril. The outcome of the present study affirms the male reproductive toxic effects of *C. zeylanicum* when applied as a therapeutic agent. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequence of *C. zeylanicum* on the sperm may be taken as an 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 and functioning of the male reproductive system nor in the reproductive organs.

Superoxide dismutase (SOD) scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. In order to act against H_2O_2 it must be conjucated with catalase or glutathione peroxidase[35]. The reduced level of catalase, glutathione peroxidase, glutathione s-tranferase and glutathione reductase might be due to the excess production of anions in response to the ethanol extract of whole plant extract of *C. zeylanicum*. It is possible that an increased rate of ROS production may inhibit the action of these antioxidant enzymes or alternatively the decreased expression of these antioxidant enzymes may cause increased oxidative stress[36]. This will result in increased LPO, decreased sperm motility, viability and function, an ultimately leads to infertility.

The present study revealed a decrease in the serum level of testosterone. This observation was similar to the earlier findings of[37-39]. 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[40,41]. In males reduction of testosterone level may impair spermatogenesis and cause male infertility. This study further observed a dose dependent increase in the serum estrogen level. This increase might probably be due to the conversion of testosterone to estrogen[42,43].

Treatment with the ethanol extract of whole plant of *C. zeylanicum* (50, 100 and 150 mg/Kg body weight) was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicated structural and functional alteration in testes, epididymis and seminal vesicle. Depletion of sperm count and sperm motility in the drug treated rats suggests alteration in sperm motility resulted in partial

infertility within 14 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[44]. 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[45].

In the present study, a significant decrease in the sperm density and motility was observed in the cauda epididymis in the entire treatment group, which lead to proven in the impairment of fertility in all the treated groups. The results presented in this study also indicated that the treatment with the ethanol extract of whole plant of *C. zeylanicum* by adult male rats reduces the number of female's impregnation. In addition, the number of implantations and the number of viable fetuses were also decreased, this could be a reflect and may be due to the decrease in sperm motility and sperm density observed in this study. Hence, this may be due to the effects of the given plant extracts on the enzymes involved in the oxidative phosphorylation.

From the present study, it can be concluded that *C. zeylanicum* is capable to suppress male fertility without altering general metabolism. Hence the possible male contraceptive efficacy of *C. zeylanicum* whole plant extract cannot be ignored paving way to the smooth development for the clinicians' interests in clinical trials towards emergence of a potent herbal male contraceptive.

Recently many laboratories are engaged in developing male contraceptives from plants[46]. 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 anti-fertility drugs from plants[47, 48]. In the present study, dose dependent treatment of *Cynoglossum zeylanicum* whole plant extract 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 extract mechanism.

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