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Research Article

EFFECT OF *OXALIS CORNICULATA* WHOLE PLANT EXTRACTS ON FERTILITY REGULATION IN FEMALE ALBINO RATS

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ABSTRACT

Aim of the present study is to find out novel fertility regulation agent from natural products. Petroleum ether and ethanol extract of whole plant of *Oxalis corniculata* (Oxalidaceae) were tested for fertility regulation studies in female albino rats at the dose level of 10 and 20 mg petroleum ether and ethanol extract per 100 gm body weight administered orally from day 1-7 of pregnant rats for antiimplantation activity and also for immature ovariectomised rats for estrogenic/antiestrogenic activity. The dose level of 10 and 20 mg of petroleum ether extract inhibited 39.71% and 76.42% implantation sites respectively. Similarly, 10 and 20 mg of ethanol extract treatment has resulted in 27.87% and 38.21% inhibition of implantation respectively. In estrogenic activity, the uterus of treated rats showed significant increase in the gravimetric and micrometric measurements, indicating its estrogenicity. When both the extracts were tested in immature rats the estrogenic activity has been confirmed by early opening of vagina, cornification of vaginal epithelial cells and increase in uterine weight. Antiimplantation activity of *O. corniculata* is due to its estrogenic nature, therefore, it may be concluded that both the activities revealed their possible fertility regulation.

Keywords: Oxalidaceae, Antiimplantation activity, Estrogenic activity, Pregnancy, Epithelial cells.

1. INTRODUCTION

Use of plant preparations and extracts for pregnancy interruption has a long - standing history among Indian physicians. Considering the side effects of hormonal contraceptives, the herbal contraceptives from indigenous plants have gained much significance in recent years. A large number of indigenous plants having such activities are recorded in ancient Indian Literature [1-4]. Indeed, recent reviews of Indian plants have been tested for their antifertility activity in laboratory animals [5-9]. Experience with current research work based on my findings with antifertility activities [10-16], the present study on *Oxalis corniculata* (whole plant) were undertaken to explore the different extracts to find their significant potentiality on fertility regulation in female albino rats.

Oxalis corniculata (Oxalidaceae) is a creeping herb commonly called as wood sorrel and available as cosmopolitan. The leaves contain oxalic acid, hot and bitter; easy to digest and a good appetizer; removes "*Kapha*" "*Vata*" [2]. The leaves are refrigerant and antiscorbutic, also cures fevers and dysentery; so, it is tested and is screened out its antifertility activity as it is mentioned in Ayuraveda. But so far, no systematic biological and chemical investigation has been carried out. Hence, in the present investigation on the solvent extracts of the whole plant of *O. corniculata* was subjected to systematic evaluation of fertility regulation in female albino rats.

2. MATERIAL AND METHODS

Whole plant of *O. corniculata* were collected the fields in and around Gulbarga during July to November and authenticated at the herbarium, Department of Botany, Gulbarga University, Gulbarga, Karnataka, India. The whole plant, including roots, stems, leaves, flowers, fruits and seeds, were shade dried, powdered and subjected to soxhlet extraction successively with petroleum ether (60-80 °C) and ethanol (95%). The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50-60 °C). The extracts were kept refrigerated until used.

2.1. Fertility regulation activities on female albino rats

2.1.1. Antiimplantation Activity

In this study, each extract was separately tested for antifertility activity in female albino rats as described by

Khanna and Choudhury [17]. Graded doses (10 mg and 20 mg/100 body weight) of the petroleum ether and ethanol extracts were prepared by suspension in Tween- 80 (1%) with distilled water. Animals used in the experiment were colony bred Wistar strain female albino rats weighing 150-200 gm. All the animals were maintained under standard husbandry conditions with food and water *ad libitum*. Vaginal smears of the female rats were studied microscopically to determine the phase of estrous cycle. Only rats with normal estrous cycle were used for estrogenic activity [18].

Rats found in the estrous phase of the cycle were caged with males of proven fertility in the ration of 2:1. Animals, which showed thick clumps of spermatozoa in the vaginal smear, were separated for the experiment and that day was designated as day 1 of pregnancy [19, 18]. The animals were divided into 5 groups consisting of 5 animals in each group. The extracts were administered orally from day 1 to 7 in 0.5 ml of Tween-80 (1%). The controls received the vehicle only. On day 10th, laparotomy was done under light ether anesthesia using aseptic condition and uteri were observed for number and size of implantations.

The abdominal incision was closed with sutures and the rats were allowed to recover and deliver full term pregnancy [18]. Those rats showing implantation sites but not delivering were again laparotomised on day 23rd, and the uteri were examined for implantation sites. Each fetus was weighed and examined for a genital distance and gross defects. The litters were allowed to grow to check for postnatal growth and congenital anomalies.

2.1.2. Estrogenic/Antiestrogenic Activity

Colony bred Wistar strain albino rats, 21 to 23 days old, weighing between 35 to 45 gm (Immature) were bilaterally ovariectomised by dorsolateral approach under light ether anaesthesia and used for estrogenicity testing for 7 days, after the operative experimental groups consisting of 6 animals were randomly divided into groups and treated as described in Table 2.

As a control, ethinyl estradiol in olive oil (1 μg/rat/day) was injected subcutaneously for 7 days. On the 8th day of the experiment all the animals were sacrificed by decapitation and uteri were dissected, cleared of surrounding tissues, blotted on fitter paper, weighed quickly on a sensitive balance and fixed in Bouin's fluid for 24 hour. The tissues were dehydrated and embedded in paraffin. The paraffin sections were at 5 mm and stained with Haematoxylin-Eosin [20] for histological observation. The diameter of uterus, thickness of endometrium and height of endometrial epithelium were measured in 20 randomly selected sections using an ocular and stage micrometer by the methods described by Deb et al [21].

2.1.3. Statistical Analysis

The statistical analysis was done to determine the significant difference of results between treated and control groups using "Student's *t* test" using software package SPSS [22]. The values were judged almost significant $P<0.05$, significant if $P<0.01$ and highly significant if $P<0.001$.

3. RESULTS

3.1. Antiimplantation Activity

The result of antiimplantation activity of extracts of *O. corniculata* at different dose levels is shown in Table 1. The maximum fertility regulation was seen with the 20 mg/100 gm dose of the petroleum ether extract. The activities of all other extract were less than 50%. During the laparotomy resorption of implants was observed with the 20 mg/100 gm dose of the petroleum ether extract as evidenced by scar marks indicating the loss of implantation in the uterine horns ($P<0.001$).

In animals treated with low dose level 10 mg/100 gm of the either the petroleum ether or ethanol extract or 20 mg/100 gm of the ethanol extract, the reduction of the number of implantation sites in the uterine horns were observed when laparotomised.

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 extracts was reversible.

3.2. Estrogenic/Antiestrogenic Activity

The result of Estrogenic/Antiestrogenic activity of petroleum ether extract when administered orally at 20 mg/100 gm caused a significant increase in the uterine weight in immature ovariectomised rats versus control ($P<0.001$) (Table 2). The uterotrophic changes such as the diameter of the uterus ($P<0.001$) thickness of the endometrium ($P<0.001$) were significantly increased when compared to control rats. The uteri of these rats were inflated and full of fluid resembling the proestrous or estrus uterus. The epithelial layer of the endometrium consisted spindle shaped cell with basal nuclei. The stroma was represented by fibroblast type of cells and was loose and oedematous.

The experimental animals showed an open vagina and an estrous smear. The number of cornified cells in the vaginal smears was considerably higher than that of controls, but notably less than of the ethinyl estradiol treated animals.

Simultaneous administration of 20 mg of petroleum ether, 20 mg ethanol extracts/100 gm body weight and ethinyl estradiol caused a highly significant increase in the uterine weight versus control ($P<0.001$). The degree of uterotrophic response was greater than that produced by ethinyl estradiol alone ($P<0.001$). It also caused a highly significant increase in uterine diameter, thickness of the endometrium and height of the endometrial epithelium versus control ($P<0.001$).

These results indicate that though both the extracts were estrogenic in nature but petroleum ether extract was more potent. The petroleum ether extract and ethinyl estradiol were synergetic in their action, as the combination of these has increased all the parameters of uterus more than their individual administration.

Table 1: Effect of petroleum ether and ethanol extracts of whole plant of Oxalis corniculata on implantation in rats when administered orally from day 1 to 7 of pregnancy

Group	Treatment	Dose (mg/100gm BW)	Rats having no implantation sites on day 10	% Inhibition of implantation	Mean No. of implants	% Inhibition implantation sites
1.	Control	Tween–80 (1%)	Nil	Nil	11.33 ± 0.33	Nil
2.	Petroleum ether extract	10	Nil	Nil	6.83 ± 0.60**	39.71**
3.	Petroleum ether extract	20	1	16.66	2.0 ± 0.30**	76.42**
4.	Ethanol extract	10	Nil	Nil	8.16 ± 0.30**	27.87**
5.	Ethanol extract	20	Nil	Nil	7.00 ± 0.36**	38.21**

*Duration: 7 days; Six animals were maintained in each group; Values are mean ± S. E.; * P < 0.01, **P < 0.001 when compared to control.*

Table 2: Estrogenic activity of petroleum ether and ethanol extracts of O. corniculata (whole plant) on immature rats

Group	Treatment	Dose (mg / 100 g BW)	Uterine weight (mg /100 g BW)	Vaginal status	Vaginal cornification
1	Control	Tween-80 (1%)	50.00±3.21	Closed	--
2	Ethinyl estradiol	1μg/rat/day	190.48**±10.01	Opened	+ To ++
3	Petroleum ether extract	20	198.21**±8.52	Opened	+++
4	Petroleum ether extract + Ethinyl estradiol	20+1μg/rat/day	242.82**±6.81	Opened	+++
5	Ethanol extract	20	098.01**±8.21	Opened	++
6	Ethanol extract + Ethinyl estradiol	20+1μg/rat/day	112.01**±12.21	Opened	+++

*Duration: 07 days; Six animals were maintained in each group; Values are mean ± S.E.; * p <0.01, ** p <0.001 when compared to control; + = nucleated epithelial cells; ++ = nucleated and cornified epithelial cells; +++ = cornified cells*

Table 3: Histometric changes in the uterus due to treatment of petroleum ether and ethanol extracts of O. corniculata whole plant in immature rats

Group	Treatment	Dose (mg/100 g BW)	Diameter of uterus (μm)	Thickness of myometrium (μm)	Thickness of endometrium (μm)	Epithelial cell height (μm)
1	Control	Tween-80 (1%)	518.66±6.50	50.50±2.83	240.26±2.68	12.51±.0.75
2	Ethinyl estradiol	1μg/rat/day	882.12±9.08**	112.00±3.21**	589.83±8.00**	22.98±2.10**
3	P. ether extract	20	816.12±12.08**	102.00±10.12**	509.81±11.97**	20.29±4.15**
4	P. ether extract + Ethinyl estradiol	20+1μg/rat/day	932.68±13.22**	190.42±8.69**	608.33±10.25**	24.26±3.00**
5	Ethanol extract	20	602.06±10.22**	87.04±13.28**	392.22±18.01**	16.41±3.98**
6	Ethanol extract + Ethinyl estradiol	20+1μg/rat/day	742.91±12.49**	103.42±20.21**	462.41±12.88**	20.32±5.27**

*Duration: 07 days; Six animals were maintained in each group; Values are mean ± S.E.; *p <0.01, **p <0.001 when compared to control.*

4. DISCUSSION

The presently available methods of female contraception, a method which is of indigenous plant origin may have particular merits such as cost effectiveness, less or nontoxic and orally bioactive. The use of plant preparation for fertility regulation is especially for prevention / interruption of pregnancy have been in practice since ancient time in India.

In the present investigation the petroleum ether and ethanol extract of the whole plant of *O. corniculata* were tested for their antiimplantation and estrogenic activities. Between the two extracts were tested, the petroleum ether at dose level of 20 mg/100 gm body weight was found to be the most potent in reducing the implantation sites and interrupting the pregnancy. The loss of implantation caused by the administration of petroleum ether extract may be due to antizygotic, blastocytotoxic or antiimplantation activity as described by Hafez [23].

The antiimplantation activity whole plant of *O. corniculata* may be due to its estrogenic activity as evidenced by significant increase in uterine diameter, thickness of the endometrium, etc. This estrogenic activity was also reflected through the cornification of the vaginal epithelial cells in immature rats. The studies of Emmens [24] show that antifertility activity is mainly due to estrogenic activity, which may cause the expulsion of ova from the tube, disrupt the luteotrophic activity of the blastocyst, disturb the functional equilibrium between endogenous estrogen and progesterone or create a non-receptive site in the uterus by changing the uterine milieu [25-27]. The histological evidence clearly supports an unfavorable uterine milieu.

In several species including non-human progesterone is essential for blastocyst implantation [28-29] and for the maintenance of pregnancy in all phases [28-31]. Inhibition of progesterone synthesis or a blockade or receptor building will result in the failure of blastocyst implantation and interruption of early pregnancy [31, 29], which might have resulted due to administration of different extracts of whole plant of *O. corniculata*.

5. CONCLUSION

However, it may be concluded that, the petroleum ether extract of whole plant of *O. corniculata* at the dose of 20 mg/100 gm body weight have more potent in resulting fertility regulation than other extract.

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