J. Phytol. Res. 18(2): 163-166, 2005

ANTISPERMATOGENIC EFFECT OF BENZENE EXTRACT OF *PLUMERIA BICOLOR* LEAVES IN MALE RATS

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The effect of benzene extract of *Plumeria bicolor* leaves was studied on testicular function in male rats. Administration of extract (500 mg/kg b.wt./day/orally) for 60 days caused a significant decline in the weights of testes ($P<0.001$), epididymides ($P<0.001$), seminal vesicle ($P<0.001$) and ventral prostate ($P<0.01$) of rats. The sperm counts and motility of cauda epididymal spermatozoa were significantly ($P<0.001$) reduced. The histomorphological picture of the testis of extract treated rats exhibited degeneration of seminiferous epithelium and impairment of spermatogenesis. Leydig cells showed atrophic changes. Thus, the benzene extract of *Plumeria bicolor* leaves has suppressive influence on spermatogenesis and testosterone biosynthesis in rats.

Keywords : Antispermatogenic; *Plumeria bicolor;* Sperm count; Sperm motility.

Introduction

Fertility regulation with natural products of plant origin has been reported in the indigenous system of medicine and appears to be an attractive alternative for a number of reasons. *Plumeria bicolor* (Family Apocynaceae) is a widely grown ornamental plant in Rajasthan, India. Traditionally many species of genus *Plumeria* are used to treat a large number of ailments. The benzene extract of *P. bicolor* leaves possesses anti-implantation effect in female rats[1]. Daily feeding of fresh green leaves of *P. alba* to male rats has been reported to cause atrophic changes in testis and arrest of spermatogenesis at primary spermatocyte stage[2]. Phytochemical analysis of the stem bark of *P. bicolor* has shown the presence of ferulic acid derivatives, plumericin and isoplumericin[3]. In the present study effects of benzene extract of *P. bicolor* leaves on testicular functions of rats were evaluated.

Materials and Methods

Plant material : P. bicolor leaves were collected fresh from the University Campus, Jaipur, India and dried under shade. The plant was identified and authenticated at the Herbarium of Botany Department, University of Rajasthan, Jaipur.

Preparation of extract : The shade dried leaves were extracted exhaustively with benzene in the ratio 1:10 (w/v) for 36 hours. The extract was concentrated under reduced pressure and low temperature and was used for the study by suspending in normal saline.

Animals and experimental protocol : Adult colony bred, healthy, male Wistar rats of proven fertility, weighing 220 - 260 g were used for this study. The animals were maintained under controlled temperature (22 ± 3°C) and constant photoperiodic conditions (12 h light/dark cycle). Commercial rat feed pellets (Lipton India, Ltd.) and water were provided *ad libitum*. The rats were randomly divided in to two groups of 7 each and treated as follows :

Group I : Vehicle (0.5 ml saline, p.o.) treated control.

Group II :Rats treated with extract (500 mg/kg b.wt./day, p.o.) suspended in normal saline (0.5 ml/rat) for 60 days.

Autopsy : After 60 days of extract treatment the rats were weighed and autopsied under mild ether anesthesia. The testes, epididymides, seminal vesicle and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed. The testes of each animal were fixed in Bouin's fluid for histopathological study.

Sperm motility and density : Cauda epididymal sperm motility and count were determined according to the method of Prasad *et al*[4].

Testicular histology : Histological sections of testis were cut at 5 µm, stained with Harris haematoxylin and eosin and observed under light microscope for histopathological effects.

Statistical analysis : All the data are expressed as mean ± SEM. Results were analyzed by Student 't' test. Values of $P<0.05$ were considered as significant.

Results and Discussion

Body and organ weights : The body weight of the control and experimental rats remained significantly unchanged while the relative weights of testes ($P<0.001$), epididymides ($P< 0.001$), seminal vesicle ($P<0.001$) and ventral prostate ($P<0.01$) declined significantly (Table 1).

Sperm density and motility : A significant ($P<0.001$) decrease in sperm motility and count was observed in the cauda epididymis of extract treated rats as compared to control rats (Table 2).

Table 1. Body and organ weights of male rats following oral administration of benzene extract of *P.bicolor* leaves.

Treatments	Body weight (g)		Organ weights (mg/100g b.wt.)			
	Initial	Final	Testes	Epididymides	Seminal vesicle	Ventral prostate
Control	230.60±	242.30±	1320±	470.2±	504.5±	481.5±
(vehicle treated)	12.80	18.40	30.8	12.5	32.2	21.7
P. bicolor extract	238.40±	229.50±	1080±	378.4±	316.2±	348.7±
(500 mg/kg b.wt./day)	16.60	17.20	32.5[b]	16.3[b]	26.8[b]	26.8[a]

Levels of significance
[a] P< 0.01 , [b] P<0.001 compared with control rats. (Values are mean ± SEM)

Table 2. Effect of benzene extract of *P. bicolor* leaves on histomorphometric characteristics of testis, epididymal sperm count and motility of rats.

Treatments	Number of Seminiferous tubules (%)		Seminiferous tubule diameter (μm)	Leydig cell nuclear diameter (μm)	Sperm count (million/ml)	Sperm motility (%)
	Normal	Abnormal				
Control	89.44±	10.55±	305.05±	6.52±	4.62±	74.30±
(vehicle treated)	2.52	2.05	9.03	0.18	0.17	3.75
P. bicolor extract	41.68±	58.31±	236.57±	4.26±	2.84±	41.42±
(500 mg/kg b.wt./day)	4.05[a]	4.05[a]	11.39[a]	0.17[a]	0.10[a]	4.10[a]

Levels of significance (Values are mean ± SEM)
[a] P<0.001

Histomorphology of testis : Histomorphological study of the testis of vehicle treated control rats showed normal spermatogenesis exhibiting presence of almost all germ cell types in the seminiferous epithelium and normal Leydig cells. In extract treated rats, testicular cytoarchitecture revealed degeneration and impairment of spermatogenesis. The diameters of Leydig cell nuclei and seminiferous tubules were reduced (P<0.001) when compared to control. There was significant increase in the number of abnormal seminiferous tubules exhibiting exfoliation and disorganization of cellular stages. The number of round and elongated spermatids and spermatozoa was also declined in affected tubules. The Leydig cells in testis of extract treated rats were atrophic (Fig. 1a, b, Table 2).

The results of the present study shows that benzene extract of *P. bicolor* leaves causes decline in epididymal sperm count and motility and impairment of spermatogenesis in seminiferous tubules.

The weights and functions of sex accessory organs is dependent on testosterone concentration. A significant decrease in the relative weights of epididymides, seminal vesicle and ventral prostate in extract treated rats indicates a suppressed level of testosterone[5].

Sperm count has been considered to be an important parameter for assessment of male fertility[6]. The depletion in sperm count in cauda epididymis reflects diminished spermatogenesis[7].

It is well established that the structural and

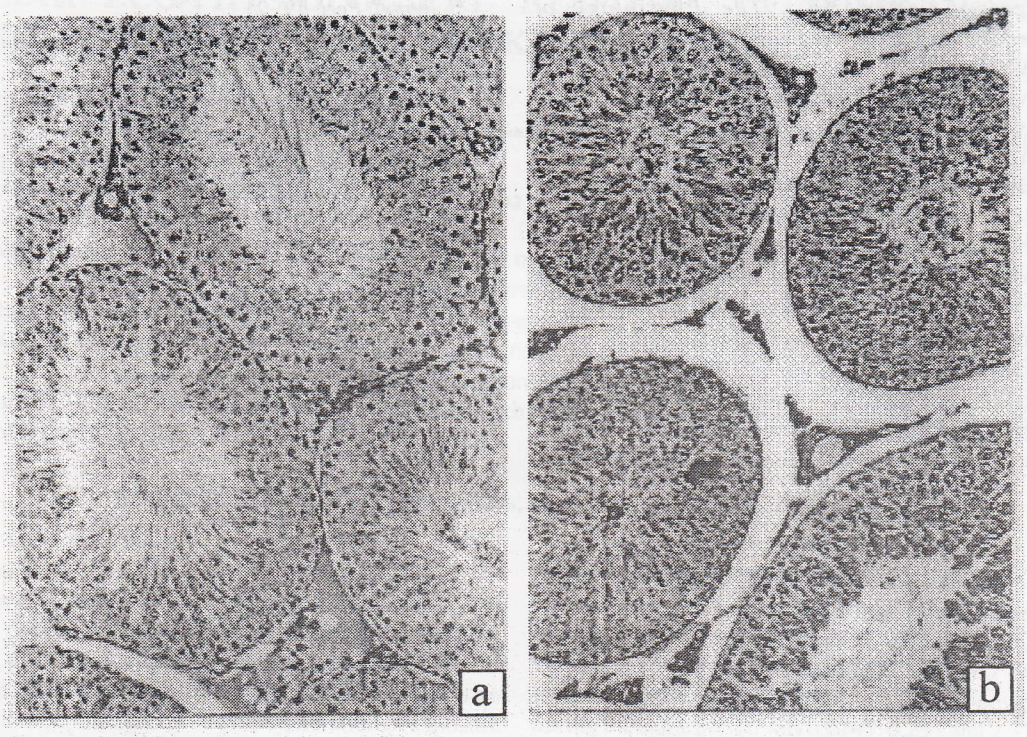

Fig. 1. Photomicrograph of testis
(a) Control rat showing normal spermatogenesis and Leydig cell morphology.
(b) *P. bicolor* extract treated rat exhibiting degenerative changes, impairment of spermatogenesis and atrophy of Leydig cells (X 100, H. E.).

functional integrity of the epididymis are dependent on androgens[8]. Significant decline in the sperm motility in cauda epididymis suggest an impairment of the androgen supply in the epididymis that adversely effects epididymal milieu and consequently sperm maturation, motility and fertilizing ability [9]. Further, the loss of motility of spermatozoa might also be associated with impairment of energy metabolism of spermatozoa [10].

The histological picture of the testis showed degenerative changes, depletion of postmeiotic germ cells. As the spermatogenesis is dependent on testosterone level via its action on the Sertoli cells[11,12], the deficiency of testosterone synthesis and secretion is expected to interfere with completion of meiosis resulting in decline in the number of spermatids and spermatozoa. A significant decline in the seminiferous tubule diameter observed in extract treated rats is very likely associated with the decline in the spermatogenic elements and spermatozoa [13]. This is also confirmed by reduced testis weight[14].

The Leydig cells are the most important source of testosterone in the mammalian testis. The decrease in nuclear diameter and atrophy of Leydig cells in extract treated rats suggests impairment of steroidogenesis [15].

The results of the present study indicates that benzene extract of *P. bicolor* leaves shows antispermatogenic effect possibly by testosterone deficiency.

Acknowledgement

The author is grateful to Prof. P. P. Bakre, Head of the Department and Prof. N. K. Lohiya, Coordinator, SAP-UGC for providing necessary facilities.

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