Physiological and Gen Study for Animals Exposed to Leaves of Plant (Eucalyptus) from Selected Locations at, Al-Nassiriy/ Iraq

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Abstract

Our study was carried out during the period from November 2018 to May 2019. 24 rats were used assigned and divided into three groups. The first group was the control group and a second group were injected with aqueous extraction of Eucalyptus leaves extract at concentrations of 100 and 150 mg /Kg experiment lasted 30 days.

Zn, Pb, Cd, and Cu were measured in the leaves of plant and after confirming the presence of these heavy metals, we exposed the laboratory animals to the Eucalyptus aqueous extract and after the end of the period rats were sacrifice and blood collected. Blood were used to measure study parameters and relative gene expression of (SOD and catalase). Moreover, antioxidants and enzymes AST and AL were measured from serum.

Results showed that the concentration of Pb in the plant was 0.19µg/g. The highest mean Zn was 14.23µg/g. The Cd concentration was 0.35µg/g and the copper concentration were 2.31µg/g from previous data we can find out that the amount of heavy metals is not really high. we would be expected low impact on animals since not high concentration found. The results of blood parameters of rats showed different outcome where it has been noticed that a significant increase in Hb, PLT and RBC at the 100mg/kg exposure and an increase in WBC in both concentrations. In addition, our results showed a significant decrease in RBC and Hb at the 150mg/Kg decrease MDA, MCV and MCH levels are decreased at both concentrations. A significant decrease in AST at 150mg/kg while the results showed a significant increase in lower concentration.

Keywords: *Eucalyptus, heavy metals, blood parameters.*

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 treatment of various diseases [1].

Environmental pollution has been a major area of concern worldwide. Environmental pollution, especially by heavy metals is one of the most important factors in the destruction of biosphere components[2]. Heavy metals remain in the soil for a long time and have a residence time ranging from a few to several hundred years [3]. Human exposure to heavy metals has increased due to excessive use of daily life and has increased in most parts of the world, particularly industrialized countries. Therefore, pollution from industrial activities is a major environmental problem requiring attention[4] .

Heavy metals are pervasive environmental toxicants that have been shown to exert oxidative stress on living systems through the production of reactive oxygen species which overwhelm the cell's capacity to maintain a reduced state. 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 [5, 6].

This study aimed was measuring heavy metals in plant Eucalyptus taken from power station and the extent of the impact of the polluted plant on laboratory animals to reveal the influence of this plant on blood parameters,

liver enzyme and gene alternation.

Method

The plant leaves Eucalyptus samples were collected from Power station in the city of Al-Nasiriyah, Thi-Qar province, Iraq. The plant brought to the lab and washed with 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 FAAS. Aqueous extract of the plant was prepared, and then laboratory rats of Rattus norvegicus species exposed to aqueous extracts of the Eucalyptus leaves that are taken from the power station at 2 concentration 100 and 150mg/kg once daily for 30 days. Blood parameters for rats were measured using hematological analyzer (Nihon Kohden). Lipid peroxidation (LPO) is determined by using the thiobarbituric acid method [7]. 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 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) [2]

Results

Figure 1 showed heavy metals in Plant extract that are taken of Power Station. Lead (Pb) concentration was (0.19 µg/g) in the plant of the power station, while Zinc (Zn) have shown the higher levels in plant (14.23µg/g). Other heavy metals are arranged according to the increase to Cu > Cd.

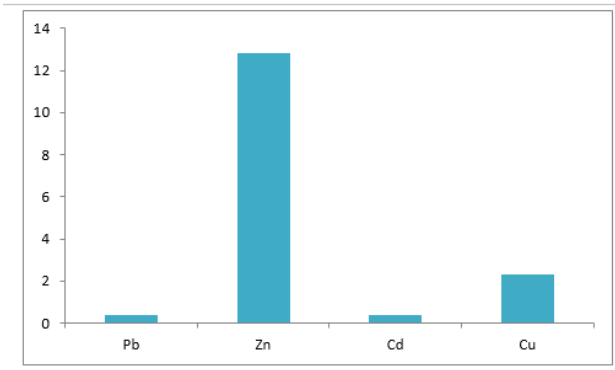

Fig (1): heavy metals in Eucalyptus (µg/gm dry wt.)

The results of the current study showed in Table (1) showed that the highest value of RBCs was in group3 and lowest in group2.As well as, showed that there is a significant increase in the level of RBC for group 2. While there was a non- significant difference in group 3 comparing to the control.

Group 3 was recorded the highest value with Hb than another group, while the lowest was group 2 . As results appeared a significant rise in hemoglobin (Hb) levels in group2 comparing to the control, as observed a non- significant difference in group 3 comparing to control group1. The highest value of WBCs was in group 3 compared with the other group, the lowest was group 2.

Interestingly, the results of the study showed that the highest value of MCV was in group 2 and the lowest value was in group 3. Moreover, the results indicated non-significant different in MCV in the groups 2 and 3

Likewise, the results presented the highest value of MCH was in group 2compared with the other groups. It also exhibited results a non-significant difference in the MCH in groups 2 and 3. Group 3 was the highest value of PLT.

Table (1): Effect of Eucalyptus extract on blood laboratory animals

	RBCs 10^ 6/ mm³	Hb g/L	WBCs mm³/10³	MCV Pg	MCH g/dl	
Group 1 (Control)	5.29 ± 0.09 0.386	12.03±0.51 0.44	5.05 ± 0.87 0.28	66.3 ± 1.22 0.79	22.35 ± 0.95 0.57	35
Group 2 (*Eucalyptus* 100 mg /Kg)	6.17 ± 0.11 0.01*	12.85±0.20 0.01*	9.42 ± 0.55 0.01*	67.15 ± 1.02 0.69	27.18 ± 0.71 0.27	25
Group 3 (*Eucalyptus* 150 mg /Kg)	7.77 ± 0.42 0.26	15.23±0.39 0.23	16.45±0.69 0.01*	61.25 ± 0.78 0.089	23.18 ± 0.91 0.79	43

♦ Values refer to mean ± SD and P value.

Group 3 was recorded the highest value with MDA than another group. MDA level a non-significant different in the groups 2 and 3 comparing to the control group1.

The present study showed that the highest value of ALT was in group 3. It also showed the highest value of AST was in group 2 table 3 .

it also observed significant differences in the enzyme AST and non-significant differences in the enzyme ALT were observed in group 2. Moreover, we found that non-significant differences in group 3 in both enzymes comparing to the control group table 3.

Table (2): Effect of Eucalyptus extract on liver enzymes.

Level of mean Zn in liver of animals that drank plant extraction higher than its values in the control group. The highest values of Zn were in group 3.

Table (3): Heavy metal concentrations in liver.

The results of the present study showed that the higher values of Zn in the group 2. The results also showed non-significant differences in groups 2 and 3.

The highest value of Pb was in group 2. The statistical analysis shows non-significant difference in groups 2 and 3.

The present study showed that the highest values of relative expression of SOD and catalase in group 3,

Table (4): Heavy metal in rat's blood.

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 [3]. Over decades, plant contamination would give a. clearer picture of pollution than in other measurements. [8]. The current study revealed that all the metals were accumulated to less concentrations by the plant studied in power station, except that of Zn. The outcome of the study come along with previous studies [9, 10]. According to Alloway (2013) the presence of heavy metals in the plant is due to plant growth in polluted soil containing heavy metal. exposing to extract of Eucalyptus showed that there was a significant increase in the RBC level in the group (2) of the power station. These outcomes may be due to medicinal plants which are rich in nutrients including minerals such as calcium, zinc and potassium [11]. Extract from the same plant exhibited a significant decrease in the RBC level in blood of animals that drank high concentration of these plants (150mg/kg). These interesting results would be due to the thyroid dysfunction and hormones that are produced from effect of toxins produced in the animal body after giving the animals a polluted plant extract. Animals hormones are playing a major influence in the direction of metabolism within body and have an indirect effect on the production of blood cells in the bone marrow [12].

As well as, exposing to Eucalyptus increased in the Hb levels in the group 2. According to Smith et al (2006) increased RBC cause increased hemoglobin [13]. Another logical reason to increase level of Hb may be because of a defect in the kidneys result of heavy metals resulting in an increase in hemoglobin [14] 150mg/kg of same plant extract taken from power station influenced differently on Hb level when significant reduction noticed after 30 days' exposure. To explain this different would be because of reactive oxygen species (ROS) that cause hemoglobin damage. It produces deposits inside the red blood cell and thus decomposes red blood cells. Likewise, it may play role in reduction of the hormone Erythropoietin and malnutrition resulting from loss of appetite causes these hemoglobin deficiency and anemia [15].

Not only that, level of WBC also influenced by Eucalyptus where level is increased. According to researchers finding, plant is content active compounds which have proven pharmaceutical effectiveness such as flavonoids, Coumarin, Glycosides and saponins [16]. These compounds are capable to activate immune system and thus be able to increase WBC. In contrast, our results showed that there are a significant decrease in levels of MCV and MCH in groups (2 and 3) from Eucalyptus 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 [17] A study done by Saidana and his colleague who reported that there is direct impact of the active compounds extracted from Suaeda plant on bone marrow of animals to generate a large number of platelets. Whereas, the reduction in PLT levels that have been noticed in the group (5) of the power station, may be due to metals ability that can

induce abnormal responses in the immune system.

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 [18]. In this investigation, a non-significant in the MDA levels in the Eucalyptus aqueous extracts. These results are in agreement with other studies which reported [19]. The influence of back to plant curcumin contains which decreases the oxidative stress [20]. In the present study, ALT and AST levels Eucalyptus that are collected from power station act differently where we noticed a significant increase AST level and decrease ALT in Group 4. Increasing the enzyme synthesis of liver cells to remove the toxic effect of metals. Customarily, the rise of ALT refers to liver damage and this disorder may occur in other organs that secrete this enzyme such as heart and muscles. Chavan and Muley (2014) explained that exposure to lead and cadmium increases the level of AST and do not affect ALT. for this reason, here we think the impact of lead and cadmium was clear on animals[21]. 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[22]]. This variation may be due to plant extracts contaminated with heavy metals taken from the power station.

The results of the current study showed that increases in the rate of relative expression SOD and catalase. The induction of SOD and catalase expression would be because of exposure to heavy metals. Activation of SOD and catalase in response to stress signals to result from a disruption of this association, releasing SOD and catalase [23]. The current study was the first study that aimed to find the connection between contaminated plant from contaminated area that placed around the power station and animals' health. Highly comminuted plant that are approved before by several of environmental scientists' group around are.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

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