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Reduction Of Cholesterol And Estimation Of Genotoxic Effects Of An Aqueous Infusion Of *Adiantum Capillus-Veneris* Leaves In Male White Rats' Testicular Tissue

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ABSTRACT

Traditional medicine usually uses many plants and herbal species in the treatment of many diseases, and it is noticeable that there are no clear indications about the toxicity and efficacy of Adiantum capillus -veneris (Acv) when used repeatedly. The potential role of Acv in preventing and reducing lipid levels after induction in rats. In this study, 4 groups for 8 weeks. A significant increase in TC, LDL-C, and AIP levels in the group fed with 2% cholesterol ($P<0.05$), whereas there was no significant difference compared to control in other groups. Testicular tissue was studied and there was a significant change in tissue with the 2% cholesterol group (capsule thick connective tissue, congested blood vessels, and scattered spermatocytes) whereas other groups showed that significant improvement of testicular tissues when treated with Acv compared with the control group. Micronucleus examined plant genotoxicity screening in bone marrow cells and showed no micronuclei in the treated groups with 100mg/kg of Acv leaves, compared to control.

Keywords: Genotoxic, Hypercholesterolemia, Testicular tissue, Aqueous infusion, Adiantum capillus -veneris

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INTRODUCTION

Hypercholesterolemia is the signs and symptoms of many risks like obesity, cardiovascular, testicular damage, Hyperlipidemia is the major cause of problems with male infertility in developed countries [1]. This occurs as a because of high Triglycerides (TG), low of high-density lipoproteins (HDL-C) and high level of very-low-density lipoproteins (VLDL-C) [2], Several studies on experimental animals showed that the common risk of infertility disease was hypercholesterolemia, which leads to tissue damage and causes many human diseases like thickening of blood vessels, testicular disorders, increased inflammation and oxidation [3], so hypercholesterolemia is the major public health problem and deserves more attention to control [4].

Spermatogenesis needs lipid and cholesterol and is the energy source for Sertoli cells and is necessary for membrane reconstruction of primary spermatocytes [5]. Increasing the size and number of adipocytes were effects male infertility and can lead to physical changes (increases in sleep apnea, scrotal temperature, and erectile dysfunction) and hormonal (increases in insulin, estrogen, and leptin level), while there is a decrease of testosterone, these changes lead to azoospermia, oligozoospermia, and low in semen volume, and the DNA fragmentation index increase [6]. Cholesterol is present in the cell sperm membranes, and they lose a high percentage of it when it passes into a female reproductive system [7]. Cholesterol plays a very important role in sperm movement because they involve it in phospholipid bilayers of the outer acrosomal membrane, and the presence and accumulation of cholesterol ester are one defect in the epididymis [8]. Also, hypercholesteremia leads to the accumulation of lipid in an epithelial apical cell and observed accumulation of cholesterol esters in the peritubular caput, this leads to the disorder of effectiveness, vitality, and movement of sperm in men and the occurrence of infertility[9].

Plants were - and still are - play an essential part in life continuity, as a food and medicine source. Many herbs were used in the treatment of heart disease, digestive and liver disorders, central nervous system disorders, Infertility, and others [10]. In the last years, drug resistance rates because of the indiscriminate use of prescription drugs. This caused scientists to search for additional sources such as medicinal plants [11]. Medicinal plants can be used and are a source of several strong and efficient medicines in different countries [12]. We now focus efforts on many herbal plants due to its ability to cause antioxidant effects [13]. *Adiantum capillus -veneris* it is a medical Moniliformopses that grows on the edges of rocks and in shady areas, and one of the most commonly used Chinese herbs [14]. It is used to treat cold and as a diuretic, fever, cough, bronchial disorders, skin diseases, liver tumors, laxative, menstrual difficulties and to increase breastfeeding and fertility [15], and as an anti-dysentery and an anti-microbial. It includes active substances like tannins, phenols, volatile oils, sugars, glues, Galic acids, and some elements such as potassium K, Ca, and Mg [16]. Adiantum capillus-veneris is an essential natural source of antioxidants because it contains large amounts of molecules that capture free radicals [15,17].

Genotoxic substances cause damage to cells' genetic material by interactions with the sequence of DNA and structure. Some chemicals affect the formation of fragile sites on the chromosome, as it can lead to cancer. In line with this statement, occupational exposure to certain pesticide mixtures correlates positively with increased genotoxic damage in individuals exposed [18]. The disparity in detoxifying capability is for the inherited polymorphisms of people in genes involved in the chemical metabolism. The different efficiency variations of DNA repair mechanisms can also be for individual variations [19]. Therefore, it is for the use of medicinal and herbal plants as an alternative to chemicals to reduce cell damage.

There is little evidence to explain the effect of hypercholesterolemia as a cause of male infertility and its treatment, so the study aimed to find out the aqueous infusion of Acv leaves in reducing of

hypercholesterolemia because of induced cholesterol on testicular tissue in male white rats, and preventing the precipitation of fats from a high-cholesterol diet by consuming aqueous infusion with cholesterol, and identify the histological changes associated with hypercholesterolemia and cell injury repair treated with the aqueous infusion and recognize the genotoxic effects of the leaves extraction or not.

MATERIALS AND METHODS

Plant collected and extraction.

Plant leaves of *Acv*, from Tikrit province in Salahadin Governorate (north-central Iraq, 34°40'50.7"N 43°39'48.5"E, elevation 137m). the specimens were classified in the Baghdad University Herbarium, Iraq, specimens were collected during April and May 2019. The plant samples were rinsed with distilled water to purify the leaves from impurities and then dried at room temperature. The crude aqueous extract of leaves was prepared by extracted in hot water infusion according to the method previously used by Newall et al. [20], and stored at 4°C until use.

Animal groups.

Experiments were conducted performed in line with the ethical guidelines adopted by the Iraqi association for animal protection and have been accepted by the Biological Society of Iraq Animal Research Ethics Committee. Adult male white rats (6 weeks old) from the College of Veterinary Medicine Animal House Tikrit University were used after one week of adaptation period in all groups. Light and Temperature (12h. light-dark cycle) with water and food *ad libitum* were maintained under environmentally controlled conditions. 20 male rats were used with weights of 160-180 g. Rats were assigned into 4 groups of 5 rats each, and an experiment lasted 8 weeks and the weight of the body was registered weekly. The 01 groups (Control) were fed with a standard diet and normal saline, group 02 Rats were fed chow containing 2% cholesterol per day, group 03 providing with a 100mg/kg b. w. of hot water aqueous infusion extract of *Acv* leaves, and group 04 were supplied with a 100mg/kg of *Acv* aqueous infusion and fed with 2% cholesterol. The final body weight fasted was weighed, and rats in all groups were killed by aortic diethyl ether anesthesia exsanguination. For examination, the serum has been isolated from blood, and immediately, testes were excised.

Biochemical test.

Total Cholesterol (TC), Triglyceride (TG), and HDL-Cholesterol (HDL-C) were obtained from heart serum blood at the end of the experiment and were determined according to the enzymatic commercially kits from BioMerix France. The concentrations of LDL-Cholesterol(LDL-C) were determined from the Friedewald equation [21], and the atherogenic index of plasma (AIP) was estimated using the equation: AIP = Log (TG/HDL-C) [22].

Testicular histology.

Specimens were removed, washed, and fixed in formalin (10%) for four h. The testicular tissue was dehydrated and paraffin-embedded, sectioned were cut at five-micrometer-thick by electric microtome (Slee, Germany). Sections of each paraffin block are prepared for Hematoxylin –Eosin stain [23].

Genotoxic analysis

Micronucleus from Bone-marrow tests was conducted according to [24]. Rats have been sacrificed through cervical dislocation. The thigh bone has been sterilized by 70% ethanol and Inject 2 ml of human plasma into the middle of the bone marrow and then collect into a test tube and centrifuged at 1000 rpm for 10 minutes, a smear was done by taking a drop of the pellets on a clean slice and discarding the supernatant. The slides were dried at room temperature and then fixed with absolute methanol for five minutes, then rinsed with distilled water then stained in May-Grunwald for 20 min and Giemsa 5% solution in 0.01 phosphate buffer adjusted to PH 6.8. The existence of polychromatic micronucleate erythrocytes (about 1000 per rat) is visually scored by a light microscope. Bone marrow cells are multinucleated when containing chromatin corpuscles with dimensions less than a third of the nucleus and stained with a higher density than the nucleus from which it was differentiated.

Statistical analysis

The differences between the groups were analyzed statistically by using the SPSS v.22 software. Data has been represented as ± SD. Statistical significance of data was calculated by the variance (one-way ANOVA) analysis plus Bonferroni's check post hoc. $P<0.05$ has been significant.

RESULTS

The effect in Lipid profile

A significant increase in TC, LDL-C, and AIP levels in group G02 ($P<0.05$), whereas there was no significant difference relative to control in other groups as shown in Table 1. There was no significant reduction in HDL-C Levels in all groups compared with control.

Table 1. Effect of hot water aqueous infusion extract of *Acv* leaves on serum lipid profile levels.

Groups	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	AIP (mmol/L)
G01	3.60 ± 0.34	1.85 ± 0.02	1.12 ± 0.02	1.60 ± 0.08	0.10 ± 0.04
G02	5.39 ± 0.61*	1.94 ± 0.18	0.87 ± 0.11	3.63 ± 0.12*	0.40 ± 0.14*
G03	3.05 ± 0.33	1.23 ± 0.02	1.30 ± 0.07	1.18 ± 0.02	0.02 ± 0.01
G04	3.15 ± 0.38	1.35 ± 0.08	1.10 ± 0.04	1.43 ± 0,03	0.08 ± 0.03

G01: Control; G02: 2% cholesterol; G03: 100mg/kg of *Acv* aqueous infusion; G04: 100mg/kg of *Acv* aqueous infusion and fed with 2% cholesterol, TC: Total Cholesterol; TG: Triglyceride; HDL-C: HDL-Cholesterol; LDL-C: LDL-Cholesterol; AIP: atherogenic index of plasma, * Significance at $p< 0.05$; Values are mean ± SD of four groups.

Effect of Acv aqueous infusion extract in Testicular tissue.

In control group G01 (Fig. 1), the seminiferous tubules were surrounded by a basement membrane, that the spermatogonium resting on it, these are one row of cells with deeply stained nuclei, the second row was the primary spermatocytes, and there were two rows of secondary spermatocytes, these are surrounded by a group of smaller cells nearby the center of seminiferous tubules called spermatids, finally, the spermatozoa present in the center of the wavy bundle

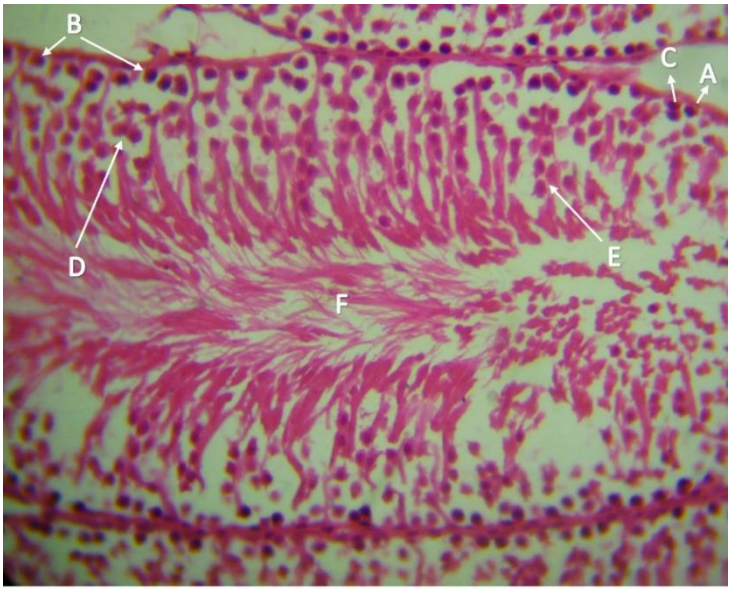

Figure 1. Testis, Seminiferous tubule, A: Basement membrane, B: Spermatogonia; C: Primary spermatocyte; D: Secondary spermatocyte; E: Spermatids; F: Spermatozoa (H&E X40).

The second group G02 which induced with 2% cholesterol, showed that the capsule of testis was thick and comprise dense connective tissue, the subcapsular area was containing the congested blood vessels and the seminiferous tubules adjacent to these blood vessels were containing scattered spermatocytes of different stages of development (Fig. 2). However, the interstitial connective tissue contained hemolyzed blood inside the lumen of blood vessels, with atrophied Leydig cells (Fig. 3).

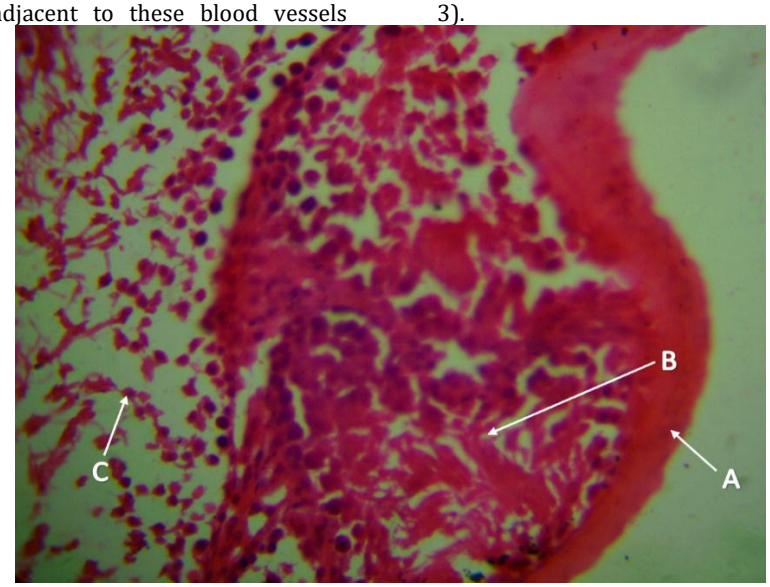

Figure 2. The capsule of the testis, A: Blood congestion of testicular blood vessel; B: Scattered of spermatocyte; C: Scattered of seminiferous tubule (H&E X40)

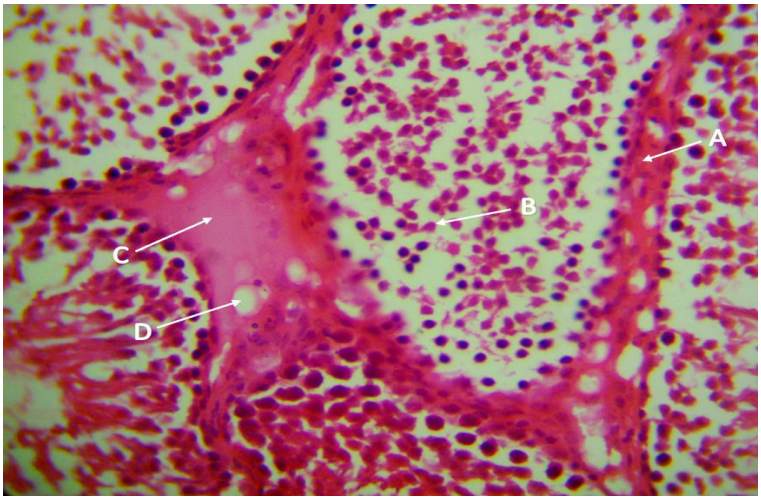

Figure 3. A: The thickened basement membrane of interstitial C.T, B: scattered spermatocytes of spermatid and degenerated sperm; C: Hemolyzed blood of blood capillaries; D: Fat droplets; (H&E X40).

Rats of 03 group which was fed 100mg/kg b.w. of hot water aqueous infusion extract of *Acv* leaves in Fig. 4. showed that the seminiferous tubule of testis was also filled with a great number of spermatozoa and the basement membrane of each tubule was thick resting on it a single row of spermatogonia, and a single row also of primary spermatocytes with two rows of secondary spermatocytes intermingled with spermatids present near the bundles of spermatozoa, present of the lame-like pattern.

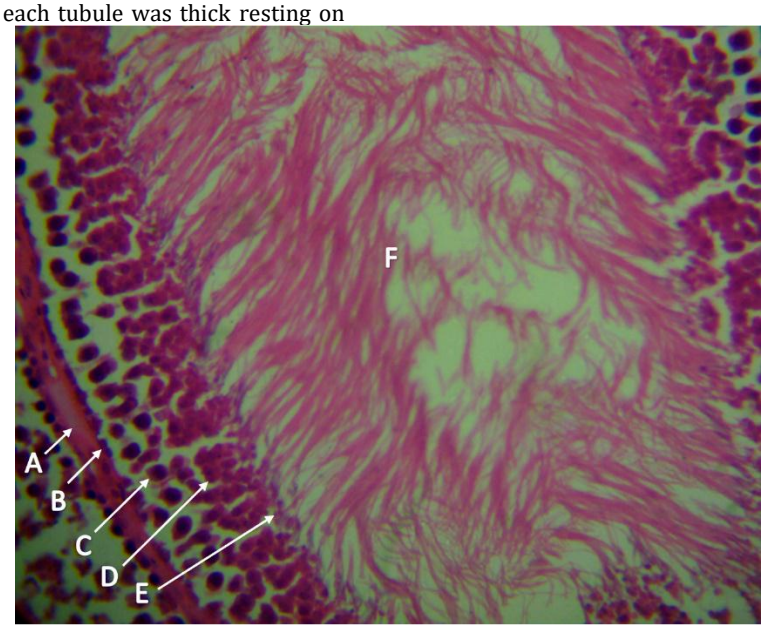

Figure 4. The seminiferous tubule of the testis; A: basement membrane of interstitial connective tissue. B: Spermatogonia; C: Primary spermatocyte; D: Secondary spermatocyte; E: Spermatids; F: Bundle of spermatozoa; (H&E X40).

In, the last group 04 which fed 100mg/kg b. w. of hot water aqueous infusion extract of *Acv* leaves with 2% cholesterol was appeared the basement membrane and the interstitial connective tissue around the seminiferous tubule were thick. The lumen of the seminiferous tubule was filled with a row of spermatogonia resting on the basement membrane, the primary and secondary spermatocytes were easily showed and groups of spermatid also seem nearby the center of the lumen of tubules which had spermatocytes, which are mostly seen in groups or masses (Fig5).

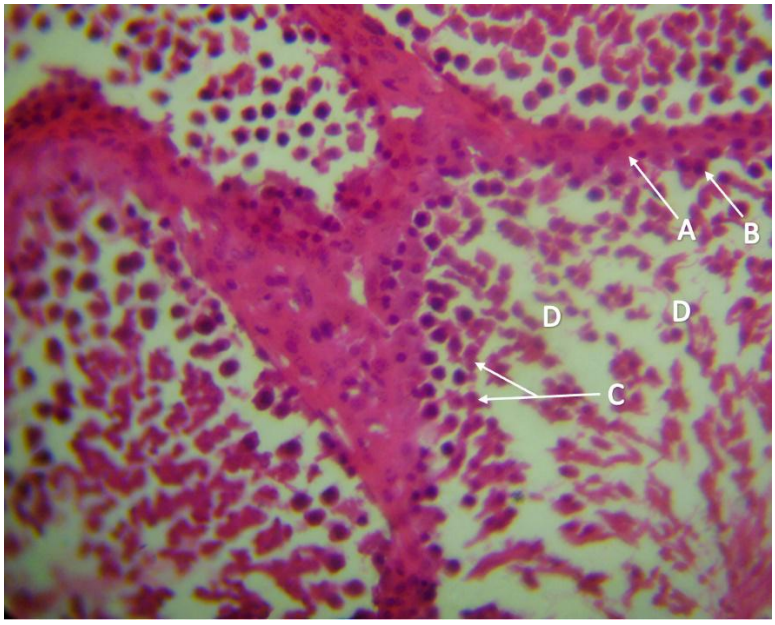

Figure 5. Thickened of interstitial C.T of A: basement membrane; B: Spermatogonia; C: Primary and secondary spermatocyte; D: Spermatid; (H&E X40).

Genotoxic Effect.

Data from the current study did not show that the G03 group was treated with the 100mg/kg of *Acv* aqueous infusion contain the polychromatic erythrocytes (PCEs) without micronuclei, (Fig. 6, A) against negative control group G01 (Fig. 6, B). All groups in this study have no significant mutagenic effects were observed.

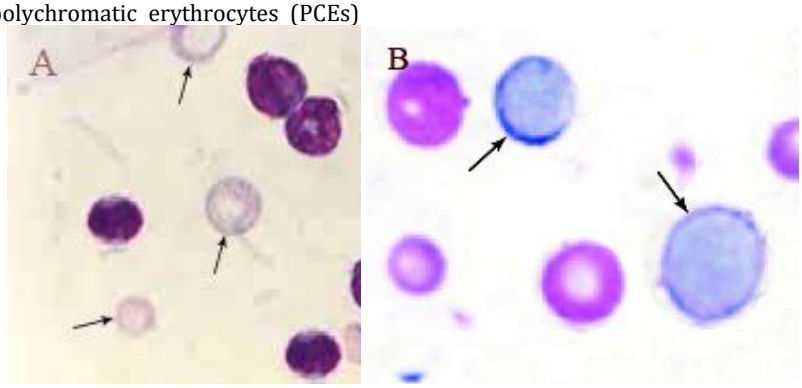

Figure 6. polychromatic erythrocytes (PCEs) from bone marrow, A: G03 group of 100mg/kg *Acv* aqueous infusion; B: G01 control group (May-Grunwald+ Gemsa 100X)

DISCUSSION

After 8 weeks of *Acv* aqueous infusion extract treatment, there were minor signs of changes in biochemical serum parameters. Table 1. shows a significant increase in TC, LDL, and AIP for the G02 group compared with the other groups and there were no significant changes in HDL-C and TG in all groups, these it is a natural result of feeding animals for 8 weeks of a diet containing 2% cholesterol which may cause an imbalance or disruption in fat metabolism and an increase in cholesterol esters[25]. Also, some changes occur in the bile absorption process, steroid excretion, and decreased bile salts, which leads to tissue changes in the bile's structure duct and bilirubin deposition in the liver [26]. The high concentration of TC and LDL-C of G02 was caused an increase of AIP risk which leads to Arteriosclerosis, blood viscosity, and blood clot formation. *Acv* aqueous infusion extract has an important affected role in the rebalancing of fat metabolism and dissolving it and reducing the weight [27].

Changes occurred in the tissue were because of hypercholesterolemia which may be forming a free radical such as hydroxyl radicals and superoxide[28], and It is also considered a risk factor for testicular dysfunction and a decrease in testosterone which leads to a decrease in the binding of luteinizing hormone (LH) with Human chorionic gonadotropin (hCG) in the testis and serum of rats induced by 2% cholesterol [29]. The results got in this study when administering the aqueous extract were shown in Fig. 4 which leads to increased testosterone in serum blood and activation of Δ5, Δ6 fatty acid desaturases, 3β-Hydroxysteroid dehydrogenase (3β-HSD), and 17β-Hydroxysteroid dehydrogenase (17βHSD) enzymes, which leads to spermatogenesis and spermiogenesis success [30]. The effectiveness of the extract in repairing testicular tissue damage maybe because it contains flavonoids, which inhibit the enzymes of oxidation, hydrogenation, and anti-inflammatory [31]. Fig. 5 were showed that the testicular tissue regained its normal state and that the aqueous infusion extract has a good effect in the repair of tissue damage and these signals indicate that the aqueous infusion is a good antioxidant [32], and the *Acv* aqueous infusion contains polyphenols such as eugenol [33], which have an

antioxidant action and this inhibits lipid peroxidation that causes damage to testicular tissue by the antioxidant effort. Thus, natural antioxidants are powerful substances capable of disabling Reactive oxygen species (ROS) and curing free radicals before they caused a defect in the tissue [34].

The evaluation of the toxic effects of medicinal plants is carried out by most studies dealing with these plants and their use with traditional medicine, lacking knowledge of their toxicity and effectiveness after long-term treatment [35]. As with many other species of medicinal plants, few histological/toxicogenic protection studies on *Acv* are performed [36]. Our data have shown that *Acv* has no genotoxic effect on rats, during the 8 weeks aqueous infusion extract treatment, no weight loss was observed among the rats treated with *Acv* at 100 mg/kg, and genotoxic examination of the PCEs showed no difference and no apparent Micronuclei. The lack of genotoxicity of this plant may be due to its possession of some active compounds such as flavonoids, tannins, and phenols that contribute to free radical scavenging activity[13].

CONCLUSION

Adiantum capillus -veneris leaves aqueous infusion extract to have a significant role as antioxidant activity against hypercholesteremia and can show the potential to maintain cholesterol homeostasis. The histological effect of testicular rats induced with 2% cholesterol there were many defects on the tissue (capsule thick connective tissue, congested blood vessels, and scattered spermatocytes) whereas other groups showed that improvement of testicular tissues when treated with *Acv* compared with the control group. Studies have not showed the effect of *Acv* in different doses to show genotoxicity when treated with laboratory animals. This study is considered the first to show the genotoxic effect of this plant on male albino rats, which explained no genotoxic effect of the *Acv* plant.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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