Research Article

ANTIFERTILITY EFFECT OF ALCOHOLIC EXTRACT OF *PLUMERIA RUBRA* ON ESTROUS CYCLE OF FEMALE ALBINO RAT

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ABSTRACT

The present work deals with antifertility effect of the alcoholic extract of *Plumeria rubra* pod in female albino rats. Pregnant rats weighing 120 to 200 gm were randomized into 4 groups [A to D]. Rats were laprotomised on 10th day of pregnancy and the live fetuses were observed in both the horns of the uterus. Rats in group A (control) were orally administered, once daily with 0.5 ml of distilled water while those in group B to D served as experimental groups and were administered 50, 100 and 200 mg/kg body weight doses of alcoholic extract of *Plumeria rubra* pods respectively. The doses were administered from 11th to 15th day of pregnancy and the rats and were allowed to go full term. The antifertility effect of alcoholic extract of *Plumeria rubra* on estrous cycle was observed to confirm the antifertility activity. The phytochemical screening of *Plumeria rubra* 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, change in the appearance of fur and mortality were not observed in the animals, at any period of the experiment. The alcoholic pod extract of *Plumeria rubra* exhibited significant post coital antifertility activity (13.46 to 100%). It was found that the extract 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 *Plumeria rubra* pods. In the present study it was observed that the alcoholic extract of *Plumeria rubra* pod extract at 200 mg/kg body weight prolonged the estrous cycle and particularly diestrus phase in the experimental animals.

Keywords: Antifertility activity, *Plumeria rubra,* pods, Estrous cycle, Female albino rat.

INTRODUCTION

The development of new fertility regulating drugs from medicinal plants is an attractive preposition. A wide variety of synthetic contraceptive are available but most of the contraceptives today are associated with some health problems like irregular menstrual cycle, migraine, frequent bleeding and other complications[1]. In such circumstances ayurvedic or ethnological drugs can be found useful. From ancient times these plants are used by the tribal for such purposes but there was no scientific basis for their use. If some of these plants are found to be scientifically valid for the purpose of contraception such as abortive, it will be beneficial for the society as a whole. Therefore, the screening of plants with antifertility activity and its effect on estrous cycle will be a useful guide towards the formulation of cheaper, affordable contraceptive with reduced toxicity. One plant that featured prominently from our ethnobotanical survey on herbal contraceptive and also claimed to be used as traditional "wash the uterus" by the tribal's of Melghat region is *Plumeria rubra*.

Plumeria rubra (Apocynaceae) is small deciduous tree with artistic branching pattern. The bark is light-gray, shining and corky. The leaves are petiolate, simple, narrow and oblong. The flowers are highly scented, red to pink, white with a patch of yellow at the center. The fruit are borne in pairs with two long, cylindrical follicle or pods. The latex of *Plumeria rubra* has been utilized in tropical regions for medicine for the treatment of itches, swellings, and fevers, it also pacifies vitiated vata, kapha, ulcers, inflammations, arthritis and constipation[2]. In the Guinas medicines are produced from root and bark of *Plumeria rubra* for the treatment of skin eruptions and abscesses, dysentery, herpes, syphilis, cough and as a purgative[3]

Therefore, the present work was undertaken to validate scientifically the antifertility role of *Plumeria rubra* pods as acclaimed by the traditional tribal user of Melghat region. But to the best of our knowledge, there is no information in the open scientific literature that has substantiated or refuted the antifertility claims of *Plumeria rubra* pods in the folklore medicine.

MATERIALS AND METHODS

Collection of plant material

The plant *Plumaria rubra* was collected from Melghat region and identified and authenticated by experts from Botany department of Government Vidarbha Institute of Science and Humanities, Amravati (M.S). A voucher specimen of the plant has been deposited in the herbarium of the department.

Preparation of extract

The pods of *Plumeria rubra* were collected, shade dried, powdered and subjected to soxhlet extraction successively with alcohol. The extract was evaporated to near dryness on a water bath, weighed and kept at 4^0 c in refrigerator until the experimental testing.

Phytochemical screening

The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as described by Thimmaiah[4].

Procurement and rearing of experimental animal

Albino rats (Wistar strain) used in the present investigation was procured from S.N. Institute of Pharmacy, Pusad (M.S). The rats were acclimatized for 15 days to the best laboratory condition (prior to experiment) and maintained on balanced diet (Trimurti lab feeds, Nagpur). Water was provided *ad libtum.*

Acute toxicity study

The animals were divided into three groups and the extract was administered orally at the doses of 500, 1000 and 2000 mg/kg body weight separately. Control rats received the vehicle only. The rats were observed for 72 hr. for behavioral changes and mortality[5].

Antifertility testing

The plant extract were tested in female albino rats for abortifacient activity by the method as described by Khanna and Chaudhary[6]**.** The vaginal smears of caged female rats of known fertility were monitored daily. Unstained material was observed under a light microscope. The proportion among the cells observed was used for determination of the estrous cycle phases[7]. The female rats 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 1st day of pregnancy. These rats were randomly distributed into 4 groups, a control group and three experimental groups of 6 animals each. On the day on the 10th day of pregnancy animals were laprotomised under light ether anesthesia using sterile condition. The two horns of uteri were examined to determine the implantation sites. There after the abdominal wound was sutured in layers. Post operational care was taken to avoid any infection.

The extract to be tested were then fed to operated pregnant rats i.e. alcoholic extract of *Plumaria rubra* (pods) at doses 50 mg/kg, 100 mg/kg, 200 mg/kg of each extract specified by an intragastric (i.g.) soft rubber catheter from day 11 up to the 15th day. The animals were allowed to go full term. After delivery the pups were counted and the antifertility activity of extract was evaluated. Litters were examined for any malformations.

Effect on estrous cycle

The ethanolic extract at 200mg/kg was found to be active amongst the three treatments in antifertility testing. Hence it was subjected to a detailed investigation for study of estrous cycle. The studies were conducted on adult female rats (140-180 gm) for 30 days. To study the estrous cycle pattern, animal showing regularity in the normal cycle were separated and chosen for further studies. Those animals showing normal estrus cycle were divided in two groups of 6 animals each; Group I- control, received distilled water (Vehicle) and Group II- treated, received alcoholic extract at dose of 200 mg/kg body weight. Vaginal smear using saline solution were taken twice daily during the entire treatment period, observation of the vaginal opening and the cell type obtained in a vaginal smear was also done. The duration of estrous cycle together with that of various phases was determined[7, 8].

All procedures with animals were conducted strictly in accordance with approved guideline regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, Ministry of Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering. All experimental protocols were met with the approval of Institutional Animal Ethics Committee registration number 1060/ac/07/CPCSEA (IAEC/7/2009).

Statistical analysis

All the data are expressed as mean±SEM. Statistical analysis was done by using paired and unpaired student´s t-test[9].

RESULTS AND DISCUSSION

Phytochemical screening

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 (Table-1).

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 mortality and changes in the behavior were observed in all treated and control groups of the rats up to a dose of 2000 mg/kg body weight. Hence one-tenth of this dose was used for antifertility testing.

Antifertility study

The alcoholic extract when evaluated for their antifertility activity, were found to exhibit significant pregnancy interceptive activity. Administration of 200 mg/kg body weight of the alcoholic extract resulted in 100% abortion and 50 mg/kg, 100 mg/kg body weight of the alcoholic extract resulted in 13.46 % and 43.63 % abortion (Table-2). This was evident from decreases in the percentage of live fetuses. While no live fetus was observed in 200mg/kg body weight of alcoholic extract. The percent resorption index increased from zero in the control animals and 100% in the 200mg/kg body weight alcoholic extract treated animals.

Table 1: Phytochemical profile of *Plumaria rubra* pod extract

Name of the plant	Alkaloids	Anthraquinone	Flavonoids	Simple phenolics	Steroids	Tannins	Saponins
Plumaria rubra	+	-	+	+	+	+	+

+ Present, - Absent

Table 2: Effect of alcoholic extract of *Plumeria rubra* (pod) on fertility of female rats when fed orally from day 11 to 15 of pregnancy

Treatment Groups	Body Weight (gm)	Drug Dose (mg/kg of body wt)	Sample Size	No. of foetus individual rats on day 10	No. of rats delivered (Litter Size)	No. of resorption in individual rats	No. of resorption In Mean±S.E	% Abortifacient activity
Group- A Control (Vehicle)	120-170	---	6	8,8,9,8,6,6	6 (8,8,9,8,6,6)	0,0,0,0,0,0	0	Nil
Group- B Alcoholic Extract	120-220	50	6	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 %
Group- C Alcoholic Extract	120-220	100	6	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 %
Group- D Alcoholic extract	120-220	200	6	14,7,10,9,9,11	6 (0,0,0,0,0,0)	14,7,10,9,9,11	10±0.96***	100%

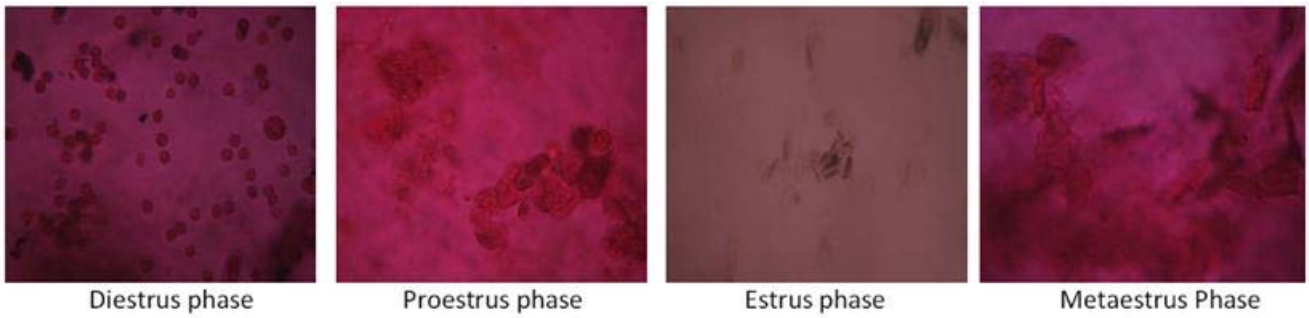
Values in Mean ± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, When compared between group.

Table 3: Effect on estrous cycle of female albino rats after the administration of 200 mg/kg alcoholic extract of *plumeria rubra* pods.

Phases	Proestrous phase (days)	Estrous phase (days)	Metaestrous phase (days)	Diestrous phase (days)	Estrous cycle (days)
Vaginal opening/ cell type obtained in a vaginal smear	25% to 40% / Epithelial cells only	Above 70% / Few cornified cells	50% to 70% / Cornified cells plus many leukocyte	50% to 70% / Leukocytes plus epithelial cells	-
Group I- Control	0.63±0.09	0.60±0.15	0.87±0.31	2.37±0.13	4.47±0.68
Group-II Alcoholic Extract 200 mg/kg	0.49±0.01*	0.55±0.05*	0.78±0.01***	3.41±0.12***	5.23±0.04*

Values in Mean±S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***p<0.001, when compared with control.

Figure 1: Vaginal smear of rat showing different phases of estrus cycle
(Magnification 40X × 10X, stained with H & E)



Effect on estrous cycle

In the present study of alcoholic extract of *Plumeria rubra* pod extract at 200 mg/kg body weight shows the prolongation of estrous cycle and diestrus phase particularly in experimental animals. The prepared smear was examined microscopically under low power for different types of cells. Four phases of the estrous cycle were identified depending upon the presence of cell types found in the smear. If majority of cells were leukocytes, then it was labeled as diestrus phase. Presence of large number of nucleated cells indicated proestrus phase. Estrus phase was confirmed when the smear showed more than 50% cornified epithelial cells. Metestrus phase was indicated by the presence of many neutrophils and scattered squamous epithelial cells in the smear (Figure 1).

DISCUSSION

The quest for naturally occurring compounds of plant origin that could be of benefit as contraceptive and fertility control agents stimulated our interest in *Plumeria rubra* pods. Plant based *in vivo* research has made significant rewarding progress in many important areas such as development of antibiotics[10], cancer[11] and is still contributing to research in reproductive medicine including hastening fetal delivery, prenatal development, pre- and post- coital contraceptives. For example, several studies have scientifically validated the use of medicinal plants such as *Carica papaya* seeds, *Garcina kola* seeds, *Bambusa vulgaris* leaves and *S. alata* as antifertility agents in rat[12-14].

Phytochemical screening has revealed many bioactive agents of plant extract coexist and canthus serves as precursors in the manufacture of drugs. For example, alkaloids which has been known for more than 2000 years to have adverse effect on pregnancy is being used by physicians either alone or in combination with oxytocics to induce abortion[14]. Furthermore, antifertility and abortifacient activities of phenolics, phytosteroids and saponins have also been sufficiently confirmed in animal models[15]. Studies on the phytochemical investigation of the various extract of the stem bark *Alangium salvifolium* also showed the presence of alkaloids, steroids, saponin, tannin and flavonoids[16].

Therefore, presence of alkaloids, phenolics, steroids and saponins in the extract of *Plumeria rubra* pods which may act either alone or in combination may be partly responsible for the observed pregnancy-terminating effects in this study.

The absence of respiratory distress, salivation, weight loss, dull eyes, diarrhea, epistasis, change in the appearance of fur, as well as mortality in the extract treated animals suggests that the *Plumeria rubra* pod extract was not clinically toxic to the female rats.

Various parameters evaluated in this study are useful indices to assess the potentials of a plant as an abortifacient. While cytotoxic agents can disrupt pregnancy possibly by interfering with the mitotic division of the fetus, chemical insults both before and after the implantation process can result in pre- and post-implantation embryonic loss[17, 18]. Therefore, the increase in the number of dead fetus as well as reduced survival ratio is an indication of the post coital antifertility activity of the *Plumeria rubra* pod extract. Such a high number of dead fetus and null survival ratio in the 200 mg/kg body weight alcoholic extract *Plumeria rubra* pod treated animals suggest a more potent antifertility activity at this dose. This finding agrees with that of the antifertility effect of *Senna alata* leaves extract in rats[19].

The increase in the post-implantation loss observed with the extract also emphasizes the antifertility or fetal resorptive properties[17]of the *Plumeria rubra* pod extract. The decrease in the number of live fetuses, following the administration of graded doses of the aqueous, alcoholic, chloroform and ethyl acetate extract of *Plumeria rubra* are indications of the possible abortifacient activity of the extract during post-implantation period. Similar observation was reported by Shibeshi, *et al*[20] following the administration of methanolic extract of *Achyranthes aspera* leaves to pregnant rats.

Experimentation with *Nelumbo nucifera* seeds showed prolonged estrus cycle and diestrus phase, suggesting its antifertility effect as prolongation of diestrus phase may explain the remote chances of the rats to get pregnant[21]. The result obtained in the study of methanolic root extract of *Rumex steudelii* also prolonged the estrus cycle and its diestrus phase in rats[22]. Similar result were obtained in the present study with alcoholic extract of *Plumeria rubra* pod extract at 200 mg/kg body weight which showed the prolongation of estrous cycle particularly diestrus phase in experimental animals.

CONCLUSION

In conclusion the study has provided evidence for the antifertility activity of all the three doses i.e. 50mg/kg, 100mg/kg and 200mg/kg body weight of alcoholic extract of *Plumeria rubra* pods. However the antifertility properties were found to be more pronounced at 200mg/kg dose of alcoholic extract and it was also substantiated by estrous cycle and particularly diestrous phase in experimental animal.

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