

Estrogenic and Anti-Estrogenic Potentials of Ethanolic Pod Extract of *Plumeria rubra* in Female Albino Rats

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Abstract: To evaluate the abortifacient activity of the ethanolic extract of *Plumeria rubra* (*P. rubra*) pod in female albino rats, the extract at the doses of 50, 100 and 200 mg/kg body weight were administered from day 11 to 15 of pregnancy and animals were allowed to go full term. The acute toxicity test of female albino rats was carried out and clinical toxicity symptoms such as respiratory distress, salivation, weight loss, dull eyes, diarrhea and change in the appearance of fur as well as mortality were not observed in the animals at any period of the experiment. The ethanolic extracts of *P. rubra* pods exhibited abortifacient activity (13.46% to 100%). The extract significantly reduced the number of live feti, whereas the resorption index and post implantation losses increased significantly. The % of abortion was found to be highest (100%) at 200 mg/kg dose of alcoholic extract of *P. rubra* pods. In ovariectomized immature young rats, the extract showed significant estrogenic effect (vaginal opening, vaginal cornification and increased uterine weight) at the dose 200 mg/kg body weight. The phytochemical screening revealed the presence of alkaloids, flavonoids, simple phenolics, steroids, tannins and saponins.

Key words: Contraceptive activity • Albino rat • Flavonoids • Estrogenic • *Plumeria rubra*

INTRODUCTION

In the last decade, there has been a great upsurge in the pharmacological evaluation of medicinal plants especially in the third world that could benefit as contraceptive and fertility control agents and be a possible replacement for hormonal contraceptives. Many plants are known to have promising contraceptive properties [1]. Therefore, the screening of plants with abortifacient activity will be a useful guide towards the formulation of cheaper, affordable contraceptive with reduced toxicity. One plant that featured prominently from ethnobotanical survey as herbal contraceptive and was also claimed to be used as traditional “wash the uterus” by the tribal’s of Melghat region is *Plumeria rubra*.

Plumeria rubra L. (Hindi: Lal champaa; English: True Frangipani) are laticiferous trees and shrubs, belong to the Apocynaceae family. 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 [2]. In India, its fruit is used as an abortifacient [3]. The decoction of the flowers of *P. rubra* is reported to be used for control of diabetes mellitus in Mexico [4]. 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 [5].

However, there is no information to substantiate or refute the abortifacient claims of *P. rubra* pods in the folklore medicine. Therefore, the present work has been undertaken to validate scientifically the abortifacient role of *P. rubra* pods as acclaimed by the traditional tribal users of Melghat region.

MATERIALS AND METHODS

Collection of Plant Material: The plant *P. rubra* was collected during the flowering period of August to

October, 2010 from Melghat region (20°51' to 21°46' N and to 76°38' to 77°33' E) of Amravati district of Maharashtra state of India, identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. DD- 1).

Procurement and Rearing of Experimental Animals:

Healthy female albino rats were procured from Sudhakar Rao Naik Institute of Pharmacy, Pusa (Maharashtra). The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hrs light and dark cycle approximately at 25±2°C. They were fed on pellets (Trimurti Lab Feeds, Nagpur) and tap water *ad libitum*. The rats were allowed to acclimatize to laboratory environment for 15 days before experimentation.

All experimental protocols were approved by the Institutional Animal Ethics Committee [registration number 1060/ac/07/CPCSEA (IAEC/7/2009)].

Preparation of the Extract: The pods of *P. rubra* were collected, shade dried, powdered and subjected to soxhlet extraction with ethanol for 48 hrs and filtered. The extract was evaporated to near dryness on a water bath, weighed and kept at 4°C in refrigerator until used for pharmacological evaluation.

Phytochemical Screening: The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as per Thimmaiah [6].

Acute Toxicity Study: The healthy female albino rats, starved for 3- 4 h were subjected to acute toxicity studies as per OECD 423 guideline [7]. The rats were observed continuously for 2 hrs for behavioral, neurological and autonomic profiles and for 24 and 72 h for any lethality or death.

Anti-Fertility Activity: The plant extracts were tested in female albino rats for abortifacient activity as per Khanna *et al.* [8]. The female rats in proestrous phase were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as day 1 of pregnancy. These rats were randomly distributed into two groups, control group and experimental groups of 6 animals each. On the day 10 of pregnancy, animals were laprotomized under

light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. Thereafter the abdominal wound was sutured in layers.

The extracts to be tested were then fed to operated pregnant rats i.e. ethanolic extract of *P. rubra* (pods) at doses of 50, 100, 200 mg/kg body weight (one tenth of the highest tolerable dose i.e 2000 mg/kg body weight) once daily by an intragastric (i.g.) soft rubber catheter from day 11 up to the 15th day of pregnancy. The animals were allowed to go full term. After delivery the pups were counted and the anti-fertility activity of extract was evaluated.

Estrogenic and Anti-Estrogenic Activity: The ethanolic extract of *P. rubra* at 200 mg/kg was found to be most active amongst the four treatments in the anti-fertility testing. Hence it was subjected to a detailed investigation for potential estrogenic and anti-estrogenic activity. The uterine weight and vaginal cornification method was employed for the estimation of estrogenic and anti-estrogenic activity [9]. Immature ovariectomized female albino rats, 21-23 days old, weighing between 35-45 gm were used. The animals were divided into four groups, consisting of six rats each.

- Group-I: Control, received 0.2 ml of distilled water orally.
- Group-II: Treated, received 0.02 mg ethinyl estradiol/kg/rat per day in olive oil orally.
- Group-III: Treated, received 200 mg ethanol extract of *P. rubra* (pod)/kg body weight in 0.2 ml of distilled water orally.
- Group-IV: Treated, received 200 mg ethanol extract of *P. rubra* (pod)/kg body weight in 0.2 ml of distilled water orally +0.02 mg ethinyl estradiol / kg /rat per day in olive oil orally.

All the above treatments were given for 7 days. On the 8th day of experiment, the animal were sacrificed by decapitation and uteri dissected out and surrounding tissues removed. The uteri were blotted on filter papers and weighed quickly on a sensitive balance and fixed in Bouin's fluid for 24 hrs. The tissue were dehydrated and embedded in paraffin. The paraffin section were cut at 5 µm and stained with hematoxylin-eosin for histological observation. The diameter of the uteri and thickness of the endometrium were measured in 16 randomly selected sections using an ocular micrometer.

Statistical Analysis: All the data were expressed as mean \pm SEM. Statistical analysis was done by using Student's t-test [10].

RESULTS

Phytochemical Screening: Preliminary phytochemical screening of the ethanolic pod extract of *P. rubra* revealed the presence of alkaloids, flavonoids, simple phenolics, steroids, tannins and saponins whereas anthraquinones were not detected.

Acute Toxicity Study: Clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Similarly no changes in the behavioral, neurological and autonomic profiles were observed in treated groups of the rats up to highest dose of 2000 mg/kg body weight.

Anti-Fertility Activity: The alcoholic extract when evaluated for their anti-fertility activity, was found to exhibit significant pregnancy interceptive activity. Administration of 200 mg/kg body weight of the alcoholic extract resulted in 100% abortion and 50 & 100 mg/kg body weight of the alcoholic extract resulted in 13.46 to 43.63% abortion (Table 1). This was evident from decrease in the percentage of live fetuses. While no live fetus was observed at the dose of 200 mg/kg body weight of

alcoholic extract. The percent resorption index increased from zero in the control animals to 100% in the 200 mg/kg body weight alcoholic extract treated animals.

Estrogenic and Anti-Estrogenic Activity: The oral administration of ethanolic extract of *P. rubra* pod at 200 mg/kg body weight caused a significant increase in the uterine weight ($P<0.001$) in immature rats as compared to the control (Table 2). However the administration of the extracts conjointly with ethinyl estradiol led to a marked increase in the weight of the uterus as compared to control, but the extent of the uterotrophic response was less than that produced by ethinyl estradiol alone. The uterotrophic changes such as, diameter of the uterus and the thickness of the endometrium ($P<0.01$) was significantly increased when compared to the control rats (Table 3). In histopathological study, the endometrial epithelium consisted of spindle shaped cells with basal nuclei and the endometrial glands were dilated. The stroma consisted of loose and edematous fibroblast type cells with edema in different experimental groups when compared with control (Fig. 1). The ethanolic extract of *P. rubra* also induced vaginal opening in experimental rats, while all the control rats had closed vaginas. The number of cornified cells in the vaginal smears were considerably higher in ethanol extract treated group (+ to ++) than those of the control (0 to +), but notably less than ethinyl estradiol treated rats (+++).

Table 1: Effect of alcoholic extract of *P. rubra* (pod) on fertility of female rats fed orally from day 11 to 15 of pregnancy Statistical Analysis by Student t- test

Treatment Groups	Body weight (gm)	Drug dose (mg/kg body wt)	No. of fetus individual rats on day 10	No. of rats delivered (Litter Size)	No. of resorption in individual rats	No. of resorption in mean \pm SEM	% Abortifacient activity
Group- 1 Control (Vehicle)	120-170	-----	8,8,9,8,6,6	6(8,8,9,8,6,6)	0,0,0,0,0,0	0	Nil
	120-220	50	11,9,7,8,9,8	6(9,8,7,8,7,6)	2,1,0,0,2,2	1.16 \pm 0.40*	13.46%
Group- 2 Alcoholic Extract of <i>P. rubra</i>	120-220	100	8,11,9,9,8,10	6(4,8,4,6,4,5)	4,3,5,3,4,5	4.0 \pm 0.36***	43.63%
	120-220	200	14,7,10,9,9,11	6(0,0,0,0,0,0)	14,7,10,9,9,11	10 \pm 0.96***	100%

Values are mean \pm SEM, n=6 in each group

Significantly different from control group * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 2: Estrogenic and anti-estrogenic potentials of the ethanolic pod extract of *P. rubra* in rats

Groups	Treatment dose (Mg/kg body wt.)	Uterine weight (Mg/100 gm body wt.)	Vaginal cornification
I	Control (distilled water)	72.83 \pm 2.28	Vagina not open (0 to +)
II	Ethinyl estradiol (0.02mg/kg)	179 \pm 2.97***	Open (+++)
III	Ethanolic extract (200 mg/kg)	104.5 \pm 4.18*** ^c	Open (+ to ++)
IV	Ethanolic extract (200 mg/kg) + Ethinyl estradiol (0.02mg/kg)	135 \pm 3.80*** ^c	Open (+++)

Statistical Analysis by Student t- test

Values are mean \pm SEM, n= 6 in each group

Significantly different from control group * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Significantly different when compared with ethinyl estradiol group ^a $P<0.05$, ^b $P<0.01$, ^c $P<0.001$

+ -nucleated epithelial cells, ++ -nucleated and cornified cells, +++ -cornified cells.

Table 3: Histological changes in the uterus and endometrium after treatment with the ethanolic Pod extract of *P. rubra* in rats

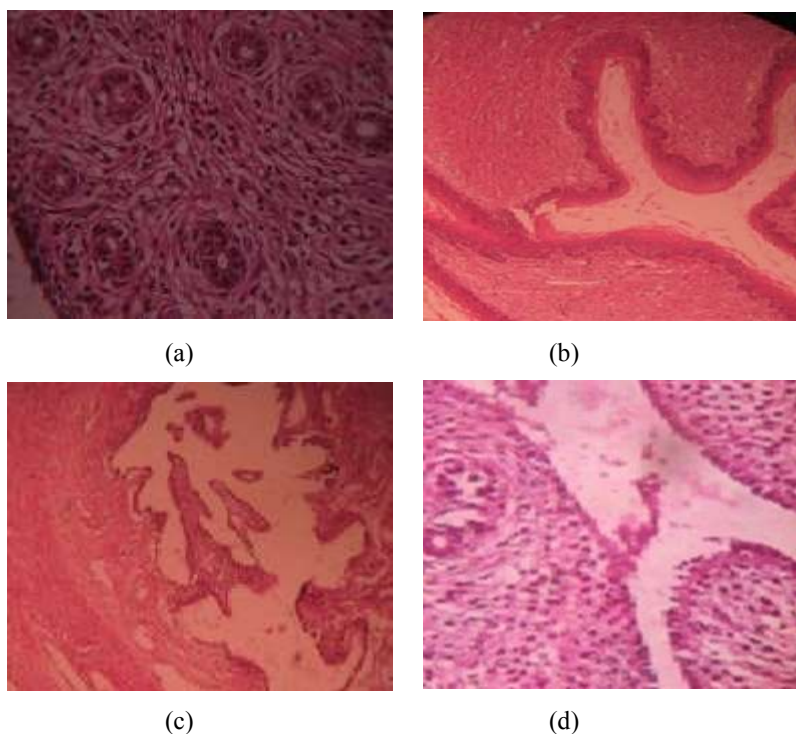
Groups	Treatment dose (Mg/kg body weight)	Diameter of uterus (μm)	Thickness of endometrium (μm)
I	Control (distilled water)	292.00 \pm 7.27	131.70 \pm 3.63
II	Ethinyl estradiol (0.02mg/kg)	514.29 \pm 6.62**	345.50 \pm 5.67*
III	Ethanolic extract (200mg/kg)	352.42 \pm 6.23** ^b	256.00 \pm 6.22** ^b
IV	Ethanolic extract (200mg/kg) + Ethinyl estradiol (0.02mg/kg)	469.59 \pm 3.89** ^a	307.00 \pm 3.93 ^a

Statistical Analysis by Student t- test

Values are mean \pm SEM, n= 6 in each group

Significantly different from control group *P<0.05, **P<0.01, ***P<0.001

Significantly different when compared with ethinyl estradiol group ^aP<0.05, ^bP< 0.01, ^cP<0.001



a: T. S. of immature ovariectomized control rat uterus

b: T. S. of uterus of immature ovariectomized rat treated with ethinyl estradiol

c: T.S. of uterus of ovariectomized rat treated with 200 mg/kg b. w. ethanolic extract of *P. rubra* pod

d: T.S. of uterus of ovariectomized rat treated with ethanyl estradiol + 200 mg/kg b. w. ethanolic extract of *P. rubra* pod

Fig. 1: Histopathological change in immature ovariectomized uterus of rat when treated with ethanolic extract of *P. rubra* pod (Photomicrograph at a Magnification of 100X)

DISCUSSION

In the present study, the oral administration of ethanolic extract of *P. rubra* tested for abortifacient properties at doses of 50, 100 and 200 mg/kg body weight produced a dose dependent adverse effect on fertility index and number of implantation in the uterine horns of the female rats, by virtue of an increase in the percentage of the post-implantation embryonic loss. These results are in agreement with those of Yakubu *et al* [11], who

reported abortifacient activity of *Senna alata* leaves. Similar result was also reported in the study of the anti-fertility activity of *Hibiscus rosa-sinensis* root [12]. Abortifacient effect might be mediated through estrogenic activity since estrogens are known to increase uterine contractibility to expel fertilized egg [13]. Large numbers of anti-fertility plant extracts are known to exhibit estrogenic activity in rats [14]. Estrogenic substances may not only cause the expulsion of ova from the fallopian tube but also disrupts the functional equilibrium between

the endogenous estrogen and progesterone which may result in failure in fertility [15]. In another study phytoestrogens have been shown to interfere in negative feedback of estrogen and indirectly alter the ovarian function which might be responsible for abortifacient activity [16]. Selected doses of ethanolic extracts were administered between days 11 to 15, since this period corresponds to the period of organogenesis [14]. In the present study also, the ovariectomized immature female rats, were orally administered the ethanolic extract of *P. rubra* pod from days 11 to 15th which resulted in increase in the uterine weight and stimulated uterine growth, suggesting estrogenic activity. These results are in agreement with those of *Derris brevipes* variety *brevipes* which exhibited estrogenic activity as shown by the significant increase in uterine weight, diameter of the uterus, thickness of the endometrium and height of endometrial epithelium and vaginal cornification in immature female rats compared with control [17]. Regular development of the entire event leading to nidation, at least in rats and mice, is chiefly under the direct command of estrogen- progesterone interplay at the cellular level [18] and a slight disturbance in this hormonal balance may result in an unfavorable endometrial environment. The pregnancy interceptive effect of the ethanol extract of *P. rubra* pod could be interpreted as due to the estrogenic nature of the plant. Estrogen is known to stimulate the content of the uterus, thereby changing the uterine milieu and creating non- receptive condition in the uterus [18]. Flavonoids isolated from *Striga lutea* and *striga orobanchioides* possess strong estrogenic activity and anti-fertility activity. In the present work the preliminary phytochemical studies indicated the presence of steroids, flavonoids and saponins in *P. rubra* ethanolic extract. Several of these compounds are known to exhibit the anti-fertility properties [19].

CONCLUSION

The ethanolic extract of *P. rubra* pods administered orally possessed estrogenic activity, which might contribute to its contraceptive effect. Further studies to identify the bioactive principles responsible for estrogenic and abortifacient activity of the extract are in progress.

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