

**PHYTOCHEMICAL, CONTRACEPTIVE EFFICACY AND SAFETY
EVALUATIONS OF THE METHANOLIC LEAVES EXTRACT OF
ACHYRANTHES ASPERA L. IN RATS**

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Abstract

In Ethiopia the control of fertility is based on the folk use of numerous traditional anti-fertility plants that has been practiced for many years. *Achyranthes aspera* is one of the plants that is used for this purpose. The efficacy and safety of many of such plants, however, have not been verified. The present study was conducted to carry out phytochemical, contraceptive efficacy on some indicators for anti-fertility activities and safety evaluations of crude extracts of *Achyranthes aspera*. The anti-fertility activity of the methanolic extract of the leaves of *Achyranthes aspera* was determined by the number of implantation sites in both horns of uterus and the number of litters after completion of one gestation period in rats. The effect of the extract on the length of estrous cycle and the weights of ovary and uterus/100g of body weight of the animal was also evaluated. Phytochemical screening revealed the presence of known anti-fertility principles such as phytosteroids, polyphenols and saponins. The methanolic leaf extract reduced significantly ($p < 0.05$) the number of litters and implantation sites in rats. The extract prolonged estrous cycle, estrous and metestrous phases ($p < 0.05$) of rats. The weight of ovary was reduced, but that of uterus was increased ($p < 0.05$). The oral LD₅₀ of the extract was found to be 9.7 g/kg in mice. The present study hinted that the methanolic extract has anti-fertility effect and is safe at the contraceptive doses.

Keywords: *Achyranthes aspera*, anti-fertility, rats, Phytochemical screening, LD₅₀.

The population of developing countries is increasing at alarming rate leading to poverty (1). Fertility control through natural products is being given great attention by WHO these days. A large number of plants are known to have anti-fertility activity (2). *Achyranthes aspera*, locally known as "Telenge" or "ambulale" is one of the traditionally used anti-fertility plants in Ethiopia. It is a stiff erect perennial herb of 1-3 feet in height with simple elliptic leaves (3). Ethnomedical information indicates that the extract of leaves, roots, seeds of the plant are used for treatment of post partum bleeding, menorrhagia, vaginal pain, placental retention, and as medical abortifacient and anti-fertility agent in women (4). The preliminary studies on leaves extract of the plant showed anti-fertility effect (5). The present study was carried out to identify the active principles from leaves crude extract of *Achyranthes aspera* and to validate further the claimed anti-fertility effects using various experimental models in rats. The oral median lethal dose (LD₅₀) of the extract was also determined.

Material and methods

Plant collection and extract preparation

The leaves of *Achyranthes aspera* were collected from Addis Ababa in November 2004. The plant was identified by a taxonomist and voucher sample (Herbarium number AA-2135) was deposited in the Herbarium of Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia. The leaves were dried under shade and ground into coarse powder which was macerated in 80% methanol for 24 - 48 hrs. and filtered with filter paper (Whatman No.1). Evaporation of the solvent using rotary evaporator gave 8.5% brownish dark sticky residue (as a percentage of dried powdered plant materials). A weighed amount of the concentrated extract was then dissolved in 2% Tween-80 solution to get the desired concentration for all experiments.

Experimental animals

Adult cyclic virgin female Wistar rats weighing 185-230g were used in various experiments. Swiss albino male and female mice weighing 27-34g were used for acute toxicity testing. All animals were housed in standard cages with uniform conditions of lighting (12h dark: 12h light cycle) and at room temperature. Animals were fed on pellet and tap water *ad libitum*. Animals were handled in this study as per the International Guidelines for handling experimental animals.

Phytochemical screening

Identification of the chemical constituents of the methanolic leaves extract was carried out by using chemical test methods and thin layer chromatography according to (6).

Pharmacological evaluations

Screening for anti-fertility effect

Two groups of matured female rats (n = 5) were used. One of the group received methanolic extract in a dose of 1g/kg body weight by gavage a week before mating. The other group received an equal volume of a vehicle (2% Tween – 80) by the same route

for the same number of days and served as a control. All animals were allowed to mate with proven fertility male rats and administration of test materials continued for 21 days. The number of litters was then determined after completion of one gestation period as described by (7, 8).

Determination of anti-implantation effect

Three groups (n=5) of matured female rats (190-200g) were used. The animals were caged at night with proven fertility males. The wet vaginal smear was examined every morning microscopically until detection of spermatozoa. The day of detection of spermatozoa was considered as day 1 of pregnancy (9). Group I and II were given the methanolic extract in a dose of 1g/kg body weight and 1.6g/Kg body weight, respectively by gavage for 10 days, and group III was given equal volume of the vehicle by the same route for 10 days.

On the 11th day of pregnancy all group of rats were sacrificed by cervical dislocation to determine the number of implantation sites in both horns of uterus as described by (8).

Effect of extract on estrous cycle

Matured female rats (n=5) weighing 195-215g were used. Vaginal smear was examined microscopically every day at constant interval of 9-10 a.m. for 21 days. The smears were classified into one of the phases of estrous cycle using the method of (10). The length of estrous cycle and the number of days spent at each stage of cycle were determined according to the method used by (11). Then, all rats received 1g/kg body weight of methanolic extract by gavage for 21 days. Vaginal smears were evaluated similarly both during administration of the extract and 21 days after cessation of dosing with the extract.

Effect of the extract on the weights of the body and genital organs.

Two groups of matured female rats (n = 5) were used. One of the groups received 1g/kg body weight of the methanolic extract by gavage for 10 days, and the other group received an equal volume of vehicle by the same volume. On the 11th day, the body weights of all rats were recorded, and the animals were sacrificed by cervical dislocation. The ovaries and uteri were carefully dissected out and weighed wet quickly on a balance with 0.0001 precision. The weights of ovary and uterus/100g of body weight were calculated for each animal and compared among groups as described by (12).

Determination of oral LD₅₀

The LD₅₀ was determined in Swiss albino mice (27-34g body weight). The animals were divided into 5 groups (n=10) each group consisting of equal number of males and females. Four dose levels ranging from 7g/kg - 12g/kg body weight were fixed and administered in a 1 ml of vehicle by gavage to mice. A control group was administered a vehicle. Any symptoms of clinical toxicity and mortality were recorded during 24hrs. Probit values of percentage mortality responses were calculated, and LD₅₀ was determined by the method of (13).

Statistical analysis

Data were analyzed using SPSS and Graph Pad Prism soft wares. All data were expressed as the mean \pm S.E.M, and the level of significance was determined by student's t-test. Values of P less than 0.05 were considered to be significant statistically.

Results

Phytochemical screening of methanolic extract using chemical tests shows the presence of saponins, polyphenolic glycosides, flavonoids and phytosteroidal compounds. However, alkaloids, tannins and anthraquinone glycosides were not detected. Chromatographic separations using thin layer chromatography (TLC) has shown the four components with better migration of the components being achieved on a mobile phase of n-Butanol: Acetic acid: water (4:1:5).

Preliminary screening for anti-fertility effect

Treatment of animals with methanolic extract resulted in a significant reduction in the mean number of litters (Table1).

Table 1. Anti-fertility effect of the methanolic leaf extract of *A. aspera*.

| Treatment | No. of animals | Litter size |
|----------------|----------------|------------------|
| Vehicle | 4 | 9.8 \pm 0.37 |
| Extract(1g/kg) | 5 | 5.6 \pm 1.63 * |

Data: Mean \pm SEM, * p < 0.05

Effect of methanolic extract on implantation

The mean number of implantation sites for rats that received 1.6 g / kg body weights of the extract was significantly lower than those of the controls (Table 2).

Table 2: Anti-implantation effect of the methanolic leaf extract of *A. aspera*.

| Treatment | No. of implantation sites |
|---------------------|---------------------------|
| Vehicle | 10.6 \pm 0.25 |
| Extract (1g/kg) | 9.4 \pm 0.68 |
| Extract (1.6 g/kg) | 6.6 \pm 0.51* |

Data : Mean \pm SEM , n= 5, * p < 0.05

The results (Table 3) show that treatment of rats for 21 days with extract significantly prolonged the estrous cycle. The duration of estrous and metestrous phase were significantly increased while the diestrous phase was shortened.

Table 3. Effect of the methanolic leaf extract of *A. aspera* on estrous cycle

| Phases (days) | Experimental group | | |
|---------------|--------------------|------------------|----------------|
| | Before treatment | during treatment | Post treatment |
| Estrous cycle | 4.27 ± 0.25 | 6.07 ± 0.32* | 4.73 ± 0.32 |
| Proestrous | 0.45 ± 0.09 | 0.64 ± 0.06 | 0.5 ± 0.16 |
| Estrous | 0.75 ± 1.47 | 2.45 ± 0.41* | 1.36 ± 0.09 |
| Metestrous | 1.22 ± 0.1 | 2.03 ± 0.2* | 1.26 ± 0.31 |
| Diestrous | 1.85 ± 0.11 | 0.95 ± 0.12* | 1.61 ± 0.16 |

Data: Mean ± SEM, n = 5, * p < 0.05

Effect of extract on weights of genital organs and body

The mean uterine wet weight and body weight were significantly increased for the test group but that of the ovary was significantly reduced (Table 4).

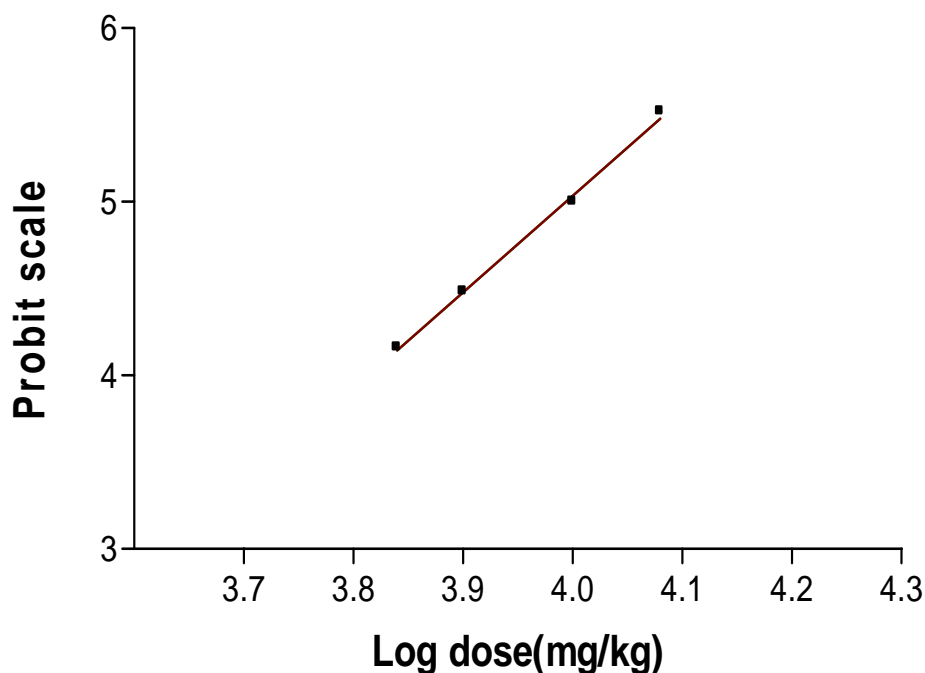
Table 4: Effect of the methanolic leaf extract of *A. aspera* on the weights of genital organs and body

| Treatment | Body wt. gain (g) | Uterine wet wt. mg (100g bwt) | Ovary wet wt. (mg/100g bwt) |
|-----------------|-------------------|-------------------------------|-----------------------------|
| Vehicle | 15.4 ± 0.74 | 177.86 ± 8.76 | 54.2 ± 1.32 |
| Extract (1g/kg) | 19 ± 1.76* | 201.34 ± 5.21* | 45.3 ± 2.79* |

Data: Mean ± SEM, n=5, * p < 0.05, wt = weight, bwt = body weight

Determination of oral LD₅₀

The oral LD₅₀ of the extract of *A. aspera* was calculated from probit scale transformed versus log dose (mg/kg) and found to be 9.7g/kg body weight (Figure1). Moreover, the mice also showed signs of acute toxicity such as decreased motor activity, convulsion, hemorrhage, diarrhea, increased respiratory rate followed by gasping and death.



Figure

1: Lethal oral doses of methanolic leaves extract of *A. aspera* in mice
Regression equation: $y = 5.61x - 17.4$, $r^2 = 0.994$,

Discussion

In phytochemical screening, the presence of phytosteroids, saponins and polyphenolic compounds may support the claimed anti-fertility effects of the test plant since the anti-fertility effects of these components was sufficiently substantiated in animal models (14). Administration of methanolic leaf crude extract to rats for 30 days significantly reduced the mean number of litters suggesting that the extract has anti-fertility effect.

Treatment of rats with higher dose for 10 days post-coitally resulted in significant reduction in the mean number of implantation sites which indicates that the extract may have anti-implantation effect. The findings are in consistent with those of (15, 9) that showed similar results in mice and rats following treatment with neem oil and *Susbania susban*, respectively. In this study, treatment of rats for 21 days showed a significant increase in the duration of estrous cycle with prolonged estrus and metestrous phases and reduced diestrus.

The results are comparable to earlier reports of (16,17) who indicated prolonged cornified phase after treatment of rats with benzene extract of flowers of *Hibiscus Rosa sinensis*, aqueous extract of *Physalis alkekengi* fruits, respectively.

Estrogenic chemicals are known to cause infertility by shortening the time of transport of egg, disrupting estrous cycle, lowering the plasmic progesterone and decreasing pregnanediol which finally stops development of endometrium (18, 19). Treatment of intact rats with methanolic extract increased uterine wet weight and body weight but decreased the ovarian wet weight. The increase in uterine wet weight and body weight might be attributed to phytoestrogenic components of the extract. In another study done on bilaterally ovariectomized rats, a positive uterotrophic effect of the extract has been observed (20). On the other hand, a decrease in the ovarian wet weight might be associated with inhibition of release of pituitary gonadotropins due to negative feed back mechanism of phytoestrogens on the pituitary hormones.

The increase in uterine wet weight of intact and ovariectomized rats might indicate the estrogenicity of the extract since estrogenic substance increase the wet weight of uterus (21). From the oral LD₅₀ value determined, relatively good safety of the methanolic extract may be inferred. In this study with animal models, it can be concluded that 80% methanolic crude leaf extract of *Achyranthes aspera* has effective contraceptive activity and reasonable safety at anti-fertility doses used, however, the study has a limitation that it was done on crude extract that contains many anti-fertility components and quantitative determination of principal active ingredient responsible for observed effects was not done. Further study on the possible mechanism as well investigation on the fractionated isolates should be pursued.

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References

1. Raka K, Gupta RS, Loyiya NK. Plants for male fertility regulation. *Phytotherapy Research* 2003; 17: 159-590.
2. Unny R, Chauhan AK, Jashi YC. A review on potentiality of medicinal plants as the source of new contraceptive principles. *Phytomedicine* 2003; 10(2):233-260.
3. Wagner WL, Herbst DR, Sohmer SH. *Manual of flowering plants of Hawaii*. Revised edition. University of Hawaii press 1999; 180-181.
4. Mathew KM. *Dictionary of Indian folk medicine and ethnobotany* 1991.
5. Desta B. Ethiopian traditional herbal drugs part III. Antifertility activity of 70 medicinal plants. *Journal of Ethnopharmacology* 1994; 44: 199-209.

6. Marini-Bettelo GB, Nicoletti M. Plant screening by chemical and chromatographic procedure under field conditions. *J Chromatogr* 1981; 218: 113-217.
7. Garg SK. Antifertility effect of some chromatographic fractions of *Daucus carota*. *Indian J. Pharmac* 1975; 7(12): 42-46.
8. Makonnen E, Rostom AAH, Assefa G, Zerihun L. Antifertility effect of *Jatropha curcas* L. seed in guinea pigs. *Ethiopian J Health Development* 1997; 11(2): 145-148.
9. Singh SP. Fertility control of female through *Susbania susban* seeds. *The Journal of Research and Education in Indian Medicine* 1990;9(4): 27-32.
10. Sheriff JA, Ravi S, Beltsos AN. Abnormal Estrous cyclicity after disruption of endothelial and inducible nitric oxide synthase in mice. *Biology of reproduction* 1999; 61: 171-177.
11. Makonnen E, Zerihun L, Rostom AAH. Antifertility activity of *Ricinus communis* seed in female guinea pigs. *East African Medical Journal* 1999; 79(6): 37-39.
12. Yamasaki K, Sawake M, Takatsuke M. Immature rat uterotropic easay of biphenol A. *Environmental Health Perspectives* 2002; 108: 12.
13. Fahrat GN, Affara NL, Gali, MHU. Seasonal changes in the composition of the essential oil extract of east Mediterranean sage and its toxicity in mice. *Toxicon* 2001; 39(10): 1601-1605.
14. Burton JL, Wells M. The effect of phytoestrogens on the female genital tract. *Journal of clinical pathology* 2002; 55: 401-407.
15. Juneja SC, Williams RS, Farrooq A. Contraceptive potential of neem oil. *J. Assist. Reprod. Genet* 1996 ; 13(7): 578-585.
16. Murthy DRK, Reddy CM, Pati SB. Effect of benzene extract on *Hibiscus Rosa sinensis* on the estrous cycle and ovarian activity in albino mice. *Biol. Pharm. Bull.* 1997; 20: 756-758.
17. Vessal M, Yazdanian M. Comparison of the effects of aqueous extract of *Physalis alkekengi* fruits and/or various doses of 17 β estradiol on rat estrous cycle and uterine glucose 6-phosphate dehydrogenase activity. *Com. Biochem. Physiol C Pharmacol Toxicol Endocrinol* 1995; 112 (2): 229-236.
18. Havranek F, Stroufova A, Kozlova J et al. On the mechanism of contraceptive action of estrogens administered after ovulation. *Cesk Gynekol* 1973; 38(8):617-619.
19. Barrett J. Phytoestrogens. *Environmental Health Perspectives* 1996; 104(5): 478-482.
20. Shibeshi W, Makonnen E, Zerihun Z , Debella A. Effect of *Achyranthes aspera* L. on fetal abortion, uterine and pituitary weights, serum lipids and hormones. *African Health Sciences* 2006; 6(2):108-112.
21. Turner DC. *General endocrinology*. 4th ed. Tokyo. WB Saunders Company 1971.