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Effects of Aqueous Leaf Extract of *Limoniastrum guyonianum* Pretreatment on Nickel Induced Acute Heamatotoxicity in Male Mice

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Abstract: The aim of this investigation was to determine the benefecial effects of aqueous leaf extract of *Limoniastrum guyonianum* and vitamin C on nickel induced hematological paramater alterations in male mice. Thirty mice were divided equally in six groups. Group 1 (control); groups 2 received intraperitoneal injection of 10mg/kg body weight of nickel sulfat for two days; groups 3, 4 and 5 received orally 50, 100 and 200 mg/kg body weight respectively of the extract, as well as group 6 received 16.6 mg/kg body weight of vitamin C for 7 days. On the 8th day, mice of groups 3, 4, 5 and 6 were injected intraperitoneally by 10mg/kg body weight of nickel sulfate and they were killed after 24 hours. Heamatological paramaters were measured. As a result of this study, nickel alone caused a significant decrease in red and white blood corpuscles (RBC and WBC), heamoglobin concentration (Hb), blood platelet (Plt) counts, packed cell volume (PCV) and mean cell volume (MCV). The aqueous extract of *Limoniastrum guyonianum* proved to be more efficient than vitamin C pretreatments in attenuating nickel induced heamatological paramater alterations. In conlusion, this plant extract was more benefecial than vitamin C in preventing the heamatotoxic effects of nickel sulfate.

Key words: Nickel · Hematotoxicity · *Limoniastrum guyonianum* · Aqueous leaf extract · Vitamin C

INTRODUCTION

Nickel is a widespread environnemental polluant, able to produce undesirable effects and/or carcinogenicity in humans and animals [1]. Nickel has a vast use in industrial processes such as painting, manufatore of olloys, coins and bateries. The nickel is absorbed from gastrointestinal tract and excreted essentially by kidney through urine and bile [2, 3]. Severe adverse heamtotoxic effects including disorders of the bone marrow, haematopoietic systems and the onset of anemia related to formation of reactive oxygen species [3-6] have been noticed in nickel exposed animals. In the last few years, phytochemicals and vitamins as natural antioxidant substances have received a great attention in health protection against acute and chronic human and animal poisining, leading to various diseases and carcinogenicity [7, 8]. Many studies have reported the protective effect of bioactive agents of herbal medicines [9-11] and vitamin C [3, 5] upon nickel toxicity in experimental animals. *Limoniastrum guyonianum* is known as an obligate halophyte widely used in traditional medicine of the north african countries, particulary in Tunisia and Algeria. As repotrted, it's able to improve human health against various diseases including infectious or parasitic and dysentry diseases [12, 13] and tumor formation [14, 15]. Indeed, *Limoniastrum guyonianum* leaf extract contains a large variety of compounds with antioxidant activities such as polyphenols, flavonoids, condenced tannin…, etc. [16, 17] that can reduce the oxidative threat due to toxicants. Therfore, our present study was carried out to assess the benefecial effect of the aqueous extract of a medicinal plant of Hodna region province of M'sila (South East of Algeria) named *Limoniastrum guyonianum* belonging to the family of Plumbaginaceae against nickel induced acute heamatotoxicity in mice compared to vitamin C.

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MATERIAL AND METHODS

Plant Material: *Limoniastrum guyonianum* was collected on April, 2014 from a region located in Bir-Alarbi, Hodna region (M'Sila city), Algeria. The plant was identified by Dr. Sarri Djamel, from the Faculty of Science, M'Sila University of Algeria. A voucher specimen, was deposited in the laboratory of the Department of Microbiology and Biochemistry at the same University.

Experimental Animals: Six groups of five healthy adult male albino mice (*Mus Musculus*) of Swiss stain, weighing from 26 to 34g were obtained from Algiers Pasteur Institute (Algeria) and were fed with laboratory stock diet (70% carbohydrate, 7% fat, 18% protein, 4% salt mixture and 1% vitamin mixture) and water *ad libitum* throughout the study. The animals were acclimated for two weeks under the same laboratory conditions of photoperiod with a relative humidity of 70%.

Preparation and Extraction of Plant Samples: Plant materials were vacuumed slightly from dust and dried at room temperature until obtaining a constant weight. The dried leaves samples were powdered and then bagged and stored in a hermetically sealed jars until required. Pratically 20g of powered leaves were imerced into 200ml of heated distilled water for 4 hours under countinous agitation. The mixture was filtered using a piece of clean, sterile Muslin cloth to remove debris and then filtered on Whatman filter paper number 3. The obtained filtrate was evaporated to dryness at 55°C in ventilated oven to obtain a dryed extract.

Acute Toxicity Study: Animals were divided randomly into six equal groups as follow: group 1: control mice, group 2: mice received intraperitoneal injection of nickel sulfate (10mg/kg body weight) for 24 hours of exposure [18], groups 3, 4 and 5: mice received by oral gavage 50, 100 and 200mg/kg body weight of aqueous plant extract (AE) respectively, group 6 receive 16.6mg/kg body weight of vitamin C [19] for 7 days. On the 8th day, mice of these groups were injected intraperitoneally with 10mg/kg body weight of nickel sulfate and killed after 24 hours of Ni injection.

Haematological Analysis: At the time of sacrifice, blood was collected into EDTA tubes for heamatological estimation. Hematological parameters: red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV%), mean cell volume (MCV) and platelet count (Plt) were estimated utilizing fully automated hematological cell counter (Coulter Swilab).

Statistical Analysis: Results were displayed as mean ± S.E.M and were subjected to statistical significance evaluation. Significance of the inter-group differences of each parameter separately were evaluated using one-way analysis of variance (ANOVA) with statistical software (GraphPad Prism, V6). The level of significance was set at $p < 0.05$.

RESULTS

Effect of Treatments on Haematological Parameters: As showed in Figures 1 and 2, nickel treatment resulted in significant (p <0.01) decrease of red (RBC) and white blood cell (WBC) counts, packed cell volume (PCV%), plateletes count, haemoglobin (Hb) concentration and mean cell volume (MCV) when compared to control group. Alike, the pretreatment of aqueous extract and vitamin C followed by nickel sulfae injection showed a significant decrease (p <0.05) of theses paramaters in comparaison with control mice. The studied paramaters were found to be improved in the combined treatments with effeciency amelioration in limoniastrum extract as compared to vitamin C along with nickel.

DISCUSSION

The hazards of toxic metals, such as nickel is able of inflicting biological damage leading to blood profile alterations [20, 21]. Vitamins C and aqueos extracts of various medicinal plants are well documented for the attenuation of oxidant mediated blood desorders in human and experimental animals [9, 22]. This study therefore was devoted to determine the benefecial effect of aqueous extract of *Limoniastrum guyonianum* and vitamin C on some heamatological indices upon nickel exposure. Our findings showed that treatment with nickel induced significant decrease in red and white blood cells (RBC, WBC), haemoglobine (Hb) concentration, packed cell volume (PCV) and mean cell volume (MCV). Therefore, the treatment with nickel sulfate induces anemia in association with significant decrease of the hematological parameters**.** In addition**,** the decrease in RBCs count, PCV% and hemoglobin concentration may be due to non- regenerative anemia arise from nickel induced direct injury of hematopoietic stem cells resulting in decreased erythrocytes, leucocytes and platelets count.

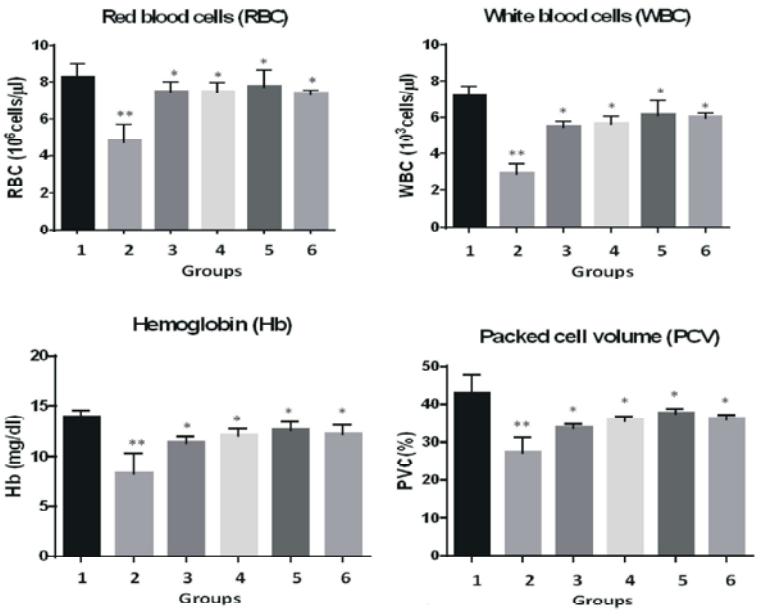

Fig. 1: Change in Red and white blood cells, heamoglobin concentration and packet cell volume of control (Group 1) and experimental mice groups. Values are given as mean ± SEM of 5 mice in each group. Significance of differences: *$p < 0.05$, **$p < 0.01$.

Treatment groups: 1- untreated control; 2- Nickel sulfate (10mg/kg); 3- Nickel sulfate + Aquous leaf extract (50mg/kg); 4- Nickel sulfate + Aquous leaf extract (100mg/kg); 5- Nickel sulfate + Aquous leaf extract (200mg/kg); 6- Nickel sulfate + Vitamin C (16,6mg/kg).

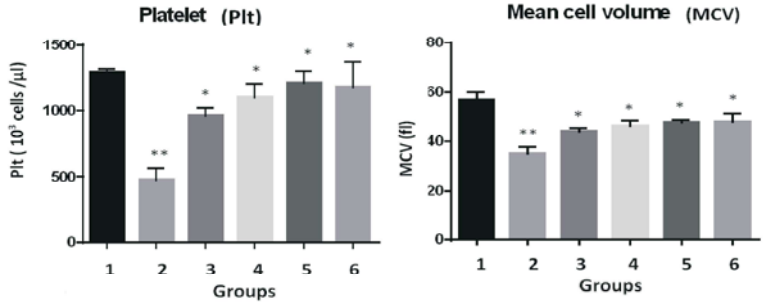

Fig. 2: Change in platelets count and mean cell volume of control (Group 1) and experimental mice groups. Values are given as mean ± SEM of 5 mice in each group. Significance of differences: *$p < 0.05$, **$p < 0.01$.

Treatment groups: 1- untreated control; 2- Nickel sulfate (10mg/kg); 3- Nickel sulfate + Aquous leaf extract (50mg/kg); 4- Nickel sulfate + Aquous leaf extract (100mg/kg); 5- Nickel sulfate + Aquous leaf extract (200mg/kg); 6- Nickel sulfate + Vitamin C (16,6mg/kg).

It was suggested that nickel may adversely affect hematopoiesis process and bone marrow activity resulting in decreased RBC and Hb which is likely be due to iron deficiency or chemically induced anemia [5, 23]. Also, nickel is found to induce oxidative injury in erythrocytes following generation of reactive oxygen species [5, 24]. The low hemoglobin concentration observed in our data may be related to disruption of some enzymes involved in the hem biosynthesis [23]. Our plant aqueous extract and vitamin C ameliorated the previous hematological parameters approximately to their normal values. However, the plant extract proved to be more efficient than vitamin C in reducing nickel heamatotoxicity following dose dependent manner. We suggest that the natural substances of leaf aqueous extract of *Limoniastrum guyonianum* used in this study exhibited an antioxidant activity in reducing the oxidative blood cell injuries, as well as they acted as good agents in activating blood cell productions and hemoglobin synthesis by stimulating bone marrow.

The present study indicated that this extract proved to be more beneficial in the treatment of Ni-heamatotoxicity than vitamin C. Furthermore, it provides biological evidence supporting the beneficial role as an antioxidant in protecting nickel induced toxic effects in hematological profile.

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