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ORIGINAL

## The Protective Effect of *Alchemilla Vulgaris* on The Reproductive System and Some Visceral Organs (Liver, Spleen) Tissues of Female Rats Exposed to High Dose of Zinc Sulphate in Drinking Water

El efecto protector de *Alchemilla Vulgaris* sobre el sistema reproductivo y algunos tejidos de órganos viscerales (hígado, bazo) de ratas hembra expuestas a altas dosis de sulfato de zinc en agua potable

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### ABSTRACT

The study examined the effects of high concentrations of zinc sulphate in drinking water on female rats' liver, spleen, and ovaries over eight weeks. The rats were divided into five groups: control, zinc-containing, zinc-sulphate-containing, zinc-sulphate-containing, and zinc-free. As for the objectives of the study, it highlighted the protective and toxic effects of *Alchemilla Vulgaris* medicinal plant on female rats' liver, spleen, and ovaries tissues from high ZnSO<sub>4</sub> concentrations in drinking water. Regarding the method of the study, it involved 30 female albino rats in a pharmacy college/university of Karbala, divided into five groups: control, treatment, treatment, and treatment, with zinc sulphate and *Alchemilla vulgaris* added to their drinking water. The data was analyzed using one-way ANOVA and SPSS 22.0 software, with four treatment means separated using a "protected" Duncan's analysis at a 0,05 level. The study reached some results, the most important of which are that there are changes in organ tissue, including necrosis in liver cells, congestion in spleen and ovaries, and an increase in progesterone. The study also found that the medicinal plant treated most liver diseases with low side effects, improved ovarian functions, and improved fertility. The study concluded that medicinal plant was used to treat most liver diseases better because its side effects were low. These plants had preventive effects against zinc excess and improved ovarian functions by improving the production of reproductive hormones in females.

**Keywords:** Zinc Sulphate; *Alchemilla Vulgaris*; Reproductive Hormones.

### RESUMEN

El estudio examinó los efectos de las altas concentraciones de sulfato de zinc en el agua potable en el hígado, la médula y los ovarios de las ratas hembras durante ocho semanas. Los ratones fueron divididos en cinco grupos: control, que contenían zinc, que contienen sulfato de zinc y que no contenían. En cuanto a los objetivos del estudio, se destacó los efectos protectores y tóxicos de la planta medicinal *Alchemilla Vulgaris* en el hígado, la médula y los tejidos ováricos de las ratas hembras debido a las altas concentraciones de ZnSO<sub>4</sub> en el agua potable. En cuanto al método del estudio, se involucraron 30 ratas albinas femeninas en un colegio/universidad de farmacia de Karbala, divididas en cinco grupos: control, tratamiento, tratamiento y tratamiento, con sulfato de zinc y *Alchemilla Vulgaris* añadidos a su agua potable. Los datos fueron analizados

utilizando el software ANOVA y SPSS 22.0 de una sola dirección, con cuatro medios de tratamiento separados utilizando un análisis “protegido” de Duncan en un nivel de 0,05. El estudio alcanzó algunos resultados, los más importantes de los cuales son que hay cambios en el tejido de órganos, incluyendo necrosis en las células del hígado, congestión en la vesícula y los ovarios, y un aumento en la progesterona. El estudio también encontró que la planta medicinal trataba la mayoría de las enfermedades hepáticas con efectos secundarios bajos, mejoró las funciones ováricas y mejoró la fertilidad. El estudio concluyó que la planta medicinal se usaba para tratar mejor la mayoría de las enfermedades hepáticas porque sus efectos secundarios eran bajos. Estas plantas tenían efectos preventivos contra el exceso de zinc y mejoraban las funciones ováricas al mejorar la producción de hormonas reproductivas en las mujeres.

**Palabras clave:** Sulfato de Zinc; Alchemilla Vulgares; Hormonas Reproductivas.

## INTRODUCTION

Environmental pollutants and their toxicity generate many problems worldwide. New pollutants stay emerging and pose severe health and systematic challenges. Water pollution is one of the biggest environmental issues causing serious problems to survive organisms<sup>(1,2)</sup> Metals are probably widely-known in the oldest toxic substances to the human and animals.<sup>(3)</sup> Heavy metals generally exist in the crust, rock, water, soil, atmosphere and biosphere, and some heavy metals in some environments may come from natural and anthropogenic sources.<sup>(4)</sup> The metal ions essential and nonessential can be toxic when present in excess.<sup>(5)</sup> Heavy metals as zinc, arsenic, cadmium, mercury and nickel have been known to possess many adverse health effects; still, heavy metals (HM) pollution continues and is even increasing in some parts of the world, in particular in less developed countries due to the uncontrolled industrialization, it has caused many kinds of the heavy metals accumulation in our organ tissue and inducing chronic toxicities.<sup>(6)</sup> Zinc is an essential mineral of exceptional biologic and public health importance, also considers as an essential trace element for plants, animals and microorganisms. It plays a role in immune function, protein and DNA synthesis and cell division, wound healing, normal growth and development, and it is the only metal which appears in all enzyme classes as a structural, regulatory or catalytic role in many enzymes.<sup>(7)</sup> Increasing pollution due to heavy metals has become a serious environmental concern, also zinc concentration in water and soil has risen as a result of human activities such as mining or production of wastewater. High concentration of Zn can affect the uptake of other nutrient elements such as Cu, Mn and Fe, and the deficiency of these elements may cause oxidative stress.<sup>(8,9,10)</sup> Herbal medicines are now used by up to 50 % of the Western population, in a substantial minority of instances for the treatment or prevention of digestive disorders. HM preparations contain many bioactive compounds.<sup>(11)</sup> HM are not pharmaceutical medications. Herbs are part of whole plants, not isolated or not synthesized chemicals. Herbal effects have to do with the synergistic action of nature's formulation. Drugs and herbs are used differently but both can be extremely beneficial when used appropriately.<sup>(12)</sup> Lady's Mantle (Alchemilla vulgaris) The common lady's mantle - A. Vulgaris- of the Rosaceae family, a perennial herbaceous plant, is common throughout virtually the whole of Europe, along with a large proportion of the European territory of the USSR and Siberia, except for the most southern regions A Vulgaris used for astringent, anti-hemorrhoidal and anti-diarrheal properties.<sup>(13)</sup> The plant infusion is used externally in the cases of wound healing and stomatitis. Also act as diuretic, depurative, intestinal antiseptic, bacteriostatic and bactericidal, tonic, anti-arthritic and cancer deterrent.<sup>(14)</sup>

## Objectives of the Study

This paper aimed at highlighting the protective effect of the medicinal plant (Alchemilla Vulgaris) against the effect of high ZnSO<sub>4</sub> concentrations in the drinking water on the liver, spleen and ovaries tissues of female's rat during 8 weeks period, and the toxic effect of high dose of ZnSO<sub>4</sub> in drinking water as well as the beneficial effects of this medicinal plant on the study organs.

## METHOD

### Experimental animals

A 30 female albino rats, weighing 150\_250 G were used in this study, The present study was conducted in the animal house in pharmacy college/ university of Karbala, rats were kept randomly in five cages in a rate of six rats per cage at room temperature and supplied with standard diet and water.

### Experimental sampling

The groups were arranged as the following: Were divided randomly into five groups (six rats for each treatment): Group1 (T1) control: six female rats were used; non-ionized water uses free zinc sulphate (ZnSO<sub>4</sub>) and Alchemilla vulgaris. Treatment 1 (T2): six female rats were used, Water used with zinc sulphate (ZnSO<sub>4</sub>) 1000 Mg / L only. Treatment2 (T3): six female rats were used, Water used with zinc sulphate (ZnSO<sub>4</sub>) 1000 Mg / L

with 200Mg/L *A. vulgaris*. Treatment3 (T4): six female-gendered rats were used, Water used with zinc sulphate ( $ZnSO_4$ ) 1000 Mg / L with 300 mg/l/L *A. vulgaris*. Treatment4 (T5): six female rats were used, Water used with 300Mg/L *A. vulgaris* only. Were Added the substances with drinking water of animals.

### Preparation of extract

The plant (Lion's foot) Aerial parts and roots were washed in water several times to remove any adhering flesh, then dried and powdered plant materials were extracted with methanol: water (80:20) mixture by continuous stirring at room temperature for eight hours. After filtration, extract was concentrated to dryness under reduced pressure and low temperature (40-50 C) on a rotary evaporator to give crude extract<sup>(15)</sup> An experimental started in 2/10/2018 Tuesday to 2/12/2018 Sunday. At the end of the first month from the experiment period (1/11/2018 Tuesday), the rats were anesthetized by using xylazine and ketamine (one from each cage randomly), Ovaries, liver and spleen of each animal were quickly removed and prepared for histological study aid of the light microscope.<sup>(16)</sup>

### Statistical analysis

Data was analyzed as one-way ANOVA using the general linear model (GLM) procedure to SPSS 22.0 software.<sup>(17)</sup> Four treatment means were separated using a "protected" Duncan`s analysis in level (0,05).

## RESULTS AND DISCUSSIONS

Our results of the current study histopathological sections of liver tissue in 1000Mg/L  $ZnSO_4$  groups that induced oxidative stress (T2), showed central vein mild congestion, feathery degeneration, and narrow sinusoids. (stain H&E) (40x). The heavy metal toxicity as zinc is their ability to bind strongly to oxygen and induce oxidative stress, through produce free radicals. In a study, ingesting 150 mg zinc sulfate tablets, leads to appear symptoms as: abdominal cramps, nausea, and vomiting.

Figure 1. shows the central vein congestion (thick arrow), feathery degeneration (thin arrow) and narrow sinusoids (blue arrow). (stain H&E). (X40). While the result of histopathological changes in T3(1000Mg/L  $ZnSO_4$  with 200Mg/L *A. Vulgaris* treatment has shown mild return back normal hepatocyte with little congestion and significant degeneration section (mild response).

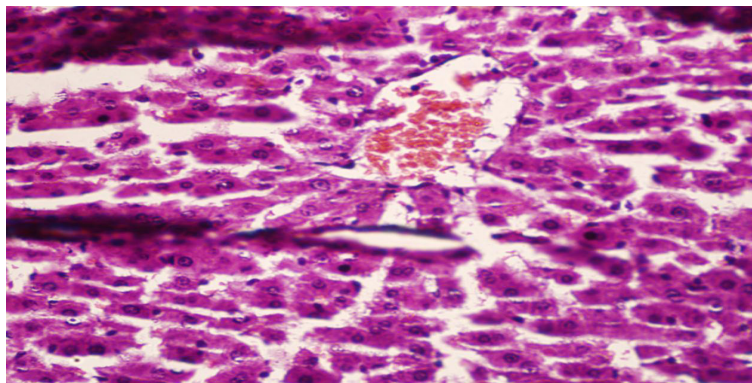


Figure 1. liver in female rats with 1000Mg/L  $ZnSO_4$  (T2) group

Figure 2 shows mild congestion (thick arrow) and mild degeneration (thin arrow). (stain H&E). (X40). Also, the results in histopathological changes in T4(1000 Mg/L  $ZnSO_4$  with 300 Mg/L *A. Vulgaris*) treatment has shown return back normal regular hepatocyte with mild degenerative and no necrosis, (mild response).

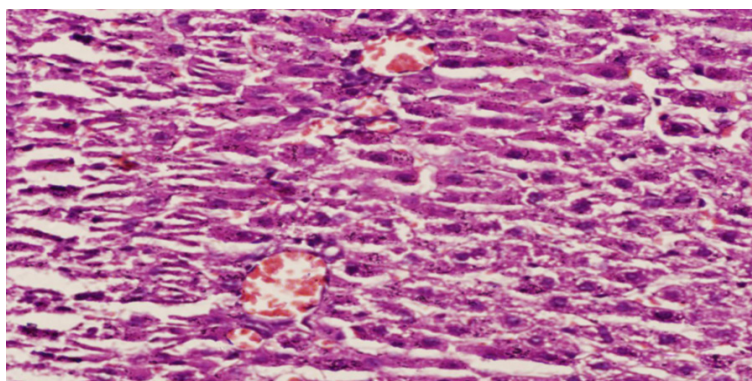


Figure 2. Liver in female rats treated with 1000Mg/L  $ZnSO_4$  and 200Mg/L *A. Vulgaris* (T3) group



Figure 3 shows normal hepatocytes (thick arrow) with mild degeneration (thin arrow). (stain H&E). (X40). The liver tissue treated with 300 Mg/L *A. Vulgaris* (T5) shows normal parenchymal tissue with no inflammation and normal architecture of central vein with mild degeneration.<sup>(18)</sup>

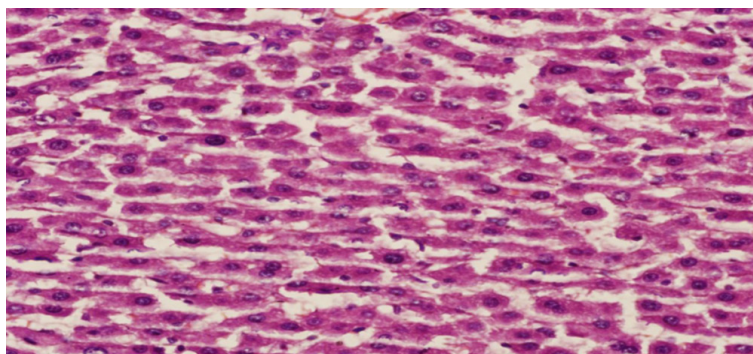


Figure 3. Liver in female rats treated with 1000Mg/L ZnSO<sub>4</sub> and 300Mg/L *A. Vulgaris* (T<sub>4</sub>) group

Figure 4 shows regular hepatocytes (normal hepatocytes) (thick arrow) and steatosis (fatty change) (thin arrow) (Stain H&E) (X40). The use of medicinal plants in a high level of antioxidant constituents has been important role as an effective therapeutic approach for hepatic damages. often of the antioxidant compounds in a typical diet are derived from plant sources and belong to different classes of compounds as phenols, flavonoids, tannins, carotenoids and vitamins which plays an important role in health protection from the risk of most diseases. This no agreement with (El-Sayed, et al, 2017) due to present little congestion in some cells of liver. Natural antioxidants from plants, the most prominent representatives of these compounds can protect the human body from free radicals. The histopathological sections of spleen tissue in 1000Mg/L ZnSO<sub>4</sub> groups that induced oxidative stress (T2), showed sever congestion of red pulp sinusoids with hemosiderin-laden macrophages.

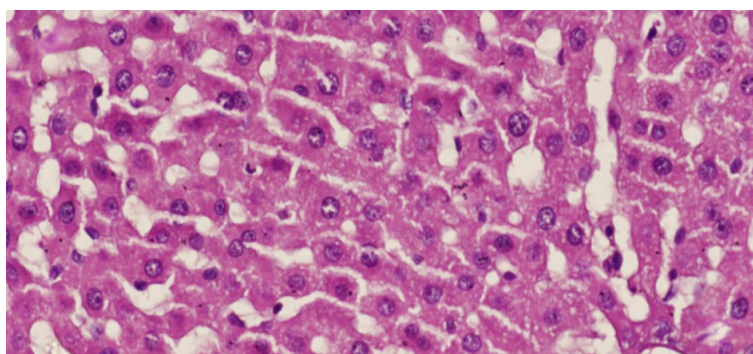


Figure 4. Liver in female rats treated with 1000Mg/L ZnSO<sub>4</sub> treated with 300Mg/L *A. Vulgaris* (T<sub>3</sub>) group

Figure 5 shows sever congestion of red pulp sinusoids (thick arrow) with hemosiderin-laden macrophage (thin arrow). (stain H&E). (X40). In a study by Samman and Roberts, 1990 leads ingestion tablets containing 150 mg of zinc sulfate, to appear symptoms in GIT. However, the result of histopathological changes in T3(1000Mg/L ZnSO<sub>4</sub> with 200Mg/L *A. Vulgaris*) treatment has shown mild hemorrhage in white pulp and hemosiderin-laden macrophage.

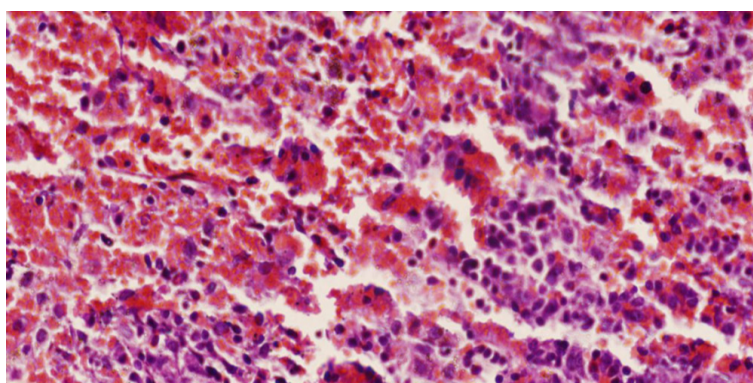
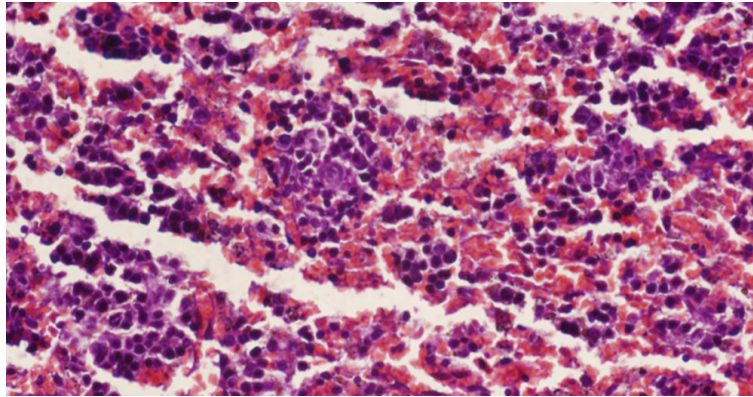


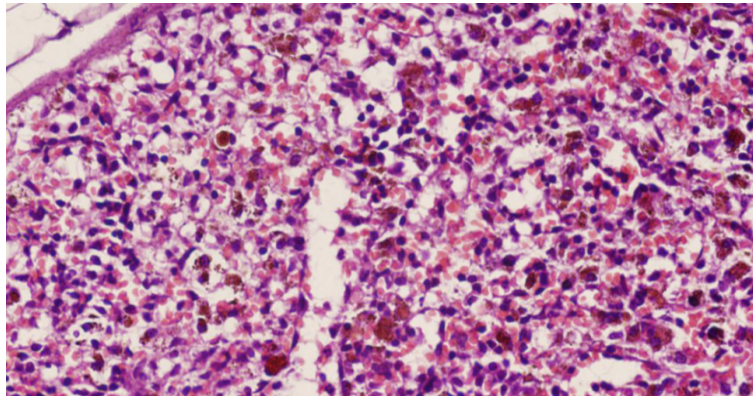
Figure 5. Spleen in female rats with 1000Mg/L ZnSO<sub>4</sub> (T<sub>2</sub>) group

Clearly, figure 6 sever congestion (thick arrow) and hemosiderin-laden macrophage (thin arrow) in white pulp. (stain H&E). (X40).



**Figure 6.** Spleen in female rats treated with 1000Mg/L ZnSO<sub>4</sub> and 200Mg/L A. Vulgaris (T3) group

Figure 7 shows that mild congestion (thick arrow) with number of hemosiderin-laden macrophage (thin arrow) in white pulp. (stain H&E). (X40). The spleen tissues treated with 300 Mg/L A. Vulgaris (T5) shows normal red pulp and white figure 8.



**Figure 7.** Spleen in female rats treated with 1000Mg/L ZnSO<sub>4</sub> and 300Mg/L A. Vulgaris (T4) group

Figure 8 shows normal red pulp (thick arrow) and normal white pulp (thin arrow) (normal tissues). (stain H&E). (X40). The paired ovaries in female rats are like the grape structures in shape, but vary in gross appearance and size, it is depending on the stages of the estrous cycle. The surface is covered by a single layer of modified peritoneal mesothelium, the ovarian surface epithelium (OSE), that supports the ovary by which is continuous with the broad ligament (mesovarium). Primordial follicle - is earliest stage of follicular growing, it is formed during early fetal development and are typically located within the peripheral cortex, direct beneath the tunica albuginea, and followed by these stages (primary, secondary, tertiary and Graafian follicles).

Then, luteinization, this term means the process following extrusion of the secondary oocyte from the Graafian follicle, the thecal cells and granulosa of the follicle remnant undergo hypertrophy and, to a lesser extent, this process occurs under the effect of luteinizing hormone (LH) and prolactin hormone, the two major luteotrophic hormones in rodents. The basement membrane is degenerating accompanying luteinization by separating the zona granulosa and theca interna, and interfering of the post-ovulatory follicle by blood vessels from the theca interna. The result is mature corpus luteum ("yellow body") formed is structure a large eosinophilic that obscures the ovarian cortico-medullary junction or may bulge out from the surface ovarian, this dependent on its location.

The histopathological sections of ovary tissue in 1000Mg/L ZnSO<sub>4</sub> (T2) group that induced oxidative stress, showed congestion in 4th and 6th weeks. At high doses, necessary elements, as copper, zinc, and selenium, could also have toxic effects on kidneys and impair reproduction. However, these results are in agreement with FEDRIP stated No histological alterations in the testes or ovaries were noted in mice fed zinc sulfate (1,110 mg zinc/kg/day) for 13 weeks. While the result of histopathological changes in T3(1000Mg/L ZnSO<sub>4</sub> with 200Mg/L A. Vulgaris) treatment has shown mild congestion section in ovary tissue with corpus luteum.<sup>(19)</sup>



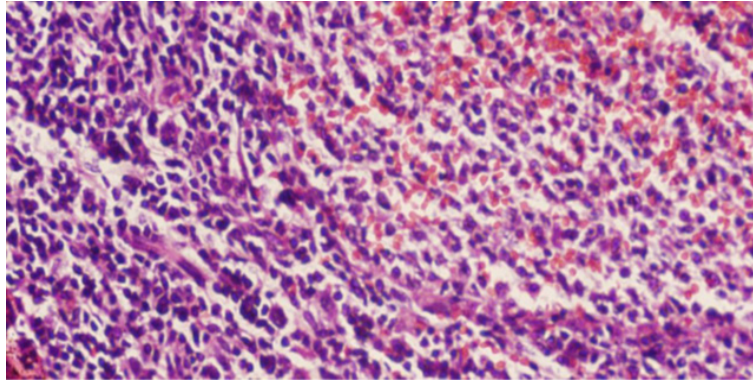


Figure 8. Spleen in female rats treated with 300Mg/L A. Vulgaris (T5) group

Figure 9 shows graafian follicle. (Stain H&E). (X40). The ovary tissues treated with 300 Mg/L A. Vulgaris (T5) shows normal ovary tissue, normal corpus luteum with significant increase in number normal primordial growing follicles.

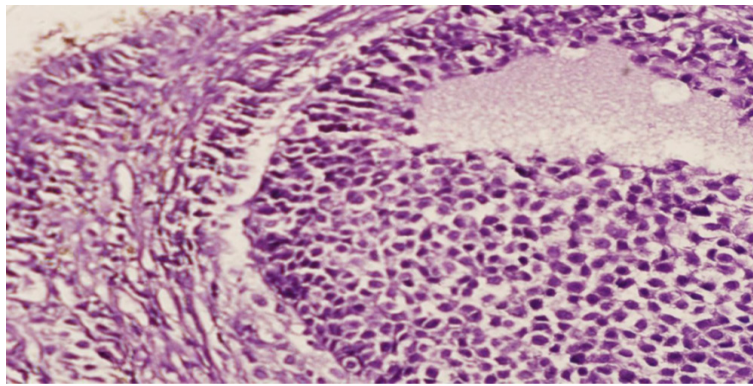


Figure 9. Ovary in female rats of T2(1000Mg/L ZnSO<sub>4</sub>) group

Figure 10 shows normal growing follicles. (Stain H&E).(X40). So, when used this medicinal plant in concentration 300Mg/L act as a stimulates the function of ovary in increase produce the primordial growing follicles in these experimental animals, this agreement with EDRAH, S. M.<sup>(20)</sup>

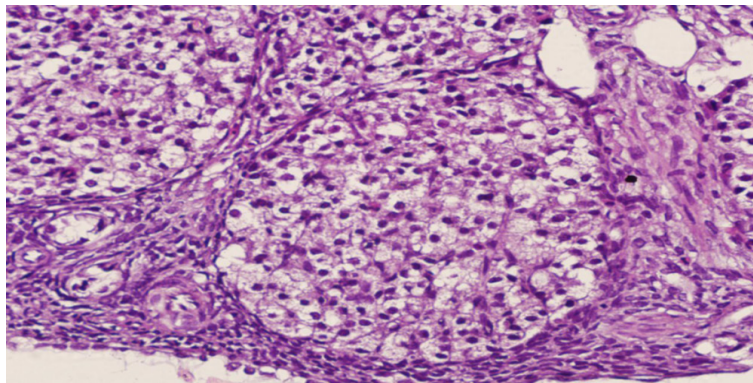


Figure 10. Ovary in female rats treated with 300Mg/L A. Vulgaris (T5) group

## CONCLUSIONS

The medicinal plant was used to treat most liver diseases better because its side effects were low. These plants had preventive effects against zinc excess and improved ovarian functions by improving the production of reproductive hormones in females.

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