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Effect of Heavy Metals in *Alhagi graecorum* on Some Physiological Parameters of Laboratory Rats

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Abstract:

The purpose of this study was measuring heavy metals in plant *Alhagi graecorum* taken from power station and the extent of the impact of the polluted plant on laboratory animals. The present study was carried out during the period from November 2017 to May 2018. eighteen rats were used assigned and divided into three groups. The first group was the control group and the second group was injected with the *A. graecorum* aqueous extract concentration 25 mg /Kg and the third group were injected with the *A. graecorum* aqueous extract concentration at 50 mg /Kg the experiment lasted 30 days.

Keywords : *Alhagi graecorum,* heavy metals, blood parameters, antioxidants, Nrf2.

Introduction:

Plants are considered the oldest friends of mankind and always play a major role in the living of human and animal and it's important to live organisms they are not just provided shelter and food, but they are also used in the treatment of various diseases (Choudhury *et al*., 2008). The study of medicinal herbs is a greatest importance in the world in general and in Iraq in particular because it overlaps with the environmental pollution caused by the wars and its aftermath of environmental damage, as well as it cause increase in rates of infection with malignant and chronic diseases such as cancer (Al-Malky, 2006).

Environmental pollution has been a major area of concern worldwide. Industrial and agricultural processes have caused an increased concentration of toxicants like heavy metals in the environment and as a result being taken up by plants or animals into their systems which cause further distribution of toxicants to the environment (Ahmad, 2002). Pollution is any substance in the environment, which causes objectionable effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death (Duruibe *et al*., 2007).

Environmental pollution, especially by heavy metals is one of the most important factors in the destruction of biosphere components (Chen *et al*., 2005). Heavy metals are pervasive environmental toxicants that have been shown to exert oxidative stress on living systems through the production of reactive oxygen species (ROS),which overwhelm the cell's capacity to maintain a reduced state (Ercal

et al., 2001). Metal-induced ROS cause damage to cellular proteins, nucleic acids and lipids, It has been clearly demonstrated that ROS interfere with the expression of a number of genes and signal transform pathways (Valko *et al*., 2006). Because ROS are oxidants by nature, they influence the oxidoreduction status and may according to their concentration cause either a positive response (cell proliferation) or a negative cell response (growth arrest or cell death) (Birben *et al*., 2012).

Alhagi graecorum Bioss is commonly known as Al-Agool, Shouk Aljemal and Camel thorn. Is a shrubby evergreen perennial suffruticose herb, erect to ascending up to 60-100 cm high very much branched with rigid spiny twigs about 1 inch long (Awmack and Lock, 2002). It was native in north Africa, the middle east and south east Europe. Also found in wide areas including Asia and Africa (El-Khatib, 2000). *A. graecorum* is customarily used in folk medicine as a remedy for rheumatic pains, bilharziasis, liver disorders, and for various types of gastrointestinal discomfort (Ullah *et al*., 2013), but there is no scientific background that supports this use.

This study aimed was measuring heavy metals in plant *A. graecorum* taken from power station and the extent of the impact of the polluted plant on laboratory animals.

2- Materials And Methods :

Preparation of plant extract the plant leaves (*Alhagi graecorum* Bioss) samples were collected from Power station in the city of Al-Nasiriyah, Thi- Qar province, Iraq . The plant brought to the lab and washed by distilled water then dried in air. After complete dryness, the plant collected in a glass container at room temperature. Heavy metals were measured in the plant extract using a flame atomic absorption spectrophotometer. Aqueous extract of plants was prepared by weighing 50g of plant powder in a flask, and 250ml of distilled water was added and mixed with a magnetic stirrer for two hours. The mixture was filtered through filter paper (Whattman No. 1). The supernatant was evaporated at 40ºC to dryness under reduced pressure in a rotary evaporator, and freeze-dried by lyophilized to obtain the dried extracts (Sheweita *et al*., 2016), and then laboratory animals *(Rattus norvegicus)* whose weight (180-210g) and aged (10-12) weeks were used. aqueous extract of the plant *A. graecorum* taken from the power station at two concentration 25 and 50 mg/kg once daily for 30 days (n = 18).

- **Group (1):** control treated with distill water for 30 days.
- **Group (2):** treated orally with (25mg/kg) of aqueous extract of (*A. graecorum)* once daily for 30 days.
- **Group (3):** treated orally with (50mg/kg) of aqueous extract of (*A. graecorum)* once daily for 30 days.

Blood parameters (RBC, WBC, Hb, MCV, MCH, and PLT) for rats were measured using hematological analyzer (Nihon Kohden). Lipid peroxidation (LPO) is determined by using the thiobarbituric acid method. In this method, MDA formed from the breakdown of polyunsaturated fatty acids is identified as the product of LPO that reacts with thiobarbituric acid (TBA), in coexisting with trichloroacetic acid (TCA) (Fong *et al*.,1973). The activity of the enzymes alanine transaminase (ALT) and aspartate transaminase (AST) in the serum was measured through the processed kit from the company Biolabo (France). The heavy metals were determined in the liver and blood rats by using flame atomic absorption spectrophotometer (FAAS). Genomic RNA was extracted from blood isolates by using RNA Extraction(Direct-zol RNA MiniPrep Zymo RNA Purification Kit (USA) and done according to company instructions in many steps. All isolates of blood were detected by Real-Time polymerase chain

reaction (RT-PCR assay) using Nrf2 gene. The reaction was performed in final amount (20µl) (Sybr green kappa Master mix 10 µL, 0.4 µl of each primer, 50X KAPA RT Mix 0.4 µL, Nuclease-free water 4.2 µL and RNA Sample Volume 5 µL) RT- PCR was performed in Thermo cycler (Eppendrof, Italy) and consisted of the following steps: 42°C for 10 minutes (Reverse transcription), 95°C for 3 minutes (Enzyme activation), 95.0°C for 15 seconds by 40 cycle (Denaturation) and the final annealing/extension step was performed at 55.0°C for 15 seconds by 40 cycle.

Table (1):.The specific primer of Nrf2

Primer	Sequence	Tm (°C)	GC (%)
Forward	5'- ATGTCACCAGCTCAAGGGCACAGTGC - 3'	52.1	45
Reverse	5'- CCATCCTCCCCGAACCTAGTT - 3'	55. 6	47.6

Reference gene primer sequence (Actb gene)

Sequence (5'->3')	Template strand	Length	Tm (°C)	GC%
Forward primer	GATCAAGATCATTGCTCCTCCTG	23	58.93	47.83
Reverse primer	AGGGTGTAAAACGCAGCTCA	20	59.89	50.00
Product length	183			

Statistical Analysis :

Statistical analysis was done using the software **(SPSS-11 -2003)** statistical package for Social Science. The results were expressed as mean ± standard deviations (mean ± SD) with P value . One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant.

3-Results:

3-1- heavy metals in Plant Extract:

Figure (1) showed the Concentrations of heavy metals in Plant extract. Lead (Pb) showed the undetectable level in the plant(*A. graecorum*). while Zinc (Zn) have shown the higher levels(16.31µg/g).Other heavy metals are arranged according to the increase to Cd > Cu.

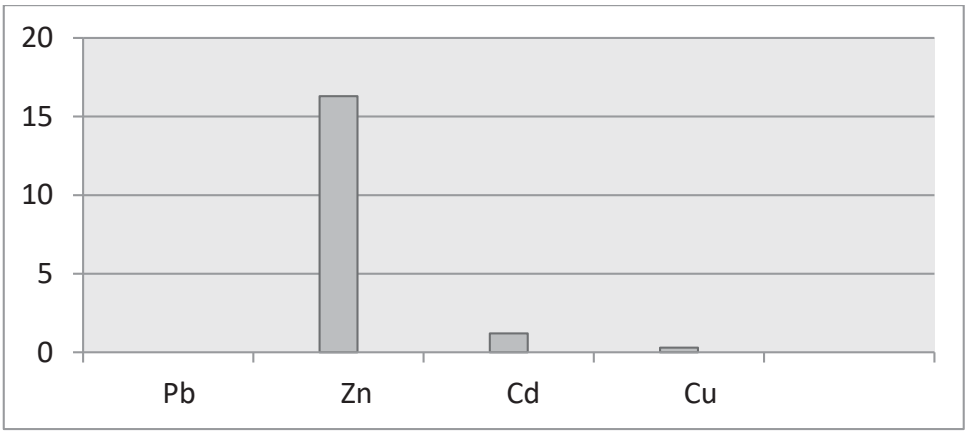

Figure (1): Concentrations of heavy metals in *A. graecorum* (µg/g dry wt.).

3-2- Heavy metal concentrations in rats liver:

Zn values in among animals injected plant extract higher than its values in the control group. The results also presented a significant increase ($p \leq 0.05$) in third group, also found decrease non- significant increase for in the group second comparing with the control group. The results too exhibited the in Cu values a significant increase ($p \leq 0.05$) third group, also recorded non- significant decrease in for second group comparing with control group. The results also showed the Pb value non- significant decrease in all groups.

Table (2): Heavy metal concentrations in rats liver.

Group	Zn	Cu	Pb
Group 1 (Control)	344 ± 9.82 0.50	109±4.41 0.06	24.7±2.23 0.38
Group 2 (*A. graecorum* 25 mg/ kg)	477 ± 108.1 0.66	104.5 ± 6.36 0.99	23.5 ±1.69 0.98
Group 3 (*A. graecorum* 50 mg/ kg)	677.5 ± 70.7 0.04*	252 ± 80.6 0.01*	20.1 ± 1.97 0.35

- Values refer to mean ± SD and P value.
- Stars (*) refer to a significant difference at ($p \leq 0.05$).
- No stars (*) refer to non-significant.

3-3- Heavy metal concentrations in rats blood :

The results also showed a significant increasing ($P \leq 0.05$) of Zn values in second group and third comparing to the control group. It was found in the table (3) that the highest values of Cu appeared, there are a significant increasing ($P \leq 0.05$) in second and third groups comparing with control group. The statistical analysis shows the Pb value non-significant increase in second and third groups .

Table (3) : Heavy metal concentrations in rats blood.

Group	Zn	Cu	Pb
Group 1 (Control)	260.5± 48.5 0.74	75 ± 30.15 0.46	21.2 ± 2.02 0.28
Group 2 (*A. graecorum* 25 mg/kg)	491.3 ± 30.75 0.02*	281.3 ± 6.71 0.01*	31.2 ± 0.84 0.11
Group 3 (*A. graecorum* 50 mg/kg)	744.8 ± 35.25 0.04*	280 ± 50.91 0.01*	31.8 ± 5.65 0.12

3-4- Effect of *A. graecorum* extract on blood parameters:

Table (4) exhibited a significant increase ($p \leq 0.05$) in RBC count in third group also show a significant decrease in second group comparing with control group. In the same table, the results indicated a significant decrease in the level of Hb in second group and show a significant increase in group third

comparing with control group 1. Furthermore, the results revealed a significant rising in level of WBCs in group third comparing with control group 1, and also a non-significant decrease in group second comparing with control group 1.

Interestingly It also showed results a significant decreasing in MCV in third group comparing with the control group1. Moreover, the results indicated non-significant decrease in MCV in the group second comparing to the control group.

Likewise, It also exhibited results a significant decreasing in the MCH in third group comparing to the control group, however, a non-significant decrease in the MCH in second group at level ($p \leq 0.05$) comparing to the control group. the a non-significant different in the PLT in second and third groups comparing with to the control group 1.

Table (4): Effect of *A. graecorum* extract on blood parameters among rats exposed for 30. days.

Group	RBCs 10^6/mm³	Hb g/L	WBCs mm³/10³	MCV Pg	MCH g/dl	PLT 10^5/ L
Group 1 (Control)	5.29 ± 0.09 0.46	12.03±0.5 0.24	5.05 ± 0.87 0.18	66.3 ± 1.22 0.29	22.35 ± 0.95 0.53	354.5 ± 2.66 0.21
Group 2 (*A. graecorum* 25 mg /Kg)	4.72 ± 0.03 0.01*	10.45±0.2 0.02*	5.40 ± 0.50 0.93	64 ± 0.66 0.12	22.05 ± 0.55 0.97	159 ± 47.64 0.09
Group 3 (*A. graecorum* 50 mg /Kg)	5.63 ± 0.03 0.08*	12.38±0.1 0.01*	9.32 ± 0.39 0.05*	62.63 ± 0.53 0.01*	21.88 ± 0.40 0.01*	333.5 ± 12.77 0.90

3-5-Effect of *A. graecorum* extract on Malondialdehyde(MDA)Concentration:

MDA level significantly increased($p \leq 0.05$) in the second and third groups comparing with control group.

Table (5): Effect of *A. graecorum* extract on MDA concentration.

Group	MDA Serum (µmol/L)
Group 1 (Control)	١.٧٤ ± 0.45 0.98
Group 2 (*A.graecorum* 25mg/kg)	3.75 ± 0.50 0.02*
Group 3 (*A.graecorum*50 mg/kg)	4.65 ± 0.37 0.04*

3-6- Effect of *A. graecorum* extract on liver enzymes:

The present study showed the a significant increase ($p \leq 0.05$) in both groups second and third comparing with control group. There are a significant increase in liver enzymes (ALT , AST) in second group comparing with other groups.

Table (6): Effect of *A. graecorum* extract on liver enzymes.

Group	ALT (U/L)	AST(U/L)
Group 1 (Control)	31.8 ± 1.05 0.36	53.58 ± 1.47 0.25
Group 2 *(A. graecorum* 25 mg/kg)	55.13 ± 0.79 0.03*	91.35 ± 1.24 0.02*
Group 3 *(A. graecorum* 50 mg/kg)	36.73 ± 0.88 0.03*	81.03 ± 1.89 0.02*

3-7- Effect of *A. graecorum* extract on relative expression (Nrf2) results:

Table (7) in the present study shows that the a significant increase($p \leq 0.05$) in second and third groups comparing with control group.

Table (7): Effect of *A. graecorum* extract on relative expression of Nrf2.

Group	Relative expression (Nrf2)
Group 1 (Control)	3.35 ±1.18 0.29
Group 2 *(A. graecorum* 25mg/kg)	5.52 ± 1.45 0.04*
Group 3 *(A. graecorum* 50 mg/kg)	5.86 ± 1.76 0.01*

4-Discussion:

Plants are considered for a long time as the most sensitive to environmental biodiversity because of their high sensitivity to the toxicity of heavy metals and effective as the first phase in the food chain that collects pollutants compared to other organisms(Radojevic and Bashkin, 2007). Over the decades, plant contamination would give a clearer picture of pollution than in other measurements (Skorbilowicz, 2009). The study revealed that all the metals were accumulated to less concentrations by the plant specie studied in power station, except that of zinc (Hussain *et al*., 2006; Rehman *et al*., 2012; Iqbal *et al*., 2013). According to Karademir *et al*.(2003) the plants have the ability to accumulate heavy metals that are necessary for growth and development including copper and zinc these results come along with present founding where noticed the high concentration of zinc compared to other elements in the plant. The amassed of Zn is maybe due to essential requirements and enzymatic metals for the plants, and it also would be necessary for proper growth and protein (Dghaim *et al*., 2015). The presence of heavy metals in the body of the plant is due to the source of these minerals from the soil contaminated with

electrical residues Power station. According to Alloway (2013) the presence of heavy metals in the plant is due to plant growth in polluted soil containing heavy metals. The tolerance of plants to different concentrations of heavy metals and their continued growth is due to the possibility of balancing the level of both enzymatic and molecular antioxidants such as peroxidase and total phenols, as well as increased secretion of cellular metabolism products such as cysteine and glutamine (Samecka and Kempers, 2001).

That accumulation of heavy metals in the liver is higher than the other organs and may be due to the position within the circulatory system, wherever it receives most of the heavy metals transported by blood (Ikemoto *et al*., 2004). The cause of high concentration of heavy metals may be related to close association metallothionein proteins (Ikem *et al*., 2003). Exposure to heavy metals can occur through drinking water, food, air, soil and dust (Ferner, 2001 and Akpor *et al*., 2014). The accumulation of minerals in the blood this can probably be attributed to various factors including in general, metabolic processes, water and food contamination, and the nature of lipid concentration in tissues (Goyer and Clarkson, 1996). This variation may be due to plant extracts contaminated with heavy metals taken from the power station.

The results showed a significant increase in the level of RBC and Hb at concentration(25 and 50 mg/kg), the main reason would be due to that *A. graecorum* plant contains high concentrations of vitamins C and B12 (Hassanein and Mazen, 2001), vitamin C stimulates the secretion of the erythropoietin hormone in the kidney to form RBC and because the kidneys need large amounts of oxygen to function and their cells are considered one of the most sensitive cells of the lack of blood supply (oxygen and nutrition) the renal capillaries secrete the erythropoietin hormone, which activates the bone marrow cells to help accelerate their reproduction and maturity, producing many of RBC (Junqueria *et al*., 2003) , vitamin B12 is considered essential for the bone marrow to produce blood cells (McDowell, 2000).

Level of White blood cells (WBC) elucidate significant increasing in the blood of animal experiment in third group. WBC level amassed due to increase activity of macrophages as a result of infection because of the contaminated plants with heavy metals. Heavy metal contaminations stimulate bone marrow to release of immature Polymorphonucler (PMN) and in large quantities to increase number of WBC, this is indicated by the (Smith *et al*., 2006).The results demonstrate a non-significant decrease in the WBC levels in second group. The decrease was among animals which injected plant extract from power station. The main reason of the reduction was because of high levels of vitamin E in the *Alhagi graecorum* plant, which has a role in refining the number of WBC because it improves status of antioxidants. Vitamin E is a line of defense against the harmful effects of free radicals such as peroxides and superperoxides for the purpose of protecting the cell membrane, and it prevents the oxidation of various materials and easy to oxidize them. This plant also having amount of vitamin A, which plays a role in activating the secretion of the hormones that are stimulating the adrenocorticotropic hormones, which affects the corticosterone hormone secreted from the adrenal gland, which stimulates the production of white blood cells, the vitamin E has a role in increasing the secretion of vitamin A in the liver and thus reduce the number of white blood cells (Shlig, 2009).

The significant increase in the MCV and MCH levels in group third. due to the rising in the values of RBC and Hb, because these blood clots affect the values of RBC and Hb. The results from current study com with agrees with (Niki and Noguchi, ٢٠٠٤). In contrast, our results showed that there are a

significant decrease in levels of MCV and MCH in groups second from plant extract . It has been proved that the reduction of MCV and MCH in blood are return to high blood lead level and low iron because of bowel perturbation this decrease causes a defect in the production of hemoglobin resulting in oxygen deficiency (Golalipour *et al*., 2007).

Moreover, PLT levels reduced in groups second and third. plant extract is consider as a rich source of natural antioxidants especially flavonoids, these compounds have anti-platelet activity (Muhammad *et al*., 2015), The previous researchers group clarified many mechanisms that explain the action of flavonoids as anti-platelets by lowering the level of intracellular calcium and the change that occurs to the metabolism of CAMP and Thromboxane A2 (Kang *et al*., 2001).

Malondialdehyde (MDA) is a final product of lipid peroxidation after exposure to ROS and many studies have used it as a marker of oxidative stress evaluation (Pan *et al*., 2010). In this investigation, a significant increases in the MDA levels in the group that treated with extract The fact is that Zinc acts as an antioxidant through various ways Zinc shows two acute and chronic antioxidation mechanisms Chronic mechanism suggests that exposure to zinc over long periods may induce other substances like metallothionein which works as an antioxidant. The acute mechanism acts in two ways, protein sulphydryls conservation and decreasing the conversion of H_2O_2 to OH (Prasad, 2014). One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress (Flora *et al*., 2008). In the present study, ALT and AST levels showed a significant increase in aqueous extract groups The hepatoprotective effect of plant extract may be due to the presence of flavone structures in the aqueous extract (Ahmad *et al*., 2010).

The results of the current study showed that increases in the rate of relative expression (Nrf2). The induction of Nrf2 expression would be because of exposure to heavy metals. Activation of Nrf2 in response to stress signals to result from a disruption of this association, releasing Nrf2 for translocation into the nucleus to affect its transcriptional activity (Stewart *et al*., 2003).

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