

Abortifacient activity of *Plumeria rubra* (Linn) pod extract in female albino rats

Dinesh Dabhadkar & Varsha Zade*

Department of Zoology, Government Vidarbha Institute of Science and Humanities, Amravati 444 604, India

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To evaluate the potential abortifacient activity of the aqueous, alcohol, ethyl acetate and chloroform extracts of *P. rubra* pod in female albino rats 50, 100 and 200 mg/kg body weight doses of each extract were administered from day 11 to 15 of pregnancy and animals were allowed to go full term. The phytochemical screening revealed the presence of alkaloids, flavonoids, simple phenolics, steroids, tannins and saponins. 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. All the four extracts of *P. rubra* pods exhibited abortifacient activity (8-100%). The extracts significantly reduced the number of live fetuses, whereas the resorption index and post implantation losses increased significantly. The % of abortion was found to be highest (100%) with 200 mg/kg dose of alcoholic extract of *P. rubra* pods.

Keywords: Abortifacient, Female albino rat, Post implantation, *Plumeria rubra*, Resorption

Rapid rise in population has caused serious problems in the economic growth and all round human development leading to poverty in developing countries like India. Family planning has been promoted through several methods of contraceptions, but due to serious adverse effects produced by synthetic steroidal contraceptions¹⁻³, attention has been focused on indigenous plants for possible contraceptive effect. 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 on herbal contraceptive and also claimed to be used as traditional “wash the uterus” by the tribal’s of Melghat region (20° 51’ to 21° 46’ N and to 76° 38’ to 77° 33’ E) of Amravati district of Maharashtra state of India is *Plumeria rubra*.

Plumeria rubra L. (Hindi: Lal champa; 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 irritation⁴. In India, however, its fruit is used as an abortifacient⁵. The decoction of the flowers of *P. rubra* is reported to be used for control of diabetes mellitus in Mexico⁶.

The Leaves of *P. rubra* are used in ulcers, leprosy, inflammations, rheumatism, bronchitis, cholera, cold and cough and as rubefacient, antibacterial, antipyretic, antifungal, stimulant etc⁷.

However, there is no information to substantiate or refute the abortifacient claims of *P. rubra* pods in the folklore medicine. Therefore, the present 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 from Melghat region and identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. DD-1).

Procurement and rearing of experimental animal—Healthy wistar strain female albino rats of about two month old and weighing 120- 200 g were procured from Institute of Pharmacy Education and Research, Wardha. The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 h light and dark cycle approximately at 25 °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 subjected to the scrutinization and approval of Institutional Animal

*Correspondent author
Telephone: 0721- 2560170
E-mail: zvarsha27@gmail.com

Ethics Committee [registration number 1060/ac/07/CPCSEA (IAEC/7/2009)].

Preparation of extract—The pods of *P. rubra* were collected, shade dried, powdered and subjected to soxhlet extraction successively with distilled water, ethanol, ethyl acetate and chloroform. The extract was evaporated to near dryness on a water bath, weighed and kept at 4 °C in refrigerator until use.

Phytochemical screening—The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as per Thimmaiah⁸.

Acute toxicity study—The healthy female albino rats, starved for 3–4 h were subjected to acute toxicity studies as per OECD 423 guideline⁹. The rats were observed continuously for 2 h for behavioural, neurological and autonomic profile and for 24 and 72 h for any lethality or death. No death was observed at highest dose (2000 mg/kg body weight) used.

Antifertility activity—The plant extracts were tested in female albino rats for abortifacient activity as per Khanna *et al.*¹⁰. The female rats in pro-estrous 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 13 groups, one control group and 12 experimental groups of 6 animals each. On the day 10 of pregnancy animals were laprotomised 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 extract to be tested were then fed to operated pregnant rats i.e. aqueous extract, alcoholic extract, ethyl acetate extract and chloroform extract of *P. rubra* (pods) at doses of 50, 100, 200 mg/kg body weight (one tenth of the highest tolerable dose) once daily by an intragastric (ig) 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 antifertility activity of extract was evaluated. The following parameters were computed: number of live and dead fetuses; % survival ratio = (number of live fetus/number of live+ dead fetus) × 100; resorption index = (total number of resorption sites/total number of implantation sites) × 100; postimplantation loss = (number of implantations-

number of live fetuses/number of implantations)×100. The anogenital distance (AGD) and crown rump length (CRL) of litters were measured by using a measuring tape. The variations in birth weight of litters and gestation period between control and experimental animal was also determined to check the abortive effect of *P. rubra*¹¹.

Histopathological studies—The ethanolic extract at 200 mg/kg dose was found most active among all the four treatments groups; hence it was subjected to detail investigation for histopathological study. After 30 days of treatment, ovaries and uterus of animals belonging to control and experimental group were dissected out and immediately fixed in 10 % buffered neutral formalin solution. After fixation, tissues were embedded in paraffin, serial sections were cut at 5 µm, stained with hematoxylin and eosin, examined for histoarchitectural changes and photographed under Olympus BX51 light microscope¹².

Statistical analysis—The data are expressed as mean±SE. Statistical analysis was done by using paired and unpaired Student's *t*-test¹³.

Results

Preliminary phytochemical screening of the pod extract of *Plumeria rubra* revealed the presence of alkaloids, anthraquinone, flavonoids, steroids, tannins and saponines whereas anthraquinone were not detected.

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 mortality and changes in the behavioural, neurological and autonomic profile were observed in treated groups of the rats up to highest dose of 2000 mg/kg body weight.

All the experimental extracts when evaluated for their abortifacient activity, were found to exhibit pregnancy interceptive activity. Administration of 200 mg/kg body weight of the alcoholic extract resulted in 100% abortion (Table 1). This was evident from decrease in the percentage of live fetuses. The 200 mg/kg body weight aqueous extract showed 48.89% live fetuses whereas the same dose of chloroform and ethyl acetate extract showed 51 and 63.64% live fetuses respectively. The percent resorption index increased from zero in the control animals to 100% in 200 mg/kg body weight alcoholic extract treated animals.

Table 1—Effect of aqueous, ethanol, ethyl acetate and chloroform extract of *P. rubra* (pod) on fertility of female rats fed orally from day 11 to 15 of pregnancy

[Values are from 6 animals in each group]

Treatment groups (dose, mg/kg body wt)		No. of foetus individual rats on day 10	No. of rats delivered (litter size)	No. of resorption in individual rats	No. of resorption (mean±SE)	Abortifacient activity (%)
Control	Gr. A	8,8,9,8,6,6	6(8,8,9,8,6,6)	0,0,0,0,0,0	0	Nil
Aqueous extract	Gr. B (50)	8,7,9,6,8,10	6(8,7,8,6,6,8)	0,0,1,0,2,2	0.83±0.40*	10.6
	Gr. C (100)	11,9,8,8,7,8	6(9,5,8,6,4,6)	2,3,2,2,3,2	2.33±0.20***	27.45
	Gr. D (200)	9,8,5,7,8,8	6(3,6,2,4,3,4)	6,2,3,3,5,4	3.83±0.60**	51.11
Ethanol extract	Gr. E (50)	11,9,7,8,9,8	6(9,8,7,8,7,6)	2,1,0,0,2,2	1.16±0.40*	13.46
	Gr. F (100)	8,11,9,9,8,10	6(4,8,4,6,4,5)	4,3,5,3,4,5	4.0±0.36***	43.63
	Gr. G (200)	14,7,10,9,9,11	6(0,0,0,0,0,0)	14,7,10,9,9,11	10±0.96***	100
Chloroform extract	Gr. H (50)	8,7,9,8,10,9	6(7,7,8,6,9,9)	1,0,1,2,1,0	0.83±0.30*	9.8
	Gr. I (100)	8,10,8,9,8,7	6(6,7,5,6,6,5)	2,3,3,3,2,2	2.5±0.22***	30
	Gr. J (200)	6,9,10,9,8,9	6(3,4,6,5,4,4)	3,5,4,4,4,5	4.16±0.30***	49
	Gr. K (50)	8,10,9,8,7,8	6(8,8,9,7,6,8)	0,2,1,1,0,0	0.66±0.33*	8
Ethyl acetate extract	Gr. L (100)	9,6,7,10,9,9	6(8,4,7,8,7,9)	1,2,0,2,2,0	1.16±0.40*	14
	Gr. M (200)	10,8,5,6,8,7	6(7,5,3,4,4,5)	3,3,2,2,4,2	2.66±0.33***	36.36

P values: * <0.05 , ** <0.01 , *** <0.001 , when compared between group.

There was a decrease in litter size with increase in the dose of herb extract in all the treatment groups. The control had the highest litter size (7.5 ± 0.50). The litter body weight recorded in animals administered with alcoholic, aqueous and ethyl acetate extract of *P. rubra* were not significantly different from control. The AGD to CRL ratio of the litter of rats administered dosed with different extract at 50, 100, 200 mg/kg body weight were similar to that of control group. Similarly, the total body length of litters at day 1 of birth also did not vary significantly from that of control. When the sex ratios of litter were determined it was found that the male sex was dominant to female sex in majority of experimental animals. The gestation period did not show any variation in extract treated group of animals as compared to control (Table 2).

The cellular organization of the ovaries of control rats presented normal features as evidenced by the presence of all types of follicles, few atretic follicles, with normal vascularity in compact stroma and a well developed graffian follicle (Fig. 1a). The dose 200 mg/kg body weight alcoholic extract of *P. rubra* pod administrated for 30 days severely affected the ovarian structure. Large number of developing as well as mature follicle underwent atresia. Some developing follicle show lysis of ova, nuclear degeneration and detachment of granulosa layer. Stroma appears fibrotic with poor vascularity (Fig. 1b).

The uterine histology of the control rat presented normal structure. The endometrium exhibited large epithelial cells having basal and middle nuclei. The uterine glands were numerous, irregular and tortuous. The uterine lumen was highly distended and the stroma was loose with normal vascularity (Fig. 2a). The administration of 200 mg/kg alcoholic extract showed absence of extensive folding of luminal epithelial and vacuolated cells with degenerating materials. It also caused great reduction in endometrial height and shrinkage of uterine glands. Stroma is compact with poor vascularity (Fig. 2b).

Discussion

The oral administration of *P. rubra* pod extract (aqueous, alcohol, ethyl acetate and chloroform) at the 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.*¹⁴ who reported abortifacient activity of *Senna alata* leaves.

Various parameters evaluated in present study are useful indices to assess the potentials of a plant as an abortifacient. While cytotoxic agents can be disrupt pregnancy possibly by interfering with the mitotic division of the fetus, chemical insults both before and

Table 2—Effect of aqueous, ethanol, ethyl acetate and chloroform extract extract of *P. rubra* (pods) on fertility of rats

[Values are mean \pm SE from 6 animals in each group]

Treatment groups (dose, mg/kg body wt)		Gestation period (days)	Litter size (No.)	Litter body weight (g)	AGD/CRL (mm)	Total body length of litter at 1 st day of birth (mm)	Sex ratio of live fetuses (male/female)	Viable fetuses (%)	Fetuses resorptions (Resorption index) (%)
Control	Gr. A (Vehicle)	22.16 \pm 0.30	7.5 \pm 0.50	4.46 \pm 0.06	1.33 \pm 0.03	62.3 \pm 0.05	24/21	100	0
	Gr. B (50)	22.33 \pm 0.20 ^a	7.16 \pm 0.40 ^{ns}	4.19 \pm 0.08 ^c	1.23 \pm 0.02 ^a	63.0 \pm 0.05 ^a	22/21	89.4	10.6
Aqueous extract	Gr. C (100)	22.16 \pm 0.46 ^{ns}	6.33 \pm 0.76 ^a	4.39 \pm 0.17 ^{ns}	1.28 \pm 0.05 ^a	63.7 \pm 0.08 ^a	22/16	72.55	27.45
	Gr. D (200)	22.00 \pm 0.25 ^{ns}	3.66 \pm 0.55 ^c	4.69 \pm 0.10 ^b	1.23 \pm 0.03 ^a	64.2 \pm 0.05 ^{ns}	13/09	48.89	51.11
	Gr. E (50)	22.83 \pm 0.39 ^a	7.5 \pm 0.59 ^{ns}	4.66 \pm 0.14 ^a	1.14 \pm 0.08 ^b	62.0 \pm 0.05 ^{ns}	22/23	86.54	13.46
Ethanollic extract	Gr. F (100)	22.33 \pm 0.33 ^b	5.16 \pm 0.65 ^a	4.69 \pm 0.26 ^a	1.45 \pm 0.11 ^{ns}	63.1 \pm 0.13 ^c	17/14	56.37	43.63
	Gr. G (200)	0	0	0	0	0	0	0	100
	Gr. H (50)	22.96 \pm 0.011 ^b	7.66 \pm 0.49 ^{ns}	6.03 \pm 0.09 ^c	1.18 \pm 0.02 ^b	59.2 \pm 0.08 ^c	25/21	90.2	9.8
Chloroform extract	Gr. I (100)	22.50 \pm 0.49 ^a	5.83 \pm 0.30 ^a	6.23 \pm 0.09 ^c	1.37 \pm 0.07 ^a	61.5 \pm 0.03 ^a	18/17	70	30
	Gr. J (200)	22.67 \pm 0.24 ^a	4.33 \pm 0.42 ^c	6.46 \pm 0.11 ^c	1.18 \pm 0.02 ^b	63.1 \pm 0.03 ^a	14/12	51	49
Ethyl acetate extract	Gr. K (50)	22.33 \pm 0.33 ^b	7.66 \pm 0.42 ^{ns}	4.36 \pm 0.15 ^{ns}	1.29 \pm 0.04 ^a	60.7 \pm 0.11 ^{ns}	23/23	92	8
	Gr. L (100)	21.88 \pm 0.09 ^{ns}	7.16 \pm 0.70 ^b	4.48 \pm 0.04 ^{ns}	1.11 \pm 0.03 ^b	61.5 \pm 0.03 ^a	23/20	86	14
	Gr. M (200)	22.67 \pm 0.54 ^a	4.66 \pm 0.56 ^b	5.58 \pm 0.07 ^c	1.52 \pm 0.11 ^{ns}	65.3 \pm 0.05 ^c	23/25	63.64	36.36

P values: ^a<0.05, ^b<0.01, ^c<0.001, when compared with control, ns= non significant.

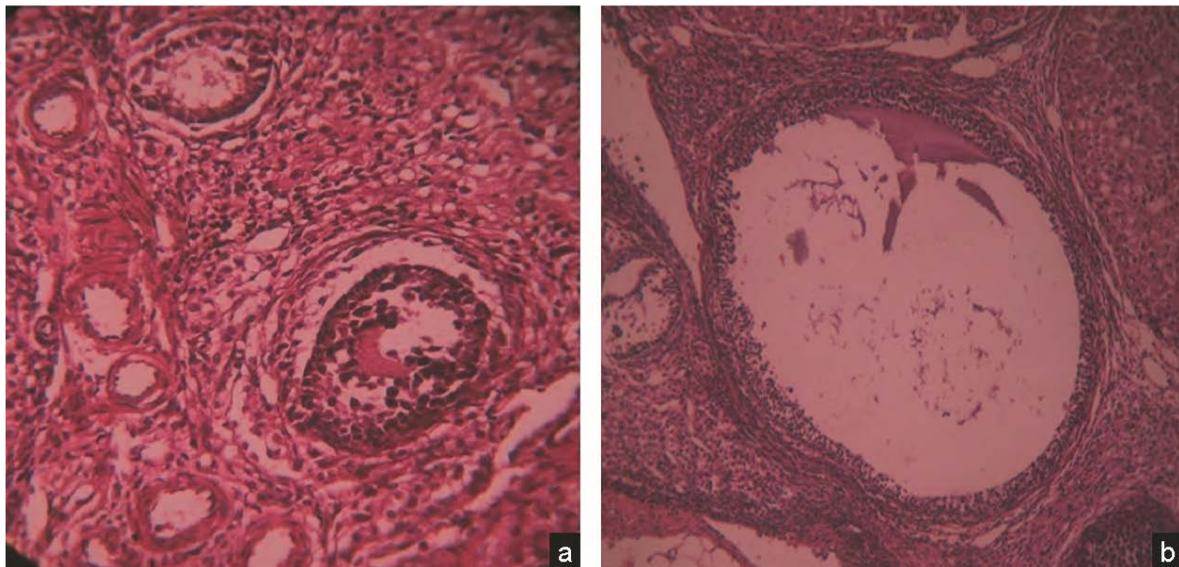


Fig. 1—Photomicrographs of ovary tissue sections of rats [(a): control rats showing normal ovarian architecture, (b): treated with 200 mg/kg body wt. dose of ethanolic extract of *P. rubra* pod. 100X]

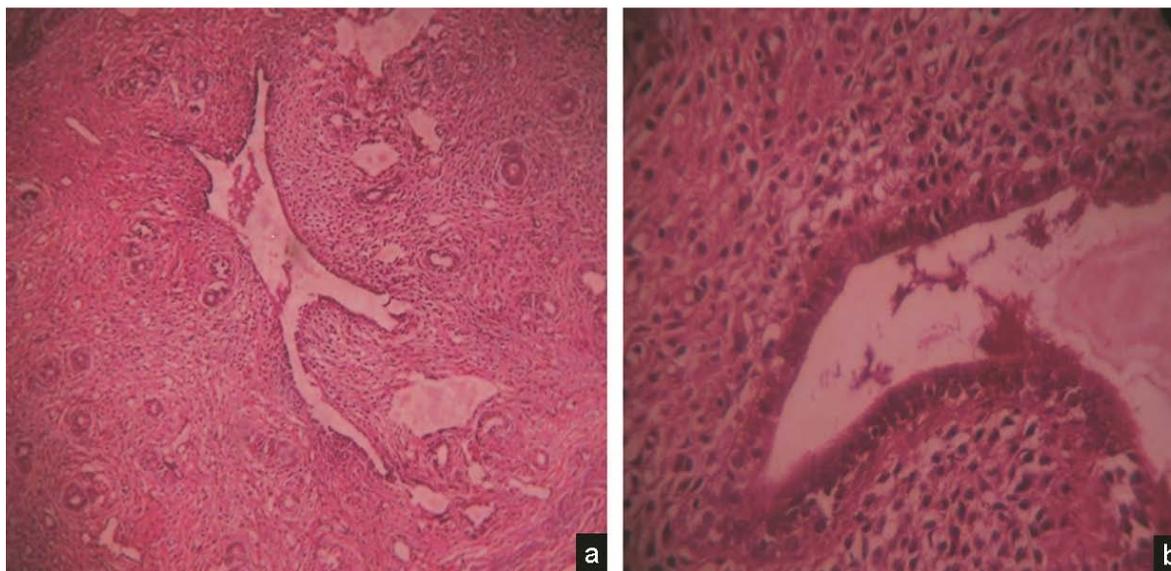


Fig. 2— Photomicrographs of uterus tissue sections of rats [(a): control rats showing normal uterine architecture, (b): treated with 200 mg/kg body wt. dose of ethanolic extract of *P. rubra* pod. 100X]

after the implantation process can be result in pre and post implantation embryonic loss¹⁵. The decrease in the number of live foetus following the administration of graded doses of the *P. rubra* pod extract is an indication of possible abortifacient activity of the extract during post-implantation period. In the present work the ethanolic extract of *P. rubra* at dose of 200 mg/kg body weight was found to possess a more potent abortifacient activity (100% efficacy). Similar observation was reported by Shibashi *et al.*¹⁶ following the administration of methanolic extract of *Achyranthus aspera* leaves to pregnant rats.

Antifertility and abortifacient activities of phenolic, phytosteroids and saponins have been sufficiently confirmed in animal models¹⁷. Therefore, presence of alkaloids, phenolics, steroids and saponins in the extract of *P. rubra* pods, which act either alone or in combination may be partly responsible for the observed pregnancy—terminating effects in the present study.

The resorption index and post-implantation loss establishes correlation between the number of implanted blastocysts and those that have not developed^{18,19}. The reduction in the resorption index and contrasting increase in the post-implantation losses consistently emphasizes the abortifacient or foetal resorptive properties of the *P. rubra* pods. Similar finding were reported by Yakubu *et al.*¹⁴ using *Senna alata* leaves. Further, the dose dependent increase in the resorption index by the extract in the present study is an indication of failure in the

development of the embryo. Such occurrences of foetal resorption suggest that interruption of pregnancy occurred after implantation of the foetus¹⁵.

The results of the present study also revealed that the plant extract was relatively non-embryotoxic as judged by the data on foetal body size and AGD/CRL ratio and the absence of any observable treatment related morphologic defects in the live fetuses, corroborating with the finding of Chukwuka *et al.*²⁰ and Abdulazez *et al.*¹¹ on *Spondias* and *Carrica* respectively.

The ovaries of treated rats showed retarded follicular growth, degenerating granulosa cells and degenerating antrum which may be due to inadequate supply of pituitary FSH. As ovulation needs increased concentration of plasma LH and FSH²¹, the content of the extract might have resulted in the inhibition of gonadotrophin release resulting in the blockage of ovulation. All follicles are apparently exposed to the same fluctuations of gonadotrophins, although not all are equally responsive, some ovulate and other becomes atretic. This shows the presence of intragonadal regulatory factors which modulate the effect of those major hormones. Two main intragonadal regulatory factors can be distinguished as intra follicular and intra ovarian²². So the biologically active substances in the extract might have suppressed follicular growth in the ovaries by interfering in the production of these intra ovarian and intra follicular regulatory factors. Thus the extract may have antiovarian effect at the doses used in the

present study. The result observed in the present study on the histology and follicular growth of the ovaries coincides with the observation of Solomon *et al.*²³ on administration of methanolic root extract of *Rumex steudelii*.

The endometrial changes in the experimental rat uterus i.e. disarrangement of endometrium, endometrial stroma and reduction in endometrial height may be due to imbalance in hormonal level caused by high level of saponins and other phytoestrogen present in *P. rubra* pod. Saponins are reported to have an antifertility effect²⁴. Similar findings were observed in albino rats using *Embelia ribes* Burm seeds²⁵.

In conclusion, all the four extracts i.e. aqueous, alcoholic, chloroform and ethyl acetate of *P. rubra* pods administered orally possess abortifacient activity. Further studies to identify the bioactive principle of abortifacient activity of the extract are in progress.

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