

**ANTIFERTILITY ACTIVITY OF ETHANOLIC AND AQUOES ROOT EXTRACT OF
AMARANTHUS SPINOSUS LINN. IN RATS**

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Summary

The aim of the present study was to evaluate the effect of aqueous and ethanolic extracts of root of *Amaranthus spinosus* Linn. (*Amaranthaceae*) in rats to explore its antifertility activity. Antifertility screenings of water and ethanolic extract of *A. spinosus* were done by the reproductive outcome in mice, antiimplantation, Abortifacient, estrogenic and anti estrogenic activity in rats. The water extract of the root of plant showed the decrease in number of implants and number of litters when compared with the ethanolic extract as the percentage of implantation failure increased and were found not significant at dose of 500mg/kg with 41.39% and 12% resorption at 500mg/kg for ethanolic and water extract respectively body weight dose level. The extract shows further more, non significant increase in uterine weight in immature ovariectomised rats. Simultaneous administrations of extracts with ethinyl estradiol cause significant antiestrogenic activity. All these observations suggest that aqueous and ethanolic extracts of *Amaranthus spinosus* Linn. have weak antifertility effect.

Key word: *Amaranthus spinosus* Linn, Antifertility, antiimplantation, abortifacient.

Introduction

Amaranthus spinosus Linn. (Amaranthaceae), is an annual or perennial herb, native to tropical America and is found throughout India as a weed in cultivated as well as fallow lands (1). In Indian traditional system of medicine (Ayurveda) the plant is used as anti-diabetic, antipyretic, laxative, diuretic, digestible, anti-snake venom, antileprotic, in blood diseases, bronchitis, piles and anti-gonorrhoeal (2-4). Some tribes in India apply *A. spinosus* to induce abortion (5).

The juice of *A. spinosus* was used by tribal of Kerala to prevent swelling around the stomach, while the leaves are boiled without salt and consumed for 2–3 days to cure jaundice (6). The Plant has a high concentration of antioxidant components (7-10). and high nutritive values due to the presence of fiber, proteins and a high concentration of essential amino acids, especially lysine (11).

A. spinosus is reported for its anti-inflammatory(12), antimalarial(13), antiandrogenic (14), immunomodulatory (15), anti-diabetic, anti-hyperlipidemic and spermatogenic activities (16), effect on hematology(17) and Biochemical changes in Epididymis (18). The betalains in stem bark of *A. spinosus* were identified as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides (19-23). It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmaterol glycoside, betaine such as glycinebetaine and trigonelline (24,25). Betalains are well known for their antioxidant, anticancer, antiviral and antiparasitosis properties (26-28). Roots are used as a laxative, emollient poultice, antimalarial, antioxidant (29) anti-inflammatory, antimicrobial, antidiuretic and used in hepatic disorders (30,31) Water extract of plant showed significant immunostimulating activity(32).

Material and Methods

Collection and identification of plant material

The plant specimens for the study were collected from the bank of the Arpa river, Bilaspur (Chhattisgarh, India) 22^o06'35.83"N and 82^o08'06.23"E and were positively identified and authenticated by the Botanist Dr. Shiddhamallaya N, Regional Research Institute (Ay.), Central Council for Research in Ayurveda and Siddha, Ashoka pillar, Jayanagar, Bangalore. A voucher specimen no. (RRCBI/mus.5-27). Care was taken to select healthy fully grown plant with normal organs. The samples of different organs were cut suitably and removed from the plant and thoroughly washed with water to remove the adherent impurities and dried in sunlight.

Processing and extraction

The shade dried root of *Amaranthus spinosus* Linn. were coarsely powdered and extracted with ethanol (99%) by continuous hot extraction method using soxhlet apparatus for 18 hrs. the ethanolic extract was concentrated using Buchi rotavapour and dried at 50-60^oC under vacuum to yield a dark green gummy solid and aqueous extract by cold maceration yield grey(33). The extract was preserved in a refrigerator.

Phytochemical screening

Identification of the chemical constituents were carried out on the powdered roots and on the methanol extract using chemical methods (40-41).

Animals

Anti-fertility test was performed on adult female Wistar rats weighing between 180-200g and Mice. They were housed in polypropylene cages and fed with standard chow diet and water ad libitum. The institutional ethical committee for animal cares and use approved all experimental procedures. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the securitization of the Institutional Animal Ethics Committee and were cleared by the same (1275/ac/09/CPCSEA).

Determination of oral LD50

Two Phase groups, each of three male mice (body weight range, 25–30 g), were treated with (Herbal extract) by oral gavage administration at a maximum dosage of 2000 mg/kg body weight. The test item was formulated in vehicle (Tween-80, 0.2% in saline). The concentrations adjusted to orally administrate 0.2 ml/10 g body weight. Animals were treated in two phases. In the first, oral doses of 100, 1000 and 2000 mg/kg of crude extracts were administered. In second the doses administered according to Lorke method (1983).

Phase 1: Three-groups of three mice per group. One dose was given to each group orally (i.e. 100, 1000 and 2000 mg/kg). The treated mice were monitored for 24 h for mortality and general behavior.

Phase 2: After 24 h fourth group of one mice each from previous group were given doses based on the findings of phase 1, orally. The mice were again monitored for 24 h. The geographic mean of the least dose that killed mice and the highest dose that did not kill mice was taken as the median lethal dose.

The animals received a single dose of the test item by oral gavage administration at 2000mg/kg body weight for Steps I and II after being fasted for approximately 18.0 hours but with free access to water. Food was provided again at approximately 3.0 hours after dosing for both the Steps.

Acute toxicity studies

The Acute oral toxicity studies were carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD-423), Ministry of Social Justice and Empowerment, Government of India.

The test substance (2000mg/kg) was administered in a single dose by oral gavage, animals were kept fasted prior to dosing (e.g. food but water was not withheld over-night). Following the period of fasting, the animals were weighed and the test substance administered. After the substance has been administered, food was withheld for a further 3-4 hours.

The animals received a single dose of the test item by oral gavage administration at 2000mg/kg body weight for the limit test.

Limit test

The limit test was primarily used because of the previous pilot experiment, which indicated that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with six animals (three animals per step).

Pharmacological screening

Effect of extracts on reproductive outcome in mice

Three groups of mature female mice (five mice/group) were selected as mentioned above. Two groups received root extract for 8 days and control group received vehicle for the same period. All the experimental mice were then allowed to mate with mature fertile male mice and the treatment was continued for 21 days. The number of litters was determined after the completion of one gestation period in all-experimental groups. The litters were allowed to grow and the growth of litters produced from the extract-administered group was compared with those of control group. The reversibility of antifertility effect of the extracts was also studied in the treated groups according to the method of Salhad et al. (34). For this study, the extracts were administered continuously for 21 days and then the extract was withdrawn. After 21 days of extracts withdrawal, animals were allowed to mate with male mice. The number of litters was determined after the completion of one gestation period.

Effect of extracts on anti-implantation activity

Proven fertile female Wistar rats, weighing between 150 and 200 g were selected and left overnight with male of proven fertile in the ratio of 3:1. The extracts were administered orally to separated group rats at the dose level of 500 mg/kg from day 1 to day 7 of pregnancy. Control animal received the vehicle (CMC 0.5%). The animals were then laparotomised on day 10 of the pregnancy under excess dose of thiopentane sodium and uteri were examined to determine the number of implantation sites(35).

Effect of extracts on abortifacient activity

Rats at day 1 of pregnancy were divided into groups, consisting of six animals in each group. The first group served as control and received vehicle only (CMC 0.5%) and rest of the groups received extracts of dose 500 mg/kg were dissolved in 0.5% carboxyl methyl cellulose, respectively from day 8th to day 14th. During the experiment animals were observed for vaginal bleeding. On 21st day, animals were laparotomised under thiopentane sodium anesthesia and observed for number of litters and percentage of resorption(35).

Effect of extracts on estrogenic and anti- estrogenic study

Colony of breed immature ovariectomised female rats (21–23 days) weighed between 150 and 200 g were used. They were divided into experimental and control groups, consisting of six animals each group. The extracts were suspended in 0.5% CMC and administered orally 7 days at the dose level of 500 mg/kg body weight. Ethinyl estradiol in olive oil 1 µg/rat per day was injected subcutaneously for 7 days in another group to induce estrous. CMC 0.5% was administered orally to the control animals. The extract at the dose level of 500 mg/kg body wt. was also administered orally along with ethinyl estradiol in olive oil at 1µg/rat per day subcutaneously to different groups of rat for the same period(36).

Statistical analysis

All values were expressed as mean±S.E.M. and data were analyzed by student's t-test.

Results

Phytochemical screening

The powdered root subject to preliminary phytochemical screening using chemical method showed the presence of alkaloid, Terpene, Glycoside, Sugar. The test for steroid, flavanoid and saponin however, showed negative result. Similar screening of the methanolic extract of the powdered roots of the plant indicated the presence of alkaloid, Terpene, Glycoside, Sugar. However steroid, flavanoid were not detected like in the powdered root.

Table 1:- Effect of extracts on reproductive outcome in mice

Group	Oestrous Cycle	Fertility	Litters Present
Control	Regular	100 % + Ve	10.42 ± 0.03
Alcoholic extract (500mg/Kg)	Irregular	40% - Ve	6.68 ± 0.10
Water extract (500mg/Kg)	Irregular	50% - Ve	5.40 ± 0.05
W.D of Alcoholic extract	Regular	100 % + Ve	9.64 ± 0.04
W.D of water extract	Regular	100 % + Ve	9.45 ± 0.31

Values are expressed as mean ± S.E.M.

P values a = P < 0.05, b = P < 0.01, when compared with normal control

Table 2:- Effect of extracts on anti-implantation activity

Treatment (Dose)	ANTI-IMPLANTATION ACTIVITY		
	No. of implants	No. of litters	Mean % anti-implantation
Control	7.33 ± 0.52	7.50 ± 0.55	Nil
Alcoholic Extract (500 mg/kg)	5.67 ± 0.52	4.33 ± 0.55	22.78
Water Extract (500 mg/kg)	5.00 ± 0.89	3.33 ± 0.82	33.61

Values are expressed as mean ± S.E.M.

P values a = P < 0.05

Table 3:- Effect of extracts on abortifacient activity

Treatment (Dose)	ABORTIFACIENT ACTIVITY		
	No. of implants	No. of litters	% Resorption
Control	7.33 ± 0.52	7.17 ± 0.40	4.12
Alcoholic Extract (500 mg/kg)	6.83 ± 0.75	6.00 ± 0.45	12.00
Water Extract (500 mg/kg)	4.50 ± 0.84	2.67 ^a ± 0.82	41.39

Values are expressed as mean ± S.E.M.

P values a = P < 0.05

Table 4:- Effect of extracts on estrogenic and anti- estrogenic study

Group	Treatment (Dose)	Uterine weight (mg/100 g body weight; mean \pm S.D)	Vaginal cornification
1	Control	70.52 \pm 4.72	NIL
2	Ethinyl estradiol (1 μ g/rat per day)	331.00 ^a \pm 8.46	+++
3	Alcoholic Extract (500 mg/kg)	134.00 \pm 9.36	+ to ++
4	Water Extract (500 mg/kg)	196.14 \pm 8.13	+ to ++
5	Ethinyl estradiol (1 μ g/rat per day)+ Alcoholic Extract (500 mg/kg)	410.67 ^b \pm 10.09	+++
6	Ethinyl estradiol (1 μ g/rat per day)+ Water Extract (500 mg/kg)	421.83 ^c \pm 5.87	+++

Values are expressed as mean \pm S.E.M.

P values a = P < 0.05, b = P < 0.01, c = P < 0.001 when compared with normal control

Table 5:- Histological changes in the uterus and endometrium after treatment with Alcoholic and Aqueous extract of *Amaranthus spinosus* linn. root

Treatment (Dose)	Diameter of uterus (μ m \pm S.E)	Thickness of endometrium (μ m \pm S.E)	Height of endometrial epithelium (μ m \pm S.E)
Control	326.92 \pm 6.14	52.28 \pm 2.02	18.6 \pm 0.55
Ethinyl estradiol (1 μ g/rat per day)	820.65 \pm 6.79	235.15 ^a \pm 10.23	51.57 \pm 2.08
Alcoholic Extract (500 mg/kg)	502.88 \pm 10.09	75.63 \pm 2.80	30.63 \pm 1.63
Water Extract (500 mg/kg)	616.57 \pm 7.75	217.93 \pm 5.75	30.02 \pm 0.99
Ethinyl estradiol (1 μ g/rat per day)+ Alcoholic Extract (500 mg/kg)	871.13 ^c \pm 11.02	283.93 ^b \pm 5.09	52.42 ^a \pm 1.56
Ethinyl estradiol (1 μ g/rat per day)+ Water Extract (500 mg/kg)	896.04 ^c \pm 11.84	306.19 ^b \pm 6.11	63.36 ^a \pm 1.78

Values are expressed as mean \pm S.E.M.

P values a = P < 0.05, b = P < 0.01, c = P < 0.001 when compared with normal control

Discussion

The water extract of the root of plant shows the decrease in number of implants and number of litters when compared with the ethanolic extract as the percentage of implantation failure increased and were found not significant supporting the result of T.Satyanarayana et al, 2008(37) at dose of 500mg/kg with 41.39% and 12% resorption at 500mg/kg for ethanolic and water extract respectively body weight dose level (Table 1).

Treatment of mice with ethanolic and water extracts decreased the mean number of litters (Table 2 and 3) suggesting the antifertility effect of the extract. The number of litters appeared to decrease more with water extract, which may suggest the aqueous extract is more effective than that of ethanolic extract. All the litters of treated mice grew up normally without showing any physical abnormality indicating that the plant is not abortifacient and teratogenic in albino mice. Absence of toxicity and lethality at the dose administered justifies the safe nature of the root extract.

Anti-implantation and abortifacient response was evident for *Amaranthus spinosus* Linn. The loss of implantation and litters caused by the *Amaranthus spinosus* Linn. may due to their anti-zygotic, blastocytotoxic activity. The ethanolic and aqueous extracts were not so potent, as the numbers of implantation sites in these cases were comparable with the control rats.

The effect of the extracts on the immature rat uterus is shown in Table 4 and Table 5. Oral administration of the extracts (500mg/kg) caused significant increase in uterine weight in immature ovariectomised rats. The uterotrophic potency was less than that of ethinyl estradiol. Both the extracts at their contraceptive dose level not significantly increased uterotrophic response when compared to control rats. The number of cornified cells in vaginal smear was considerably higher (+ to ++) than that of the control, but notably less than that of ethinyl estradiol. Simultaneous administration of extracts caused a non-significant increase in uterine weight, but the extent of the uterotrophic response was less than that produced by ethinyl estradiol alone. The extract therefore has weak estrogenic activity at their contraceptive dose level when given alone. However, along with ethinyl estradiol, they exhibited strong anti estrogenic property. In immature female rats, extracts exhibited definite estrogenic activity at their contraceptive dose. Hence, the antiimplantation activity of these extracts may be due to endogenous estrogen and progesterone levels. 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. It is interesting to note that aqueous extract and ethanolic extract possess around 40% and 59% of the estrogenic efficacy of ethinyl estradiol and thus may reduce some of the unwanted side effects of estrogens. It appears that the both extracts have non-significant estrogenic activity when given alone. However, both the extract did not show any anti-estrogenic activity when given along with ethinyl estradiol at the tested dose.

The presence of phytosterols and polyphenols in both the powdered root and in the methanolic extract could be responsible for the antifertility effect (38, 39). These classes of compounds may attribute for the claimed antifertility effect of plant as supported by the pharmacological tests. Further investigations should, however, be pursued to see the possible effect of the isolated compounds.

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