

## EFFECT OF *CITRULLUS COLOCYNTHIS* ON FUNCTION OF CAUDA EPIDIDYMIS AND ACCESSORY REPRODUCTIVE ORGANS OF MALE RATS

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### ABSTRACT

In the present study, the effects of administration of methanolic extract of *Citrullus colocynthis* on cauda epididymis and accessory reproductive organs i.e. vas deferens, seminal vesicle and ventral prostate of male rats (*Rattus norvegicus*) were investigated. Methanolic extract of *C. colocynthis* was fed orally to male albino rats at the dose levels of 75 and 150 mg/kg b.wt./day for 60 days. Oral administration of *C. colocynthis* caused a significant decrease in epididymal and accessory organs weight. The sperm motility, sperm concentration and fertility were significantly reduced at both the dose levels. The level of protein and sialic acid also declined. The histoarchitecture of the epididymis and accessory organs i.e. vas deferens, seminal vesicle and ventral prostate in the rats receiving *C. colocynthis* methanolic extract

showed highly degenerative changes with increased intertubular space. Lumen contains few sperms along with cellular debris in cauda epididymis and vas deferens. Intertubular stroma is disrupted with few connective tissues. Eosinophilic secretion present in seminal vesicle and ventral prostate was also decreased in treated animals. The extract alters epididymal milieu and affects the functioning of spermatozoa. Therefore *C. colocynthis* extract might be encountered as an efficient and competent male antifertility agent, but further studies are called for understanding the exact mechanism.

**Key words:** Accessory reproductive organs; sperm motility; sialic acid; degenerative changes; antifertility agent.

## INTRODUCTION

In the developing countries like India, over population is one of the serious problems. Control of population growth is very important in populated countries. For this, these countries require developing family planning programs. Involvement of men in these programs is generally lesser than women, which may be due to the limited contraceptive choices for them [1]. So, development of new contraceptive methods for males is required. Various chemical substances and plant extracts have been tested but due to side effects produced by chemical agents, plants used in folk remedy may be good choice to look for new contraceptive agents [2].

In recent years, use of ethnobotanical information in medicinal plant research has gained considerable attention in segments of the scientific community [3]. The use of medicinal plants and their products for regulation of fertility in India and other countries is still continuing [4]. Until recently, plants were important sources for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived directly or indirectly from plants [5, 6]. There are several medicinal plants associated with male antifertility potential [7, 8].

*C. colocynthis* (L.) Schrad. is used to treat constipation, oedema, bacterial infections, cancer, diabetes and as an abortifacient. Antidiabetic, antifungal, antibacterial, hypolipidaemic and local anaesthetic activity of different plant parts extracts of *C. colocynthis* have also been reported. In view of all these medicinal properties, the present study has been designed to investigate the effect of methanolic extract of *C. colocynthis* on epididymal and other sex organs function and fertility of male albino rats.

## MATERIALS AND METHODS

### Plant collection

The plant material was collected from the University of Rajasthan 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.

### Preparation of Plant extract

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.

### **Animal model**

Experiment was carried out by using healthy adult male albino rats (*Rattus norvegicus*) of Wistar Strain of an average body weight 150-200 gms with proven fertility. Animal colonies were developed by breeding animals under normal husbandry conditions.

### **Experimental design**

The rats were randomly divided into three groups. One group served as a control while the other two groups received *C. colocynthis* treatment at dose levels of 75 mg/kg rat and 150 mg/kg rat respectively for 60 days.

### **Sperm motility and density**

The epididymis was removed immediately after anesthesia and known weight of cauda epididymis was gently teased in a specific volume of physiological saline (0.9% NaCl) to release the spermatozoa from the tubules. The sperm suspension was examined within five minutes after their isolation from epididymis. The results were determined by counting both motile and immotile sperms in at least ten separate and randomly selected fields. The results were finally expressed as percent motility. The sperm density was calculated in testes and epididymis in million per ml as per dilution [9].

### **Fertility test**

The mating exposure test of all the animals was performed (male female ratio 1:3), five days prior to sacrifice period. The vaginal plug and presence of sperm in the vaginal smear was checked for positive mating. The mated females were allowed to complete the gestation period. The numbers of pups delivered, litter size and fertility percentage were recorded.

### **Body and organ weights**

The initial and final body weights of the animal were recorded. Then epididymis, seminal vesicle, vas deferens and ventral prostate were dissected out and weighed accurately up to milligram level.

### **Tissue Biochemistry**

Protein [10], glycogen [11], sialic acid [12] and cholesterol [13] were estimated in cauda epididymis, vas deferens, seminal vesicle and ventral prostate.

### Epididymal and accessory organs histology

Cauda epididymis, vas deferens, seminal vesicle and ventral prostate 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  $\mu$ m thickness, and stained with hematoxylin and eosin (H&E) for light microscopic examination.

### Statistical Calculations

The data obtained from the above experiments were subjected to statistical analysis. All the values were expressed in terms of mean  $\pm$  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

### Effect of *C. colocynthis* on the weight of the reproductive organs (Table 1)

A significant decrease ( $p \leq 0.01$  and  $p \leq 0.001$ ) was observed in weights of epididymis, vas deferens, seminal vesicle and ventral prostate after administration of methanolic extract of *C. colocynthis* at dose levels of 75mg and 150mg in comparison to controls (Table 1).

**Table 1: Epididymis and accessory sex organ weight analysis after administration of *C. colocynthis* for 60 days**

Treatment	Epididymis	Vas Deferens	Seminal Vesicle	Ventral Prostate
	mg/100g body wt.			
Group I (Control)	478.18 $\pm$ 8.92	143.46 $\pm$ 6.58	432.26 $\pm$ 12.32	343.52 $\pm$ 18.25
Group II (75 mg/kg b.wt./day)	438.32* $\pm$ 9.66	114.25* $\pm$ 8.08	385.80* $\pm$ 11.74	302.72 <sup>ns</sup> $\pm$ 12.23
Group III (150 mg/kg b.wt./day)	395.26** $\pm$ 7.35	107.52** $\pm$ 6.33	342.42** $\pm$ 15.68	275.12* $\pm$ 11.77

(Mean  $\pm$  SEM of 10 Animals)

Group II and III compared with group I

ns = non-significant

\* = significant ( $P < 0.01$ )

\*\* = highly significant ( $P < 0.001$ )

### Effect of *C. colocynthis* on sperm dynamics and fertility of treated rats

The sperm density in cauda epididymis decreased significantly ( $P \leq 0.001$ ) after *C. colocynthis* administration. A severe impairment of sperm motility in cauda epididymis was also observed. Control rats showed 100% positive fertility in the mating exposure test while the rats exposed to 75 mg dose level and 150 mg/kg b.wt./day showed 25% and 60% negative fertility respectively (Table 2).

**Table 2: Sperm dynamics after administration of *C. colocynthis* for 60 days**

Treatment	Sperm motility (%)	Sperm density (million/ml)	Fertility (%)
	Cauda epididymis	Cauda epididymis	
Group I (Control)	67.43 $\pm 2.15$	52.35 $\pm 1.63$	100%(+)ve
Group II (75 mg/kg b.wt./day)	58.61* $\pm 2.19$	45.26* $\pm 1.58$	25%(-)ve
Group III (150 mg/kg b.wt./day)	44.35** $\pm 1.69$	37.42** $\pm 0.98$	60%(-)ve

(Mean  $\pm$ SEM of 10 Animals)

Group II and III compared with group I

ns = non-significant

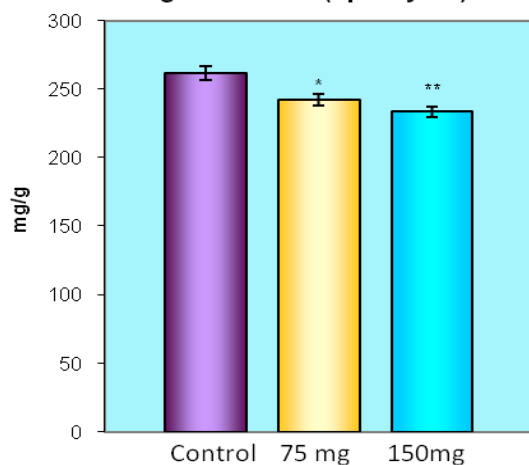
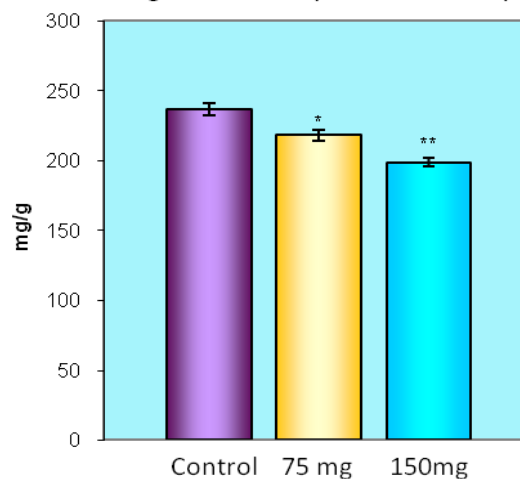
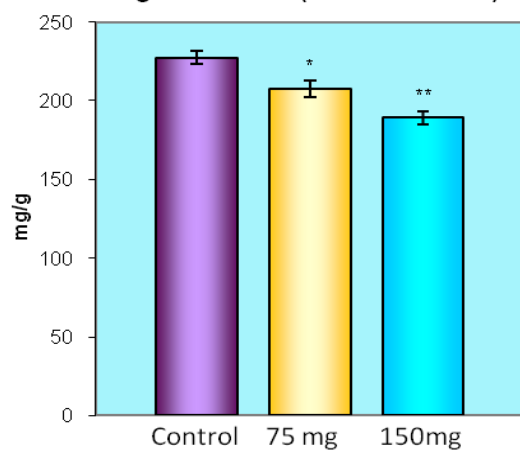
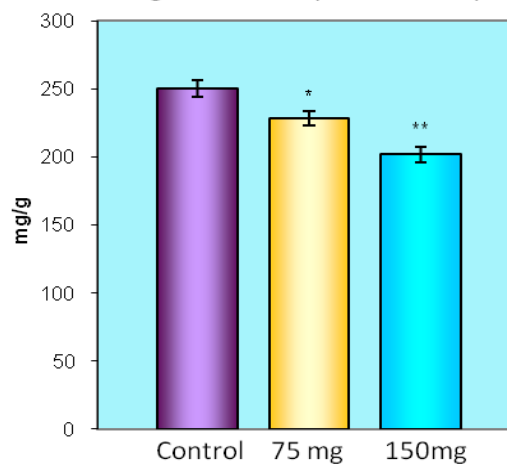
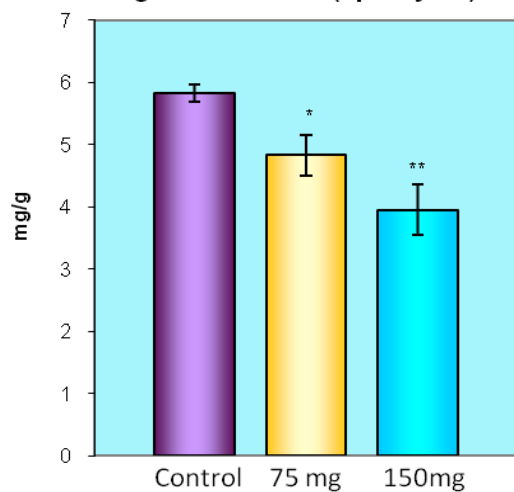
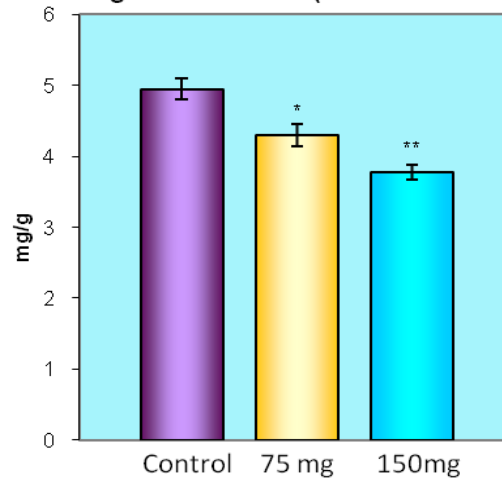
\* = significant ( $P < 0.01$ )

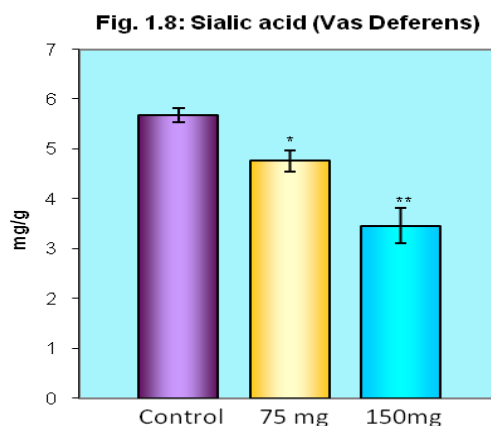
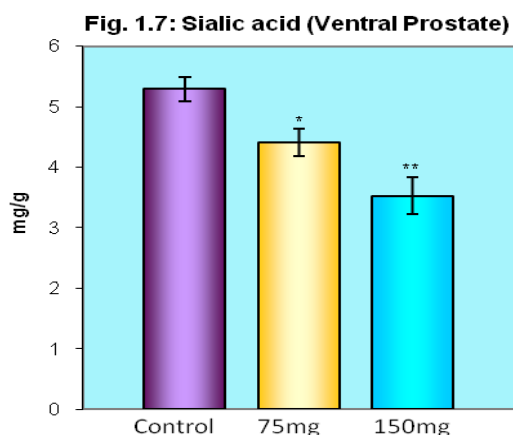
\*\* = highly significant ( $P < 0.001$ )

### Biochemical changes in epididymis and other accessory sex organ after administration of methanolic extract of *C. colocynthis*

The protein level in cauda epididymides, seminal vesicle, vas deferens and ventral prostate decreased significantly ( $p \leq 0.01$  and  $p \leq 0.001$ ) in *C. colocynthis* (methanolic extract) treated rats at dose level of 75 and 150 mg/kg (Fig. 1.1-1.4).

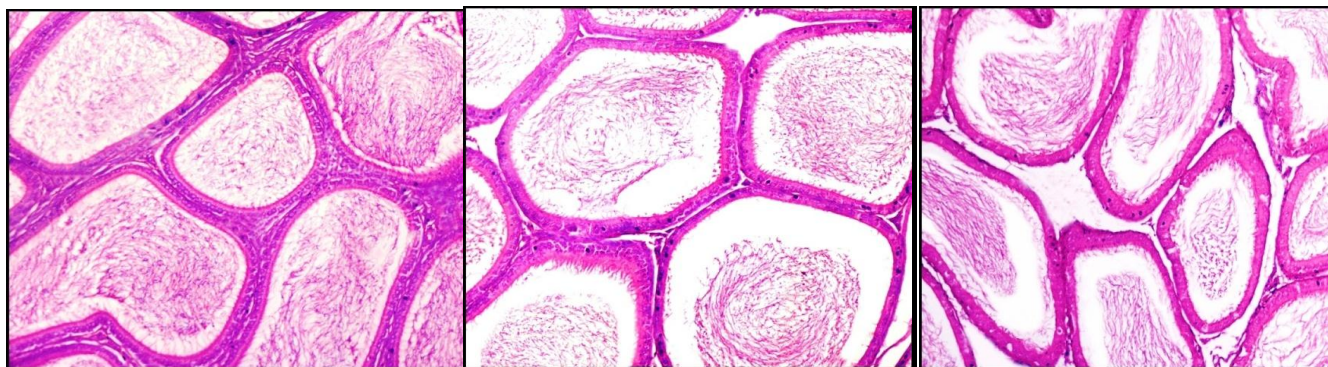
Rats administered with 75 and 150 mg/kg methanolic extract of *C. colocynthis* exhibited significant reduction ( $p \leq 0.01$  and  $p \leq 0.001$ ) in the sialic acid contents of cauda epididymides, seminal vesicle, vas deferens and ventral prostate at both dose levels (Fig. 1.5-1.8).

**Fig. 1.1: Protein (Epididymis)****Fig. 1.2: Protein (Seminal Vesicle)****Fig. 1.3: Protein (Ventral Prostate)****Fig. 1.4: Protein (Vas Deferens)****Fig. 1.5: Sialic acid (Epididymis)****Fig. 1.6: Sialic acid (Seminal Vesicle)**



### Histology

Administration of *C. colocynthis* extract showed adverse effect on histoarchitecture of epididymis and other accessory sex organ. The histoarchitecture of cauda epididymis of treated rats showed slight degenerative changes. Degenerated epithelial wall of tubules can be observed with short stereocilia and few spermatozoa (Fig. 2.1-2.3).



**Figure 2.1**

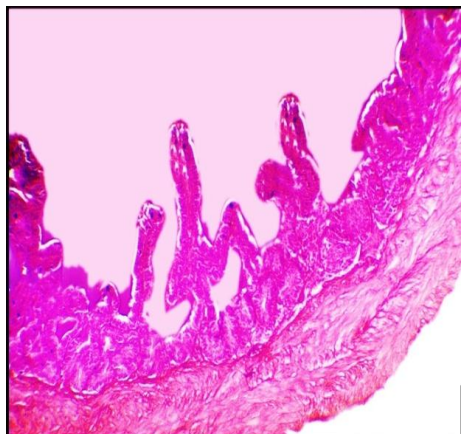
**Figure 2.2**

**Figure 2.3**

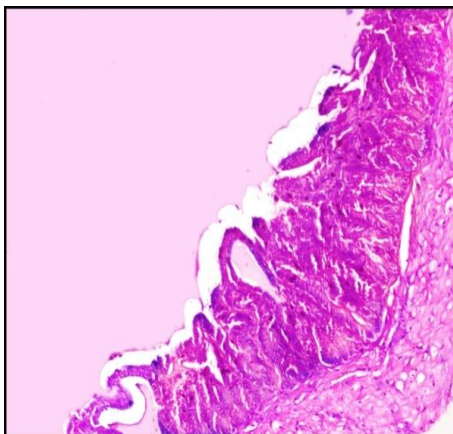
**Figure 2.1:** Microphotograph of cauda epididymis of control rat exhibiting large and compact tubules lined with pseudostratified epithelial cells with stereocilia. The lumen filled with mature spermatozoa. Intertubular stroma is filled with blood vessels and connective tissue. **Fig. 2.2:** The histoarchitecture of cauda epididymis of rats treated *C. colocynthis* (75 mg/kg.b.wt./day) show slight degenerative changes. Few spermatozoa are present in the lumen. Slightly increased intertubular space is seen. **Fig. 2.3:** Methanolic extract of *C. colocynthis* (150 mg/kg.b.wt./day) caused reduction in size of tubules along with decline in the luminal content is prominent. Degeneration in the intertubular stroma and blood vessels is visible.



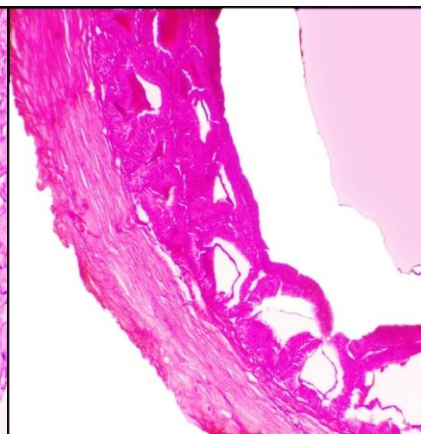
Administration with *C. colocynthis* showed some degenerative changes in seminal vesicle with reduced weight, epithelial thickness and reduced secretion in the lumen. The histoarchitecture of seminal vesicle of rats treated with *C. colocynthis* showed slight degenerative changes with detached muscular layer at some places. Lumen contains less secretion compared to control (Fig. 3.1-3.3).



**Figure 3.1**



**Figure 3.2**



**Figure 3.3**

**Figure 3.1: Histoarchitecture of seminal vesicle of control rat exhibits normal muscular layer consisting of inner circular and an outer longitudinal muscle fibre layers with mucosal folds of columnar epithelial cells extended into lumen. Lumen is filled with eosinophilic secretion. Fig. 3.2: Seminal vesicle of 75 mg/kg.b.wt./day treated rats showing slightly degenerative changes in muscular layer at some places. Lumen contains less secretion compared to control. Fig. 3.3: The seminal vesicle of treated rats (150 mg/kg.b.wt./day) exhibits atrophy in the epithelium or degenerated and disorganized epithelium and reduction in eosinophilic secretion in the lumen.**

After administration with *C. colocynthis* extract, ventral prostate showed decreased weight and altered histology. The ventral prostate of treated rats exhibited degenerated epithelial lining of the alveoli with scanty secretion. Damaged interstitial stroma can also be seen (Fig. 4.1-4.3).



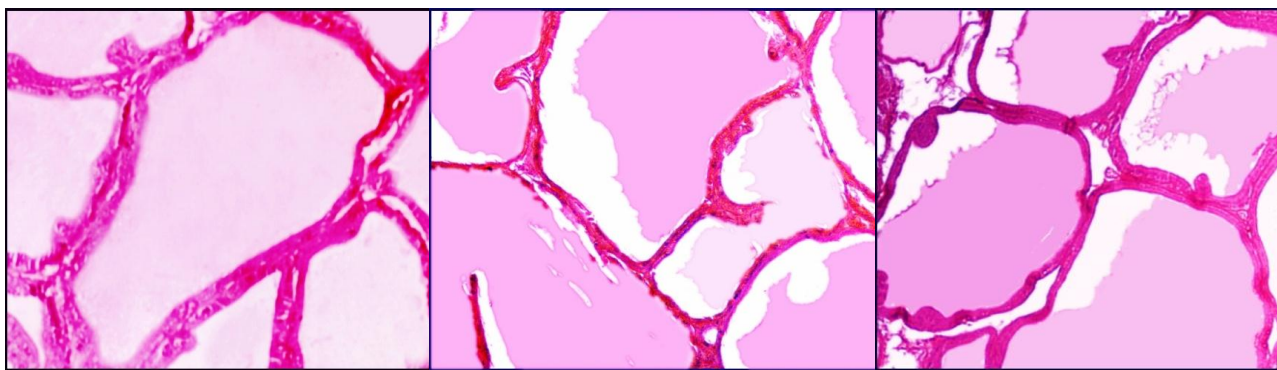


Figure 4.1

Figure 4.2

Figure 4.3

**Figure 4.1:** Microphotograph of ventral prostate of control rat showing alveoli with well developed cuboidal epithelium. Lumen is filled with eosinophilic secretion and interalveolar spaces with connective tissue and blood vessels. **Fig. 4.2:** The histoarchitecture of ventral prostate of rats treated with 75 mg/kg.b.wt./day *C. colocynthis* show alveoli with slightly degenerated epithelium. Less eosinophilic secretion is present in the lumen of alveoli. **Fig. 4.3:** The ventral prostate of treated rats (150 mg/kg.b.wt./day) exhibiting degenerated epithelial lining of the alveoli with scanty secretion. Damaged interstitial stroma can also be seen.

*C. colocynthis* extract caused degenerative changes in histoarchitecture of vas deference with slight degenerative changes in the epithelium and muscular layer of vas deferens. The vas deferens of treated rats showed degenerated epithelium with reduced size and detached from circular muscle layer. Less stereocilia was prominently visible. Lumen contains some cellular debris (Fig. 5.1-5.3).

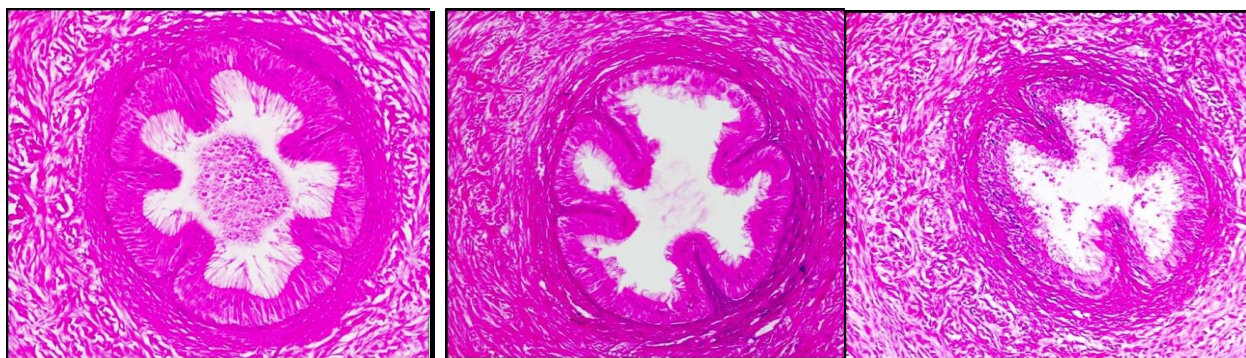


Figure 5.1

Figure 5.2

Figure 5.3

**Figure 5.1:** Microphotograph of vas deferens of control rat exhibits folds of lamina propria. Thick muscular layer consist of inner circular layer and outer longitudinal layer. The lumen contains pseudostratified columnar epithelium with long stereocilia along with normal spermatozoa. **Fig. 5.2:** The vas deferens of treated rats (75 mg/kg.b.wt./day methanolic extract of *C. colocynthis*) exhibiting slightly degenerated epithelial lining along with fused stereocilia and few spermatozoa. Folds of epithelium lining are reduced. **Fig. 5.3:** Histoarchitecture of vas deferens of rats treated with 150 mg/kg.b.wt./day methanolic extract of *C. colocynthis* showing degenerated epithelium with reduced folds and degenerated columnar epithelium. Lumen contains some cellular debris.

## DISCUSSION

The present data showed that the oral administration of *C. colocynthis* to male rats at dose level of 75 and 150 mg/kg b.wt for 60 days brought about a highly significant loss in epididymis, seminal vesicle, ventral prostate and vas deferens weights. 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 [14]. The reduction in the weight of accessory sex organs also reflects antiandrogenic nature of plant extract [15-18]. A dose-dependent lowering of cauda epididymal sperm motility and density suggested an undersupply of testosterone to the epididymis, thereby possibly causing impaired epididymal function, as it is known that the structure and function of the epididymis is dependent on androgens [19, 20]. The decrease in fertility potentials reported after the treatment of male rats has been attributed to impairment in sperm motility and viability [21]. Protein is involved in almost every physiological system. The growth rate of any organ is proportional to its protein content [22]. Reduced epididymal protein content could be correlated with the absence of spermatozoa in the lumen [23-25] since the luminal fluid of epididymis contains a number of proteins [26, 27] some of which remain bound to spermatozoa. Further, the decreased level of protein in seminal vesicle and ventral prostate are indicative of reduced secretion. This may be due to reduction in number and activity of Golgi complex or reduction in number of secretory granules [28, 29]. Sialic acid acts as a 'lubricant' to facilitate the movement of sperm and to reduce friction among spermatozoa [30]. Oral administration of *C. colocynthis* to male rats caused a significant decrease in sialic acid content. It is likely that the extract might be interfering with the synthesis and/or release of sialic acid from the principal cells of the epididymal epithelium [31]. Reduced sialic acid content of seminal plasma caused deteriorating effects on the structural integrity of sperm cells [32] and also affected metamorphosis and the maturational stages of spermatids [33, 16].

The histoarchitecture of reproductive organs of treated rats showed degenerative changes. The epididymis is essential for the storage, maturation, and transport of sperm. It has a smooth muscular coat, which aids in moving the sperm with peristaltic waves toward the vas deferens. Effect of administration of *C. colocynthis* extract on epididymis resulted in decreased motility and fertility [34]. The gradual decrease in tubular diameter, regression of epididymal epithelium and changes in composition of epididymal plasma may be due to less secretion of androgen [35, 36].

Seminal vesicles function is important for chromatin stability [37, 38]. Its secretory epithelium produces the bulk of the seminal vesicle fluid which assists in a number of ways to insure fertility [39]. Administration with *C. colocynthis* showed some degenerative changes in seminal vesicle with reduced weight, epithelial thickness and reduced secretion in the lumen, which may be adverse effect of reduced androgen level as a result of treatment with plant extract [40, 41]. Vas deferens is involved in sperm transport and seminal emission. Administration of *C. colocynthis* extract caused degenerative changes in histoarchitecture of vas deferens with slight degenerative changes in the epithelium and muscular layer along with the absence of spermatozoa in the lumen also may be due to low supply of androgen to vas deferens [42, 43]. Ventral prostate plays key role in male reproduction and its secretion is essential for the normal function of spermatozoa [44]. After administration with *C. colocynthis* extract, ventral prostate showed decreased weight and altered histology. The secretory material of prostate also reduced which indicates changes in the function of Leydig cells of target organs [45].

In the present study, administration of *C. colocynthis* extract suggests marked alterations in the epididymal structures along with other accessory male reproductive organs. Therefore, it can be concluded that *C. colocynthis* is capable to suppress the fertility in male rats.

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