Proc. Indian natn. Sci. Acad. B **44**, pp. 1-6 (1978)
Reproductive Physiology

EFFECTS OF *MALVAVISCUS CONZATTII* GREENM FLOWER EXTRACT ON THE MALE REPRODUCTIVE ORGANS OF BAT (*RHINOPOMA KINNEARI* WROUGHTON) : A CONFIRMATION

by V. P. DIXIT *and* S. K. BHARGAVA, *Reproduction Physiology Section, Department of Zoology, University of Rajasthan, Jaipur* 302 004

(Recieved 27 *June*, 1977; *after revision* 3 *November*, 1977*)*

1. Chronic administration of *M. conzattii* flower extract (50 mg/day for a period of two weeks) caused testicular lesions resulting in a mass atrophy of the spermatogenic elements. 2. Epididymides were shrunken and the lumen was devoid of spermatozoa. 3. Serum protein, cholesterol, lipids, transaminases enzymes (SGOT, SGPT) and phosphatases were in normal range. 4. Normal functioning of liver, kidney and general metabolic activities could be established. 5. Leydig cell impairment and relative decrease in accessory sexual organ weight points out the deficient androgen production following the administration of *M. conzattii* flower extract.

INTRODUCTION

The need of cheap, safe, effective and easily administrable oral contraceptive is very well recognized. Many hormonal preparations are available for the purpose but they are not free from side effects. Hence the search for a suitable product from indigenous medicinal plants, which could be effectively used in place of the " Pill ", is being actively pursued.

The flowers of *Hibiscus rosa sinensis* (family Malvaceae) are attributed to possess contraceptive properties in Ayurvedic literature, Materia Medica and folklores. Batta and Santhakumari (1971); Kholkute *et al.* (1972), Kholkute and Udupa (1974) have observed antifertility properties of this flower. Recently Kholkute and Udupa (1976) reported anti-estrogenic properties of *H. rosa sinensis* in bilaterally ovariectomized immature albino rats.

No other member of the family Malvaceae has yet been worked out for its antifertility action in males. A study of acute or chronic testicular effects of *M. conzattii* flower extract seemed desirable.

Investigations into the effects of prolonged treatment with *M. conzattii* flower extract in gerbil (*Meriones hurrianae* Jerdon) and house rat (*Rattus rattus* Rufescens) have led to the observation that the flower extract produces a selective effect on the male reproductive system (Dixit, 1977). This prompted us to study the testicular function in a variety of mammalian species including hibernating mammals.

Materials and Methods

Rhinopoma kinneari *(Wroughton)*

In the rat tailed bat, the testis is at its peak from late January till the end of April in the arid zone region of Rajasthan, India (Kumar, 1965). The bats were caught from the surroundings of Amber fort in the month of May, 1977. The healthy males, weighing 23 ± 3 g were housed in wire cages in groups of twenty. They were acclimatized in the laboratory for at least 7 days. The bats were administered orally with *M. conzattii* flower extract 50 mg/day for a period of two weeks. An equal number of males received 0·2 ml. of distilled water each day and served as controls.

The total extract of *Malvaviscus conzattii* Greenm flowers was prepared by extracting the freshly collected dried and powdered flowers by Soxhalation. For Soxhalation 100 g of flower was taken in Soxhalate and extracted with rectified spirit. Extract was taken in 1 litre flask filled with vacuum-distillation and solvent was removed under reduced pressure and controlled temperature (55 to 60°C). The total alcoholic extract was administered as aqueous suspension. The extracts were administered orally by specially designed hypodermic syringe needle.

The animals were sacrificed by rapid decapitation after termination of the treatment. At necropsy, the testes, sex-accessories, adrenal and thyroid glands were removed, weighed and inspected for edema, haemorrhages or other gross abnormalities. The testes and epididymides were fixed in Bouin's fluid, embedded in paraffin, cut at 6 μ and stained with haematoxylin-cosin or with PAS. Fifty seminiferous tubules appearing circular in section were traced with a camera lucida at $\times 80$. Two perpendicular diameters of each circular tracing were measured, averaged and expressed in terms of mean tubular diameter. Student's 't' test was applied in comparing means. The measurement of the diameter of the 100 Leydig cell nuclei was carried out on four sections from each testicle with camera lucida drawings at $\times 800$.

Hepatic and general metabolic activities were followed with determination of SGOT, SGPT, serum alkaline/acid phosphatase, cholesterol, lipids, proteins, blood sugar, blood urea, haemoglobin and packed cell volume (Mohun & Cook, 1957; Fiske & Subbarow, 1925; Oser, 1965; Folch *et al.*, 1957; Lowry *et al.*, 1951). The standard techniques were used for most of the clinical parameters.

Observations

In the males, the testes are permanently intra-abdominal and a scrotum is absent. The spermatogenic cycle is completed in the intra-abdominal testes. Sperms are stored within the cauda epididymis, which is quite enlarged. The accessory reproductive organs of the males, i.e. the ampullary, prostate, and the bulbo-urethral glands of Cowper, were enlarged.

M. conzattii flower extract administration by oral route did not cause loss in body weight, whereas the relative testicular weight of flower extract treated bats decreased drastically. The adrenal and thyroid gland weight did not change. The accessory sex organs were reduced in size (weights not recorded).

The seminiferous tubules presented marked degenerative changes and were lined by one or two cell layers (Figs. 1 and 2). Spermatogonia decrease in number. Degenerative changes in the spermatocytes, such as giant cell formation or chromatolytic changes in pachytene spermatocytes, and occasional degenerating spermatocytes could be found. Shrinkage of the seminiferous tubule and Leydig cell nuclear diameter was conspicuous (Table I).

TABLE I

Changes in body weight, the weights of testes, adrenal and thyroid glands together with seminiferous tubule and Leydig cell nuclear diameter of Rhinopoma kinneari *treated with* Malvaviscus *flower extract*

Group No.	Treatment		Body wt. (g)	Testes	Adrenal	Thyroid	Seminiferous tubule diam. (μm)	Leydig cell nuclear diameter (μm)
				weight (mg.)				
1.	Control	(25)	23±3	45±7	2·8±0·2	2·1±0·3	167±4	10·7±0·3
2.	*Malvaviscus* flower extract [50 mg/day for 2 weeks]	(30)	19±5	32±4*	2·8±0·3	1·9±0·2	124±7*	8·6±0·2*

*$P < 0·01$ compared with control.
Figures in parentheses represent the number of animals examined.
Values are mean ± SE.

Epididymides

M. conzattii flower extract caused degenerative changes in the epididymal histology. The luminal epithelium was reduced and the stereocilia were absent. The lumen was devoid of spermatozoa (Figs. 3 and 4).

Serum/Blood Analysis

Total protein, cholesterol and lipids were in normal range (Table II). Blood sugar, urea, serum transaminases (SGOT and SGPT) alkaline/acid phosphatase were at the control levels (Table II).

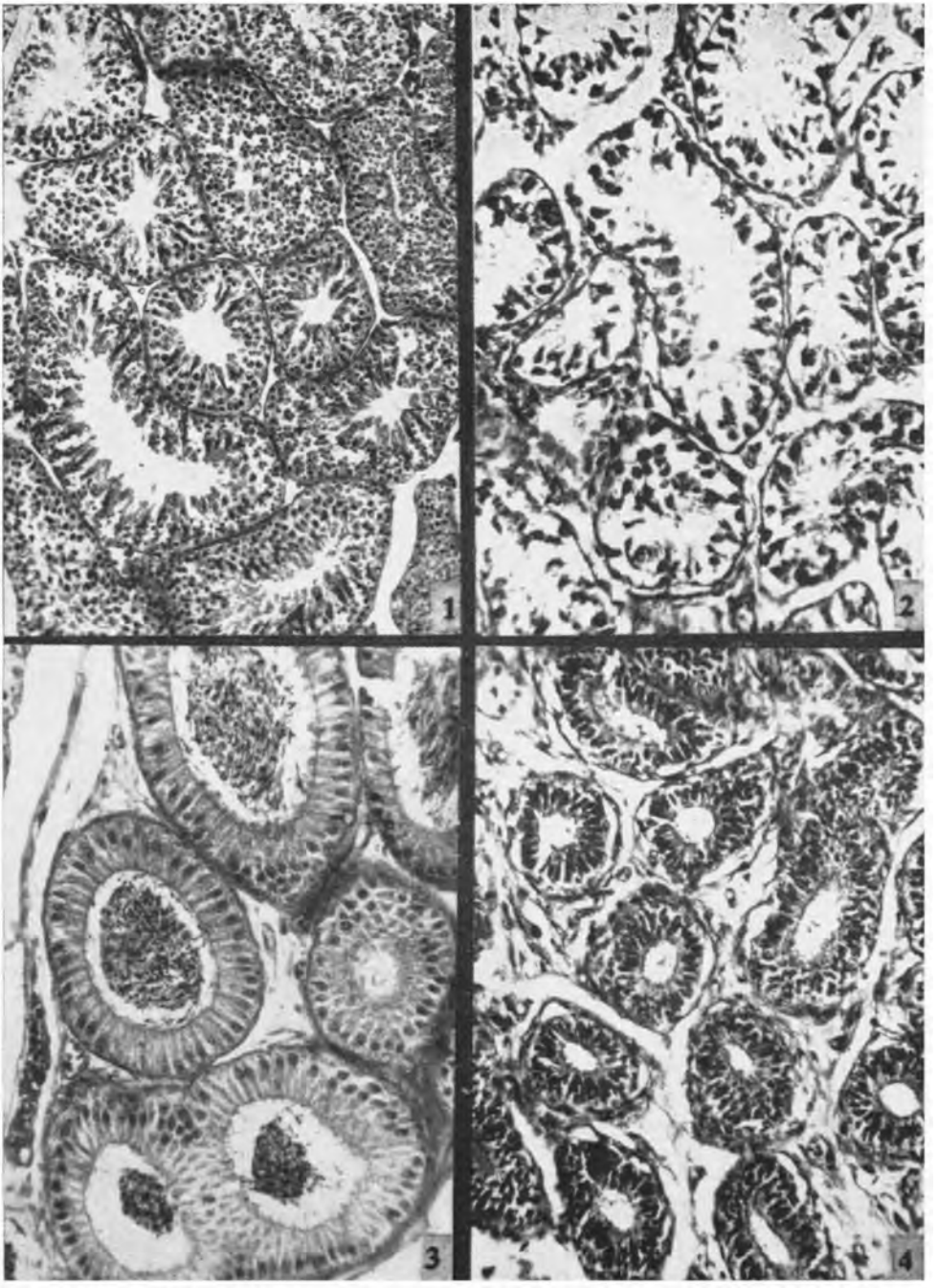
Haematological Studies

Red cells — $5·97 \times 10^6/mm^3$; Haemoglobin — 12·5 g/100 ml ; Packed Cell Volume (PCV : Haematocrit value) — 29·6% ; Leucocytes — $10,500/mm^3$.

Differential Leucocyte Count

Neutrophils — 67%; Lymphocytes — 30%; Monocytes — 2%; Eosinophils — 1%; Basophils — 0%.

Pathological examination of the liver taken at biopsy did not show any damage. The architecture was microscopically normal.



FIGS. 1-4. 1, Section of a testis from a control bat. The seminiferous tubules show full spermatogenesis. × 100 HE; 2, Section of a testis after *M. conzattii* flower extract administration (50 mg/day for 2 weeks). Note the degenerative changes. × 100 HE; 3, Section of a control caput epididymides of bat. The lumen is full of spermatozoa. × 200 HE; 4, Section of a caput epididymides after *M. conzattii* flower extract administration. Note the absence of spermatozoa. × 100 HE.

TABLE II

Serum analysis of normal/flower extract treated bats

	Protein Cholesterol Lipids (mg/100 ml serum)			Alkaline Phosphatase *	Acid	SGOT	SGPT	Blood sugar (mg/100 ml)	Blood urea (mg/100 ml)
Control values	5670±211	187±13	9±1·5	6·7±0·5	2·5±0·3	37±5	43±7	65·6±3·7	36·3±2·5
Malvaviscus flower extract (50 mg/day for 2 weeks)	4607±131	223±11	7±0·7	9·8±0·3	2·8±0·5	63±9	54±11	75·7±5·8	31·4±3·7

*μg alkaline phosphorus/hour/ml of serum.

Biochemical estimations : mean of six determinations ± SE.

DISCUSSION

The most important finding in the present work is the high selectivity of lesions inflicted by *M. conzattii* flower extract in the gonad of male bat. The testes were severely but selectively injured. The degenerative changes in the spermatocytes were followed by pyknosis and chromatolysis. Administration of alkylating agents such as nitrofuran (Nelson & Steinberger, 1953) and of thiophene (Steinberger, Boccabella & Nelson, 1956) produce a specific type of damage characterized by a spermatogenic arrest at the stage of primary spermatocyte. *M. conzattii* flower extract administration follow a similar pattern. The impairment of Leydig cell function was evidenced by its reduced nuclear diameter. This may lead to deficient androgen production.

Eisalo *et al.* (1964) reported reversible elevations of serum transaminase levels in women using oral contraceptives. *M. conzattii* flower extract administration for a period of two weeks did not produce any appreciable change in serum transaminase, alkaline/acid phosphatase and serum cholesterol and serum lipid levels. Histopathological examination of the liver did not show any damage.

Our data weigh in favour of normal functioning of liver, kidney and general metabolic activities as revealed by clinically important parameters [serum transaminases (SGOT, SGPT), phosphatases, blood sugar and blood urea, haemoglobin, hematocrit].

Haematological studies did not reveal any deviation from the normal values except a slight increase in total leucocyte counts.

CONCLUSION

M. Conzattii flower extract administration induced an antifertility state without altering general metabolic activities.

ACKNOWLEDGEMENT

I am indebted to Dr R. S. Mathur, Chairman of the Department of Zoology, for providing the facilities. The investigation was supported by Indian Council of Medical Research (Grant No. 5/10/42/75-RB), New Delhi, India.

REFERENCES

Batta, S. K. & Santhakumari, G. (1971). The antifertility effect of *Ocimum santum* and *Hibicus rosa sinensis*. *Indian J. med. Res.*, **59**, 777–781.

Dixit, V. P. (1977). Effects of *Malaviscus* flower extract on the testicular function of house rat (*Rattus rattus* Rufescens) and gerbil (*Meriones hurrianae* Jerdon) : A biochemical study. *Indian J. exptl. Biol.*, **15**, 506–509

Eisalo, A., Jarvinen, P.A. & Luukkainen, T. (1964). Hepatic impairment during the intake of contraceptive pills : clinical trials with postmenopausal women. *Brit. Med. J.*, **2**, 426-427.

Fiske, C. H. & Subbarow. Y. (1925). The colorimetric determination of phosphorus *J. biol. chem.*, **66**, 375–400.

Folch, J., Lees, M. & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. chem.*, **226**, 497-509.

Kholkute, S. D., Chaterjee, S. & Srivastava, D. N. (1972). Antifertility effect of the alcoholic extract of Japa *(Hibiscus rosa sinensis)*. *J. Res. Indian Med.*, **7**, 72–73.

Kholkute, S. D. & Udupa, K. N. (1974). Antifertility properties of *Hibiscus rosa sinensis*. *J. Res. Indian Med.*, **9**, 99–102.

———(1976). Antiestrogenic activity of *Hibiscus rosa sinensis* Linn. flowers. *Indian J. exptl. biol.*, **14**, 175–176.

Kumar, T. C. A. (1965). Reproduction in the rat tailed bat *Rhinopoma kinneari*. *J. Zool.*, **147**, 147–155.

Lowry, O. H., Rosebrough, M. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. *J. biol. Chem.*, **193**, 265–275.

Mohun, A. F. & Cook, J. J. Y. (1957). Simple methods for measuring serum levels of the glutamic oxalacetic and glutamic pyruvic transaminases in routine Laboratories. *J. Clin. Path.*, **10**, 394–399.

Nelson, W. O. & Steinberger, E. (1953). Effects of nitrofuran compounds on the testis of the rat. *Fed. Proc.*, **12**, 103 (abstr.)

Oser, B. L. (1965). In: Hawk's *Physiological Chemistry* (14th ed.), p. 246. McGraw Hill, New York.

Steinberger, E., Boccabella, A. & Nelson, W.O. (1956). Cytotoxic effects of 5-chloro-2-acetylthiophen (Ba-11044) on the testis of rat. *Anat. Rec.*, **125**, 312 (abstr.)