Toxic Effects of Prolonged Ni (II) and Cr (VI) Exposure in Male Mice on Bone Marrow and Some Hematological Parameters

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Abstract

Heavy metals hazard which can causes toxicological effects on many organs in animals and humans, therefore the purpose of the present study was to investigated the pathological lesion of Nickel (Ni II) and Chrome (Cr VI) metals on some hematological parameters in which heavy metal causes disorder in hematopoietic cell renewal system, which the results showed significant decrease ($P\leq0.01$) in the red blood cells (RBC) count for all doses of Ni and Cr elements, significant decrease ($P\leq0.05$) in the monocytes cell counts only at intermediate and high dose of Ni and Cr elements while significant increase ($P\leq0.01$) of granulocytes cell counts only at high dose for both Ni and Cr elements. Although the results demonstrated there was no any significant difference on platelets for all doses of Ni. On other hand no any significant difference on platelets only at the lowest dose of Cr while, high significant differences ($P\leq0.01$) on platelets at both intermediate and high dose of Cr. Also the results showed significant decrease ($P\leq0.05$) in the bleeding time and clotting time for all groups treated with different doses of both Ni and Cr elements. Dramatic histological lesion for both elements found in the tissue of bone marrow such as degenerate, necrotic and loosely arranged of tissue.

التأثيرات السمية الناتجة من التعرض الى عنصر النيكل (II) والكروم (VI) في ذكور الفئران على نخاع العظم وبعض المعايير الدموية

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الخلاصة: تشكل المعادن الثقيلة خطرا كبيرا ومسببة للعديد من التأثيرات السمية للعديد من الاعضاء في الأنسان والحيوان. لذلك هدفت هذه الدراسة الى توضيح الاضرار المرضية الناتجة من عنصري النيكل والكروم على بعض المعايير الدموية حيث تسبب هذه المعدن اثر بالغ على الخلايا المكونة للدم . اذ بينت النتائج وجود انخفاض معنوي ($P\leq0.01$) في اعداد خلايا الدم الحمراء ولكل المجاميع المعاملة بجرعات مختلفة من عنصري النيكل والكروم مقارنة مع مجموعة السيطرة. كما اظهرت النتائج انخفاض معنوي ($P\leq0.05$) في اعداد خلايا monocytes عند الجرعتين المتوسطة والعالية لكلا العنصرين مقارنة مع مجموعة السيطرة بينما اظهرت النتائج ارتفاع معنوي ($P\leq0.01$) في اعداد خلايا granulocytes فقط عند الجرع العالية من عنصري النيكل والكروم, بالرغم من عدم وجود اي فرق معنوي في اعداد الصفيحات الدموية لجميع الجرع الخاصة بعنصر لنيكل من جانب اخر بينت النتائج ارتفاع معنوي (($P\leq0.01$) في اعداد الصفيحات لكلا الجرعتين المتوسطة والعالية من عنصر الكروم. كما اظهرت النتائج اختلاف معنوي في كل من زمن التخثر وزمن النزف ($P\leq0.05$) لكلا العنصرين ولجميع المجاميع المقارنة مع مجموعة السيطرة. اوضحت لنتائج وجود اضرار نسيجية حقيقية لكلا العنصرين على نخاع العظم تمثلت بالتنخر والتنكس وفقدان التنسج.

Introduction

Nickel element is the most abundant in the earth crust constitute % 3. It can be used in many industrial processes [1]. Ni Know as essential element for vary physiological events at low concentration but it's very toxic at high concentration. Ni can reach to animals and human by food chains [2; 3]. Exposure to this element leads to formation free radical then causes damage of cell by increased activity of lipid peroxidase (LPO) while inhibited glutathione peroxidase (GSH-Px) and catalase (CAT) activity also reduce glutathione (GSH) contents as scavenger in antioxidant system [4]. Chrome is one of environmental pollution resulted from different industrial processes because it widespread used in metal plating, manufacturing industries, ferrochrome production, tanneries and other industries [5]. There is two oxidation state of chromium: trivalent chromium (Cr-III) often resulted from natural source and hexavalent chromium (Cr-VI) resulted from industrial source. (Cr-VI) have been shown carcinogenic and toxic effects on both animals and humans in which was a cause DNA damage like DNA-crosslinks and DNA single strand breaks and many other disorders [6]. Blood acts as an internal transporter and important tool to assess organism's health and it is one of the major routes for absorption of environmental pollutant, also it is the most important parameters for evaluation of physiological status and response of the whole organisms [7].Bone marrow is one of the most important and largest organs in the body, it's found within central cavity of long and axial bone composed of hematopoietic cells and fat tissue surrounded by blood sinuses interspersed within bone trabecular Bone marrow is major hematopoietic organs responsible for the production blood elements such as erythrocytes, granulated and non-granulated leucocytes and platelets [8]. Heavy metals and different type of pollution have adversely effects on mesenchyme stem cells (MSCs) also can damage blood vessels of bone marrow [9]. Heavy metals can causes disorder in the circulation of blood cells by increase hemolysis which is known to shorten the life-span. Lead effect on cellular proliferation, maturation and differentiation in the bone marrow also affect on iron metabolism inside red cell which is cause hemoglobin synthesis porphyria also a hemolytic and sideroblastic anemia [10].The blood is an important liquid connective tissue circulation through the body it have an important role by carried out oxygen and other material to various tissues, then remove carbon dioxide and different waste in order to maintains the health status of an organisms [11]. Ni (II) and Cr (VI) elements induced alteration in the hematological parameters such as effect on erythrocyte membrane protein and lipid bilayer, also heavy metals can linked with plasma protein like albumin and membrane of red blood cells and activated ROS and metallothioneins and this resulted from oxidative stress and damage in erythrocytes and different organs [12].

Material methods

Forty two immature mice (10-15 g) were divided randomly into 7 groups of 6 mice each animal were bred in the animal house of Pharmacy College, Karbala University. The experiment starts from February to March. Each group was housed in a separate in plastic cages measuring 30×12×11 cm. under control temperature, 22±2 Cº. Group 1 served as control received tap water, group 2 (20 mg/kg of $Nicl_2$), groups 3 and 4 received (40 and 60

mg/kg of $NiCl_2$) while group 5 (20 mg/kg of $K_2Cr_2O_7$), groups 6 and 7received (60 and 100 mg/kg of $K_2Cr_2O_7$) all groups received doses orally by methods of [13]. Bleeding time (BT) and Clotting time (CT) were estimated by Duke's method as summarized by [14]. For evaluation of histological lesions of the tissue of bone marrow the femur was taken after sacrificing of animals and remove muscle then saved at formalin (10%) to prepared by method of [15].

Statistical Analysis: The software SAS program .USA/version 9 (2004) was used to analyses the data of present work by using complete random design (CRD), then compared the differences between the averages using the test of less significant difference (LSD) [16].

Results The table (1) showed RBC count for all these doses were high significant differences as compared to the control group when treated with potassium dichromate. Lymphocytes count for intermediate and high doses were high significant differences as compared to the control group. Also monocytes cells showed significant differences for both intermediate and high dose as compared to the control group. However a high significant difference of granulocytes counts only at the highest does. Bleeding and clotting time showed significant differences for all doses as compared to the control group. The result of present study showed high significant differences in RBC and lymphocytes counts for all doses of Nickel chloride as compared to the control group. While only the highest dose has significant differences in granulocytes count as compared to the control group. As well as bleeding and clotting time showed significant differences for all doses as compared to the control group table (2). The histological examination of bone marrow section (Figure 1) showed the normal histological appearance with adequate all blood cellular precursors. Consecutive administrated of potassium dichromate and nickel chloride causes histological alteration in the studied organ such as necrosis, decrease numbers of megakaryocytes and degeneration (Figure 2, 3, 4, 5).

Table (1): The effect of different doses of $NiCl_2$ (II) on some blood parameters.

parameters		Concentrations Mg/Kg				
		CO	20	60	100	LSD
WBC	Lym.	4.47±0.28*	3.72±1.00	2.02±0.51	1.96±0.22	1.69
	Mon.	1.161±0.106**	0.736±0.118	0.463±0.092	0.291±0.030	0.426
	Gran.	0.02±0.01*	0.05±0.00	0.07±0.4	0.33±0.16	0.24
Platelets		734.4±102.63	700.2±95.44	683.6±69.18	565.4±36.74	230.26
Bleeding time		4.638±0.410**	5.793±0.434	8.288±0.310	8.883±0.310	0.426
Clotting time		4.226±0.275**	4.953±0.298	6.876±0.410	8.165±0.506	0.24

* Significant difference (P≤0.01), ** significant difference (P≤0.05), lym. (Lymphocytes), mon.(monocytes), Gran.(granulocytes), LSD(least significant differences). RBC& Platelets =Cell/10^6/mm^3, WBC= Cell/10^3/mm^3, Bleeding & Clotting time =minutes, mean ± stander error.

Table (2): The effect of different doses of $K_2Cr_2O_7$ (VI) on some blood parameters.

parameters		Concentrations Mg/Kg				
		CO	20	40	60	LSD
WBC	Lym.	4.47±0.28*	2.62±0.56	2.17±0.50	0.96±0.18	1.19
	Mon.	1.161±0.106**	0.845 ± 0.069	0.716±0.100	0.346±0.062	0.411
	Gran.	0.02±0.01*	1.80±0.28	1.81±0.90	3.50±0.93	1.90
Platelets		734.4±102.63*	581.03±85.30	424.1±58.12	358.3±61.76	266.77
Bleeding time		7.49 ± 0.419**	6.78 ± 0.383	6.485±0.377	4.638±0.410	0.41
Clotting time		4.226±0.275**	5.666±0.267	7.51±0.246	8.123±0.470	0.411

* Significant difference (P≤0.01), ** significant difference (P≤0.05), lym. (Lymphocytes), mon.(monocytes), Gran.(granulocytes), LSD(least significant differences). RBC& Platelets =Cell/10^6/mm^3, WBC= Cell/10^3/mm^3, Bleeding & Clotting time =minutes, mean ± stander error.

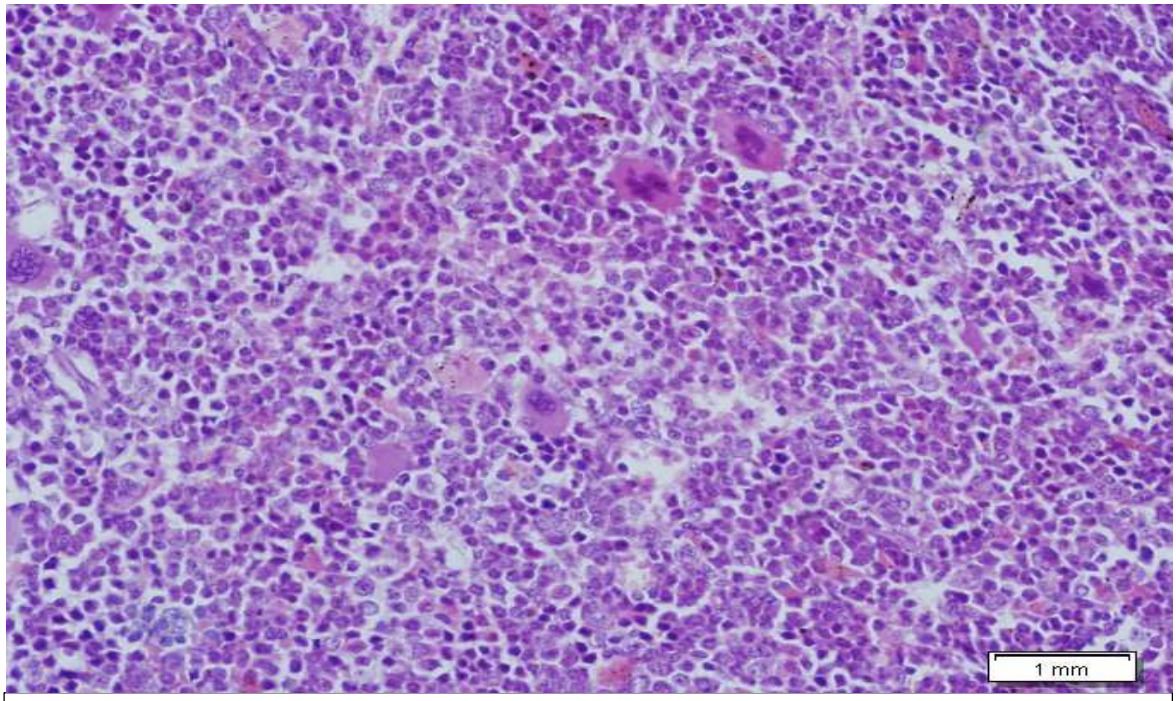

Fig.(1) Cross histological section of bone marrow from intact male mice showed the normal structure include cellular marrow with adequate all blood cellular precursors (H&E.stain,X200).

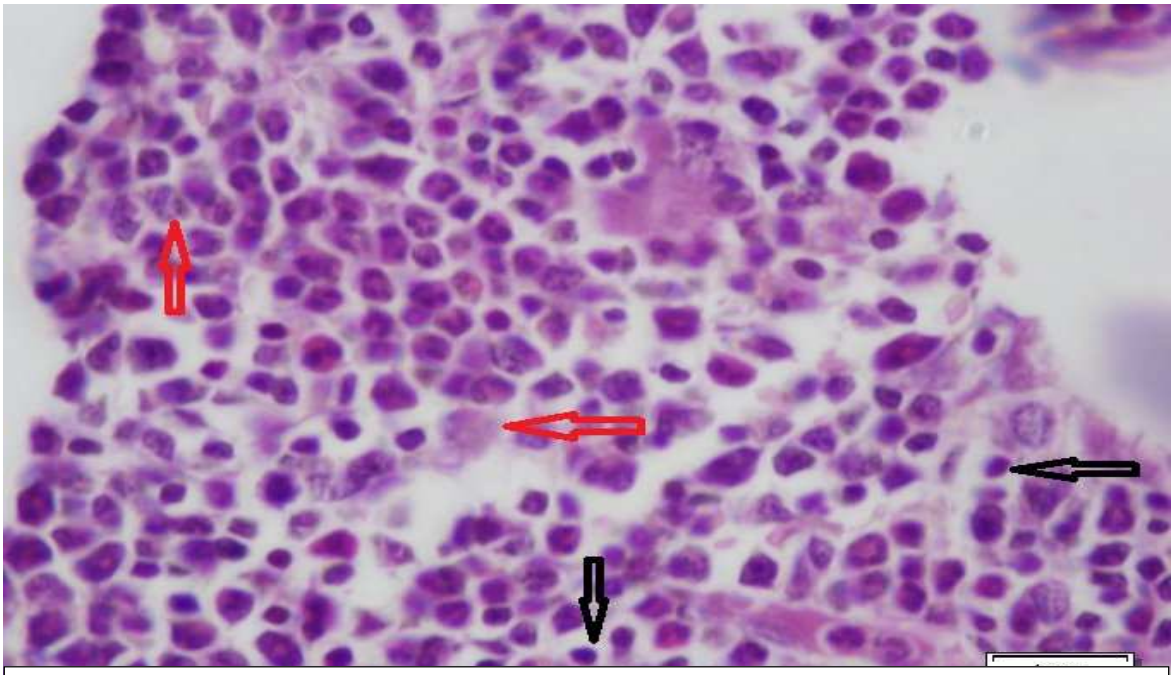

Fig.(2) Cross histological section of bone marrow of male mouse treated with 100 mg/kg K2Cr2O7 showed relatively cellular marrow but with decreased numbers of megakaryocytes, frequent apoptosis (black arrows),scattered degenerated cells (H&E.stain,X400).

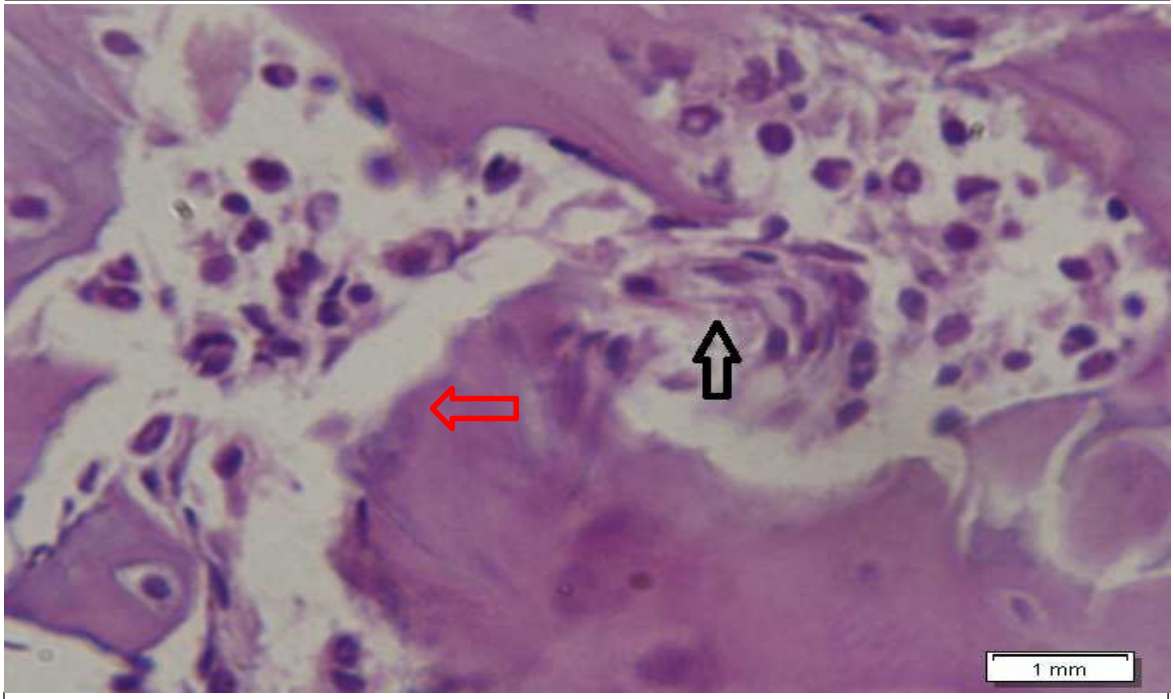

Fig.(3) Cross histological section of bone marrow of male mouse treated with 60 mg/kg K2Cr2O7 showed Marked decreased cellularity, and megakaryocytes, frequent necrosis (red arrows), and fibroblastic cells (black arrows) (H&E.stain,X400).

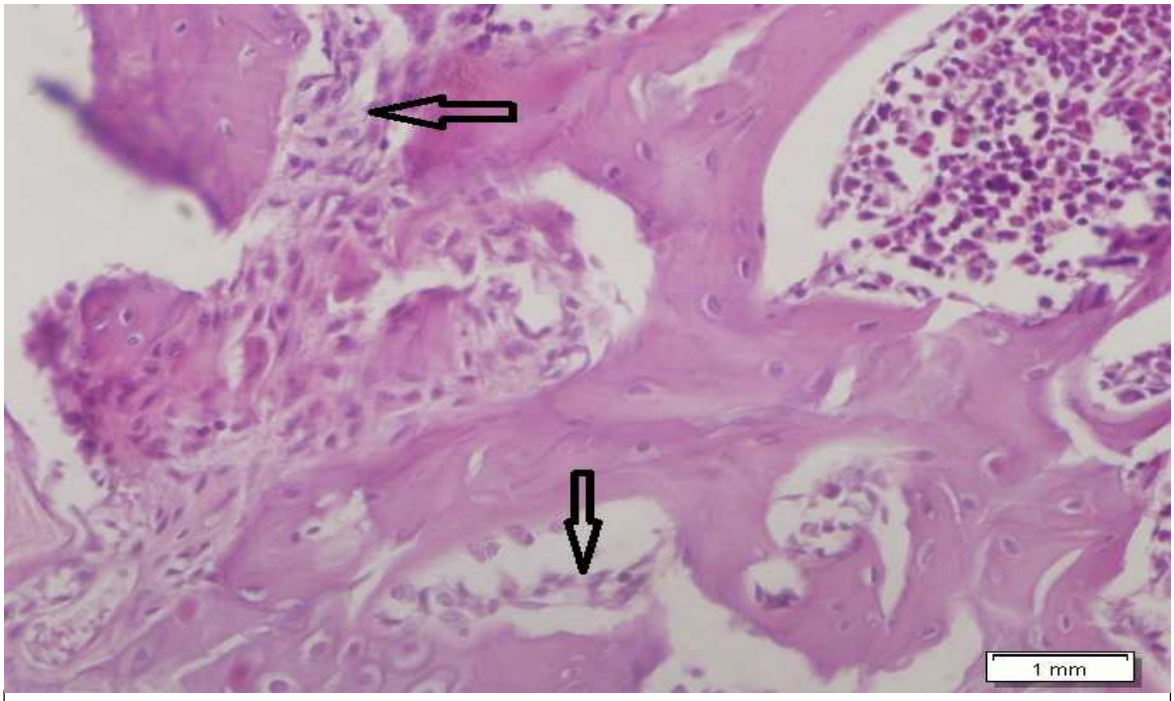

Fig.(4) Cross histological section of bone marrow of male mouse treated with 60 mg/kg $NiCl_2$ showed bone marrow with decreased cellularity, focal reactive fibrosis (black arrow),decreased megakaryocytes (H&E.stain,X200).

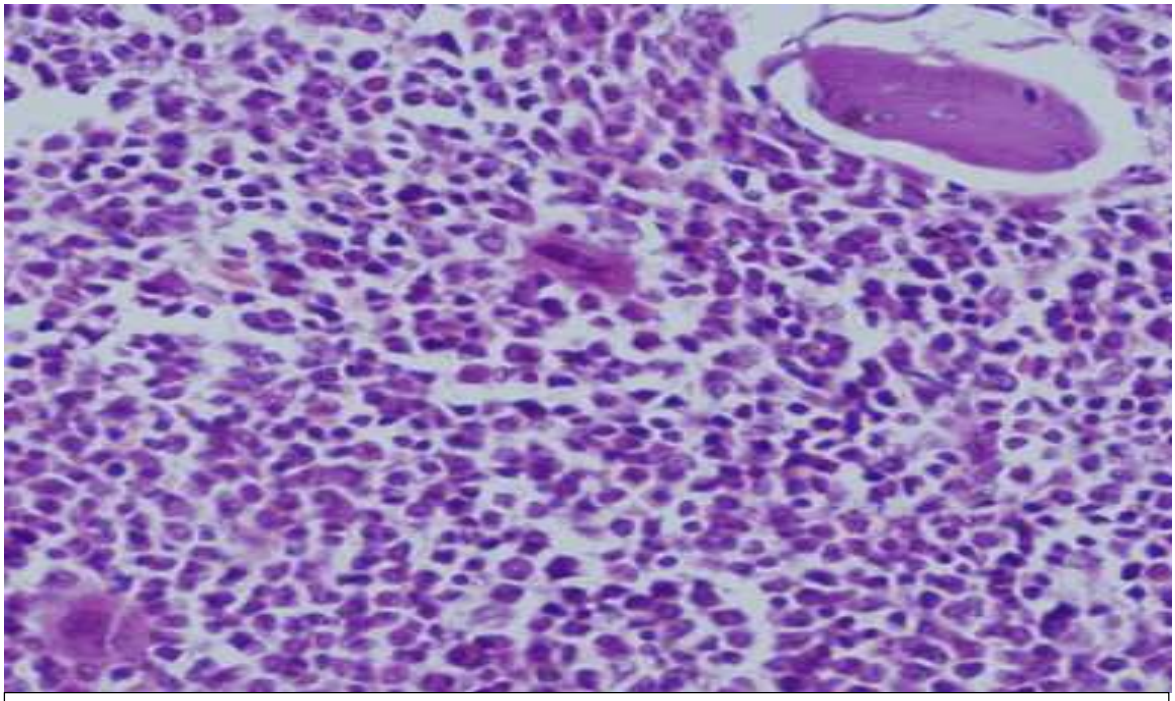

Fig.(5) Cross histological section of bone marrow of male mouse treated with 40 mg/kg $NiCl_2$ showed bone marrow with relatively normal cellularity ,adequate blood cellular precursors (H&E.stain,X400).

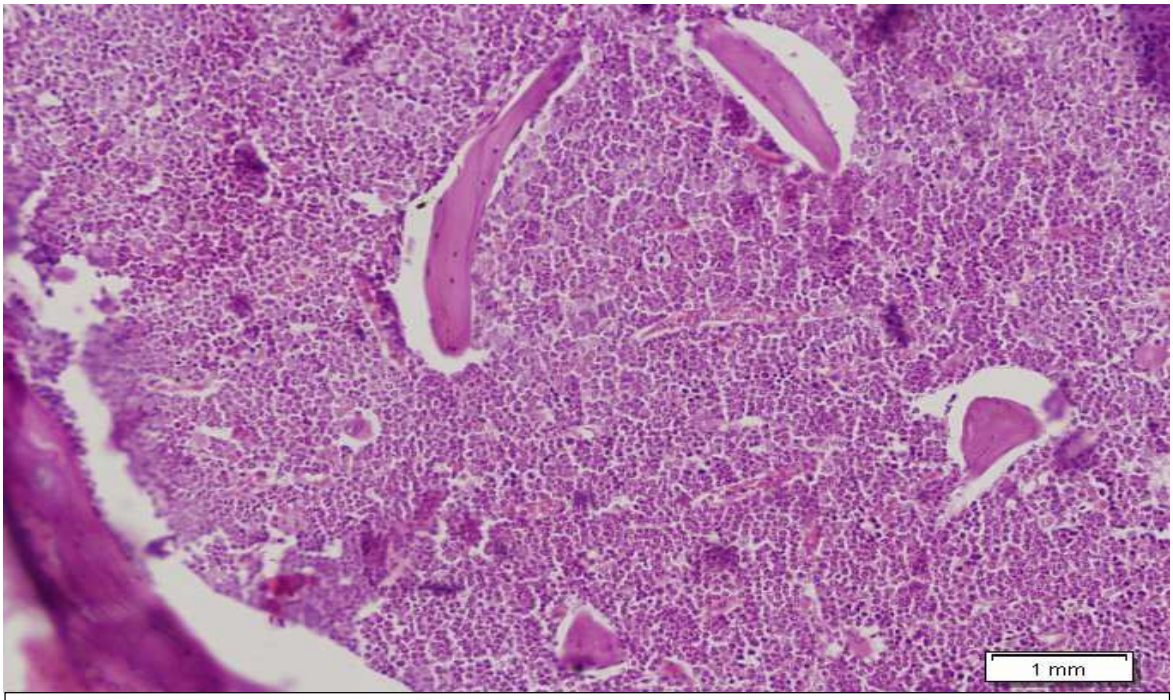

Fig.(6) Cross histological section of bone marrow of male mouse treated with 20 mg/kg $NiCl_2$ showed bone marrow with relatively normal cellularity ,adequate blood cellular precursors (H&E.stain,X100).

Discussion

The results obtained from the present study showed that when male mice exposed to different doses of $K_2Cr_2O_7$ or $NiCl_2$ caused significant differences ($P \leq 0.01$) in most of hematological parameters (Table 1, 2).The reasons behind decrease blood parameters may be due to many factors such as: impaired intestinal absorption of iron [17], or may be due to inhibition of erythropoiesis and sever damage occur at stem cell within bone marrow or by destruction of red blood cells by hemolysis [18, 19]. Also the result of present study showed significant differences ($P \leq 0.01$) in BT (Bleeding Time) and CT (Clotting Time) of groups treated with different doses of heavy metals as compared to the control group. This can attribute to many reasons: first reason may be due to inhibition of calcium pumps work [20], which causes very decrease in secretion of calcium ion which is essential in making the coagulation process. The second cause may be due to inhibition of plasma protease enzyme that initiator for coagulation process and located on cell membranes [21].Serious damage found in the histological examination of bone marrow in mice resulted from many toxic effects occurred on the stem cells. These findings are in agreement with findings by [12] who found that the decrease of lymphocyte counts either by decrease production or increase consumption, while decline in RBC and Hb possible return to the reduce formation of succinyl and glycine pool of amino acids both are required in the initial stage of the heme biosynthesis [22]. [23] Found a decrease in WBC, RBC, Hbg and PCV for male rats treated with Cr and Ni elements may be resulted by injury of hematopoietic stem cells then induced anemia, many other study show

the same effects such as results obtain by [24]. The present results in agreement with [25] who found decrease in RBC, Hb, PCV and WBC may be due to hemolysis in the erythrocyte membrane then led to sever anemia, leucopenia (a reduction in the number of WBC), and thrombocytopenia (deficiency of platelets in the blood). The present results in agreement with another study founded by [26]. The present study in agreement with [27] demonstrated increase in CT in mice treated with Nickel resulted from increased activity of lipid peroxidase; damage of DNA and altered in sulfhydryl and calcium homeostasis also disorder in antioxidant system of defense. Also the present data in agreement with finding by [28] that showed increase BT and CT in albino rats treated with mercuric chloride probably is due toxic effects on haemopoietic operation which cause decrease and destruction in the level of platelet counts. However the present result in agreement with [11] demonstrated the increased in BT and CT can result from liver disorder because the important clotting factors such as fibrinogen and prothrombin. The present data in agreement with finding by [29] that showed reduce in the activity of bone marrow of male rats causes reduce in all type of blood cell after treated with Nickel sulfate. The results of the present study in agreement with what found by [30] that showed sever damage in the tissue of bone marrow of rat after treated with lead acetate. Bone marrow damage by lead acetate was thought to be caused by oxidative stress [31]. [32] Showed acute histological hanges in the cell of bone marrow of rat resulted from chromosome aberrations after administration with $NiCl_2$.

Conclusion:

In conclusion, based on the results observed in the present study and discussion the male mice treated with different doses of $K_2Cr_2O_7$& $NiCl_2$ causes toxic effects on hematological parameters also induced many lesions on histological section of bone marrow by inducing necrosis and degenerations to the many cells.

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