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A PRAGMATIC ANTIFERTILITY ASSESSMENT OF METHANOLIC BARK EXTRACT OF *PLUMERIA RUBRA (L.)* IN MALE ALBINO RATS

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ABSTRACT

Fertility control is an issue of global and national health concern, many study have been done for the male contraception. The use of medicinal plants in different sorts of disease including fertility problem is widespread throughout the world. Though considerable progress has been made the development of highly effective, acceptable and reversible methods of contraception among females, progress possibilities on males are still sluggish and limited. With recent advancement towards a better understanding of male reproductive physiology there is a need to develop new contraceptive modalities for male. *Plumeria rubra*, a plant belonging to the family Apocynaceae and it is commonly known as Lal Champa in India and is one of the widely used folk medicinal plants. The aim of the present study was to investigate the antifertility activity of *Plumeria rubra* bark methanolic extract orally administered in adult male albino rats. The plant extract were obtained from *P. rubra* as an effective ideal male contraceptive agent. There was reduction in the weight of testes and accessory sex organs may be due to the hormonal imbalances. The results of the present study indicate that the methanolic bark extract of *Plumeria rubra (L.)* have significant antifertility activity and it suppresses the process of spermatogenesis which can lead to infertility in male albino rats. As a whole it can be postulated from study that the extract appears to be promising male contraceptive agent in order to develop the potential of herbal medicine.

KEYWORDS: *Antifertility, Epididymis, Leydig cells, Sertoli cells.*

1. INTRODUCTION

In the developing countries like India over population is one of the most serious problems and world population would be reached about 9.2 billion by the year 2050. The last Indian census [2011] revealed that the population of India in 2011 was 1,210,193,422. Over population effects on poverty, environmental degradation, depletion of Natural resources, rise in unemployment. Modern reproductive biomedicine has provided several preventive and effective methods of contraceptives for fertility control in male and female but none of which is very safe and without any serious side effects.

Fertility control is very essential for maintaining satisfactory standards in the developing countries. Inexpensive, safe and effective as well as universally acceptable contraceptive is very much needed in present scenario. Fertility regulation has therefore become the major concern for the people all around the world. Hence, various methods are being used to reduce the total fertility rate in both men and women. Though, the search for an orally active, safe and effective plant preparation is yet to be needed for regulation of fertility due to incomplete fertility inhibition or side effects.

Besides rapid progress and development of science in medicine field, faith in and popularity of traditional methods and natural products have not decreased. There are a huge number of studies which supports the antifertility effects of plants and their product. Plant preparations play an important role in fertility regulation. The effect of either synthetic or natural products on the male reproductive health in the last decades has been of growing concern. A number of plant species have been tested for fertility regulation beginning about 50 years ago. The role of these indigenous plant products in the induction of male and female fertility in experimental animals has drawn the attention of researchers over the turn of the century.

Plumeria is grown as an ornamental plant in India, Indonesia, Philippines and South Africa. *Plumeria rubra*, a plant belonging to the family Apocynaceae and it is commonly known as Lal Champa in India, True Frangipani in English, Red Paucipan, Red Jasmine, Temple Tree, Mexican Plumeria, Red Plumeria, Pagoda Tree. Plumeria rubra is small deciduous tree. The bark is light-gray, shining and corky. The leaves are pointed, petiolate, simple, narrow and oblong and ranges from 2 to 4 inches wide and 8 to 12 inches long. The flowers are tubular, expanding into a “pinwheel” of five petals that averages 2-3 inches in diameter and are highly scented, red to pink. The fruit are borne in pairs with two long, cylindrical follicle or pods. Various species of this plant are used as medicine for the cure of many diseases and used as

antipsychotic, diuretic or antitumouragents. The plant has also been reported to have antifertility (Sharma, G., et al., 2011) and anticancerous activity. Some species of this plant are used in ulcers, leprosy, inflammations and rubifecient (Kardono et al. 1990). Decoction of the bark is used as counter irritant on the gum for tooth ache. The juice is rubifecient in rheumatic pain (Tembare et al. 2012) used as decoction for controlling dysentery & diarrhea during summer season (Begun et al. 1994). The decoction of bark and roots of P. rubra is traditionally used to treat asthma, ease constipation, promote menstruation, reduce fever and the latex is used to soothe irritation (Wiart, C., 2002). In India, its fruit is used as an abortifacient (Zaheer, Z., et al. 2010). The decoction of the flowers of *P. rubra* is reported to be used for control of diabetes mellitus in Mexico (Bobbarala, V., et al. 2009) and the flowers have antioxidant property as well as hypolipidmic property. The leaves of *P. rubra* are used in ulcers, leprosy, inflammations, rheumatism, bronchitis, cholera, cold and cough and as rubefacient, antibacterial, antipyretic, antifungal, stimulant etc (Kirtikar, K.R. and B.D. Basu, 2004). The pods of P. rubra are used in antifertility. The shoot of P. rubra is used as antifertility, rheumatism, diarrhea and veneral disease.

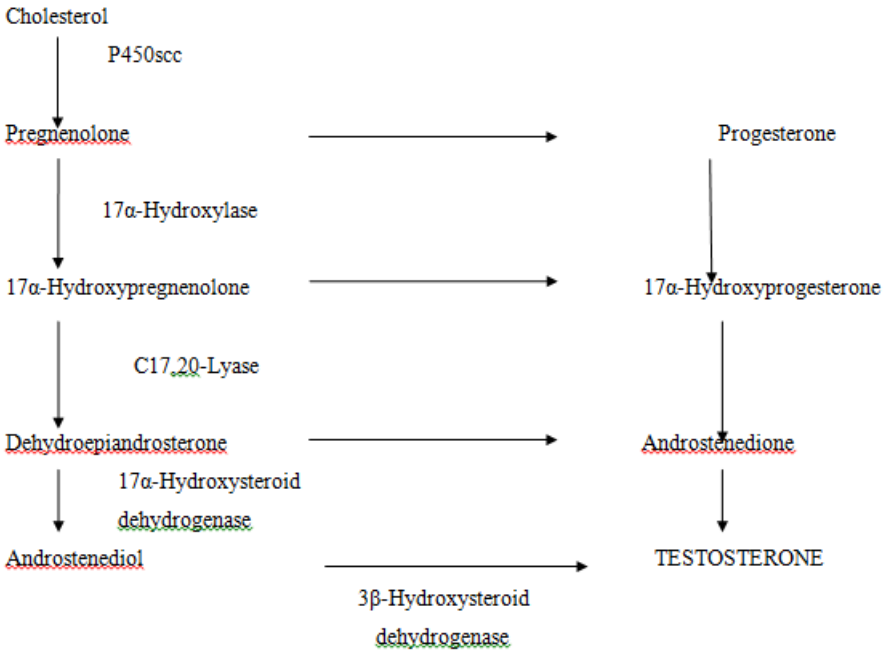
The Pods of *P. rubra* shows abortifacient potential in female albino rats as it contains alkaloids, phenolic, steroids and saponins, which act either alone or in combination, may be partly responsible for observed pregnancy terminating effects in present study (Dabhadkar and Varsha, 2012). Whereas the bark of Plumeria rubra consists scopoletin (coumarin) (Chen and Zang, 1991). β-Sitosterol (phytosterols) (Tohar Norista, 2006), glycosides, plumieride, plumeric acid, β-sitosterol, lupeol, amyrin and fulvoplumierin. Plumericin, isoplumericin, 4-hydroxy acetophenone, plumieride, coumarylplumieride and protoplumericine are found to present in the bark of the plant. Flowers contain essential oil. Roots contain fulvoplumierin, plumericin and three new compounds -isoplumericin, β-dihydroplumericin and β-dihydroplumericinic acid (Choudhary et al., 2014), Plumieride (triterpens esters) (Gupta et al., 2006), fulvoplumericin (triterpens esters) (Gupta et al., 2008) which leads to reduction of testosterone level in male albino rats. Plumieride, an iridoid has also been reported to show the antidermantophytic activity (Tiwari et al. 2002) and antifertility activity (Gupta et al., 2004).

1.1 BIOSYNTHESIS OF TESTOSTERONE HORMONE

Steroid hormones are synthesized from the enzymatic modification of cholesterol. Cholesterol is the precursor of biosynthesis of steroidal hormones as testosterone.

Testosterone is derived from cholesterol. The plant contains Beta-sitosterol which is similar to cholesterol. It might help reduce cholesterol levels by limiting the amount of cholesterol that is able to enter the body so this affects the production of testosterone in the Leydig cells which reduces the spermatogenesis process.

Testicular Biosynthetic Pathway of Testosterone



Therefore the present study was conducted to investigate the antifertility effect of the *P. rubra* bark methanolic extract in male albino rats.

2. MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIAL

The stem bark of the *Plumeria rubra* was obtained from Mansarovar, Jaipur during the flowering period of July to October. It was authenticated morphologically in the Department of Botany, University of Rajasthan, Jaipur. A voucher specimen has been preserved in the laboratory for future reference. ***RUBL NO. 211562.***

2.2 PREPARATION OF EXTRACT

The bark of *Plumeria rubra* was separated from the stem of plant collected. The sample was dried in an uninhabited room for four weeks at room temperature. Dried sample was ground into powder mechanically, using manual grinder. The 250g of plant powder was soaked in 500ml of 100% methanol and kept at room temperature for 24 hours. The mixture was then

vigorously shaken and soxhlet for extraction (Ahmad et al., 2009) in a soxhlet apparatus for 35-40 hours (7-8 hours for 5 days) with 100% methanol. The extract was filtered through whatman No.1 filter paper and methanol was distilled off under reduced pressure, where dark greenish (*P. rubra*) mass was obtained. The extract was soluble in distilled water.

2.3 EXPERIMENTAL ANIMALS

The sexually mature, healthy, proven fertile male albino rats (*Rattus norvegicus*) of 'Wistar strain' weighing between 150 - 200 g were used in the present study. The animals were housed and bred in polypropylene cages under standard environmental conditions at temperature (25±2°C) and light and dark (12:12h) in the animal house of Department of Zoology, university of Rajasthan, Jaipur. Rats were feed with standard pellet diet and water ad libitum. The animals were divided in to 4 groups of 6 animals each.

All the experimental procedures and protocols used in this study were reviewed in accordance with the guidelines of the CPCSEA.(***1678/GO/a/12/CPCSEA Dated 09-01-2013***).

2.4 ACUTE TOXICITY STUDY

For the determination of acute toxicity study adult male albino rats weighing between 150-200 g were taken. Further they were divided into 4 groups of two animals each. Rats were fasted for 6 hours with only access to water ad libitum before experimental study. The methanolic extract of *Plumeria rubra* was dissolved in distilled water and administered orally with the doses viz. 50, 100, 200 mg/kg b.wt according to the recommendation of OECD/OCED Guidelines (OECD/OCDE, 2001). The animals were observed continuously for 72 hr. for mortality or any abnormality and for behavioural changes. There is no sign of toxicity and behavioural changes were observed even with the dose level 200mg/kg of methanolic extract of *Plumeria rubra*.

2.5 STUDY DESIGN

P. rubra bark extract was dissolved in distilled water and administered orally to male albino rats. Animals were divided into 4 groups 6 animals in each –

- Group I: Intact control vehicle treated (Distilled water) for 60 days.
- Group II: *P. rubra at* the dose level of 50 mg/kg body weight for 60 days.
- Group III*: P. rubra* at the dose level of 100 mg/kg body weight for 60 days.
- Group IV: *P. rubra* at the dose level of 200 mg/ kg body weight for 60 days.

A suspension of the extract was prepared in sterile distilled water (100 mg/ml) before administration. The required drug was administered orally.

2.6 FERTILITY TEST

After 55th days of drug treatment, the male rats were cohabited with the normal cycling virgin female weighing between 150-200g in the ratio of 1:2 in proestrus cycle for overnight. On the next day in early morning the vaginal smear was taken to check sperms for positive matting. The mated female rats were separated and allowed to deliver at full term and numbers of litters were counted. Percentage fertility was calculated as number of pregnant female rats divided by the number of mated females multiplied by 100.

2.7 AUTOPSY SCHEDULE

After 24 hours of last treatment the final body weight was recorded and the animals were sacrificed by decapitation.

2.8 BODY WEIGHT AND REPRODUCTIVE ORGANS WEIGHT

Body weight of control and experimental animals were recorded during the experimental period. After 24 hrs of the last dose treatment the final body weight was recorded and the animals were sacrificed by decapitation. Testes, epididymis, vas deferens, seminal vesicle and ventral prostate were dissected out, trimmed off extraneous fat and connective tissue and weighed accurately on a torsion balance. One testis from each animal was fixed in bouin's fixative for histopathology and the other testis from each animal was processed for biochemical studies.

2.9 STATISTICAL ANALYSIS

Data were expressed as Mean ± SEM. Student's t test was used for statistical comparison. The analysis was performed manually at the significance levels of $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.

3. RESULTS

3.1 BODY WEIGHT, REPRODUCTIVE AND VITAL ORGAN WEIGHTS

The Body weights of *P. rubra* bark extract treated groups did not reveal any significant change when compared to the respective control groups. The significant ($p<0.05$) decrease in the weights of testes, epididymis, seminal vesicles and ventral prostate in the treated groups at 50,100,200 mg/kg body weight doses when compared to the control groups. Vital organ

weight including heart, liver, kidney and adrenal gland remain unchanged in the treated groups when compared with control groups. (**Table 1**).

Table 1: Difference in mean body weight (in gms) and reproductive organs weight (in mg/100 g b.wt.) following treatment with P. rubra bark extract in male Wistar albino rats.

Treatment	Body Weight		Testes	Epididymides	Seminal Vesical	Ventral Prostate	Vas Deferens	Liver	Heart	Kidney	Adrenal
	Initial	Final									
GroupI control	165.16 ±7.10	238.4 ^{ns} ±12.06	1229.82 ±12.5	479.08 ±6.84	459. 2 ±7.28	341. 55 ±6.58	152. 02 ±12.01	3959.23^{ns} ±425.7	406.33^{ns} ±23.93	785.05^{ns} ±8.64	24.35^{ns} ±1.8
Group II 50mg/kg b.wt.	169.21 ±7.38	179.62^{ns} ±6.36	947.52 ±10.34*	388.38 ±3.70*	407. 48 ±6.72*	312. 67 ±4.40*	135. 3 ±11.37*	3520.83^{ns} ±247.65	385.23^{ns} ±24.76	779. 3^{ns} ±4.94	24.17^{ns} ±1.2
GroupIII 100mg/kg b.wt.	168.70 ±6.18	177.59^{ns} ±5.38	914.68 ±9.02**	329.98 ±3.35**	389. 36 ±5.76**	289. 8 ±3.69**	118. 27 ±9.35**	3163.05^{ns} ±377.1	370.31^{ns} ±19.65	778.15^{ns} ±4.58	22.85^{ns} ±1.55
Group1V 200mg/kg b.wt.	170.34 ±7.48	180.46^{ns} ±5.08	861.05 ±7.67***	316.06 ±3.08***	365. 28 ±4.13***	256. 17 ±2.85***	112. 45 ±7.36***	3271.2^{ns} ±278.05	366.35^{ns} ±22.4	775.73^{ns} ±5.30	23.20^{ns} ±0.75

Level of Significance:- ns-non significant, * P≤0.05, **P≤0.01and ***P≤0.001

Group I: Control (Animals were orally administered distilled water); Group II: Animals were orally administered with 50 mg/kg b.wt./day for 60 days; Group III: Animals were orally administered with 100 mg/kg b.wt./day for 60 days; group IV: Animals were orally administered with 200mg/kg b.wt. for 60 days.

3.2 FERTILITY TEST

The oral administration of methanolic bark extract of *P. rubra* (50, 100 and 200 mg/Kg body weight) for 60 days to male albino rats causes a significant decrease ($p < 0.05$) in the number of females impregnated by male treated rats. Treated group 50mg/kg b.wt. showed 58 percent -ve fertility, 100mg/kg b.wt. showed 64 percent -ve fertility and group 200mg/kg b.wt. showed complete 100 percent -ve fertility. (***Table- 2).***

Table 2: Effect of methanolic bark extract *of P. rubra* on the percentage of fertility of male Albino rats.

Treatment	Male : Female	Fertility (%)
Group I control	1:3	100
Group II 50mg/kg b.wt.	1:3	58-ve
GroupIII 100mg/kg b.wt.	1:3	64-ve
Group IV 200mg/kg b.wt.	1:3	100-ve

4. DISCUSSION

It has been postulated the use of some plants as male contraceptive (Ashok et al. 2004, Gupta et al. 2006). In order to be useful, these plants should exert localized effects on the reproductive system. Without systemic alteration that may alter many others physiological functions (Gupta et al. 2000, Sharma and Jocob 2001, Venma et al., 2002). Medicinal plants have been of age remedies for human diseases they contain components of therapeutic values (Nostro et al., 2000; Britto and Gravelin, 2011).

The plant extract were obtained from *P. rubra* as an effective ideal male contraceptive agent. There was reduction in the weight of testes and accessory sex organs may be due to the hormonal imbalances. All these organs play an important role in the maturation and mobility of the sperm and formation of semen. The critical role of the epididymis in the maturation of spermatozoa is well established in mammals. Moreover, testosterone plays a pivotal role in sexual maturation, behavior and maintenance of accessory sex organs (Mann and Ludwak-Mann, 1981; Jean–Faucher et al., 1984; Luke and Coffey, 1994; Ojeda and Urbanski, 1994). Significantly decline in testicular weight was observed in all treated groups which is due to the absence of post-meiotic cells i.e. spermatids and the spermatozoa. Testosterone is produced by Leydig cells (Chung et al., 2011). Testosterone plays a fundamental role in the maintenance of spermatogenesis (O'Donell et al., 2006; Mishra and Singh, 2009). Spermatogenesis is the process of male gamete production, where in the spermatogonia transfer into highly specialized matured by gonadotropins and testosterone in mammals (Jones, 1991). Previous studies point out potential role of plant extract in reducing number of spermatogenic cells and spermatozoa through the antispermatogenic effects (Harisha et al., 2012). Accessory sex organs are androgen dependent and thus reflect the availability of androgen. The availability of androgen is directly related to the weight of accessory sex organs. The extract at different dose levels causes reduction in weights of accessory sex organs which indicate the atrophy of glandular tissue and also a reduction in secretary ability, thus reflecting the decreased level of testosterone. The weight of accessory sex organs was mainly due to the hormone deficiency. The plant extract reported to cause reduction in testicular and epididymal weight via a direct or indirect effect mediated by suppression of pituitary gonadotropin. One hypothesis is that the active ingredient(s) of the extract may alter the pituitary gonadotropins hormones i.e. leutinizing hormone and follicle stimulating hormone (Kusemuji et al., 2010).

Testicular weight loss could be due to the reduced protein contents in the testes of treated groups. The low level of testicular protein are usually indicative of inhibition of spermatogenesis (Dixit and Bhargava, 1983). Similar result was reported by Vijay kumar et al., (2004), Chinoy et al., (2005), Chinoy and Mehta (1999).

5. CONCLUSION

The results of the present study indicate that the methanolic bark extract of *Plumeria rubra (L.)* have significant antifertility activity and it suppresses the process of spermatogenesis which can lead to infertility in male albino rats. In conclusion, the extract appears to be promising male contraceptive agent in order to develop the potential of herbal medicine. However, more detail and extensive studies would be required to find out the exact nature of the component showing anti-fertility activity and by further planning studies in human. Further studies are planned in this direction.

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