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Evaluation of Anti-Fertility Activity of Ethanolic Extract of *Cassia fistula* (Linnaeus) Leaf on Male Albino Rats

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ABSTRACT

In the present study, antifertility effect of ethanolic extract of *Cassia fistula* leaf on the fertile male albino rats was studied. The sperm count was significantly decreased when treated with extract. Similarly, the sperm vitality, sperm motility were also decreased after the treatment of *Cassia fistula* extract. The abnormalities of sperm were also increased in the *Cassia fistula* extract treated rats. Thus the *C. fistula* may be used as a male antifertility agent.

Keywords: Male antifertility activity; *Cassia fistula* leaves

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INTRODUCTION

The extra ordinary growth of the world population stands as one of the significant events of the modern era to thing over. Present estimates are the population to reach 8-12 billion before the end of the 21st century. During each lecture hour, more than 10,000 new people enter the world, a rate of 3 individuals per second[1]. At present India's population is second to that of china[2]. Population growth resulted from rapidly lowered death rates (particularly infant and child mortality rates), combined with sustained high birth rates success in reducing death rate is attributable to several factors increases in food production and distribution, improvements in public health (water and sanitation) and in medical technology (vaccines and antibiotics), along with gains in education and standard of living within many developing nations[3]. India like other developing countries is faced with the dilemma of a high birth rate and a declining death rate. This is a vicious circle, not easy to break.

Fertility control is an issue of global and national public health concern. Current methods of contraception result in an unacceptable rate of unintended pregnancies. Contraceptive vaccines, and inhibitors of spermatogenesis and sperm motility, provide a potential for non hormonal male contraceptive. Use of antifertility agent is one of the methods in controlling human population. In recent years there has been concern about the use of plant products in affecting fertility of humans. India has vast resources of natural products people have been using many of the medicinal plants for inducing abortion and permanent sterility[4]. A large number of herbal drugs are used to control fertilization with considerable success. Although the use of these drugs has a sound tradition, their place has yet to be validated in therapeutics using the current methodology. Scientific studies are therefore required to judge actual efficacy, mode of action and other limitations to widen the scope of these drugs, if they are provided to be really effective[5].

Thus the present study was undertaken to find out the antifertility effect of golden shower (*Cassia fistula*) leaves on male albino rats.

MATERIAL AND METHODS

Plant material:

Golden shower (*Cassia fistula* Linnaeus*)* leaves were selected for the present study. Fresh leaves of *Cassia fistula* are collected from local village; they were dried under shade and powdered.

Test system and design of experiments:

Male albino rats (IEC Reg. No. 418/01/A/dt.4/6/2001) have been used as test system. Rats were divided into 3 groups. In each group consisted of 4 rats. The average body weights of the rats

were 200g ± 10 g.

Group I: Control group, the rats fed with normal pellet feed (Sai Durga feeds and food, Bangalore).

Group II: The rats were orally administered with phosphate buffer (1ml/day/rat) and normal Pellet feed.

Group III: The rats were orally administrated with ethanol extract of *Cassia fistula* leaves by force feeding for 48 days.

Ethanol extraction of *Cassia fistula*:

200 grams of dried powder was continuously extracted in ethanol using Soxhlet apparatus for 24 hours. The extract was then dried using rotary vaccum evaporator and it was kept in a vaccum desiccator for 24 hours. 60 mg/ml of stock solution was prepared mixing 600 mg of powder mixture in 10 ml of phosphate buffer. Herbal extract was administrated to group III (60mg/kg of body weight of rat) through infant feeding tube (0.8 mm) for 48 days.

Evaluation of antifertility effect:

Antifertility effect of ethanolic extract of *Cassia fistula* leaves was evaluated by studying the spermatological changes and histological changes in testis and cauda epididymis of rats.

Spermatological changes:

Sperm morphology, sperm count, sperm motility, sperm vitality and sperm abnormalities were studied[6].

Harvest and preparation for analysis:

The rats were sacrificed after 48th days of treatment, dissected out the testis and washed in physiological saline. Semen was collected from cauda epididymis by cutting one end of cauda epididymis.

Sperm smear:

A drop of dilute sperm was smeared on a clean slide. It was fixed in methanol and stained in Giemsa`s stain and observed under microscope to study the sperm abnormalities.

Sperm counts:

For making sperm counts the diluting fluid was prepared[6]. After thorough mixing, a deep of dilute semen was transferred to a neubauer counting chamber and a cover slip was over laid and observed under a compound microscope.

Assessment of sperm motility:

Grades of motility:

It was scored based on the relative number of sperm in any of the following grades *viz.*, Rapid

linear progressive motility (+++), slow or sluggish linear progressive motility (++) and immotile(0).

Assessment of vitality:

Vitality is testing whether the sperm are alive or dead using eosin and nigrosis stain.

Histological studies:

The rats were sacrificed and the testis and epididymis were removed. The tissues were fixed in Bouin's fluid overnight and embedded in paraffin wax and sections were cut at 3µ thickness in a rotary microtome. Haematoxylin and eosin stains were used for staining. The sections were observed under a microscope, under bright field illumination, and chosen areas were photographed.

RESULTS AND DISCUSSION

Spermatological studies

Sperm counts, viability, motility and structural integrity of sperm are essential pre-requisits, for them to the successful infertilization[7].

Sperm count:

The sperm counts were observed about 788.00X 10^5/ ml in the normal rats while it was drastically deceased about 343 x 10^5/ml in the *Cassia fistula* extract treated rats (Table 1). Lower count of spermatozoa in the ethanol extract of *Cassia fistula* leaf treated rats may be due the presence of some essential spermicidal compounds.

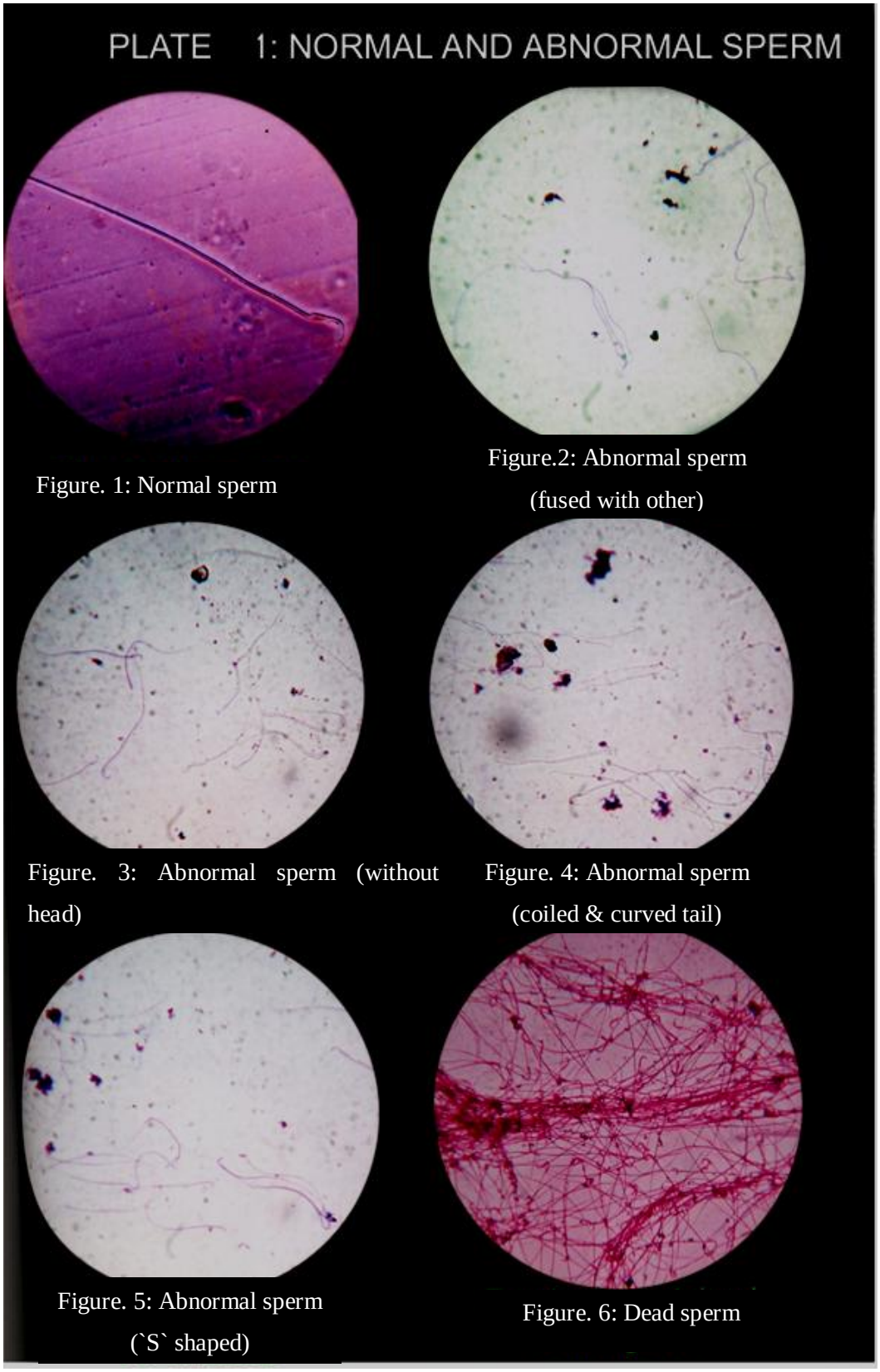
Sperm motility:

Grades of sperm motility are given in table 1. In the normal rats 67.30% of sperm belonged to rapid progressive category, 27.84 % under sluggish category and only 4.67% under immotile category. In contrast, sperm motility was drastically decreased in the *Cassia fistula* leaf extract treated rats (7.46% rapid motility, 32.89% sluggish motility and immotile sperms 59.63%). The percentage immotile sperms were observed in more numbers in the *Cassia fistula* extract treated, when compared to the normal rats. It indicated that *Cassia fistula* extract interfered with sperm motility. Immotility of sperm may be due to structural defects of the flagellum, microtubular abnormalities of defective mitochondria[8, 9].

Sperm abnormalities in treatment rats:

Several abnormalities in the sperms of ethanol treated rats were observed (Figures 1-6). Most of the sperm had head and tail separated. The next stage major abnormality was fused sperm (figure.2). In this case, two sperm had their flagella fused at various points and over varied length and in different patterns. The next abnormality was double headed sperm and without

head (Figure.3). The major abnormality was coiled tail (Figure. 4), several patterns of coiling especially 'S' shaped sperm (Figure.5) were observed.



Figures 1-6: Normal and Abnormalities In The Sperm

Sperm vitality:

Data on the sperm vitality of Group I, Group II and Group III are given in table 1. In control rats, 92.53% of the sperm were alive similarly in the buffer treated rats have 89.59% of live sperms. On the other hand, the *Cassia fistula* leaf extract treated rats shown only 12.54% of sperms in live conditions. Notable decreases in vitality of sperm (Large number of dead sperms showed in figure 6) were observed in the *C. fistula* leaf extract treated rats. It is due to the integrity and functional activities of sperm membrane are crucial for viability and the physiological changes that occur at the sperm surface during the fertilization process[10].

Wet weight of the testis:

The average wet weight of left testis about 0.85g/100g of body weight and the right testis about 0.93g/100g of body weight were observed in the normal rats. In the *Cassia fistula* extracted treated rats, the total weight of both testes significantly decreased to 0.39 g/100g of body weight (left testis), and 0.46g/100g of body weight (right testis) when compared to control rats (Table 1). The effect of abnormalities of the sperms and maximum decrease in testis weight might be either caused directly by the herbal extract of *Cassia fistula* or due to the indirect manifestation through altered epididymal epithelial function.

Table 1: Spermatological studies showed the effect of ethanolic extract of *C. fistula* on male albino rats.

Spermatological Parameters		**Group**		
		Group I (control)	**Group II (Buffer treated)**	**Group III (Extract treated**
Sperm count (Mean)		788 x 10^5/ml	726 x 10^5/ml	343 x 10^5/ml
Sperm motility	Mean percentage of Rapid Progressive Motility (+++)	67.30	78.86	7.46
	Mean percentage of slow Progressive Motility (++)	27.84	14.40	32.89
	Mean percentage of Immotile Progressive (0)	4.67	6.3	59.63
Vitality of sperm	Percentage of Live sperm	92.53	89.59	12.54
	Percentage of Dead sperm	7.68	11.44	87.45
Wet weight of testes	Left testis (g/100g of body weight)	0.8541	0.986	0.3984
	Right testis (g/100g of body weight)	0.9342	1.013	0.4697
Wet weight of epididymis	Left Epididymis (g/100g of body weight)	1.33	1.13	0.93
	Right Epididymis (g/100g of body weight)	1.10	0.95	0.88

Wet weight of the epididymis:

The wet weight of epididymis was also markedly reduced after the 48 days treatments of *C. fistula* leaf extract (Table 1). The epididymis provide a suitable environment for the

morphological and biochemical changes in spermatozoa[11]. Physiological and biochemical integrity of epididymis are dependent on androgen[12, 13]. The deficiency of androgen causes a marked reduction in tubular diameter a general regression of epididymal epithelium a rapid decline in the number of spermatozoa within the cauda epididymis and changes in the composition of epididymis plasma[14]. The reduction in fertility could be attributed to decrease in size of caudaepididymis[15]. It may be due to the presence of some essential spermicidal compounds present in the extract of *C. fistula*[15].

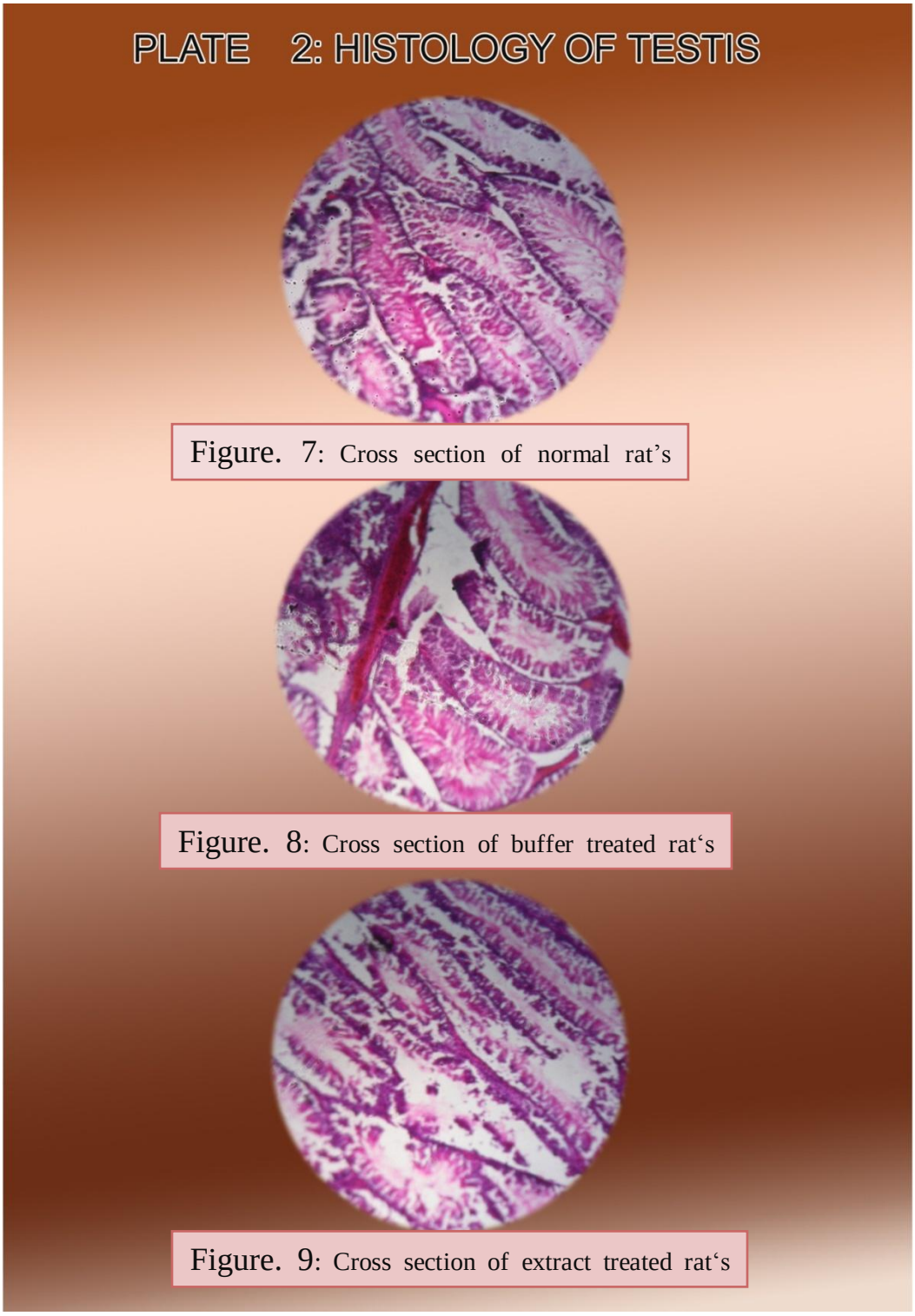
Histological studies

Changes in histoarchitecture of testis:

Histoarchitecture of testes are shown in (Figures 7-9). There were no changes in the histology of testes of normal rats (Figure. 7) and buffer treated rats (Figure. 8). The following changes were observed in the extract treated rat's testis (Figure. 9). In most of the tubules, the seminiferous epithelium was depleted, and the cells were less coherent. The lumen was invariably decreased. The seminiferous epithelium appeared decreased in height. Large vacuoles spaces appeared in the epithelium. The apical portion of the sertoli cells appeared in the epithelium. The apical portion of the sertoli cells appeared detached or sloughed off and even among the parts which were intact the germinal elements beyond leptotene spermatocytes were partially or totally exfoliated, which was the basis for vacuole formation, the basal portion of the sertoli cells was also not compact, their nuclei appeared pycontic and cytoplasm highly vacuolated. The leydig cells were depleted, their nuclei appeared pycontic and cytoplasm vacuolated. In several tubules, the epithelium was further more depleted, though elongated spermatids were still prevalent, but in the lumen stiff sperm tails and sloughed off sertoli cells along with attached spermatogenic elements were present. The leydig cells effect was more acute. The diameters of the seminiferous tubules decreased when compared with those of control animals. The majority of semiferous tubules of treated rats where characterized by the presence of degenerating germ cells and contained vacuoles of varying sizes. The number of germ cells in all stages of the seminiferous tubule decreased in treated rats compared to those of controls. In treated rats, the number of labelled spermatogonia in the different stages of seminiferous tubule cycle was lower than that of control rats.

Changes in histoarchitecture of epididymis:

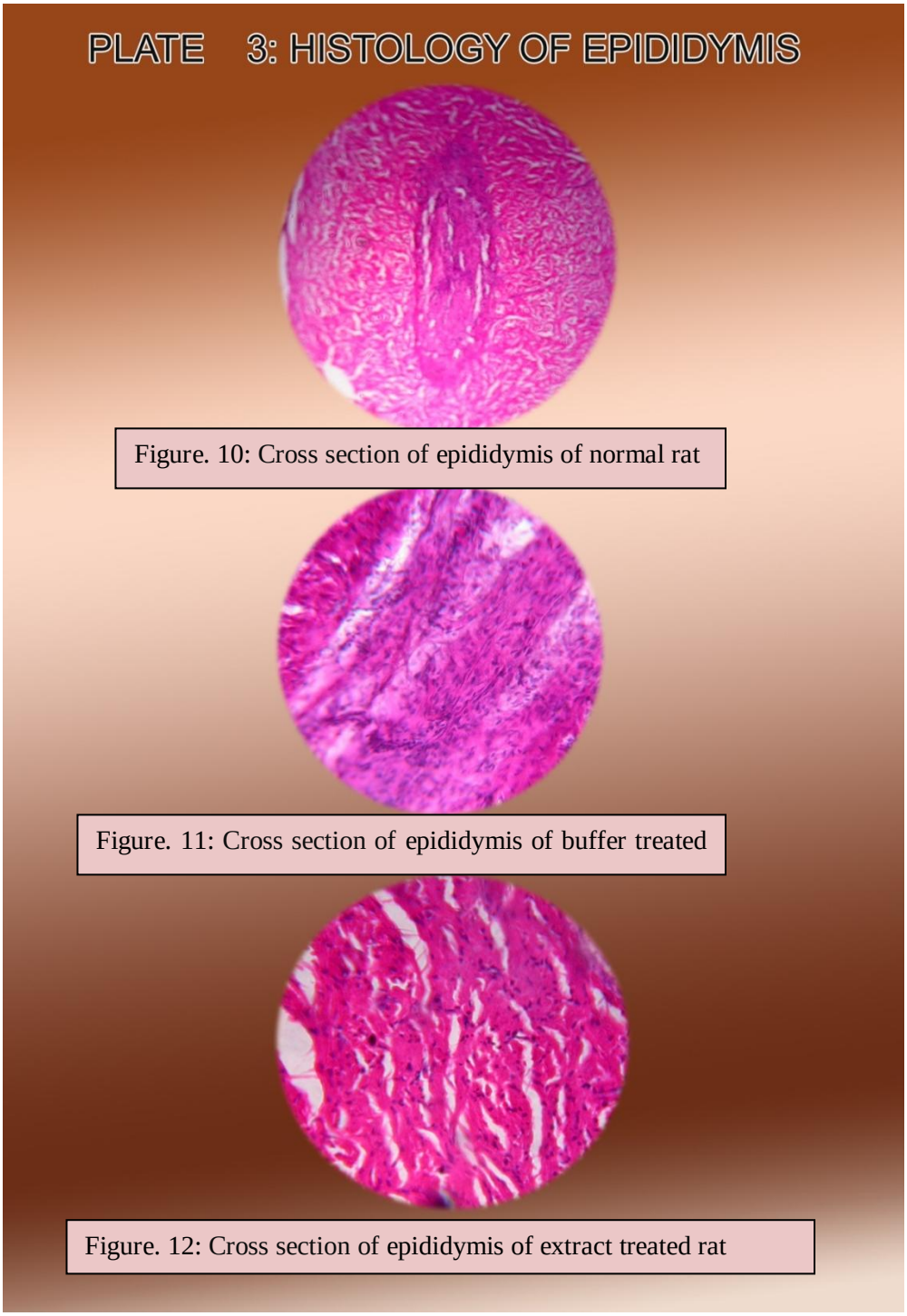
Histoarchitechture of epididymis are shown in figures 10 to12. There were no changes in the histology of epididymis of normal rats (Figure. 10) and buffer treated rats (Figure. 11). The following changes were observed in the extract treated rat's epididymis (Figure. 12).



Figures 7-9: Histo architecture of Testes of Experimental Rat Groups

In the treated group rats, the tubule sections were still compact, but the epithelium was here and there vaculated. More dramatic change was observed in the lumen. The lumen content was depleted and more dispersed in such a way as to press on the stereo-cilia. The lumen contained, in addition to spermatozoa, round as well as partially elongated spermatids and tall and stiff cell

fragments, presumably apical portions of the sertoli cells.



Figures 10-12: Histo architecture of Epididymis of Experimental Rats

It is known that the structure and function of the epididymis is dependent on androgen[16]. 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. This impaired epididymal function may also be due to the reduced activity of the testes, which affects

the normal passage of testicular fluid into the epididymis[17]. Treatment with *cassis fistula* possibly inhibited the activity of ATP in spermatozoa by uncoupling oxidative phosphorylation from the respiratory chain and preventing phosphorylation of adenosine diphosphate to adenosine triphosphate, thus rendering the spermatozoa immotile. Structural integrity of the acrosomal membrane of sperm is dependent upon sialic acid[18], an alteration in its content may also lead to a change in the motility and fertilizing ability of the spermatozoa, thereby rendering these animals infertile[19].

CONCLUSION

The sperm counts were tremendously decreased. The sperm motility was extensively inhibited and thus more immotile sperms were found. The vitality of sperm was also decreased. The sperm had undergone various abnormalities. The wet weight of testis and epididymis were markedly reduced. Thus the present study concluded that the ethanolic extract of *C. fistula* leaf have potent male antifertility effect on albino rats.

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