Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2011; 3(2): 41-44

Research Article ISSN 0975 1556

Evaluation of Antifertility activity from Stem Part of *Ocimum gratissimum* in Acetone extracts

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ABSTRACT
Acetone extract of Stem of *Ocimum gratissimum* was screened for the antifertility activity in proven fertile female albino rats at the doses 100, 200 and 500 mg/kg b.wt./day. Oral administration of the extract to mated female rats on days 1-5 of pregnancy resulted in a decline in the fertility index, numbers of uterine implants and live fetuses in a dose dependent manner as was confirmed by laparotomy on day 15 of pregnancy. The extract (100 mg/kg b.wt.) exhibited weak estrogenic activity when given alone and tested in immature bilaterally ovariectomized female albino rats, but exhibited slight antiestrogenic activity when administration along with estradiol valerate (0.1 mg/kg b.wt.). Blood sugar and haematological parameters were within normal range. Thus, the results of the present study indicate that the acetoneextract of *Ocimum gratissimum* stem possesses pregnancy terminating effect by virtue of anti-implantation activity.

Keywords: *Ocimum gratissimum*, antifertility, female rats.

INTRODUCTION

The quest for the oral contraceptive agent that can control human fertility is as old as recorded history. Although a variety of synthetic contraceptive agents are available, but these cannot be used continuously because of their side effects. So, natural plant substances possessing mild inherent estrogenic and antiestrogenic properties offer themselves as an effective nonconventional source of contraception with less deleterious side effects. *Ocimum gratissimum* Linn. (Hindi-Amaltas; English-Golden Shower or Indian Laburnum), a medium sized tree belonging to the family - Caesalpiniaceae, is widely cultivated throughout India as an ornamental tree. *Ocimum gratissimum* has been used extensively in the folklore medicine for the treatment of a variety of diseases. [1-2] Pharmacologically the plant has been investigated for its antibacterial [3], anti-diabetic [4], hypocholesterolaemic [5], hepatoprotective [6], antitumour [7], laxative [8] and antioxidant [9] effects. The plant is rich in phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives. [10] Ethanolic extract of fruits of this plant has been reported to possess anti-implantation and estrogenic effect in rats. [11] The previous study in our laboratory showed that postcoital administration of aqueous extract of Stem of *Ocimum gratissimum* at the dose 500 mg/ kg b. wt./day prevented pregnancy in all the treated female rats by virtue of antiimplantational activity. [12] Because of the wide range of therapeutic efficacies of *Ocimum gratissimum* plant and since the active principles present in various extracts of it may be different, the present study, therefore, proposes to evaluate the post-coital antifertility efficacy of acetone extract of Stem of *Ocimum gratissimum* in female rats and also to investigate its hormonal profile in immature bilaterally ovariectomized female rats in order to gain insight into its possible mechanism of action.

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MATERIALS AND METHODS

Plant material and extraction

Ocimum gratissimum pods were collected during the season and were thoroughly dried in the shade. The plant was authenticated at the Department of Botany, CRC group, Hyderabad, AP. The Stem separated from the shade dried pods were ground to coarse powder (1000 g) and was subjected to soxhlet extraction with acetone (B.P. 60-80). The crude extract thus obtained was concentrated under reduced pressure and low temperature. The residue obtained was then utilized for evaluating antifertility efficacy by suspending in appropriate volume of olive oil. The yield of the extract was 14% of starting crude material.

Experimental animal

Colony bred, adult albino Wistar rats (weighing 170-200 g) for antifertility studies and immature female rats (21-24 days old). All the experimental procedures were performed

according to the guidelines for the care and use of experimental animals and approved by the Institutional Ethical Committee for Animals Care and Use, CRC group Dr. Yuvaraj Head, for bioassay studies were used as experimental animal model. All the animals were housed in standard laboratory conditions (temperature 22 ±3^{o}C and 14 h light/10 h dark cycle) with free access of food (Lipton India Ltd) and tap water *ad libitum*.

Dose and route of administration

The animals of Group I received vehicle (olive oil, 0.2 ml/rat) only and served as control. Animals of Group II, III and IV received crude acetoneextract of *Ocimum gratissimum* at 100, 200 and 500 mg/kg b.wt./day (suspended in olive oil) doses, respectively, once a day from day 1-5 *post coitum* (*pc*). The extract was administered orally by using a curved needle and a tuberculin syringe.

Antifertility study

For the antifertility study, only normal cycling proestrous or estrous female rats were caged over-night with males (2:1 ratio) of proven fertility. Next morning, insemination was confirmed by the presence of the vaginal plug and spermatozoa in the vaginal smear. This day of mating was designated as day 'Zero' of pregnancy. These mated females were isolated, weighed and divided into four groups of seven animals each. In order to confirm if implantation occurred following mating, all the control and treated female rats were sacrificed on day 15 *pc* under mild ether anaesthesia and their body weights were recorded. Blood samples for hematological studies were collected directly from the cardiac puncture. [13] During autopsy, both the uterine horns were examined for the number of implantation sites, live or dead / resorbed fetuses. Embryos with bright reddish aspect and clear margins were considered to be normal and those with dull blue colour, no clear margin, smaller in size and with some surrounding exudate were considered to be resorbing. The ovaries were excised and examined for the number of fresh corpora lutea using a stereomicroscope. The uterine horns were removed and trimmed of fat. These uterine horns with embryonic contents intact were quickly weighed on an electric pan-balance to the nearest milligrams. The fetuses were removed from the uterine horns and suitable parts of these uterine horns were fixed in Bouin's fixative for histological observations in future.

Hematology

The counts of RBC and WBC, Hemoglobin and hematocrit values were determined from the blood collected directly from the heart of rats receiving 500mg/kg b.wt. extract at the time of scarification. [13]

Hormonal profile / Estrogenic and antiestrogenic activity

Crude acetoneextract of the test substance was subjected to standard bioassay procedures for assessment of estrogenic or antiestrogenic activity in terms of the rat uterotrophic assay. [14] Immature female rats (21-24 days old) were taken for bioassay studies. These animals were bilaterally ovariectomized by dorsolateral approach under light ether anaesthesia and semisterile conditions and after a rest period of seven days, these were randomly divided into four groups of five rats each and treated as follows:

Group I: Control group, receiving olive oil only (0.2 ml/rat/day), orally.

Group II: Estradiol valerate (0.1 mg/kg b.wt./day), intramuscularly (i.m.).

Group III: Extract alone (100 mg/kg b.wt./day), orally.

Group IV: Extract (100 mg/kg b.wt./day, orally) + Estradiol valerate (0.1 mg/kg b.wt./day, i.m.), conjointly. All these rats received treatment twice daily for 3 consecutive days. These treated rats were sacrificed 24 hours after the last dose administration. Their body weights were recorded. Uteri were carefully dissected out, freed from adherent tissues, blotted on filter paper and were weighed quickly to nearest milligrams on digital balance. Condition of vaginal opening was also recorded.

Uterine luminal epithelial cell height

Haematoxylin-eosin stained slides were observed microscopically for luminal epithelial cell height. One hundred luminal epithelial cells from 25 sections were measured with occular micrometer at X400. Two diagonal and one median length was measured, averaged and expressed as mean epithelial cell height and were then calibrated with a stage micrometer.

Statistical analysis

All the values are expressed as the mean ± SEM for 7 rats per group. Data were analysed statistically by Student's t-test and $p< 0.05$ was considered as statistically significant.

RESULTS

Antifertility activity

Table 1 summarizes the data obtained in the fertility study after postcoital administration of acetone extract of Stem of *Ocimum gratissimum*. In the control group, all the mated female rats were pregnant. Oral administration of acetoneextract of Stem of *Ocimum gratissimum* at the doses 100, 200 & 500 mg/kg b.wt. to the female rats from day 1-5 *pc* impaired fertility substantially in terms of quantal pregnancy and number of uterine implants. The quantal pregnancy rate in rats receiving 100 and 200 mg/kg b.wt./day extract doses was declined to 42.86% and 28.57%, respectively. However, the dose (500 mg/kg b.wt./day) of the extract also exhibited only 28.57% quantal pregnancy as two of the females showed the presence of implantation sites on day 15 *pc*. The number of total uterine implantation sites and viable fetuses showed a dose-dependent decrease by virtue of increase in the percentage of the pre-implantation embryonic loss rate. The total number of healthy corpora lutea in control and extract treated rats remained significantly unchanged.

Ponderal changes (Body and Uterine weight)

Administration of crude acetoneextract of Stem of *Ocimum gratissimum* orally at different doses from day 1-5 *pc* did not produce any significant change in the maternal body weight, but did produce a significant decline in relative uterine weight when compared with controls
(Table 2).

Hematology

A statistically non-significant change in the RBC and WBC counts, hemoglobin and hematocrit values was observed (data not shown).

Hormonal profile

Table 3 shows the results of uterine bioassay studies of acetoneextract of Stem of *Ocimum gratissimum* in bilaterally ovariectomized immature rats. Oral administration of the extract (100 mg/kg b.wt./twice daily) to ovariectomised immature female rats produced a slightly significant ($p<0.05$) increase in the uterine wet weight. However, the extract did not induce premature opening of the vagina, thus, suggesting mild estrogenic activity of the extract. But when the extract was administered conjointly with estradiol valerate (EDV,

Table 1: Effect of acetone extract of stem of *Ocimum gratissimum* on implantations in female rats from Day 1-5 Post-Coitum

Group	Treatment dose (mg/Kg b.wt.)	No. of pregnant rats (Fertility index)1	No. of implantation sites in individual rats	No. of reabsorbing fetuses	No. of viable/live fetuses	No. of Corpora lutea	% Preimplantation loss2	Postimplantation or Reabsorption rate3	Birth rate5
Control (0.2ml olive oil/rat)	-	7 (100)	(10,11,13,13,12,13,12)	0	84	105	20	0	80
Acetone	100	3 (42.86)	(0,10,0,4,0,13, 0)	12	15	101	73.27	44.44	14.85
	200	2 (28.57)	(0,10,0, 0,12, 0, 0)	10	12	100	78	45.45	12
	500	2 (28.57)	(0,0,8,0,0,10, 0)	9	9	102	82.53	50	8.82

1Quantal pregnancy or fertility index (%) =(No. of pregnant animals/No.of mated animals) x100 2Preimplantation loss (%) = [(Total no. of corpora lutea – Total no. of implantation)/Total no. of corpora lutea] x 100 3Postimplantation loss (%) or Reabsorption rate(%) = [(Total no. of implantation – Total no. of viable fetuses)/Total no. of implantation] x 100
4Birth rate = no. of live fetuses/ no. of corpora lutea x 100

Table 2: Effect of acetone extract of stem of *Ocimum gratissimum* on the body and relative uterine weight of female rats

Group	Treatment dose (mg/Kg b. wt.)	Initial body wt. (g)	Final body wt. (g)	uterine weight (g/100 g b. wt.)
Control (0.2ml olive oil/rat)	-	198 ± 5.83	215 ± 12.65	6.45 ± 0.27
Experimental (acetone extract)	100	174 ± 17.49	182 ± 15.62	2.52 ± 1.35*
	200	170 ± 13.04	176 ± 7.48	2.51 ± 1.47*
	500	180 ± 7.07	184 ± 5.09	2.47 ± 1.34*

[Values are mean + SEM]
Level of Significance when compared with control rats : $p < 0.05$

Table 3: Showing estrogenic and antiestrogenic activity of petroleum ether extract of stem of *Ocimum gratissimum* in bilaterally ovariectomized immature female rats

Group	Treatment group	uterine weight (mg/100g b. wt.)	vaginal opening	Luminal epithelial cell height (µm)
I.	Control (0.2ml olive oil/rat)	38.36 ± 1.63	Closed	10.45 ± 0.32
II.	Estradiol valerate (0.1mg/kg b.wt.)	529.36 ± 23.91aaa	open	42.50 ± 0.29aaa
III.	acetone extract (100mg/kg b.wt.)	45.50 ± 1.41*	Closed	16.72 ± 0.37***
IV.	acetone extract (100mg/kg b.wt.) + Estradiol valerate (0.1mg/kg b.wt.)	308.50 ± 13.99###	open	35.64 ± 0.24###

[Values are mean ± SEM]1. Group II and group III treated rats compared with Group I rats.
aaa $p < 0.001$, *$p < 0.05$, *** $p < 0.001$ 3. Group IV treated rats compared with Group II rats.
$p < 0.001$ (Highly significant)

0.1 mg/kg b.wt./twice daily), it significantly ($p<0.001$) prevented the estrogen induced uterotrophic effect, thus, reflecting antiestrogenic nature of the extract in presence of a strong estrogen.

Uterine luminal epithelial cell height

The extract when administered alone to ovariectomized rats induced a mild stimulation of all the uterine constituent elements and a significant ($p<0.01$) increase in the uterine luminal epithelial cell height when compared with ovariectomized control only, thus, showing the estrogenic nature. But when the extract was administered along with a strong estrogen ie. estradiol valerate (EDV), it showed antagonism of EDV induced hypertrophy of the uterine constituent elements and a highly significant ($p<0.001$) decline in the uterine luminal epithelial cell height in comparison to EDV alone treated rats (Group II).

DISCUSSION

In the present study oral administration of acetoneextract of *Ocimum gratissimum* stem at the doses 100, 200 and 500 mg/kg orally from day 1 to 5 pc, produced a dose dependent adverse effect on fertility index (quantal pregnancy) and number of implantations in uterine horns of the female rats by virtue of an increase in the percentage of the pre-implantation embryonic loss. The antifertility effect of aqueous extract of *Ocimum gratissimum* has been reported earlier by our laboratory. [12] The present findings indicate that acetone extract of stem of *Ocimum gratissimum* also possess significant antifertility activity as it interfered with steroidal conditioning of the uterus and renders it hostile to ovum implantation. [15] A significant decrease in relative uterine weight after postcoital administration of the extract was observed on day 15 *pc* in comparison to control. This decrease in uterine weight was correlated with decrease in the number of implantation sites and viable fetuses in the uterine horns. [16-17] As, the uterine weight in pregnant rats also serves as an index of uterine decidualization and a significant decrease in uterine weight indicates suppression of decidual changes in uterus. [18] In the present investigation, a non-significant change in the total erythrocyte and leucocyte counts, hemoglobin and hematocrit values following oral therapy of acetone extract of stem of *Ocimum gratissimum* suggests non-toxic action of the extract on general body metabolism. These results are in agreement with the reports of Mutreja *et al* [19] who also reported a nonsignificant change in haematological parameters after administration of alcoholic extract of Stem of *Nelumbo nucifera*. In bioassay test, crude acetone extract of *Ocimum gratissimum* stem showed mild estrogenicity when treated alone but when treated conjointly with estradiol valerate it produced estrogen antagonistic effects. Thus, the pregnancy interceptory effect of the extract of the test plant might be due to its antiestrogenic nature. Thus, like other relatively weak estrogenic substances it behaved as an antiestrogen in the presence of relatively more potent estrogen by possibly affecting the uterine estrogen receptor binding. A number of

plants possessing antiestrogenic activity have also been reported to interrupt pregnancy. [20-23] Antiestrogens with weak estrogenic activity administered early in pregnancy may interfere with implantation by altering the normal pattern of hormonal conditioning of uterus required for conception. [24] Furthermore, many of the synthetic non-steroidal compounds having estrogen agonist-antagonistic property have also shown postcoital antifertility effects. [25-26] Experimental studies also indicate pregnancy blocking effect of certain synthetic estrogen agonist-antagonist compounds. [27-28] Pre-implantation losses can arise due to disruption of events which are prerequisite for fertilization or impairment in the production of cytokines, growth factor and various types of adhesion molecules either by the developing blastocyst or by the uterine epithelium around the site of implantation. Therefore, one possible explanation of anti-implantation effect of the extract can be explained by pre-implantational embryonic loss due to accelerated embryonic transport which is an estrogen mediated process. In conclusion, the present study suggests that the antifertility activity of the acetone extract of Stem of *Ocimum gratissimum* is probably due to its antioestrogenic property. The action of estrogen on the uterus, which is essential for implantation, is antagonized by the extract. Hence, an unfavourable environment is created in the endometrium checking nidation. While the hematological studies performed in extract treated rats did not reflect any adverse effect.

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