

Evaluation of Medicinal Properties of *Hibiscus rosa sinensis* in Male Swiss Albino Mice

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ABSTRACT

The present study was undertaken to evaluate the medicinal properties of *Hibiscus rosa sinensis* on male mice. For this Swiss albino mice were orally administered *Hibiscus rosa sinensis* (500 mg/kg of body weight) and effect of the treatment on, reproductive organ cholesterol level and glucose level. Recovery study was also carried out. The treatment caused reduction in the weight of testis, epididymis and evaluation of sperm count indicates that sperm density decreased significantly. Histologically, testis in mice treated with the plant extract showed alteration in the seminiferous tubules and alteration include decrease in thickness and density of germinal epithelium and hypertrophy in majority of cells moreover lumen shows negligible presence of sperms in the treated animal as compared to control. The treatment group had decrease in levels of testosterone. Crude extract of blooms of *Hibiscus rosa sinensis* possess hypoglycemic and hypocholesterolemic potentially. The alterations caused in the above endpoints by the plant extract were reversible and by 60 days of the treatment withdrawal, the parameters recovered to control levels. The results in Swiss albino mice thus suggest that *Hibiscus rosa sinensis* treatment causes reversible suppression of spermatogenesis, Cholesterol level and glucose level.

Keywords: *Hibiscus rosa sinensis*, Seminiferous tubules, Germinal epithelium, Hypertrophy, Testosterone, spermatogenesis.

INTRODUCTION

In India, the use of different parts of medicinal plant to cure specific ailments has been in vogue from ancient times. The earliest mention of medicinal use of plants has been noticed in "Rig Veda". These days herbalism is emerging as an alternative medicine since it makes healthcare affordable for all. It is believed that the mixture of several crude extracts, when used in formulation enhances the beneficial effects through synergistic amplification and diminishes any possible adverse effects. Williamsons further emphasized this concept that a whole or partially purified extract of a plant offers advantage over a single isolated ingredient. [1] The plant *Hibiscus rosa sinensis* (Malvaceae) commonly known as Chinesis Hibiscus or tropica.

Hibiscus rosa sinensis is a native of china and is a potent medicinal plant. It is a common Indian garden perennial shrub. [2] And often planted as a hedge or fence plant. *Hibiscus rosa sinensis* flower decoctions are used in India and Vanuatu as aphrodisiacs, for menorrhagia, uterine haemorrhage and for fertility control. [3] It possesses anticomplementary, antidiarrhetic and antiphlogistic activity.

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Hibiscus rosa sinensis flower showed antispermatogenic androgenic antitumor and anticonvulsant activities [4-8] and antitumor, antihypertensive, antioxidant, antiammonemic. [9-13] Leaves and flowers also possess hypoglycemic activity. [14-15] The present investigation is the first ever study undertaken to find the effect of crude extract on reproductive hormone, diabetes and on Cholesterol level.

MATERIAL & METHOD

Animal-Adult Swiss albino mice of the weight 28-32 g were used under investigation. Mice were maintained under hygienic condition in well ventilated room with 12 h photoperiod (8 am to 8 pm) and were fed pelleted food (Hindustan lever); drinking water available *ad libitum*. Animals in each group were housed in polypropylene cages, with dry rice husk as the bedding material. General health condition and body weight of animals were monitored regularly during the entire tenure of the experiment. Animals were maintained according to the Guidelines of Institutional Animal Ethics Committee.

Test Material - Crude Extract of the Flower prepared by Homogenizing petals in water.

Drugs and Chemical - All the drugs and chemical used in this experiment were purchased from Hi media, Qualigen, CDH. The chemicals were of analytical grade.

Induction of Diabetes

Animals were injected freshly prepared alloxan monohydrate in sterile normal saline at a dose of 150 mg/kg of body

weight, intraperitoneally. ^[16] The animals shown blood glucose level >200 mg/dl after 48 h were considered as diabetic.

Experimental Design

The animals were randomly allocated into five groups (10 mice each) and treated as follows:

Groups Treatment (Dose)

Ist Control

IIInd Alloxan Treated (150mg/kg of BW)

IIIrd Alloxan + *H.rosa sinensis* Treated (500mg/kg of BW)

IVth *H. rosa sinensis* at 500mg/kg of BW for reproductive system

Vth *H. rosa sinensis* at 500 mg/kg of BW for Cholesterol level.

Aqueous extract of *Hibiscus rosa sinensis* was suspended in sterile distilled water and administered orally, with the help of an oral feeding needle. Control received equivalent volume of sterile distilled water. The dose of *Hibiscus rosa sinensis* was selected by small experimentation and by checking its LD₅₀. Mice were sacrificed in regular interval of 10 days from the day of commencement of dose till 30 days. Then mice were kept for recovery. Blood was collected, and serum was prepared and stored at -20°C until further use.

Organ Weight

Testis and epididymis were dissected out, blotted free of blood, adhering tissues and weighed.

Sperm Count

Caudal epididymal sperm count was assessed in haemocytometer and expressed as 10⁶/ml of suspension. ^[17]

Histological Studies

For Histological studies, testies and epididymus were randomly selected from left or right side of mice from fourth group and control. Then fixed in Bouin's fluid, dehydrated in graded ethanol series, cleared in benzene and embedded in paraffin. Tissue were sectioned at 5µ, and the sections were stained with eosin and haematoxylin.

Blood Glucose Level

Blood Glucose level was detected checked by Glucometer.

Biochemical estimation

Quantitative Biochemical estimation of cholesterol in blood serum. ^[18]

Statistical Analysis

All data were represented in statistical form and student - T test analyses were carried out to determine the levels of significance between control and experimental groups.

RESULTS

BW and Organ weight

There is no significant difference was observed in BW of *Hibiscus rosa sinensis* control and treated mice and they were found healthy in throughout period of investigation. Weight of testes and epididymis showed significant reduction in comparison to control. After withdrawal of treatment mice were kept for recovery period of 2 months and it was found there is recovery in weight of testes and epididymis of treated mice (Table 1).

Sperm Density

Significant reduction in sperm density was found from 10th day onwards and was observed till 30 days and the mice were recovered after withdrawal of treatment (Fig 1).

Histology

Histological examination of testies of treated and control animals indicate that though there is no decrease in diameter of seminiferous tubules, in the treated animals, but the

thickness and compactness of germinal epithelium is significantly lost and most of the cells of the germinal epithelium have undergone hypertrophy. The process of spermatogenesis is highly disrupted and the accumulation of sperms within the lumen is almost negligible but after the withdrawal of treatment it was found that there is recovery of treated mice (Fig 6). While the epididymides of untreated control mice showed normal histologic features. Hematoxylin stained sections through Days 10, 20, 30 and 90 of mouse epididymus. After dosing of *H.rosa sinensis*, 500mg/kg BW/day showing normal appearance of the segment, except that the lumen is empty and stroma appears increased after 10th, 20th and 30th day treatment. And after recovery of treatment it was found that both control and treated mice have normal appearance (Fig 7).

Testosterone Level

Assay of testosterone in the serum by ELISA also indicates the fall in density of sperms and that of testosterone level are correlated to one another (Fig 2).

Blood Glucose Level

Blood Glucose level was observed after fasting and PP and it was found that it is showing significant decrease after 20 days treatment in fasting while it is showing significant decline after 10 day onwards in PP (Fig 3, 4).

Cholesterol Level

Blood Cholesterol Level Decreased from 10th day only and it was showing significant decline till 30th day treatment. And after withdrawal of treatment it was observed that level was recovered as of control (Fig 5).

DISCUSSION

The result of present study show that *Hibiscus rosa sinensis* treatment did not cause alterations in the body weight but weight of testis and epididymis showed a significant reduction in treated animals. And there was no decrease in diameter of seminiferous tubules. But the thickness and compactness of germinal epithelium have undergone hypertrophy. The process of spermatogenesis is highly disrupted and the accumulation of sperms with in the lumen is almost negligible. Flower of *Hibiscus rosa sinensis* posses antifertility property which has been reported long back in Ayurvedic text. However, in the last decade it was pointed out that the alcoholic extracts of the flowers of *Hibiscus rosa sinensis* decrease spermatogenic elements of testis and epididymal sperm count at a dose of 125 and 250 mg/kg of BW. ^[8] The present study also observed similar changes in the seminiferous tubules of testis and sperm density though the dose was much higher in the present study. Sperm density declined significantly. As it was reported in case of *Bougainvillea spectabilis* crude extract showing changes in sperm density as well as alterations in seminiferous tubules was found. ^[19] Assay of testosterone in the serum by ELISA also indicated the fall in density of sperms and that of testosterone level are correlated to one another because reduction in the number of spermatozoa in *Hibiscus rosa sinensis* extract treated mice appeared to be due to the suppressive effect of the treatment on spermatogenesis as sperm number recovered to control level after recovery of the process following cessation of the treatment.

Earlier Kholkute, has described that antifertility action of *Hibiscus rosa sinensis* is season dependent. He also reported that it causes significantly inhibitory action on spermatogenesis in mediated via pituitary gland without affecting pituitary adrenal and pituitary thyroid function in male

Table 1: Weight of testis and epididymis in mice after *H. rosa sinensis* treatment (500 mg/kg of BW) and following recovery from treatment.

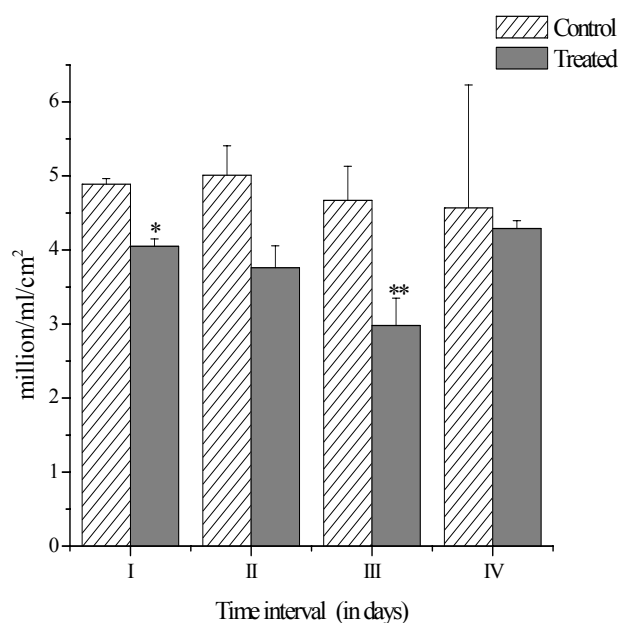
Time Interval	Control (mg/100gm BW)			Treated (mg/100 gm BW)		
	BW(g)	Testies	Epididymis	BW(g)	Testies	Epididymis
10	30.00±0.0	582.23±3.2	425.47±3.5	30.00±0.81	565.56±1.3	380.29±2.25
20	30.00±0.0	597.33±4.7	419.21±1.2	30.00±.40	533.19±1.9*	352.01±3.2
30	30.00±0.0	580.21±2.1	415.41±1.8	30.75±.82	510.02±2.5**	341.19±1.5**
90 ^a	30.00±0.0	585.66±1.51	420±3.7	30.80±.40	493.44±3.65**	325.51±4.33**

The values are expressed as mean ± SD for 10 animals (n=10) per group.

a Treatment was discontinued after 30 days, and animals were sacrificed 60 days after withdrawal of treatment.

* indicates statistically significant at $p < 0.001$.

** Indicates statistically significant at $p < 0.005$.

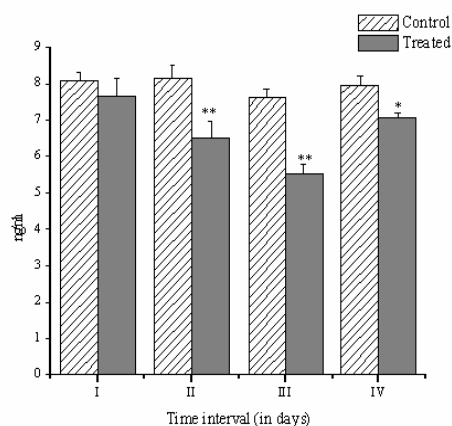
Fig. 1: Sperm density in mice after *Hibiscus rosa sinensis* treatment (500mg/kg of BW) following recovery from treatment

The values are expressed as mean ± SD for 10 animals (n=10) per group.

IV Treatment was discontinued after 30 days, and animals were sacrificed 60 days after withdrawal of treatment.

* indicates statistically significant at $p < 0.001$.

** Indicates statistically significant at $p < 0.005$.

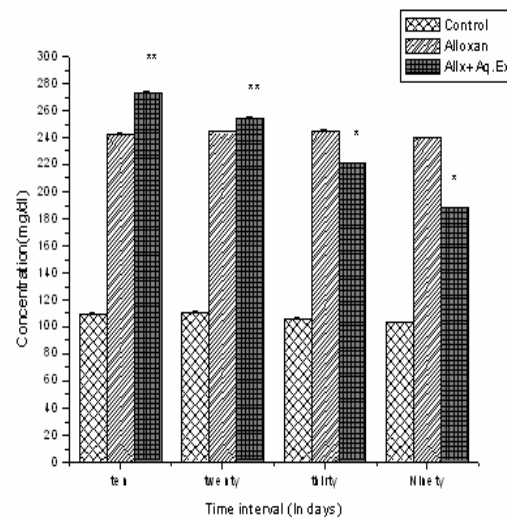
Fig 2: Testosterone level in mice after *Hibiscus rosa sinensis* treatment (500mg/kg of BW) following recovery from treatment

The values are expressed as mean ± SD for 10 animals (n=10) per group.

IV Treatment was discontinued after 30 days, and animals were sacrificed 60 days after withdrawal of treatment.

* indicates statistically significant at $p < 0.001$.

** Indicates statistically significant at $p < 0.005$.

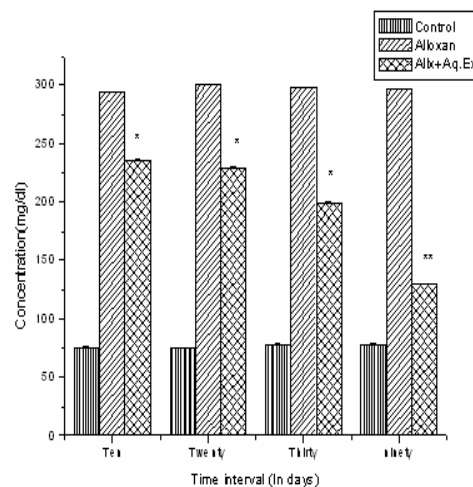
Fig 3: Blood glucose level after pp in mice after *Hibiscus rosa sinensis* (500mg/kg of B.W) and following recovery from treatment.

The values are expressed as mean ± SD for 10 animals (n=10) per group.

Ninety Treatment was discontinued after 30 days, and animal were sacrificed 60 days after withdrawal of treatment

*Indicates statistically significant at $p < 0.001$.

** Indicates statistically significant at $p < 0.005$.

Fig 4: Blood glucose level after fasting in mice after *Hibiscus rosa sinensis* (500mg/kg of B.W) and following recovery from treatment.

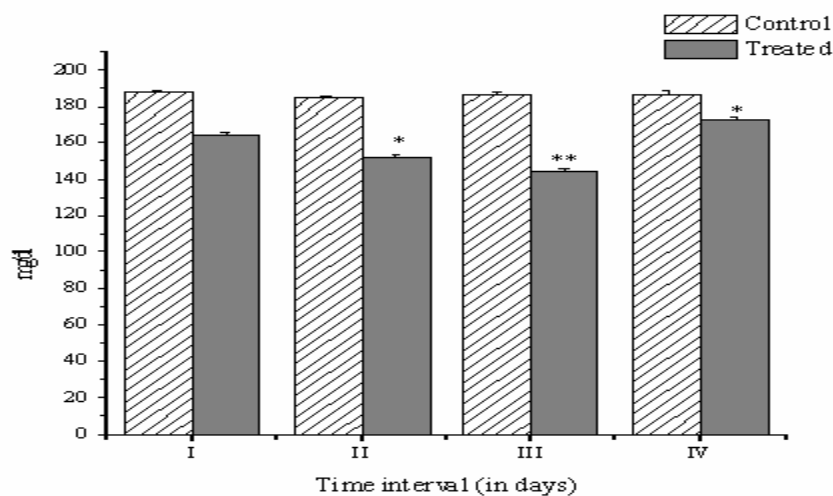
The values are expressed as mean ± SD for 10 animals (n=10) per group.

Ninety Treatment was discontinued after 30 days, and animal were sacrificed 60 days after withdrawal of treatment

*Indicates statistically significant at $p < 0.001$.

** Indicates statistically significant at $p < 0.005$.

Fig 5: Cholesterol level in mice after *Hibiscus rosa sinensis* treatment (500mg/kg of BW) and following recovery from treatment.



The values are expressed as mean \pm SD for 10 animals (n=10) per group.

IV Treatment was discontinued after 30 days, and animals were sacrificed 60 days after withdrawal of treatment.

* indicates statistically significant at $p < 0.001$.

** Indicates statistically significant at $p < 0.005$.

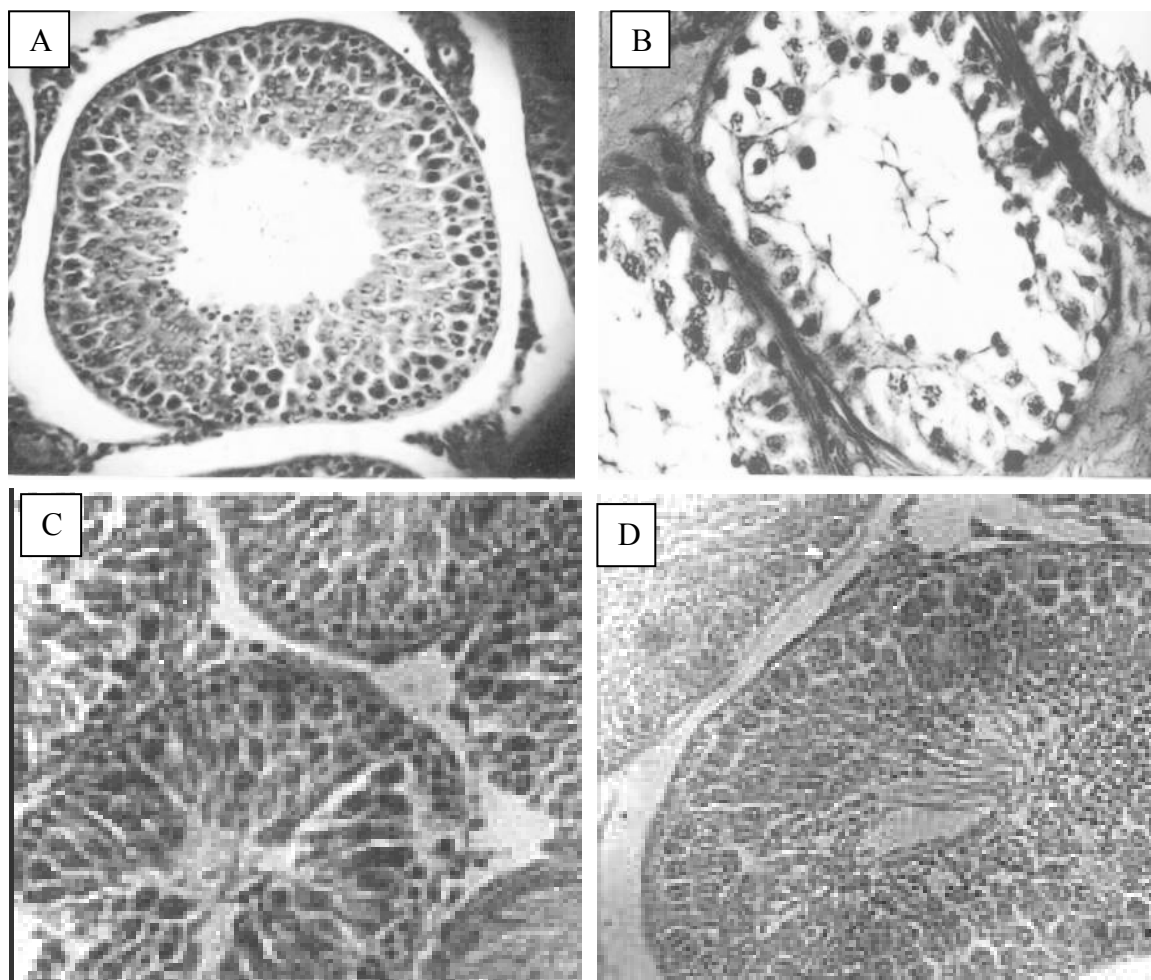


Fig. 6: Cross Section of Testis of the control and treated mice. (A) Control showing normal appearance of seminiferous tubules. (B) After *H.rosa sinensis* treatment, 500mg/kg BW for 30 days showing Decrease in compactness of germinal epithelium and sperm number was almost negligible in Lumen. (C) Control was showing normal appearance after recovery period also. (D) After withdrawal of treatment recovery was observed.

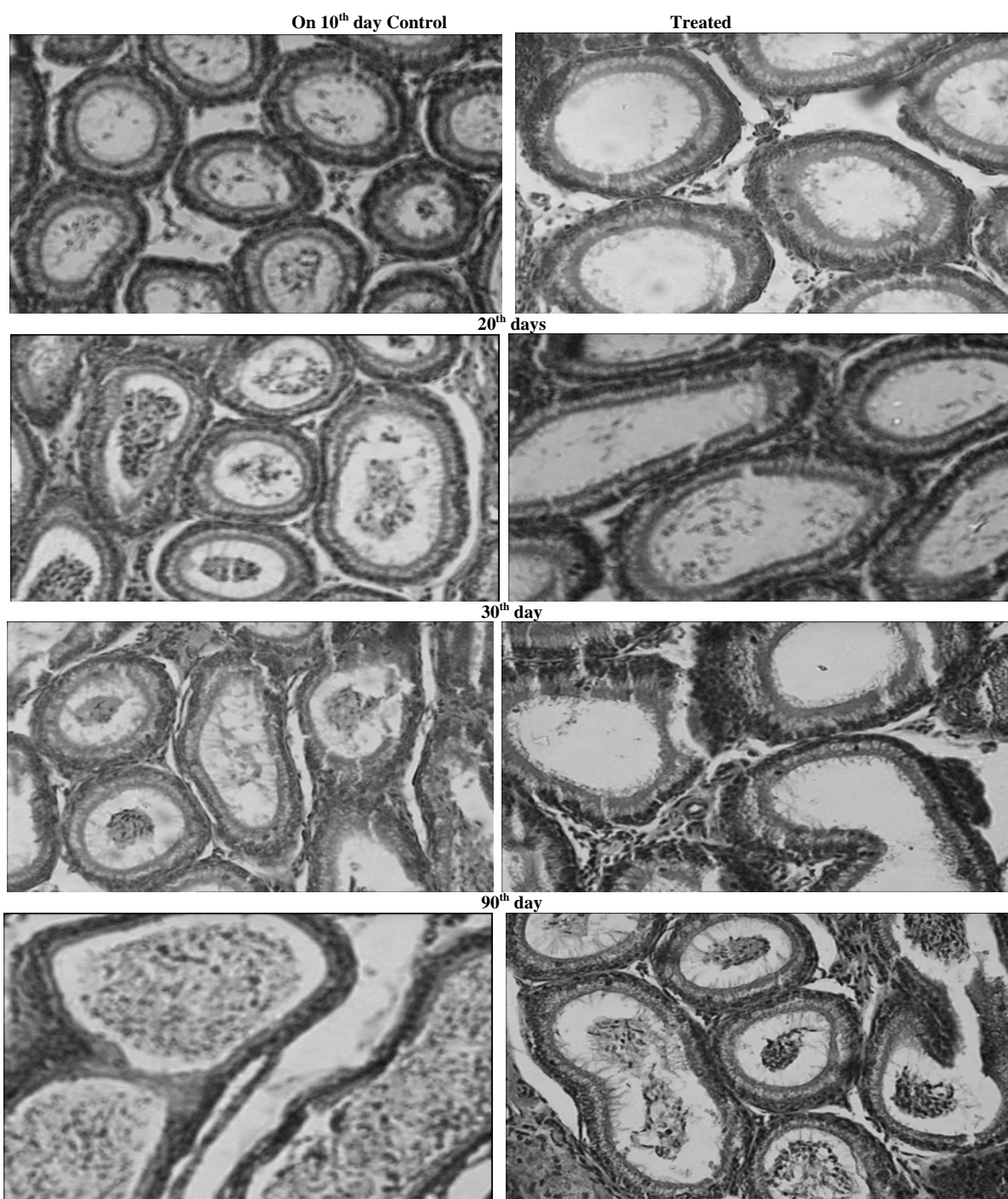


Fig. 7: Hematoxylin stained sections through Days 10, 20, 30 and 90 of mouse epididymus .Control mice showing normal histological features. After dosing of *H.rosa sinensis*, 500mg/kg BW/day showing normal appearance of the segment, except that the lumen is empty and stroma appears increased after 10th, 20th and 30th day treatment. And after recovery of treatment it was found that both control and treated mice have normal appearance.

albino rats. [20]

In second phase of study, deals with the evaluation of glucose level in the blood after PP and fasting. From data it is clear that crude extract of *Hibiscus rosa sinensis* blooms causes a significant decrease in level of glucose in alloxan induced diabetic mice at a dose of 500 mg/kg of BW after 10th day treatment as compared to alloxan treated mice. This observation supporting the report of Sachdewa and Khemani, suggesting that oral intake of *Hibiscus rosa sinensis* blooms causes hypoglycemia even in normal animal. [15] Earlier, it has been assigned hypoglycemia property to aqueous extract of *Hibiscus rosa sinensis* concerned, evidence suggest that hibiscus mucilage rich in flavonol bioside, in general posses antidiabetic property. [14]

In third phase of study, Cholesterol level of blood quantitatively estimated in control as well as treated animals. After analysation of data, it is inferred that oral administration of *Hibiscus rosa sinensis* flowers decrease blood cholesterol level significantly just after 10th day. Decline was upto 25%. A decline of serum cholesterol and triglyceride levels 22% and 32 % in the flower crude extract treated animals reported by Sachdewa and Khemani is in concordance with the present analysation. [15] Moreover it was observed that the mucilage obtained from the plant of genus *Hibiscus* has hypocholesterogenic properties. [21-22] In conclusion our result of Swiss albino mice suggests that *Hibiscus rosa sinensis* treatment at doses and durations employed in the present study caused marked alteration in

the male reproductive organs and that alteration are reversible after cessation of treatment. Treatment also had a reversible effect on Cholesterol level and diabetes.

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