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ESTROGENIC AND PREGNANCY INTERCEPTORY EFFECTS OF *ACHYRANTHES ASPERA* LINN. ROOT

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Abstract

Achyranthes aspera Linn. (Amaranthaceae) is an abundant indigenous herb in India. It is traditionally being used as an abortifacient. Four successive solvent extracts of the root were screened for antifertility activity in female albino rats. The chloroform and ethanol extracts exhibited 100% anti-implantation activity when given orally at 200 mg/kg body weight. Both the extracts at the dose of 200 mg/kg body weight also exhibited estrogenic activity. Histological studies of the uterus were carried out to confirm this estrogenic activity.

Keywords: *Achyranthes aspera*, Antifertility, Anti-implantation, Estrogenic, Uterotropic.

Introduction

A. aspera Linn. (Amaranthaceae) is distributed as weed throughout India, tropical Asia. The decoction of the whole plant is reputed to possess diuretic properties, a water extract is given for pneumonia, the dried plant is used to treat children for colic and also as an astringent in gonorrhoea treatment (Kirtikar and Basu, 1975). It is used by traditional healers for the treatment of fever, especially malarial fever, dysentery, asthma, hypertension and diabetes (Girach and Khan, 1992; Tang and Eisenbrand, 1992; Bhom, 1992). The root extract is well reputed for its pronounced insect molting hormonal activity (Banerji and Chadha, 1970). The root of *A. aspera* is reported to have application in infantile diarrhea and cold (Borthakur and Gowswami, 1995) while dry leaves are employed against asthma (Singh, 1995). Leaf extracts are reported to possess hypoglycemic, thyroid-stimulating and antiperoxidative properties (Akthar and Iqbal, 1991; Tahiliani and Kar 2000). The alcoholic extract of the plant is reported to have antiinflammatory activity (Vetrichelvan and Jagdeesan, 2003). The seed of the plant is reported to enhance the immunity of catla (Rao and Chakrabarti, 2005).

In ancient Indian literature it is claimed to possess abortifacient activity (Basu, 1946). The ethanol extract of the root was found to have spermicidal action *in vitro* and *in vivo* studies (Sandhyakumari et al., 2002; Paul et al., 2006). The aerial parts of the plant is reported to prevent pregnancy in adult female rats (Wadhwa et al., 1986). A careful survey of the literature revealed that no female antifertility activity has so far been carried out on of the root of *A. aspera*. The present paper deals with the antifertility screening of various extracts of the root of *A. aspera* Linn. in female albino rats and to authenticate the traditional use.

Materials and Methods

Plant material

The root of *A. aspera* was collected from the campus of Guru Jambheshwar University, Hisar, during March and April 2002. The plant (with seeds) 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.

Preparation of plant extracts

The root of the plant was coarsely powdered (500 g) and was extracted successively with petroleum ether (60°-80°C, 2l), chloroform (2l), ethanol (95%, 2l) and distilled water (2l) by cold maceration process. The extracts were concentrated to dryness under reduced pressure and controlled temperature (50-60 °C). The extracts were stored in refrigerator. The extracts were prepared in 1% acacia, suspended in distilled water and they were administered at a dose of 200 mg/kg body weight orally.

Animals

Colony-bred female albino rats (Wister strain), weighing (150-200 g), were used for antifertility testing. Immature colony-bred female albino rats (Wister strain), 21-23 days old, were used for the study of estrogenic activity. All the animals 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.

Post-coital antifertility testing

Vaginal smears from each rat were monitored daily, only rats with normal estrous cycle were selected. Anti-implantation activity was determined as described by Khanna and Chaudhary (1968). Rats found in proestrus phase of cycle were caged with males of proven fertility, in the ratio of 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 divided into five groups containing six rats in each group. The extracts were administered at 200 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

The chloroform and ethanol extracts were found to be most effective of the extracts of *A. aspera*, hence they were subjected to a detailed investigation for potential estrogenic and antiestrogenic activity. Colony-bred immature female albino rats, 21-23 days old, weighing between 35 and 45 g, were bilaterally ovariectomized by dorsolateral approach under light ether anesthesia and sterile conditions. They were divided into six groups, consisting of 6 rats each. The first group served as a control and received vehicle only (acacia 2 %). The second group received ethinyl estradiol in olive oil, 1µg/rat per day, subcutaneously. The third group and fourth group received ethanol and chloroform extracts at a dose of 200 mg/kg body weight, respectively. The fifth and the sixth groups received, in addition to ethinyl estradiol, a test dose of the ethanol and chloroform extracts at 200 mg/kg body weight, respectively. 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 haematoxylin-eosin for histological observations. The diameter of the uteri and thickness of the endometrium were measured in 16 randomly selected sections using an ocular micrometer.

Statistical analysis

All the results are expressed as mean \pm standard error. Data was analyzed by one way ANOVA. The results were judged significant if $P < 0.05$.

Results

Post- coital antifertility activity

The anti-implantation activity is expressed as percentage of animals showing absence of implantation in uteri when laparotomised on day 10 of pregnancy. The ethanol and chloroform extracts at the dose of 200 mg/kg body weight inhibited pregnancy in all rats. However, both the doses of petroleum ether and distilled water extract were found to be ineffective and the number of implantation sites in these cases was comparable with the control rats (Table 1).

Table 1: Effect of various extracts of *A. aspera* root on implantation in rats when fed orally from days 1 to 7 of pregnancy

Treatment	Dose (mg/kg body weight)	No. of rats having no implantation sites on day 10	Mean number of implants \pm S.E.	% of rats having on day 10 no implantation sites
Control		Nil	8.60 \pm 0.61	Nil
Petroleum ether extract	200	Nil	6.50 \pm 0.70	Nil
Chloroform extract	200	6	Nil	100
Ethanol extract	200	6	Nil	100
Distilled water extract	200	Nil	7.33 \pm 0.52	Nil

Each group consisted of six rats.

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 deliver of normal litters, indicating that the action of the extracts was reversible.

Estrogenic activity

The estrogenic effects of the ethanol and chloroform extracts are shown in Tables 2 and 3. Oral administration of the ethanol and chloroform extracts at 200 mg/kg body weight caused a significant increase in uterine weight in immature rats (versus control, $P < 0.004$, $P < 0.001$). The uterotrophic potency, as shown by the weight of the uterus, is about 55% of that of the ethinyl estradiol in case of ethanol extract and 65% that of ethinyl estradiol in case of chloroform extract. The uterotrophic changes, such as the diameter of the uterus and thickness of the endometrium 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.

It appears that the ethanol and chloroform extract have estrogenic activity at 200 mg/kg dose but did not show any antiestrogenic activity at the similar dose.

Discussion

In the present study, the root of *A. aspera* was tested for its anti-implantation and estrogenic properties. Among the four extracts tested, at the dose of 200 mg/kg body weight, the ethanol and chloroform extracts at 200 mg/kg body weight dose were more potent in their anti-implantation activity, as 100% of the rats failed to show any implantation sites. However, the ethanol and distilled water extracts were inactive, as the number of implantation sites in these cases was comparable with the control rats.

The loss of implantation caused by ethanol and chloroform extract may be due to antizygotic, blastocytotoxic or anti-implantation activity (Hafez, 1970). The ethanol and chloroform extracts also exhibited estrogenic activity as shown by the significant increase in uterine weight, diameter of the uterus, thickness of endometrium and vaginal epithelial cornification in immature rats.

Table 2: Estrogenic activity of the ethanol and chloroform extracts of *A. aspera* Linn. root.

Treatment (dose)	Uterine weight (mg/100 g body weight; mean \pm S.E.)	Vaginal cornification
Control	58.06 \pm 0.003	Nil
Ethinyl estradiol (1 μ g/rat per day)	141.00 \pm 0.03*	+++
Chloroform extract (200 mg/kg)	78.66 \pm 2.79* ^o	+ to ++
Ethanol extract (200 mg/kg)	92.12 \pm 8.71** ^{oo}	+ to ++
Ethinyl estradiol (1 μ g/rat per day) + Chloroform extract (200 mg/kg)	230.17 \pm 14.15*** ^{ooo}	+++
Ethinyl estradiol (1 μ g/rat per day) + Ethanol extract (200 mg/kg)	219.00 \pm 22.44* ^{oooo}	+++

+ nucleated epithelial cells; ++ nucleated and cornified cells; +++ cornified cells.

* $P < 0.001$; when compared to control. ** $P < 0.004$; when compared to control.

*** $P < 0.0001$; when compared to control.

^o $P < 0.003$; when compared to ethinyl estradiol. ^{oo} $P < 0.003$; when compared to ethinyl estradiol.

^{ooo} $P < 0.005$; when compared to ethinyl estradiol. ^{oooo} $P < 0.05$; when compared to ethinyl estradiol.

Table 3: Histological changes in the uterus and endometrium after treatment with ethanol and chloroform extract of *A. aspera* Linn. root.

Treatment (dose)	Diameter of uterus (μ m \pm S.E.)	Thickness of endometrium (μ m \pm S.E.)
Control	355.80 \pm 1.68	111.40 \pm 0.82
Ethinyl estradiol (1 μ g/rat per day)	658.00 \pm 3.05*	280.00 \pm 1.88*
Chloroform extract (200 mg/kg)	560.00 \pm 3.10*** ^{oo}	235.00 \pm 1.96* ^{oo}
Ethanol extract (200 mg/kg)	493.30 \pm 3.01** ^o	218.33 \pm 2.58* ^{oo}
Ethinyl estradiol (1 μ g/rat per day) + Chloroform extract (200 mg/kg)	860.00 \pm 3.57* ^o	530.00 \pm 2.12* ^{oooo}
Ethinyl estradiol (1 μ g/rat per day) + Ethanol extract (200 mg/kg)	860 \pm 2.95* ^{ooo}	438.33 \pm 1.98* ^{ooo}

* $P < 0.0001$; when compared to control. ** $P < 0.005$; when compared to control.

*** $P < 0.0003$; when compared to control.

^o $P < 0.005$; when compared to ethinyl estradiol. ^{oo} $P < 0.05$; when compared to ethinyl estradiol.

^{ooo} $P < 0.001$; when compared to ethinyl estradiol. ^{oooo} $P < 0.0001$; when compared to ethinyl estradiol.

It is well known that for implantation exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility (Psychoyos, 1966). The compound of hormonal values usually disturbs the hormonal milieu in the uterus and provokes and infertility effect. In this study, the histological evidence of the uterus treated with petroleum ether and chloroform extracts clearly supports an unfavorable uterine milieu. Therefore, the anti-implantation activity may be due to estrogenic activity, causing the expulsion of ova from the tube, disrupting the luteotrophic activity of the blastocyst (Anderson, 1972). It is interesting to note that these extracts possess around 60% of the estrogenic efficacy of ethinyl estradiol and thus may reduce some of the unwanted side effects of estrogens.

Simultaneous administration of ethinyl estradiol and ethanol extract caused a significant increase in the uterine weight when compared with control ($P < 0.001$), the degree of uterotrophic potency was more than that produced by ethinyl estradiol alone ($P < 0.05$). It also caused a highly significant increase in uterine diameter, thickness of the endometrium (versus control $P < 0.0001$). Simultaneous administration of ethinyl estradiol and chloroform extract caused a significant increase in the uterine weight when compared with control ($P < 0.0001$), the degree of uterotrophic potency was more than that produced by ethinyl estradiol alone ($P < 0.005$). It also caused a highly significant increase in uterine diameter, thickness of the endometrium (versus control $P < 0.0001$).

It appears that the ethanol and chloroform extracts have significant estrogenic activity when given alone. However, both the extracts did not show anti-estrogenic activity when given along with ethinyl estradiol at the tested dose.

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