

PHYTOCHEMICAL AND CONTRACEPTIVE PROPERTY OF *SIDA ACUTA* BURM FI. IIN. IN ALBIO RATS

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Running title: CONTRACEPTIVE PROPERTY OF *SIDA ACUTA*

Abstract: Petroleum ether, chloroform and ethanol extracts of *Sida acuta* leaves were subjected for phytochemical studies. All the extracts showed the presence of carbohydrates and fatty acids in common. In addition the petroleum ether extract contains alkaloids, steroids, flavones and anthocyanins and in ethanol extract contains alkaloids, steroids, glycosides, amino acids, proteins, saponins, flavones, anthocyanins and phenolic compounds. All the extracts were tested for antiimplantation activity in female albino rats from day 1 to day 7 of pregnancy at the dose level of 50mg and 100mg/kg body weight. Of these three extracts, the ethanolic extract was found to be most effective in causing significant antiimplantation activity. The petroleum ether extract was found to possess estrogenic activity when tested in immature ovariectomised female albino rats. The adverse effects on fertility are reversible upon withdrawal of the extract treatment is observed.

Keywords: *Sida acuta*; contraceptive, antiimplantation, pregnancy; estrogenic

Introduction

In recent years there has been considerable interest in plants with potential contraceptive properties. Kirtikar and Basu^[1], Nadakarni and Nadakarni^[2] and Chopra *et al.*,^[3] have reported many such plants. Research on Indian medicinal plants with contraceptive property has been exhaustively reviewed by Kamboj and Dhawan^[4], Satyavati^[5], Kamboj^[6], and Bhargava^[7], but so far no single plant is available, which can safely caused be used prevent pregnancy. Although few plants have shown promising results in

preventing pregnancy but they have failed in the course of other investigations. With all these consequences, the research is still continued to search out potent contraceptive plants.

Sida acuta Burm FI. Ind. (Malvaceae) has been used in varied ailments in Indian medicine^[1-2],^[6]. In the Gold Coast this plant is used to cure venereal disease. The leaves when bruised are shiny and are put on the hands of midwives when they are about to remove dead children from the womb. They are frequently used to cause abortion. The whole plant of *S. acuta* possessing antifertility activity^[8]. Hence, the present study has been undertaken to evaluate the effects of various extracts of *S. acuta* leaves in female albino rats.

Material and methods

The healthy and disease free *Sida acuta* leaves were collected from in and around the Gulbarga University campus during the month of August to October 2006 and authenticated at the herbarium, Department of Botany, Gulbarga University, Gulbarga, Karnataka, India.

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The shade dried leaves were powdered and subjected to cold extraction successively and separately non-polar to polar solvents i.e., petroleum ether (B. P. 60-80°C), chloroform and ethanol (95 %). The powdered leaves (3kg) were separately added into Erlmeyer Flasks with a known amount of solvent rotatory shaker for 48 hours. Then the material obtained was filtered by Whatman filter paper No: 40. The extracts were concentrated to dryness in a flash evaporator (Buchi) under reduced pressure and controlled temperature (40-60°C). On evaporation, extracts were preserved in refrigerator. The dried mass was considered as the extract and individually screened for contraceptive property in albino rats. For administration to test animals the extracts were macerated in Tween-80 (1 %) and resuspended in distilled water for their complete dissolution.

The presence of various chemical constituents in plant extracts were determined by preliminary phytochemical screening as described by Harnborne and Kokate ^[9-10].

Healthy, colony bred, virgin adult female albino rats of Wistar strain (150-200 g) were maintained under controlled standard animal house conditions of temperature, relative humidity and light/dark cycle, fed with commercial balanced diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*.

Antiimplantation activity

Proven fertile female Wistar strain rats, with normal estrous cycle ^[11] were selected for this study. Antifertility activity was determined in female albino rats as described by Khanna and Choudhury ^[12]. Rats found in the estrous phase of the cycle were caged with males of proven fertility in the ratio of 2:1. Animals, which showed thick clumps of spermatozoa in the vaginal smear on the next day, were separated for the experiment and that day was designated as day 1 of pregnancy ^{[13],[11]}. These animals were divided into 7 groups consisting of 6 animals in each group. The group I received vehicle only and served as control. Groups II and III received petroleum ether extract at doses of 50 and 100mg/kg body weight respectively. Group IV and V received chloroform extract at doses of 50 and 100mg/kg body weight respectively. Group VI and VII received ethanol extract at doses of 50 and 100 mg/kg body weight respectively. All the above treatments were given from day 1 to 7 of pregnancy and on day 10, laparotomy was performed under light ether anesthesia using aseptic condition and uteri were observed for number and size of implantation sites.

The abdominal incision was closed with sutures and the rats were allowed to recover and deliver full term pregnancy ^[11]. Those rats showing implantation sites but not delivered were again laparotomised on day 23, 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 postnatal growth and congenital anomalies.

Estrogenic/antiestrogenic activity

The ethanol extract was found to be most active extract of *S. acuta*, hence, it was subjected for detailed investigation for potential estrogenic/antiestrogenic activity. Bilaterally ovariectomised immature female rats (Wistar strain) of 25-30 days old, weighing between 30-40g were divided into 6 groups, each consisting of 6 animals. The Group I received vehicle only and served as control. Group II received ethinyl estradiol 1µg/rat/day in olive oil subcutaneously. Group III and IV received petroleum ether extract at the doses of 50 and 100mg/kg body weight respectively. And group V and VI received 1µg/rat/day in olive oil + petroleum ether extract at doses of 50 and 100mg/kg body weight respectively. All the above treatments were given for 7 days. On the 8th day of the experiment all the animals were sacrificed by decapitation and uteri were dissected, cleared from surrounding tissues, blotted on filter paper, weighed quickly on a sensitive balance and fixed in Bouin's fluid for 24 hours. The tissues were dehydrated and embedded in paraffin. The paraffin sections were cut at 5µm and stained with Haematoxylin –eosin ^[14] for histological observations. 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, ^[15]. The statistical analysis was done to determine the significant difference of results between treated and control groups following the methods using as described by Snedcor and Cochran ^[16]. All the values were statistically analyzed by using Students 't' test. The values were judged almost significant if [$P<0.05$], significant if [$P<0.01$] and highly significant if [$P<0.001$].

Results

Phytochemical screening

The results of phytochemical screening of *S. acuta* shown in [Table 1] and reveals the presence of alkaloids, steroids, carbohydrates, flavones, anthocynins and fatty acids in petroleum ether extract, carbohydrates and fatty acids in chloroform extract and alkaloids, steroids, carbohydrates, glycosides, amino acids and proteins, saponins, flavones, anthocynins, fatty acids and phenolic compounds in ethanol extract.

Antiimplantation activity

Among the three extracts of *S. acuta* evaluated for antiimplantation activity, the ethanol extract was found to be more effective. The ethanol extract at a dose of 50mg/kg body weight inhibited pregnancy in 6/6 rats with mean number of implants 6.25 ± 0.75 [$P<0.001$]. The same extract at a dose of 100mg/kg body weight significantly inhibited pregnancy 3/6 rats with mean number of implants 5.4 ± 0.20 [$P<0.001$]. Chloroform extract at both the doses inhibited pregnancy in 2/6 rats with mean number of implants 10.7 ± 1.00 and 9.00 ± 0.57 respectively. However, both the doses of petroleum ether extract was

found to be ineffective as the number of implantation sites in these cases were comparable with the control rats.

No toxic effect was observed in the animals and their pups either by gross visual examination or in the weight of experimental animals. Soon after the parturition all the experimental animals exhibited normal estrous cycle and on breeding they underwent normal pregnancy and delivered normal litters. The antiimplantation activity was reversible on withdrawal of the treatment of the extract and the complete recovery was observed within 10-15 days in the both cases.

Estrogenic/antiestrogenic activities of the ethanol extract [Table 2 & 3]

The oral administration of the ethanol extract at 50mg and 100mg/kg body weight caused a significant increase in the uterine weight in immature rats when compared to control, [$P < 0.001$]. The uterotrophic changes such as the diameter of the uterus [$P < 0.001$] and thickness of the endometrium [$P < 0.001$] were significantly increased when compared with control rats. The uteri of these rats were inflated and full of fluid resembling the proestrous or estrous uterus. The epithelial layer of the endometrium consisted spindle shaped cells with basal nuclei. The treated rats 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 that of the ethinyl estradiol treated rats.

Simultaneous administration of ethanol extract and ethinyl estradiol caused a highly significant increase in the uterine weight (compared to 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 myometrium and height of the endometrial epithelial cells compared to control [$P < 0.001$]. These results indicate that the ethanol extract of *S. acuta* leaves contain bioactive compounds which can show estrogenic activity.

Discussion

Currently the available methods of female contraception, is that of indigenous plant origin may have particular merits such as cost effectiveness, less or non toxic and orally bioactive. In the present investigation, the petroleum ether, chloroform and ethanol extracts of leaves of *S. acuta* were tested for their phytochemical, antiimplantation and estrogenic activity. Among the three extracts tested, the ethanol extract at dose level of 100mg/kg body weight was found to be the most potent in reducing the implantation sites. The loss of implantation caused by the administration of ethanol extract may be due to antizygotic, blastocytotoxic antiimplantation activity as described by Hafez^[17].

Implantation in the rat depends on the completion of basic sequence of events occurring both at the fertilized egg and endometrium. The endometrium needs 48 hours period of progesterone preparation and presence of estrogen at the end, leading to the formation high sensitive decidualised

endometrium^[18]. The requirement of progesterone is in milligrams whereas the estrogen requirement is in micrograms. The imbalance in this hormonal level is results in failure of implantation^[19-22]. The compound of hormonal values usually disturbs the hormonal milieu in the uterus and provokes infertility effects. In this study, the histological evidence of the uterus treated with ethanol extract clearly supports an unfavorable uterine milieu. Therefore, the above observation suggests that antiimplantation activity may be due to estrogenic activity which is causing the expulsion of ova from the tube and disturbing the luteotrophic activity of the blastocyst^[23-24].

Testing the ethanol extract further evidences of the estrogenic activity. Administration of the ethanol extract to immature ovariectomised rats has caused significant increase in the uterine weight, diameter of uterus, thickness of endometrium and myometrium by increasing the height of endometrial epithelial cells and vaginal epithelial cornification. It appears that the ethanol extract of *S. acuta* leaves at both the doses have strong estrogenicity.

It is well documented that estrogen secretion during pregnancy is much lowered when compared to progesterone, as the former is in the range of nanogram and later is in microgram^[25-26]. In the present study, the ethanol extract of *S. acuta* leaves has proved to possess estrogenic activity, the imbalance caused in progesterone and estrogen levels might be the reason for interruption of pregnancy. Withdrawal of these treatments to adult rats has resulted in normal reproductive activities.

The phytochemical studies of *S. acuta* leaves revealed that presence of several constituents in ethanol extract. Since various flavonoids have been reported to possess contraceptive property^{[23], [12], [25]}, the antiimplantation activity of the ethanol extract of *S. acuta* leaves might be due to the presence of flavonoids or other constituents, which possess estrogenic activity.

Table: 1: Antiimplantation activity of various extracts of *S. acuta* leaves in pregnant rats.

Group	Treatment	Dose (mg/kg body weight)	No. of rats without implantation sites on day 10	Mean no. of implants $\pm$ S.E.	% of rats having implantation sites on day 10	% rats delivered on full term
I	Control	Tween-80 (1%)	Nil	13.66 $\pm$ 0.33	Nil	100
II	Petroleum ether	50	Nil	11.5 $\pm$ 0.57	19.48	100
III		100	Nil	10.75 $\pm$ 0.64	26.80	100
IV		50	Nil	10.75 $\pm$ 1.00	26.80	100
V	Chloroform	100	Nil	9.00 $\pm$ 0.57*	34.12	100
VI		50	02	6.25 $\pm$ 0.75**	48.76	000
VII	Ethanol	100	03	5.45 $\pm$ 0.20***	56.04	000

M $\pm$ S.E. = Mean $\pm$ Standard error

Duration: 07 days, received the treatment from day1 to 7 and laparotomized on day 10 of pregnancy.

Six animals were maintained in-group

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared with control

Table: 2: Effect of ethanol extract of *S. acuta* leaves on uterine wet weight, vaginal opening and cornification of vaginal epithelial cells in bilaterally ovariectomized immature rats

Group	Treatment	Dose (mg/kg b. w.)	Uterine wet weight (mg/100g)	Vaginal opening and cornification
I	Control	Tween-80 (1%)	108.55 ± 8.22	-/-
II	Ethinyl estradiol	1µg/rat/day	145.55 ± 2.55***	6/6
III	Ethanol	50mg	131.71 ± 3.86***	6/6
IV	Ethanol	100mg	137.77 ± 3.81***	6/6
V	Ethinyl estradiol + Ethanol	1µg/rat/day + 50mg	140.55 ± 7.97***	6/6
VI	Ethinyl estradiol + Ethanol	1µg/rat/day + 100mg	149.44 ± 3.79***	6/6

M±S.E. = Mean ± Standard error

Duration: 07 days, six animals were maintained in each group

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared with control

Table: 3: Histometric changes in the uterus due to the administration of ethanol extract of *S. acuta* leaves in bilaterally ovariectomized immature rats.

Group	Treatment	Dose (mg/kg b. w.)	Diameter of uterus (μm)	Thickness of myometrium (μm)	Thickness of endometrium (μm)	Epithelial cell height (μm)
I	Control	Tween-80 (1%)	263.86 ± 4.55	21.60 ± 1.83	38.76 ± 1.22	21.86 ± 1.25
II	Ethinyl estradiol	1μg/rat/day	685.92 ± 7.75***	120.78 ± 3.21***	230.8 ± 7.71***	45.98 ± 1.76***
III	Ethanol	50mg	410.76 ± 2.65**	55.87 ± 2.26*	75.8 ± 2.76**	29.9 ± 1.10*
IV	Ethanol	100mg	580.68 ± 6.25***	64.34 ± 2.76***	178.8 ± 2.54***	38.2 ± 4.34**
V	Ethinyl estradiol + Ethanol	1μg /rat/day + 50mg	728.86 ± 9.55***	86.6 ± 3.73***	220.1 ± 3.83***	29.0 ± 1.59**
VI	Ethinyl estradiol + Ethanol	1μg/rat/day + 100mg	770.8 ± 11.0***	131.5 ± 4.10***	263.8 ± 4.18***	44.2 ± 1.25***

M±S.E. = Mean ± Standard error

Duration: 07 days, six animals were maintained in each group

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared with control

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