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ANTI- IMPLANTATION EFFECT OF *PLUMERIA BICOLOR AND KIGELIA PINNATA* EXTRACTS IN FEMALE RATS

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Post coital antifertility activity of stem bark extracts of two plants, *Plumeria bicolor* (Apocynaceae) and *Kigelia pinnata* (Bignoniaceae) was studied in female rats. The rats received extracts orally at two different doses i.e. 25 and 50 mg/day/rat from day 1 to 5 *post coitum*. The quantal pregnancy rate in female rats receiving chloroform extract of *Plumeria bicolor* (60, 40%) and alcoholic extract of *Kigelia pinnata* (50, 30%) stem barks were significantly declined. However, complete prevention of pregnancy was not achieved at the doses used in both the plant extracts treated rats. The decline in quantal pregnancy indicates anti-implantation effect of the extracts.

Keywords : Anti- implantation; *Kigelia pinnata; Plumeria bicolor; Post coital;* Quantal pregnancy.

Introduction

Post coital contraceptive agents are used to prevent pregnancy after unprotected intercourse or contraceptive accidents, the so called "morning after pill" or emergency contraception. The use of many plants and herbs for preventive or abortive purposes of fertility control has been prevalent in India for many centuries.

Plumeria bicolor (Family-Apocynaceae) is widely grown ornamental plant in Rajasthan, India. Certain species of *Plumeria* genus have been reported to possess antidiabetic[1], anti-implantation and abortifacient activity in female rats[2-4]. Phytochemical analysis of *Plumeria bicolor* stem bark has shown the presence of two new ferulic acid derivatives, 34-hydroxy tetratriacontanyl ferulate and 34-0-acetyl tetratriacontancyl ferulate along with plumericin and isoplumericin[5].

Kigelia pinnata D.C. (Family-Bignoniaceae) is widely grown in gardens and on road side in plains of India. Ethanolic and acetone extracts of the plant have been reported to possess anti-implantation (60-70%) effect in female rats[6]. The heartwood of the root has been reported to be used as dressing for ulcer, syphilis, rheumatism and as purgative[7]. *Kigelia pinnata* stem and root bark extracts have also been reported to possess antibacterial and antifungal[8], antitrypanosomiasis[9] and anti *Plasmodium falciparum* effects[10]. Crude dicholoromethane extract of stem bark and fruit also showed cytotoxic activity *in vitro* against cancer cell lines[11]. From the root and stem bark of this plant, 2(1-hydroxy ethyl) - napthol[2,3b]-furan-4,9-quinone, isopinnatal, Kigelinol and isokigelinol has been isolated and identified. Beside these active compounds octacosanol, lapachol, kigelin, stigmasterol, rhodamine β and β-sitosterol and also present in root heart wood[7,12].

In the present investigation anti-implantation activity of stem bark of two plants namely *Plumeria bicolor* and *Kigelia pinnata* have been observed.

Materials and Methods

Plant collection and extraction: The stem barks of *P. bicolor* and *K pinnata* were collected from the University campus and authenticated at the herbarium of Department of Botany, University of Rajasthan, Jaipur.

Shade dried stem bark (1.5 kg) of *P. bicolor* was coarsely powdered and exhaustively extracted with chloroform for 36 hours and filtered. The filterate was concentrated under reduced pressure and dark waxy mass (20 g) was obtained. This was suspended in olive oil and used for treatement.

Shade dried and coarsely powdered

stem bark of *K. pinnata* (2 kg) was extracted exhaustively with ethyl alcohol on a steam bath for 36 hours. The extract was filtered and concentracted under reduced pressure whereby a dark brown waxy mass (15g) was obtained. The extract was suspended in olive oil and used for treatment.

Animals : Colony bred adult female and male albino Wistar rats (150-200g) of proven fertility were used in the present study. All the rats were housed in a standard laboratory conditions (temperature 22° ± 3°C and 14h light/10h dark cycle) with free acess of pellet food (Lipton India Ltd., Bangalore) and tap water *ad libitum.*

Experimental Protocol : Normal cycling proestrous/estrous female rats were caged with males in the ratio of 2 :1 respectively. Successful mating was verified by the presence of spermatozoa in the vaginal smear taken every morning. The day on which a sperm positive vaginal smear was obtained was designated day 1 of pregnancy (pc). These mated female rats were randomly divided into five groups each having 10 rats.

Group I : Vehicle (0.5 ml olive oil, po.) treated control rats.

Group II : Rats treated orally with *P. bicolor* stem bark extract (25mg/rat/day) from day 1-5 *post coitum* (pc).

Group III : Rats treated orally with *P. Bicolor* stem bark extract (50mg/rat/day) from day 1-5 *post coitum* (pc)

Group IV : Rats orally exposed to *K . pinnata* stem bark extract (25mg/rat/day) from day 1-5 *post coitum* (pc)

Group V : Rats orally exposed to *K . pinnata* stem bark extract (50mg/rat/day) from day 1-5 *post coitum* (pc)

Autopsy : In order to confirm if implantation occurred following, all the rats were sacrificed on day 14 *post coitum* (pc) under mild ether anesthesia and their body weights were recorded. During autopsy both the uterine horns were examined for total number of implantation sites, live or dead/degenerated fetuses. The ovaries were examined for the number of fresh corpora lutea using a stereoscopic microscope. The uterine horns were removed and were fixed in Bouin's fluid for histopathological study in future.

Results and Discussion

The results of fertility performance of control and experimental female rats are shown in Table 1. In control group all the mated females were pregnant with mean number of implantations and live fetuses as 9.90 ± 0.35 and 9.30 ± 0.33 respectively. The quantal pregnancy rate in mated female rats receiving 25 and 50mg/rat/day *P. bicolor* extract from day 1-5 *post. coitum* were reduced to 60% and 40% respectively. There was no significant changes in the mean numbers of implantation sites, live fetuses and percentage of pre and post implantation losses in females treated with 25mg/rat/day *P. bicolor* extract. However, in higher dose group (50mg/rat/day), the average numbers of implantations and live fetuses were significantly declined. These pregnant females exhibited higher rate of preimplantation loss ($P<0.01$) as compared to controls.

Oral treatment of ethanolic extract of *K. pinnata* stem bark at the 25 and 50mg/day/rat dose regimen also exhibited a decline in pregnancy rate to 50% and 30% respectively. The mean numbers of implantations and live fetuses, percentage of pre and post implantation losses in 50mg/day/rat group were comparable to those of controls. However, in lower dose (25mg/day/rat) *K. pinnata* extract treated females, the mean number of implantations were slightly less ($P<0.05$), while the pre-implantation loss was increased ($P<0.05$) significantly. The average number of corpora lutea in rats of all the treated groups were similar to those of the controls.

The results of the present study indicates that administration of crude

Table 1. Fertility performance of female rats after oral administration of extracts of *P.bicolor* and *K. pinnata stem* barks from day 1-5 *post-coitum* (pc.)

Group	Treatment	Quantal pregnancy %	No. of *Corpora lutea*	No. of Implantations	No. of live embryos	Pre-implantation loss %	Post-implantation loss %
Group I	Control	10/10 = 100%	11.20±0.53	9.90±0.35	9.30±0.33	10.82±2.70	5.54±2.62
Group II	*P. bicolor* (25mg/rat/day)	6/10 = 60%	12.00±0.58	9.83±0.54	8.66±0.49	17.56±4.46	11.48±4.00
Group III	*P. bicolor* (50mg/rat/day)	4/10 = 40%	11.50±0.65	7.5±0.29***	6.50±0.50***	33.77±6.11**	13.39±5.13
Group IV	*K.pinnata* (25mg/rat/day)	5/10 = 50%	11.80±0.86	8.80±0.37*	7.80±0.66	24.43±3.97*	11.67±5.30
Group V	*K.pinnata* (50mg/rat/day)	3/30 = 30%	12.0±1.15	8.66±0.67	8.00±0.58	25.39±12.99	7.50±3.82

Levels of significance when compound with control
* P<0.05, ** P<0.01, *** P<0.001

Values are expressed as means ± SEM

Quantal pregnancy =(No. of females pregnant / No. of mated females) x 100
Pre-implantation loss = [(No. of *corpora lutea* -No. of implantations) / No. of *corpora lutea*] x100
Post-implantation loss =(No. of dead embryos / No. of implantations) x 100

chloroform extract of *Plumeria bicolor* and ethanolic extract of *Kigelia pinnata* stem barks suppressed quantal pregnancy in mated female rats by virtue of their anti-implantation effects. Our results are consistent with the finding of Dhir[4] who also observed anti-implantation and estrogen like action of alcoholic extract of *Plumeria rubra* leaves in rats. Prakash *et al.*[6] also reported that ethanolic and acetone extracts of *Kigelia pinnata* possess anti-implantation effects (60-70%) in female rats.

Pregnancy interceptory substances of plant origin usually produce thir effect by virtue of their inherent hormonal activity[13]. They may exert their antifertility activity by interfering with post-ovulatory events like transport, fertilization and development of eggs or preparation of the uterus for nidation by conjectually disturbing the delicate hormonal balance that exist between endogenous estrogen and progesterone[14-17].

Pre-implantation losses can also arise due to an impairment in the production of cytokines, growth factors and various types of adhesion molecules either by the developing blastocyst or by uterine epithelium around the site of implantation[18-20].

Beside these, several other possible mechanisms, such as stress, or anxiety can also induce an increase in pre-implantation losses[21]. The detailed study of the mechanism of antigestogenic effects of these extracts is under progress.

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