

Study of the Effect of *A. graveolens* Seeds Ethanolic Extract on the Histological Structure of Some Organs of the Reproductive System in Male Experimental Rats

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Abstract: Medicinal plants have been widely used to enhance or regulate fertility in males. The purpose of our study is to evaluate the effects of methanol extract derived of celery (*Apium graveolens* L.) on the histological structure of some organs of the reproductive system in male experimental rats. This study was conducted on eighty experimental male rats (Sprague Dawely strain) weighing about 220 g each were used throughout the study and randomly assigned to four experimental groups of 20 rats each. Group I received normal saline (0.5 mL/kg) and serves as control. Group II is the vehicle groups. Group III and IV-gavaged daily for 30 days with 1 mL of the ethanol extract at doses of 213 mg/kg and 425 mg/kg body wt. After 30 days of treatments, under light ether anesthesia 24 h after the last treatment all rats were sacrificed and some organs as seminiferous tubules, tests, liver and kidney surgically removed weighed and a part of each was fixed in 10% formaldehyde for histological processes. The results of the effect of *A. graveolens* seeds ethanolic extract on diameter of seminiferous tubules showed that the treated groups showed a remarkable decrease in the diameter of seminiferous tubules in compression with control and vehicle groups. Light microscopic examination of hematoxylin and eosin stained sections of control and vehicle groups showed that the testicular tissues have many seminiferous tubules showing germinal epithelium with normal spermatogenesis process occur. The treated groups (213 mg/kg) showed complete loss of germinal cells in addition to reduction of the spermatogenesis process compared to the control group while at 425 mg/kg dose level sections showed complete loss of germinal cells and severely damage in the seminiferous tubules. However, results of the effect of *A. graveolens* seeds ethanolic extract on percentage of normal and abnormal seminiferous tubules are presented in a marked decrease in the percentage of normal seminiferous tubules appeared in both treated groups from 11 and 20% for 425 and 213 mg/kg, respectively, compared to the control group 57% and vehicle group 54%. Moreover, the abnormal tubules in both treated groups increased compared to the control and vehicle groups. Examination of histological sections through the liver of the control and vehicle groups showed normal hepatocytes that were arranged into hepatic plates; also both treated groups do not show any change. Histological examination of the cortex region of the kidney of the control and vehicle groups showed normal intact glomeruli and both proximal and distal tubules. Moreover, the treated groups at two dose level do not show any change. From the present study we conclude that the ethanolic extract of *A. graveolens* seed may act as antifertility agent. This is supported by the results that showed a decrease in the fertility parameters (sperm motility and sperm count), testosterone level, protein content of the testes, weight of the testes and seminal vesicle, diameter of seminiferous tubules and fertility rate. Histology of the testes of the treated groups showed a complete loss of the germ cells with arrest in the spermatogenesis process. Also, the 425 mg/kg treated group showed severe damage in the seminiferous tubules compared to the control and vehicle groups. On the other hand, histological examination of both liver and kidney of the treated groups does not show any signs of changes. These indicate that the ethanolic extract of *A. graveolens* seed may not have any signs of toxicity. Examination of histological sections through the liver of the control and vehicle groups showed normal hepatocytes that were arranged into hepatic plates; also both treated groups do not show any change. Histological examination of the cortex region of the kidney of the control and vehicle groups showed normal intact glomeruli and both proximal and distal tubules. Moreover, the treated groups at two dose level do not show any change. From the present study we conclude that the ethanolic extract of *A. graveolens* seed may act as antifertility agent. This is supported by the results that showed a decrease in the fertility parameters (sperm motility and sperm count), testosterone level, protein content of the testes, weight of the testes and seminal vesicle, diameter of seminiferous tubules and fertility rate. Histology of the testes of the treated groups showed

a complete loss of the germ cells with arrest in the spermatogenesis process. Also, the 425 mg/kg treated group showed severe damage in the seminiferous tubules compared to the control and vehicle groups. On the other hand, histological examination of both liver and kidney of the treated groups does not show any signs of changes. Examination of histological sections through the liver of the control and vehicle groups showed normal hepatocytes that were arranged into hepatic plates; also both treated groups do not show any change. Histological examination of the cortex region of the kidney of the control and vehicle groups showed normal intact glomeruli and both proximal and distal tubules. Moreover, the treated groups at two dose level do not show any change. These indicate that the ethanol extract of *A. graveolens* seed may not have any signs of toxicity.

Key words: *A. graveolens* seed, fertility, toxicity, seminiferous tubules, histological examination, proximal

INTRODUCTION

Herbal drug therapy is a common practice adopted in folk and alternative medicine and has been used in the treatment of various disorders, since, ancient times (Nabavizadeh *et al.*, 2009). According to Ekor (2014), about 80% of individuals from developing countries use traditional medicine for their primary healthcare need (Ekor, 2014). Jordan is a country rich in flora regarding the number of plant species (Oran, 2014). It was recorded that 20% of the total flora of Jordan is medicinal plants (Oran and Al-Eisawi, 2015) which are used in folk medicine and can be used in pharmaceutical industry. Despite the deficiency in the evidence-based safety and efficacy of herbal medicine, herbal drug utilization has been increasing in the developing countries including Jordan (Akour *et al.*, 2016). According to a survey conducted by Bardaweel (2014), 92% of males with infertility problems in Jordan resort to herbalists to treat their problems (Bardaweel, 2014).

Many drugs could be beneficially or adversely affected the reproductive functions (Al-Snafi *et al.*, 2003; Al-Snafi *et al.*, 2007; Fathi *et al.*, 2010; Al-Snafi *et al.*, 2011; Al-Snafi *et al.*, 2013, 2014; Al-Snafi, 2013a-c; Al-Snafi, 2014; Al-Snafi, 2015a-c; Al-Gazi *et al.*, 2016; Al-Snafi *et al.*, 2015a, b). Furthermore, a wide range of medicinal plants also exerted reproductive effects. In more previous studies Al-Snafi (2015a-c), it mentioned that many plants possessed reproductive effects on both males and females reproductive systems these plants included *Achillea santolina*, *Ailanthus altissima*, *Alhagi maurorum*, *Allium cepa*, *Althaea rosea*, *Ammannia baccifera*, *Anthemis nobelis*, *Anethum graveolens*, *Arachis hypogaea*, *Arctium lappa*, *Asclepias curassavica*, *Asplenium trichomanes*, *Avena sativa*, *Bacopa monniera*, *Bryophyllum calycinum*, *Caesalpinia crista*, *Calendula officinalis*, *Calotropis procera*, *Carum carvi*, *Capsella bursa-pastoris*, *Carthamus tinctorius*, *Chenopodium album* and Date palm. These studies represented a second part of medicinal plants affected the functions of reproductive systems in males and females. The hydroalcoholic extract (300 mg/kg/day intraperitoneally, for 20 days) of *Achillea*

santolina caused histological alterations in the seminiferous tubules included disorganized germ epithelium, exfoliation of immature germ cells, germ cell necrosis and increased number of metaphases in germinal epithelium of seminiferous tubules in mice. The researchers concluded that *Achillea santolina* exerted antispermatogenic effect. The hydroalcoholic extract (300 mg/kg/day intraperitoneally, for 20 days) of *Achillea santolina* caused histological alterations in the seminiferous tubules included disorganized germ epithelium, exfoliation of immature germ cells, germ cell necrosis and increased number of metaphases in germinal epithelium of seminiferous tubules in mice. The researchers concluded that *Achillea santolina* exerted antispermatogenic effect (Al-Snafi, 2013a-c). Other study showed that the aqueous extract of *Arctium lappa* L. roots enhanced sexual behavior in male rats. Oral administration of *Arctium lappa* L. roots extract at 600 and 1,200 mg/kg body weight significantly increased the frequencies of mount intromission and ejaculation frequency ($p < 0.05$). Administration of the extract also reduced the post-ejaculatory interval (Matsumoto *et al.*, 2006; JianFeng *et al.*, 2012; Farnsworth *et al.*, 1975; Al-Snafi, 2014). Other findings showed that the *Bacopa monniera* extracts caused reversible suppression of spermatogenesis and fertility. The treatment caused reduction in motility and viability of the sperms and reduced the number of spermatozoa in cauda epididymidis and testis and caused alterations in the seminiferous tubules in mice (Singh and Singh, 2009; Al-Snafi, 2013a-c). *Capsella bursa-pastoris*, dried and ground was added at rates of 20 and 40% to the stock diet of male and female mice, found that at the 40% level, both materials impeded ovulation and produced temporary infertility in males and females (Al-Snafi, 2015a-c). The effects of aqueous extract of *Carthamus tinctorius* was tested on mouse spermatogenesis. Histopathological criteria such as epithelial vacuolization, sloughing of germ and detachment were significantly decreased in *Carthamus tinctorius* L. treated mice ($p < 0.001$). *Carthamus tinctorius* extract induced formation of multinucleated giant cells in the germinal epithelium. It also caused a significant decrease in seminiferous tubule

diameter, somniferous epithelium height and maturation arrest ($p < 0.001$). Accordingly, *Carthamus tinctorius* extract has toxic effects on mouse testicular tissue and it was recommended to be use with caution with reproductive problem (Mirhoseini *et al.*, 2012; Al-Snafi, 2015a-c). The effect of ethanol Extract of *Cistanche Tubulosa* (Schenk) R. Wight stem (CTE) was studied on hormone levels and testicular steroidogenic enzymes in rats. It appeared that the administration of CTE (0.4 and 0.8 g/kg) increased sperm count (2.3 and 2.7 folds) and sperm motility (1.3 and 1.4 folds) and decreased the abnormal sperm (0.76 and 0.6 folds), respectively. The serum level of progesterone and testosterone in rats was also increased by CTE administration ($p < 0.05$) (Wang *et al.*, 2016). The weights of seminal vesicle and prostate gland of castrated young rats were significantly increased by administration of alcohol soluble extract from the decoction of *Cistanche tubulosa*. The phagocytic function of intra-abdominal macrophage in mice was activated by the decoction of *Cistanche tubulosa* (Zong *et al.*, 1996). Chaturvedi *et al.* (2003) Chaturvedi M, Mali PC and Ali Esmail Al-Snafi, founded that a crude 50% ethanol extract of *Citrullus colocynthis* Schrad was administered orally to male albino rats for evaluation of antifertility effects. The animals were divided into five groups: group A was a vehicle-treated control group; treatment groups B-D received 100 mg/kg/day *Citrullus colocynthis* extract for periods of 20, 40 and 60 days, respectively and group E animals received the extract at dose of 100 mg/kg/day for 60 days followed by 60 days of recovery. For androgenicity evaluation of the extract, the animals were divided into four groups: group F animals were castrated 30 days before the experiment to serve as controls and group G-I were subjected to castration 30 days before the experiments, followed by administration of fruit extract (100 mg/kg/day po), testosterone propionate (0.01 mg/rat/alternate days) and fruit extract along with testosterone propionate, respectively, for 30 days. Significant reduction of cauda epididymis sperm motility and density, number of pups, fertility and circulatory levels of testosterone were observed in all treatment groups. The weights of testes, epididymis, seminal vesicle and prostate were significantly decreased in groups B-D. The weights of all organs in the different groups of the androgenicity study were markedly decreased in group F when compared with group A in group G when compared with group F and in group I when compared with group H and increased in group H when compared with group F. The serum testosterone levels also showed a similar pattern. The concentration of testicular cholesterol was significantly elevated while protein, sialic acid, acid and alkaline phosphates concentrations were decreased. The histoarchitecture of the testes showed degenerative changes in the seminiferous epithelium, arrest of

spermatogenesis at the secondary spermatocyte stage, cytolysis and the lumen filled with eosinophilic material. Histometric parameters (except Sertoli cell) revealed that the nuclear area and the number of round spermatids were markedly altered. All altered parameters restored to normal in group E. No changes were observed in body weight, litter size, hematology and serum biochemistry. The researchers concluded that 50% ethanol extract of *Citrullus colocynthis* showed an antiandrogenic nature, thereby reduced infertility in male albino rats (Chaturvedi *et al.*, 2003; Al-Snafi, 2016). The effect of administration of aqueous extract of entire plant of *Cynodon dactylon* for 30 days on reproductive hormones and reproductive organ weight of female was studied in Wistar rats. Administration of the extract produced significant increase ($p < 0.001$) in the serum estradiol concentration whereas follicle stimulating and luteinizing hormones were significantly ($p < 0.001$) reduced. Furthermore, a significant increase ($p < 0.001$) in the weight of the uterus and significant decrease in the weight of the ovaries ($p < 0.001$) was observed in the treated group when compared to the control group. In addition, the estrous cycle was found to be irregular and disturbed (Nayanatara *et al.*, 2012a, b). The previous reviewed studies showed that the effects of the medicinal plants on the functions of reproductive systems in males and females to be utilize in medical applications as a result of effectiveness and safety. The purpose of our study is evaluation the effect of *A. graveolens* seeds ethanolic extract on the histological structure of some organs of the reproductive system and some hormones in male experimental rats.

MATERIALS AND METHODS

Plant processing: Brown carmocar seeds of *A. graveolens* were purchased from the local market (Amman). The seeds were planted in the greenhouse of the Department of Biological Sciences, Faculty of Science University of Jordan. The plant was taxonomically identified by direct comparison with authentic sample and with the help of Prof. Dawoud Al-Eisawi, Department of Biological Sciences, University of Jordan. A voucher specimen (No. APO-05) was deposited at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan. *A. graveolens* seeds (3 kg) were finely, powdered and infused using hot water overnight. Plant materials were then extracted by Soxhlet apparatus using 96% ethanol for 2 h. The solvent was then distilled off under reduced pressure below 50°C using Rotavapor. The dark brown residual extract which equals to 188 g was kept in the refrigerator at 4°C until use. The yield of the ethanol extract was 6.26%.

TLC screening: Plant extracts were applied to pre-coated TLC silica gel plates (silica gel 60 F 254, AluGram,

Table 1: Phytochemical screening of ethanol extracts of *Apium graveolens* L.

Compounds	Ethanol extract
Flavonoids	+++
Coumarins	++
Alkaloids	-
Tetranoides	+++

Germany) developed in appropriate solvent systems and visualized using different reagents according to the type of secondary metabolites under investigation. Chromatograms were examined before and after spraying under UV and daylight to detect the presence of flavonoids, coumarins, alkaloids and terpenes Table 1.

Determination of the median Lethal Dose (LD50): The toxicity of the *I. viscosa* extract was evaluated by the calculation of intraperitoneal (i.p) LD50 which determines the dose that kills 50% of animals. The LD50 in rats was determined to evaluate the proper treatment dose to be used in this study. For the LD50 determination, BALB/c male mice (weight 20-25 g) were obtained from the Animal House of Al-Ahliyya Amman University. The LD50 for the *Apium graveolens* L. extract was determined according to the method by Alawi and Jeryes. Mice were divided into eight groups (ten mice each). The doses of the plant extract ranged from 200-1000 mg/kg and were given i.p. Animal behavior was carefully observed for 2 h and the number of dead mice was counted in the experimental groups after 24 h.

Toxicity and fertility: In this part of the study, 9 weeks male Wistar rats were used. A total of twenty-four rats were randomly divided into three groups (eight rats each): group I (high dose group) received 82.95 mg/kg (1/10 of the LD50 of the acetone extract of *I. viscosa*). Group II (low dose group) rats received 41.475 mg/kg (1/20 of the LD50 of the acetone extract). Group III (control group) received the vehicle of the acetone extract (100 μ L of Dimethyl Sulfoxide (DMSO) per rat daily). Rats received their i.p treatments once a day and the treatment period lasted for sixty consecutive days in accordance with the WHO protocol (1983).

Animal model: This study was conducted on eighty experimental male rats (Sprague Dawley strain) weighing about 220 g each were used throughout the study and randomly assigned to four experimental groups of 20 rats each. Group I received normal saline (0.5 mL/kg) and serves as control. Group II is the vehicle groups. Group III and IV gavaged daily for 30 days with 1 mL of the ethanol extract at doses of 213 and 425 mg/kg body wt. After 30 days of treatments, under light ether anesthesia 24 h after the last treatment all rats were sacrificed and some organs as seminiferous tubules, tests, liver and kidney surgically removed weighed and a part of each was fixed in 10% formaldehyde for histological processes.

Experimental design: The eighty rats were randomly assigned to 8 experimental groups of 20 rats each: group I received normal saline (0.5 mL/kg) and serves as a control. Group II is the vehicle groups, group III and IV-gavaged daily for 30 days with 1 mL of the ethanol extract at doses of 213 and 425 mg/kg body wt. After 30 days of treatments, under light ether anesthesia 24 h after the last treatment all rats were sacrificed and some organs as seminiferous tubules, tests, liver and kidney surgically removed weighed and a part of each was fixed in 10% formaldehyde for histological processes.

These experiments complied with the guidelines of our animal ethics committee which was established in accordance with the internationally accepted principles for laboratory animal use and care.

Blood sample collection: Blood sample collection by the end of each experiment, the rats were reweighed, starved for 24 h and sacrificed under chloroform anesthesia. About 5 mL of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 min before centrifuging using a bench top centrifuge (Cenformix). The remaining blood sample was put in an EDTA bottle for hematological determinations.

Assessment of sperm motility and testes: One of caudal epidermal and testis was taken immediately and minced into two halves by a sharp blade, one half was taken and immersed in one mL of physiological saline and this solution was kept in 37°C. After gentle mixing a drop of this solution. It was taken on a Neubauer chamber and then assessed for sperm motility as percent this was determined by counting both motile and nonmotile spermatozoa in different fields. All the solutions and instruments that were used in this experiment were kept in an incubator at 37°C (Mali *et al.*, 2002).

Assessment of sperm count: The control and treated rats were sacrificed by cervical dislocation under light ether anesthesia and the following measurements were recorded, testicular weight, body weight, epididymis and testicular sperm count for treated and control groups. The excised left testes and epididymis from each rat was put in 20 mL of normal saline (0.9% sodium chloride) and homogenized for sperm count. The epididymis was put in 15 mL of normal saline (0.9% sodium chloride) and homogenized for epididymis sperm count. Sperm count was performed according to the method of Amann and Lambaise as follows: testis and epididymis for each rat were sectioned by disposable blade in 20 mL of normal saline in Petri dish, then minced using manual glass homogenize, the homogenate was placed in a hemocytometer chamber epididymis sperm count were evaluated and expressed as number of sperm per gram of epididymis, testicular sperm were calculated and expressed as a number of spermatids per gram of

testis. The estimate was of Daily Sperm Production (DSP) in testis per day and per gram of testis each day “efficiency” estimate on calculated based on a factor of 6.1 which is the duration of the somniferous cycle during which developing spermatozoa in the spermatid stage.

Histopathological study: Tissues were cut into small pieces (5×5×5 mm) and immersed in 3.5% formalin solution until they were used. Tissues were dehydrated by several solutions of ascending concentrations of ethanol starting from 70-100% ethanol over two-hour period for each solution. Then other two cycles in xylene were applied over 2 h period using paraffin as dissolving solution. Subsequently, the tissues were infiltrated by using paraffin wax in an oven at temperature of 53-55°C for 2 h. The tissues were embedded in paraffin wax at 53°C by the use of metal blocks. Then by the use of rotary microtome the tissues were sectioned at 5 µm. Some sections were stained with hematoxylin and eosin as described below. The other sections were stained with immunohistochemistry stains according to the procedure mentioned in section 2.8. Staining by hematoxylin and eosin was fulfilled by placing the sections in two different xylene solutions, 5 min each. Then they were hydrated through different descending concentrations of ethanol solutions (100, 90, 80 and 70%) for 3 min. After placing them in tab water, they were impregnated in Mayer’s hematoxyline solution (5 min). Subsequented with running tab water (5 min), eosin (3 min), ethanol solutions (70, 80, 90, 95, 100, 100%) 1 min each, two cycles of xylene solutions (2 min each), a drop of DPX was placed on the section then covered with a cover slip and examined under light microscope.

Hormonal analysis: All plasma samples were assayed for hormones using Enzyme-Linked Immunoassay Methodology (ELISA) and the absorbency was read at 450 nm as described previously. The ELISA Kits were obtained from Dia Metra (Italy).

Statistical analysis: The results were expressed as mean±standard deviation. Differences between control and experimental groups were estimated using students

t-test analysis. Within-group comparisons were performed by analysis of variance using the ANOVA test. Differences were considered significant, if p<0.05.

RESULTS AND DISCUSSION

Chromatograms were examined before and after spraying under UV and daylight to detect the presence of flavonoids, coumarins, alkaloids and terpenes Table 2. Upon gavaging the animals in control and vehicle groups with 1 mL of the solutions, the number of survived animals were recorded after 24 h of treatment. Animals were gavaged with one mL of the prepared doses; 30, 40, 50, 60, 70, 80, 85, 90, 95, 100 mg/20 g. After 24 h of injection, the dead and survived animals were recorded. Changes in body weight in all groups are shown in Table 3. Our data in Table 3 and Fig. 1 showed the two dose of *A. graveolens* seeds ethanolic extract 425 and 213 mg/kg, respectively, decreased the level of testosterone compared to the group I which received normal saline (0.5 mL/kg) and serves as a control and to group II which concedes the vehicle groups. The results of this study showed that from the statistical point of view, substantial increases were seen in diameter of seminiferous tubules and the spermatocytes, spermatogonia and spermatozooids (sperms in the cauda epididymis) counts in the treatment groups 3 and 4 in comparisons with the control group (p≤0.05 and p≤0.001, respectively) On the other hand, the number of spermatids was significantly increased in the treatment group 3 (213 mg/kg of celery extract) vs. control group (p≤0.05). Moreover, the mean seminiferous tubules diameter (µm) was increased in both experimental groups 2 and 3 but it was only statistically significant in group 3 when compared to control group (p≤0.05) Table 4. After 30 days of treatment with methanol extract of celery, the testis volume was increased significantly in both groups 3 and 4 in comparison with the control group (p≤0.001). Moreover, the weights of testes, cauda epididymis and vas deferens were increased but only the weight of epididymis in high dose group (425 mg/kg methanol extract of celery) was increased statistically significant (p≤0.05). The results of the effect of *A. graveolens* seeds ethanolic extract on diameter of seminiferous tubules showed that the treated groups showed a remarkable decrease in the

Table 2: Result of the LD50 experiment

Dose (mg/20 g)	30	40	50	60	70	80	85	90	95	100
Total number of the group	7	7	7	7	7	7	7	7	7	7
Number of animals died	0	1	2	1	3	1	1	6	6	7
Number of animals survived	7	6	5	6	4	6	6	1	1	0
Sg	42	35	29	24	18	14	8	2	1	0
DS	0	1	3	4	7	8	9	15	21	28
Sg+DS	42	36	32	28	15	22	17	17	22	28
Mortality = DS/(Sg+DS) X 100%	0	3	9	14	28	36	53	88	95	100

Sg: Number of mice survived at this dose and higher doses, DS: Number of mice died at this dose and lower doses

Table 3: Testosterone concentration in control, vehicle and treated groups TA

Groups	Testosterone concentration (pg/mL serum)
(Mean±SD)	
Control	(8.4±0.9)
Vehicle	(7.0±2.8)
(213 mg/kg)	(2.9±1.0)*
(425 mg/kg)	(2.1±0.9)*

*Significant value

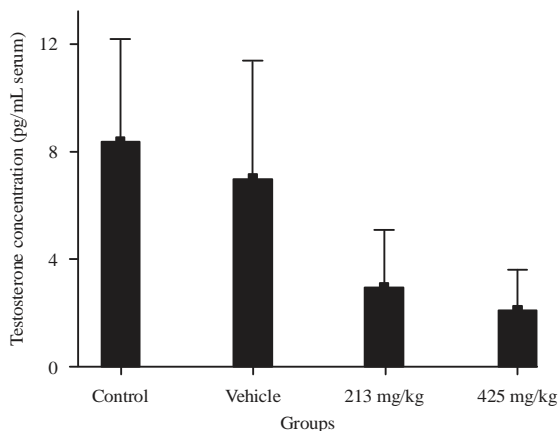


Fig. 1: Effect of *A. graveolens* extracts on testosterone concentration

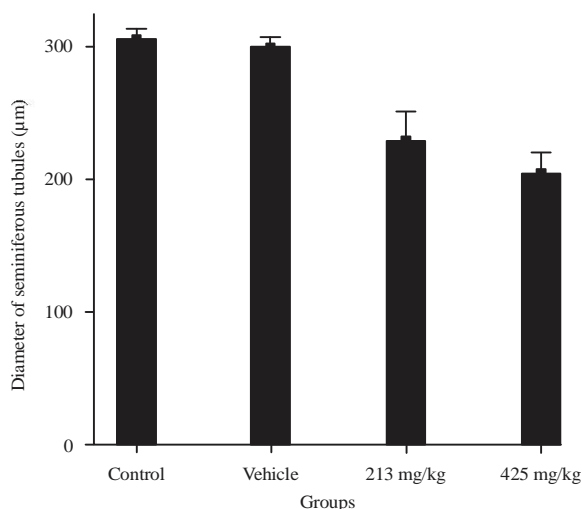


Fig. 2: Effect of *A. graveolens* extracts on seminiferous tubules diameter

diameter of seminiferous tubules from 204.8 and 229.4 µm for 425 and 213 mg/kg, respectively, to 304.9 and 298.8 µm for control and vehicle groups. Light microscopic examination of hematoxylin and eosin stained sections of control and vehicle groups showed that the testicular tissues have many seminiferous tubules showing germinal epithelium with normal spermatogenesis process occur Fig. 3-7. The treated

groups (213 mg/kg) showed complete loss of germinal cells in addition to reduction of the spermatogenesis process compared to the control group Fig. 8 and 9 while at 425 mg/kg dose level sections showed complete loss of germinal cells and severely damage in the seminiferous tubules Fig. 10 and 11. However, results of the effect of *A. graveolens* seeds ethanolic extract on percentage of normal and abnormal seminiferous tubules are presented in Table 4. A marked decrease in the percentage of normal seminiferous tubules appeared in both treated groups from 11 and 20% for 425 and 213 mg/kg, respectively, compared to the control group 57% and vehicle group 54%. Moreover, the abnormal tubules in both treated groups increased compared to the control and vehicle groups Fig. 13. Examination of histological sections through the liver of the control and vehicle groups showed normal hepatocytes that were arranged into hepatic plates; also both treated groups do not show any change (Fig. 14-21). Histological examination of the cortex region of the kidney of the control and vehicle groups showed normal intact glomeruli and both proximal and distal tubules. Moreover, the treated groups at two dose level do not show any change (Fig. 22-29).

Herbal preparation has been used in many parts of the world, since, ancient times. In recent years, their popular alternative to modern medicine has increased considerably even in developing countries (Anquez-Traxler, 2011). However, despite lack of sufficient information on the precise nature of constituents used and absence of properly documented experimental, clinical or pharmacological data, people generally, continue to equate herbal medicine with safety. So, a proper characterization, standardization, safety and efficacy evaluation of herbs is needed (De Smet, 1995). *A. graveolens* reported to have many different medical properties as described in literature review but the effect of the *A. graveolens* seeds extract on male reproductive system has not been previously investigated despite its use in folklore medicine as aphrodisiac (Malviya *et al.*, 2016). So, to clear its effect on fertility parameters (sperm motility and sperm count), testes and seminal weight, biochemical, hormonal, fertility rate, number of viable fetuses and their weights, number of reabsorption sites and histological studies of the testes, liver and kidney were investigated. Also, studying the hematological and serum biochemical profiles may clarify some of the toxicological effects of this plant extract.

In the present study the two doses of the extract were chosen to investigate the effect, of *A. graveolens* seed ethanol extracts on male fertility, based on determination of the lethal dose of the seed extracts (85/20 g). Two doses were chosen (425 and 213 mg/kg) and were administered by oral gavage needle. Since, the whole

Table 4: Normality of seminiferous tubules and their diameter

Groups	Seminiferous tubules diameter (μm)	Percent of normal seminiferous tubules (%)	Percent of abnormal seminiferous tubules (%)
Control (mean \pm SD)	(304.9 \pm 12.7)	(57 \pm 13.5)	(42 \pm 13)
Vehicle (mean \pm SD)	(298.8 \pm 12.2)	(54 \pm 12.7)	(46 \pm 13)
(213 mg/kg) (mean \pm SD)	(229.4 \pm 20.2)*	(20 \pm 14.5)*	(80 \pm 15)*
(425 mg/kg) (mean \pm SD)	(204.8 \pm 21.1)*	(11 \pm 11.7)*	(89 \pm 12)*

* The mean differences are significant at the 0.05 level

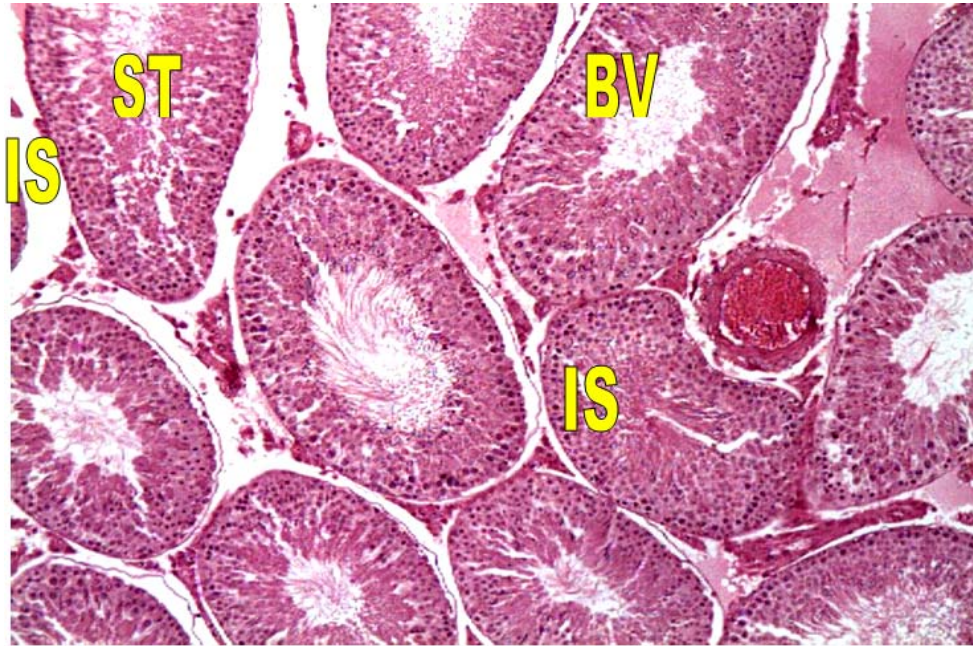


Fig. 3: Control testis showing normal Seminiferous Tubules (ST) and Blood Vessel (BV) filled with red blood cells entrapped between them. Also, Leydig cell is entrapped in the Interstitial tissue (IS) between three seminiferous tubules (Magnification: 200X)

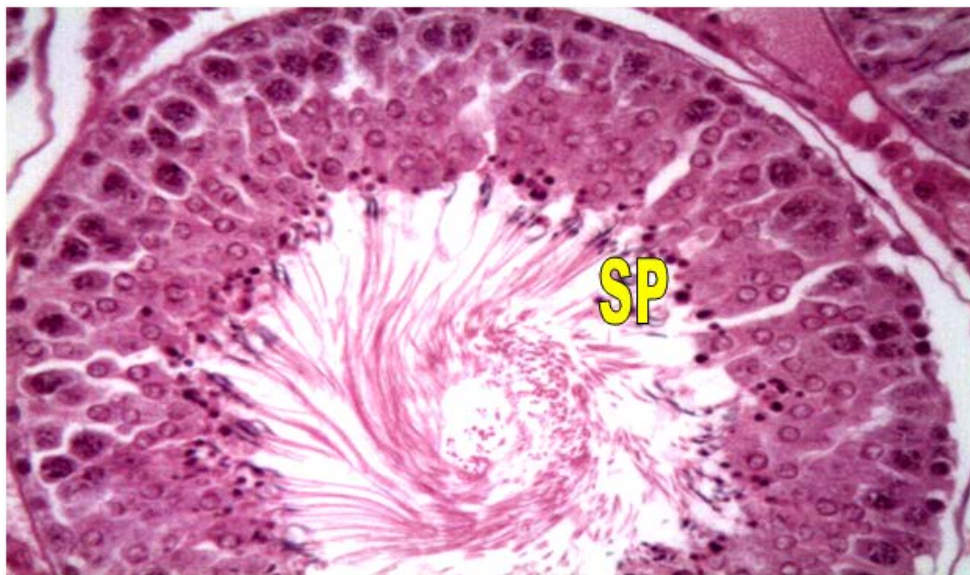


Fig. 4: Control testis showing seminiferous tubules containing germinal cells of all stages of maturation and Spermatozoa (Sp) (Magnification: 400X)

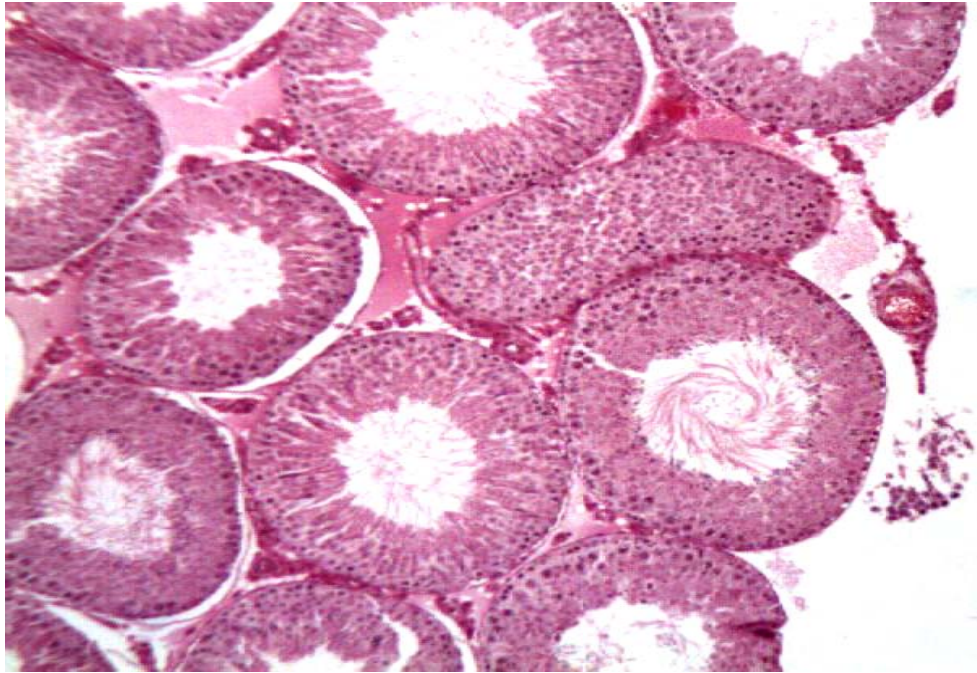


Fig. 5: Vehicle testis showing seminiferous tubules containing germinal cells of all stages of maturation and spermatozoa (Magnification: 200X)

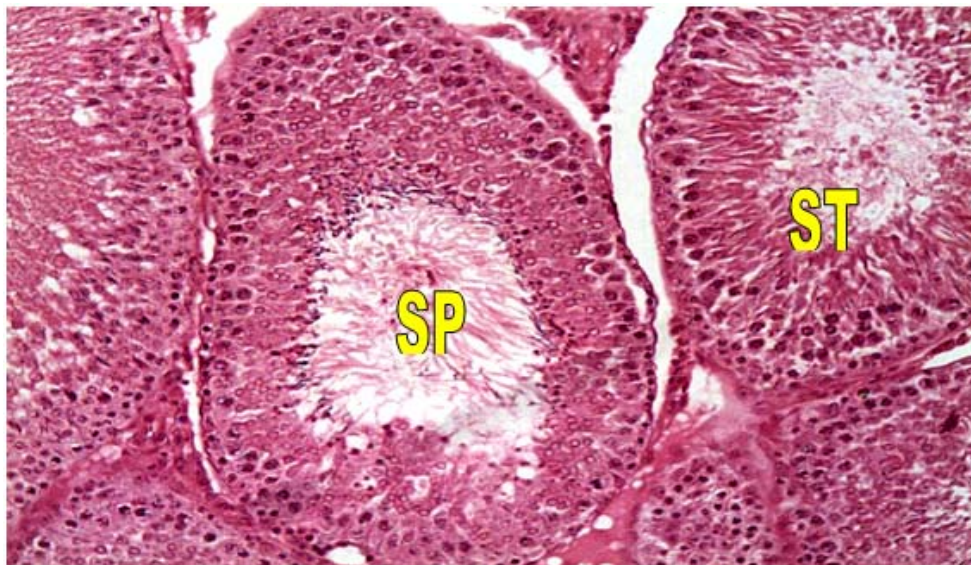


Fig. 6: Vehicle testis showing normal Seminiferous Tubules (ST) containing germinal cells of all stages of maturation and Spermatozoa (Sp). (Magnification: 400X)

spermatogenesis process requires 53 days out of which the spermatozoa spend the last 6 and 7 days in the final transit through epididymis, so, oral administration of ethanolic extract of *A. graveolens* seed to male rats for a period of sixty days will cover one complete spermatogenesis cycle (Kitajima *et al.*, 2003).

Due to, oral administration of the extract a decrease in the body weight was observed between the treated and the control groups. The exact mechanism of action for this extract in the present study is not known. However, several studies have shown that agents could cause body weight reduction through several proposed mechanisms.

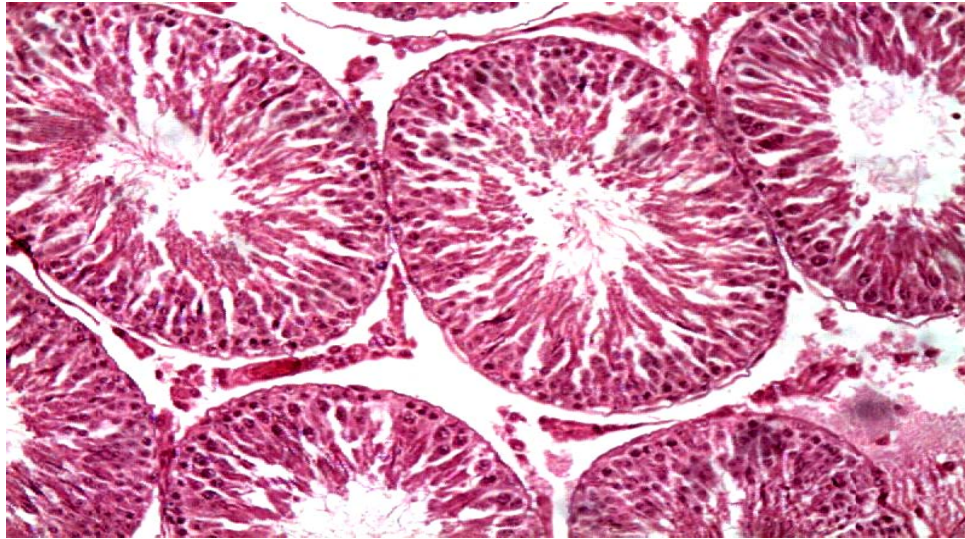


Fig. 7: Testis of treated group (213 mg/kg) showing seminiferous tubules with germinal epithelium containing different stages of maturation (Magnification: 200X)

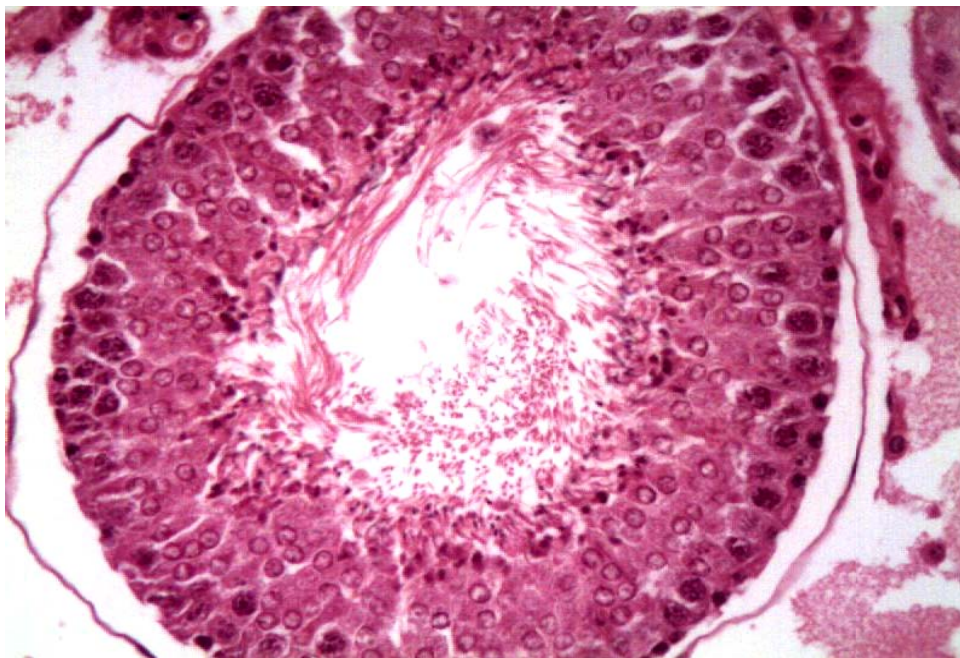


Fig. 8: Testis of treated group (213 mg/kg) showing seminiferous tubules with germinal epithelium containing different stages of spermatogenesis but less than the control (Magnification: 400X)

These include: stimulation of fat mobilization inhibition of lipoproteins lipase activity increasing energy expenditure, inhibition of absorption of nutrients from the gastrointestinal tract, suppression of the appetite and reduction of food intake (Dyer, 1994; Janqueira and Carneiro, 1983). Liver and adrenal glands did not show any changes in their weights

compared to the control. However, the kidneys showed a significant reduction in their weight at both 425 and 213 mg/kg dose levels in contrast to the control group. Salama (2001) reported that celery seeds juice is strong diuretic and they are not recommended when acute kidney problems exist (Salama, 2001). Some other studies indicated that D-Limonene

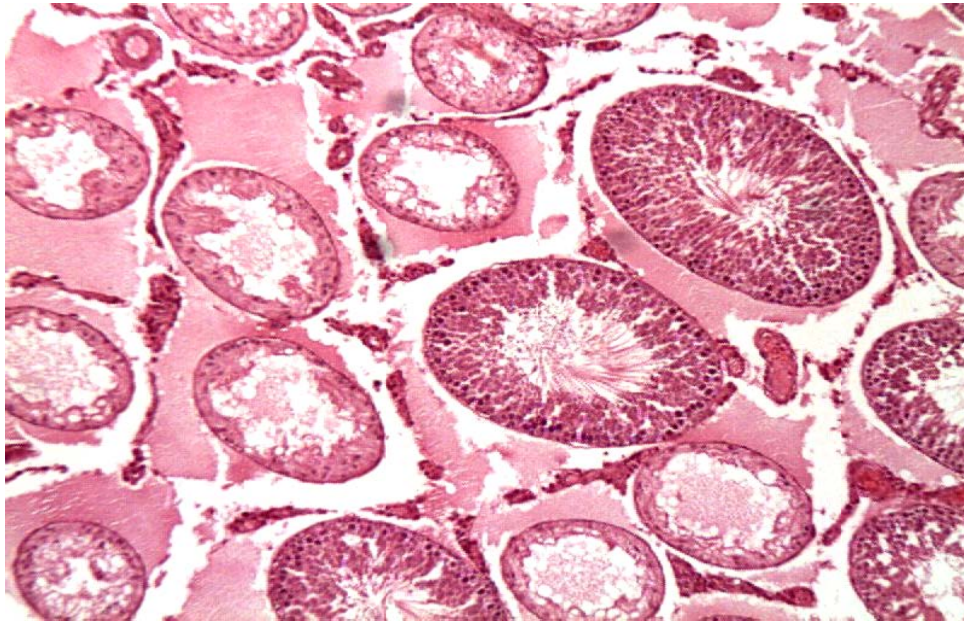


Fig. 9: Testis of treated (425 mg/kg) rats showing damaged seminiferous tubules with loss of germinal cells leaving only supportive cells (Magnification: 200X)

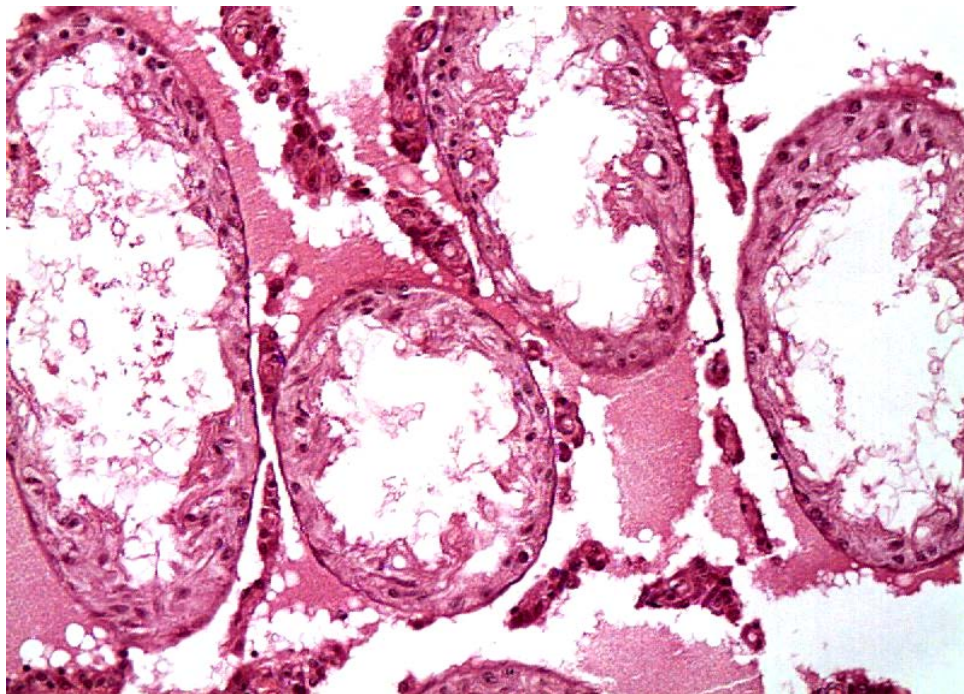


Fig. 10: Testis of treated (425 mg/kg) rats showing damaged seminiferous tubules with loss of germinal cells leaving only supportive cells (Magnification: 400X)

(monocyclic monoterpene) which is a compound, found in the Celery seeds induced hyaline nephropathy. Moreover, seeds can stimulate urine excretion in the

laboratory animals (Kobasi, 1993). In the present study, the weight of the testes of both treated group 425 and 213 mg/kg showed a significant reduction,

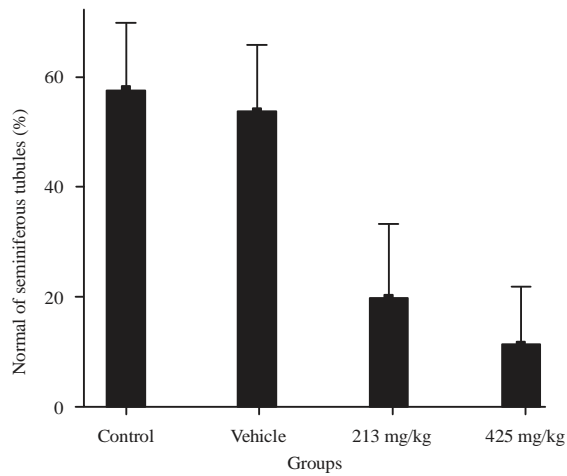


Fig. 11: Effect of *A. graveolens* extracts on seminiferous tubules normal

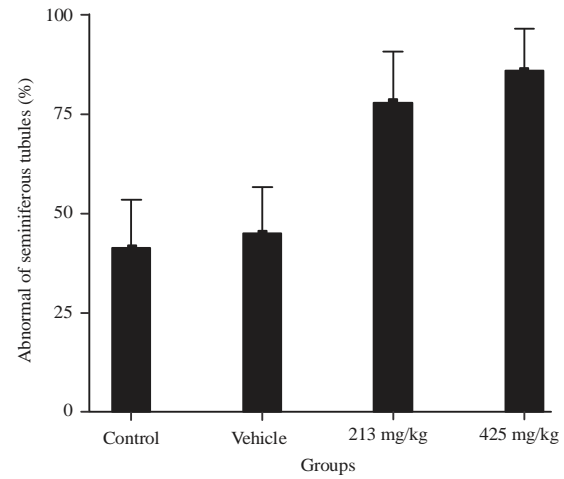


Fig. 12: Effect of *A. graveolens* extracts on seminiferous tubules abnormal

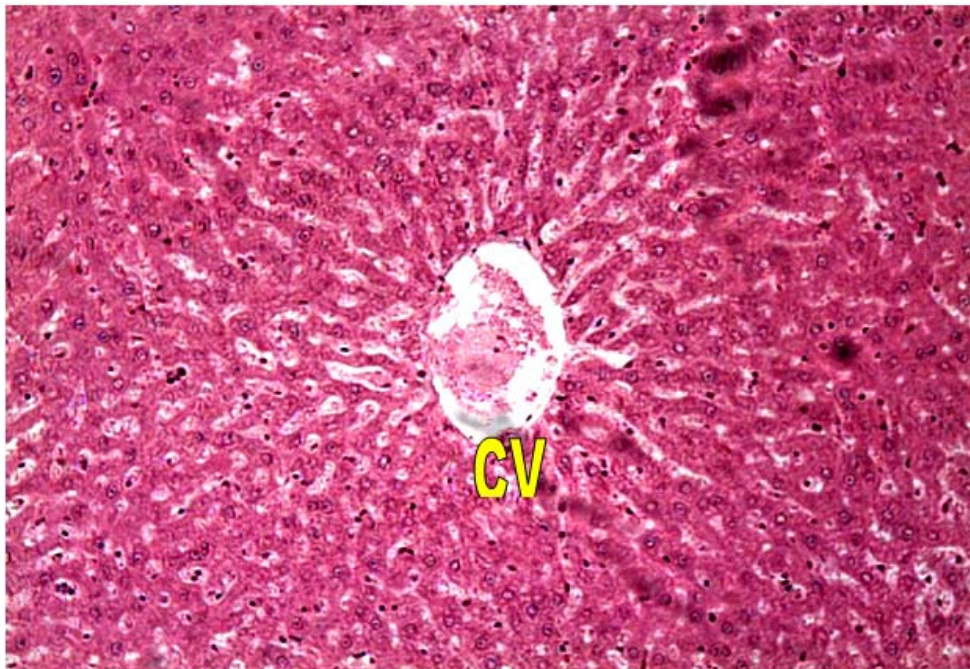


Fig. 13: Liver of untreated rat showing Central Vein (CV) surrounded by hepatocytes (Magnification: 200X)

compared to vehicle and control groups. Testes are the sites where hormonal production occurs by the different cells. These hormones are required for various functions in the body such as: secondary sexual functions and controlling the feedback mechanism for the hypothalamus and pituitary gland to control the secretion of gonadotropins LH and FSH (O'Donnell *et al.*, 2001). So, any changes in weight or morphology of the testis may indicate the upset of balance among the hormones and malfunction may appear.

A significant reduction in the seminal vesicle weight was shown by both treated groups 425 and 213 mg/kg compared to the control group. Accessory sex organs secretions are important in the completion of spermatozoa formation. One of these is seminal vesicle secretions which are important for semen coagulation, sperm motility, stability of sperm chromatin and suppression of immune activity in female reproductive tract. Moreover, the development of seminal vesicle is highly dependent on androgens in which a decrease in serum testosterone was associated with reduction in secretory activity of the

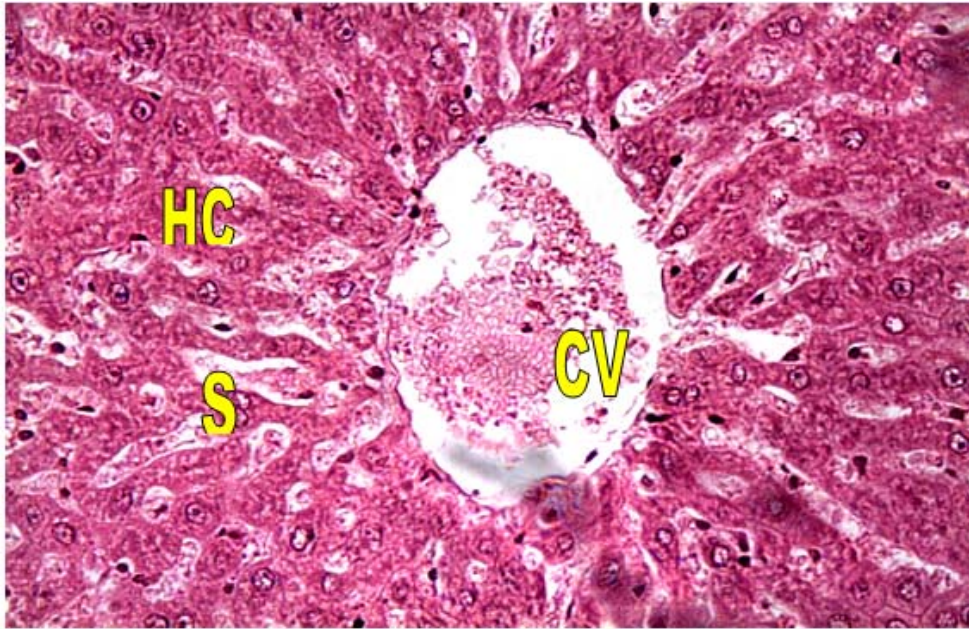


Fig. 14: Liver of untreated rat showing Central Vein (CV) and liver cords of Hepatocytes (HC) separated by Sinusoids (S) containing red blood cells (Magnification: 400X)

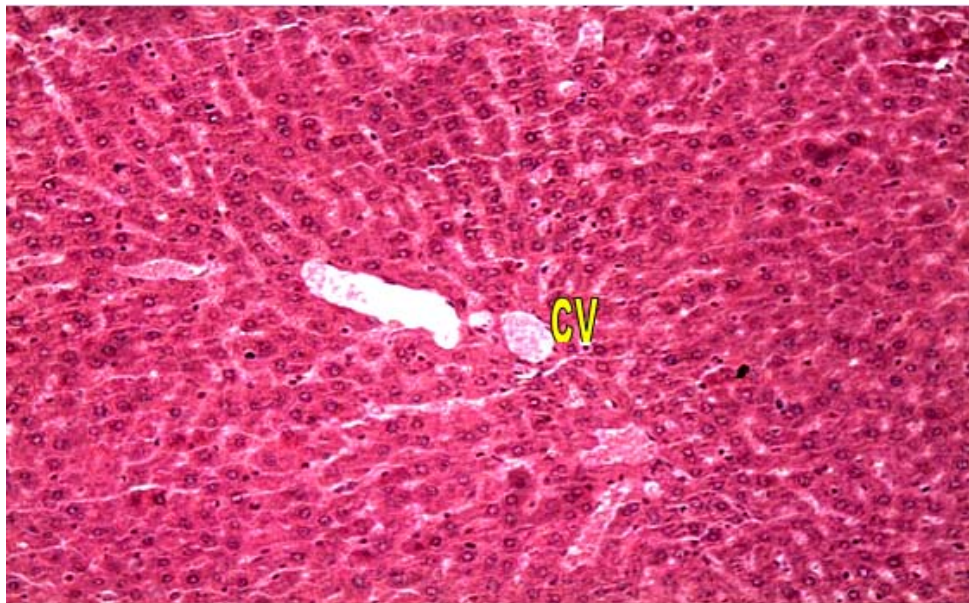


Fig. 15: Liver of vehicle group showing Central Vein (CV) surrounded by hepatocytes (Magnification: 200X)

seminal vesicle and a decrease in seminal vesicle weight (Tavana *et al.*, 2012). So, any reduction in the weight of seminal vesicle may indicate impairment in production of the androgen.

Treated groups, 213 and 425 mg/kg, of the ethanolic extract of *A. graveolens* exhibited a significant reduction

in sperm counts compared to the control and vehicle groups. This reduction may be explained through the results obtained by Sultana *et al.* (2005). In which they stated “modulation of metabolic transcription machinery and profound suppression of the rate of cell proliferation gives ample evidence of efficacy of methanolic extract of

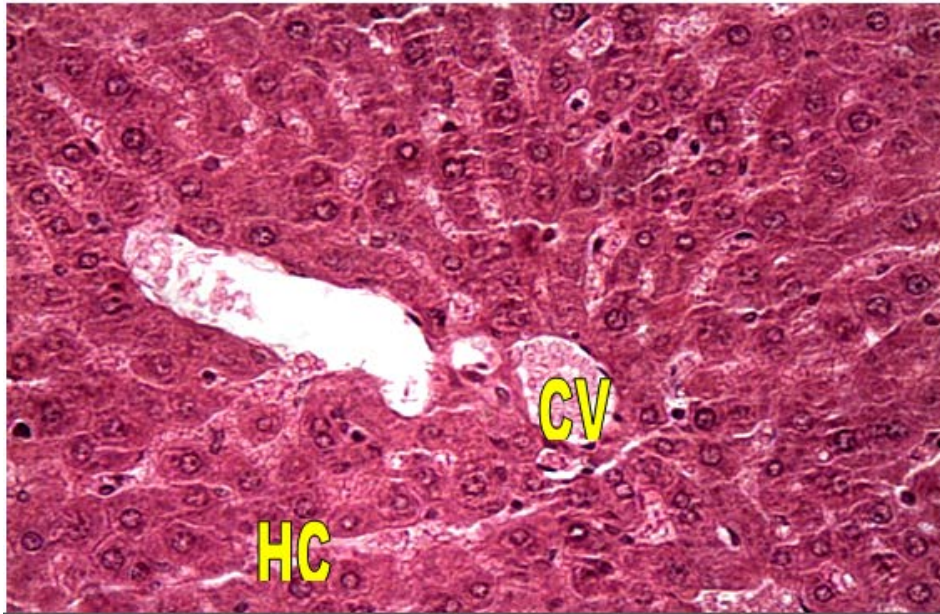


Fig. 16: Liver of vehicle group showing Central Vein (CV) and liver cords of Hepatocytes (HC) (Magnification: 400X)



Fig. 17: Liver of treated group (213 mg/kg) showing Central Vein (CV) surrounded by hepatocytes (Magnification: 200X)

celery seeds as potential anticarcinogenic and antiproliferative agent". This means that would cause reduction in spermatogenesis. In addition, this may be due the presence of certain compounds in the studied extract and the actions are not discovered yet. The methanolic extract of *Plumeria bicolor* stem was reported to have the same action in sperm count where a histological

observation of the treated groups showed a significant reduction in testicular cell population (Gupta *et al.*, 2004; Mali *et al.*, 2002) stated that the 50% ethanolic extract of *Martynia annua* root causes a reduction in sperm count and this due may be to reduction in both LH and testosterone concentrations. This reduction in LH at testicular level results in Leydig cell dysfunction, there by

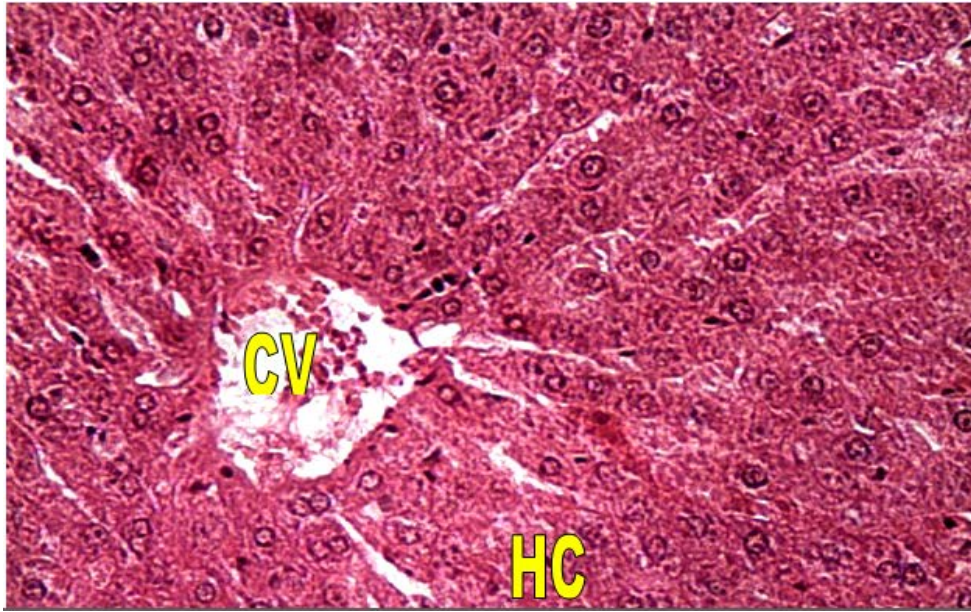


Fig. 18: Liver of treated group (213 mg/kg) showing Central Vein (CV) and liver cords of Hepatocytes (HC) (Magnification: 400X)

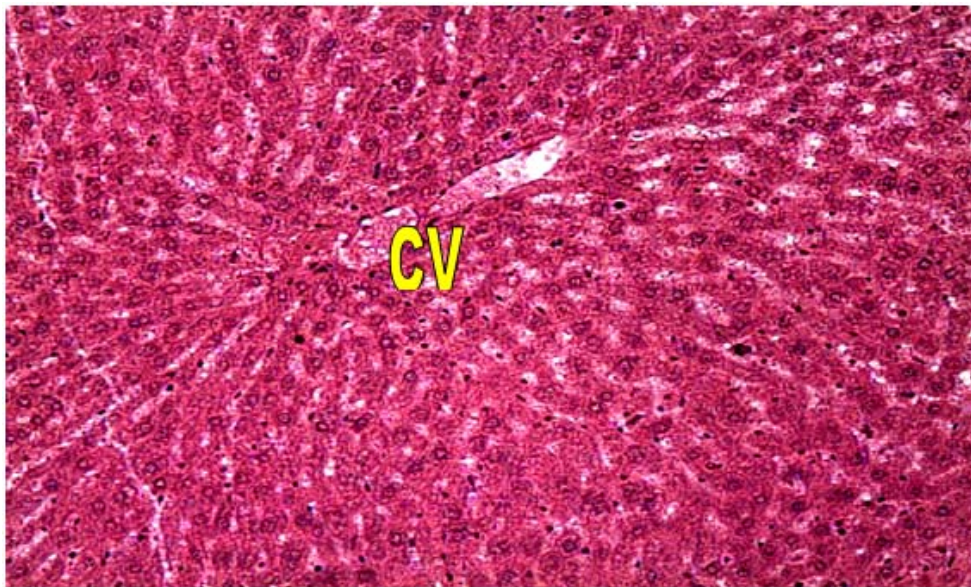


Fig. 19: Liver of treated group (425 mg/kg) showing Central Vein (CV) surrounded by hepatocytes (Magnification 200X)

resulting in decline in testosterone secretion which is responsible for diminished spermatogenesis. In the present study there was a marked decrease in testosterone concentration in the treated groups related with the decreasing with the testicular protein content in the treated groups compared to the control and this was

proven, so, any modifications in level of proteins are always taken as indicators of toxicity of the given extract. This also reflects changes in testicular enzymes. Similar effect was shown by the methanol stem extract of *Saracostemma acidum* (Venma *et al.*, 2002). Many kinds of proteins are found in testes and one of these is

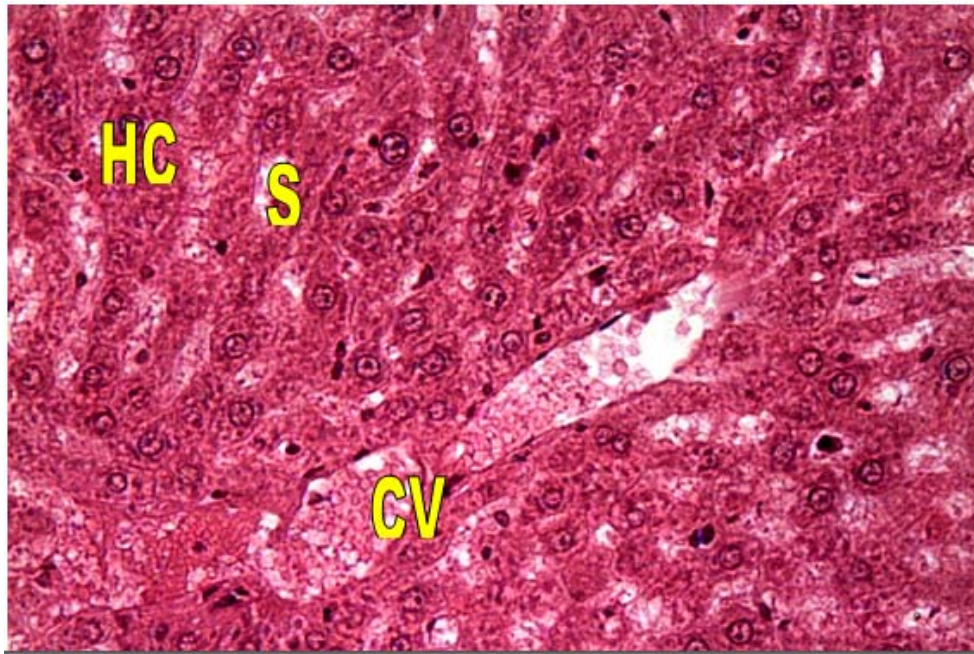


Fig. 20: Liver of treated group (425 mg/kg) showing Central Vein (CV) and liver cords of Hepatocytes (HC) separated by Sinusoids (S) containing red blood cells (Magnification: 400X)

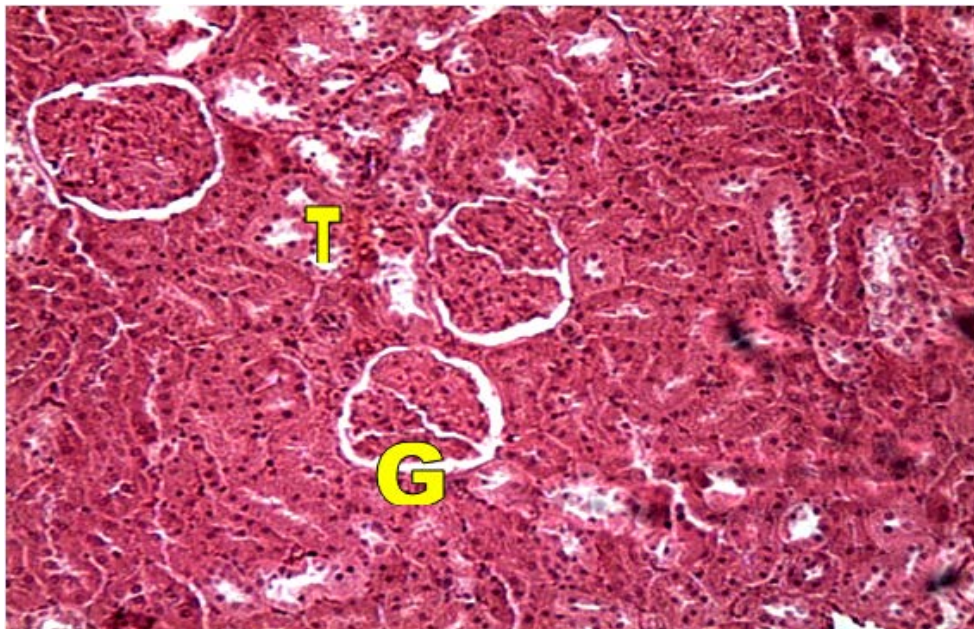


Fig. 21: Kidney of untreated group showing Glomeruli (G) surrounded by Tubules (T) (Magnification: 200X)

Androgen-Binding Proteins (ABP) which are synthesized by Sertoli cells and regulate the availability of testosterone, dihydrotestosterone and estradiol to germ cells in the seminiferous tubular fluid. These proteins prevent reabsorption of sex steroids and ensure their

continued presence for sperm need in epididymis fluid. Also, regulate the inhibitory effect of estradiol on Leydig cell testosterone synthesis (Robaire *et al.*, 2006). Another protein that is found in the testes is the Phosphatidyl Ethanolamine Binding Protein (PEBP). This is considered

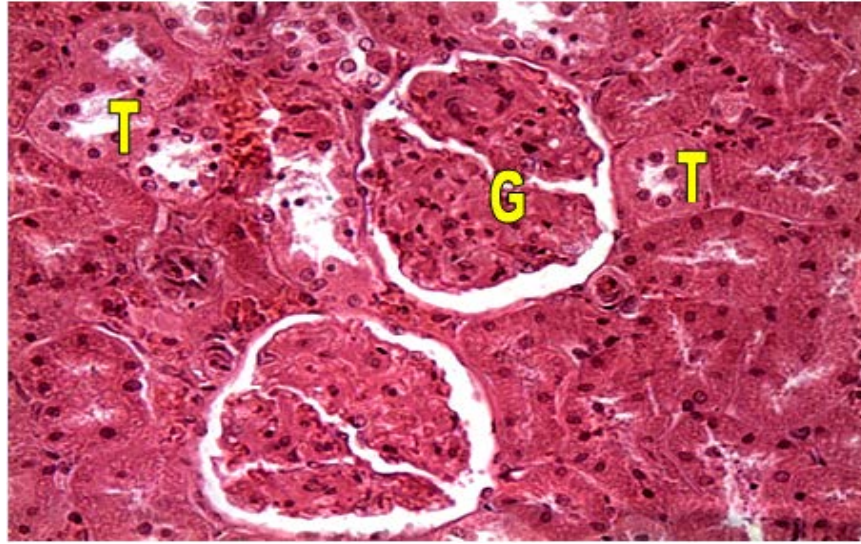


Fig. 22: Kidney of vehicle group showing Glomeruli (G) surrounded by Tubules (T) (Magnification: 200X)

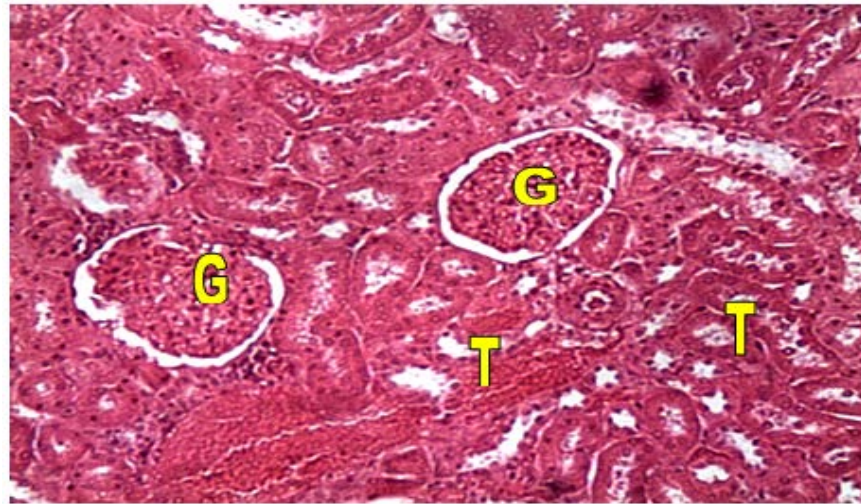


Fig. 23: Kidney of vehicle group showing Glomeruli (G) surrounded by Tubules (T) (Magnification: 400X)

as a major secretory product of haploid testicular germ cells. Where it is believed to contribute to stabilization and establishment of membrane domains on the sperm surface. This play a similar role in the testes (Berne *et al.*, 2004).

At both concentrations of treated groups a marked decrease in free testosterone concentrations was observed relative to the control and vehicle groups. Testicular testosterone is important in the regulation of many diverse body functions like: bone and muscle development, red blood cell turn over and maintenance of male sexual characteristic (sexual drive and growth of body hair). Also, it is needed for necessary quantitative maintenance of spermatogenesis (Rommerts, 1988). So, any changes in

the level of testosterone will have a direct effect on spermatogenesis process and this will be reflected on the sperm count. Substances that affect fertility, especially, those that decrease testosterone serum levels will cause a decrease in sperm count or as well. For instance, the methanolic extract of *Albizia lebbek* bark causes a decrease in Leydig cells and reduction in sperm density (Gupta *et al.*, 2004). Another example is the effect of the leaf ethanolic extract of *Colebrookia opposiyifolia* which causes a reduction in testosterone serum level. As a result, loss of germ cells as well as depletion in testicular protein content was observed (Gupta *et al.*, 2004).

A remarkable decrease in the diameter of the seminiferous tubules was noticed from 204.8 μm for



Fig. 24: Kidney of treated group (213 mg/kg) showing Glomeruli (G) surrounded by Tubules (T) (Magnification: 200X)

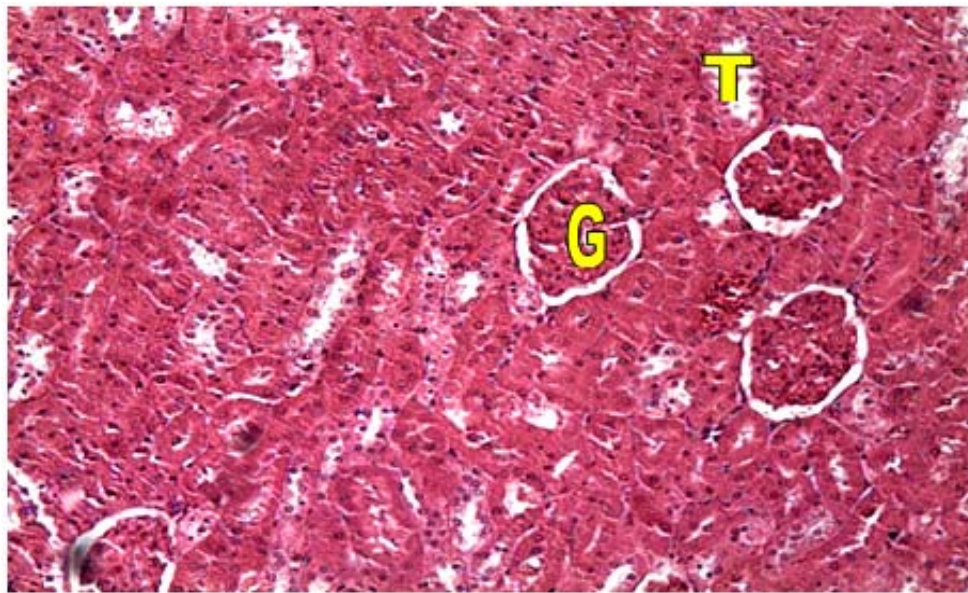


Fig. 25: Kidney of treated group (213 mg/kg) showing Glomeruli (G) surrounded by Tubules (T) (Magnification: 400X)

425 mg/kg and 229.8 for 213 mg/kg to 304.9 μm for the control and 298.8 for vehicle groups. Also, this reduction was accompanied with wide damage in the seminiferous tubules components. Moreover, seminiferous tubules diameter is the best primary assessment of spermatogenesis process, since, the tubules and germinal elements account for approximately 90% of the wet weight of the normal rat testis. So, any

modifications in seminiferous tubules diameter will be attributed to the spermatogenic arrest and this will be reflected negatively on sperm count (Shereen Cynthia D'Cruz *et al.*, 2010) reported that the sections of the testes of the treated animals with the ethanolic extract of *Martynia annua* root showed a reduction in the size of seminiferous tubules and arrest in spermatogenesis process at secondary spermatocyte stage.

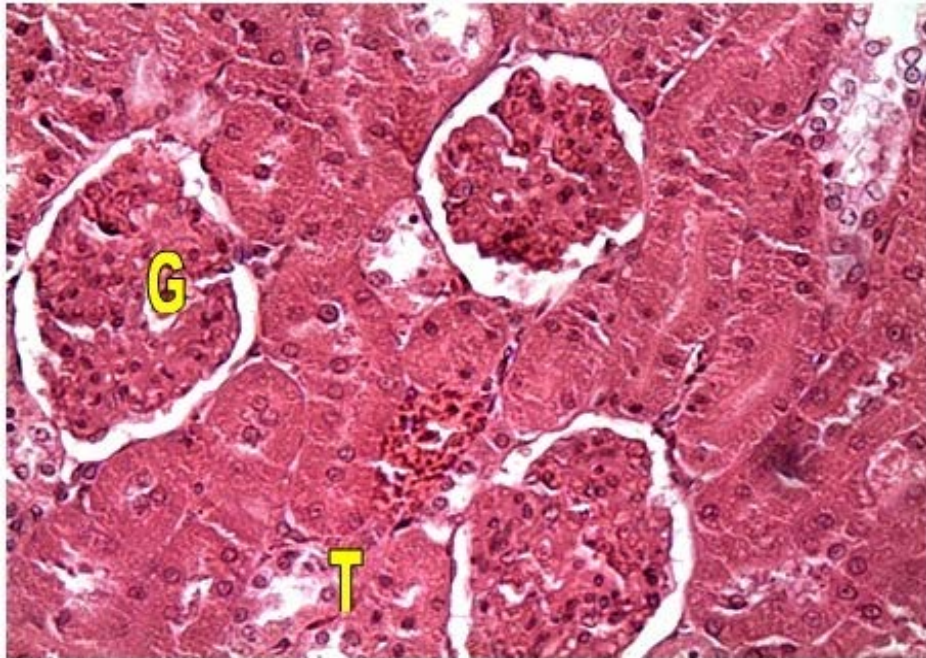


Fig. 26: Kidney of treated group (425 mg/kg) showing Glomeruli (G) surrounded by Tubules (T) (Magnification: 200X)

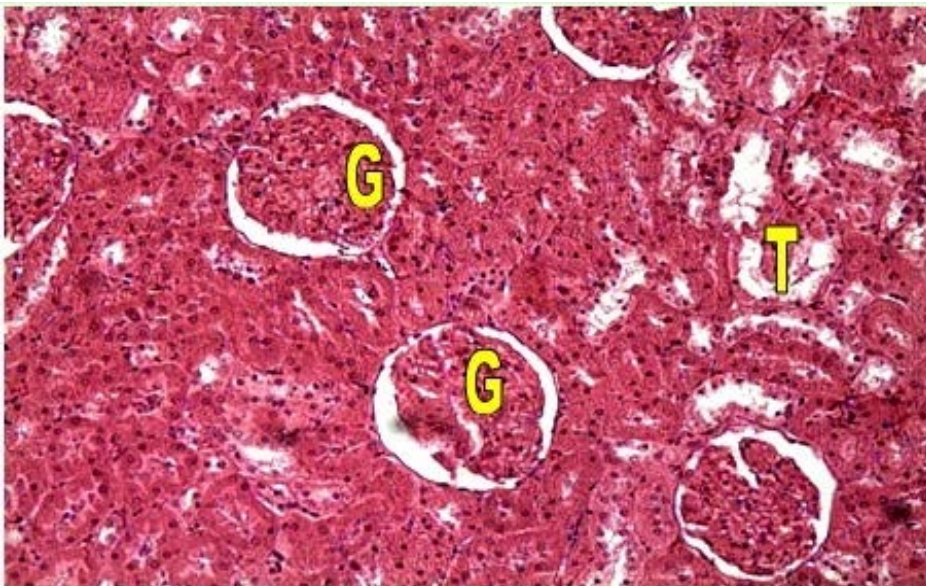


Fig. 27: Kidney of treated group (425 mg/kg) showing Glomeruli (G) surrounded by Tubules (T) (Magnification: 200X)

Sections through the testes of control and vehicle groups showed normal circular or oval seminiferous tubules with normal spermatogenesis process and without any signs of damages all over the section. The treated groups (425 and 213 mg/kg) showed total loss of germinal cells with severely damaged seminiferous tubules. Also, both treated groups shown a significant reduction in the

percentage of the normal seminiferous tubules that consist of germinal epithelium with all stages of maturation including spermatozoa, compared to the control and vehicle groups.

The obtained results were in full agreement with previous investigations such as: decrease in sperm count, protein content and testosterone level reported that

differentiation of primordial germ cells in to spermatogonia and the consequent appearance of spermatogenic cycles are under the control of gonadotropins and of testosterone. Such control being possibly mediated by Sertoli cells which regulate cell cycle kinetics and influence both spermatogonia and preleptotene spermatocyte.

Both treated groups exhibited a marked decrease in sperm motility. At the two concentrations used, 425 and 213 mg/kg sperm motility was found to be 32.4 and 46.5%, respectively, compared to 88.4 and 84.8% for the control and vehicle groups, respectively. Also, the sperms of treated groups were observed to have an aggregation from the head region. This inhibition may be due to low level of ATPase activity or ultra structure defects of sperms which cause serious repercussion on sperm motility and fertility rate. The methanolic extract of *Nyctanthes arbortristis* stem showed similar effect where this extract caused a significant decreasing in sperm motility and sperm count (Gupta *et al.*, 2004). The obtained results had shown that females mated with treated males attained a significant decreasing in the fertility rate from 83% for both the control and vehicle increase in resorption sites. Also, reductions in fetuses weights were observed in the normal females that mated with treated males. Similar results were observed on the effect of petroleum ether extract of the leaves of *Mentha arvensis* in male albino mice, at oral dose 10 and 20 mg/mouse per day for different periods up to 60 days. The reduction in the number of offspring of the treated male mated with normal females showed a dose-duration dependence. Negative fertility was observed in both dose regimens after 60 days of treatment. This was accompanied with a significant decrease in the weight of the testis, cauda epididymal sperm count, motility and normal morphology of the spermatozoa. No alteration in hematological parameters: red blood cells count, white blood cells count and PCV in the treated groups compared to that in control groups. Similar effect was shown by the bark chloroform extract of *Quassia amara* and the 70% methanolic extract of *Saracostemma acidum* stem (Venma *et al.*, 2002). Examining the histology of the cortical region of kidney in the treated groups showed normal intact glomeruli and tubules without any sings of changes compared to the control and vehicle groups. This indicated that the ethanolic extract of *A. graveolens* seed might not have any toxic effect. Moreover, Salama (2001) had reported that celery seeds juice is a strong diuretic and is not recommended when acute kidney problems exist. Some other studies indicated that D-limonene (monocyclic mono terpene) which is a compound, found in the celery seeds induced hyaline nephropathy.

Histological examination of the liver of both treated groups showed normal structure compared to the control and vehicle groups. Where Singh and Singh (2009)

reported that the methanolic extract of celery showed a significant hepatoprotective activity compared to the paracetamal and thioacetamide groups in rats. This was also reported by Bahar *et al.* and who experimentally proved that this activity of this extract was due to presence of flavone and diterpene.

No marked changes in the concentrations of GOT and GPT enzymes were observed in this study. This indicates that *A. graveolens* seed extract may not cause any toxic effect on the body. However, Batra and Sharma, reported that the isolated flavonoid, apigenin, acts as inhibitory agent against tumor formation in the liver cells. Similar result was reported in effect the aqueous of *Carica papaya* seeds on liver function enzymes. No changes in the concentration of these enzymes were observed.

CONCLUSION

From the present study we conclude that the ethanolic extract of *A. graveolens* seed may act as antifertility agent. This is supported by the results that showed a decrease in the fertility parameters (sperm motility and sperm count), testosterone level, weight of the testes and seminal vesicle, diameter of seminiferous tubules and fertility rate.. Histology of the testes of the treated groups showed a complete loss of the germ cells with arrest in the spermatogenesis process. Also, the 425 mg/kg treated group showed severe damage in the seminiferous tubules compared to the control and vehicle groups. On the other hand, histological examination of both liver and kidney of the treated groups does not show any signs of changes. Also, there was neither alteration in the hematology parameters nor liver function enzymes. These indicate that the ethanolic extract of *A. graveolens* seed may not have any signs of toxicity.

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