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Antifertility Activity of the Ethanolic Extract of *Cassia occidentalis*, *Derris brevipes* Variety *Brevipes* and *Justicia simplex*

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Abstract: The powdered roots of *Derris brevipes* variety *coriacea* and its ethanolic extract were screened for antifertility activity in proven fertile female rats at 200 and 600 mg/kg body weight, respectively following an oral dose on day 1 to 7 of pregnancy. Both doses of the root powder of Derris brevipes variety coriacea showed 50% anti-implantation activity and the number of litters born were reduced significantly. The ethanolic extract exhibited 40% anti-implantation activity, when given orally at 600mg/kg body weight. The rats, which continued their pregnancy, did not deliver any litters after their full term. Hence, the combined antifertility (anti-implantation and abortifacient) activity of the ethanolic extract was 100%. The results suggest that the ethanolic extract possesses more abortifacient type effect than the anti-implantation activity. The ethanolic extract also exhibited weak estrogenic activity when given alone when tested in immature ovariectomised female albino rats. But, when given along with ethinyl estradiol, it exhibited slight antiestrogenic activity. Histological and biochemical estimations confirmed this activity.

Key words: *Derris brevipes* variety *brevipes* · Antifertility · Anti-implantation · Estrogenic

INTRODUCTION

Efforts are being made to develop antifertility products from plants. Ethnomedical literature contains thousands of references to the use of plants for a variety used by the laity since ages. Traditional physicians in and around Kotagiri village near Ootacamund, use a mixture of powdered roots of *Cassia occidentalis* (family: Caesaloineae), *Derris brevipes* variety *brevipes* (family: Papillionaceae) and *Justicia simplex* (family: Acanthaceae) to control fertility. Administration of this mixture for a few days after menstruation prevented conception during that month without any toxic effects and among the three roots, *Derris brevipes* variety *brevipes* is the most potent. (Personal communication with Vaidyas). Literature survey revealed that leaves of *Cassia lanceolata* [1] ethanolic extract of fruits of *Cassia fistula* [2-3] rotenone isolated from the roots of *Derris elliptica* [4] and justicisaponin isolated from *Justicia simplex* [5] possesses antifertility properties. But, so far no antifertility activity has been carried out on *Derris brevipes* variety *brevipes* and a mixture of root powders of *Derris brevipes* variety *brevipes*, *Cassia occidentalis* and *Justicia simplex*. Hence, we were interested to submit the mixture of equal quantities of the combined root powders of these three plants, as being used by the Vaidyas, for antifertility testing. Along with this the root powder of *Derris brevipes* variety *brevipes* amd its ethanolic extract were also subjected for a detailed antifertility screening. The other portion of the uterus was homogenized with ice-cold distilled water in a pre-cooled mortar and pestle to contain 10 mg of tissue/ml. The homogenate was centrifuged in cold at 3000 rpm for 15 min and the supernatant was used for the estimation of glucose, cholesterol and alkaline phosphatase using the standard methods [6]. Statistical analysis was carried out using Student's *t*-test.

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MATERIALS AND METHODS

Animal Stock: Albino Swiss mice weighing 35 to 65 g were used for the acute toxicity study. 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. The rats and mice were maintained under standard husbandry conditions with food and water *ad libitum*.

Acute Toxicity Study: The animals were divided into two groups of six mice per cage. Group 1 received 5 ml of saline by oral route. Group 2 received 6000 mg/kg of the powdered roots of *Derris brevipes* variety *coriacea* (p.o.). The animals were observed for physical signs of toxicity for 24 h.

Post-Coital Antifertility Testing: Vaginal smears from each rat were monitored daily and the 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 five groups containing six rats in each group. The extract was administered at 200 and 600 mg/kg body weight orally from day 1 to 7 of pregnancy. The powdered drug was also administered at 200 and 600 mg/kg body weight orally from day 1 to 7 of pregnancy. Control rats received the vehicle (distilled water). On day 25, 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: The ethanolic extract at 600 mg/kg was found to be active amongst the four 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 the estimation of estrogenic and antiestrogenic activity. Immature ovariectimized female albino rats, 21–23 days old, weighing between 35 and 45 g were used. They were divided into four groups, consisting of six rats each. The first group served as a control and received vehicle only (Tween-80, 1%). The second group received ethinyl estradiol in olive oil, 0.02 mg/kg/rat per day, orally. The third group received the ethanol extract at a dose of 600 mg/kg body weight. The fourth group received, in addition to ethinyl estradiol, a test dose of the ethanol extract at 600 mg/kg body weight. All the above treatments were given for 10 days. On the 10th 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. The diameter of the uteri and thickness of the endometrium were measured in 16 randomly selected sections using an ocular micrometer.

Cytotoxity Studies: The in vitro effect of the ethanolic extract of *Derris brevipes* variety *coriacea* was studied on normal African green monkey kidney (Vero) cells. The ethanolic extract was dissolved in dimethyl sulphoxide (DMSO) and the volume was made up to 10 ml with DMEM to obtain 1 mg/ml concentration. Vero cell lines were provided by National Center for Cell Sciences, Pune, India. Stock cells were cultured in minimum essential medium with 2% fetal calf serum supplemented with glutamine at 37°C in an atmosphere of 5% CO_2 and 95% humidity. The medium was changed every 3 days. Monolayer cultures of Vero cells were trypsinized and the cells were plated out at 6 x 10^3 cells/well in 96 well microtitre plate. The cell growth was found to be exponential during 2-3 days in the medium. The cultured cells were incubated with the ethanolic extract at 1000, 800, 600, 400, 200 and 100 µg/ml. Control cells were incubated with DMSO (final concentration, 0.2%) at 37°C, 5% CO_2. Cell viability counts were made by Trypan blue dye exclusion test [7]. The percentage viability was calculated and plotted against concentration to get the IC_{50} values.

RESULTS

Acute Toxicity Studies: No mortality and changes in the behavior were observed in all the treated and control groups of mice up to a dose of 6000 mg/kg body weight. Hence, one-tenth of this dose, i.e. up to 600 mg/kg body weight, was used for antifertility testing.

Table 1: Post-coital antifertility activity of combined root powder of three plants, root powder of Derris brevipes variety brevipes and its ethanolic extract when fed orally from day 1 to 7 of pregnancy

Treatment(a)	Dose,mg/kg body weight(b)	No. of corporateamean ± S.E.(c)	No. of rats having no implantation sites on day 10/ No. used (d)	Anti-implantation Activity %(e)	No. of litters born mean±S.E.(f)
Control	-	100.4±0.41	0/6	0	9.83±0.38
Combined	200	9.19±0.33	0/6	0	7.83±0.5
root powder	600	9.49±0.24	0/6	0	7.49±0.64
Derris brevipes	200	9.00±0.42	3/6	50	8.24±1.65*
variety brevipes	600	9.29±0.53	3/6	50	8.00 ± 0.79**
Ethanolic extract	200	9.18±0.87	0/6	0	8.19±0.47
	600	9.78±0.42	4/10	40	4.69±1.41***

*P < 0.05, **P < 0.01, ***P < 0.001, when compared with control

Table 2: Estrogenic and Estrogenic Activity of the Ethanolic Extract of Roots of Derris brevipes variety brevipes

Treatment(dose, mg/kg body weight)	Uterine weight mg/100 g body weightmean±S.E.	Vaginal cornification
Control (Tween-80, 1%)	32.71±0.96	Vagina not open(0 to +)
Ethinyl estradiol (0.02 mg/kg)	129.94±9.11	Open (+++)
Ethanolic extract (600 mg/kg)	77±6.31	Open (+ to ++)
Ethinyl estradiol (0.02 mg/kg) +ethanolic extract (600mg/kg)	119±6.01	Open (+++)

*P < 0.01 when compared with control, +, nucleated epithelial cells; ++, nucleated and cornified cells, +++, cornified cells. * P\< 0.001, when compared with control

Table 3: Histological Changes in the Uterus and Endometrium after Treatment with the Ethanolic Extract of Roots of Derris brevipes variety brevipes

Treatment(dose, mg/kg body weight)	Diameter of uterus(mm)	Thickness of endometrium(μm)	Height of endometrial epithelium(μm)
Control (Tween-80, 1%) (-)	1.44±0.06	227.20±9.73	15.38±1.23
Ethinyl estradiol (0.02 mg/kg)	2.69±0.17*	388.86±14.48*	35.09±3.07*
Ethanolic extract (600 mg/kg)	1.97±0.13*	291.11±13.69*	27.53±1.81*
Ethinyl estradiol (0.02 mg/kg) +ethanolic extract (600 mg/kg)	2.52±0.13*	353.51±16.78*,**	32.42±3.51*

*P < 0.001, when compared with control ** P < 0.05, when compared with ethinyl estradiol

Table 4: Biochemical Changes in the Uterus after Treatment with the Ethanolic Extract of Roots of Derris brevipes variety brevipes

Treatment(dose, mg/kg body weight)	Glucosemg/100gm	Cholesterolmg/100gm	Alkaline PhosphataseIU/100 gm
Control (Tween-80, 1%)	0.93±0.29	4.98±0.15	0.41±0.02
Ethinyl estradiol (0.02 mg/kg)	1.53±0.09	6.55±0.93**	0.80±0.06*
Ethanolic extract (600 mg/kg)	1.18±0.31	5.11±0.71	0.59±0.12
Ethinyl estradiol (0.02 mg/kg) (+)ethanolic extract (600 mg/kg)	1.29±0.08	5.81±0.36	0.77±0.12

*P < 0.001, when compared with control **P < 0.05, when compared with control ***P < 0.05, when compared with ethinyl estradiol

Post-Coital Antifertility Activity: The anti-implantation activity is expressed as the percentage of animals showing absence of implantations in uteri when laparotomised on day 10 of pregnancy (Table 1). The combined root powder of the three plants at both doses used and the ethanolic extract of the roots of the *Derris brevipes* variety *brevipes* at 200 mg/kg body weight did not show any anti-implantation activity. However, the root powder of *Derris brevipes* variety *brevipes* at both doses used and its ethanolic extract at 600 mg/kg body weight exhibited significant anti-implantation activity (versus control P < 0.05 and 0.001, Table 1). All six treatments reduced the number of litters born significantly confirming the antifertility activity of the plants used. This may be due to the resorption of the implantation sites after day 10 or due to abortion. However no vaginal bleeding was observed. Rats treated with the ethanolic extract at 600 mg/kg body weight did not deliver any litters. Laparotomy of these rats on day 25 showed the resorption of the implantation sites. Hence, it showed 100% antifertility (anti-implantation as well as abortifacient) activity. All six treatments didn't alter

the number of corporalutea, which was similar to those of the controls. The litters born to the experimental and control animals showed no morphological defects and no appreciable changes in their weights. Hence, the treatments do not exhibit any teratogenic effects.

Estrogenic and Antiestrogenic Activity: The effect of the ethanolic extract of *Derris brevipes* variety *brevipes* on the immature rat uterus is shown in Table 2, Table 3 and Table 4. Oral administration of the ethanolic extract at 600 mg/kg body weight caused a significant increase in uterine weight in immature ovariectomised rats (versus control $P < 0.001$). The uterotrophic potency as shown by the weight of the trophic changes such as the diameter of the uterus, thickness of the endometrium and height of endometrial epithelium were significantly ($P<0.001$) increased in the uterine content of glucose, cholesterol and alkaline phosphatase was observed (versus control, $P <0.05$ and 0.01). The uteri of these rats were inflated and fluid filled, resembling the proestrous/estrous uterus. The epithelium of the endometrium consisted of spindle shaped cells with basal nuclei. The stroma consisted of fibroblast type cells, loose and oedematous. The ethanolic extract also induced vaginal opening and the smear showed proestrous or estrous conditions, while all the control rats had closed vaginas. The number of cornified cells in the vaginal smears was considerably higher (+ to ++) than those of the controls (0 to +), but notably less than those for ethinyl estradiol treated rats (+++). Simultaneous administration of ethinyl estradiol and the ethanolic extract caused a highly significant increase in uterine weight (versus control, $P < 0.001$), but the extent of the uterotrophic response was less than that produced by ethinyl estradiol alone. It also caused a significant increase in uterine diameter, thickness of the endometrium and height of endometrial epithelium when compared with control rats ($P < 0.001$). There was also a significant increase in glucose, cholesterol and alkaline phophatase content of the uterus of these rats (versus control, $P<0.05$). However, the extent of all these changes was less than that of only ethinyl estradiol treated rats and significant only in case of cholesterol ($P<0.05$). Therefore, the ethanolic extract showed estrogenic activity when given alone, but exhibited slight antiestrogenic activity when givn along with ethinyl estradiol. There was no significant difference between the body weights and the weights of adrenal glands in the control and treated animals. The histological pictures of the adrenal glands of all these treatments were normal indicating the non-toxic nature of the extract.

Cytotoxic Studies: The IC50 observed with the ethanolic extract against normal Vero cell lines after 72 h culture is 795 μg/ml.

DISCUSSION

In the present study, the combined root powder of equal quantities of *Cassia occidentalis*, *Derris brevipes* variety *brevipes* and *Justicia simplex*, the root powder of *Derris brevipes* variety *brevipes* and its ethanolic extract were tested for antifertility properties at doses of 200 and 600 mg/kg body weight. The combined root powder of the three plants did not show any anti-implantation activity. But the number of litters born due to this treatment was significantly less than that of controls. This indicates abortiacient nature of the roots of these plants. Similar effect was observed with 200 mg/kg dose of the ethanolic extract of the roots of *Derris brevipes* variety *brevipes*. Both doses of the root powder of *Derris brevipes* variety *brevipes* showed 50% anti-implantation activity and also a significant reduction in the number of litters born. Hence, there was no correlation between the dose tested and the activity. Its ethanolic extract at a dose of 600 mg/kg body weight exhibited 40% anti-implantation activity and none of these rats delivered any litters. Hence, it showed 100 % antifertility (anti-implantation as well as abortifacient) activity. These results suggest that the ethanolic extract possess more abortifacient type effect than the anti-implantation activity. The study clearly reveals that the ethanolic extract is effective before and after the implantations occurred. But, from the present studies, it is not possible to know that on which days of pregnancy, the extract is more effective. Further studies are required where, the ethanolic extract given after implantation on different periods helps to know its effects on early or later stages of pregnancy. The ethanolic extract of *Derris brevipes* variety *brevipes* also exhibited estrogenic activity as shown by the significant increase in uterine weight, diameter of the uterus, thickness of the endometrium and height of endometrial epithelium and vaginal cornification in immature female rats compared with control. The treatment also caused a significant increase in the uterine content of glucose, cholesterol and alkaline phosphatase. In a typical experiment like this estrogen is known to stimulate the contents of these in the uterus, thereby changing the uterine milieu and creating non-receptive conditions in the uterus [8]. The extract acted as a weak estrogen when given alone, but when given along with ethinyl estradiol it exhibited slight antiestrogenic nature. This shows that the extract

acted as competitive, antagonist to the much more potent ethinyl estradiol. Oestrogen is necessary for implantation and nidation. Plant products exhibiting estrogenic activity and producing antifertility effects are known in the literature. The ingestion of 200, 400 and 800 mg/kg of ethanolic extract of *Saliva fruticosa* from D_{1-6} of pregnancy didn't cause pregnancy failure, but reduced the number of viable fetuses and increased the number of resorptions in female pregnant rats [9]. Vasicine isolated from *Adathoda vasica* showed potent abortifacient and uterotonic effects in guinea pigs [10]. Flavonoids isolated from *Striga lutea* and *Striga orobanchioides* possessed strong estrogenic and antifertility properties [11-12]. The IC_{50} value observed in the cytotoxic studies of the ethanolic extract may be mainly due to its estrogenic activity. Rotenone, isolated from the roots of *Derris elliptica* when given on day 6-15 of pregnancy showed significant antifertility effects in rats [4]. Our studies also confirm the antifertility nature of *Derris brevipes* variety *brevipes*, another plant belonging to the same genus. Preliminary phytochemical studies indicated the presence of steroids, terpenoids, flavonoids and glycosides in the ethanolic extract. Since several of these compounds are known to exhibit antifertility activity [5, 11, 13] the similar effect of the ethaolic extract of *Derris brevipes* variety *brevipes* might be due to the presence of such compounds. In conclusion, these plants in general and *Derris brevipes* variety *brevipes* in particular, merit further investigation for detailed antifertility activities especially at higher doses and also identification of their active constituents.

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