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Effect of *achyranthes aspera* L. on fetal abortion, uterine and pituitary weights, serum lipids and hormones

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Abstract

Back ground: The practice of traditional medicine for the control of fertility in rural Ethiopia is based on folk use of numerous anti-fertility herbs and *Achyranthes aspera* is one of these used for this purpose. Many plants are known to possess anti-fertility effect through their action on hypothlamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs resulting in inhibition of ovarian steroidogenesis.

Objectives: The present study focused to investigate the effect of methanolic leaves extract of *Achyranthes aspera* L. on some indicators for anti-fertility activities such as abortifacient, estrogenesity, pituitary weight, and ovarian hormone level and lipids profile in female rats, in attempt to validate the traditional claim.

Methods: The abortifacient effect of the methanolic extract of the leaves of *Achyranthes aspera* was determined by counting the dead fetuses *in vivo*. Effect on estrogenesity was assessed by taking the ratio of the uterine weight to body weight. The ratio of the pituitary weight to body weight was also calculated. The effect of the extract on the level of ovarian hormones and lipid profile was evaluated using electrochemiluminescence immunoassay.

Results: The extract showed significant ($p<0.05$) abortifacient activity and increased pituitary and uterine wet weights in ovarectimized rats. The extract, however, did not significantly influence serum concentration of the ovarian hormones and various lipids except lowering HDL at doses tested.

Conclusion: The methanolic leaves extract of *Achyranthes aspera* possesses anti-fertility activity, which might be exploited to prevent unwanted pregnancy and control the ever-increasing population explosion.

Key words: *Achyranthes aspera*, female rats, hormones, lipids.

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Introduction

Overpopulation is becoming a global problem causing much pressure on economic, social and natural resources. Control of fertility with hormonal preparations containing estrogen and progesterone has been proved to be effective. The safety of long term use of these contraceptives, however, is controversial. To this effect, World Health Organization has given much attention in the search for safe, affordable and socially acceptable alternatives. Part of this vital work has focused upon folk use of anti-fertility herbs. Plants with estrogenic property can directly influence pituitary action by peripheral modulation of LH and FSH, decreasing secretion of these hormones and blocking ovulation[1]. On the other hand, plants with anti-estrogenic activities intercept in the process of synchronized development of ovum and endometrium, still others have abortifacient or anti-progestational effects[2, 3]. It is well established that plants action on ovarian-uterine axis can provoke change in the pattern of reproductive cycles[4].

Achyranthes aspera L., locally known as "Telenge or ambulale" is one of the traditionally used anti-fertility plants in the indigenous health care delivery system of Ethiopia. It is a stiff erect perennial herb of 1-3 feet with simple elliptic leaves. The extracts of leaves, roots, and seeds of the plant have been used for control of fertility, in placental retention, and in postpartum bleeding[5]. The preliminary study on leaves extract of the plant had shown some anti-fertility effect[6]. The objective of the present study was to investigate the effect of methanolic leaves extract on fetal abortion, uterine and pituitary

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weights, gonadal horomones, serum lipids and in female rats in attempt to further validate scientifically the traditional claim.

Materials and methods

Plant collection and extract preparation

Fresh 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, ground into course powder and macerated in 80% methanol for 24 - 48 hrs. It was then filtered with filter paper (Whatman No. 1). The solvent was removed by using rotary evaporator. Further concentration of the extract was made by heating and evaporation of the solvent kept in water bath at 40 ^{0}C which finally gave a brownish dark sticky residue. The concentrated extract was weighed and dissolved in Tween - 80 to get the desired concentrations for all experiments.

Experimental animals

Female Wistar strain rats were used in all experiments. All animals were housed in standard cages in uniform lighting (12h dark. 12h light cycles) 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.

Determination of abortifacient activity

Three groups of mature virgin female rats (n= 5) weighing 195-225g were employed in this experiment. The rats were allowed mating with males of proven fertility at night. The vaginal smears were examined every morning for detection of spermatozoa. The day on which spermatozoa were detected in vaginal smears was considered day 1 of pregnancy[7]. On the 15th day of pregnancy groups I and II were given the methanolic leaves extract by gavage in a single dose of 3 g/kg and 5.5g/kg body weight, respectively and group III (control) was given an equal volume of vehicle (2% Tween - 80) by the same route. All animals were observed daily and autopsied 48 hours after dosing. Then the number of dead and viable fetuses was evaluated as described by Gebrie *et al* [8].

Determination of estrogenicity

Uterotrophic bioassay of bilaterally ovariectomized (OVX) female rats weighting 150-170g was used to determine estrogenicity of the extract[9]. Two groups of OVX rat models (n=5) were considered. After 10 days of ovariectomy the first group received 1 g/kg body weight of methanolic leaves extract by gavage for 7 days. The other group (control) received by the same route an equal volume of vehicle (2% Tween - 80) by the same route for the same number of days. On the 8th day, body weights were recorded, and all rats were sacrificed by cervical dislocation. The uterus was carefully dissected and removed with its luminal fluid and weighed quickly on a balance with 0.0001 precision. The ratio of the uterine weight to body weight was calculated for each animal by using the method of Yamasaki *et al* [10].

Effect of the extract on pituitary weight

The experiment was done on two groups of female rats (n=6) with body weight ranging between 150 to 175g. One of the groups was given methanolic leaves extract at a dose of 1g/kg body weight by gavage for 15 days, while another group was given an equal volume of vehicle (2 % Tween- 80) by the same route for the same duration. At day 16, all rats were deeply anesthetized with diethyl ether and, thoracic cavity was opened and brains were fixed with perfusate solution composed of 4% formalin in 0.1 M phosphate buffered saline by applying the gravity method of Miki *et al*.[11]. After perfusing the brain, the skull was opened, and the pituitary was carefully detached from the brain tissue and weighed wet on a balance with 0.0001 precision. Then the ratio of weight of pituitary to body weight was calculated according to the method of Choundhary *et al*[12].

Effect of the extract on hormones and lipid profile

Two groups of female rats (n=5) weighing 205- 230 were used. The first group was given 1g/ kg body weight of methanolic leaves extract by gavages for 14 days as described by Benie *et al* [13]. The control group was given an equal volume of vehicle (2% Tween - 80) by gavage for the same duration. On the 15th days, all rats were anesthetized with ether, and blood was directly collected by puncture of the right atrium. The blood was allowed to coagulate and centrifuged at 3000 rpm for 10 minutes. The serum was collected and stored at – 20 ^{0}C until analysis. The sera were analyzed for progesterone and estradiol by using electrochemiluminescence immunoassay (ECLIA). At the same time sera were also analyzed for total cholestrol, triglyceride, LDL and HDL concentrations by using enzymatic colorimetric methods (SEAC ch 16)

Statistical analysis

Data were analyzed by using SPSS and Graphpad prism softwares. all the data were expressed to the mean value ±S.E.M and the level of significance were determined

by student's t-test. A probability level less than 5% ($p<0.05$) was considered statistically significant difference between test and control groups for measured values.

Results

Table 1 shows that the methanolic leaves extract of *Achyranthes aspera* significantly ($P<0.05$) reduced survival of fetuses at the higher dose (5.5g/kg). It, however, did not show significant abortifaccient activity at the lower dose (3g/kg). The extract significantly ($p<0.05$) increased the uterine wet weight compared to the controls (Table 2). As shown in Table 3, the mean wet weight of pituitary was significantly ($p<0.05$) higher for the test group. The mean serum concentration of estradiol and progesterone for the extract treated group were not significantly higher than that for the control (Table 4). The extract was observed to have significant ($p<0.05$) hypolipidemic effect only on HDL (Table 5).

Table1 : Abortifacient activity of the methanolic leaf extract of *A. aspera* in rats.

Treatment	Fetuses		
	Live	Dead	Survival (%)**
Control	8.8 ± 0.37	0.600 ± 0.25	91.6±2.13
3 g/kg bwt extract	7.8 ± 0.37	14.0±0.51	85.5±5.03
5.5 g/kg bwt extract	6.8±0.86*	3.00±0.71*	68.9±7.89*

Data: Mean ± SEM, * $P<0.05$, n=5, Survival = Live (live + dead) × 100

Table 2:The effect of the methanolic leaves extract of *A. aspera* on the uterine weight of OVX rats

Treatment	Uterine wet weight (g)	Relative uterine wet wt. (mg /100g bwt)
Control	0.1784± 0.0514	89.43± 26.65
1g/kg bwt extract	0.3980 ± 0.0138*	213.75± 4.090*

Data Mean ± SEM,* $p<0.05$, n=5

Table 3: Effect of the methanolic leaves extract of *A. aspera* on pituitary weight of female rats

Treatment	Pituitary wet wt(g)	Relative pituitary wet wt. (mg/100g bwt)
Control	0.0248±0.0008	13.66±0.2800
1g/kg bwt extract	0.0326±0.0020*	18.11±1.040*

Data: Mean± SEM, *$p<0.05$, n=6

Table 4: Effect of the methanolic leaves extract of *A. aspera* on ovarian steroids of rats

Treatment	Estradiol (pg/m1)	Progesterone(pg/ml)
Control	7.22±0.97	18.24±5.45
lg/kg bwt extract	13.17±0.00	22.48±4.45

Data: Mean ±SEM, n =5

Table 5. Effect of the methanolic leaves extract of *A. aspera* on serum lipid profile of female rats

	Treatment	
Lipid parameters (mg/dl)	Control	1g/kg bwt extract
Total cholesterol	78.20 ±3.54	74.8±4.16
Triglyceride	60.4±13.2	50.8±2.27
LDL	16.96±3.20	20.04±4.37
HDL	56.6±3.88	44.6 ±2.44*
LDL:HDL	0.32±0.08	0.46±0.11

Data: Mean ± SEM,* $p<0.05$, n=5

Discussion

Administration of the methanolic extract at high dose (5.5 kg per body weight) caused a significant change in the number of both live and dead fetuses and fetal survival percentage indicating the possible abortifacient activity of the exact during post-implantation period.

In the rat uterus bioassay, significant increase in uterine wet weight of bilaterally ovariectomized rats shows uterotrophic activity of the methanolic extract suggesting possibly estrogenicity in rats. In other study we observed cornification of vaginal epithelial cells which is another evidence for estrogenicity and the results are in consistent with the trends for uterine weight gain (unpublished data).

Typical estrogenic compounds possess ability to increase the uterine wet weigh and induce cornification and opening of vagina in immature rats[14]. It appears that abortifacient effect and anti-implantation effect of the extract (unpublished data) observed in adult matured female rats might be mediated through estrogenic activity since estrogens are known to increase uterine contractility to expel fertilized eggs. Reproductive and general metabolic effects in mature and immature rats are manipulated with the ingestion of phytoestrogenic substance, and produce effects similar to those of gonadal steroid 17 b-estradiol[15]. The increase in wet weight of pituitary in the extract treated rats might be associated with estrogenic components of the extract. The results are comparable to those described by Choudhary *et al*[12], where administration of methanolic leaves extract of *Cleistanthus collinus* and *Terminalia bellirica* to rats increase the pituitary weight. Treatment of rats with methanolic extract did not produce a significant increase in serum concentration of estradiol and progesterone. Plant estrogens are known to inhibit enzymes involved in steroidogenesis. These compounds, however, are estrogenic per se, and may thus replace endogenous estrogens[16]. In another study[17] phytoestrogens have been shown to interfere in estrogen negative feedback by binding to estrogen receptors in anterior pituitary or hypothalamus and indirectly alter ovarian steroidogenesis.

Administration of the extract to rats did not produce significant hypolipidemic effects except on high density lipoproteins (HDL). Phytoestrogens are known to reduce serum cholesterol by binding to steroids in the lumen and excreting them into faeces, resulting in reduced gonadal steroid biosynthesis through effects on cholesterol availability or the activity of the side chain cleavage enzyme [18-19]. The insignificant hyploipidemic effect of methanolic extract in the present study might be related to low potency of estrogenic components.

In conclusion, the present study hints that the methanolic leaves extract of *Achyranthes aspera* has anti-fertility effect and proves its traditional claim, which might be exploited to prevent unwanted pregnancy and control the ever-increasing population growth. Further studies, however need to be pursued on the fractionated isolates in order to come up with the pure active anti-fertility component (s).

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