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THE PROTECTIVE EFFECT OF *ALCHEMILLA VULGARIS* ON THE REPRODUCTIVE SYSTEM AND SOME VISCERAL ORGANS (LIVER, SPLEEN) OF FEMALE RATS EXPOSED TO HIGH DOSE OF ZINC SULPHATE IN DRINKING WATER

Shaimaa K. Mohammed* and Mehdi A. Ali

College of Veterinary Medicine, University of Kerbala, Iraq
*e-mail : Mohammedme8989@gmail.com

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ABSTRACT : This study was aimed investigate the effect of high $ZnSO_4$ concentrations in the drinking water on the liver , spleen and ovaries of females rat during eight weeks period. And the protective effect of the medicinal plant (*Alchemilla vulgaris*) against this toxicity. Also the beneficial effects of this medicinal plant on the study organs. A total of 30 early mature, 2-3 months old, apparently healthy, female albino rats initially weighing 150-250 G were used in this study. The present study was conducted in the animal house in Pharmacy College, University of Kerbala. Rats were distributed randomly in fife cages in a rate of six rats per cage at room temperature and supplied with diet and water. The groups were arranged as the following: were divided randomly into five groups (six rats for each treatment): Group 1 (T_1) control: six female rat were used, Non-ionized water uses free zinc sulphate ($ZnSO_4$) and *Alchemilla vulgaris*. Treatment 1 (T_2): six female rat were used, water used with zinc sulphate ($ZnSO_4$) 1000 Mg/L only. Treatment 2 (T_3): six female rat were used. Water used with zinc sulphate ($ZnSO_4$) 1000 Mg /L with 200Mg/L *A. vulgaris*. Treatment 3 (T_4): six female rat were used, Water used with zinc sulphate($ZnSO_4$) 1000 Mg / L with zinc sulphate ($ZnSO_4$) 1000 Mg/L with 300MG/L *A. vulgaris*. Treatment 4 (T_5): six female rat were used, water used with 300Mg/L *A. vulgaris* only.

Were added the substances with drinking water of animals. Blood samples were collected in different periods of the study. The results of the current study showed a significant increase ($p \leq 0.05$) in progesterone of the (T5), Non-significant ($p \leq 0.05$) in progesterone of the (T_2, T_3 &T_4), significant increase ($p \leq 0.05$) in FSH & LH of the (T_4), compare with other groups , estrogen was increase significantly($p \leq 0.05$) in (T5), Non-significantly ($p \leq 0.05$) within other groups, respectively compare with the control (T1), The blood HCT, HGB, MCH, MCHC, MCV & PLT were non-significantly ($p \leq 0.05$) between all groups respectively; however, RBC was increase significantly ($p \leq 0.05$) in the (T_3) compare with (T_2), RBC was conjoined significantly ($p \leq 0.05$) in (T_4) & (T_5), but significant decreased ($p \leq 0.05$) in (T2) groups, on enzymes study (GOT & GPT),Non-significant ($p \leq 0.05$) GOT of the all groups, while GPT was significant decreased ($p \leq 0.05$) in T_4 and increase significantly in T_5 compare with other groups and control (T1). Also, WBC, GRNA, LYM and MID non-significant ($p \leq 0.05$) in all groups. In conclusion, used the medicinal plant for treatment most liver diseases better with low side effects by protective effects of these plant against excessive zinc and improvement of ovary functions and improvement fertility.

Key words : Zinc sulphate, *Alchemilla vulgaris*, reproductive hormones, medicinal plants, heavy metals.

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 issue causing serious problems to survive organisms (Uddin, 2017; Oehmen *et al*, 2006).

Metals are probably widely-known in the oldest toxic substances to the human and animals (ElOkle *et al*, 2014). 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 (Qadir *et al*, 2014). The metal ions essential and nonessential can be toxic when present in excess (Tennstedt *et al*, 2009).

Heavy metals as zinc, arsenic,cadmium, mercury and nickel. have been known to possess many adverse health effects; still, 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 (Chen *et al*, 2009).

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 an structural ,regulatory or catalytic roles in many enzymes (Egwurugwu *et al*, 2013).

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 (Alkorta *et al*, 2004; Fernandez *et al*, 2012; Singh *et al*, 2016).

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 (Langmead *et al*, 2001). 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 (Shinde *et al*, 2012). 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 (Smolyakova *et al*, 2012). *A. vulgaris* used for astringent, anti-hemorroidal and anti-diarrheal properties. The plant infusion is used externally in the cases of wound healing and stomatitis (Neagu *et al*, 2015). Also act as diuretic, depurative, intestinal antiseptic, bacteriostatic and bactericidal, tonic, anti-arthritic and cancer deterrent (Hamid *et al*, 2017).

Aims of study according to the above, this study was conduct on the effect of high $ZnSO_4$ concentrations in the drinking water on the liver, spleen and ovaries of females rat during 8 weeks period ,And the protective effect of the medicinal plant (*Alchemilla vulgaris*) against the toxic effect of high dose of $ZnSO_4$ in drinking water. Also the beneficial effects of this medicinal plant on the study organs.

MATERIAL AND METHODS

Experimental animals

A total of 30 early mature, 2-3 months old, apparently healthy, female albino rats initially weighing 150–250 G were used in this study. The present study was conducted in the animal house in Pharmacy College, University of Kerbala. Rats were kept randomly in fife 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):Group 1 (T_1) control: six female rat were used, non-ionized water uses free zinc sulphate ($ZnSO_4$) and *Alchemilla vulgaris*. Treatment 1 (T_2): six female rats were used,Water used with zinc sulphate ($ZnSO_4$) 1000 Mg/L only.Treatment 2 (T_3): six female rat were used, water used with zinc sulphate ($ZnSO_4$) 1000 Mg/L with 200Mg/L *A. vulgaris*. Treatment 3 (T_4): six female rat were used,Water used with zinc sulphate($ZnSO_4$) 1000 Mg / L with 300Mg/L *A. vulgaris*.Treatment 4 (T_5): six female rat 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, than 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^{0}C) on a rotary evaporator to give crude extract (El-Hadidy *et al*, 2019).

Started experimental period 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), blood was collected by cardiac puncture. first portion of blood sample were put into clean and dry tube with anticoagulant (1Mg EDTA/5Ml blood) and immediately used for estimation of some blood parameters (RBCs, HCT, HGB, MCH, MCHC, MCV, WBCs, GRAN, LYM, MID, GRA%, LYM%, MID% and Platelet) by using automatic blood counter apparatus in laboratory.other part of blood The serum was carefully separated into dry clean gel tubes and kept frozen till analysis at -20°C.

Complete blood count analyses: analysis blood picture Automatically in apparatus in laboratory analysis begins when a well mixed whole blood sample is placed on a rack in the analyzer. The instrument utilizes flow cells,

Table 1 : The effect of zinc sulphate and *Alchemilla vulgaris* on hormones of the study (Mean±SE).

Parameter / Treatment	Estrogen	Progesterone	FSH	LH
T1	50.05±7.73A	28.78±5.70AB	0.10±0B	0.14±0.01B
T2	52.59±9.16A	11.21±3.15 B	0.13±0.007B	0.19±0.01B
T3	49.85±2.68A	10.99±1.53B	0.11±0.01B	0.17±0.01B
T4	48.93±8.63A	17.62±6.87B	1.32±0.29A	1.17±0.26A
T5	69.11±16.42A	44.35±11.86A	0.12±0.009B	0.15±0.04B

Different letters represent a significant difference at (p≤0.05).

however, RBC was increase significantly (p≤0.05) in the (T_3) compare with T_2, RBC was conjoined significantly (p≤0.05) in T_4 & T_5, but significant decreased (p≤0.05) in T_2 groups (Table 2).

Non-significant (p≤0.05) GOT of the all groups , GPT was significant decreased (p≤0.05) in T_4 and increase significantly in T_5 compare with other groups and control (T_1) (Table 3).

Table 2 : The effect of zinc sulphate and *Alchemilla vulgaris* on RBC, HCT, HGB, MCH, MCHC, MCV & PLT of the study(Mean±SE).

Parameter / Treatment	RBC	HCT	HGB	MCH	MCHC	MCV	PLT
T1	6.44±0.47ABC	40.33±3.58A	13.05±1.49A	18.76±0.26A	30.31±0.82A	62.65±1.27A	514.5±93.44A
T2	6.001±0.10BC	37.56±1.49A	12.71±1.19A	18.68±0.27A	30.50±0.62A	62.38±1.59A	546.8±94.51A
T3	7.08±0.22A	45.20±2.67A	13.25±0.43A	17.80±0.58A	29.43±1.14A	63.60±2.47A	636.0±76.56A
T4	5.56±0.43AB	37.96±2.70A	10.92±0.87A	18.31±0.68A	28.63±1.51A	66.62±3.69A	712.5±65.13A
T5	6.51±0.18AB	39.35±2.33A	11.90±0.34A	18.8±0.12A	29.17±1.02A	63.20±2.48A	640.7±83.95A

Different letters represent a significant difference at (p≤0.05)

photometers and apertures in order to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood (Wheeler, 1998).

Biochemical analyses of serum

Serum protein profiles: Serum samples were used for the determination of (total protein, albumin, globulin, GOT, GPT, estrogen, progesterone, LH and FSH) according to the methods described by a manual of laboratory training book by laboratory in Kerbala.

Statistical analysis

Data was analyzed as one-way ANOVA using the general linear model (GLM) procedure to SPSS 22.0 software (Delwiche *et al*, 2012). Four treatment means were separated using a "protected" Duncan`s analysis in level (0.05).

RESULTS AND DISCUSSION

Our results of the current study showed a significant increase(p≤0.05) in progesterone of the (T_5), non-significant (p≤0.05) in prog. Of the (T_2, T_3 &T_4), significant increase (p≤0.05) in FSH & LH of the (T_4), compare with other groups, estrogen was increase significantly (p≤0.05) in (T_5), non-significantly (p≤0.05) in other groups, respectively compare with the control (T_1) (Table 1).

The blood HCT, HGB, MCH, MCHC, MCV & PLT were non-significantly (p≤0.05) in all groups respectively;

Table 3 : The effect of zinc sulphate and *Alchemilla vulgaris* on liver enzymes GOT & GPT of the study (Mean±SE).

Parameter / Treatment	GPT	GOT
T1	182.33±15.43A	45.50 ±4.27AB
T2	182.17 ±9.09 A	44.83 ±3.78 AB
T3	176.67 ±29.43 A	50.33 ±4.31 AB
T4	192.83 ±15.08 A	39.66 ±1.85 B
T5	185.00 ± 5.50 A	54.16 ±4.61 A

Different letters represent a significant difference at (p≤0.05).

Also, WBC, GRNA, LYM and MID Non-significant (p≤0.05) in all groups (Table 4).

The increment of progesterone and decrement of estrogen in T_2 (imbalance between them) may be due to the zinc interfere with hormone (estrogen) receptors which capable decrease estrogen that leads to disrupt biosynthesis of hormones. Our results are agreement with Georgescu *et al* (2011), Oðuz *et al* (2014).

Also, the improvement of ovary hormones may be attributed to increase activity of enzymes led to increase production of hormones and improvement the fertility (Eshak *et al*, 2018; Özbilgin *et al*, 2015).

The decrement RBC number may be excessive zinc led to copper deficiency through lower the copper absorption in small intestine led to inhibit the transport iron and result in anemia (Rawi *et al*, 2015; Fischer *et al*, 2005; AL-Diwan *et al*, 2010).

Table 4 : The effect of zinc sulphate and *Alchemilla vulgaris* on WBC, GRAN, LYM & MID of the study (Mean±SE)

Parameter / Treatment	WBC	GRAN	LYM	MID	GRA%	LYM%	MID%
T1	5.91± 0.61A	0.50± 0.12A	5.18± 0.52A	0.51± 0.05A	4.31± 1.93A	89.37± 2.07A	6.41±0.45A
T2	5.42± 0.47A	0.55± 0.07A	4.66± 0.41A	0.33± 0.06A	8.59± 0.63A	87.01± 1.89A	6.05±0.68A
T3	7.45± 0.46A	1.80± 1.36A	6.53± 0.47A	0.50± 0.05A	6.88± 0.93A	85.87± 2.23A	6.28±1.01A
T4	7.50± 1.77A	0.85± 0.39A	5.95± 1.30A	0.61± 0.11A	9.64± 1.88A	84.49± 3.49A	6.76±1.60A
T5	6.80± 1.98A	0.60± 0.45A	5.55± 1.46A	0.58± 0.15A	6.02± 2.71A	86.41± 2.69A	7.86± 0.52A

Similar letters represent non-significant difference at ($p \leq 0.05$).

The decrement of some liver enzymes and improvement of the liver functions may be attributed to antioxidetive effect of flavonoids compound in medicinal plant, against free radical accumulation by excessive zinc supplementation with drinking water may be this plant rich with phenolic compounds, flavonoids and tannins (Samani *et al*, 2018; Said *et al*, 2011; Ansari and Maiti, 2018; Ho *et al*, 2012).

CONCLUSION

Used the medicinal plant for treatment most liver diseases better because have low side effects by protective effects of these plant against excessive zinc, and improvement of ovary functions through improvement the production of reproductive hormones in female.

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