

ORIGINAL ARTICLE

# Acute and subacute toxicity of *Ammi visnaga* on rats

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## ABSTRACT

*Ammi visnaga* (Av) is a source of khellin where a tea made from the fruit of this plant was used as herbal medicine for kidney stones in Egypt. In the present research, the acute and subacute toxicity studies with oral intake of 150, 300 and 600 mg/kg of Av seed ethanolic extract in rats were done. In acute toxicity test, 4 groups of rats (n = 6/group: 3 males and 3 females) were chosen and the first control group received tap water, while the other three groups received Av seed ethanolic extract dissolved in tap water at doses of 150, 300, and 600 mg/kg, and general behavior, adverse effects, and mortality were recorded for up to 14 days. In subacute toxicity study, 72 rats (36 males and 36 females) were divided into 4 major groups; group I received tap water (control group), while animals in groups II, III, and IV (test groups) received oral intake of Av seed ethanolic extract dissolved in tap water at doses of 150, 300 and 600 mg/kg bwt, respectively. Each of this major group was subdivided consequently into 3 subgroups (n = 6/group: 3 males and 3 females) where brain tissue, blood sample, body and organs weights were recorded at the beginning and then after two and four weeks of the experiment for the determination of hematological, biochemical and histopathological changes in tissues (liver, kidney, brain, spleen, heart, testis and ovary). With regard to acute toxicity, Av seed ethanolic extract did not induce any toxic effects or death or any organ toxicity. In subacute toxicity study; oral intake with Av seed ethanolic extract did not reveal any change in body and organs weights, hematological parameters, serum glucose and cholesterol, brain neurotransmitters, liver and kidney functions, male and female hormones. In conclusion, Av seed ethanolic extract is nontoxic to liver, kidney, brain, spleen, heart, testis and ovary.

**KEY WORDS:** *Ammi visnaga*; Apiaceae; acute toxicity; subacute toxicity; rats

## Introduction

In the last decade, the medicinal plants represent an important and rich source of new synthetic drugs. There is huge number of the world's populations depending upon medicinal plants as an alternative and complementary drugs therapy for many known diseases. The most common plants uses involve application of plant extracts, which contain numerous of molecules with well-known biological effects (Yakob *et al.*, 2012). The medicinal plants are commonly used world-wide without informing about their possible unhealthy or toxic effects, the World Health Organization has recommended that traditional plants used for the treatment of diseases need further scientific investigation on their toxic side effects (WHO, 2008). The

medicinal plants contain bioactive ingredients which act as defense mechanisms against many diseases but the plant itself may be toxic in nature (Roch *et al.*, 2001). So, the medicinal uses of herbs have been restricted by a lack of defined chemical classification, dose treatment, and well-known toxicity data to evaluate plant safety (Denga *et al.*, 2013). Consequently; it is very urgent and important to evaluate the toxicity of the medicinal plants used in medicinal and pharmacological applications.

*Ammi visnaga* (Av) belongs to family Apiaceae. Av is medically applied for prevention and treatment of urinary lithiasis and consequently Av represents an alternative and complementary medical application. Av is used in medical usage against the crystallization of calcium oxalate in kidney (Kachkoul *et al.*, 2018). It is a flowering plant that is traditionally known by numerous names depending on the different areas of cultivation and out of these names bisnaga and khella were used. It is cultivated in North Africa, Europe and Asia, but it can be cultivated throughout the world. Av plant is annual herb up to 80 centimeters in height. The plant leaves are

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20 centimeters long and are often oval or sometimes triangular in shape. The inflorescence, like other Apiaceae family, forms multiple umbel of white flowers. The fruit forms a compacted oval-shape (less than 3 millimeters long). Khellin is a main constituent obtained from Av and other Ammi species while khellin has a diuretic effect. Another important effect of khellin is relaxation of the smooth muscle but this function is limited due to its side effects (Waltenberger *et al.* 2016). Herbicidal activity was observed and recorded for both khellin and visnagin, constituents of Av. This herbicidal activity of khellin and visnagin was not a light-dependent effect. Both khellin and visnagin induced photosynthetic efficiency decrease, membrane destabilization, decline in cell division and death (Travaini *et al.*, 2016). Khellin showed very fast mortality effect for the larvae of *Culex quinquefasciatus* in various development stages so 18 minutes of khellin action induced a 54.3% mortality rate of the larvae within 24 hours (Pavela *et al.*, 2016). The famous and medically applicable two synthetic derivatives of khellin are amiodarone and cromoglycate. These two synthetic derivatives of khellin have fewer side effects so they are used in modern medicine. Av also contains visnagin as a chemical constituent (Vanachayangkul *et al.*, 2010). In Egypt, a tea from Av fruit is famously used as a popular herbal remedy for kidney stones.

This research was designated to study the acute toxicity (determination of  $LD_{50}$ ) and subacute toxicity (clinical changes, hematological, biochemical and histopathological changes) in rats after daily oral administration with *Ammi visnaga* though the period of 28 days.

## Materials and methods

### Materials

All kits reagents used in this research were purchased from Bio-diagnostic Kits reagents through local Egyptian supplier with the exception of testosterone, dehydroepiandrosterone sulfate,  $3\beta$ -hydroxysteroiddehydrogenase,  $17\beta$ -estradiol and estradiol- $17\beta$ -stearate kits that were purchased from Biosource Inc, Belgium. Further, the kits reagents used in this research include dehydroepiandrosterone sulfate and  $3\beta$ -hydroxysteroiddehydrogenase kits that were achieved from EURO/DPC Ltd., United Kingdom while kits reagents used in the current research such as  $17\beta$ -estradiol and estradiol- $17\beta$ -stearate kits were purchased from Steraloids Inc., USA. All kits reagents used in this study were manufactured in 2018.

### Plant material

In this research, *Ammi visnaga* (Av) seeds were delivered from Horticulture Department, Ministry of Agriculture, Dokki, Cairo, Egypt. The seeds were crushed, pulverized, and weighed in sequence to prepare extraction.

### Preparation of seed ethanolic extract

Av seeds were air-dried in an oven at  $40^{\circ}\text{C}$  for 4 days and then the dry seeds were cut and pulverized. The dried

seeds (500 g) were placed in 1000 ml of distilled boiling water and kept at room temperature for 15 minutes to yield dried powder. Then the dried powder was macerated for 7 days using 70% ethanol as a solvent. The solvent was then eliminated by a rotary vacuum evaporator under reduced pressure to obtain total alcoholic extract. The alcoholic extract obtained is lyophilized and represents a yield of 15% of the dry seeds extracted. Evaporation process of the extract was done to dryness to give dried seed total alcoholic extract (150 g) according to the method of Chopra *et al.* (1986).

### Animals

Albino rats of *Sprague Dawley* strains ( $130\pm 10$ g, 10-weeks old) of both sexes were obtained from the animal house of the National Research Centre, Dokki, Cairo, Egypt and were kept in special plastic cages. The animals were maintained on a commercial balanced diet and tap water. The laboratory animal conditions were followed (Guide for the Care and Use of Laboratory Animals, 2011) such as 12 hours light/12 hours dark cycle, room temperature =  $26\text{--}30^{\circ}\text{C}$ , humidity = 40–70%, Number of rats=3 rats /one cage and each group comprised 2 cages (3 males/cage and 3 females/cage). The animal room ventilation maintained the temperature for rats within 68 to  $79^{\circ}\text{F}$  and minimized temperature fluctuations. All experimental procedures were done following the acceptance of the ethics committee of National Research Centre and in accordance with recommendations for the proper care and use of laboratory animals (NIH publication no 85:23 revised 1985).

### Experimental design

#### Determination of $LD_{50}$ of Av

There were 36 rats of both sexes (18 males and 18 females) used. The  $LD_{50}$  of Av seed ethanolic extract was determined in mg/kg body weight (bwt) for adult rats (Behrens & Karber, 1953), where  $LD_{50} = DM - \Sigma Z.D/m$ ,  $DM$  is the highest dose used,  $Z$  is the number of dead rats from two successive doses divided by two,  $D$  is the difference between two successive doses,  $m$  is the number of rats in each group. The doses chosen for such study were 5%, 10% and 20% of the  $LD_{50}$  of Av seed ethanolic extract (Koriam *et al.*, 2010).

#### Acute toxicity study

Animals were divided into 4 groups ( $n=6$  rats/group: 3 males and 3 females) were followed OECD 423 guideline (OECD, 2001). Control group rats received tap water while the other three groups received Av dissolved in tap water at doses of 150, 300, and 600 mg/kg. The above doses was selected on the basis of  $LD_{50}$  of Av. Animals were observed closely for first 6 hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Animals were under more investigation for the period of 14 days and the number of rats that died within the study period was noted (Gautam *et al.*, 2012).

#### *Subacute toxicity study*

The repeated doses (28 days) for oral toxicity studies were carried out in rats according to the OECD test guideline 407 (OECD, 2008). Seventy-two rats (36 males and 36 females) were used and divided into 4 major groups; group I received tap water (control group), whereas rats in groups II, III, and IV (test groups) received Av dissolved in tap water at doses of 150, 300 and 600 mg/kg bw/d, respectively. Each of this major group was subdivided consequently into 3 subgroups (n=6 rats / group: 3 males and 3 females) where brain tissue, blood sample, food consumption, water intake, body and organs weights were measured and recorded at the beginning and then after two and four weeks of the experiment. The Av seed extract dose was taken daily by oral gavage in the volume of 5 ml of tap water/kg bw/d, once daily for 28 consecutive days. Any death or abnormal clinical signs among animals during the experimental period were observed daily.

#### **Biochemical analysis**

Hemoglobin (Hb) concentration was determined by the method described by Van Kampen & Zijlstra (1961). Hematocrit (Hct) value, Red blood cells (RBCs) and white blood cells (WBCs) were carried out according to the method of Rodak (1995). Serum glucose was measured according to the enzymatic colorimetric method described by Trinder (1969). Allain *et al.* (1974) method was applied to determine serum total cholesterol while Reitman & Frankel (1957) method was used to calculate serum aspartate and alanine aminotransaminases (AST & ALT) activities. The colorimetric method of Kind & King (1954) was used to determine serum alkaline phosphatase (ALP) while Walter and Gerard (1970) method was applied to calculate serum total bilirubin. The method of Gornall *et al.* (1949) was used to calculate serum total protein while Drupt (1974) method was used to estimate serum albumin. The enzymatic method of Patton & Crouch (1977) was applied to estimate serum urea while kinetic method of Houot (1985) was used to calculate serum creatinine. The method of Kabasakalian *et al.* (1973) was applied to determine serum uric acid while Ciarlone & Smudski (1977) method was used to estimate serotonin (5-hydroxytryptamine; 5-HT), norepinephrine (NE), and dopamine (DA) in brain cerebral cortex. Maruyama *et al.* (1987) method was applied to estimate serum testosterone (Ts) and Dehydroepiandro-sterone sulfate (DHEA-SO<sub>4</sub>) concentrations. Talalay (1962) method was applied to determine serum 3 $\beta$ -hydroxysteroiddehydrogenase (3 $\beta$ HSD) level while Vihma *et al.* (2001) method was used to estimate serum 17- $\beta$ -estradiol (17 $\beta$ -E) and estradiol-17- $\beta$ -stearate (E-17 $\beta$ -s) levels.

#### **Histopathological investigation**

Tissue samples of brain, liver, kidney, spleen, heart and ovary tissues were fixed at 10% neutral formalin solution while testes tissues were fixed in Bouin's fluid and then processed for routine embedding in paraffin. Blocks

were sectioned at a thickness of 5  $\mu$ m and stained with hematoxylin and eosin for histopathological examination under microscope for any cellular damage or change in morphology of that particular tissue.

#### **Statistical analysis**

The results obtained in this research were tabulated as mean  $\pm$  standard error mean (SEM). The variances between different groups were assessed by ANOVA using the SPSS 13 software package for Windows followed by *post-hoc* testing. The inter-group comparisons were performed using the least significant difference (Tukey) test; significance at  $p \leq 0.05$ .

## **Results**

#### **The LD<sub>50</sub> of Av**

The LD<sub>50</sub> of Av was equal to 3000 mg/kg body weight, so 5%, 10% and 20% of the LD<sub>50</sub> of Av seed extract equal to 150, 300 and 600 mg/kg, respectively.

#### **Acute toxicity study**

Av seed extract administrated at doses 150, 300 and 600 mg/kg bw/d respectively by oral gavage into normal male and female rats did not induce any death or toxic symptoms in treated rats. All animals were normal throughout the study and survived until the end of the 14-day experiment period.

#### **Subacute toxicity study**

##### *General observations*

No mortality and clinical changes were observed in both males and females during the test. Both male and female animals did not reveal any disturbance in animal behavior or other physiological activities.

##### *Food consumption and water intake*

No difference in food consumption and water intake were found between control and treated rats throughout the period of 28 day.

##### *Body and organs weights*

Slight changes were observed in animal body and organs weights in all the Av-treated animals in comparison with control rats after 28 successive days of Av oral intake.

##### *Hematological parameters, serum glucose and cholesterol*

It was obvious that Av seed extract did not induce any change in Hb, Hct, RBCs, WBCs, serum glucose and total cholesterol in all the treated animals compared to control group after 28 days of study period (see Table 1).

##### *Liver function*

It was clear that Av seed extract did not induce any change in serum AST, ALT, ALP, total bilirubin, total protein and albumin in all the treated animals compared to control group after 28 days of study period (see Table 2).

*Kidney function and brain cerebral cortex neurotransmitters*

It was observable that Av seed extract did not induce any change in serum urea, creatinine and uric acid, as well as, tissue serotonin, norepinephrine and dopamine in all the treated animals compared to control group after 28 days of study period (see Table 3).

*Male and female hormones*

It is clear that Av seed extract did not induce any change in serum Ts, DHEA-SO<sub>4</sub>, 3βHSD, 17β-E and E-17β-s activities on treated male and female animals compared to control group after 28 days of study period (see Table 4).

*Histopathological results*

Histological structure of the liver in all treated rats was comparable with normal pattern of the organ in controls (Figure 1A). No changes of hepatic lobular architecture and hepatocytes were observed (Figures 1B, C&D).

Histological structure of the kidney in all treated rats was comparable with normal pattern of the organ in controls (Figure 2A). No changes of glomeruli and the renal tubules architecture were detected (Figures 2B, C&D).

Histological structure of the brain in all treated rats was comparable with normal pattern of the organ in controls (Figure 3A). No changes of the normal brain structure architecture were identified (Figures 3B, C&D).

Histological structure of the spleen in all treated rats was comparable with normal pattern of the organ in controls (Figure 4A). No changes within macrophages in the red pulp with normal structure architecture were found (Figures 4B, C&D).

Histological structure of the heart in all treated rats was comparable with normal pattern of the organ in controls (Figure 5A). No changes in cardiac muscle fibers, acidophilic cytoplasm and centrally located nuclei were observed (Figures 5B, C&D).

Histological structure of the testis in all treated rats was comparable with normal pattern of the organ in controls (Figure 6A). No changes in seminiferous tubules and germ cells architecture were identified (Figures 6B, C&D).

Histological structure of the ovary in all treated rats was comparable with normal pattern of the organ in controls (Figure 7A). No changes in small follicles and large follicles architecture were reported (Figures 7B, C&D).

**Discussion**

Orally taken medicinal plants without any ordinary dose in correlation with lack of sufficient scientific research on their medical safety attract more attention regarding their toxicity (Saad *et al.*, 2006). There are no published studies on Av toxicological data after subacute exposure of its seed extract.

In acute toxicity study, the oral administration of Av seed ethanolic extract at 150, 300 and 600 mg/kg bw/d respectively did not show any observable toxic effects in rats in terms of any deaths or abnormal symptoms which points to it being nontoxic and safe in rats. In subacute

**Table 1.** The effect of Av on blood parameters, serum glucose and cholesterol

| Parameters                                    |              | Day 0        | Day 14       | Day 28       |
|---|--------------|--------------|--------------|--------------|
| Hb (gm/dl)                                    | Control      | 13.79±0.45   | 14.12±0.27   | 15.24±0.61   |
|   | Av 150 mg/kg | 13.62±0.37   | 13.84±0.61   | 14.28±0.45   |
|   | Av 300 mg/kg | 14.18±0.60   | 14.68±0.37   | 15.16±0.29   |
|   | Av 600 mg/kg | 14.25±0.83   | 14.90±0.56   | 15.38±0.70   |
| Hct (%)                                       | Control      | 43.81±1.50   | 45.16±1.82   | 46.41±2.13   |
|   | Av 150 mg/kg | 40.56±1.73   | 42.18±1.73   | 43.26±1.90   |
|   | Av 300 mg/kg | 41.38±1.92   | 44.50±2.04   | 45.28±1.74   |
|   | Av 600 mg/kg | 45.12±2.07   | 46.34±1.85   | 47.02±1.64   |
| RBCs (10 <sup>6</sup> cells/mm <sup>3</sup> ) | Control      | 5.90±0.46    | 5.75±0.62    | 5.95±0.38    |
|   | Av 150 mg/kg | 5.45±0.35    | 6.20±0.93    | 5.80±0.56    |
|   | Av 300 mg/kg | 5.60±0.69    | 5.95±0.81    | 6.15±0.42    |
|   | Av 600 mg/kg | 5.95±0.58    | 6.10±0.84    | 6.25±0.95    |
| WBCs (10 <sup>3</sup> cells/mm <sup>3</sup> ) | Control      | 9.45±0.75    | 9.60±0.91    | 9.85±0.53    |
|   | Av 150 mg/kg | 9.25±0.48    | 9.85±0.67    | 10.15±0.82   |
|   | Av 300 mg/kg | 9.50±0.69    | 9.95±0.90    | 10.20±0.74   |
|   | Av 600 mg/kg | 9.65±0.75    | 9.70±0.86    | 9.85±0.63    |
| Serum glucose (mg/dl)                         | Control      | 108.12±15.25 | 104.92±17.34 | 109.40±14.18 |
|   | Av 150 mg/kg | 106.65±13.21 | 103.76±15.80 | 107.24±16.35 |
|   | Av 300 mg/kg | 110.00±10.93 | 107.00±12.91 | 109.24±13.95 |
|   | Av 600 mg/kg | 107.85±14.18 | 110.20±16.15 | 108.52±17.45 |
| Total cholesterol (mg/dl)                     | Control      | 122.8±3.6    | 124.1±4.0    | 119.8±2.5    |
|   | Av 150 mg/kg | 119.2±4.1    | 123.5±5.2    | 120.7±4.6    |
|   | Av 300 mg/kg | 124.0±2.9    | 118.9±3.7    | 123.2±5.8    |
|   | Av 600 mg/kg | 117.9±5.3    | 119.2±4.1    | 121.8±3.9    |

Values are expressed as mean ± SEM of 6 rats in each group

**Table 2.** The effect of Av on liver function

| Parameters              |              | Day 0      | Day 14     | Day 28     |
|-------------------------|--------------|------------|------------|------------|
| Serum AST (U/l)         | Control      | 19.81±3.95 | 20.23±4.21 | 19.56±3.57 |
|                         | Av 150 mg/kg | 18.93±4.26 | 19.40±3.64 | 20.13±4.10 |
|                         | Av 300 mg/kg | 20.15±2.74 | 19.35±4.04 | 18.75±2.91 |
|                         | Av 600 mg/kg | 19.28±3.47 | 18.86±3.79 | 19.73±4.15 |
| Serum ALT (U/l)         | Control      | 9.27±3.23  | 8.69±4.06  | 9.42±3.61  |
|                         | Av 150 mg/kg | 8.69±2.82  | 9.14±4.02  | 8.90±3.57  |
|                         | Av 300 mg/kg | 9.17±3.51  | 8.76±2.80  | 9.35±3.94  |
|                         | Av 600 mg/kg | 9.60±2.94  | 9.39±4.17  | 9.43±2.75  |
| Serum ALP (U/l)         | Control      | 210.5±6.81 | 209.0±4.93 | 211.2±5.24 |
|                         | Av 150 mg/kg | 208.7±5.43 | 211.5±6.26 | 208.4±4.90 |
|                         | Av 300 mg/kg | 209.2±6.14 | 207.5±5.81 | 210.0±6.34 |
|                         | Av 600 mg/kg | 212.0±5.96 | 208.5±4.82 | 209.1±5.67 |
| Total bilirubin (mg/dl) | Control      | 0.56±0.08  | 0.55±0.04  | 0.57±0.09  |
|                         | Av 150 mg/kg | 0.54±0.06  | 0.57±0.08  | 0.59±0.07  |
|                         | Av 300 mg/kg | 0.58±0.09  | 0.56±0.05  | 0.60±0.08  |
|                         | Av 600 mg/kg | 0.57±0.07  | 0.54±0.09  | 0.58±0.06  |
| Total protein (g/dl)    | Control      | 8.72±0.54  | 8.90±0.37  | 9.14±0.72  |
|                         | Av 150 mg/kg | 9.14±0.72  | 8.85±0.52  | 9.23±0.69  |
|                         | Av 300 mg/kg | 8.56±0.49  | 8.74±0.60  | 8.95±0.56  |
|                         | Av 600 mg/kg | 9.24±0.63  | 8.82±0.46  | 9.15±0.80  |
| Serum albumin (g/dl)    | Control      | 5.08±0.49  | 5.14±0.37  | 5.26±0.67  |
|                         | Av 150 mg/kg | 5.26±0.62  | 4.93±0.59  | 5.34±0.90  |
|                         | Av 300 mg/kg | 4.89±0.37  | 4.95±0.81  | 5.19±0.57  |
|                         | Av 600 mg/kg | 5.31±0.59  | 5.13±0.47  | 5.20±0.64  |

Values are expressed as mean ± SEM of 6 rats in each group

**Table 3.** The effect of Av on kidney function and brain neurotransmitters.

| Parameters                  |              | Day 0     | Day 14     | Day 28    |
|-----------------------------|--------------|-----------|------------|-----------|
| Urea (mg/dl)                | Control      | 26.5±2.83 | 28.1±2.48  | 27.5±3.16 |
|                             | Av 150 mg/kg | 28.2±3.27 | 26.9±2.74  | 29.0±3.84 |
|                             | Av 300 mg/kg | 27.0±2.54 | 29.1±2.96  | 25.8±2.69 |
|                             | Av 600 mg/kg | 25.9±1.96 | 28.90±3.52 | 27.3±2.50 |
| Creatinine (mg/dl)          | Control      | 0.75±0.08 | 0.70±0.07  | 0.68±0.09 |
|                             | Av 150 mg/kg | 0.69±0.06 | 0.74±0.08  | 0.71±0.05 |
|                             | Av 300 mg/kg | 0.72±0.09 | 0.68±0.06  | 0.74±0.07 |
|                             | Av 600 mg/kg | 0.78±0.05 | 0.72±0.09  | 0.76±0.06 |
| Uric acid (mg/dl)           | Control      | 8.21±0.43 | 7.95±0.68  | 8.15±0.36 |
|                             | Av 150 mg/kg | 7.87±0.62 | 8.27±0.59  | 7.96±0.52 |
|                             | Av 300 mg/kg | 7.92±0.50 | 8.17±0.42  | 8.25±0.71 |
|                             | Av 600 mg/kg | 8.34±0.71 | 7.98±0.69  | 8.13±0.45 |
| Serotonin (g/g tissue)      | Control      | 0.56±0.05 | 0.55±0.03  | 0.57±0.06 |
|                             | Av 150 mg/kg | 0.54±0.04 | 0.56±0.05  | 0.55±0.07 |
|                             | Av 300 mg/kg | 0.57±0.06 | 0.55±0.03  | 0.56±0.05 |
|                             | Av 600 mg/kg | 0.58±0.08 | 0.57±0.07  | 0.59±0.04 |
| Norepinephrine (g/g tissue) | Control      | 2.49±0.19 | 2.46±0.16  | 2.48±0.15 |
|                             | Av 150 mg/kg | 2.46±0.17 | 2.48±0.14  | 2.45±0.19 |
|                             | Av 300 mg/kg | 2.50±0.14 | 2.45±0.17  | 2.47±0.16 |
|                             | Av 600 mg/kg | 2.48±0.23 | 2.47±0.20  | 2.50±0.24 |
| Dopamine (g/g tissue)       | Control      | 0.27±0.01 | 0.26±0.04  | 0.28±0.03 |
|                             | Av 150 mg/kg | 0.25±0.03 | 0.24±0.02  | 0.26±0.04 |
|                             | Av 300 mg/kg | 0.28±0.04 | 0.27±0.03  | 0.25±0.02 |
|                             | Av 600 mg/kg | 0.26±0.02 | 0.28±0.05  | 0.27±0.03 |

Values are expressed as mean ± SEM of 6 rats in each group

**Table 4.** The effect of Av on males and females hormones.

| Parameters             |              | Day 0      | Day 14     | Day 28     |
|------------------------|--------------|------------|------------|------------|
| T <sub>s</sub> (ng/ml) | Control      | 5.98±0.42  | 6.14±0.53  | 5.86±0.69  |
|                        | Av 150 mg/kg | 5.75±0.59  | 5.84±0.68  | 6.14±0.47  |
|                        | Av 300 mg/kg | 6.12±0.70  | 5.95±0.43  | 6.08±0.56  |
|                        | Av 600 mg/kg | 6.09±0.42  | 5.87±0.67  | 6.95±0.51  |
| 3βHSD (U/l)            | Control      | 74.36±3.67 | 70.86±4.12 | 75.16±3.49 |
|                        | Av 150 mg/kg | 76.12±4.54 | 73.40±3.75 | 78.01±4.86 |
|                        | Av 300 mg/kg | 71.95±3.80 | 75.16±4.09 | 72.56±3.98 |
|                        | Av 600 mg/kg | 73.46±3.69 | 71.50±2.86 | 76.30±4.21 |
| DHEA'S (μg/dl)         | Control      | 197.5±23.7 | 200.1±21.8 | 198.2±24.9 |
|                        | Av 150 mg/kg | 201.2±26.9 | 196.8±24.6 | 199.7±19.8 |
|                        | Av 300 mg/kg | 195.7±28.5 | 199.0±25.9 | 201.4±27.1 |
|                        | Av 600 mg/kg | 202.0±25.3 | 198.5±27.0 | 200.0±24.9 |
| 17β-E (pmol/l)         | Control      | 276.4±32.7 | 281.7±28.9 | 278.2±27.6 |
|                        | Av 150 mg/kg | 275.0±28.1 | 278.4±31.2 | 276.7±31.2 |
|                        | Av 300 mg/kg | 278.4±27.9 | 279.0±29.4 | 280.3±28.5 |
|                        | Av 600 mg/kg | 280.3±30.5 | 282.0±33.6 | 281.2±31.4 |
| E-17β-s (pmol/l)       | Control      | 122.8±20.5 | 124.0±24.1 | 125.0±21.9 |
|                        | Av 150 mg/kg | 120.6±19.7 | 121.7±23.4 | 123.5±25.7 |
|                        | Av 300 mg/kg | 123.1±23.2 | 125.0±26.0 | 127.0±19.2 |
|                        | Av 600 mg/kg | 124.0±19.6 | 123.9±25.1 | 126.2±27.4 |

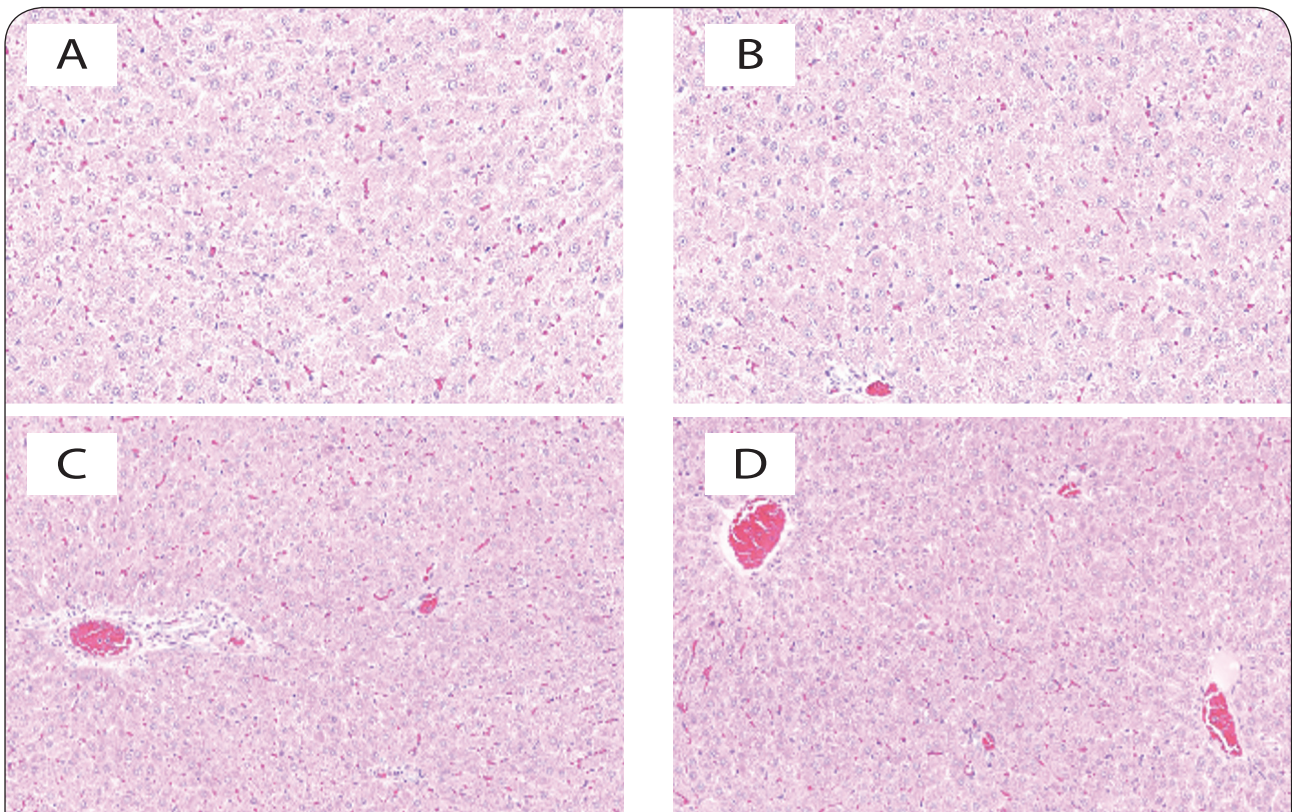
Values are expressed as mean ± SEM of 6 rats in each group

toxicity research, animals that were orally administrated with 150, 300 and 600 mg/kg bw/d respectively of Av for 28 days did not show any mortality, body and organ

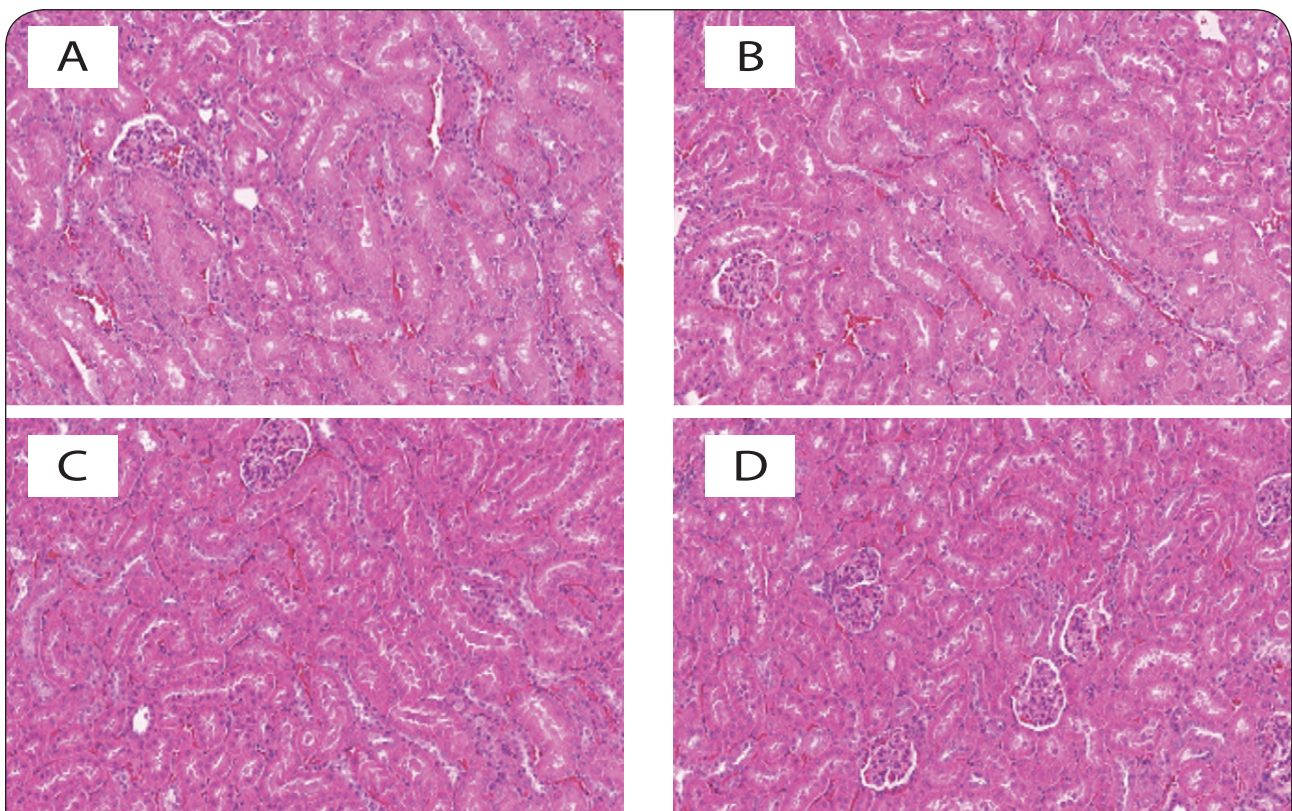
weight, biochemical or histopathological changes in animal organs such as liver, kidney, spleen, heart and testis that support the safety protocol of Av seed extract. Subacute research did not reveal any biochemical change in liver, kidney, brain, heart, spleen, testis and ovary in both male and female rats after oral intake of 600 mg/kg bw/d Av seed ethanolic extract for 28 successive days. The hematopoietic system/bone marrow is one of the most sensitive targets for any toxic compounds or drugs and it is an important guide of any physiological and pathological changes in both man and animal (Mukinda & Syce, 2007). In subacute research, the hematological parameters are associated with toxic results since any variation in hematological system is a sensitive index for any human toxicity if the data obtained from animal studies are translated (Olson *et al.*, 2000). Subacute study of both male and female animals with daily oral intake of Av seed extract for 28 successive days showed small or minor variations in some biochemical and hematological parameters.

The main organ for metabolism, including drugs, is liver. The hepatic tissue is a place of cholesterol removal or degradation and, in the same time, liver is a main place of cholesterol synthesis. In this explanation, liver controls glucose synthesis and increase free glucose discharge from liver glycogen stores (Anderson & Borlak, 2008). Minor variations were recorded in both glucose and cholesterol levels in this research. Consequently, Av seed ethanolic extract had minor effect on the lipid and carbohydrate metabolism in both male and female animals. Further, drugs showing any toxicity affect the transaminases aspartate aminotransferase (AST) and alanine amino transferase (ALT) in liver which are well known enzymes used as good indicators of liver function (El Hilaly *et al.*, 2004) and biomarkers predicting possible toxicity (Rahman, 2001). The elevation or increase in serum AST and ALT enzyme activities indicate the destruction occurring in parenchymal hepatic tissue/cells (Anderson & Borlak, 2008). In this research, serum AST and ALT levels did not reveal any variation related to increase even at the higher dose (600 mg/kg bw/d) of Av seed extract compared to control group. The serum AST is originally from cytoplasm and mitochondria and consequently any variance in AST level give an indication of hepatic destruction that lead to discharge of AST from liver into serum (Mukinda & Eagles, 2010). Thus, no observed increases in both AST and ALT hepatic enzymes support the evidences that Av seed ethanolic extract subacute administration did not induce any change in hepatic cells or metabolism in both male and female animals.

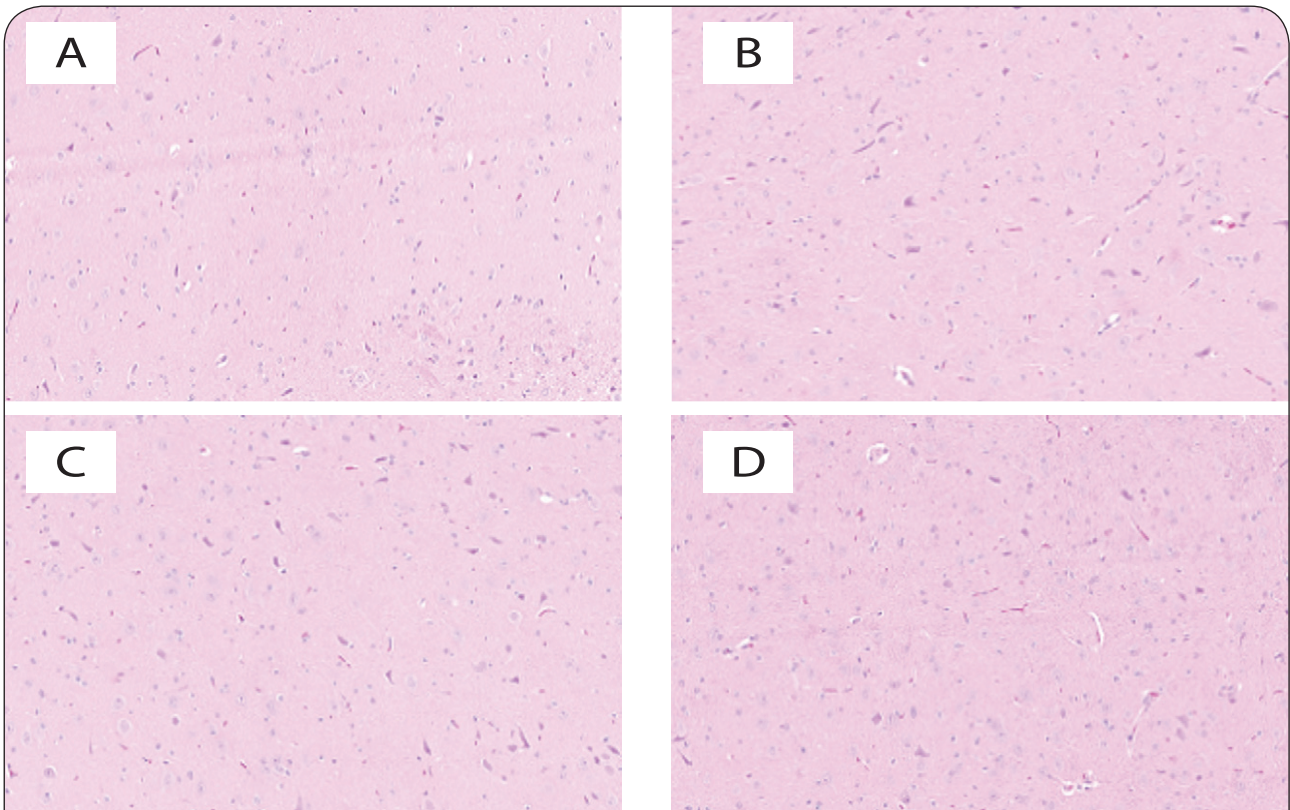
The Av seed ethanolic extract did not show any histopathological alterations in hepatic, nephrotic and splenic tissues which indicate no observable effect on reticulo-endothelial system. Av seed ethanolic extract neither showed any observable variances in the weight of the organs or color of organs nor affected the histopathological changes in organs like heart, brain, testis and ovary indicating minimum increasing toxic effects on reproductive, cardiac and brain tissues.



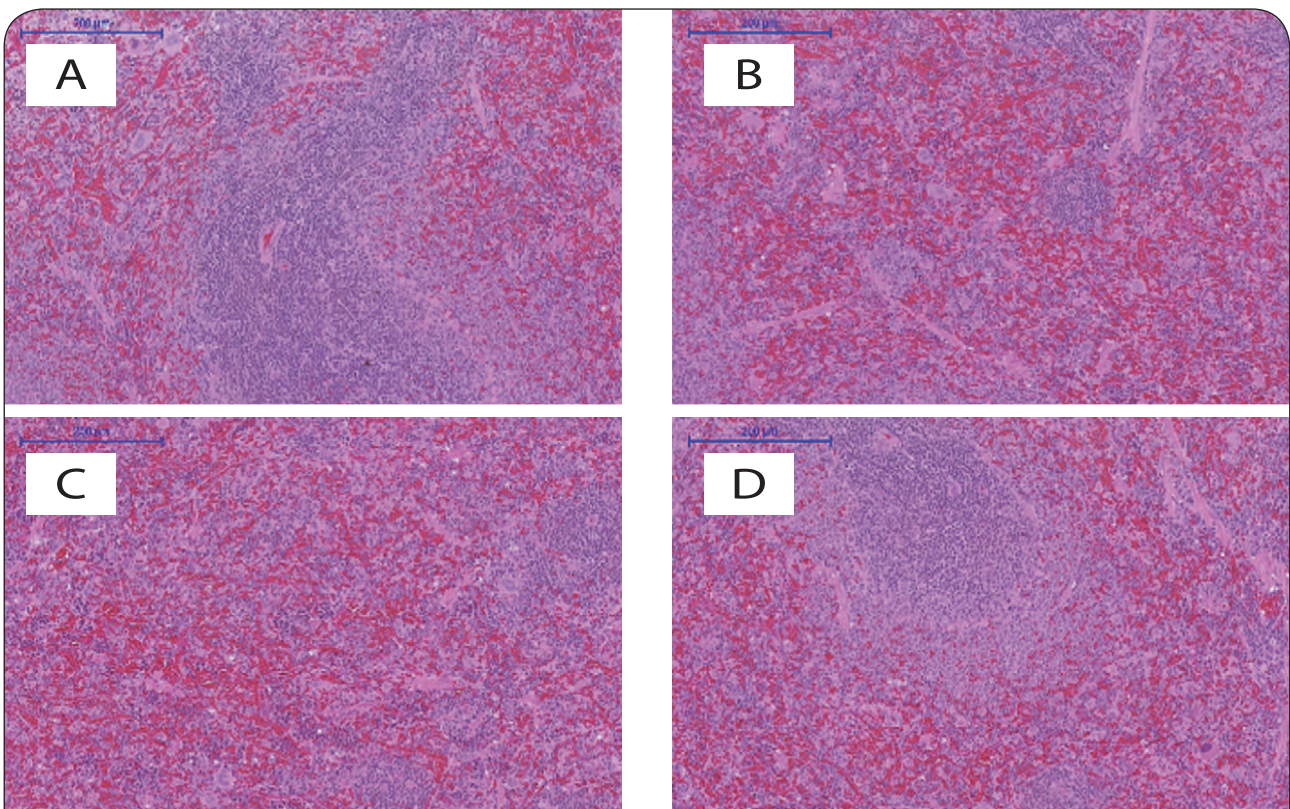
**Figure 1.** Histology of liver (H&E, 100x) of control and Av-treated animals. (A) Section of liver from control animals revealed normal architecture and hepatic cells with granulated cytoplasm; [(B), (C), and (D)] liver from Av (150, 300, and 600 mg/kg)-treated animals exhibited normal architecture of hepatocytes and hepatic cells with granulated cytoplasm.



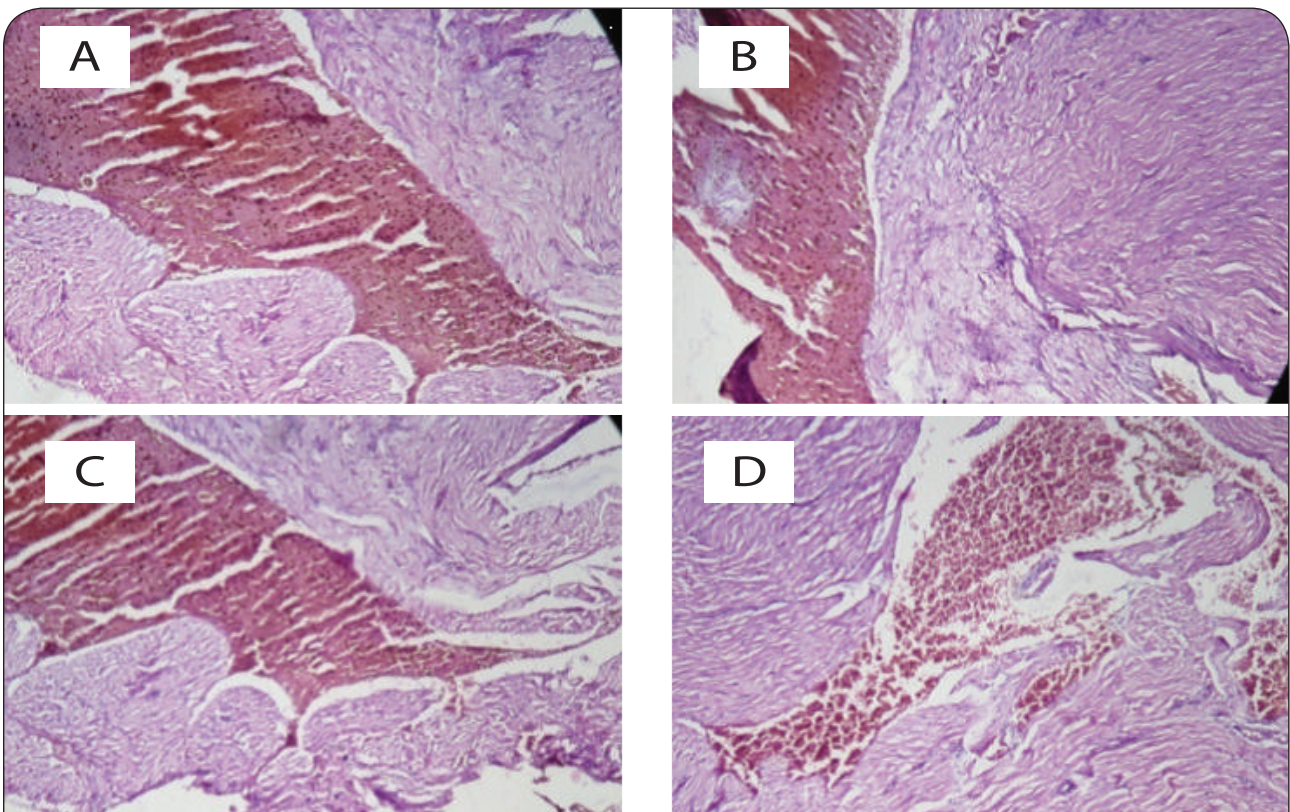
**Figure 2.** Histology of kidney (H&E, 100x) of control and Av-treated animals. (A) Section of kidney from control animals showed normal size of glomeruli with normal tubules; [(B), (C), and (D)] kidney from Av (150, 300, and 600 mg/kg)-treated animals exhibit normal size of glomeruli with normal tubules.



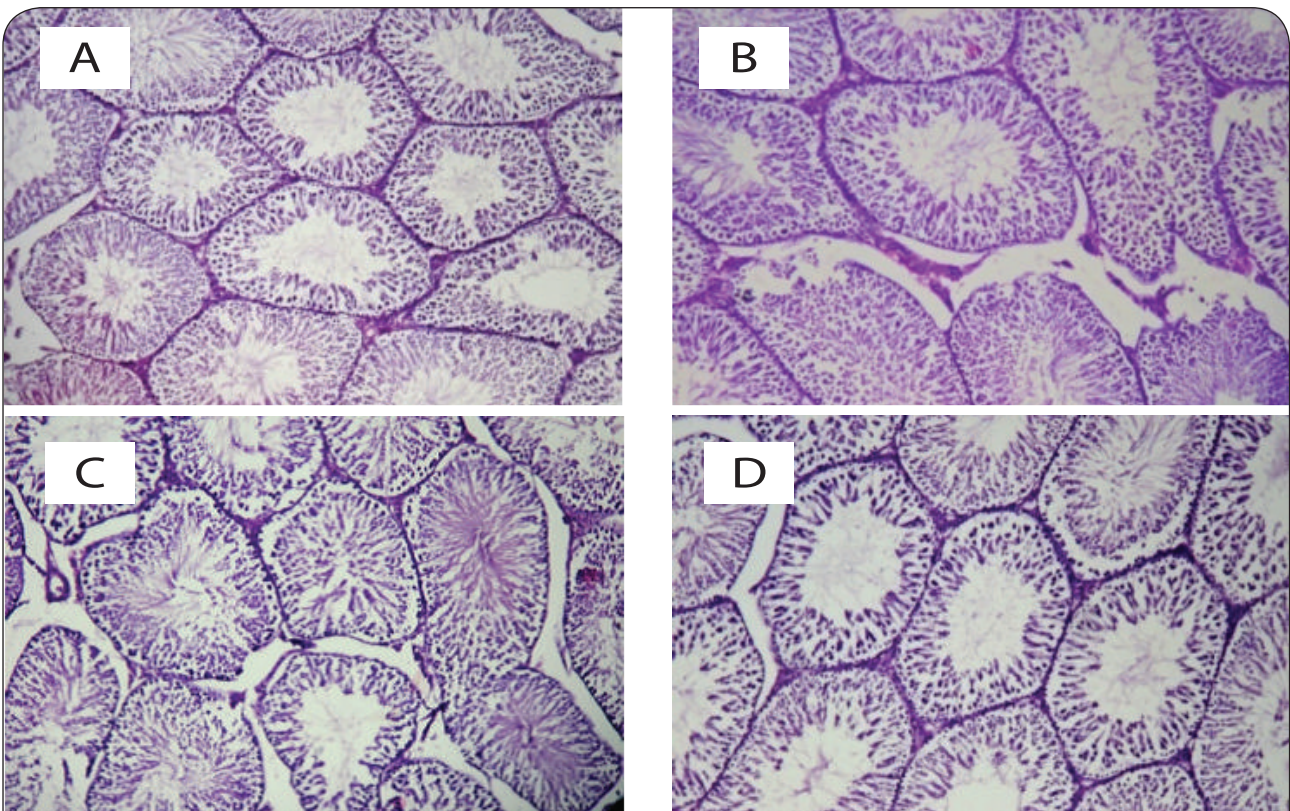
**Figure 3.** Brain sections of control rats showing the highly active nerve cells that have huge nuclei that relatively pale-stained (A). [(B), (C), and (D)] brain from Av (150, 300, and 600 mg/kg)-treated animals exhibit normal brain structure looks like control.



**Figure 4.** Histology of spleen (H&E, 100x) of control and Av-treated animals. (A) Section of spleen from control animals showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp; [(B), (C), and (D)] spleen from Av (150, 300, and 600 mg/kg)-treated animals exhibit normal hemosiderin pigment predominantly within macrophages in the red pulp with normal structure.

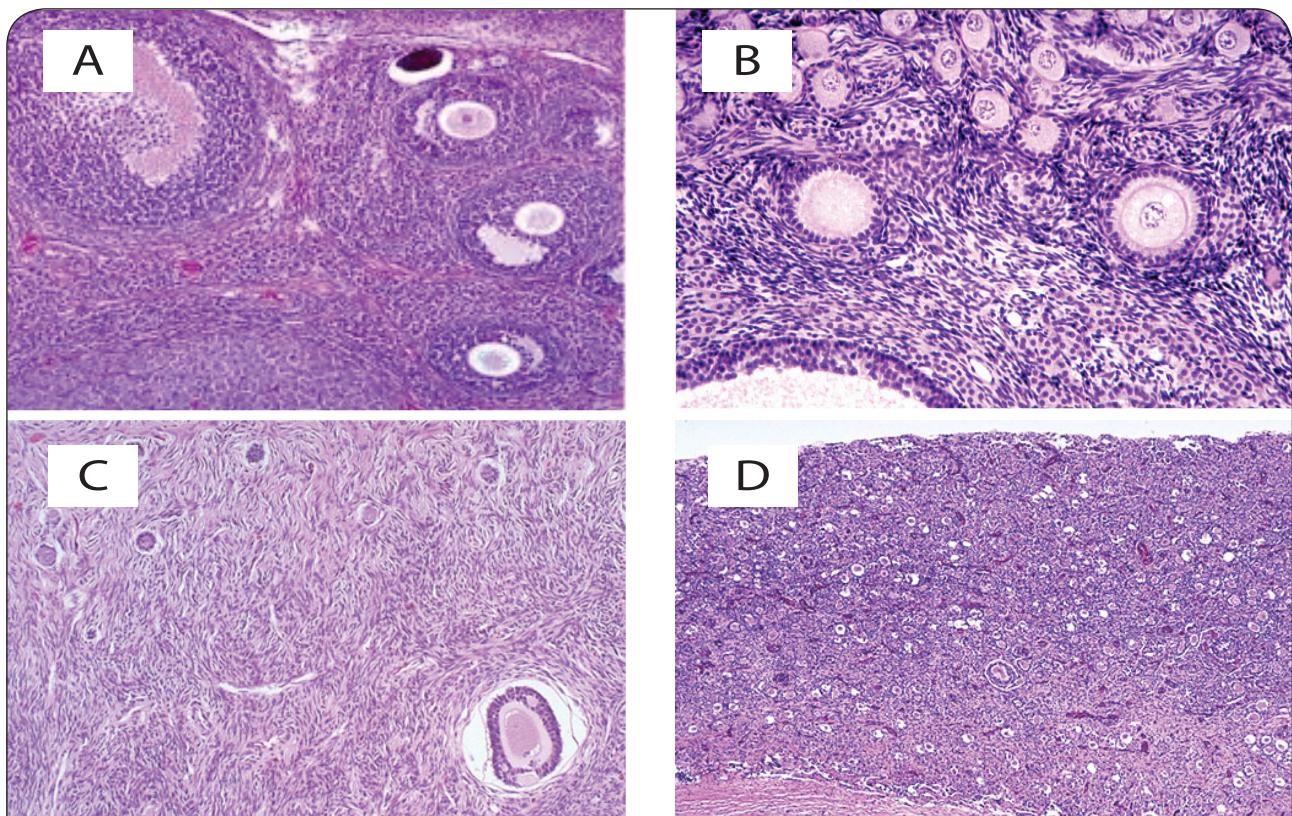


**Figure 5.** Histology of heart (H&E, 100x) of control and Av-treated animals. (A) Section of heart from control animals showed normal muscle fibers with acidophilic cytoplasm and centrally located nuclei; [(B), (C), and (D)] heart from Av (150, 300, and 600 mg/kg)-treated animals exhibit normal muscle fibers with acidophilic cytoplasm and centrally located nuclei.



**Figure 6.** Histology of testis (H&E, 100x) of control and Av-treated animals. (A) Section of testis from control animals showed well-layered seminiferous tubules with germ cell; [(B), (C), and (D)] testis from Av (150, 300, and 600 mg/kg)-treated animals exhibit normal seminiferous tubules with germ cell.





**Figure 7.** Histological of ovary (H&E, 100x) of control and experimental group of animals. (a) Section of ovary from control animal showed normal small follicles and large follicles; ((B), (C) and (D)) ovary from Av (150, 300, and 600 mg/kg)-treated exhibit normal small follicles and large follicles in the histology.

Finally, no observable alteration occurred in kidney function parameters represented by no variance in serum urea, creatinine and uric acid levels as well as histological changes in Av seed ethanolic extract-treated nephrotic tissues.

## Conclusion

The study provides valuable data on the acute and sub-acute effects of *Ammi visnaga* in male and female rats. The doses of 5, 10 and 20% corresponding to 150, 300 and 600 mg/kg bw/d of Av did not cause any mortality or toxic effects in the acute toxicity study on rats. No significant clinical, hematological, biochemical, histopathological changes or levels of some hormones were observed in treated animals versus controls in 28-day oral toxicity study.

## Conflict of interest statement

The authors declare that there are no conflicts of interest.

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