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Original Article

Post-Coital Antifertility Activity of *Hibiscus rosa-sinensis* Linn. roots

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Ancient literature mentions the use of a number of plants/preparations for fertility regulation. Some local contraceptive agents have also been described in Ayurvedic and Unani texts. Documented experiments or clinical data are, however, lacking. Therefore, the present study was undertaken to explore the antifertility and estrogenic activity of ethanolic extract of the roots of *Hibiscus rosa-sinensis* Linn. A strong anti-implantation (inhibition 100%) and uterotropic activity was observed at the dose level of 400 mg/kg body weight. Histological studies were carried out to confirm this effect.

Keywords: anti-implantation – estrogenic – *Hibiscus rosa-sinensis* – uterotropic

Introduction

Hibiscus (Malvaceae) is a genus of herbs, shrubs and trees. Its 250 species are widely distributed in tropical and subtropical regions of the world and are reported to posses various medicinal properties viz; antitumor, antihypertensive, antioxidant, antiammonemic (1–5). About 40 species are found in India. *Hibiscus rosa-sinensis* Linn. is a native of China and is a potent medicinal plant. It is a common Indian garden perennial shurb (6) and often planted as a hedge or fence plant. Traditionally this drug is attributed to antifertility activity in Ayurvedic literature (7). The flowers have been reported to posses anti-implantation and antispermatogenic activities (8,9). The petroleum ether extracts of the leaves and flowers have been shown to potentiate hair growth *in vivo* and *in vit*ro (10). Leaves and flowers also possess hypoglycemic activity (11,12). The mucilage of the leaf has anticomplementary activity (13). The extracts of *Hibiscus rosa-sinensis* have also been shown a protective effect against the tumor promotion stage of cancer development (14). The anthocynidin from the petals of the plant have protective effect against carbon tetra chloride-induced acute liver damage (15). The present investigation is the first ever study undertaken to find the unexplored anti-implantation and uterotropic activity of the roots of *Hibiscus rosa- sinensis*, using ethanol, being highly polar, so as to extract the maximum phytoconstituents present in the roots.

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Materials and Methods

Plant Collection and Preparation of Extract

The roots of *Hibiscus rosa-sinensis* were collected from Hisar during August 2002. The plant was authenticated by Dr M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. Herbaria are made and their voucher specimen retained in the department for future references.

The shade-dried roots of the plant were coarsely powdered (500 g) and were extracted with ethanol (95%, 3l) in a soxhlet extractor for 72 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (50–60°C) to yield a reddish brown solid (52 g). The extract was stored in refrigerator. A suspension of this was prepared in distilled water using acacia (1%).

Rats and Mice

Colony-bred female albino rats (Wistar strain), weighing (150–200 g), were used for antifertility testing. Immature colony-bred female albino rats (Wistar strain), 21–23 days old, were used for the study of estrogenic activity. Adult albino mice of either sex were used for acute toxicity studies. The rats and mice were maintained under standard husbandry conditions with food and water *ad libitum*. The Institutional Ethical Committee for Animal Cares and Use approved all experimental procedures.

Acute Toxicity Studies

Acute toxicity was carried out as described by Turner (16). Adult albino mice of either sex were divided into three groups containing five animals in each group. The mice were fasted for 18 h with water *ad libitum*. The suspensions prepared as above were administered orally at two different doses 2000 and 4000 mg/kg body weight, respectively, to different groups of mice separately. Control mice received the vehicle (acacia 1% p.o.) only. The animals were observed for 72 h for behavioral changes and mortality.

Post-coital Antifertility Testing

Vaginal smears from each rat were monitored daily, only rats with normal estrous cycle were selected. Rats found in proestrus phase of cycle were caged with males of proven fertility, in the ratio 2:1 and examined the following morning for evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy and those rats were divided into three groups containing six rats in each group. The extract was administered at 200 and 400 mg/kg body weight orally from day 1 to 7 of pregnancy. Control rats received the vehicle (acacia 1% p.o.). On day 10, laparotomy was performed under light ether anesthesia and semisterile conditions. The uteri were examined to determine the number of implantation sites.

Estrogenic and Antiestrogenic Activity

Ethanol extract at 400 mg/kg was found to be active amongst the two treatments in post-coital antifertility testing. Hence, it was subjected to a detailed investigation for potential estrogenic and antiestrogenic activity.

The uterine weight and vaginal cornification method was employed for this assay (17). Colony-bred immature ovariectimized female albino rats, 21–23 days old, weighing between 35 and 45 g, were used (18–22). They were divided into four groups, consisting of six rats each. The first group served as a control and received vehicle only (acacia 1%). The second group received ethinyl estradiol in olive oil, 1 μg/rat per day, subcutaneously. The third group received the ethanol extract at a dose of 400 mg/kg body weight. The fourth group received, in addition to ethinyl estradiol, a test dose of the ethanol extract at 400 mg/kg body weight. All the above treatments were given for 7 days. On the 8th day, the rats were sacrificed by decapitation, the 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 h. The tissues were dehydrated and embedded in paraffin. The paraffin sections were cut at 6 μm and stained with hematoxylin–eosin for histological observation (23). The diameter of the uteri and thickness of the endometrium were measured in 16 randomly selected sections using an ocular micrometer.

Statistical Analysis

All results were expressed as mean ± standard error. The data was analyzed using Student's *t*-test. The results were judged significant if $P < 0.05$.

Results

Acute Toxicity Studies

No mortality and changes in the behavior were observed in all treated and control groups of the mice up to the dose of 4000 mg/kg body weight. Hence, one-tenth of the doses were used for antifertility testing.

Post-coital Antifertility Activity

The anti-implantation activity is expressed as percentage of animals showing absence of implantation in uteri when laparotomized on day 10 of pregnancy. The ethanol extract given orally to the rats at a dose of 400 mg/kg exhibited a very potent anti-implantation activity since no implants, in all the treated animals, were observed, indicating 100% anti-implantation activity (Table 1).

No toxic effects were observed either by gross visual examination or in the weight of animals. After discontinuation of treatment, all the animals were mated. This resulted in pregnancy and delivery of normal litters, indicating that the action of the extract was reversible.

Estrogenic Activity

The estrogenic effects of the ethanol extract are shown in Tables 2 and 3. Oral administration of the ethanol extract at 400 mg/kg body weight caused a significant increase in uterine weight in immature rats (versus control, $P < 0.001$). The uterotrophic potency, as shown by the weight of the uterus, is about 62.7% of that of the ethinyl

Table 1. Effect of ethanol extract of *Hibiscus rosa-sinensis* Linn. roots on implantation in rats when fed orally from days 1 to 7 of pregnancy[a]

Treatment	Dose	No. of rats having no implantation sites on day 10	Mean number of implants ± SE	% of rats having no implantation sites on day 10
Control	0.5 ml/rat	Nil	8.60 ± 0.61	Nil
Ethanol extract	200 mg/kg	1	7.23 ± 0.44	16.66
	400 mg/kg	6	0	100

[a]Each group consisted of six rats.

Table 2. Estrogenic activity of the ethanol extract of *Hibiscus rosa-sinensis* Linn. Roots

Group	Treatment (dose)	Uterine weight (mg/100 g body weight; mean ± SE)	Vaginal cornification
I	Control (0.5 ml/rat)	58.06 ± 0.003	Nil
II	Ethinyl estradiol (1 μg/rat per day)	141.00 ± 0.03*	+++
III	Ethanol extract (400 mg/kg)	88.40 ± 0.04*^{oo}	+ to ++
IV	Ethinyl estradiol (1 μg/rat per day) + Ethanol extract (400 mg/kg)	202.15 ± 0.01*^{o}	+++

+, nucleated epithelial cells; ++, nucleated and cornified cells; +++, cornified cells.
$^{oo}P<0.02$ when compared with ethinyl estradiol.
$^{o}P<0.05$ when compared with ethinyl estradiol.
$^{*}P<0.001$ when compared with control.

Table 3. Histological changes in the uterus and endometrium after treatment with ethanol extract of *Hibiscus rosa-sinensis* Linn. roots

Treatment (dose)	Diameter of uterus (μm ± SE)	Thickness of endometrium (μm ± SE)
Control	355.80 ± 1.68	111.40 ± 0.82
Ethinyl estradiol (1 μg/rat per day)	658.00 ± 3.05*	280.00 ± 1.88*
Ethanol extract (400 mg/kg)	438.30 ± 1.30**^{ooo}	228.60 ± 2.65*^{o}
Ethinyl estradiol (1 μg/rat per day) + ethanol extract (400 mg/kg)	777.50 ± 3.03*^{oo}	358.30 ± 2.45*^{oo}

$^{*}P<0.001$ when compared with control.
$^{**}P<0.01$ when compared with control.
$^{oo}P<0.05$ when compared with ethinyl estradiol.
$^{o}P<0.02$ when compared with ethinyl estradiol.
$^{ooo}P<0.001$ when compared with ethinyl estradiol.

estradiol. The uterotropic changes, such as the diameter of the uterus ($P<0.01$) and thickness of the endometirum ($P<0.001$) were significantly increased when compared with control rats. The uteri of these rats were inflated and full of fluid resembling the proestrous/estrous uterus. The epithelium of the endometrium consisted of spindle-shaped cells with basal nuclei. The stroma consisted of loose and edematous fibroblast-type cells. The treated rats showed open vaginas. Examination of the vaginal smears of treated rats revealed predominantly cornified and nucleated epithelial cells. However, their number was less than in ethinyl estradiol-treated rats.

Simultaneous administration of ethinyl estradiol and ethanol extract caused a significant increase in the uterine weight, uterine diameter and thickness of the endometrium (versus control $P<0.001$). When compared with ethinyl estradiol, there was significant increase in uterine weight, thickness of endometrium and diameter of uterus ($P<0.05$). It appears that the ethanol extract has estrogenic activity, but no antiestrogenic activity at 400 mg/kg dose.

Discussion

In the present study, the roots of *Hibiscus rosa-sinensis* were tested for their anti-implantation and estrogenic properties. The loss of implantation caused by ethanol extract may be due to antizygotic, blastocytotoxic or anti-implantation activity (24).

In ovariectimized immature female rats, oral administration of the ethanol extract of *Hibiscus rosa-sinensis* roots increased the uterine weight and stimulated uterine growth, suggesting estrogenic activity. It is known that administration of estrogen has uterotropic effects in ovariectomized immature female rats and mice (25,26). Such effects are associated with growth and proliferation of endometrial microvilli on the apical surface as well as increase in cell number.

The pregnancy interceptive effect of the ethanol extract of *Hibiscus rosa-sinensis* root can be interpreted as due to the estrogenic nature of the plant. Regular development of all the events leading to nidation, at least in rats and mice, is chiefly under the direct command of

estrogen–progesterone interplay at the cellular level (27) and a slight disturbance in this hormonal balance may result in an unfavorable endometrial environment.

Pre-implantation losses can also arise due to disruption of events that are prerequisite for fertilization or 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 (28,29).

Plant products exhibiting estrogenic activity and producing antifertility effects are known in literature, viz the ingestion of 200, 400 and 600 mg/kg of ethanol extract of *Salvia fructosa* from day 1–6 of pregnancy did not cause pregnancy failure, but reduced the number of viable fetuses and increased the number of resorptions in female pregnant rats (30). Vasicine, isolated from *Adathoda vasica* showed potent abortifacient and uterotonic effects in guinea pigs (31). Flavonoids isolated from *Striga lutea* and *Striga orobanchiodes* possessed estrogenic and antifertility property (32). Preliminary phytochemical studies indicated the presence of steroids, amino acids and carbohydrates in the ethanol extract. Since some of these compounds are known to exhibit antifertility activity (33), the effect of the extract might be due to the presence of such compounds.

In conclusion, the present study suggests that the ethanol extract of *Hibiscus rosa-sinensis* root posseses anti-implantation activity, and the estrogenic property of the extract may be responsible, at least partly, for this anticonceptive effect.

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