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Estrogenic and Progestational Activity of Roots of *Rubia cordifolia*

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The estrogenic and progestational activity of the fractionated precipitate of root of *Rubia cordifolia* were studied on estrous cycle and implantation in old female albino rats. It enhances heat phase in the estrous cycle and exhibited estrogenic and progestational activity without exhibiting any toxic manifestation.

Key Words: *Rubia cordifolia*, Estrogenic activity, Progestational activity, Implantation.

INTRODUCTION

Several hundreds of plants have been found to exhibit estrogenic activity or to contain estrogenically active compounds. This plant derived non-steroidal compounds with estrogen- like biological activity, have been defined as phytoestrogens. *Rubia cordifolia* (L.) (Rubiaceae) is also known as Manjishtha (Hindi). This plant, a climber herb, which reaches a length of about five meters and has many small prickles on the stem. The root stocks are perennial; the roots, long and cylindrical with the thin red bark. Herbal extracts are frequently used in traditional Indian medicines for the treatment of hormonal disturbances and the number of poly-herbal formulations (Evecare, Mensodil, Septlin) are used to treat the amenorrhea and menopause. The root of this plant is one of the common ingredients of herbal preparation as antiinfective agent[1-4]. The alcoholic extract of root of this plant is reported to inhibit platelet aggregation[5] and possess antitumor activity[6,7]. Literature also documents the amelioration of stress or induced immuno deficiency by antioxidants[8-11]. The present study deals with the effect of insoluble fraction of ethyl acetate of root part of the plant on estrous cycle and hormonal profile in experimental animals.

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EXPERIMENTAL

The roots of *Rubia cordifolia* (L.) (Rubiaceae) were collected from Kalimpong, Darjeeling, West Bengal, India in the month of March in 2005. A voucher specimen was deposited in Central National Herbarium, Botanical Survey of India and Shibpur Howrah (CES 13-5/2005/121) and authenticated by Dr. H.J. Choudhary. The roots were collected and dried under shade and powdered in a mixer grinder and stored in a closed container and brought to the college for further use.

Preparation of the extract: Powdered roots were defatted with petroleum ether (60-80ºC) and then thoroughly extracted with methanol in soxhlet apparatus, the extract was concentrated, then fractionated with ethyl acetate to get the insoluble precipitate of *R. cordifolia* (RCPP) which is a reddish brown colour (yield: 4.3 %). On preliminary phytochemical screening the *Rubia cordifolia* precipitate (RCPP) showed positive results for Shinoda reaction (flavonoid), Dragendroff's reaction (alkaloid) and Borntrager,'s reaction (anthraquinone). RCPP made into suspension (SRCPP) with distilled water using carboxymethyl cellulose (CMC) (0.5 %) as suspending agent.

Test animals: Old female albino rats weighing between 150-160 g, inbred in animal house, maintained at room temperature (25 - 28ºC) on a 14:10 h light and dark cycle and relative humidity of about 60 ± 5% RH were used. They were fed on a standard pellet diet (Lipton India Ltd.) with free access to water under hygienic conditions. Experiments were performed in accordance with current guidelines of CPCSEA; letter no 918/ac/05/ cpcsea, BRNCP, Mandsaur, India.

Drugs: Oestradiol valerate and progesterone were obtained from Pune Chemicals Pvt. Limited Company, Pune, Maharastra, India and groundnut oil (Sriram Oil Mill, Mandsaur and M.P. India) was purchased from the local market. The oestradiol valerate and progesterone were suspended in groundnut oil (0.05 mL).

Pharmacological evaluation

Effect on estrous cycle: Twelve adult healthy old female albino rats (150-160 g) were acclimatized to animal's house conditions for one week before starting of the experiment and divided into two groups (control group and test group) of six animals in each group. SRCPP (100 mg/Kg body weight) was fed orally through rubber catheter once daily throughout the duration of the experiment (18 d) to the test group of animals whereas the animals of the control group received distilled water containing 0.5% CMC as placebo. Changes in the phases in the estrous cycle were monitored carefully by vaginal simear method[12] and these appearance indices of different phases were calculated[13] (Table-1).

TABLE-1
OCCURRENCE OF DIFFERENT PHASES IN ESTROUS CYCLE OBSERVED FOR ALL THE ANIMALS IN CONTROL AND SRCPP GROUP FOR THE TOTAL PERIOD OF 18 d FOLLOWING TREATMENT WITH SRCPP

Phase	Control group	SRCPP group	Appearance index
Estrus	15	31	+53.33
Metestrus	14	23	-14.29
Diestrus	30	29	-13.39
Pro-estrus	13	18	-15.38

Effect on implantation: The old female rats, (150-160 g each) showing less number of copious spermatozoa in the vaginal smear, were caged with male rats of known fertily on the evening of proestrous. The presence of copious spermatozoa in the vaginal smear in the following morning was counted as day first of the pregnancy. The SRCPP was administered to the pregnant rat orally form day 1st to day 10th of pregnancy. Laprotomy was performed on day 11 under light chloroform anesthesia and the number of implantation sites in the uterine horn was recorded. Animals with at least one normal foetus were considered as pregnant. The abdomen was sutured carefully as rats were returned to their cages. After parturition, the number of litters was counted. The delivered pups were observed for at least, one month for any gross malformations. A group of pregnant rats, which received vehicle, only served as control (Table-2).

TABLE-2
IMPLANTATION ACTIVITY OF SRCPP IN ALBINO RATS

Treatment	Dose mg/kg p.o.	No of pregnant rats/total no of rats	No of implantation sites (Mean)	Percentage activity
Control	-	4/6	6.52 ± 0.33	65.00
SRCPP	250	6/6	9.16 ± 0.40	91.60

Estrogenic activity: These were determined on old female rats (40-50 g). Animals were divided into four groups, each group comprising six animals. The dose of SRCPP selected on the basis of lethal dose. The first group served as control received the treatment through sub cutaneously (s.c.) of groundnut oil (0.05 mL), second group received oestradiol valerate 0.1 µg/rat/d s.c. in groundnut oil (0.05 mL) as a standard drug. The third group received orally SRCPP at the dose of 250 mg/kg. The fourth group received the combined above-mentioned doses of oestradiol valerate and SRCPP through s.c and oral, respectively. The treatment was given for 3 d and 24 h after the last treatment, the rats were sacrificed. The uteri excised, cleared of the adhering tissues and weighed (Table-3).

TABLE-3
ESTROGENIC ACTIVITY OF SRCPP IN MATURED FEMALE ALBINO RATS

Treatment	Dose	Body weight (g)	Weight of uterus (mg/100 g body weight)
Control	0.05 mL groundnut oil	42.12 ± 0.770	54.52 ± 0.372
Oestradiol valerate	0.1 µg/rat/day s.c.	43.16 ± 1.190	304.06 ± 0.689
SRCPP	250 mg/kg p.o.	43.18 ± 1.078	123.02 ± 0.720
Oestradiol valerate+ SARc	0.1 µg/rat/d s.c. + 250 mg/kg p.o.	43.00 ± 0.810	217.76 ± 1.126

Progestational activity: These were assessed by pregnancy maintenance test in the pregnant rat overiectomized on the 12th day of the pregnancy[14]. Overiectomized rats which born normal implantation were regrouped into four groups of six rats in each group and the first group served as control received the treatment through sub cutaneously (s.c.) of 0.05 mL of groundnut oil, second group received progesterone 2 mg/rat/day s.c in groundnut oil (0.05 mL) as a standard drug. The third group received orally SRCPP at the dose of 250 mg/kg. The fourth group received the combined above-mentioned doses of progesterone and SRCPP through s.c and oral, respectively. Treatment was given from day 12 to day 19 of pregnancy. Autopsy of the rats was performed on day 20 and the number of live foetus was recorded. The results were expressed as the percentage of foetal survivals (Table-4).

$$\text{Foetal survival (\%)} = \frac{\text{No of live foetus}}{\text{Total number of implantation sites}} \times 100$$

TABLE-4
PROGESTATIONAL ACTIVITY OF SRCPP IN PREGNANT FEMALE ALBINO RATS' OVERIECTOMIZED ON DAY 12 OF PREGNANCY

Treatment	Dose	No of implantation sites (mean)	No of live foetuses (mean)	Foetal survival (%)
Control	-	9.16 ± 0.30	0.00 ± 0.00	0
Progesterone	2 mg/rat/d s.c.	9.00 ± 0.36	7.34 ± 0.16	81.5
SRCPP	250 mg/kg p.o.	9.00 ± 0.51	8.00 ± 0.25	88.8
Progesterone+ SRCPP	2 mg/rat/d s.c. + 250 mg/kg p.o.	9.16 ± 0.40	8.97 ± 0.49	97.9

RESULTS AND DISCUSSION

It is evident from Table-1 that SRCPP increases the regularity of the estrous cycle of the rats, which there is random occurrence of the heat period (estrous phase). Its appearance index is +53.33, which accounts for the increase of the desire of the old female to mate with males. When administered in the dose of 250 mg/kg p.o. it significantly acted as an implantation agent (Table-2).

Table-3 presents its estrogenic activity in old female albino rats. The mean uterine weight of the control group was 54.52 ± 0.372 mg/100 g body weight and estradiol valerate at the dose of 0.1 µg/rat/d s.c. in groundnut oil produced very significant increase in the uterine weight compared to the control value. SRCPP given by oral route at the dose of 250 mg/kg produced significant change in the uterine weight indicating the presence of estrogenic activity. The significant change in the uterine weight was also observed when SRCPP and estradiol valerate were given together as compared to the value obtained with estradiol valerate treatment alone.

The presence of any progestational activity is also evident with SRCPP. (Table-4), since SRCPP sustained the pregnancy of the old female rats overiectomized on the day 12 of the pregnancy. SRCPP seams to possess potent progestational activity, since it synergies the effect of progesterone (Group IV). The foetal survival per cent with progesterone is 81.5 whereas with the combination it is 97.9% (Table-4).

From the aforesaid results it is clear that SRCPP has good estrogenic potential in *R. cordifolia* due to the presence of flavonoids as much as it possesses strong implantation and progestational activity.

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