Abortifacient Effect of *Amaranthus viridis L.* Aqueous Root Extract on Albino Rats

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ABSTRACT :
Amaranthus viridis L. is a commonly growing herb, mainly used as leafy vegetable. The vegetable is a medicinal food used in urinary problems. Roots are known for its antifertility activity in Ayurveda. The term antifertility many times is used loosely in ethnic literature denoting abortifacient, antiimplantation and antiovulatory activity. Aqueous root extract was administered orally at the dose of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight respectively for 5 days from 11-15 days of pregnancy to female albino rats. The results show that the antifertility effect is expressed as abortifacient activity which is dose dependent increasing with higher dose.

Keywords: *Amaranthus viridis*, Antifertility, Abortifacient, Aqueous extract.

INTRODUCTION:

Ethnopharmacological information is an important component in both traditional health systems and for future medicine development. Biodiversity-rich countries, indigenous cultures with their knowledge of the use of bioresources as medicines and companies that seek to discover new therapeutics through medicinal plants and traditional knowledge are on the way sharing common interests.

Indigenous herbal treatment is a part of the culture and dominant mode of therapy in most of the developing countries. A large part of population among the developing countries especially in rural and forest areas rely on traditional medicines for their primary health care. Traditional knowledge on herbal medicine since the time of Great physician '*Charaka*' has led to the discovery of many important drugs of modern age (Uniyal et al. 2002). These traditional phytoremedies with a considerable extent of effectiveness are socially and economically accepted. International trade on medicinal plants is therefore increasing rapidly mainly as a result of intensified adoption of crude extracts for self medication by the general public in the developed countries. Today about 65% of the Indian population depends on the traditional system of medicine (Timmermans 2003). About one-third of the modern pharmaceutical preparations also have botanical origin.

Amaranthus viridis L. (Amaranthaceae) grows commonly on wastelands along the gattars and in cultivated fields throughout the year. It is probably of American origin, naturalized in India. The tender shoot tips are often used as pot-herb.

Plant is used in snakebite. Roots are used as antifertility agent in ayurveda. Leaves emollient, paste applied on scorpion sting. The tender tops are cooked and are eaten by the people in urinary problems (Kirthikar and Basu, 1935; Nadkarni, 1995; Jain, 1991; Chopra et al., 1996; Agrawal, 1997; Kaushik and Dhiman, 1999). Roots are pounded, mixed with one glass of sugar candy solution (100gm) and given to a 1-4 months pregnant lady once daily for inducing abortion in Orissa (Satapathy and Panda 1992). In Bangladesh the plant is used as analgesic and antipyretic in traditional systems of medicine (Yusuf et al. 1994). In Nepal vegetable made of green leaves is given to cure diarrhea, seeds and flowers used in gastric problems and seed powder roasted in ghee is given to reduce pregnancy pains (Turin 2003). Leaves are used as vermifuge, anti-inflammatory for urinary tract and in veneral diseases in Brazil (Anonymous 1988; Agra et al. 2007).

Root material was selected to study the antifertility activity. Since in traditional medicine either crude drug powder or aqueous extract/paste is used, here also only aqueous extract was used for experimentation to evaluate the traditional claim.

MATERIAL AND METHODS:

Roots of *A. viridis* were collected from Melghat (District Amravati, MS), washed thoroughly and shade dried. Dried

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material was powdered for preparing extract and phytochemical analysis and for the study of minerals ash was prepared at 550°C.

Phytochemical Studies:

The material was screened for the presence of 16 bioactive molecules following standard methods (Peach and Tracey 1979; Harborne 1973; Evans 1997). Mineral profile was studied as per Johanson (1940); quantitative estimation was done following titrimetric (Gupta and Varshney 1997) and flame photometric methods.

Animal Experimentation:

Preparation of Extract – Fifty gm of powder was taken in about 100-150 ml distilled water and soxhlated for 24 hrs at 50°C. After cooling the extract was collected, evaporated on a water bath to dryness and stored at room temperature. The extract was mixed in double distilled water for the treatment.

Female albino rats (Wistar strain) of age between 11-14 weeks were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad and allowed to acclimatize in the animal house. Animals were maintained and housed in wire mesh cages under standard environmental conditions. They were feed with pellet diet and water *ad libitum*. The animal room was well ventilated with a temperature range of 25-27°C under day/night 12-12 hour photoperiod. All experiments were carried out in a quiet laboratory setting with ambient illumination and temperature close to those of the animal house. The procedures with animals were conducted strictly in accordance with approved guidelines by the Institute's Animal Ethical Committee regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering, and in addition, the number of rats used was kept at a minimum. The protocol was approved by the Institutional Animal Ethical Committee Registration No. 817/04/AC/CPCSEA (IAEC/2/2005-06).

Method by Khanna and Chaudhury (1968) was adopted with the modification for the abortifacient activity of aqueous extracts of plants.

Female albino rats (Wistar strain) weighing 150-200gms of proven fertility were used to assess abortifacient activity. Vaginal smears from each rat were monitored daily. Only the rats with normal oestrous cycle were selected for the experiment.

Female rats of proestrus phase were kept with male rats of proven fertility for mating in a ratio of 2:1. The females were examined in the following morning for evidence of copulation. Animals exhibiting thick clumps of spermatozoa in vaginal smears were separated from male partner. The day when spermatozoa were detected in the vaginal smear was considered as day one of gestation. The separated pregnant rats were divided into four groups of six rats each. On the 10th day laparotomy was carried out under light ether anesthesia and semisterile condition. A small incision was given in the lower abdomen. Both horns of the uterus were observed for the presence of number of implantation sites and number of corpora lutea in ovary. The abdomen was sutured and animals left in cages to allow the term. Female albino rats of proven fertility were divided into following 4 groups of 6 each:

The various groups were treated as follows:

Group I - Control : Distilled water (Vehicle)

Group II - Aqueous extract (50mg/kg) body weight

Group III - Aqueous extract (100mg/kg) body weight

Group IV - Aqueous extract (150mg/kg) body weight

The extracts were administered orally with the help of catheter from 11th to 15th day of gestation. The control animals received only vehicle (Distilled water). The number of litters delivered were counted and compared with the number of implantation sites and percentage abortifacient activity was calculated using following formula.

$$\% \text{ Resorption} = 100 - \left(\frac{\text{Number of Rats Delivered}}{\text{Number of Implantation Sites}} \times 100 \right)$$

Statistical Analysis:

The results were analyzed as per Mungikar (1997) using Microsoft Excel 2007. A one-way ANOVA was employed for comparison among the four groups.

RESULTS & DISCUSSION:

Amaranthaceae are characterized by presence of saponins, betalains, oxalates and potassium nitrates (Cronquist 1981). Very little is known about the root chemistry of *A. viridis*. During present investigation alkaloids, flavonoids, unsaturated steroids, saponins, triterpenoids and polyoses were found to be present in root tissue imparting various biological properties; while, anthraquinones, anthracene glycosides, simple phenolics, tannins, leucoanthocyanins, cardenolides, emodines and polyurenoids were found to be absent. Tissue is rich in sodium (15.37 mg /gm) and iron (15.9 mg /gm) while potassium, calcium and phosphorus were found to present in low quantity (1.92, 1.74 and 7.0 mg/gm respectively). Sulphur, magnesium, chlorine, aluminium and manganese were also detected. Amasterol has been reported which inhibits the seed germination and seedling growth in lettuce. Sterol-spinesterol is also reported (Rastogi and Mehrotra, 2004). Flavonoids rutin and quercetin reported by Ashok Kumar et al. (2009).

The number of average resorption in control group is nil, while it gradually increased to (8.39%), (22.96%) and (42.62%) due to 50, 100 and 150 mg/kg treatments of *Amaranthus viridis* extracts respectively. The decrease in the number of rats delivered was significant at $p = 0.01$ when the treatment concentration was 150mg/kg. The variation among the individuals was also found to be significant, with lower value of variance ratio ($F = 2.40$) as compared to that obtained for treatments ($F = 31.84$).

Methanolic extract of whole plant is shown to produce antinociceptive and antipyretic activity (Bagepalli et al., 2009). Methanolic extract of whole plant exhibits anthelmintic property (Ashok Kumar et al., 2010). Ahmed et al. (2013) found the leaves and seeds to be antioxidant and antimicrobial. Aqueous extract of leaves is found to possess anti-inflammatory activity (Macharla et al. 2011). However, antifertility activity of root has been tested here for the first time.

Table 1: Abortifacient effect of *Amaranthus viridis* L.

Sr. No.	Group & Treatment	Animals Used	Body Wt. (gm)	No. of Implantation Sites in Individual Rat on Day 10	No. of Rats Delivered	% Abortifacient Activity
1	Control (Vehicle)	6	150-200	8, 8, 9, 8, 6, 6	8, 8, 9 8, 6, 6	Nil
2	Aqueous Extract (50mg/Kg)	6	150-200	9, 8, 8, 6, 7, 8	8, 7, 8 6, 6, 7	8.69%
3	Aqueous Extract (100mg/Kg)	6	150-200	9, 8, 10, 9, 9, 8	7, 7, 9, 6, 7, 5	22.64%
4	Aqueous Extract (150mg/Kg)	6	150-200	9, 6, 9, 7, 8, 8	4, 4, 7 3, 5, 4	42.55%

Table 2: Percent abortificient effect of *Amaranthus viridis* on individual female rats

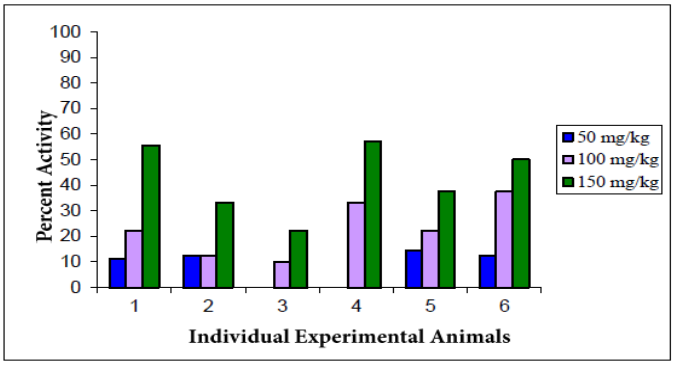
Treatment Group Animals	Percent Resorption After Treatment			
Sr. No.	Control	50 mg/kg body wt.	100 mg/kg body wt.	150 mg/kg body wt.
1	0.000	11.110	22.220	55.560
2	0.000	12.500	12.500	33.330
3	0.000	0.000	10.000	22.220
4	0.000	0.000	33.330	57.140
5	0.000	14.290	22.220	37.500
6	0.000	12.500	37.500	50.000

Table 3: Statistical Analysis

Groups	Count	Sum	Average	Variance
Control	6.000	0.000	0.000	0.000
50 mg/kg body wt.	6.000	50.396	8.399	43.346
100 mg/kg body wt.	6.000	137.777	22.962	119.495
150 mg/kg body wt.	6.000	255.753	42.625	192.246

Source of Variation	Df	SS	MSS	F
Replicate	5	790.0223	158.0045	2.404725
Treatment	3	6277.092	2092.364	31.84442
Error	15	985.5875	65.70583	
Total	23	8052.702		
S.E.	4.679951			
C.D. 5 %	9.968296			
C.D. 1 %	13.80586			

(Df = Degrees of freedom; SS = Sum of squares; MSS = Mean sum of squares;
F = Equality in variances; S.E. = Standard error; C.D. = Critical difference)



Graph: Percent abortifacient activity in individual experimental animals.

CONCLUSION:

The root of *A. viridis* L. is abortifacient. The activity was found dose dependent and individual specific. The maximum result produced exhibited 57.14% abortifacient activity. Though activity was found to increase with increase in dose, responses of individual animals showed significant range at each dose level. Also it was observed that increase in activity with dose was not linear within the animals of single group. At lowest dose level 30% animals did not respond to the treatment; at next higher dose the range of activity was from 10% to 37.5% and with highest dose range was between 22.2% to 57.14% (Graph). The drug therefore can be called to be more individual specific in its action and not showing generalized response.

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