Original Article

Contraceptive Efficacy of *Citrullus colocynthis* Methanolic Extract in Male Rats

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ABSTRACT

To study the effects of *Citrullus colocynthis* on testicular function and fertility, 70% methanolic extract of *C. colocynthis* was fed orally to male albino rats at the dose levels of 75 and 150 mg/kg body wt/day for 60 days. Oral administration of *C. colocynthis* caused a significant decrease in testicular weight and sperm concentration. The level of serum testosterone, LH and FSH also declined and spermatogenesis was impaired. The histoarchitecture of the testes in the rats receiving *C. colocynthis* methanolic extract showed degenerative changes and disruption of spermatogenesis and the number of spermatogonia remained significantly decreased. Thus the antifertility effects of *C. colocynthis* seemed to be mediated by disturbances in structure and testicular function including leydig and Sertoli cells resulting in an alteration in physio-morphological events of spermatogenesis.

Keywords: Testicular weight, Sperm concentration, Testosterone, Spermatogenesis, Degenerative changes, Antifertility.

INTRODUCTION

The quest for the oral contraceptive agent that can control human fertility is continue since ancient times[1]. Although a variety of synthetic contraceptive agents are available, but because of their side effects these cannot be used continuously[2]. Scientists and researchers throughout the world are realising the importance of avoiding the harmful effects of synthetic compounds, and thus cooperating in efforts to design new and effective contraceptives from compounds of plant origin[3]. Use of ethnobotanical information in medicinal plant research has gained considerable attention in recent years, especially in segments of the scientific community[4]. The use of herbs is very common in developing countries, particularly in rural communities. However, an increase in the use of plants

has been observed in metropolitan areas of developed countries during the last decade[5].

Citrullus colocynthis (L.) Schrad is an important medicinal plant belonging to the family of Cucurbitaceae. *C. colocynthis* contain large amounts of phenolics and flavonoids that have antioxidant activities[6]. It has a beneficial effect on improving the glycemic profile without severe adverse effects in type II diabetic patients[7]. It was used as purgative, anthelmentic, antipyretic, carminative, cures tumours, leucoderma, asthma, jaundice, enlargement of spleen, tuberculous glands of the neck, elephantiasis and ulcers, also reported that fresh fruit and seeds are eaten as laxative and for removing kidney stones[8].

The therapeutic efficacy of *C. colocynthis* extract in treatment of human diseases has been established. Thus the aim of this study was to investigate the effect of methanolic extract of *C. colocynthis* on various male fertility parameters to evaluate its possible contraceptive efficacies in wistar strain male rats.

MATERIAL AND METHODS

Plant collection

The plant material was collected from the university campus and authenticated at the Herbarium, Department of Botany, University of Rajasthan, Jaipur in comparison with the pre existing voucher specimen (RUBL 20689). Stem and leaves of *C. colocynthis* were shed dried and powdered.

Plant crude extract preparation

Shed dried stem and leaves of *C. colocynthis* were powdered and extracted with 70% methanol for 36-48 hours by Soxhlet extraction method. The extract was filtered and then methanol was separated under reduced pressure to obtain a solid mass. Various doses of *C. colocynthis* methanolic extract were administered orally for 60 days.

Animals and animal treatment

Healthy adult male albino rats (*Rattus norvegicus*) of Wistar Strain of an average body weight 150-200 gms with proven fertility have been employed for experimentation. The animals were kept in clean polypropylene cages covered with chrome plates grills and maintained under controlled environmental conditions (12-h light: 12-h dark). The animals were mostly maintained on standard pellet diet procured from Ashirwad Industries, Chandigarh (India) and occasionally on germinated/ sprouted gram and wheat seeds as an alternative feed. They were given clean water *ad libitum*. The guidelines for care and use of animals for scientific research (INSA, 2000) were strictly followed throughout the course of investigation.

Experimental design

Doses were freshly prepared by making a suspension of the methanolic extract of *Citrullus colocynthis* was daily made in distilled water for administration and administered forcibly through mouth by pearl point needle during the study duration. Animals were equally distributed into three treatment groups, each consisting of 10 animals:

Group I: Animals of this group were given sterile distilled water alone orally for 60 days. This group was serves as vehicle treated control.

Group II: Animals of this group were administered with methanolic extract of *Citrullus colocynthis* at the oral dose of 75 mg/kg/ body wt/day, for 60 days.

Group III: Animals of this group were administered with methanolic extract of *Citrullus colocynthis* at the oral dose of 150 mg/kg/ body wt/day, for 60 days.

Body and organ weight measurement

After the last day of treatment, the body weight of each animal was recorded and

they were killed along with control animals. Blood was collected in sterile tubes by cardiac puncture. Testes of each animal were excised from the surrounding tissue and blotted free of blood for weighing.

Sperm density determination

Total number of sperms were counted using haemocytometer after further diluting the sperm suspension from testis. The sperm density was calculated in million per ml as per dilution[9].

Fertility Test

The mating exposure test of all the animals was performed. They were cohabited with proesterous females in the ratio 1:3. The vaginal plug and presence of sperm in the vaginal smear was checked for positive mating. Females were separated and resultant pregnancies were noted, when dam gave birth. The number and size of litters delivered were recorded. Fertility was calculated in control as well as in treated groups.

Serum biochemistry

Serum was isolated and stored for detection of protein content[10], total cholesterol[11], phospholipids[12], triglyceride[13], acid phosphatase and alkaline phosphatase[14].

Hematology

The blood samples were collected from the heart and analyzed for blood urea[15], blood sugar[16], hematocrit level[17], RBC and WBC[18].

Tissue Biochemistry

Protein[10], Glycogen[19], Sialic acid[20] and Cholesterol[11] were estimated in testis.

Testicular histology

Testes of rats of all experimental groups were fixed in Bouin's fixative for at least 48 h, processed by paraffin wax impregnation method, cut using a rotary microtome at 5 µm thickness, and stained with hematoxylin and eosin (H&E) for light microscopic examination.

Hormone analysis

Serum concentration of total testosterone, FSH and LH of control and treated groups were measured by radioimmunoassay (RIA)[21].

Statistical Calculations

The data obtained from the above experiments were subjected to statistical analysis. All the values were expressed in terms of mean ± SEM. The data were analyzed statistically by using Student's "t" test and the significance of differences was set at $P < 0.01$ and $P < 0.001$.

RESULTS

Testicular weight, Sperm Dynamics and Fertility

A significant decrease was observed in weight of testes after administration of methanolic extract of *C. colocynthis* at dose levels of 75mg ($p \leq 0.01$) and 150mg ($p \leq 0.001$) in comparison to controls (Table 1). The testicular sperm density also decreased significantly ($P \leq 0.001$) after *C. colocynthis* administration. Control rats showed 100% positive fertility in the mating exposure test while the rats exposed to 75 mg and 150 mg/kg b. wt/day dose level showed 25% and 60% negative fertility respectively (Table 1).

Effect of *C. colocynthis* on Serology of treated rats

No significant changes were observed in serum total protein, phospholipid, triglyceride, total cholesterol, acid phosphatase and alkaline phosphatase levels after administration of methanolic extract of *C. colocynthis* at 75 and 150 mg/kg dose levels (Table 2).

Effect of *C. colocynthis* on Haematology of treated rats

After administration of methanolic extract of *C. colocynthis*, the levels of total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin, haematocrit, blood sugar and blood urea revealed a non-significant change in 75 and 150 mg/kg b. wt. treated rats in comparison to control animal (Table 3).

Biochemical changes in testes after administration of methanolic extract of *C. colocynthis*

The protein, glycogen and sialic acid contents in testes decreased significantly ($p \leq 0.01$ and $p \leq 0.001$) while testicular cholesterol increased significantly ($p \leq 0.01$ and $p \leq 0.001$) in *C. colocynthis* (methanolic extract) treated rats (Fig. 1-4).These changes were in dose dependent manner.

Serum concentration of total testosterone, FSH and LH

Rats administered with methanolic extract of *C. colocynthis* exhibited significant ($p \leq 0.01$ and $p \leq 0.001$) reduction in the level of serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in comparison to control (Fig. 5-7).

Testicular histology

In the histological study of testis, seminiferous tubules of control animals showed clear organization of cells at various stages of spermatogenesis with clear spermatozoa maturation occurring near the lumen (Fig. A). In treated rats, the histoarchitecture of testes of treated rats showed seminiferous tubule with some degenerative changes. Interstitial space was increased between the seminiferous tubules. The number of spermatogonia and spermatocytes were also decreased (Fig. B and C).

DISCUSSION

In the present study oral administration of methanolic extract of *C. colocynthis* at the doses 75 and 150 mg/kg orally for 60 days produced a dose dependent adverse effect on fertility of male rats. 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[22]. The decreasing weight of the reproductive organs in the extract-treated male rats clearly indicated that the extract caused structural and functional alteration in the reproductive organs[23]. Sperm count is one of the most sensitive tests for spermatogenesis and it is highly correlated with fertility. Low sperm count and inactive spermatozoa are always responsible for infertility in males[24-26]. Reduced sperm count in testes and epididymis may also be due to inhibition of meiotic division of spermatocytes[27,28] or may be due to the suppressive effect of the extract on spermatogenesis, while alterations in sperm motility and viability might have resulted from disturbances in epididymal function[29,30].

In the present study, reduction in the number of fertile males was observed and fertility depleted after 60 days of treatment with *C. colocynthis* extract. The decrease in fertility potentials reported after the treatment of male rats has been attributed to impairment in sperm motility and viability[31]. A non-significant change in the total erythrocyte and leucocyte counts, hemoglobin and hematocrit values following oral administration of methanolic extract of *C. colocynthis* suggests non-toxic action of the extract on general body metabolism[32]. The unchanged level of serological parameters in the present study is indicative of the clinical non-toxicity of the extract.

The biochemical parameters are useful ‘marker’ indices of male reproductive

health and function. Protein biosynthesis is a key factor for testicular development and spermatogenesis. The low levels of testicular protein are usually indicative of inhibition of spermatogenesis[33,34]. A significant increase in cholesterol level was observed after *C. colocynthis* treatment. This increase in the concentration of cholesterol in testes of extract-treated male rats may reflect reduced conversion of cholesterol to testosterone[35] which resulted in accumulation of cholesterol in the testes, and thus impaired spermatogenesis[36]. The structural integrity of the acrosomal membrane is dependent upon sialic acid; the reduced sialic acid content might alter the structural integrity of acrosomal membrane, ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa[37,38]. *C. colocynthis* extract treated rats showed a significant decline in glycogen content. Reduced glycogen reflects decrease in the number of postmeiotic germ cells, which are thought to be the sites of glucose metabolism. A decrease in glycogen content could also be due to increased glycogenolysis[39-41].

Testosterone, an important androgen, plays a pivotal role in maturation, spermatogenesis and the maintenance of accessory sex organs[42,43,34]. *C. colocynthis* treatment caused a significant decrease in the testosterone level of treated rats. The significant reduction of testosterone level in blood indicates the reduction of androgen level in treated animal. 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)[44]. The reduction in the serum level of testosterone could probably be due to the decrease of serum levels of FSH, LH and prolactin which are essential for the gonadal development and steroidogenesis in rats[45,46]. The decrease in the serum concentrations of FSH, LH and testosterone in the treated animals clearly indicates the action of extract on the secretion of pituitary gonadotropins and inturn in the testosterone biosynthesis in the testis and reproductive organs.

CONCLUSION

C. colocynthis extract might be encountered as an efficient and competent male contraceptive as the methanolic extract of *C. colocynthis* decrease sperm count and fertility. *C. colocynthis* treatment also caused a significant decrease in the testosterone, LH and FSH levels of treated rats and some histopathological changes were observed in seminiferous tubules of the testis. Thus, it can be concluded that the methanolic extract of *C. colocynthis* has antifertility effects and could be a promising contraceptive in male. However, further studies are required for understanding its mechanism of action.

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Table 1. Testicular weight, Sperm dynamics and fertility analysis after administration of *C. colocynthis*

Treatment	Testicular weight	Sperm density (million/ml)	Fertility (%)
	mg/100g body wt.	Testes	
Group I (Control)	1206.04± 32.56	4.65 ± 0.38	100% (+) ve
Group II (75 mg/kg b. wt/day)	1086.28*± 23.45	3.41*± 0.19	25% (-) ve
Group III (150 mg/kg b. wt/day)	965.75**± 27.12	2.43**± 0.139	60% (-) ve

(Mean ±SEM of 10 Animals) Group II and III compared with group I
ns = non-significant
* = significant (P<0.01)
** = highly significant (P<0.001)

Table 2. Serum analysis after administration of *C. colocynthis* for 60 days

Treatment	Total Protein	Phospholipid	Triglyceride	Total-Cholesterol	Acid Phosphatase	Alkaline Phosphatase
	mg/dl				u/l	
Group I (Control)	14475.28 ± 124.74	135.42 ± 10.12	115.94 ± 8.55	120.27 ± 5.72	9.25 ± 1.08	85.20 ± 5.10
Group II (75 mg/kg b. wt/day)	14308.46 ns ± 112.92	121.47 ns ± 11.06	119.36 ns ± 10.24	134.16 ns ± 6.24	8.72 ns ± 2.12	82.56 ns ± 4.76
Group III (150 mg/kg b. wt/day)	14062.15 ns ± 107.19	110.24 ns ± 9.45	123.22 ns ± 9.26	137.20 ns ± 5.05	8.40 ns ± 1.45	81.62 ns ± 6.32

(Mean ±SEM of 10 Animals) Group II and III compared with group I
ns = non-significant
* = significant (P<0.01)
** = highly significant (P<0.001)

Table 3. Blood analysis after administration of *C. colocynthis* for 60 days

Treatment	Total erythrocyte count (TEC)	Total leukocyte count (TLC)	Haemoglobin	Haematocrit	Blood sugar	Blood urea
	million/mm^3	cells/mm^3	gm%	%	mg/dl	
Group I (Control)	6.24± 0.21	7367.75 ± 134.12	15.19± 0.63	47.55 ± 0.59	94.57 ± 0.68	45.12 ± 0.43
Group II (75 mg/kg b. wt/day)	5.89 ^{ns}± 0.37	7191.97 ^{ns} ± 126.66	15.45 ^{ns} ± 0.54	48.13 ^{ns} ± 0.60	93.01 ^{ns} ± 0.71	43.64 ^{ns} ± 0.49
Group III (150 mg/kgb. wt/day)	5.35 ^{ns}± 0.35	7121.39 ^{ns} ± 119.92	14.83 ^{ns} ± 0.35	48.23 ^{ns} ± 0.58	92.20 ^{ns} ± 0.81	43.34 ^{ns} ± 0.72

(Mean ±SEM of 10 Animals) Group II and III compared with group I
ns = non-significant
* = significant (P<0.01)
** = highly significant (P<0.001)

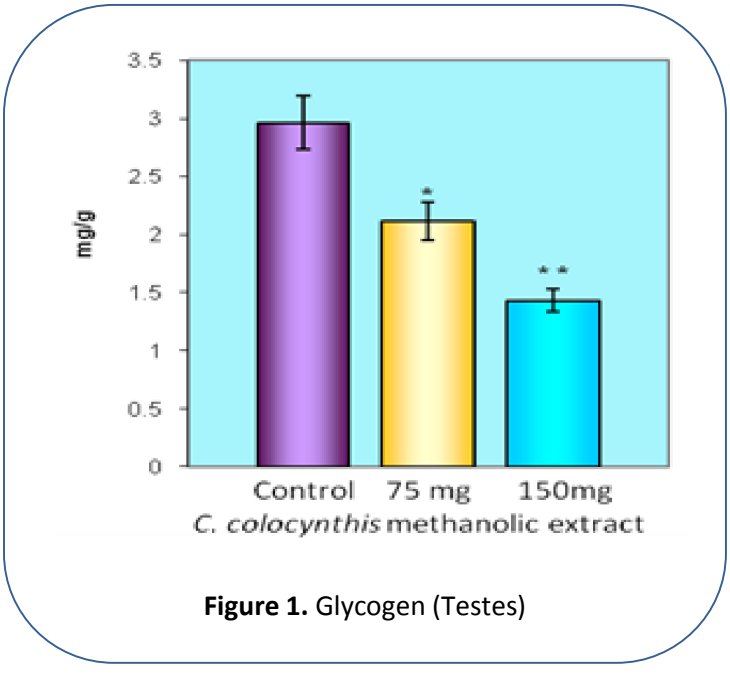

Figure 1. Glycogen (Testes)

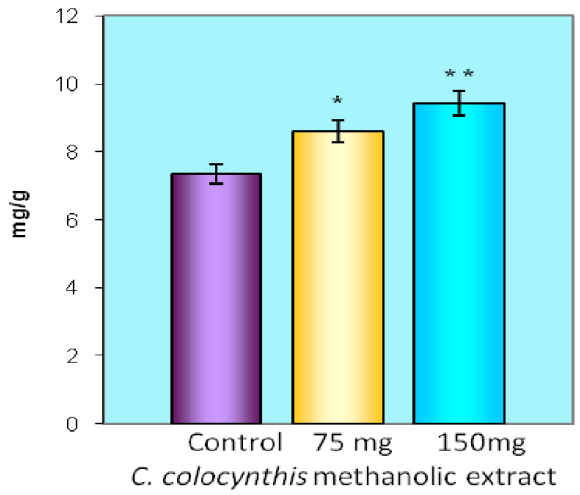

Figure 2. Cholesterol (Testes)

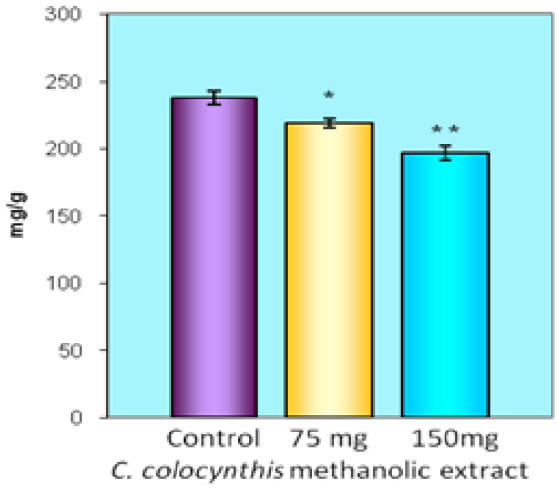

Figure 3. Protein (Testes)

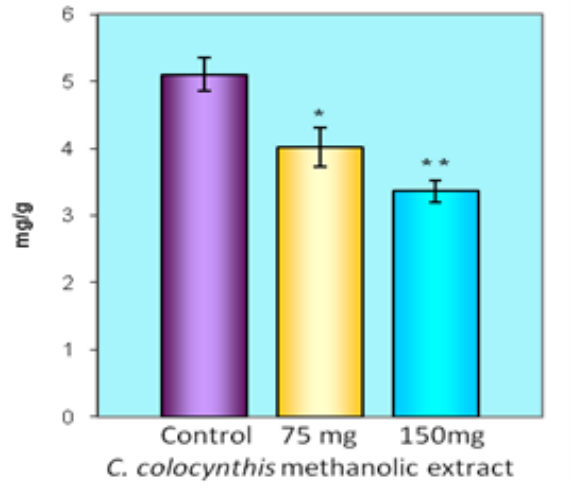

Figure 4. Sialic acid (Testes)

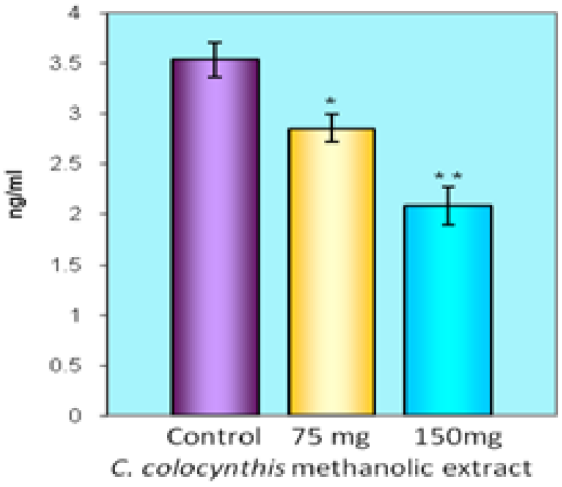

Figure 5. Serum testosterone

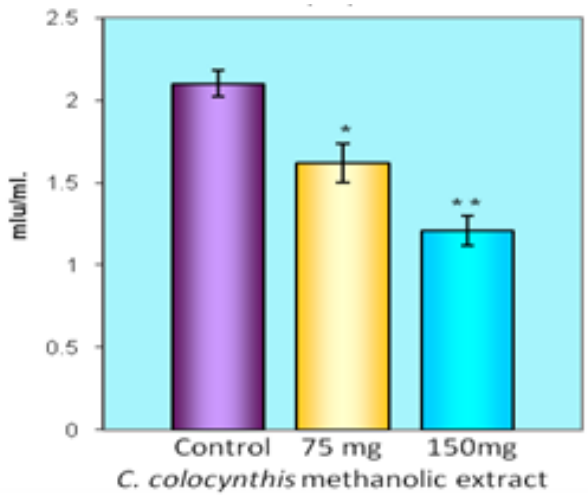

Figure 6. Serum luteinizing hormone (LH)

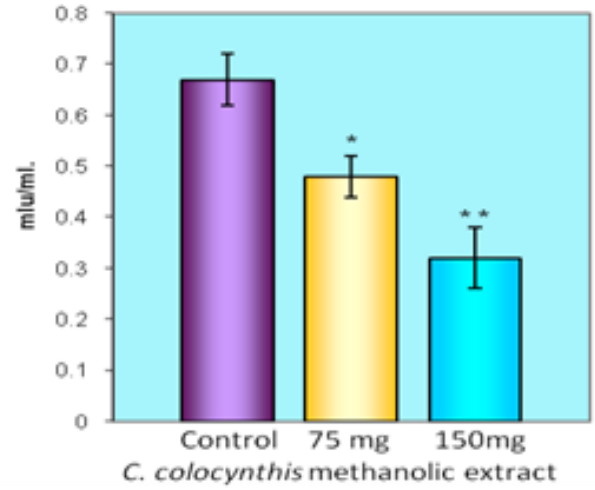

Figure 7. Serum follicle stimulating hormone (FSH)

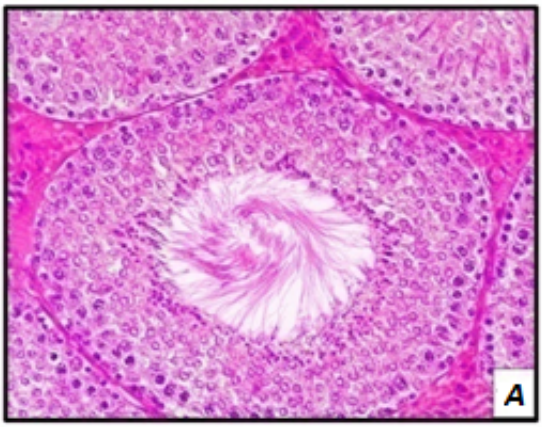

Fig. A. Group I (Control)

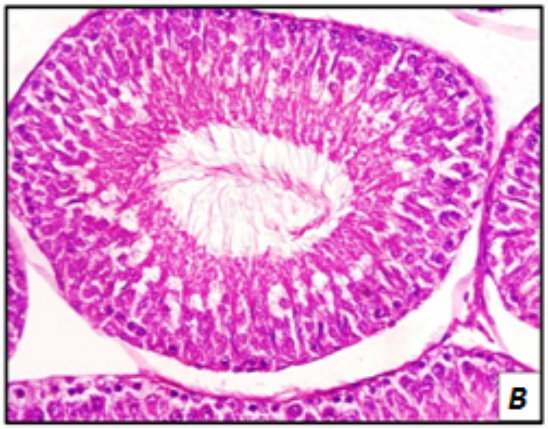

Fig. B. Group II (75 mg/kg b. wt.)

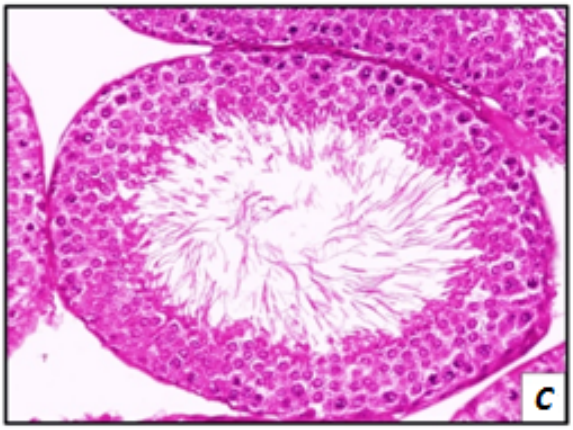

Fig. C. Group III (150 mg/kg b. wt.)

Figure. (A) Micro-photograph of control rat testes showing all the successive stages of spermatogenesis i.e. normal morphology of seminiferous tubules. Lumen is filled with sperm. Leydig cells are also present; (B) Histoarchitecture of testes of treated rats (75 mg/kg. b. wt /day *C. colocynthis* extract) showed seminiferous tubule with some degenerative changes. Interstitial space increased between the seminiferous tubules. The number of spermatogonia and spermatocytes also decreased; (C) Histoarchitecture of testes of rats treated with 150 mg/kg. b. wt/day *C. colocynthis* extract showed degenerative changes with disruption of spermatogenesis. It also exhibited increased interstitial space and irregular epithelium loosened at some places with decreased number of spermatogenic elements and lumen contains cellular debris. Lumen with less sperms is seen. (H X E 200X)