

Research Article

Evaluation of Anti-fertility Potential of Aqueous Extract of *Bougainvillea spectabilis* Leaves in Swiss Albino Mice.

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ABSTRACT

Population explosion is a major problem it is raising tremendously; this may affect drastically the economic growth. So family planning has been promoted through several methods of contraception. A wide variety of synthetic contraceptive agents are available, but these cannot be used continuously due to their side effects. Thus the present study was to evaluate the effect of *Bougainvillea spectabilis* on fertility of male and female mice. Swiss Albino mice were orally administered with aqueous crude extract of the leaves of *Bougainvillea spectabilis* (800 mg/kg of body weight/day, for 30 days), and effect of crude extract on reproductive organs and fertility was investigated. The treatment Caused decrease in weight of testis, sperm count and the histological examination revealed a reduction in the size of seminiferous tubules along with the thickness of germinal epithelial cells, though some of the epithelial cells and interstitial cells of leydig showed hypertrophy. Moreover, the lumen of the tubules was found to be devoid of sperms. On the other hand, in treated females no histological changes could be observed. The only marked change, which could be visualized, was the slight disruption of estrous cycle having prolonged metaestrus phase, which has been increased by 145.28 %. The overall duration of estrous cycle had been extended by more than one day. The hormonal profile was also influenced by the *Bougainvillea spectabilis* extract. The testosterone and estrogen levels were significantly decreased.

Keywords: *Bougainvillea*, estrogen, metaestrus, seminiferous tubules, sperms, testis, testosterone.

INTRODUCTION

Control of population growth is very important in populated countries. Control is an issue of global and national public health concern. Current methods of contraception result in an unacceptable rate of unwanted pregnancies and having side effect also. Thus there is a need to replace these agents by safe and effective agents such as plant based contraceptive agents. Many plants/plant extracts have been used as antifertility agents in folklore and traditional medicines without producing apparent toxic effects.^[1-5] As part of this research programme, we present in this paper antifertility efficacy of leaves of the plant *Bougainvillea spectabilis* *Bougainvillea spectabilis* (family Nyctaginaceae) is the most common evergreen ornamental woody plant inhabiting in warmer climate. The leaves of *Bougainvillea spectabilis* are reported to have medicinal properties viz. anti-diabetic^[6-7] hepatoprotective^[8], antiviral^[9] insecticidal activities^[10], antibacterial^[11] etc. D-pinitol (3-o-methyl-chiroinositol) an active constituent has been isolated from leaves of *Bougainvillea spectabilis*^[6]. Keeping all these reports in view, fact encouraged us to take up the leave of *spectabilis* investigation specifically on the reproduction capabilities of both the sexes.

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MATERIAL AND METHODS

Animals

Adult (age 12-14 weeks) Swiss albino mice weighing 30 g were used in the investigations. Mice were maintained under hygienic conditions in well ventilated room with 12 h photoperiod (8 am to 8 pm) temperature maintained at 25 ± 2°C. All the animals were fed twice a day with animal pellet feed (Hindustan lever Limited, Mumbai) and also supplemented either with bread, spinach leaves, soaked black grams or dahlia. Tap water was provided *ad libitum*. Animals in each group were housed in polypropylene cages. General body weight of the animals was monitored regularly during the entire tenure of the experiment. Animals were maintained according to the guideline of Institutional animal ethics committee.

Plant material

Fresh healthy leaves of *Bougainvillea spectabilis* were collected from the plants grown Vidyapith's campus. These were oven dried and powdered. Powdered leaves were concentrated under reduced pressure and then dissolved in distilled water for administration to mice.

Experimental protocol

The animals were randomly selected for investigation. 20 mice selected for study were divided into two groups of 10 animals each (5 males and 5 females).

Groups Treatment

I Untreated control

II *Bougainvillea spectabilis* at 800mg/kg BW for 30 days

Aqueous extract of *B. spectabilis* was suspended in sterile distilled water and administered orally with the help of oral feeding needles. Control receives an equivalent volume of sterile distilled water in similar manner. The dose of *B. spectabilis* selected on the basis of a pilot study. After recording final BW at the end of the treatment animals were sacrificed. Blood was collected and serum was prepared and stored at -20°C until further use.

Body weight and Organ weight

Body weight recorded before and after treatment. Testis, ovary were dissected out, blotted free of blood, adhering tissues were removed and weighed.

Sperm count

After cessation of treatment, the mice were sacrificed. Their testes and epididymis were removed. Epididymis was minced in dissociation solution (Normal Saline with sodium azide [0.25 %]). The mixture was then allowed to settle for 1 min after gentle swirling to resuspend the sperms. 1 ml sample was taken and stained by drop of eosin solution (1 % eosin Y in water) followed by incubation for 45 – 60 min at room temperature. Sperm number were counted using hemocytometer 11 and expressed as 10^6 ml^{-1} of suspension.^[12]

Evaluation of female fertility potential

For the study of estrous cycle, vaginal smear of all the females was examined twice a day till the completion of the treatment. By introducing 2-3 drops of physiological saline in vagina of mice. Then final drop obtained in the dropper was taken on the slide and studied under the high power of the student microscope.^[13]

Hormonal Assay

The level of testosterone was measured in serum by ELISA testosterone and estrogen standard kits (Biochem. Immuno systems Ltd., Italy). The serum samples were extracted from the blood samples. Blood was collected through orbital sinus of both the groups.

Histological studies

For Histological studies, Testis and ovary were randomly selected from left or right sides of the mice in each group. Portion of the testis and ovary were fixed in bouin's fixative. These were dehydrated in graded ethanol series. Cleared in benzene and embedded in paraffin. Then these were sectioned at 5μ using rotatory microtome. Then stained with haematoxylin-eosin, examined and photographed (X200).

Statistical analysis

The data were statistically analyzed using student's t-test. The values were expressed as mean $\pm$ S.D.

RESULTS

Oral administration of aqueous extract of *Bougainvillea* leaves in treated mice revealed the effect on gonads of male and female albino mice.

Genital organ weights and pH

In the first phase of study, the evaluation of body weight, pH and weight of gonads was done in both control and treated groups, after 30 days of treatment to the second group with crude aqueous extract of *Bougainvillea* leaves. The results are summarized in bodyweight but significant decline in the weight of gonads of treated animals as compared to control in both the sexes. Similarly, the pH of gonads was found to be significantly decreased in treated males and females (Table 1)

Sperm count

The caudal epididymal sperm count of the experimental mice significantly declined (87.13 %) after treatment with aqueous extract of *Bougainvillea* leaves (Table 1).

Female fertility potential

The estrous cycle was found to be irregular and disturbed. Almost all the treated females showed prolonged metaestrus phase. Because of it the duration of cycle was extended from 105.2 ± 1.24 h in control to 130.0 ± 0.71 in treated animals (Table 2). It was revealed that there was an increase of duration of every phase of the estrous cycle except proestrus phase. The most significant change has been due to 145.28 % increase in metaestrus phase along with 75.44 % in estrus and 11.28 % in diestrus phase (Table 2, Fig. 1).

Hormonal Assay

The third phase of study, which dealt with the quantification of steroidal hormones in both the sexes by ELISA, found significant declined in the titer of testosterone in males and estrogen in females after the treatment (Table 3, Fig. 2).

Histological studies

In the last phase of study, histological slides of gonads in both sexes were prepared. The histological examination of slides revealed that in males the diameter of seminiferous tubules and the thickness of germinal epithelial cells are drastically reduced (Fig. 3 B). Moreover, in all the treated animals the lumen of seminiferous tubules, were found to be devoid of sperms and the interstitial cells of leydig and some of the germinal epithelial cells were hypertrophied, in comparison to control mice. However in females, no significant changes in the histology of ovary of treated animals could be observed with respect to controls (Fig. 3 C and D).

DISCUSSION

In past, many reports have suggested that the use of plant extract affecting the reproductive physiology of the animals. Much interest, however, has been shown in recent years, to control male fertility by using plants.^[14-15] Therefore, several plants have been evaluated for their antifertility potential in the hope of developing a contraceptive for use in men.^[16-22] Some of the plants like *Hibiscus rosa sinensis*, *Ocimum sanctum* and *Allium cepa* possessing antidiabetic properties affect the reproduction of males^[23-28] and females.^[29-31, 4] The most common plants affecting female fertility include *Rivea hypocrateriformis*. It was revealed an irregular estrous cycle with shortened estrus and metaestrus, and with lengthened proestrus.^[32] Aqueous extract of seeds of *Cassia fistula* are shown to possess antifertility properties.^[31, 4, 33] It has suggested that crude extract of plants is more beneficial as compared to the isolated ingredient.^[33] But the present study suggests that the crude extract intake must be recommended with care as there might be number of plants, not reported so far, having both antidiabetic and antifertility properties. The results of this study revealed that the extract of *Bougainvillea* leaf which is recommended for controlling diabetes⁶ had shown adverse affect on its reproductive organs. Male mice have shown more degeneration of their gonads in comparison to the female mice. Fertility potential test revealed that oral administration of *B. spectabilis* aqueous extract had extended the reproductive cycle of female mice by 1-2 days with prolonged metaestrus and decrease in serum estrogen level whereas in male total count of sperm decreased along with a decrease in titer of testosterone. Histologically it was found that reduction in diameter of seminiferous tubules, thickness of germinal epithelium and

Table 1: Effect of *Bougainvillea* leaf extract on sperm count, pH, and weight of testes and ovary

Treatment	Body Weight	Sperm count in cauda epididymus (10^6 ml^{-1})	pH		Reproductive organ weight (in grams)	
			Testis	Ovary	Testis	Ovary
Control (Group 1)	24.4 $\pm$ 0.7483	5.05 $\pm$ 0.36	8.3 $\pm$ 0.074	8.1 $\pm$ 0.08	0.123 $\pm$ 0.01	0.0113 $\pm$ 0.001
Treated (Group 2)	25.4 $\pm$ 0.50991	0.65 $\pm$ 0.06*	7.9 $\pm$ 0.26*	7.5 $\pm$ 0.14*	0.0875 $\pm$ 0.002*	0.0074 $\pm$ 0.001*

The values are expressed as mean $\pm$ SD for five animals (n=5) per group.

* indicates statistically significant at $p < 0.001$.

Table 2: Effect of *Bougainvillea* leaf extract on estrous cycle of Female albino mice

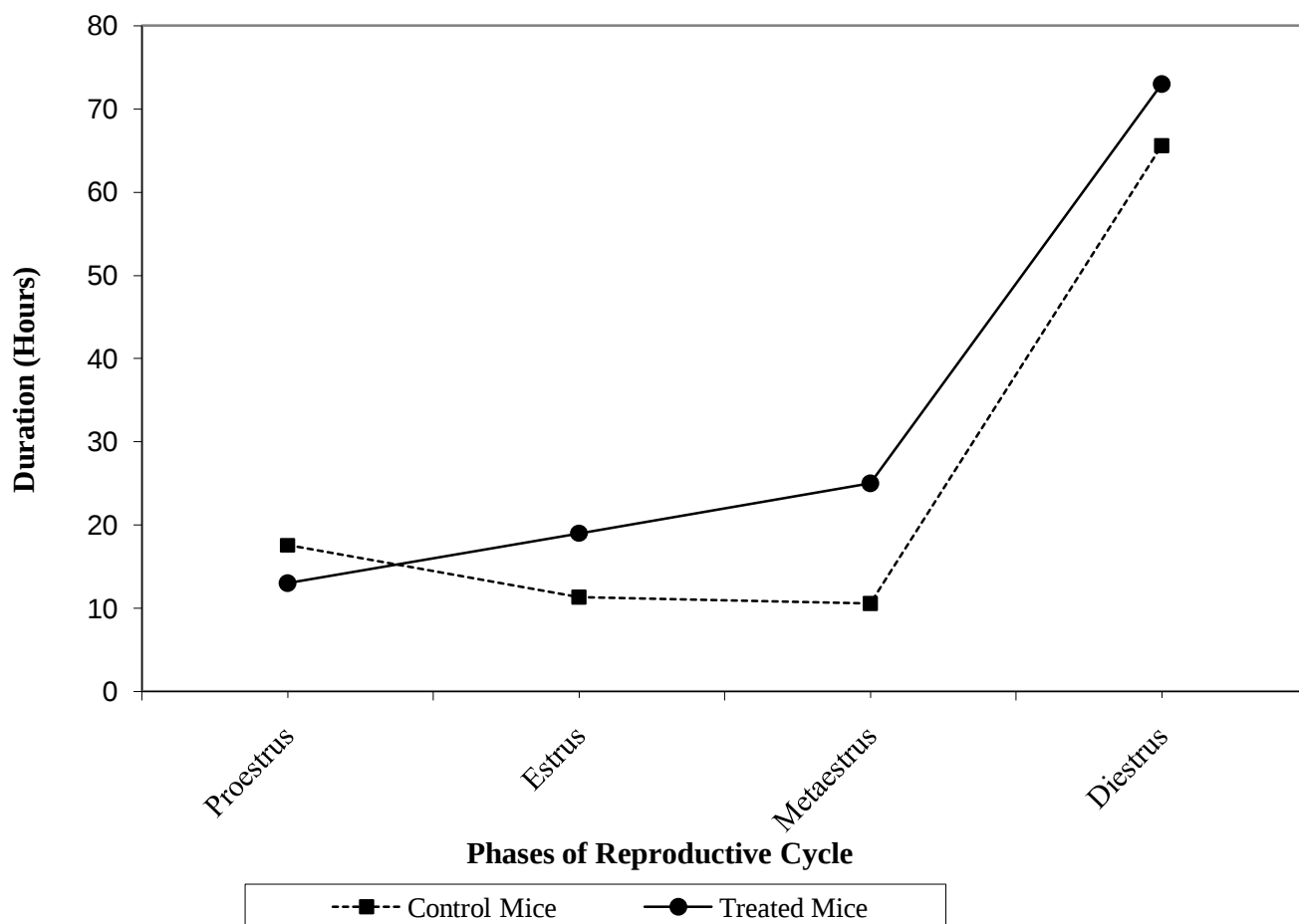
Reproductive cycle of female mice	Control (Group 1) (h)	Treated (Group 2) (h)	% Change in treated mice
Proestrus	17.6 $\pm$ 0.81	13.0 $\pm$ 0.45	- 26.41
Estrus	11.4 $\pm$ 0.87	19.0 $\pm$ 0.23	75.44
Metaestrus	10.6 $\pm$ 0.40	25.0 $\pm$ 0.45	145.28
Diestrus	65.6 $\pm$ 1.81	73.1 $\pm$ 0.40	11.43
Total period of cycle	105.2 $\pm$ 1.24	130.0 $\pm$ 0.71	23.57

Table 3: Hormonal profile of control and *Bougainvillea* treated animals

Treatment	Titer of testosterone (ng ml^{-1})	Titer of estrogen (pg ml^{-1})
Control (Group 1)	1.6 $\pm$ 0.1	83.4 $\pm$ 2.3
Treated (Group 2)	0.7 $\pm$ 0.25*	55.4 $\pm$ 3.25*

The values are expressed as mean $\pm$ SD for five animals (n=5) per group.

* indicates statistically significant at $p < 0.001$.

**Fig. 1:** Comparison of the duration of various phases of estrous cycle in control and treated mice

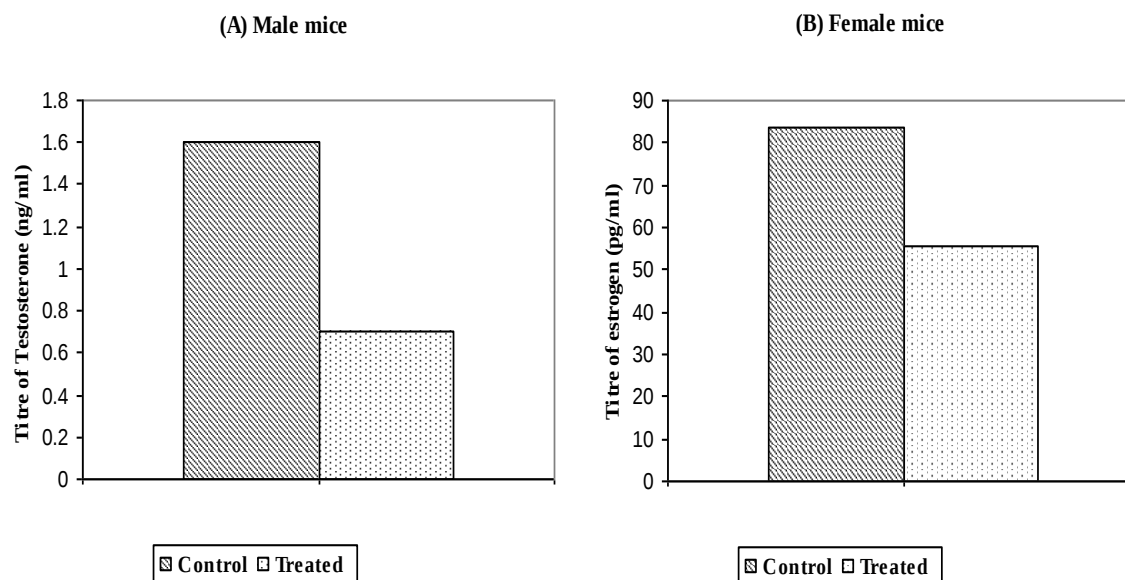


Fig. 2: Comparison of the titer of hormonal profile of (A) male (B) female albino, control and treated mice

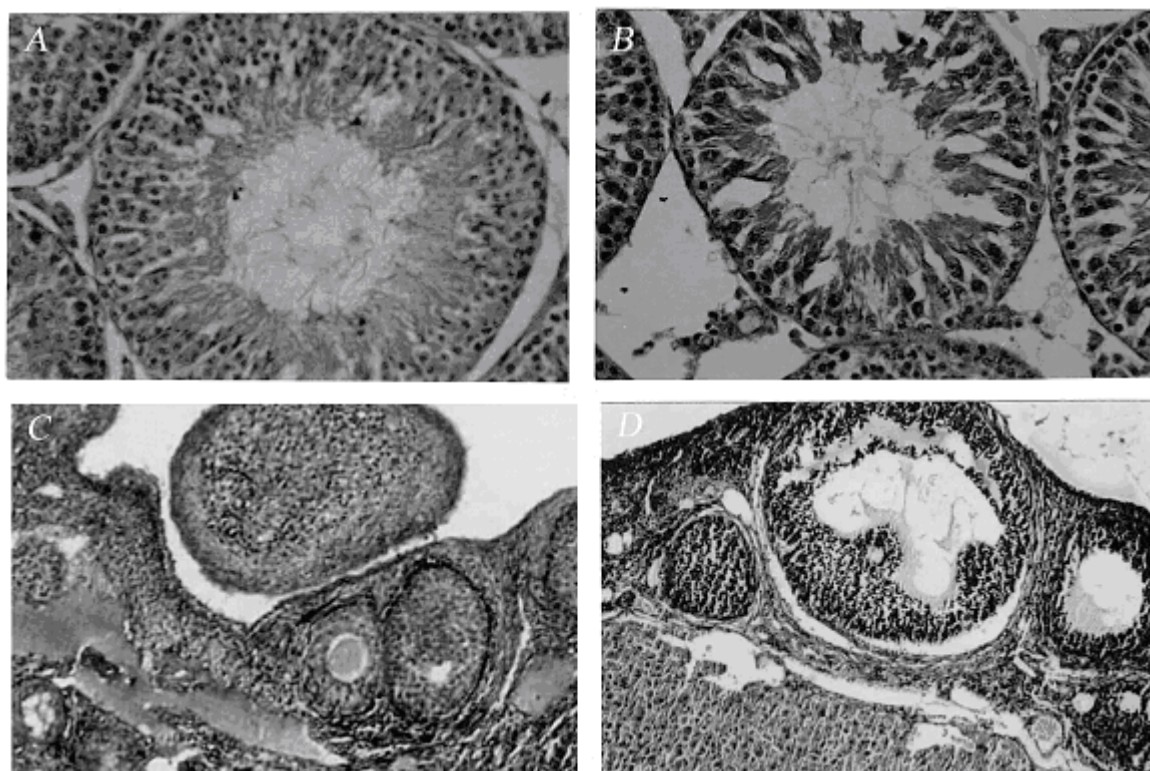


Fig. 3: Cross sections of the testis and ovary of the control and treated mice (A) T.S. testis of control (B) T.S. testis of treated mice (C) T.S. ovary of control (D) T.S. ovary of treated mice

absence of sperm in the lumen of tubules in treated animals. Besides this some of the interstitial cells of leydig and germinal epithilium were found to be hypertrophied. But in case of female no significant changes were found in histological test.

This investigation gave a clue of adverse pharmacological effect of the mechanism of action of the leaves extract of *B spectabilis* on male and female reproductive organs. Drugs obtained from plants is no doubt cheaper but crude extract intake must be recommended with care as it may control one aliment but may effect other physiological phenomena in the body.

ACKNOWLEDGEMENTS

We would like to thank Prof. Aditya Shastri (Vice Chancellor, Banasthali University) for providing financial support.

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