

Antifertility Activity of Methanolic Extract of *Cuminum Cyminum* Seed on Male Albino Rats

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Abstract

In the present study, an attempt was made to evaluate the antifertility activity of methanol extract of *Cuminum cyminum* seed on male albino rats. Methanolic extract of *Cuminum cyminum* seed was administered orally to adult male albino rats at the dose of 100 mg/rat/ day for 60 days. On 61st day, the rats were killed; the testes and accessory reproductive organs were removed and weighed. The organs were processed for biochemical estimations; sperm motility and sperm density in cauda epididymis were also observed. The result revealed that the *Cuminum cyminum* seed extract treatment caused a significantly ($P < 0.05$ and $P < 0.01$) decreased in weight of testes and accessory reproductive organs, whereas body weight did not show any significant changes than control. Sperm motility as well as sperm density significantly ($P < 0.01$) decreased, which resulted in total suppression of fertility. Significant reduction was also observed in biochemical estimation of testicular and cauda epididymal protein, sialic acid, glycogen and seminal vesicular fructose, whereas cholesterol content of testes was increased. A marked diminution in the germ cell population was noticed, production of spermatids declined significantly ($P < 0.01$), Seminiferous tubular diameter and Leydig cell nuclear area showed notable reduction ($P < 0.05$ and $P < 0.01$). The Sertoli cells count and its cross-sectional surface area was also significantly reduced ($P < 0.01$). Therefore, methanolic extract of *Cuminum cyminum* seed causes impairment of testicular function and affect spermatogenesis on male rats.

Keywords: *Cuminum cyminum*, Testicular cell population, Sperm motility, Sperm density, Leydig cells

Introduction

The genus *Cumin* belongs to family *Umbliferae*. It is distributed in E. Asia and in India, it is found wild in the forest of Madhya Pradesh, Rajasthan, Uttarpradesh and in Sub-Himalayan tracts. The aromatic dried fruits commonly called Cumin seed are used as spices and condiment. Abortifacient activity of the seed has been investigated by few workers (Choudhury and Haq, 1980; Garg, 1976). Al-Khanis and Parmar, (1988) reported that the anti-implantation and abortifacient activity of the aqueous extract of *Cuminum cyminum* on female rats. Balamurugan et al., (2009) suggested that the antifertility activity of ethanolic extract *Cuminum cyminum* on female male rats. Bhargava, (1988) reported that the oestrogenicity of *C. cyminum* seed on ovariectomised rats. Therefore, the present study was undertaken to evaluate the antifertility effect of methanolic extract of *Cuminum cyminum* seed on adult male rat.

Materials and Methods

Experimental animals

In the present study healthy and matured male albino rats were used. Wister strain albino male rats weighed 150-200gms were purchased from animal house centre of “Jawaharlal Nehru Institute of Post Graduate Medical Educations and Research” (JIPMER), Pondicherry.

Acclimation

The experimental animals were maintained in Tarson's Poly Proylene cages (3”×12” ×8”) with metal grill tops under natural day length. Fresh and dried husk was used as bed material. Husk bed was changed daily and water bottles were properly cleaned and water was changed everyday to maintain the hygiene. Animals were acclimated to the laboratory condition for 10-15 days before starting the experiments. The rats were fed with standard diet of Sai Durga Feeds, Bangalore and provided with water *ad libidum*.

Collection of *Cuminum cyminum* seeds

The *Cuminum cyminum* seeds were purchased from local herbal store from authenticated Dr. S. Mani, Sidha Clinic, Pudukottai, Tamil Nadu, India. The seeds were air dried in a dry indoor place and were powdered using a mixer.

Preparation of Extract

The powdered seeds were extracted with 70% methanol in a Soxhalet apparatus and obtain a solid viscous brown mass, which is “Crude extract”. This crude extract was used for present study.

Study protocol

Adult male rat were divided into 2 groups of 10 rats each.

Group – I: Rats received 1 ml of Olive oil for 60 days and served as control rats.

Group – II: Rats received methanolic extract of *Cuminum cyminum* seeds dissolved in

1ml olive oil (100 mg/kg/b.wt) for 60 days.

Fertility test

Fertility test in both groups (Group I and Group II) was observed from 55 to 60 days. Male rats from control and extract treated group were caged overnight with normal proestrous females in the ratio of 1:2 for normal mating. Presence of sperm in the vaginal smear confirmed the positive mating and the day was taken as an index of Day-I of gestation. The implantation sites of mated females were checked on day 16th by laparotomy.

Autopsy schedule

The male rats were weighed and killed under ether anesthesia after 24 hours of the last drug administration. Blood was collected by cardiac puncture and serum was separated by centrifugation at 3000rpm, and reproductive organs (testes, epididymis, seminal vesicle and ventral prostate) were removed, cleared off fats connective tissues and weighed on an electronic balance and kept at -20°C for biochemical estimation.

Sperm analysis

The motility of cauda epididymal sperm was determined with haemocytometer and sperm density was assessed in testes and cauda epididymis in Neubauer's counting chamber by the method of Prasad *et al.*, 1972.

Biochemical analysis

The estimation of protein (Lowry *et al.*, 1951) and sialic acid (Warren, 1971) were performed in testis and epididymis. Glycogen (Montgomery, 1981), cholesterol (Zlatkis *et al.*, 1953) in testes and fructose (Foreman *et al.*, 1973) in seminal vesicle were also estimated. Serum was analyzed to estimate the total cholesterol (Zlatkis *et al.*, 1953), total protein (Lowry *et al.*, 1951), phospholipids (Radin, 1981) and ascorbic acid (Roe *et al.*, 1943).

Quantitative analysis

Quantitative evaluation of cell population was based on the calculation made for each cell type per cross tubular section, only round section of spermatogonia, spermatocytes and Sertoli cells were counted using X800. At least 20 round tubular cross sections were counted for each stage of spermatogenesis. These crude counts were corrected by using Abercrombie's correcting factor. Interstitial cell types such as fibroblast mature and degenerating Leydig cells were estimated applying differential counts which were statistically verified by the binomial distribution. Mean seminiferous tubular diameters were determined by measuring and tracing an average of 100 selected seminiferous tubules. The Leydig cell nuclei area and Sertoli cell area were measured at X800.

Statistical analysis

Mean and standard error of mean [SEM] were calculated and the significance of difference was analyzed by applying Student "t" test.

Result

Body and accessory organs weight

No significant changes were observed in the total body weight and ventral prostate in methanolic extract of *Cuminum cyminum* seed treated rats than control. However, there was significant reduction in the weight of testis ($P < 0.01$), epididymis ($P < 0.01$) and seminal vesicle ($P < 0.05$) when compared to control rats (Table-1).

Sperm motility of the cauda epididymis significantly decreased ($P < 0.01$) and sperm density of testis and cauda epididymis reduced significantly ($P < 0.01$) which resulted in the suppression of male fertility by 100% negative (Table-2).

Administration of *Cuminum cyminum* seed extract caused an effective inhibition of spermatogenesis. There was a significantly ($P < 0.01$) reduced in most of the cell types of seminiferous tubules than control rats. Population of sertoli cells, preleptotene, pachytene, secondary spermatocytes and rounded spermatids were reduced by 26.68%, 58.51 %, 54.21 %, 38.70 % and 64.29 % respectively. Spermatogonial population did not show any significant alteration when compared to control (Table-3).

Cross sectional surface area of Sertoli cells showed a significant ($P < 0.01$) reduction than control rats. Number of mature Leydig cells was decreased significantly ($P < 0.01$) where as degenerating cell number was increased when compared to control rats. Significantly ($P < 0.01$) decreased in the seminiferous tubular diameter and Leydig cell nuclear area than control rats (Table- 4).

Biochemical study showed that the significant changes were observed in the treatment of *Cuminum cyminum* extract treated rats than control rats. Protein content of testes and epididymis were significantly ($P < 0.01$) decreased after the extract treated rats than control rat. Content of sialic acid was significantly decreased in testes and epididymis ($P < 0.01$) in the extract treated rats when compared to normal healthy rats. The level of glycogen was significantly ($P < 0.01$) decreased in testis in the extract treated animals, whereas the level of cholesterol in testes was significantly ($P < 0.01$) increased than control rats. Fructose level of seminal vesicle was significantly ($P < 0.05$) reduced when compared to control rats (Table-5).

Table1: Effect of methanolic extract of *Cuminum cyminum* seed in body and accessory organs weight.

Accessory organs weight (mg/ 100g/ b.wt)					
Treatment	Body weight (g)	Testes	Epididymis	Seminal vesicle	Ventral prostate
Group-I Control	232 ±6.50	1403 ±32.00	612.15 ±11.20	561.00 ±22.50	306.50 ±2.02
Group-II <i>Methanolic extract of Cuminum cyminum</i> (100 mg/rat/day)	212 ±10.00	1354 ±15.00**	596.50 ±5.00**	531.75 ±25.18*	287.99 ±1.80

Values are mean ± SEM (n=10);

P* < 0.05 and P** < 0.01 vs control

Table2: Effect of methanolic extract of *Cuminum cyminum* seed in sperm dynamics and fertility test.

Sperm density (million/ml)				
Treatment	Sperm motility % (Cauda epididymis)	Cauda Epididymis	Testes	Fertility test (%)
Group-I Control	71.00 ±2.50	45.57 ±1.42	4.42 ±0.44	100 (+) ve
Group-II Methanolic extract of <i>Cuminum cyminum</i> (100 mg/rat/day)	61.04 ±1.25 **	39.20 ±0.70 **	3.95 ±0.11 **	100 (-) ve

Values are mean ± SEM (n=10);

P^{**} < 0.01 vs control

Table3: Extract of *Cuminum cyminum* seed in testicular cell population.

Treatment	Testicular cell counts (number/10 cross section)					
	Sertoli cell	Spermatogonia	Preleptotene	Pachytene	Secondary spermatocyte	Round Spermatid
Group-I Control	2.81±0.02	6.85±1.20	20.23±1.78	29.27±1.09	46.55±1.00	36.55±3.25
Group-II Methanolic extract of <i>Cuminum cyminum</i> (100 mg/rat/day)	2.66±0.04 **	6.42±0.52	18.42±0.98 **	27.43±0.78 **	43.55±2.30 **	33.15±2.25 **
	(-) 26.68%		(-) 58.52%	(-) 54.23%	(-) 38.77%	(-) 64.25%

Values are mean ± SEM (n=10);

P^{*} < 0.05 and P^{**} < 0.01 vs control

Table4: Effect of *Cuminum cyminum* seed extract on Leydig Cell Differential Counts and Testicular histometry.

Treatment	Leydig Cell Differential Counts			Testicular histometry		
	Fibroblast	Mature	Degenerated	Seminiferous Tubular Diameter (µm)	Leydig Cell Nuclear Area (µm)	Sertoli Cell Area (µm)
Group-I Control	53.00 ± 3.60	98.70± 0.26	56.00± 2.80	267±9.00	11.13± 0.05	48.97± 3.20
Group-II Methanolic extract of <i>Cuminum cyminum</i> (100 mg/rat/day)	56.30±2.90 *	82.55±3.65 **	80.15±2.60 **	248±7.50 **	9.88±0.12 **	45.55±2.00 **

Values are mean \pm SEM (n=10);
 $P^* < 0.05$ and $P^{**} < 0.01$ vs control

Table5: Extract of *Cuminum cyminum* seed in testicular and epididymal tissue biochemistry.

Treatment	Protein (mg/g)		Sialic acid (mg/g)		Glycogen (mg/g)	Cholesterol (mg/g)	Fructose (mg/g)
	Testes	Cauda epididymis	Testes	Cauda epididymis	Testes	Testes	Seminal vesicle
Group-I Control	204.42 \pm 4.44	255.25 \pm 3.25	4.62 \pm 0.16	5.30 \pm 0.15	3.88 \pm 0.10	7.80 \pm 0.30	4.20 \pm 0.19
Group-II Methanolic extract of <i>Cuminum cyminum</i> (100 mg/rat/day)	195.00 \pm 2.22 ^{**}	243.00 \pm 5.45 ^{**}	4.45 \pm 0.03 ^{**}	5.12 \pm 0.10 ^{**}	3.51 \pm 0.07 ^{**}	12.10 \pm 0.50 ^{**}	4.00 \pm 0.12 [*]

Values are mean \pm SEM (n = 10);
 $P^* < 0.05$ and $P^{**} < 0.01$ vs control

Discussion

Animals are treated with methanolic extract of *Cuminum cyminum* seeds showed a notable depression of spermatogenesis. There is significant ($P < 0.01$) reduction in testes weight, which can be attributed for the loss of germ cell (Venkatesh et al., 2002; Thakur et al., 2009).

In the present study, significant ($P < 0.01$) reduction of seminiferous tubule diameter reflects tubular shrinkage, which may occur due to cell death or sloughing of epithelial cells (Goldberg et al., 1997). Significantly ($P < 0.01$) reduced in the weight of accessory reproductive organs directly supports the reduced availability of androgens (Comhaire, 1994). Since male accessory organs are essential under the control of androgens through their specific receptors and androgenic deprivation in the rat prostate up regulates the concentration of transcripts for the androgen receptors (Kumar et al., 1997).

Marked inhibition of sperm motility may be due to low level of ATP content (Comhaire et al., 1983). Slight reduction due to alteration in the metabolism of the testes has serious repercussion on sperm motility and fertility rate, since normal internal milieu of epididymis is necessary for proper maturation of sperm (Chinoy, 1997). Spermatogenesis requires functional integrity and cooperation of the Sertoli cells as they occupy the full thickness of the seminiferous tubules and are in close contact with germinal cells. Secretory activity of Sertoli cells i.e., ABP (Androgen

Binding Protein) production is modulated by germinal cells particularly by pachytene and early spermatids (Martin, 1993). Alteration of Sertoli cells affects the production of ABP, which in turn leads to the arrest of spermatogenesis. There is also evidence that the disturbance of Sertoli functions results in the damage of spermatogenesis (Gupta, 2006). In this results, showed low counts of Sertoli cells and some structural changes in Sertoli cell after administration of extract of *Cuminum cyminum* seeds (Table 3). Similar results have been observed with *Albizia lebbek* pod extract (Born et al., 1988).

In the interstitial compartment of extract treated rats there appeared to be severe depletion of the number of mature Leydig cells. Leydig cells maintain concentration of testicular testosterone in testicular fluid surrounding the seminiferous tubules. Degenerative changes in Leydig cells effect on functional ability of these cells to synthesize testosterone, which is required for maintenance of spermatogenesis (Gupta et al., 2004). The present result is showed that testicular function would be altered by reduced protein content (Sarkar et al., 1997). Result revealed that protein and sialic acid content of epididymis reduced significantly ($P < 0.01$) (Table 5). The principle cells of the epididymis are responsible for the synthesis of proteins and sialic acid, which are directly poured into the epididymal lumen (Robaire and Hermo, 1988). Reduction in testicular sialic acid either by due to absence of spermatozoa or reduced androgen production could affect metamorphosis and maturational stages of spermatid (Hinton and Palladino, 1995).

According to Du Toit, (1996), it is also reported that the bounded sialic acid and sperm ATP concentration negatively correlated with sperm motility. The glycogen content in the testicular cell indicates energy storage. Sertoli cells and spermatogonia often contain glycogen and secrete substrate from the blood and provide source of reserve carbohydrates for seminiferous tubular cells and the glycogen level is found to be directly proportional to the steroid hormones (Jahan *et al.*, 2009).

In this study, glycogen content was significantly ($P < 0.01$) decreased in testes after the administration of *Cuminum cyminum* seeds extract treated rats may reduce the energy source for spermatogenic activity which might have resulted in spermatogenic arrest (Venkatesh et al., 2002). Similar results have been revealed by Pathak and Prakash, (1989). High accumulation of cholesterol in the testes in the extract treated rats may be due to decreased steroidogenesis (Hall, 1994). Curry and Atherton (1990) reported that the reduction in seminal fructose might be due to reduced synthesis and secretion of circulating androgens.

Conclusion

The methanolic extract of *Cuminum cyminum* seeds is effective in suppressing the spermatogenesis with the changes in structural activity of Sertoli cell, seminiferous tubules and Leydig cell. Thus, the present investigation shows that the *C. cyminum* extract exerts antifertility activity in male albino rats. Further studies are recommended to isolate active compounds responsible for antifertility activity.

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