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***Research Article** (Open Access)*

HISTOLOGICAL CHANGES IN THE TESTIS OF ALBINO RAT TREATED WITH AQUEOUS EXTRACT OF *AMARANTHUS SPINOSUS*

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ABSTRACT
Amaranthus spinosus is a tropical plant, used traditionally as a vegetable and in folk lore medicine for the treatment of various ailments. The present study was carried out to investigate the antifertility effect of aqueous extract of *Amaranthus spinosus* on testis in male albino rat. Male wistar rats were received aqueous extract of *Amaranthus spinosus* at 125, 250, 500 and 1000 mg/kg body weight per day orally for 60 days. A portion of testis was used for histological studies and biochemical markers i.e. cholesterol and lactate dehydrogenase (LDH). For histology, the tissue was fixed in Bouin's fixative, dehydrated in various grades of ethanol, cleared in benzene, infiltrated and embedded in paraffin wax for sectioning. The sections of 6 □m thickness, were stained with hematoxylin and eosine. The histological architecture of testis showed regression of seminiferous tubules and degeneration of spermatogenesis. Significant reduction was observed in cholesterol and LDH after 60 days of treatment period. The effects were dose dependent and more noticeable at higher doses but were recovered to normal levels after 90 days of treatment withdrawal.

Keywords: *Amaranthus spinosus, Testis, Antifertility, Wistar Rats, Cholesterol, Lactate Dehydrogenase*

INTRODUCTION
Amaranthus spinosus Linn. (Family: Amaranthaceae) is an annual or perennial herb, native to tropical America and is widely distributed throughout the tropics and warm temperate regions of Asia as a weed in cultivated as well as fallow lands (Mishra *et. al.*, 2012). In India, it is commonly known as ''Kate Wali Chaulai (Kanatabhajii)" and used as vegetable and cultivated throughout India. It is widely used in folk lore medicinal system of India and traditionally utilized as a diuretic, antidiabetic, antipyretic, anti-snake venom, antileprotic and anti-gonorrheal drug (Kirtikar and Basu, 2001).
Scientific studies carried out on extracts of *Amaranthus spinosus* showed anti-diabetic (Bavarva and Narasimhacharya, 2013), antioxidant (Barku *et al.*, 2013), anti-cholesterolemic (Girija *et al.*, 2011), antipyretic (Ashok *et al.*, 2010), anti-inflammatory (Olumayokun *et al.*, 2004), spermatogenic (Sangameswaran and Jayakar, 2008), antitumor (Joshua *et al.*, 2010), antifertility (Jhade *et al.*, 2011), immuno-stimulatory (Lina *et al.*, 2005), anti-malarial (Hilou *et al.*, 2006), and hepatoprotective (Ilango *et al.*, 2010) activities. The aqueous extract showed dose dependent changes in haematological and biochemical parameters in male rats (Bhande and Wasu, 2016)[a,b], Methanolic extract and 50% ethanolic extract showed effects on hematology (Srivastava *et al.*, 2011; Olufemi *et al.*, 2003).
Moreover, the root Juice of *Amaranthus spinosus* reported to induce antifertility in tribes located in Kerala (Priya *et al.*, 2002). The Nepalese and some tribes in India have been reported to use it to induce abortion (Azhar-ul-huq *et al.*, 2004; Mali *et al.*, 2006, Tayade and Patil, 2005). The combined extract of *A. spinosus* roots and *D. biflours* seeds (DBS) showed biochemical changes in the epididymis (Murugan *et al.*, 1993). However, there are no reports on the effects of *A. spinosus* on male reproductive function. Thus, present study has been carried out to explore the effects of aqueous extract of *Amaranthus spinosus* on histology of testis in male albino rats.

MATERIALS AND METHODS

Test Material

Amaranthus spinosus as a whole plant was collected from in and around places of Nandurbar district in Maharashtra state and identified in Department of Botany of our institution.

Extraction procedure

Whole plant was shade dried and coarsely powdered. Hundred gram of powder was refluxed with 600 ml of water at 100^{o}C for 24 hours. The extract was filtered through double layer 100 µm nylon wire mesh and concentrated at 50^{o}C to obtain crude aqueous extract.

Animals

Normal healthy male Wistar rats (*Rattus norvegicus*) weighing 200-240g were used in the present investigation. The animals were maintained as per the Guidelines for Care and Use of Animals for Scientific Research (Indian National Science Academy, 2000) in Department of Zoology, in group of three animals in polypropylene rat cages under 12:12 hrs. light-dark schedule and fed with rat pellet diet and water was provided ad libitum.

Experimental Design

The animals were divided into five groups consisting 10 animals in each group. Group I served as vehicle treated control and received 2 ml of distilled water. The animals of Group II, Group III, Group IV, and Group V was administered orally with aqueous extract of *Amaranthus spinosus* at 125, 250, 500 and 1000 mg/Kg bw/day respectively for 60 days. Following completion of respective treatment schedule, all the animals were withdrawn from the treatment for a further period up to 90 days. Five animals from each group were sacrificed on next day, following the 60th day of treatment and 90 days of treatment withdrawal.

Histology of Testis

The testes from each rat were removed, weighed and fixed in Bouin's fixative, dehydrated in various grades of ethanol, cleared in benzene, infiltrated and embedded in paraffin wax for sectioning. The sections, of 6 µm thickness was stained with hematoxylin and eosine.

Tissue Biochemistry

Androgen sensitive biochemical markers viz., cholesterol (King and Wolten, 1959), and lactate dehydrogenase (LDH) (Bergmeyer, 1965) was estimated quantitatively using the homogenates of the testis portion.

Statistical analysis

Student's t-test was employed for the statistical comparison.

RESULTS AND DISCUSSION

In the present study, the effect of aqueous extract of *Amaranthus spinosus* on the histology of testis of albino rats has been investigated. Dose dependent significant reduction ($p<0.01$) was observed in the weight of testis after 60 days of treatment. After 90 days of treatment withdrawal, the weight of testis was comparable statistically to that of control animals at dose level of 250 (Group III) and 500 mg/Kg bw/day (Group IV). However, continued significant reduction ($p<0.01$) was observed in the weight of testis in animals of Group V receiving aqueous extract of *A. spinosus* at 1000 mg/Kg bw/day (Table 1). This indicates the irreversible effect of extract at higher dose regimen. Dose dependent decrease in the weight of testis has been reported in the experimental animals treated with different extracts of plants / parts. The aqueous extract of *Boerhavia diffusa* L. leaves for sixty days to male Wistar rats showed dose dependent significant decreases in the weight of the testes of treated rats compared to control. (Adenubi *et al.*, 2010). The testis of the control animals showed round or oval seminiferous tubules with the epithelium containing Sertoli cells and germ cells of various stages covering the complete spermatogenesis. Sertoli cells showed closer association with elongated spermatids. Lumen contained mature spermatozoa. The interstitium occupied with distinct Leydig cells (Fig. A). The administration of aqueous extract of *Amaranthus spinosus* at 125 (Group II) and 250 mg/Kg bw/day (Group III) did not show any appreciable

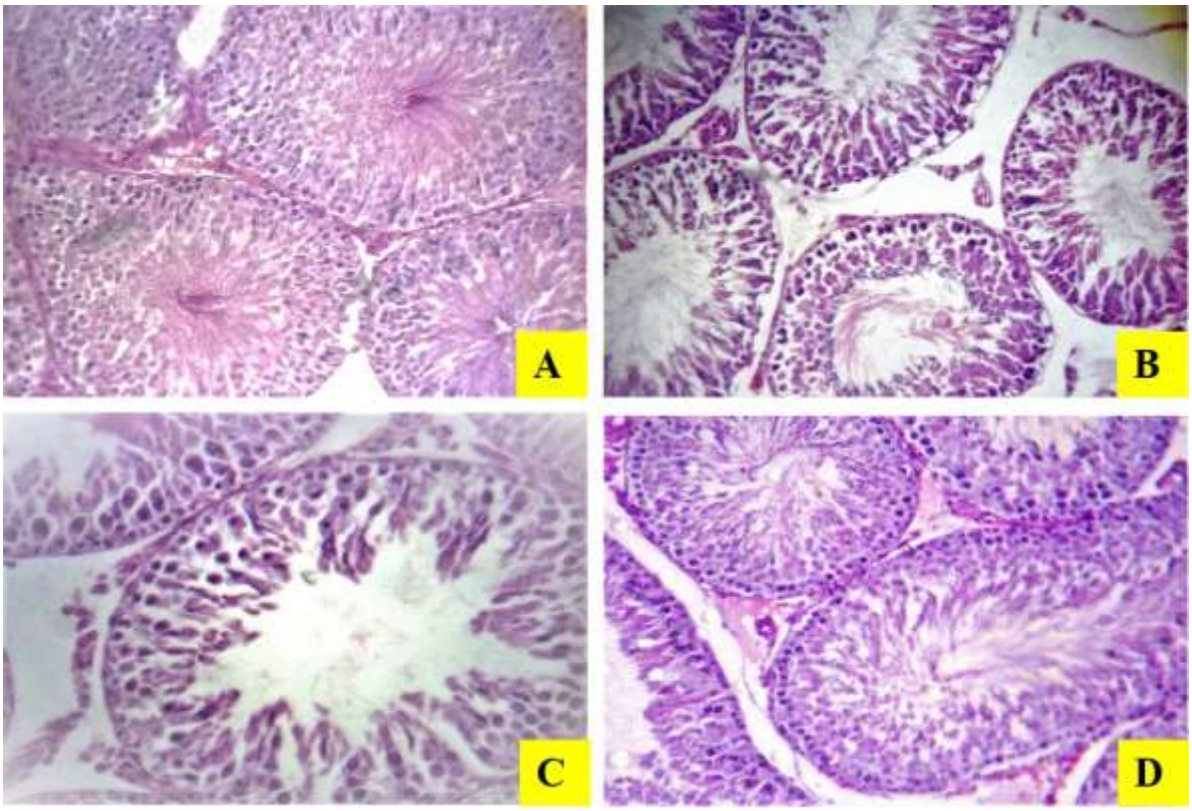

Figure A: Histology of the testis in control rat. The seminiferous tubules are round or oval showing all the stages of spermatogenesis. Lumen contain spermatozoa and interstitium contain Leydig cells. X100
Figure B: Histology of the testis in rat treated with aqueous extract of *Amaranthus spinosus* at 500 mg/kg.bw/day for 60 days. Slight degeneration in the stages of spermatogenesis. Reduction in the number of spermatozoa in the lumen. X100
Figure C: Histology of the testis in rat treated with aqueous extract of *Amaranthus spinosus* at 1000 mg/kg.bw/day for 60 days. Seminiferous tubules are loosely arranged with reduction in the size. Spermatogenesis is disrupted and lumen contains lesser number of spermatozoa. X400
Figure D: Histology of the testis in rat following 90 days of treatment withdrawal. Resumption of Spermatogenesis with lumen containing spermatozoa. The histological features are comparable to control animals. X100

gross or histological changes in the testis. However, at dose level 500 mg/Kg bw/day, the seminiferous tubules are not compactly arranged. There was slight degeneration in the stages of spermatogenesis and reduction in the number of spermatozoa (Fig. B). In 1000 mg/Kg bw/day group, the disruption of spermatogenesis was evident. The seminiferous tubules appeared regressed and the basal lamina appeared thick. Degeneration of seminiferous epithelium, showing vacuolization in sertoli cells and germ cells (Fig. C). Similar outcome was obtained after sub-chronic administration of aqueous extract of *Hibiscus sabdariffa* calyx in rats (Orisakwe *et al.*, 2004). Adenubi *et al.*, (2010) observed a dose dependent degeneration of the germinal epithelia of seminiferous tubules and spermiostasis. The aqueous extract of stem of *Fadogia agrestis* resulted into dose dependent lesions on seminiferous tubules ranging from mild distortion, destruction of sperm cell to complete destruction of spermatogenic cells (Yakubu *et al.*, 2007). Also, significant decrease in the weight of testis and disruption of spermatogenesis was observed in male

rats treated with the compounds, MCP I and ECP I, isolated from the seeds of *Carica papaya*, at 50 mg/kg bw/day, for a period of 360 days (Lohiya *et al.*, 2005).

Following 90 days of treatment withdrawal, the histological architecture of testis of 500 mg/Kg bw/day treatment group animals was comparable to that of control animals, while in 1000 mg/Kg bw/day treatment group, the diameter of seminiferous tubules was restored to some extent and the stages of spermatogenesis was noticeably recovered to normal levels (Fig. D).

The levels of Cholesterol and LDH of testis in control animals were 5.96±0.46 mg/g and 28.2±2.5 U/mg respectively. There were no appreciable changes in LDH and cholesterol in Group II and III. However, significant reduction in the level of LDH and highly significant reduction in cholesterol was observed at dose level 500 and 1000 mg/Kg bw/day. Following treatment withdrawal up to 90 days, levels of Cholesterol and LDH was comparable to that of control animals (Table 2). Significant reduction of cholesterol was observed in the animals at dose level 500 mg/kg bw/day and 1000 mg/kg bw/day. It is remarkable to note that, the disturbed process of spermatogenesis in the testis is correlated with the elevated levels of cholesterol. The role of cholesterol in spermatogenesis and male fertility is well established. It is well known that the process of spermatogenesis is androgen dependent and cholesterol is the major precursor for steroidogenesis (Gwynne and Strauss, 1982; Sedes *et al.*, 2018). Moreover, Sertoli cells has important role in every step of cholesterol metabolism, including cholesterol uptake, efflux, storage and recycling and also maintain cholesterol homeostasis through reverse cholesterol transport (Shi *et al.*, 2018). It is evident in the present study that there is vacuolization in sertoli cells and germ cells at 1000 mg/kg bw/day. Hence, it could be accomplished that the aqueous extract of *Amaranthus spinosus*, at higher dose level affects the levels of testicular cholesterol thereby disturbing the steroidogenesis resulting in disruption of spermatogenesis.

In conclusion, administration of aqueous extract of *Amaranthus spinosus* showed dose dependent decrease in the weight and disturbed architecture of testis which correlates with the levels of cholesterol, after 60 days of treatment period. The effect was more noticeable at higher doses but was recovered to normal levels after 90 days of treatment withdrawal. The effects might be due to elevated levels of cholesterol which might influence the testosterone levels, affecting the normal process of spermatogenesis. Further studies are required at molecular level to establish the exact mechanism of drug action.

Table 1: The weight of Testis in rats treated orally with aqueous extract of *Amaranthus spinosus*. (Values with SEM of 5 animals)

Treatment Schedule	**Testis (mg/100g bw)**			
	125 mg/Kg bw/day	**250 mg/Kg bw/day**	**500 mg/Kg bw/day**	**1000 mg/Kg bw/day**
Control	694±2.70	694±2.70	694±2.70	694±2.70
Treatment 60 days	691±3.24	686±4.2*	678.4±4.4***	668.4±9.04***
TW 90 days	696.4±3.9	690.6±7.5	687.6±7.52	681±5.54**

TW – Treatment withdrawal (* p<0.05, ** p<0.01, *** p<0.001)

Table 2: Biochemical markers of Testis of rats treated orally with the aqueous extract of *Amaranthus spinosus* (Values with SEM of 5 animals)

Treatment Schedule	Cholesterol (mg/g)				LDH (U/mg)			
	125 mg/Kg bw/day	250 mg/Kg bw/day	500 mg/Kg bw/day	1000 mg/Kg bw/day	125 mg/Kg bw/day	250 mg/Kg bw/day	500 mg/Kg bw/day	1000 mg/Kg bw/day
Control	5.96±0.46	5.96±0.46	5.96±0.46	5.96±0.46	28.2±2.5	28.2±2.5	28.2±2.5	28.2±2.5
Treatment 60 days	5.84±0.24	5.30±0.29 *	4.9±0.29**	4.52±0.41** **	27.7±2.3	26.66±2.1	25.1±1.2*	23.98±1.2*
TW 90 days	6.08±0.48	5.76±0.52	5.38±0.48	5.14±0.38*	29.04±1.72	27.06±2.0	26.56±1.6	26.04±1.9

TW – Treatment withdrawal (* p<0.05, ** p<0.01, *** p<0.001)

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