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Antifertility effect of methanolic extract of aerial plant parts of *Calotropis gigantea* L. in male albino wister rats

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Abstract
The effects of methanolic extract of aerial plant part of *Calotropis gigantea* on reproduction have been studied on male rats. The study was divided into four groups of 10 animals each. The first group (I) received vehicle alone to serve as control. The second and third (II and III) of animals were administered the root extract daily at 100 mg/kg body weight, and 200 mg/kg body weight, respectively, for a period of 60 days. Animals of group IV served as recovery group and treated with 100 mg dose for 60 days and kept of recovery period of 30 days. Significant decreases in the weights of testes, Epididymis, seminal vesicle and ventral prostate were observed. A dose related reduction in the testicular sperm count, epididymal sperm count and motility, number of fertile males, ratio between delivered and inseminated females and number of pups were observed. Significant reduction in serum concentration of luteinizing hormone and testosterone were observed. No distinct change in serum FSII concentration was recorded. The final body weights of all groups were elevated markedly. Results reveal that methanolic extract of *Calotropis gigantea* could be used as potential source of anti-fertility agent.

Keywords: Methanolic extract, *Calotropis gigantea*, sperm count, antifertility agent

1. Introduction

The herbal medicines occupy distinct position right from ancient period. Many higher plants accumulate extractable organic approaches substances in quantities sufficient to be economically management of diseases. *C. gigantea* is very common weed found throughout plains and lower hills of Rajasthan. Chemical investigations of this plant has shown the presence of various chemical constituents such as toxic glycosides calotropin, uscharin and calotoxin, cardenolides, flavonoids, and saponins [1]. Traditionally *Calotropis* is used alone or with other medicines [2] to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea [3]. The objective of this work is to determine the effects of methanolic extract of aerial plant parts *C. gigantea* on some reproductive and biochemical parameters in male Wistar rats.

2. Materials and Methods

2.1 Animals, Grouping and Experimental Design

30 (3 weeks old) sexually mature male albino Wistar rats, bred and maintained at the Experimental Animal House, Department of Zoology, University of Rajasthan, were used for this study. There were 4 groups (of 10 rats each) designated as Group I, Group II, Group III and Group IV; 10 rats served as control and 10-10 rats served as test animals.

2.2 Preparation of *Calotropis gigantea* extract

Aerial plant parts (containing leaves, stem, flowers and fruits) of *C. gigantea* plant were collected from different places of Jaipur city and were identified at the Department of Botany, University of Rajasthan, Jaipur. Plant parts were shed dried for one week at 28°C and macerated and 50gm powdered material was soxhlet extracted with 50ml of methanol and later marc was collector and filtered. The filtrate was air dried and concentrated. At the time of administration dose prepared by dissolving 1gm concentrated extract in 10%DMSO.

2.3 Administration of *Calotropis gigantea*

Dose were administered orally at a dosage of 100 mg/gm body weight to group I and 200 mg/gm body weight to group II once daily using a 5ml oral cannula for 60 days. The control animals in each group received 10% DMSO for the same number of days. Animals of group IV served as recovery group and treated with 100 mg dose for 60 days and kept of recovery period of 30 days.

2.4 Sacrification schedule
Twenty-four hours after their last dose, the rats were weighed and sacrificed under light ether anesthesia.

2.5 Parameters Studied
2.5.1 Body and Organs weight
The weights of the animals were noted prior of experiment and before scarification. Twenty-four hours after their last dose, the rats were sacrificed under light ether anesthesia. The testes, seminal vesicles, epididymis and ventral prostate were dissected out, cleared of adhering fat and extraneous tissue before weighing on single pan balance.

2.5.2 Fertility Test
Fertility test of individual rat was accessed prior to the experiment and after 55 days of treatment. To assess fertility of each male rat was cohabitated with proestrous females in 1:2 ratio, vaginal smear was reported every morning for positive mating and number of litters delivered was noted.
Sperm motility and Sperm density in cauda epididymis ware reported [4].

2.5.3 Tissue Biochemistry
The testis, cauda epididymis, seminal vesicles were freezed for the analysis of protein [5], sialic acid [6] cholesterol [7], glycogen [8], and fructose [9] contents.

2.5.4 Serum Hormonal Assay
Testosterone, FSH and LH were analysed [10].

2.5.5 Testicular histology
One (right) of the two testes of each animal was fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5 mm, stained with Harris' hematoxylin and eosin and observed under a light microscope.

2.6 Ethical aspects
The study was approved by the ethical committee of the University Department of Zoology, Jaipur, India. Indian National Science Academy, New Delhi (INSA, 2000) guidelines were followed for maintenance and use of the experimental animals.

2.7 Statistical analysis
Data were expressed as Mean ± SEM.

Table 1: Changes in body and organs weight of male rats treated with methanolic extract of C. gigentea

Treatment	Initial B. wt. (gm.)	Final B. wt. (gm.)	Testes (mg/100gm b. wt.)	Epididymis (mg/100gm B. wt.)	Seminal vesicle (mg/ 100gm B. wt.)	Ventral Prostate (mg/100gm B. wt.)	Kidney (mg/100gm B. wt.)	Heart (mg/100gm B. wt.)	Liver (mg/100gm B. wt.)	Adrenal (mg/100gm B. wt.)
Group-I Control Vehicle treated	134.375 ±2.397	158.375 ±2.744	780.247 ±6.735	520.286 ±3.216	447.605 ±4.282	167.362 ±1.310	644.038 ±3.055	405.196 ±3.885	1342.537 ±6.476	7.265 ±0.171
Group-II 100mg/kg b.wt / day	137 ±1.311	159.375 ±1.564	708.945 ±2.557*	516.142 ±1.024	406.546 ±2.307*	138.172 ±1.931*	664.290 ±2.179	411.016 ±6.880	1338.292 ±1.664	7.352 ±0.128
Group-III 200mg/kg b.wt / day	135.375 ±4.21	160.5 ±2.824	648.839 ±2.398**	512.649 ±4.605	399.35 ±2.307**	124.556 ±1.198**	673.165 ±4.016	390.331 ±331	1354.288 ±6.550	7.56 ±0.110
Group-IV Recovery (withdrawal of 30 days	135.22 ±3.214	159.264 ±0.924	778.982 ±3.541	519.234 ±9.234	444.971 ±3.632	167.120 ±6.333	644.213 ±6.246	400.165 ±5.216	1345.265 ±2.065	7.541 ±0.215

Data are expressed as Mean ±S.E,* Significant (*P*≤0.05), ** Highly Significant (*P*≤0.01).

Table 2: Sperm Dynamics and Fertility test of male rats treated with methanolic extract of C. gigentea

Treatment	Sperm Motality (%)	Sperm Density Cauda(million/ mm^3)	Fertility (%)
Group-I Control Vehicle treated	68.4775±1.121	60.1337±0.994	91.75%
Group-II 100mg/kg b.wt / day	64.412±1.004*	57.895±0.520*	45% (-) *
Group-III 200mg/kg b.wt / day	62.345±1.005**	52.1137±0.794**	37.3% (-)**
Group-IV Recovery (withdrawal of 30 days	67.345±1.95	60.985±3.95	62.5% (-)

Data are expressed as Mean ±S.E, * Significant (*P*≤0.05), ** Highly Significant (*P*≤0.01).

Table 3: Biochemical changes in Tissue of male rats treated with methanolic extract of C. gigentea

Treatment	Protein (mg/gm)					Sialic acid (mg/gm)		
	Testis	Epididymis	Seminal Vesicle	Vas-deferens	Ventral Prostate	Testis	Epididymis	Seminal vesicle
Group-I Control Vehicle treated	244.258±6.006	269.249±6.092	241.123±5.394	234.230±0.123	181.272±7.888	5.77±0.123	5.65±0.108	5.26±0.088
Group-II 100mg/ kg b.wt / day	230.73±1.444*	250.597±2.618	217.594±2.425*	228.129±6.241	170.206±0.885	4.37±0.246*	4.94±0.152*	4.37±0.173*
Group-III 200mg/ kg b.wt / day	223.272±0.888**	239.652±1.368*	206.43±1.115**	225.3690±0.645*	157.953±1.143	4.22±0.111**	4.382±0.104**	4.2±0.094**
Group-IV Recovery (withdrawal of 30 days)	244.124±0.672	268.144±0.574	240.957±1.24	231.245±0.894	176.999±4.648	5.71±6.482	5.569±3.154	5.92±3.469

Data are expressed as Mean ±S.E, Significant (*P*≤0.05), ** Highly Significant (*P*≤0.01).

Table 4: Biochemical changes in Tissue of male rats treated with methanolic extract of C. gigentea

Treatment	Cholesterol (mg/gm)			Glycogen (mg/gm)			Ascorbic acid (mg/gm)	Fructose (mg/gm)
	Testis	Liver	Heart	Testis	Liver	Heart	Adrenal	Seminal Vesicle
Group-I Control Vehicle treated	7.702±0.344	9.307±0.395	7.308±0.402	7.321±0.419	9.307±0.395	5.76±0.444	5.32±0.523	5.322±0.218
Group-II 100mg/kg b.wt / day	6.643±0.210*	8.854±0.395	7.217±0.410	7.498±0.402	8.854±0.344	6.505±0.212	5.96±0.429	4.892±0.214*
Group-III 200mg/kg b.wt / day	6.16±0.084**	9.042±0.063	7.188±0.120	7.411±0.107	9.042±0.063	5.619±0.164	5.66±0.153	4.601±0.049**
Group-IV Recovery (withdrawal of 30 days)	6.987±6.541	9.3±0.694	7.295±4.125	7.297±0.111	9.295±6.999	5.70±1.987	5.32±2.114	5.207±0.947

Data are expressed as Mean ±S.E, * Significant (*P*≤0.05), ** Highly Significant (*P*≤0.01).

Table 5: Hormonal assay of male rats treated with methanolic extract of C. gigentea

Treatment	Testosterone	LH	FSH
Group-I Control Vehicle treated	2.61±0.060	2.208±0.067	1.171±0.121
Group-II 100mg/kg b.wt / day	2.311±0.078*	1.927±0.011	0.901±0.018
Group-III 200mg/kg b.wt / day	1.842±0.0512**	1.777±0.001	0.791±0.007*
Group-IV Recovery (withdrawal of 30 days)	2.357±3.064	2.00±0.364	1.169±6.297

Data are expressed as Mean ±S.E,* Significant ($P \leq 0.05$), ** Highly Significant ($P \leq 0.01$).

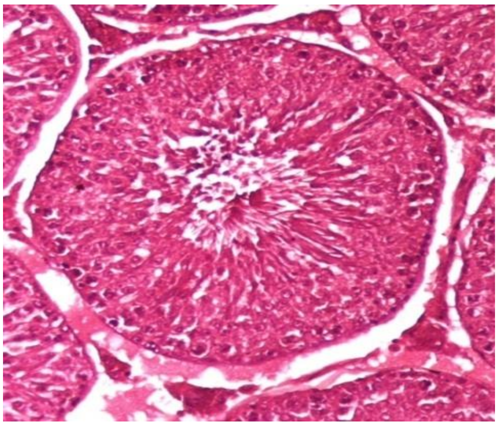

Fig 1: Photomicrograph of testis of a rat of group I (vehicle treated control) showing normal features with normal features. H & E×/400.

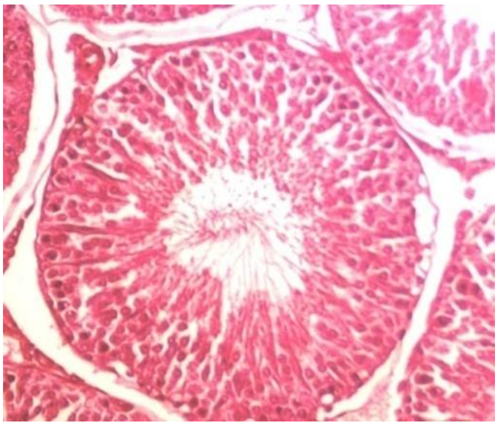

Fig 2: Photomicrograph of an affected region of testis of a rat of group II (100 mg/kg body weight, po) after 60 days of treatment. H & E ×/400.

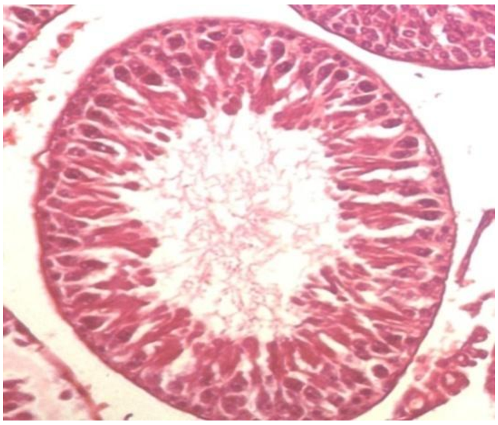

Fig 3: Photomicrograph of testis of a rat of group III (200 mg/kg body weight, po) after 60 days of treatment showing reduced seminiferous tubular diameter and cellular damage of testes.

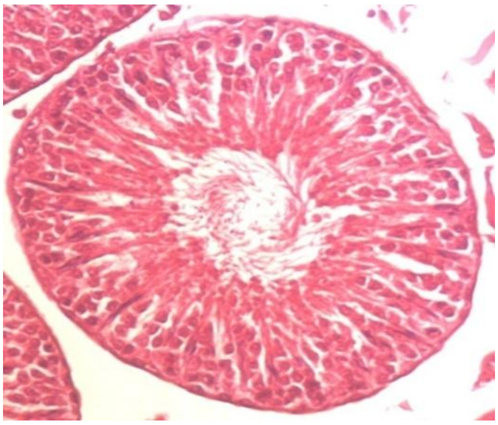

Fig 4: Photomicrograph of testes of rat of group IV (100 mg./kg./wt. extract) after 60 days of treatment further 30 days recovery period showing normal cellular structure as compare to group I.

3. Results

3.1 Body and organ weight

The final body weights of all groups increased markedly when compared with their respective initial body weights. The final body weights of group II (I00 mg/kg b.wt. po), and group III (200 mg/ kg b.wt. po) significantly increased when compared with the final body weight of group I (vehicle treated control) animals ($P \leq 0.05$). A great decline in the weights of testes, seminal vesicle, vas deferens and ventral prostate (expressed in mg/200 gm of body weight; $P \leq 0.01$) were observed in all treatment groups when compared with group I animals but weight of liver and adrenal increased significantly in group III and IV but decrease in group II. Weight of heart increase in group II but slightly decreased in group III and IV (Table 1).

3.2 Fertility Test

The number of fertile males decreased in all treatment groups, leaving 2, 3 and 1 still fertile after 60 days of treatment, respectively, in groups II, III and IV. The ratio between delivered and inseminated females (5/10, 4/10 and 2/10 animals versus 10/10 animals in group I), and the number of pups (53, 26 and 14 pups versus 87 pups in group I) dropped after 60 days of treatment. However, no significant difference was observed in the litter size of the females in any group. Spermatozoa with shortened and thinned flagella were present in the semen found in the vaginal smears of females, which were cohabited with the treated males. All delivered pups were normal and healthy.

A significant decrease in percent cauda epididymal sperm motility was evident in groups II, III and IV animals when compared with group I animals. After 60 days of treatment only 64.41, 62.34($P \leq 0.05$) and 67.34($P \leq 0.01$), respectively, of spermatozoa versus 68.47 of spermatozoa in group I were found to be motile. The sperm counts from the cauda epididymis and testes were also diminished significantly in all treatment groups (P0.01). Fertility (%) were declined 45% in group I ($P \leq 0.05$) and 31.3% ($P \leq 0.01$) in group II (Table 2) but increased in recovery group IV 62.5%.against control group I (91.75%).

3.3 Serum Biochemical analysis

The protein level in testis in group II is 230.73 mg/gm ($P \leq 0.05$) and group III is 223.27mg/gm ($p \leq 0.01$), epididymis ($p \leq 0.01$), seminal vesicle in group II is 217.594mg/gm ($p \leq 0.05$) and in group III is 206.43mg/gm ($P \leq 0.01$) and vas deferens in group III is 225.369 mg/gm ($p \leq 0.05$), was significantly declined after administration of methanolic extract of *C. gigantea*. In the treated rats sialic acid level were significant decrease in testis ($p \leq 0.01$), epididymis ($p \leq 0.01$) and seminal vesicle ($p \leq 0.01$) (Table-3). In the testis, level of glycogen ($p \leq 0.05$) and cholesterol ($p \leq 0.01$) were significant decreased (Table-4). In the seminal vesicle, level of fructose ($p \leq 0.01$) was decreased.

3.4 Serum Hormonal Assay

Significant decline in serum testosterone levels was observed in all treatment groups when compared with group I (vehicle treated control). The respective levels in groups II, III and IV were 2.311, 1.842 and 2.357 mg/ml versus 2.61 mg/ml in group I. Serum LH and FSH levels were significantly reduced in treatment group (Table 5).

3.5 Testicular histology

The testes of group I (vehicle treated control) animals showed normal features with successive stages of transformation of the seminiferous epithelium into spermatozoa. Leydig cells were situated in between the tubules (Fig. 1). Histopathological examination of the testis after 60 days of treatment showed a clear correlation between the dose and the severity of lesions of the seminiferous epithelium. In rats treated with 100 mg/kg, po (group II) some lesions were observed and affected only a few tubules (Fig. 2). The dose of 200 m g/kg, po (group III) produced diffuse changes of the tubules (Fig. 3). Since the differences among the doses were more quantitative than qualitative, only a general description of the findings related to all treatment groups is given. The seminiferous tubules appear reduced in size with a frequently filled eosinophilic material but with normal lamina propria. In general, diminished spermatogenesis was evident at secondary spermatocyte stage. Pachytene spermatocytes were undergoing degeneration. Disorganization and sloughing of immature germ cells were visible. The nuclei became pyknotic. Leydig cells revealed signs of atrophy. Contrary to this, no morphological changes were observed in the sertoli cells.

4. 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. Similar work was observed in *C. procera* [11]. Gupta *et.al.* (1990) isolated novel compound Calatropin from roots of *C. procera* and observed antifertility effect in gerbil and rabbit. Antifertility and functional alteration in genital organs of male Swiss albino mouse [12] have also been studied previously. In the present study, the administration of methanolic extracts of *C. gigantea* do not exhibit 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. A significant weight reduction was reported in the testes, caudal epididymis. It may be due to deficiency of androgen, which was not enough produced for maintaining the weight of gonads and accessories [13, 14]. Methanolic extract of *C. gigantea* affected the maturation of the spermatozoa in the male rats, which might be a probable cause of decreasing in the mean total sperm count. The decrease in the sperm counts is indicating direct or indirect effect of *C. gigantea* extract on spermatogenesis as well as spermigenesis. The production of the sperm cells (speratozoa) 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 [15]. FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in leydig cells of the testis [16]. The observations of the present study, confirm to those already reported and studied with various plant extract [17-19]. 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 [20] which might have affected the fertility in treated rats. The decrease of serum levels of LH causes reduction in the serum level of testosterone. Leyding cells secrete testosterone by the stimulatory effect of LH [21, 22, 23]. Methanol soluble extract at 100 and 200 mg/kg BW caused marked degenerative changes in the histological appearance of testes and epididymis. Such histological alterations have also been described in other reports [24, 25] All the above statements indicating about male infertility symptoms. Antifertility activity of *C. gigantea* has been attributed due to the presence of certain phytochemical compound such as stigmasterol, psoralon, gigantein, sapononis, tannins etc. [26]. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequence of *C. gigantea* on the sperm may be consider as the plateform for further study.

5. Conclusion

Maximum studies focus on female contraceptives rather than male contraceptives. Oral male contraceptive pills are comparatively few and far fetched. The present study provides informational data about anti-fertility effects of methanolic extract of *C. gigantea* on male rats which can be a mile stone for further studies on development of orally effective, cheap, safe and reversible contraceptives for male.

6. Acknowledgements

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