

An Assessment of Ant fertility Efficacy of Ethanol Extract of *Celastrus Paniculatus* Seed in Male Albino Rats

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Abstract: Crude ethanolic extract of *Celastrus paniculatus* L. seeds was administered to adult male albino rats for the evaluation of its antifertility effect. Aqueous solution of the extract, when administered orally for 45 days to cover the major portion of spermatogenesis at a dose level of 250 mg/kg body weight by oral route caused significant inhibition of spermatogenesis. There was a significant decrease in essential as well as accessory reproductive organs weight ($p < 0.05$). Decrease in cauda epididymal sperm count and motility was noted. Cytoplasmic droplets in the mid-piece of sperms were seen, which is a sign of arrival of immature spermatozoa in cauda epididymis region. Testicular histology revealed arrest of spermatogenesis at the secondary spermatocyte stage. Early stages of maturation (primary and secondary spermatocytes) showed nuclear pyknosis and vacuolization with the presence of round cells and necrotic material in the tubular lumina. Biochemical estimations of marker testicular enzymes, lactate dehydrogenase (LDH) and γ -glutamyl transpeptidase (γ -GT) showed an increase whereas sorbitol dehydrogenase (SDH) activity was significantly decreased ($p < 0.05$). These results suggest that *C. paniculatus*, may induce male infertility in rats, therefore, should be evaluated further as a potential antifertility agent.

Key word: *Celastrus paniculatus*, antispermatogenic, testicular enzymes, anti-fertility.

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I. Introduction

The expedition for the oral contraceptive agent that can control human fertility is not a new concept. Even though a broad array of synthetic contraceptive agents are available for use, these cannot be used incessantly due to their reported adverse side-effects. Due to this reason people are tracing back to age old traditional way of using plant medicines, which have lesser known or no side-effects. Regulation of male fertility by using materials of plant origin has become an area of active research.^{1,2,3}

Celastrus paniculatus (CP, Family-Celastrace; common name in Hindi-Malkangini, Jyotishmati) is an ancient Indian ayurvedic botanic, used for its immense human health benefits. Tribal system of medicine in different regions of India has claimed the use of its in treatment of wide range of ailments⁴ and seed oil is used as neuro tonic due to its beneficial effects on memory and intelligence.⁵ In ayurvedic medicine this plant is known for its other biological effects and is used for treating a variety of ailments, such as gout, rheumatism and others.⁶ The several phytochemicals are reported to present in CP such as sesquiterpenes, alkaloids celastrine, celapanine, celapanigine, celapagine polyalcohol, triterpenoid sterols *etc.*. So far different parts of CP including flowers, leaves and seeds have been evaluated for a variety of pharmacological actions.⁷ Seeds of CP were found to have hypolipidemic and antioxidant activities.^{8,9} It is also used as a nerve tonic¹⁰, sedative, and tranquilizer¹¹ and also as a cure for irregular menstrual cycles.¹² Preliminary findings with CP seeds oil have shown a possible antifertility effect on the testis and associated sex organs.^{13,14}

Though the seeds of CP have been screened for different pharmacological activities, no systematic study has been reported to explore its effects in male fertility regulation. This prompted us to investigate further its possibility as a potential agent for male fertility regulation. The present study involved estimation of certain marker testicular enzymes and testicular histology in the seed-extract treated rats. This investigation was also aimed at the assessment of the alteration produced, if any, in the reproductive system, fertility and behavior of male rats after oral administration of ethanolic extract of CP seeds.

II. Materials and Methods

Plant extract preparation: Seeds of CP authentically verified were obtained from the Botany Department of Lucknow University. Seeds were air dried under shade and powdered. The powdered seeds were extracted with 90% ethanol at 65°C for 24-48 hrs using a Soxhlet extractor. The total extract (19.8% w/v) thus obtained was concentrated in vacuo at reduced pressure and temperature. The extract was found to be freely soluble in water.

The required doses were freshly prepared at the time of administration by dissolving the extract in requisite quantities of water.

Animals: Colony –bred adult male albino rats (Sprague-Dawley strain) of proven fertility weighing between 200-250 grams were used for present investigation. Feed and water provided *ad libitum*, and the animals were maintained under standard laboratory conditions for the study.

Pilot Study: The animals were divided into two groups: control (1 ml distilled water/day/rat) and treated (CP seed extract, 250 mg/kg body weight/day x 45 days) group. Here, the dose level was selected on an exploratory basis. The experiment revealed that significant anti-spermatogenic activity was present at this dose level (vide infra).

Definitive Study: The extract was given at a daily dose of 50, 150, 250 and 350 mg/kg body weight orally with the help of feeding canula. In this study, 4 doses of the test material were used for: 1) Reconfirmation of the findings of the pilot study, and for 2) Establishing a dose- response relationship. Body weights of control and experimental animals were recorded before and after the treatment. The animals were sacrificed by exsanguinations under light ether anesthesia 24 h after the termination of each experimental schedule. Testes and accessory sex organs were removed and their weights were recorded.

Fertility test: Fertility test of individual male animal was done after completion of the treatment period. Males from each group were caged separately with regularly cycling females of proven fertility, in the ratio 1:2. Next morning after the mating exposure, the presence of spermatozoa in the vaginal smear of the female animal was taken as day '0' (Zero) of pregnancy. Mated females were laprotomized on 15th day of post-coitum, the number of corpora lutea, and the number of implantation and/or resorption sites (if any), were recorded.

Sperm motility and count: Motility and number of cauda epididymal spermatozoa in control and treated rats were assessed by using a haemocytometer (Neubaur's Chamber). Motile spermatozoa were calculated per unit area and expressed as % sperm motility.¹⁵ Sperm counts were recorded as million/ml of suspension.¹⁶

Enzymes estimation: A portion of the testis was homogenized (1:9) in 0.2 M Tris/ HCl buffer, pH 7.0, having 0.1% cetyltrimethylammonium bromide using Potter Elvehjem homogenizer for the estimation of sorbitol dehydrogenase (SDH).¹⁷ In the same homogenate lactate dehydrogenase (LDH) was also estimated.¹⁸ Another portion of the testis was homogenized (1:9) in 0.05 M Tris, HCl Buffer, pH 7.4, for the assay of γ -glutamyl transpeptidase (γ -GT).¹⁹ Protein content of the sample was estimated according to the method of Lowery et al.²⁰ The enzymes activities have been expressed as specific activities (Units/mg protein).

Data Analysis: Data obtained were analyzed statistically by students 't'-test and values were expressed as Mean \pm SE. $p < 0.05$ considered as significant.

III. Results

Body Weight: There was a pronounced reduction in body weights of rats after CP (250 mg/kg b.wt.) extract treatment as compared to their control group (Table 1).

Organs Weight: Statistically significant decrease in weight of reproductive organs viz. testis, epididymis, seminal vesicle and prostate were found while coagulatory gland weight exhibited statistically insignificant change (Table 1).

Mating Behavior and Fertility: Incidence of mating was similar among the treated and control animals at all the dose levels. However, extract treatment reduced the fertility of male rats in a dose related manner (Table 2), and no implantation sites were seen at 250 and 350 mg/kg dose levels. After termination of treatment reversal of fertility was noted within 30 days.

Sperm Count, Morphology and Motility: The total number and motility of cauda epididymal spermatozoa reduced significantly in treated animals, as compared to control group (Table 3). The presence of cytoplasmic droplets in the mid piece of cauda epididymal sperms was also observed.

Testicular Enzymes: The specific activities of marker testicular enzymes viz. LDH and γ – GT increased significantly in testis of CP treated animals, after 45 days treatment. The activity of testicular SDH decreased significantly after treatment (Table-4).

Testicular Histology: All the seminiferous tubules in the control animals showed normal spermatogenesis (Fig.1) and those of the animals treated with CP for 45 days exhibited diminished spermatogenesis. The rats with decreased spermatogenesis showed maturation arrest at the stage of secondary spermatocytes with absence of spermatozoa and spermatids in the seminiferous tubules. Early states of maturation such as primary and secondary spermatocytes showed nuclear pyknosis and vacuolization with presence of round cells and necrotic materials in the lumen of tubules. Interstitial tissue, basement membrane of seminiferous tubules, Sertoli cells and spermatogonia were not affected by treatment. Vascularity of organ also remained unaffected by the treatment. (Fig. 2, 3 and 4)

IV. Discussion

Medicinal plants have been valuable for humans since prehistoric days. The present data shows that the ethanolic extract of CP seeds suppresses sperm counts and causes lesions on the seminiferous tubules. In this study, the decrease in sperm motility and total epididymal sperm count caused by extract treatment is directly responsible for loss of fertility of male rats, since forward and progressive movements and adequate concentration are necessary for penetration of spermatozoa through cervical mucus and cumulus cell mass to fertilize the ova.²¹ It has also been observed in our experiments that crude extract administration has got an effect on embryo resorption. The mechanism of extract induced embryo resorption is not traced out, but could be due to physiological/genetic alteration in the spermatozoa.

Histoarchitecture of seminiferous tubules of rats treated with seeds extract showed a progressive damage in their germinal epithelium and terminated into an arrest in spermatogenesis along with regression of reproductive organs weight. Further arrest of spermatogenesis resulted in to the formation of multinucleated giant cells in testis. Similar impairments of spermatogenesis characterized by germ cell depletion and exfoliation have been noted by earlier researchers by *C. paniculatus* seeds oil.^{22,23} and also under other experimental conditions like cryptorchidism, hypophysectomy *etc.*^{22,24,25} Further the observed vacuolization and resultant desquamation of the germinal epithelium in treated rats testis is due to the reaction of Sertoli cells to the damaged process of spermatogenesis.¹⁸ Following the experimentally induced spermatogenic arrest, the accessory male sex organs also showed morphological regression, which was probably due to an impairment of normal steroidogenic function.^{26,27} and the subsequent regressive changes in androgen dependent sex organs.²² Certain other plant products are also reported to induce spermatogenic arrest in a similar manner.^{3,28,29} Increase in testicular lipid has been reported along with the spermatogenic arrest after treatment with *C. paniculatus* seed extract treatment.^{22, 23} Similar lipid accumulation has also been reported after *Calotropis procera*³⁰, hypophysectomy³¹ and cryptorchidism.²⁴ Probably CP extract reduced the conversion process of testicular lipids into androgens with a resultant accumulation of lipids on the testis, and the simultaneous regression in androgen dependent accessory sex organs.¹⁴ Similar to our study spermatogenic impairment and subsequent regression on the accessory glands due to different experimental conditions such as hypophysectomy, testosterone depletion and antiandrogen treatment had also been reported.^{14, 32.}

The marker enzymes studies are associated with specific cell types of testis so that is possible to correlate the changes in testicular enzymes activities with changes in respective cell types.³³ As seen in present study, a significant decrease in specific activities of SDH, marker for post meiotic germ cells, indicate the depletion of these cells after the treatment.^{34,35} Significant increase in specific activity of LDH, is a sign of degenerative germinal epithelium.³² increased activity of γ -GT, a marker of Sertoli cells,³⁶⁻³⁸ may indicate that these cell types are not affected by the treatment. However, due to massive enrichment of the surviving cell type caused by depletion of affected cells population and also by a marked reduction in testicular weight, alteration in marker enzyme activity of unaffected or less affected cells likely to be observed. Conclusion obtained from the above discussion that the Sertoli cells are not target for CP extract and that this acts directly on germ cells. Changes in specific activity of SDH and LDH after 45 days of treatment, indicates that spermatogenesis is unable to proceed beyond meiosis. However, the spermatids depletion was not due to an effect on Sertoli cells. Results of present study indicate that CP exerts a direct action on germ cells with no damage to Sertoli cell. The arrest of spermatogenesis occurs predominantly at the spermatid stage by gossypol (known anti spermatogenic effect) whereas the arrest was noted at spermatocyte stage in case of CP. One of the earliest signs of gossypol action in rat is Sertoli cell damage³⁹ while CP does not exert any adverse effect on Sertoli cells, spermatogonia and early spermatocytes and may have reversal of fertility in treated animals. Thus CP may hold promise for development of plant origin male contraceptive.

V. Conclusion

In literature it is documented that plant showed the maximum pharmacological activities when tested on extracts obtained from polar solvents like methanol and ethanol.⁴⁰ In our study the extractive value of the CP seed for alcohol was found to about 19.8% w/v. therefore we selected alcoholic extract for the *in vivo* study in animals. Preliminary phytochemical screening of the extract also indicated presence of alkaloids and oil has

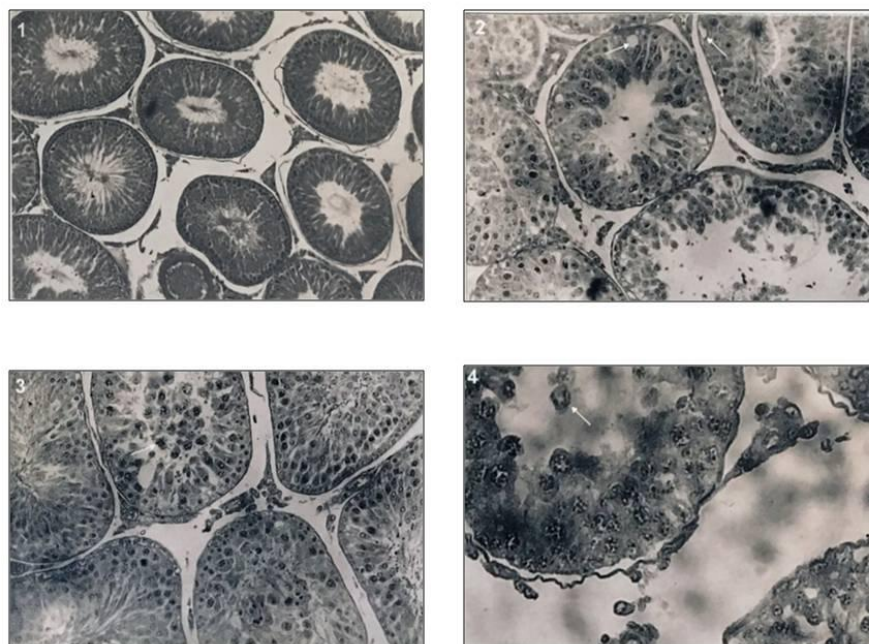
been reported for fatty acids.⁴⁰ In future, to have an insight in its mechanism of action at the hormonal level it is necessary to study the Leydig cell function and serum level of testosterone and gonadotropins. It is also essential to establish the genotoxic potential and reversibility of the anti-spermatogenic effect of the CP extract before it may be selected for development as a male anti-fertility agent. Further work on these aspects is in progress.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Reference

- [1]. Bajaj VK, Gupta RS. Review on research of suppression male fertility and male contraceptive drug development by natural products. *Nat Prod Commun.* 2013; 8(8):1183-1193.
- [2]. Sharma N, Jacob D. Assessment of reversible contraceptive efficacy of methanol extract of *Mentha arvensis* L leaves in male albino mice. *J Ethnopharmacology.* 2002; 80 (1): 9-13.
- [3]. Cordero-Martínez J, Aguirre-Alvarado C, Guzmán-Soriano JG, Sánchez-Arroyo CE, Flores-Alonso JC, Rodríguez-Páez L. Effects of aqueous crude extract of *Echeveria gibbiflora* on mouse sperm function. *Syst Biol Reprod Med.* 2016; 62(5):343-352.
- [4]. Monojit D, Reshmi P, Harshita K, Nishteswar K. Medico ethnobotanical perspectives of *Jyotismati* (*Celastrus paniculatus* Willd): A herbal tranquilizer. *J Drug Deliv Ther.* 2012; 2: 8-11.
- [5]. Nadkarni KM. *Indian materia medica.* Mumbai: Popular Prakashan; 2007. p. 296.
- [6]. Sharma P. *Charak Samhita.* Vol. IV. Varanasi: Chaukhambha Orientalia; 2012. p. 201.
- [7]. Ahmad F, Khan RA, Rasheed S. Preliminary screening of methanolic extracts of *Celastrus paniculatus* and *Tecomella undulata* for analgesic and anti-inflammatory activities. *J Ethnopharmacol.* 1994; 42:193-198.
- [8]. Mathur NT, Varma M, Dixit VP. Hypolipidaemic and antiatherosclerotic effect of *Celastrus paniculatus* seed extract in cholesterol fed rabbits. *Indian Drugs.* 1993; 30:76-82.
- [9]. Kumar MH, Gupta YK. Antioxidant property of *Celastrus paniculatus* willd: A possible mechanism in enhancing cognition. *Phytomedicine.* 2002; 9:302-311.
- [10]. Aryabishak A. "Oil of *Celastrus paniculatus* as a nervine tonic, sedative and diuretic." In: *Sastu-Sahitya Publication, 9th Ed.* Ahmedabad, India, 1939; 334.
- [11]. Joglekar G, Balwani JH. Review of tranquilizing effects of *Celastrus paniculatus*. *J Indian Med Association* 1967; 1: 190.
- [12]. Nadkarni AK. *Indian Materia Medica.* Edn 3, Vol. I, Popular Prakashan, Bombay, 1976, pp. 296.
- [13]. Bidwai P.P, Wangoo D. Effect of an oil extract from the seeds of *Celastrus paniculatus* on the testis of albino rats. *Journal of Andrology.* 1985; (suppl) 6:18.
- [14]. Zhen QS, YE X, Wei ZJ. Recent progress in research on *Tripterigium* : a male antifertility plant. *Contraception.* 1995; 51(2): 121-129.
- [15]. Freund M, Carol B. Factors affecting haemocytometer counts of sperm concentration in human semen. *J Rep Fertility.* 1964; 8: 149-155.
- [16]. Adelman MM, Eileem MC. *Atlas of Sperm morphology,* ASCP press. 1936.
- [17]. Bishop DW. 1968. Sorbitol dehydrogenase in relation to Spermatogenesis and fertility. *J Rep Fert* 17: 410.
- [18]. Yokoe Y, Hall PF. Testicular Phospholipids: I. Action of follicle stimulating hormone (FSH) upon the biosynthesis of phospholipids by rat testis. *Endocrinology.* 1970; 86: 18-28.
- [19]. Roomi MW, Goldberg M. 1981. Comparison of γ – glytamyl transferase induction by phenobarbital in rat, guinea pig and rabbit. *Biochem. Pharmacol.* 30(12): 1563-1571.
- [20]. Lowry OH, Rosenbrough MJ, Farr AB, Randall RJ. Protein measurement with folin phenol reagents. *J of Biol Chem.* 1951. 193 (1): 265-275.
- [21]. Aitken RJ, Sultan M, Warner P, Richardson DW. Relationship between the movement characteristics of human spermatozoa and their ability to penetrate cervical mucus and zona-free hamster oocytes. *J. Reprod Fert.* 1985; 73(2):441-449.
- [22]. Wangoo D, Bidwai PP. Antispermatic effects of *Celastrus paniculatus* Seed extract on the testis of albino. *Fitoterapia.* 1988; 59: 377.
- [23]. Bidwai PP, Wangoo D, Bhullar N. Antispermatic action of *Celastrus paniculatus* seed extract in the rat with reversible changes in the liver. *J Ethnopharmacol.* 1990. 28(3):293-303.
- [24]. Fleegeer JL, Bishop JP, Gomes WR, Van Demark NK. Testicular lipids I.: Effects of unilateral cryptorchidism on lipid classes. *J Rep and Fert.* 1968; 15: 1-7.
- [25]. Fransworth NR and Waller DP. Current status of plant products reported to inhibit sperm. *Research Frontiers in Fertility Regulation.* 1983; 2(1): 1-16.
- [26]. Saksena SK, Salmons RA. Antifertility effects of gossypol in male hamsters. *Fertil Steril.* 1982; 37(5): 686-690.
- [27]. Shandilya L, Clarkson TB, Adams MR, Lewis JC. Effect of gossypol on reproductive and endocrine functions of male cynomolgus monkeys (*Macaca fascicularis*). *Biology of Reproduction* 1982; 27: 241-252.
- [28]. Singh N, Singh SK. Aqueous fruit extract of *Mimusops elengi* causes reversible suppression of spermatogenesis and fertility in male mice. *Andrologia.* 2016; 48(7): 807-816.
- [29]. Verma HP, Singh SK. Antifertility efficacy of *Coccinia indica* in male mice and its possible mechanisms of action on spermatogenesis. *Gen Comp Endocrinol.* 2017.15; 241:89-99.
- [30]. Garg A. Effect of *Aak Calotropis procera* (Ait) R.Br. Flowers extract on testicular function of Indian desert male Gerbil *Meriones hurrianae* Jordan: A Biochemical histological study. *Ind J Exp Biol.* 1979; 17: 859-862.
- [31]. Vassault A. In: *Methods of enzymatic analysis* (Eds. Bregmeyer 118) 1983.
- [32]. Brandes D. Fine structure and cytochemistry of male sex accessory organs. In: "Male accessory sex organs, structures and functions in Mammals." Brandes D (Ed.), Academic Press, N.Y., USA and London, UK.P. 1974; 17-113.
- [33]. Sherins RJ, Hodgen GD. Testicular γ – glutamyl Transpeptidase and index of Sertoli cell function in man. *J. Rep. Fert.* 1976. 48: 191-193.
- [34]. Bishop DW. Sorbitol dehydrogenase in relation to Spermatogenesis and fertility. *J Rep Fert.* 1968; 17: 410.
- [35]. Mills NC, Means RA. Sorbitol dehydrogenase of rat testis, changes of activity after hypophysectomy and following gonadotropic hormone administration. *Endocrinology* 1972; 91(1): 147-156.

- [36]. Niemi M, Setchell BP. Gamma glutamyl transpeptidase in the vasculature of the rat testis. *Biology of Reproduction* 1986; 35(2): 385-391.
- [37]. Steinberger A, Steinberger E. The testis (Eds. Johnson AD, Gomes WR, Vandemark NL). Vol. II, Academic Press, New York. 1977. P. 363.
- [38]. Steinberger A, Steinberger E. Hormonal Control of spermatogenesis, In: SJ Segal, R Crozeier, PA Corman and PG Condlifte (Eds). *The Regulation of Mammalian Reproduction*. Charles C. Thomas, Springfield. 1973. P. 139.
- [39]. Qian SZ, HU JH, Ho LX, Sun MX, Huang YZ, Fong Jh. The first clinical trial of gossypol on male antifertility. In Turnei P (Ed.), *Clinical Pharmacology & Therapeutics*, McMillan, New York. 1980. P. 489.
- [40]. Kulkarni YA, Agarwal S, Garud MS. Effect of Jyotishmati (*Celastrus paniculatus*) seeds in animal models of pain and inflammation. *J Ayurveda Integr Med*. 2015; 6(2):82-88.



Legends to the Figures

- Fig. 1: Seminiferous tubules of control rat showing the normal stages of spermatogenesis. Hematoxylin and Eosin. (x40)
- Fig. 2: Testis of treated rats showing vacuolization in the seminiferous epithelium and spermatids and spermatozoa are not seen in lumen of these tubules. Hematoxylin and Eosin. (x100).
- Fig. 3: Seminiferous tubule of treated rats showing multinucleated giant cells in lumen. Hematoxylin and Eosin. (x100).
- Fig. 4: Magnification of seminiferous tubule of treated rats showing multinucleated giant cells in lumen. Hematoxylin and Eosin. (x400).

Table 1: Effect of *Celastrus paniculatus* treatment on body weight and reproductive organs weight or rats

Group	Body weight (grams)		Reproductive organs weight (grams)				
	Initial	Final	Testis	Epididymis	Seminal vesicle	Ventral prostrate	Coagulating gland
Control	214.17±3.7	235±3.5	A 1.64±0.09 R 0.69±0.04	0.54±0.01 0.24±0.01	0.55±0.04 0.23±0.02	0.26±0.03 0.11±0.01	0.15±0.01 0.06±0.01
<i>Celastrus paniculatus</i> (250mg/kg b.wt.)	208.50±4.7	184.0±3.0**	A 0.87±0.02** (47%)	0.62±0.20* (15%)	0.36±0.16** (34%)	0.21±0.01* (20%)	0.13±0.01 ^{NS} (12%)
			R 0.47±0.01** (32%)	0.34±0.1** (44%)	0.19±0.01* (16%)	0.12±0.01 (2%)	0.07±0.01 (13%)

Data represent Mean ±SEM (N=6)

*P≤0.05 and **P≤0.01 considered to be statistically significant. ^{NS}P≥0.05 considered as statistically insignificant

A: absolute weight, R: relative weight

Table 2: Effect of *Celastrus paniculatus* on fertility of male rats

Group	No. of successful mates (Total no used4)	No. of mated females (total no. used 8)	Corpora lutea sites	Implantation sites		
				Total	Normal	Resorbed
Control	4	7	14.16±0.55	8.5±1.25	5.16±0.89	3.33±1.24
<i>Celastrus Paniculatus</i> (250mg/kg b.wt.)	3 (75%)	6	14.16±0.51	9.6±1.29	-	9.6±1.20

Data represent Mean ±SEM (N=6)

Table 3: Effect of *Celastrus paniculatus* on %sperm motility and sperm count of rats

Group	%sperm motility	Total sperm count (per epididymis)x10 ⁶
Control	87.17±1.68	224.00±14.73
<i>Celastrus paniculatus</i> (250mg/kg b.wt.)	64.33±1.58**	83.30±6.15**

Data represent Mean ±SEM (N=6);

*P≤0.05 and **P≤0.01 considered to be statistically significant

^{NS}P≥0.05 considered as statistically insignificant

Table 4: Effect of *Celastrus paniculatus* on marker testicular enzymes of rats

Group	Enzymes		
	Sorbitol dehydrogenase	Lactate dehydrogenase	γ-glutamyl transpeptidase
Control	4.41±0.16	250.60±18.50	39.56±5.7
<i>Celastrus paniculatus</i> (250mg/kg b.wt.)	3.64±0.26* (18%)	479.01±12.19** (92%)	59.57±1.1** (51%)

Data represent Mean ±SEM (N=6);

*P≤0.05 and **P≤0.01 considered to be statistically significant. ^{NS}P≥0.05 considered statistically insignificant
Enzymes activities are expressed as specific activities (n moles of substrate oxidized or product formed/min/mg protein)

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