# Final Report of the work done on the Minor Research Project

**Project Title**

# Scientific evaluation of antifertility effect of *Amaranthus spinosus* in albino rat


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Annexure -III

**Report of the work done: (From January, 2013 to September, 2015)**

**Project Title:**

**Scientific evaluation of antifertility effect of *Amaranthus spinosus* in albino rat**

**Introduction:**

India is well known for its floral diversity and is rich in medicinal plant wealth. Since ancient times, mankind has used medicinal plants to cure diseases and relieve physical sufferings. Because of better cultural acceptability, better compatibility with the human body, lesser side effects and effectiveness, many traditional medicines is now an accepted fact. In India around 20,000 medicinal plant species have been recorded (Dev, 1997) and more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000). According to the World Health Organization (WHO) as much as 80% of world's population depends on traditional medicine for their primary health care needs and up to 90% of the developing world relies on the use of medicinal plants. The primary population is well acquainted with the use of medicinal plants by traditional medical treatments of tribals and rural communities (Chopra et al., 1958).

In United States, about 100 plant derived compounds serve as drugs. Several valuable drugs, such as digitoxin, degoxine, morphine, codein, paparevin, atropine, reserpine, colchiceines etc, many of them being cardiotonic agents and hence for long term use with free of toxicity have been developed from the herbal sources based on the ethnomedical information (Farnsworth and Waller, 1982; Farnsworth et al., 1983). Many of the Indian plants are reported to possess antifertility activity as antiovulatory, antiimplantation, abortifacient, spermicidal, antiandrogenic or antispermatogenic. The leaves, flowers, fruits and seeds of several plants are known to possess estrogen or antiandrogen like substances which act on the reproductive system of male or female thus inhibiting fertility (Nadkarni, 1954; Delaszlo and Henshaw, 1954; Farnsworth and Waller, 1982; Ray et al., 1991; Lohiya et al., 2001). The plant world is still under scrutiny to identify a possible herbal contraceptive for long term use without adverse side effects.

Although, number of antifertility plants are mentioned in the folklore history and tribals and rural people are still using them, many plants needs scientific investigations to find out their active principals and mode of action and *Amaranthus spinosus* is one among them.

*Amaranthus spinosus*, commonly known as spiny amaranth, prickly amaranth or thorny amaranth. It is native to the tropical Americas, but it is present on most continents as an introduced species and sometimes a noxious weed. It can be a serious weed of rice cultivation in Asia (Caton et. al., 2004). The plant is astringent, diaphoretic, diuretic, emollient and febrifuge. It is used internally in the treatment of internal bleeding, diarrhoea and excessive menstruation. It is also used in the treatment of snake bites. Externally, it is used to treat ulcerated mouths, vaginal discharges, nosebleeds and wounds. The root is emmenagogue and galactogogue. It is used in the treatment of menorrhagia, gonorrhoea, eczema and colic. The seed is used as a poultice for broken bones. Jhade, et. al., 2009 have reviewed and reported that the preliminary work on *Amaranthus spinosus* showed Antiprotozoal activity, Anti-inflammatory activity, Antioxidant properties, Anti-malarial activity, Analgesic properties, Immuno-modulatory properties, Haematological Properties, Antifertility activity, Anti-diabetic, anti-hyperlipidemic and spermatogenic effects. It possesses potent anthelmintic activity (Ashok kumar, 2010), significant and dose dependant antiinflammatory activity also central and peripheral analgesic activity (Zeashan, et. al., 2009). Tribes in Rajasthan uses leaves for urinary disorder orally as a diuretic (Meena and Rao, 2010).

The significance of antifertility property of *Amaranthus spinosus* as folklore has been well documented. Juice of the roots of *Amaranthus spinosus* is mixed with rice washed water and administered orally for three days after menstrual periods 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 (Azahar-ul-huq, et. al., 2004). Decoction of whole plant given orally is reported to induce abortion in tribes located in Jalgaon, Dhule and Nandurbar districts in North Maharashtra (Mali, et al., 2006, Tayade and Patil, 2005).

**Objective of the project:**

1) Isolation of the active compounds from the leaves / whole plant of *Amaranthus spinosus.*
2) Antifertility effects, reversibility and toxicological evaluation of the isolated compounds.
3) Functional status of testis, epididymis, seminal vesicle and prostate to define the mode of action.

**Test Material:**

*Amaranthus spinosus* as a whole plant was collected from in and around places of Nandurbar district. The plant was authenticated by Dr. S. K. Tayade, Head Department of Botany of our college. The whole plant (except roots) was then subjected to extraction procedure as follows.

**Extraction procedure:**

The whole plant was shade dried for one week and coarsely powdered in an electrical grinder. Hundred gram of powder was refluxed with 600 ml. of double distilled water at 60$^{o}$C for 24 hours. The extract was filtered through double layer 100 μm nylon wire mesh and concentrated at 50$^{o}$C to obtain 200 ml slurry of crude aqueous extract. The slurry was stored in a refrigerator at 4$^{o}$C until administration. To obtain the exact concentration of water solubilized components, 10 ml of the extract was concentrated to obtain residue. The yield of concentrated and dried residue was 0.8 gm. Thus, yield of extract was 8 % of the starting raw material.

**Experimental Design:**

Male albino rats, *Rattus norvegicus* (Wistar strain) of approximately 3 months old, weighing 200 - 250 g are procured from R. C. Patel College of Pharmacy, Shirpur. The animals were used for experimentation after acclimatization for two weeks. The animals were maintained in animal house facility, in group of three animals in polypropylene rat cages under 12:12 hrs. light-dark schedule and fed with rat pellet diet and water is provided ad libitum.

The animals were divided into the following five groups; each consisting of 10 animals. Each animal was treated orally with the aqueous extract of *Amaranthus spinosus* as given bellow.

Group I - Treated with distilled water at 0.5 ml/Rat/day orally for 60 days (Control)

Group II - Treated with aqueous extract at 25mg/Rat/day (i.e. 125 mg/kg. Body Weight/day) orally for 60 days

Group III - Treated with aqueous extract at 50mg/Rat/day (i.e. 250 mg/kg. Body Weight/day) orally for 60 days

Group IV - Treated with aqueous extract at 100mg/Rat/day (i.e. 500 mg/kg. Body Weight/day) orally for 60 days

Group V - Treated with aqueous extract at 200mg/Rat/day (i.e. 1000 mg/kg. Body Weight/day) orally for 60 days

The concentration of extract (slurry) used was 80 mg / ml. Before administration, each dose i.e. 25 mg, 50 mg, 100 mg and 200 mg was either dissolved in 0.5 ml of distilled water or concentrated to get required dose regimen and administered orally with the help of oral feeding needle connected to 1 ml disposable syringe.

**Observations and Results:**

**Body and organ weight:**

The body weight and the weights of brain, thyroid, heart, lungs, liver, spleen, adrenal, kidney, testis, epididymis, seminal vesicle free of coagulating gland and ventral prostate excised free of adhering tissues were recorded after 60 days of treatment period and after 90 days of treatment withdrawal.

The body weight of the vehicle treated control animals was 256±2.28 g and the weight of vital organs viz. brain, thyroid, heart, lungs, liver, spleen, adrenal and kidney were 1.73±0.02, 0.040±0.01, 0.758±0.02, 1.38±0.01, 4.0±0.10, 0.674±0.02, 0.18±0.02 and 1.76±0.11 g respectively.

In the aqueous extract treated animals (Group II, III, IV and V), there was no significant change observed in the body weight and organ weights after 60 days of treatment.

Following treatment withdrawal up to 90 days, the body weight and organ weights of the animals was comparable statistically to that of control animals (Table 1A, 1B, 1C and 1D).

The reproductive organ weights viz., Testis, epididymis, seminal vesicle and ventral prostate of vehicle treated control animals was 694±2.70, 184±3.39, 238±1.58, 129±0.83 mg/100 g body weight.

There was significant reduction ($p<0.01$) observed in the weights of testis and seminal vesicle in treatment Group III. The reduction in weight of testis, epididymis, seminal vesicle and ventral prostate was highly significant ($p<0.001$) in treatment Group V after 60 days of treatment.

After 90 days of treatment withdrawal, the weights of reproductive organs of Group III and IV animals were comparable statistically to that of control animals. However, continued significant reduction ($p<0.01$) was observed in the weight of testis and seminal vesicle (Table 1A, 1B, 1C and 1D).

Thus, there was no appreciable changes observed in body weight and vital organ weights of animals in the treatment groups treated with aqueous extract of *Amaranthus spinosus*. However, the weights of reproductive organs viz., testis and seminal vesicle were reduced at 500 mg/kg. bw/day while at higher dose i.e. 1000 mg/kg bw/day, the weight of testis, epididymis, seminal vesicle and ventral prostate were reduced significantly, recovered after 90 days of treatment withdrawal.

**Biochemical Investigations:**

**Reproductive tissue biochemistry:**

Androgen sensitive biochemical markers viz., cholesterol (King and Wolten, 1959), and lactate dehydrogenase [LDH] (Bergmeyer, 1965) of testis, sialic acid (Svennerholm, 1960), and α–glucosidase of epididymis, fructose of seminal vesicle and acid phosphatase [ACP] of ventral prostate (WHO, 1992) were estimated quantitatively using the homogenates of the respective tissues.

The levels of Cholesterol and LDH of testis, Sialic Acid and α-Glucosidase of epididymis and Fructose of seminal vesicle and Acid phosphatase (ACP) of ventral prostate of the control animals were 5.96±0.46 mg/g, 28.2±2.5 U/mg, 6.84±0.44 mg/g, 98.28±4.0 mgPNP/mg protein, 3 6.94±0.74 mg/g and 5.32±0.64 B.U. respectively.

There were no appreciable changes in Cholesterol, LDH, Sialic Acid, α-Glucosidase, Fructose and ACP in group II and III for 60 days. However, there was significant reduction in the cholesterol, fructose and ACP in the animals of group VI and V after 60 days of treatment period.

Following treatment withdrawal up to 90 days, levels of Cholesterol, LDH, Sialic Acid, α-Glucosidase, Fructose and ACP in group IV animals was comparable to that of control animals. However, the levels of fructose and ACP did not show complete recovery in group V animals (Table 2A, 2B, 2C and 2D).

Thus, biochemical marker of testis, seminal vesicle and ventral prostate viz., cholesterol, fructose and ACP respectively showed reduction in the levels at 500 and 1000 mg/kg.bw/day after 60 days. The cholesterol was recovered completely but fructose and ACP did not restored significantly in 1000 mg/kg.bw/day treatment group. The epididymal marker viz., sialic acid and α-Glucosidase showed significant change only at 1000 mg/kg.bw/day which were recovered after treatment withdrawal.

**Sperm Parameters:**

The cauda epididymis was chipped in 1 ml of sperm diluting fluid/ normal saline and the clear fluid was used for the analysis of sperm concentration, motility, viability and abnormality (WHO, 1999).

The sperm concentration was calculated by hemocytometric method using Neubauer's haemocytometer at 1:19 dilution. The per cent motility, viz., rapid linear progressive, slow linear progressive, vibratory and non motile, per cent viability was assessed through eosin-nigrosin staining method and per cent normal/abnormal spermatozoa through Rapid Pap$^{TM}$ staining kit (Biolab Diagnostics Pvt. Ltd., India), was assessed under phase contrast microscope.

**Sperm concentration:**

The sperm concentration of vehicle treated control animals was 25±3.03 million/ml.

Gradual reduction in the sperm concentration was observed with the increasing dose in group III, IV and group V respectively after 60 days of treatment

period. Following treatment withdrawal after 90 days, the sperm concentration reached the control levels (Table 3A, 3B, 3C and 3D).

**Sperm motility:**

The per cent motility of spermatozoa of vehicle treated control animals was 77±4.02.

There was no significant change in sperm motility of treatment groups II and III. However, the sperm motility was decreased significantly ($p<0.001$) in the treatment groups IV and V. After 90 days of treatment withdrawal the sperm motility was regained up to the control levels (Table 3A, 3B, 3C and 3D).

**Sperm viability:**

The per cent viable spermatozoa of vehicle treated control animals was 57±4.3.

There was no significant change in sperm viability of treatment groups II and III. However, there was statistically significant ($p<0.001$) decline in the sperm viability of treatment groups IV and V. After 90 days of treatment withdrawal the sperm viability was regained up to the control levels (Table 3A, 3B, 3C and 3D).

**Sperm abnormality:**

The per cent abnormal spermatozoa of vehicle treated control animals was 24±3.2.

There was significant increase ($p<0.01$) in the abnormal spermatozoa of treatment group III and the increase was highly significant ($p<0.001$) in group IV and V. After 90 days of treatment withdrawal the sperm abnormality was regained up to the control levels (Table 3A, 3B, 3C and 3D).

Thus, there was dose dependent decrease in the sperm concentration, motility and viability while increase in percent abnormal spermatozoa. The levels were highly significant at 1000 mg/kg.bw/day. All the sperm parameters were regained after 90 days of treatment withdrawal.

**Toxicological Investigations:**

Blood samples were collected by cardiac puncture and used for routine haematology and serum clinical chemistry.

**Hematology:**

Total red blood corpuscles [RBC], white blood corpuscles [WBC] (Lynch et al., 1969), haemoglobin (Crosby et al., 1954) and red cell indices viz., PCV, MCV, MCH and MCHC (Natelson, 1951) was recorded.

Total RBC, WBC, Hemoglobin, PCV, MCV, MCH and MCHC levels from the blood of vehicle treated control animals were $8.22\pm0.14x10^6/mm^3$, $10.6\pm0.36x10^3/mm^3$, 15.8±0.22 g/dl, 38.5±0.17 %, 46.9±0.60 $\mu^3$, 19.3±0.53 μ.μg and 41.1±0.70 % respectively.

Treatment with aqueous extract for 60 days did not show appreciable changes in all these parameter at 125 mg, 250 mg, and 500 mg /kg body weight/ day. However there is significant reduction ($p<0.001$) in RBC, hemoglobin, PCV and MCHC in the treatment group V, while significant increase ($p<0.001$) in WBC and MCV at 1000 mg/kg body weight/ day. The levels of MCH did not show alterations in all the treatment group.

After 90 days of treatment withdrawal, all the hematological parameters regained to control levels (Table 4A, 4B, 4C and 4D).

**Clinical chemistry:**

Total protein, glucose, cholesterol, creatinine, serum glutamate pyruvate transaminase [SGPT], serum glutamate oxalate transaminase [SGOT], lactate dehydrogenase [LDH], bilirubin, urea, triglycerides [TGL] was estimated using reagent kits (Biolab Diagnostics Pvt. Ltd., India),.

The levels of protein glucose, cholesterol, creatinine, SGPT, SGOT, bilirubin, urea and Triglycerides (TGL) levels in the serum of vehicle treated control animals were 7.34±0.11 g/dl, 98.8±4.6 mg/dl, 121±6.2 mg/dl, 0.942±0.05 mg/dl, 34.68±2.7 IU/L, 71.98±1.9 IU/L, 12.02±0.3 IU/L, 18.09±0.35 mg/dl and 63.16±3.4 mg/dl respectively.

Protein levels showed dose dependent significant increase while, cholesterol showed decreased levels in treatment group III, IV and V. SGPT, SGOT and urea were significantly increased in group IV and V. However, Glucose showed significant decrease only in the treatment group V. Creatinine and bilirubin did not show any change in the levels of all the treatment groups after 60 day.

Following treatment withdrawal, after 90 days, all the serum clinical parameters recovered to control levels (Table 4A, 4B, 4C and 4D).

Thus hematological parameter showed significant changes at higher dose level i.e. 1000 mg/kg.bw/day after 60 days. The levels of protein, cholesterol, SGOT, SGPT, urea and triglycerides showed dose dependent changes. The alteration in the levels of clinical parameters was restored after 90 days of treatment withdrawal.

**Histological Investigations:**

A portion of the reproductive organ viz., testis, cauda epididymis, seminal vesicle and ventral prostate were used for histological studies. 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. The sections of 6 μm thickness, were stained with hematoxylin and eosine.

**Testis:**

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.1a).

The administration of aqueous extract *Amaranthus spinosus* at 125 and 250 mg/kg. bw/day did not show any appreciable gross or histological changes in the testis. However, in animals at 500 mg/kg. bw/day treatment group, the seminiferous tubules are not compactly arranged. There was slight degeneration in the stages of spermatogenesis and reduction in the number of spermatozoa (Fig.1b). In 1000 mg/kg.bw/day group, the disruption of spermatogenesis was evident. The diameter of seminiferous tubules was reduced to great extent. The seminiferous tubules appeared

regressed and the basal lamina appeared thick. Degeneration of seminiferous epithelium, showing vacuolization in sertoli cells and germ cells (Fig.1c).

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. 1d).

Thus, administration of aqueous extract of *Amaranthus spinosus* showed dose dependent regression of seminiferous tubules and degeneration in spermatogenesis 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.

**Epididymis:**

The epididymis of the control animals, particularly the cauda epididymis, where storage and maturation of spermatozoa takes place, showed normal epididymal tubules and intertubular elements. The basal and principal cells of the epithelium showed pseudostratified appearance with stereocilia in the apical cells. The basal cells were short cuboidal, while the principal cells were columnar with stereocilia. The nuclei of the basal cells situated at the base of the cells, while that of principal cells arranged in the center. The lumen contained packed spermatozoa (Fig. 2a).

Treatment with aqueous extract of *Amaranthus spinosus* at 125 and 250 mg/kg.bw/day for 60 days did not show appreciable changes in the histology of epididymis. The pseudostratified epithelium showed normal appearance. The shape and size of basal and principle cells were normal and the lumen of epididymal tubules was packed with spermatozoa. In animals treated with 500 mg/kg.bw/day, there was increased intertubular space. However, the epithelium and the basal and principal cells of the epithelium appeared normal. The lumen contained relatively less spermatozoa, arranged in loose configuration in most of the tubules (Fig 2b). In 1000 mg/kg.bw/day treatment group, there was an increased intertubular element. Most of the tubules were empty and few showed less number of spermatozoa in lumen. Degeneration of pseudostratified epithelium was also observed (Fig. 2c).

Following treatment withdrawal, after 90 days, there was recovery of control features. The epididymal epithelium appears normal with the lumen containing spermatozoa (Fig.2d).

Thus, administration of aqueous extract of *Amaranthus spinosus* causes degeneration of epididymal architecture at higher dose i.e.1000 mg/kg.bw/day. Notable changes were, increase in intertubular elements and decrease in the spermatozoal mass in the lumen of epididymis in higher doses. Withdrawal of treatment recovered the changed histological feature to control quality.

**Seminal Vesicle:**

The seminal vesicle of the control animals showed the outer muscular region lined by secretory epithelium. The epithelium was tubular, cryptic and thrown into many folds. The cells of the epithelium were columnar with basal nucleus and the cytoplasm appeared granular. The central lumen and the cryptic lumen contained eosinated secretory material (Fig. 3a).

Treatment with aqueous extract of *Amaranthus spinosus* at 125 and 250 mg/kg.bw/day for 60 days did not show appreciable changes in the histology of seminal vesicle. The secretory epithelium appears normal and lumen was filled with secretory material. In animals at 500 mg/kg.bw/day, the height of the secretory epithelium was reduced and there was decreased secretory content (fig 3b). In 1000 mg/kg.bw/day treatment group, there was contraction of seminal vesicle and atrophy of epithelium and the lumen was devoid of secretory material (Fig. 3c).

Following 90 days of treatment withdrawal, the histological structure of seminal vesicle was regained (Fig. 3d).

Thus, animals treated with 125 and 250 mg/kg.bw/day for 60 days, did not show changes in the histological structure of seminal vesicle. However treatment with higher doses i.e. 500 and 1000 mg/kg.bw/day, the degenerative changes were observed which were regained after 90 days of treatment withdrawal.

**Prostate:**

The structure of the prostate of the control animals showed a typical lobular structure with several prostatic follicles. Each follicle was lined by columnar

epithelium. In the principal cells, the nucleus situated at the central region and the cytoplasm contained eosinated granules. Intertubular region was occupied by connective tissue (Fig. 4a).

Following treatment with 125 and 250 mg/kg.bw/day for 60 days, no appreciable changes was observed in the prostate histological structure. However, in animals treated with 500 mg/kg.bw/day, the size of prostatic follicles was decreased (Fig. 4b). In 1000 mg/kg.bw/day group, along with decrease in the size of follicles, the secretory material was also reduced (Fig. 4c).

The changes in the prostatic histology were recovered after 90 days of treatment withdrawal Fig. 4d).

Thus, higher doses i.e. 500 and 100 mg/kg.bw/day made degenerative changes in the protate which were recovered after 90 days of treatment withdrawal.

**Fertility Test**

The control and treated animals were exposed to proven fertile female rats after 60 days treatment period and following 90 days of treatment withdrawal at 1:2 ratio, to assess the libido, mounting behaviour and fertility. Success of mating was confirmed by appearance of spermatozoa in the vaginal smear in the successive morning.

The fertility test in vehicle treated control animals showed normal viable offsprings (7.0±2.3). In 125 and 250 mg/kg.bw/day groups, animals showed normal sexual desire mounting behaviour and fertility and the litter size was 6.0±1.5. While, 500 mg/kg.bw/day group animals showed decrease in the libido and the litter size was decreased (4.0±2.9). In 1000 mg/kg.bw/day the litter size was significantly decreased (1.0±0.7). However, Normal viable offsprings without mortality, morbidity or visible teratogenic syndrome were resulted after 90 days of treatment withdrawal.

Thus, it is clear that there was no effect on the fertility of male rats treated with 125 and 250 mg/kg.bw/day. However, in higher doses i.e. 500 and 1000 mg/kg.bw/day, the libido of animals was decrease along with significant reduction in the litter size. This may be because of significant decrease in the sperm concentration and increase in the abnormality of sperm.

**Conclusion:**

The present work was undertaken to investigate the anitfertility effects of *Amaranthus spinosus* in male albino rats. The aqueous extract of *Amaranthus spinosus* showed mild antifertility effect at 500 mg/kg.bw/day and significant antifertility effect at 1000 mg/kg.bw/day in male albino rats. The toxic effects were also observed only at higher doses. Mortality and morbidity was not recorded. On the basis of histological architecture, it was observed that, the functional status of testis and accessory glands viz. seminal vesicle and prostate was disturbed. However, the effects were reversible after 90 days of treatment withdrawal. The antifertility effects may be due to decrease in the testosterone levels which affect the normal process of spermatogenesis. Further studies are required on molecular level to know the exact mode of action of aqueous extract of *Amaranthus spinosus*.

## Tables:

## Body and organ weight:

### Table 1A: The body weight and organs weight in rats treated orally with aqueous extract of *Amaranthus spinosus* @ 125 mg/Kg body wt./day (Values with SEM of 5 animals)

| Treatment Schedule | Body Weight (g) | Brain (g) | Thyroid (g) | Heart (g) | Lungs (g) | Liver (g) | Spleen (g) | Adrenal (g) | Kidney (g) | Testis (mg/100g bw) | Epididymis (mg/100g bw) | S V (mg/100g bw) | V P (mg/100g bw) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 256±2.28 | 1.73±0.02 | 0.040±0.01 | 0.758±0.02 | 1.38±0.01 | 4.0±0.10 | 0.674±0.02 | 0.18±0.02 | 1.76±0.11 | 694±2.70 | 184±3.39 | 238±1.58 | 129±0.83 |
| **Treatment 60 days** | 258±1.58 | 1.75±0.02 | 0.043±0.01 | 0.768±0.01 | 1.40±0.02 | 4.1±0.11 | 0.686±0.04 | 0.22±0.04 | 1.9±0.12 | 691±3.24 | 179±7.34 | 234±4.7 | 126.8±6.30 |
| **TW 90 days** | 261±7.2 | 1.78±0.06 | 0.050±0.01 | 0.782±0.04 | 1.44±0.04* | 4.5±0.37* | 0.742±0.04* | 0.23±0.04* | 2.0±0.14* | 696.4±3.9 | 187.4±9.57 | 244±5.44 | 134.4±4.36* |

**TW – Treatment withdrawal, SV – Seminal vesicle, VP – Ventral prostate (* p<0.05)**

### Table 1B: The body weight and organs weight in rats treated orally with aqueous extract of *Amaranthus spinosus* @ 250 mg/Kg body wt./day (Values with SEM of 5 animals)

| Treatment Schedule | Body Weight (g) | Brain (g) | Thyroid (g) | Heart (g) | Lungs (g) | Liver (g) | Spleen (g) | Adrenal (g) | Kidney (g) | Testis (mg/100g bw) | Epididymis (mg/100g bw) | S V (mg/100g bw) | V P (mg/100g bw) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 256±2.28 | 1.73±0.02 | 0.040±0.01 | 0.758±0.02 | 1.38±0.01 | 4.0±0.10 | 0.674±0.02 | 0.18±0.02 | 1.76±0.11 | 694±2.70 | 184±3.39 | 238±1.58 | 129±0.83 |
| **Treatment 60 days** | 259.4±1.01* | 1.748±0.02 | 0.0416±0.001 | 0.776±0.01 | 1.402±0.017 | 4.1±0.14 | 0.704±0.01* | 0.21±0.03 | 2±0.27 | 686±4.2* | 174.2±7.46* | 232±3..8* | 121.2±3.76* |
| **TW 90 days** | 260±6.7 | 1.77±0.04 | 0.0496±0.007 | 0.818±0.06 | 1.476±8.8* | 4.5±0.53* | 0.752±0.07 | 0.232±0.39* | 2.34±0.36* | 690.6±7.5 | 181.2±6.24 | 235.4±4.3 | 124±4.5 |

**TW – Treatment withdrawal, SV – Seminal vesicle, VP – Ventral prostate (* p<0.05)**

**Table 1C: The body weight and organs weight in rats treated orally with aqueous extract of *Amaranthus spinosus* @ 500 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Body Weight (g) | Brain (g) | Thyroid (g) | Heart (g) | Lungs (g) | Liver (g) | Spleen (g) | Adrenal (g) | Kidney (g) | Testis (mg/100g bw) | Epididymis (mg/100g bw) | S V (mg/100g bw) | V P (mg/100g bw) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 256±2.28 | 1.73±0.02 | 0.040±0.01 | 0.758±0.02 | 1.38±0.01 | 4.0±0.10 | 0.674±0.02 | 0.18±0.02 | 1.76±0.11 | 694±2.70 | 184±3.39 | 238±1.58 | 129±0.83 |
| **Treatment 60 days** | 262.2±3.31* | 1.746±0.02 | 0.0422±0.001 | 0.746±0.02 | 1.42±0.04 | 4.28±0.27 | 0.71±0.03 | 0.22±0.04 | 2.1±0.27 | 678.4±4.4*** | 169.4±6.4** | 223±3.2*** | 116±7.0** |
| **TW 90 days** | 263±6..9 | 1.816±0.05* | 0.0488±0.007 | 0.786±0.044 | 1.47±0.07* | 4.66±0.46* | 0.744±0.04* | 0.252±0.04* | 2.42±0.37** | 687.6±7.52 | 183.2±6.70 | 232.6±4.49* | 124.6±5.00 |

**TW – Treatment withdrawal, SV – Seminal vesicle, VP – Ventral prostate (* p<0.05, ** p<0.01, *** p<0.001)**

**Table 1D: The body weight and organs weight in rats treated orally with aqueous extract of *Amaranthus spinosus* @ 1000 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Body Weight (g) | Brain (g) | Thyroid (g) | Heart (g) | Lungs (g) | Liver (g) | Spleen (g) | Adrenal (g) | Kidney (g) | Testis (mg/100g bw) | Epididymis (mg/100g bw) | S V (mg/100g bw) | V P (mg/100g bw) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 256±2.28 | 1.73±0.02 | 0.040±0.01 | 0.758±0.02 | 1.38±0.01 | 4.0±0.10 | 0.674±0.02 | 0.18±0.02 | 1.76±0.11 | 694±2.70 | 184±3.39 | 238±1.58 | 129±0.83 |
| **Treatment 60 days** | 265.8±6.49* | 1.754±0.07 | 0.0464±0.00 | 0.74±0.05 | 1.418±0.03 | 4.2±0.45 | 0.722±0.04 | 0.23±0.04 | 2.2±0.42 | 668.4±9.04*** | 159.8±6.36*** | 211.8±6.04*** | 109±.22*** |
| **TW 90 days** | 259±4.2 | 1.794±0.05* | 0.049±0.01* | 0.804±0.04 | 1.48±0.04* | 4. 88±0.57* | 0.73±0.05 | 0.256±0.04* | 2.52±0.43** | 681±5.54** | 177.4±6.40 | 229±4.4** | 120.2±6.30* |

**TW – Treatment withdrawal, SV – Seminal vesicle, VP – Ventral prostate (* p<0.05, ** p<0.01, *** p<0.001)**

**Biochemical Investigations:**

**Reproductive tissue biochemistry:**

**Table 2A: Reproductive tissue biochemistry of rats treated orally with the aqueous extract of *Amaranthus spinosus* @ 125 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Cholesterol (mg/g) | LDH (U/mg) | Sialic Acid (mg/g) | α-Glucosidase (mgPNP/mg protein) | Fructose (mg/g) | ACP (B.U.) |
|---|---|---|---|---|---|---|
| Control | 5.96±0.46 | 28.2±2.5 | 6.84±0.44 | 98.28±4.3 | 6.94±0.74 | 5.32±0.64 |
| Treatment 60 days | 5.84±0.24 | 27.7±2.3 | 6.76±0.60 | 97.12±3.28 | 6.74±0.63 | 5.2±0.56 |
| TW 90 days | 6.08±0.48 | 29.04±1.72 | 6.68±0.34 | 98.1±2.1 | 7.04±0.47 | 5.5±0.46 |

**TW – Treatment withdrawal**

**Table 2B: Reproductive tissue biochemistry of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 250 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Cholesterol (mg/g) | LDH (U/mg) | Sialic Acid (mg/g) | α-Glucosidase (mgPNP/mg protein) | Fructose (mg/g) | ACP (B.U.) |
|---|---|---|---|---|---|---|
| Control | 5.96±0.46 | 28.2±2.5 | 6.84±0.44 | 98.28±4.3 | 6.94±0.74 | 5.32±0.64 |
| Treatment 60 days | 5.30±0.29***** | 26.66±2.1 | 6.32±0.31 | 96.22±2.9 | 6.08±0.26***** | 4.50±0.37***** |
| TW 90 days | 5.76±0.52 | 27.06±2.0 | 6.54±0.79 | 97.36±3.5 | 6.86±0.60 | 5.2±0.56 |

**TW – Treatment withdrawal (* $p<0.05$)**

**Table 2C: Reproductive tissue biochemistry of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 500 mg/Kg body wt./day (Values wtih SEM of 5 animals)**

| Treatment Schedule | Cholesterol (mg/g) | LDH (U/mg) | Sialic Acid (mg/g) | α-Glucosidase (mgPNP/mg protein) | Fructose (mg/g) | ACP (B.U.) |
|---|---|---|---|---|---|---|
| Control | 5.96±0.46 | 28.2±2.5 | 6.84±0.44 | 98.28±4.3 | 6.94±0.74 | 5.32±0.64 |
| Treatment 60 days | 4.9±0.29** | 25.1±1.2* | 5.7±0.71* | 92.62±2.1* | 5.38±0.44** | 4.26±0.27** |
| TW 90 days | 5.38±0.48 | 26.56±1.6 | 5.94±0.98 | 96.06±2.58 | 5.92±0.73 | 4.86±0.63 |

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

**Table 2D: Reproductive tissue biochemistry of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 1000 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Cholesterol (mg/g) | LDH (U/mg) | Sialic Acid (mg/g) | α-Glucosidase (mgPNP/mg protein) | Fructose (mg/g) | ACP (B.U.) |
|---|---|---|---|---|---|---|
| Control | 5.96±0.46 | 28.2±2.5 | 6.84±0.44 | 98.28±4.3 | 6.94±0.74 | 5.32±0.64 |
| Treatment 60 days | 4.52±0.41*** | 23.98±1.2* | 5.42±0.54** | 90.84±2.3** | 4.86±0.51*** | 3.58±0.25*** |
| TW 90 days | 5.14±0.38* | 26.04±1.9 | 5.66±0.59 | 91.12±5.3 | 5.6±0.4** | 3.98±0.46** |

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

**Sperm Parameters:**

**Table 3A: Sperm parameters of rats treated orally with the aqueous extract of *Amaranthus spinosus* @ 125 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Sperm density (mill/ml) | Sperm motility (%) | Sperm viability (%) | Abnormal sperm (%) |
|---|---|---|---|---|
| Control | 25±3.03 | 77±4.02 | 57±4.3 | 24±3.2 |
| Treatment 60 days | 23.8±3.7 | 73±4.1 | 52±4.8 | 29±3.1 |
| TW 90 days | 28±5.2 | 76±4.9 | 55±4.1 | 27±1.72 |

**TW – Treatment withdrawal**

**Table 3B: Sperm parameters of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 250 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Sperm density (mill/ml) | Sperm motility (%) | Sperm viability (%) | Abnormal sperm (%) |
|---|---|---|---|---|
| Control | 25±3.03 | 77±4.02 | 57±4.3 | 24±3.2 |
| Treatment 60 days | 19±1.7* | 69±2.7* | 50±2.3* | 34±3.2** |
| TW 90 days | 22±2.4 | 73±3.2 | 53±3.7 | 30±3.2 |

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

**Table 3C: Sperm parameters of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 500 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Sperm density (mill/ml) | Sperm motility (%) | Sperm viability (%) | Abnormal sperm (%) |
|---|---|---|---|---|
| Control | 25±3.03 | 77±4.02 | 57±4.3 | 24±3.2 |
| Treatment 60 days | 16±1.8** | 46±5.6*** | 34±4.4*** | 57±3.7*** |
| TW 90 days | 21±2.4 | 67±3.7 | 46±3.5 | 30±1.04c** |

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

**Table 3D: Sperm parameters of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 1000 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Sperm density (mill/ml) | Sperm motility (%) | Sperm viability (%) | Abnormal sperm (%) |
|---|---|---|---|---|
| Control | 25±3.03 | 77±4.02 | 57±4.3 | 24±3.2 |
| Treatment 60 days | 12±2.3*** | 34±6.6*** | 23±2.4*** | 73±4.3*** |
| TW 90 days | 19±2.5* | 63±5.2** | 46±4.4* | 30±2.3* |

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

**Toxicological Investigations:**

**Hematology:**

**Table 4A: Hematology of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 125 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | RBC ($10^6$/mm$^3$) | WBC ($10^3$/mm$^3$) | Hemoglobin (g/dl) | PCV (%) | MCV ($\mu^3$) | MCH ($\mu.\mu g$) | MCHC (%) |
|---|---|---|---|---|---|---|---|
| Control | 8.22±0.14 | 10.6±0.36 | 15.8±0.22 | 38.5±0.17 | 46.9±0.60 | 19.3±0.53 | 41.1±0.70 |
| Treatment 60 days | 8.18±0.08 | 10.7±0.71 | 15.5±0.37 | 38.4±0.24 | 46.9±0.27 | 19.0±0.57 | 40.6±0.84 |
| TW 90 days | 8.36±0.15 | 10.4±0.50 | 15.7±0.86 | 38.0±0.41 | 46.0±1.16 | 18.7±1.09 | 40.9±1.93 |

**TW – Treatment withdrawal**

**Table 4B: Hematology of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 250 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | RBC (106/mm3) | WBC (103/mm3) | Hemoglobin (g/dl) | PCV (%) | MCV ($\mu^3$) | MCH ($\mu.\mu g$) | MCHC (%) |
|---|---|---|---|---|---|---|---|
| Control | 8.22±0.14 | 10.6±0.36 | 15.8±0.22 | 38.5±0.17 | 46.8±0.60 | 19.3±0.53 | 41.1±0.70 |
| Treatment 60 days | 8.16±0.12 | 10.9±0.29 | 15.4±0.32 | 38.6±0.12 | 47.1±0.78 | 18.9±0.62 | 40.9±0.38 |
| TW 90 days | 8.20±0.15 | 10.8±0.50 | 15.6±0.86 | 38.8±0.41 | 47.4±1.16 | 19.2±1.09 | 40.8±1.93 |

**TW – Treatment withdrawal**

**Table 4B: Hematology of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 500 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | RBC (106/mm3) | WBC (103/mm3) | Hemoglobin (g/dl) | PCV (%) | MCV ($\mu^3$) | MCH ($\mu.\mu g$) | MCHC (%) |
|---|---|---|---|---|---|---|---|
| Control | 8.22±0.14 | 10.6±0.36 | 15.8±0.22 | 38.5±0.17 | 46.8±0.60 | 19.3±0.53 | 41.1±0.70 |
| Treatment 60 days | 7.96±0.33 | 11.4±0.68 | 15.2±0.19** | 37.7±0.16*** | 47.2±0.34 | 19.2±0.72 | 40.8±0.50 |
| TW 90 days | 8.11±0.26 | 10.9±0.54 | 15.5±0.37 | 37.9±0.40* | 46.7±1.33 | 19.5±0.44 | 40.9±0.70 |

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

**Table 4B: Hematology of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 1000 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | RBC (106/mm3) | WBC (103/mm3) | Hemoglobin (g/dl) | PCV (%) | MCV ($\mu^3$) | MCH ($\mu.\mu g$) | MCHC (%) |
|---|---|---|---|---|---|---|---|
| Control | 8.22±0.14 | 10.6±0.36 | 15.8±0.22 | 38.5±0.17 | 46.8±0.60 | 19.3±0.53 | 41.1±0.70 |
| Treatment 60 days | 6.50±0.22*** | 13.7±0.88*** | 13.1±0.41*** | 35.6±0.62*** | 54.8±2.20*** | 20.1±1.10 | 35.9±0.66*** |
| TW 90 days | 7.58±0.53* | 11.4±0. 86* | 15.4±0.53* | 37.2±1.28* | 49.2±3.71* | 20.8±2.09 | 49.9±1. 86 |

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

**Clinical chemistry:**

**Table 5A: Serum clinical biochemistry of rats treated orally with the aqueous extract of *Amaranthus spinosus* @ 125 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Protein (g/dl) | Glucose (mg/dl) | Cholesterol (mg/dl) | Creatinine (mg/dl) | SGPT (IU/L) | SGOT (IU/L) | Bilirubin (IU/L) | Urea (mg/dl) | TGL (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 7.34±0.11 | 98.8±4.6 | 121±6.2 | 0.942±0.05 | 34.68±2.7 | 71.98±1.9 | 12.02±0.3 | 18.09±0.35 | 63.16±3.4 |
| Treatment 60 days | 7.48±0.62 | 97.2±6.5 | 119±5.7 | 0.914±0.02 | 37.4±3.7 | 73.12±2.6 | 11.84±0.64 | 18.96±1.2 | 62.4±3.4 |
| TW 90 days | 7.42±0.58 | 98±3.1 | 120±2.5 | 0.926±0.05 | 35.4±5.1 | 72.6±1.9 | 11.92±0.60 | 18.88±1.03 | 63±5.8 |

**TW – Treatment withdrawal**

**Table 5B: Serum clinical biochemistry of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 250 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Protein (g/dl) | Glucose (mg/dl) | Cholesterol (mg/dl) | Creatinine (mg/dl) | SGPT (IU/L) | SGOT (IU/L) | Bilirubin (IU/L) | Urea (mg/dl) | TGL (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 7.34±0.11 | 98.8±4.6 | 121±6.2 | 0.942±0.05 | 34.68±2.7 | 71.98±1.9 | 12.02±0.3 | 18.09±0.35 | 63.16±3.4 |
| Treatment 60 days | 8.3±0.45** | 95.4±6.1 | 98.46±6.4*** | 0.938±0.03 | 39.56±4.2 | 80.8±5.0 | 11.98±0.35 | 19.9±0.86 | 56.31±1.6 |
| TW 90 days | 7.9±0.56 | 96.8±5.4 | 105.14±6.4** | 0.954±0.02 | 35.4±3.3 | 76.6±4.3 | 11.86±0.45 | 19.5±0.74 | 61.24±3.6 |

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

**Table 5C: Serum clinical biochemistry of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 500 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Protein (g/dl) | Glucose (mg/dl) | Cholesterol (mg/dl) | Creatinine (mg/dl) | SGPT (IU/L) | SGOT (IU/L) | Bilirubin (IU/L) | Urea (mg/dl) | TGL (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 7.34±0.11 | 98.8±4.6 | 121±6.2 | 0.942±0.05 | 34.68±2.7 | 71.98±1.9 | 12.02±0.3 | 18.09±0.35 | 63.16±3.4 |
| Treatment 60 days | 9.26±0.76*** | 91.6±6.9 | 95.8±8.6*** | 0.948±0.04 | 41.96±1.96** | 80.16±5.5** | 12.78±0.26 | 22.44±0.86*** | 51.7±6. 8** |
| TW 90 days | 8.16±0.63* | 95±8.0 | 112.6±5.7* | 0.954±0.04 | 40.36±3.6* | 79. 80±6. 8* | 12.68±0.44 | 19.66±1.16* | 57.06±4.6* |

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

**Table 5D: Serum clinical biochemistry of rats treated orally with aqueous extract of *Amaranthus spinosus* @ 1000 mg/Kg body wt./day (Values with SEM of 5 animals)**

| Treatment Schedule | Protein (g/dl) | Glucose (mg/dl) | Cholesterol (mg/dl) | Creatinine (mg/dl) | SGPT (IU/L) | SGOT (IU/L) | Bilirubin (IU/L) | Urea (mg/dl) | TGL (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 7.34±0.11 | 98.8±4.6 | 121±6.2 | 0.942±0.05 | 34.68±2.7 | 71.98±1.9 | 12.02±0.3 | 18.09±0.35 | 63.16±3.4 |
| Treatment 60 days | 10.02±0.19*** | 84.08±0.58*** | 90.8±2.5*** | 0.934±0.02 | 53.24±1.7*** | 98.98±5.9*** | 13.02±0.21 | 26.1±0.61*** | 41.82±2.02*** |
| TW 90 days | 8.36±0.82* | 92.72±4.02* | 107±7.5* | 0.95±0.04 | 39.34±3.23* | 79.76±6.15* | 13.12±0.46 | 22.72±3.9* | 53.16±5. 8* |

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

**Figures:**

**Fig. 1a: 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.**

**Fig. 1b: 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**

**Fig. 1c: 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. X100**

**Fig. 1d: 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**

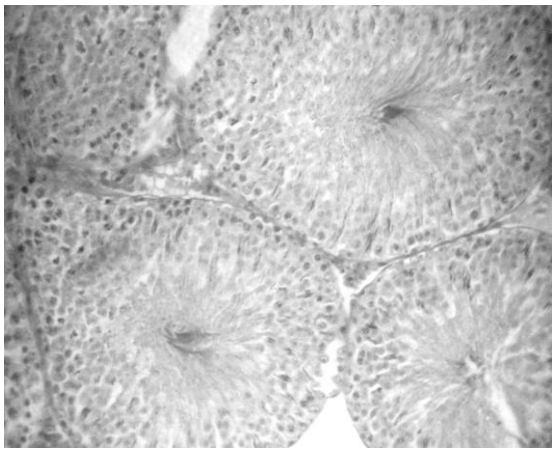

Fig.1a

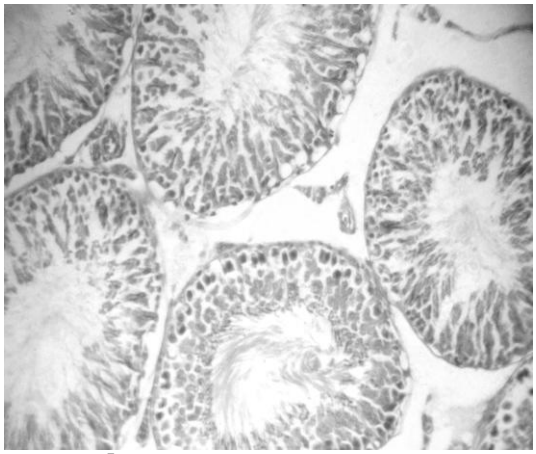

Fig.1b

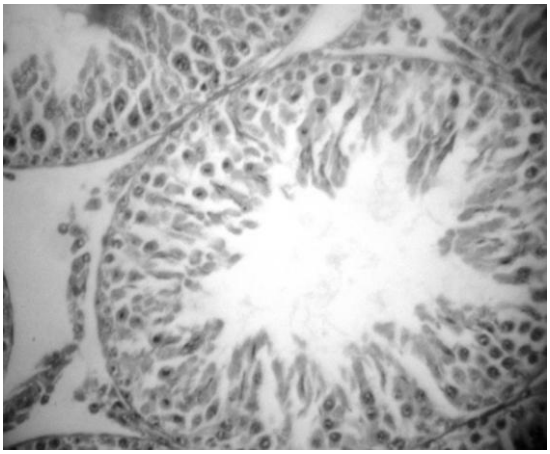

Fig.1c

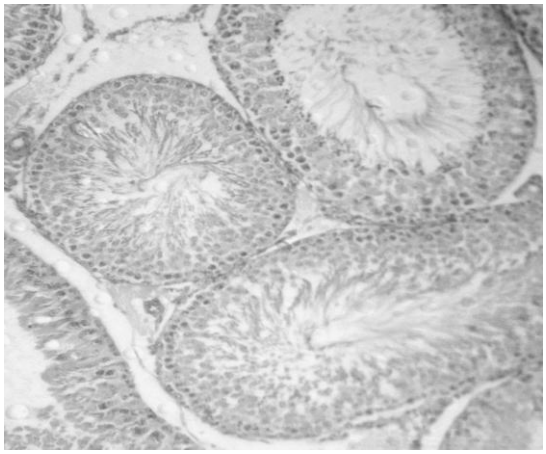

Fig.1d

**Fig. 2a: Histology of the epididymis in control rat. The epithelium is well defined with principal and basal cells. The lumen is packed with spermatozoa. X100.**

**Fig. 2b: Histology of the epididymis in rat treated with aqueous extract of *Amaranthus spinosus* at 500 mg/kg.bw/day for 60 days. The lumen shows fewer spermatozoa. X100**

**Fig. 2c: Histology of the epididymis in rat treated with aqueous extract of *Amaranthus spinosus* at 1000 mg/kg.bw/day for 60 days. The intertubular elements occupy more space. The lumen of few tubules is devoid of spermatozoa. X100**

**Fig. 2d: Histology of the epididymis in rat following 90 days of treatment withdrawal. The epithelium is normal with lumen containing spermatozoa. X100**

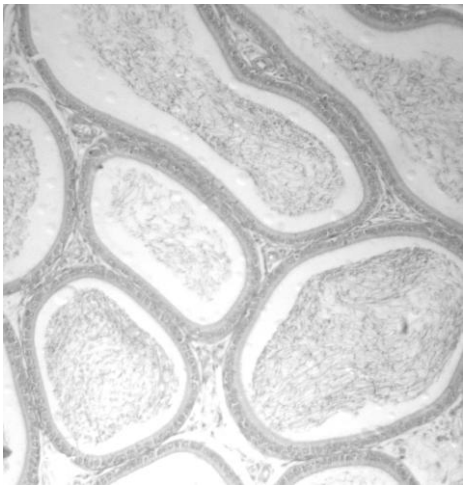

Fig.2a

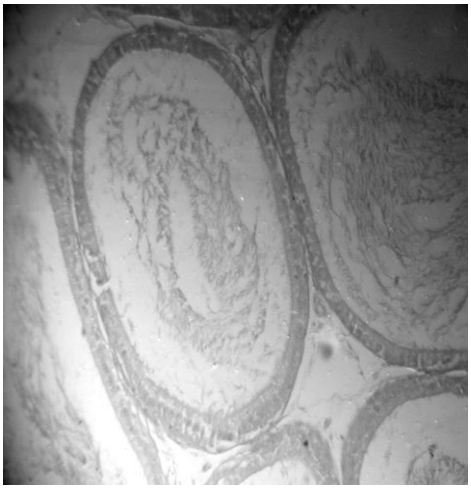

Fig.2b

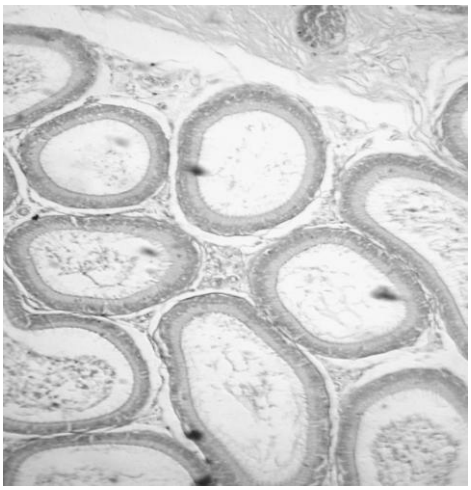

Fig.2c

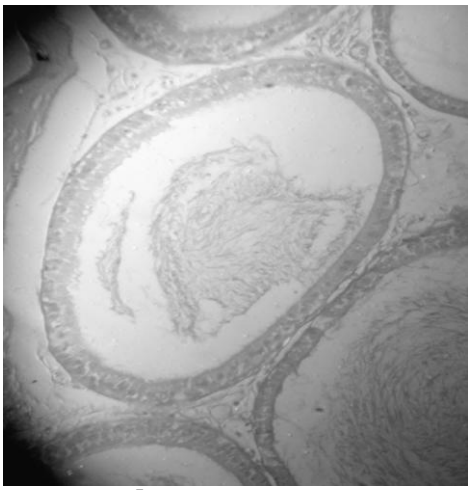

Fig.2d

**Fig. 3a: Histology of the seminal vesicle in control rat showing cryptic epithelium. The central lumen contained secretory material. X100**

**Fig. 3b: Histology of the seminal vesicle in rat treated with aqueous extract of Amaranthus *spinosus* at 500 mg/kg.bw/day for 60 days. The central lumen shows decreased secretory content. X100**

**Fig. 3c: Histology of the seminal vesicle in rat treated with aqueous extract of *Amaranthus spinosus* at 1000 mg/kg.bw/day for 60 days. The epithelium shows degenerative changes. The central lumen is devoid of secretory content. X100**

**Fig. 3d: Histology of the seminal vesicle in rat following 90 days of treatment withdrawal. The epithelium is normal with lumen containing secretory material. X100**

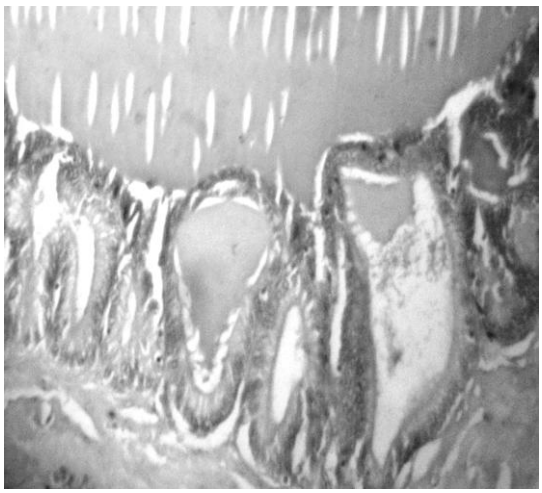

Fig.3a

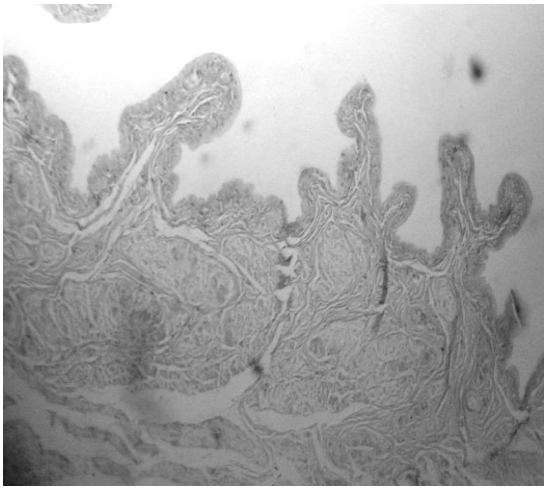

Fig.3b

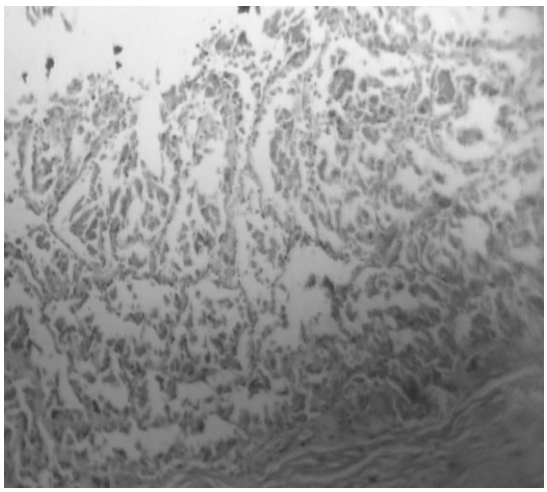

Fig.3c

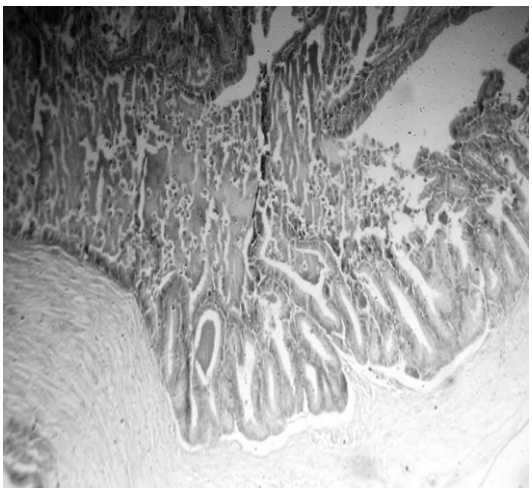

Fig.3d

**Fig. 4a: Histology of the ventral prostate in control rat showing typical lobular structure lined with columnar epithelium with secretory material in the center. X100**

**Fig. 4b: Histology of the ventral prostate in rat treated with aqueous extract of *Amaranthus spinosus* at 500 mg/kg.bw/day for 60 days. The lumen of the prostatic follicles shows less secretory material. X100**

**Fig. 4c: Histology of the ventral prostate in rat treated with aqueous extract of *Amaranthus spinosus* at 1000 mg/kg.bw/day for 60 days. The secretory material is scanty in the lumen. X100**

**Fig. 4d: Histology of the ventral prostate in rat following 90 days of treatment withdrawal. The histological features are comparable to control animals. X100**

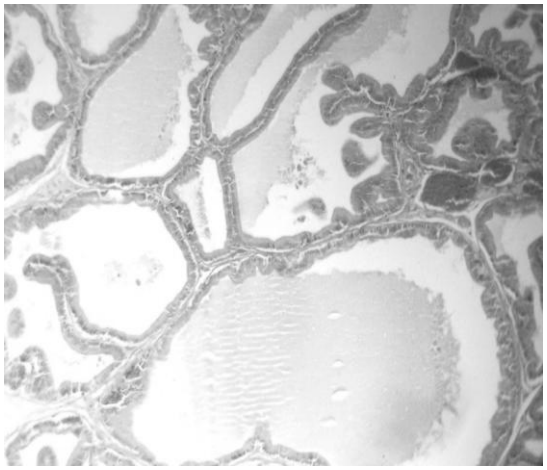

Fig.4a

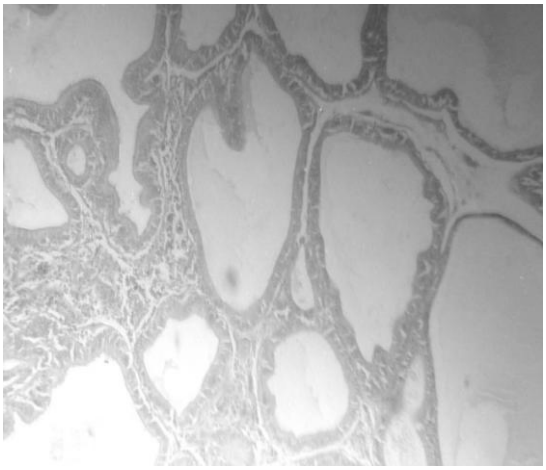

Fig.4b

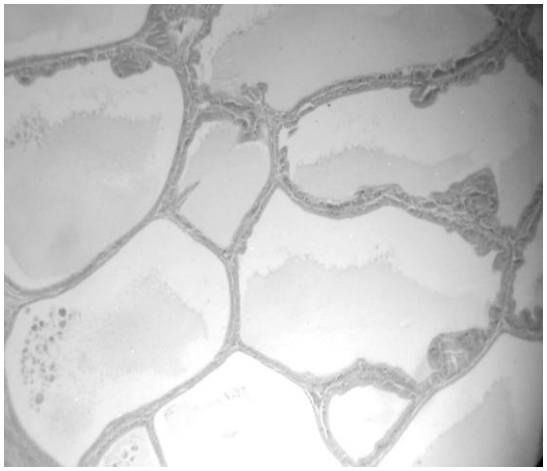

Fig.4c

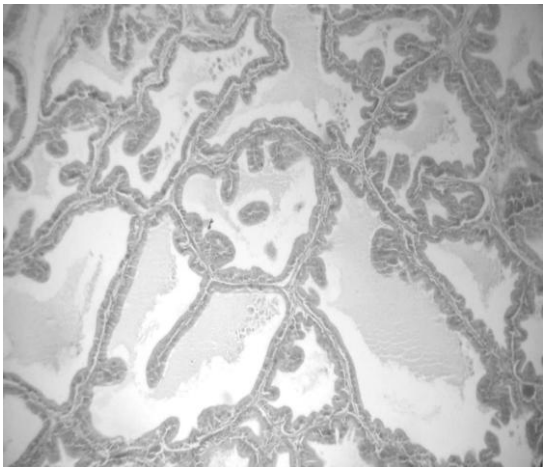

Fig.4d

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