



Haematological effects of ethanolic extract of *Blepharis linariifolia* alone or co-administered with aluminum chloride in rats

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Abstract

Background and objectives: The indigenous medicinal plants in Sudan form an important component of the natural wealth of the country. Most of these plants have been used indiscriminately by many local populations for managing various diseases without actually knowing how relief is brought about or its safety/toxicity risks. The effect of oral administration of *Blepharis linariifolia* ethanolic extract alone and/or co-administered with aluminium chloride at different doses on haematological profile in white albino rats was investigated in this study.

Materials and Methods: The animals were divided into five groups of five animals each. Group A: Control, Group B, C were administered orally by AlCl₃ alone (0.5 mg/Kg BW), plant extract (400 mg/ml), plant extract (200 mg/ml), respectively, throughout the experiment which continued for consecutive 14 days and 28 days Group E was administered with plant extract (400 mg/ml) concurrently with AlCl₃ for consecutive 14 days and 28 days. The blood parameters measured were: White Blood Cells count (WBCs), Red Blood Cells count (RBCs), Haemoglobin concentration (Hb), Haemocrit value (HCT), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Platelets (PLT) and Lymphocytes (LYM).

Results: Treatment of rats (B group) with AlCl₃ after 14 days caused significant difference at P<0.05 and P<0.01 in total RBC count, Hb, HCT and MCV. Whereas after 28 days caused a significant difference in WBC and MCV. Treatment of rats (C group) with 400 mg/ml/Kg BW of plant extract after 14 and 28 days caused significant difference at P<0.05 in the WBC count and Hb values relative to their respective control. Administration of rats (D group) with 200 mg/ml of plant extract plus AlCl₃ did not produce any significant change (P<0.05) on the RBC and factors relating to it (Hb, HCT, MCV, MCH and MCHC) on 14 days. Interestingly, administration of the plant extract 200 mg/ml plus AlCl₃ produced significant alterations only in the platelets. Administration of 200 mg/ml plant extract plus AlCl₃ for 28 days produce significant change (P<0.05) on the RBC, Hb and HCT. There was no significant difference (P<0.05 and P<0.01) after treatment with 400mg/ml extract plus AlCl₃ for 14 days between the two control group and the treated (E group) for RBC, Hb, HCT, MCV, MCH, MCHC and LYM, and at P<0.01 for WBC. The PLT values were significantly different at P<0.05 and P<0.01 between the control and treated group after 14 and 28 days.

Conclusion: uptake of ethanolic extract of *B. linariifolia* alone or co-administered with AlCl₃ had mitigated aluminum chloride – induce anemia and raised the values of blood indices of anemia, almost, to their normal levels.

Keywords: haematological profile; toxicity; *Blepharis linariifolia*; aluminum chloride; rats

1. Introduction

Many people are relying on herbal medicines for health care, because the other treatment options available are more expensive and are often associated with serious side effects. Therefore, there should be scientific documentation of information on the safety/toxic risk potentials of plants. One of such plants is *Blepharis linariifolia*. It has been widely used by many localities in Africa including Sudan in venereal diseases such as syphilis. Various workers had shown that haematological investigations among others could be used to evaluate the health status of an animal (de Gruchy, 1976) ^[1].

The aim of the present research work were to assess the effect of ethanolic extract of *Blepharis linariifolia* (200 mg/ml and 400 mg/ml) on the treatment of aluminum chloride – induced anemia in white albino rats when administered for 14 and 28 days.

2. Materials and Methods

2.1 Materials

2.1.1 Animals

White albino rats weighing between 100-120 g were used for the studied experiments. They were housed in standard rat cages under laboratory conditions with 12:12 h light /dark cycle at 30 + 2 C. The animals were allowed to acclimatize for 2 weeks. The experiment was conducted in accordance with the guidelines of the US National Institute of Health (NIH) on the care and use of laboratory animals.

2.1.2 Plants

The whole plant of *Blepharis linariifolia* were collected in September 2014 from Omdurman North, Khor Omer. Plant was

washed and dried in shades. The dried plant was blended into powder and was stored in clean container prior extraction.

2.2 Methods

2.2.1 Plant Extraction

One hundred grams of dried whole plant of *B. linariifolia* were extracted by Soxhlet Apparatus for 6 hours with 70% ethanol. The extract was filtered and the solvent was evaporated with Rota-evaporator and then was dried at room temperature for two weeks.

2.2.2 Blood collection

Two ml of blood samples were collected by using haematocrit capillary tubes from retro-orbital plexus of each rat (Khanna *et al.*, 1992). Each blood sample was placed in ethylene diamine tetra-acetic acid (EDTA) container.

2.2.3 The Experimental Protocol

The animals were divided into five groups of five animals each. Group A: Control. Group B (denoted B-1 and B-2) was administered orally by AlCl₃ (0.5 mg/Kg BW) as stated by Weber (2002) throughout the experiment which continued for consecutive 14 days (B-1) and 28 days (B-2). Group C (denoted C-1 and C-2) was administered orally by Plant extract (400 mg/ml) for consecutive 14 days (C-1) and 28 days (C-2). Group D (denoted D-1 and D-2) was administered with plant extract (200 mg/ml) concurrently with AlCl₃ for consecutive 14 days and 28 days. Group E (denoted E-1 and E-2) was administered with plant extract (400 mg/ml) concurrently with AlCl₃ for consecutive 14 days and 28 days.

Collected blood were used for the measurement of blood parameters using automated Haematology Analyzer (Sysmex Apparatus of the type 8999). The blood parameters measured were: white blood cells count (WBCs), red blood cells count

(RBCs), haemoglobin concentration (Hb), haemocrit value (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets (PLT) and lymphocytes (LYM).

2.2.4 Statistical Analysis

Student t test was used for comparison between (mean \pm standard deviation) values of AlCl₃ – treated group and control group and each of them with the mean values of the group treated with AlCl₃ plus *B. linariifolia* (400 mg/ml), the group provided with AlCl₃ plus *B. linariifolia* (200 mg/ml) and the group provided with *B. linariifolia* (400 mg/ml). The significance is taken at P<0.05 and P <0.01. t-test parameter was performed using SPSS 20.0 Software for Windows.

3. Results and Discussion

The haematological effect of ethanolic extract of *B. linariifolia* was evaluated in male albino rats during 14 and 28 days administrations of aluminum chloride alone (0.5 mg/ml/Kg bw), plant extract at 400 mg/ml /Kg bw, 200 mg/ml plus aluminum chloride and 400 mg/ml plus aluminum chloride, orally. Parameters evaluated include WBC, RBC, Hb, HCT, MCV, MCH, MCHC, PLT and LYM.

3.1 The effect of AlCl₃ alone on haematological parameters in albino rats

The effect of AlCl₃ alone on haematological parameters of albino rats after treatment for 14 and 28 days are shown in the Table 1. Treatment of rats (B-1 group) with AlCl₃ alone after 14 days caused significant difference at P<0.05 and P<0.01 in total RBC count, Hb, HCT and MCV. The oral administration of AlCl₃ alone after 28 days (B-2 group) caused a significant difference (P<0.0 and P<0.01) in WBC and MCV. Also caused a significant difference (P<0.05) in Hb, MCH and MCHC.

Table 1: The effect of AlCl₃, plant extract and AlCl₃ plus plant extract on haematological parameters in male rats

Parameters	Control	AlCl ₃ alone After 14 days	AlCl ₃ alone After 28 days	400 mg/ml extract after 14 days	400 mg/ml extract after 28 days	AL Cl ₃ plus 200 mg/ml extract after 14 days	AlCl ₃ plus 200 mg/ml extract after 28 days	AL Cl ₃ plus 400 mg/ml extract after 14 days	AlCl ₃ plus 400 mg/ml extract after 28 days
WBC (X10 ⁹ /ul)	6.44 \pm 2.997	8.66 \pm 2.436	12.32 \pm 00*,**	10.52 \pm 3.01*	11.6 \pm 3.3176*	10.92 \pm 3.973*	5.2 \pm 5.069	9.2 \pm 1.56*	8.48 \pm 1.633
RBC (X10 ⁶ /ul)	7.87 \pm 0.304	5.52 \pm 0.187*,**	8.04	8.758 \pm 0.866*,**	8.44 \pm 0.63	7.624 \pm 0.30	5.83 \pm 1.786*	7.818 \pm 0.369	7.55 \pm 1.1466
Hb (g/dl)	13.46 \pm 0.21	12.74 \pm 0.187*,**	14.94*	14.18 \pm 0.432*,**	14.525 \pm 0.574*,**	13.2 \pm 0.18	11.234 \pm 0.106*	13.16 \pm 0.677	13.34 \pm 2.131
HCT (%)	44.14 \pm 1.60	41.34 \pm 1.72*,**	46.96*	47.44 \pm 1.98*,**	47.125 \pm 3.0598	43.05 \pm 1.771	32.97 \pm 10.152*	44.375 \pm 1.269	42.96 \pm 7.158
MCV (fl)	56.1 \pm 0.977	59.48 \pm 1.333*,**	58.4*,**	54.18 \pm 1.25*	55.9 \pm 0.868	56.24 \pm 1.632	56.5 \pm 1.916	55.9 \pm 1.15	56.76 \pm 1.443
MCH (pg)	17.14 \pm 0.69	18.34 \pm 0.372	18.58	16.22 \pm 0.63*	17.28 \pm 0.834	17.18 \pm 0.554	17.07 \pm 2.098	16.95 \pm 0.580	17.64 \pm 5.235
MCHC (g/dl)	30.52 \pm 1.13	30.84 \pm 0.680	31.82	29.92 \pm 0.687	30.85 \pm 1.60	30.58 \pm 0.759	31.57 \pm 0.777	30.28 \pm 0.576	31.1 \pm 0.418
PLT (X10 ³ /ul)	1083 \pm 192.283	950.4 \pm 59.584	1118	889.4 \pm 273.54	1067.75 \pm 99.28	829.4 \pm 98.865*	622.7 \pm 500.5	683.8 \pm 146.64*,**	739.4 \pm 72.3*
LYM (%)	48.92 \pm 7.09	41.66 \pm 7.618	44.66	52.52 \pm 7.84	44.575 \pm 11.092	46.86 \pm 9.755	45.75 \pm 19.163	41.22 \pm 8.963	46.1 \pm 4.729

Values are expressed as mean + SD, N=5, * for P=0.05 and ** for P= 0.01

3.2 The effect of *Blepharis linariifolia* extract (400 mg/ml) on haematological parameters in albino rats

The effect of plant extract at 400 mg/ml/Kg BW on the haematological parameters of albino rats after treatment for 14 and 28 days are shown in the Table 2. Treatment of rats (C-1 group) with 400 mg/ml/Kg BW of plant extract after 14 days caused significant difference at P<0.05 in the WBC, RBC, Hb, HCT, MCV and MCH values relative to their respective control,

while treated rats were showed no significant (P<0.05) change in MCHC, PLT and LYM values relative to their respective control. Treatment of rats for 28 days caused significant (P<0.05) difference in the WBC and Hb values relative to their respective control, while no significant (P<0.05) change in RBC, HCT, MCV, MCH, MCHC, PLT and LYM values relative to their respective control.

Table 2: The effect of the AlCl₃ alone on haematological parameters in albino rats compared to representative control (A Group) when administered daily for 14 and 28 days

Blood Parameter	B-1 Group				B-2 Group			
	Change	Change %	P=0.05	P=0.01	Change	Change %	P=0.05	P=0.01
WBC	Increment	34.4	NS	NS	Increment	91.3	S	S
RBC	Decrement	29.9	S	S	Increment	2.2	NS	NS
Hb	Decrement	5.3	S	S	Increment	11.0	S	NS
HCT	Decrement	6.3	S	S	Increment	6.4	NS	NS
MCV	Increment	6.0	S	S	Increment	4.1	S	S
MCH	Increment	7.0	NS	NS	Increment	8.4	S	NS
MCHC	Increment	1.0	NS	NS	Increment	4.3	S	NS
PLT	Decrement	12.2	NS	NS	Increment	3.2	NS	NS
LYM	Decrement	14.8	NS	NS	Decrement	8.7	NS	NS

3.4 The effect of *Blepharis linariifolia* extract (200 mg/ml) plus AlCl₃ on haematological parameters after treatment of rats for 14 and 28 days

Administration of 200 mg/ml of plant extract plus AlCl₃ did not produce any significant change (P<0.05) on the RBC and factors relating to it (Hb, HCT, MCV, MCH and MCHC) on 14 days

investigated rats Table 3. Interestingly, administration of the plant extract 200 mg/ml plus AlCl₃ produced significant alterations in the platelets. Administration of 200 mg/ml plant extract plus AlCl₃ produce significant change (P<0.05) on the RBC, Hb and HCT, and did not produce any significant change (P<0.01) on the all blood parameters and on WBC, MCV, MCH, MCHC, PLT and LYM at P<0.05 on 28 days of investigated rats.

Table 3: The effect of the *Blepharis linariifolia* extract (400 mg/ml) on haematological parameters in albino rats compared to representative control (A Group) when administered daily for 14 and 28 days

Blood Parameter	C-1 Group				C-2 Group			
	Change	Change %	P=0.05	P=0.01	Change	Change %	P=0.05	P=0.01
WBC	Increment	63.4	S	NS	Increment	80.1	S	NS
RBC	Increment	11.3	S	S	Increment	7.2	NS	NS
Hb	Increment	5.3	S	S	Increment	7.9	S	S
HCT	Increment	7.5	S	S	increment	6.8	NS	NS
MCV	Decrement	3.4	S	NS	decrement	0.4	NS	NS
MCH	Decrement	5.4	S	NS	Increment	0.8	NS	NS
MCHC	Decrement	2.0	NS	NS	Increment	1.1	NS	NS
PLT	Decrement	17.9	NS	NS	decrement	1.4	NS	NS
LYM	Increment	7.4	NS	NS	Decrement	8.9	NS	NS

Table 4: The effect of the *Blepharis linariifolia* extract (200 mg/ml) plus AlCl₃ on haematological parameters in albino rats compared to representative control (A Group) when administered daily for 14 and 28 days

Blood Parameter	D-1 Group				D-2 Group			
	Change	Change %	P=0.05	P=0.01	Change	Change %	P=0.05	P=0.01
WBC	Increment	69.6	S	NS	decrement	19.3	NS	NS
RBC	Decrement	3.2	NS	NS	decrement	25.9	S	NS
Hb	Decrement	1.9	NS	NS	decrement	16.6	S	NS
HCT	Decrement	2.5	NS	NS	decrement	25.3	S	NS
MCV	increment	0.2	NS	NS	increment	0.7	NS	NS
MCH	Increment	0.2	NS	NS	decrement	0.4	NS	NS
MCHC	Increment	0.2	NS	NS	increment	3.4	NS	NS
PLT	Decrement	23.4	S	NS	decrement	42.5	NS	NS
LYM	Decrement	4.2	NS	NS	decrement	6.5	NS	NS

Table 5: The effect of the *Blepharis linariifolia* extract (400mg/ml) plus AlCl₃ on haematological parameters in albino rats compared to representative control (A Group) when administered daily for 14 and 28 days

Blood Parameter	E-1 Group				E-2 Group			
	Change	Change %	P=0.05	P=0.01	Change	Change %	P=0.05	P=0.01
WBC	Increment	42.9	S	NS	increment	31.7	NS	NS
RBC	Decrement	0.6	NS	NS	decrement	4.1	NS	NS
Hb	Decrement	2.2	NS	NS	decrement	0.9	NS	NS
HCT	Increment	0.5	NS	NS	decrement	2.7	NS	NS
MCV	decrement	0.4	NS	NS	increment	1.2	NS	NS
MCH	Decrement	1.1	NS	NS	Increment	2.9	NS	NS
MCHC	Decrement	0.8	NS	NS	Increment	1.9	NS	NS
PLT	Decrement	36.9	S	S	decrement	31.7	S	NS

LYM	Decrement	15.7	NS	NS	decrement	5.8	NS	NS
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3.5 The effect of *Blepharis linariifolia* extract (400 mg/ml) plus AlCl₃ on haematological parameters in albino rats

There was no significant difference ($P < 0.05$ and $P < 0.01$) between the two control groups and the treated group for RBC, Hb, HCT, MCV, MCH, MCHC and LYM, and at $P < 0.01$ for WBC on 14 day. The PLT values were significantly different at $P < 0.05$ and $P < 0.01$ between the control and treated group. Administration of 400 mg/ml plant extract plus AlCl₃ did not produce any significant change ($P = 0.5$ and $P = 0.01$) in all investigated blood parameters (except PLT at $P 0.05$) on 28 day.

In the present study, the decrease MCV, MCH and MCHC, in rats administered AlCl₃ alone refer to the type of anemia (microcytic - hypochromic anemia).

Jung-Hoon Jea *et al.*, (2005) [2] studied the decline in RBC count, hemoglobin concentration and HCT presumably reflects erythrocyte hemolysis and due to either an increase in the rate at which hemoglobin concentration may be destroyed or a decrease in the hemoglobin synthesis. Decrease in hematocrit is attributable to the reduction in RBC count caused either destruction or reduction in size.

As inferred from other reports, two mechanisms were suggested for the prevention of aluminum toxicity by *B. linariifolia*: First the plant extract inhibited or reduced Al absorption from intestine, and the second: aluminum overload might modulate gastrointestinal iron absorption and hinder the cellular uptake and use of iron for hemoglobin synthesis (Cannata *et al.*, 1991; Ganchev *et al.*, 1998) [3,4], and *B. linariifolia* corrected this toxic effect of Al by enhancing iron absorption, cellular uptake and use of iron for hemoglobin synthesis. The proposed mechanisms appear to involve inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization (Kaiser and Schwartz, 1985; Ganchev *et al.*, 1998; Han *et al.*, 2000) [5, 4, 6].

In a different studies, it were confirmed Al overload accumulation in all tissue to be the cause of anemia and it had led to AL-Zheimer s disease when accumulated in the tissue of the brain. Furthermore, disturbances in the distribution pattern of trace elements: zinc, copper and iron together with lipid peroxidation in plasma and erythrocytes were also suggested as a mechanism of aluminum-induced anemia in rats (Guo *et al.*, 2004) [7].

The calculated blood indices MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals (Coles, 1986) [8]. The non- significant effects on these indices relating to RBC suggest that there was no effect on the average size of RBC (microcytes) and also in the haemoglobin weight per RBC. This implies that the plant extract 200 mg/ml plus AlCl₃ does not possess any potential of including anemia throughout the 28 days period of administration.

Other indices that relate to WBCs were significantly altered; an indication of pathological condition which may imply challenge on the immune system by the plant extract 200 mg/ml plus AlCl₃. The significant increase in WBC following the administration of the plant extract /AlCl₃ indicates a boost in the immune system. Such effects may also be due to increase in vascular permeability. The non-significant effect of the plant extract 200 mg/ml plus AlCl₃ on the RBC and indices relating to it (Hb, PCV, MCV, MCH and MCHC) throughout the experimental period is an

indication that there was no destruction of matured RBC s and no change in the rate of production of RBC s (erythropoiesis). It further shows that the extract /AlCl₃ does not have the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996; Sanchez-EL-Sner *et al.*, 2004) [9, 10].

The non-significant effect on the RBC and Hb also implies that there was no change in the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following the extract /AlCl₃ administration since RBC and Hb are very important in transferring respiratory gases (de Gruchy, 1976) [1]. The results obtained from the present study, referred to the type of anemia caused by AlCl₃ exposure in male rats, which is microcytic - hypochromic anemia. Anemia that is partially due to a shortened life span of circulating erythrocytes and reduced RBCS production in bone marrow. This Shortened RBCS survival and expected premature elimination of circulating erythrocytes, however, could be explained by much mechanism, one of these is the oxidative stress caused by AlCl₃ in increase production of free radicals, decrease catalase activity, and decrease the erythrocyte ATP concentration (AL-Hashem, 2009; Newairy *et al.*, 2009) [11, 12].

All or some of these deleterious effects of AlCl₃ on RBCs membrane caused increased membrane fragility, increased RBCs destructions. The reduced level of hemoglobin content in rats administered AlCl₃ can be associated with RBCs hemolysis which confirmed by reduced RBCs count in the present study, or disturbances in heme biosynthesis.

Aluminum-induced haematological alterations leading to microcytic hypochromic anemia in albino rats of this study are similar to previous reports on the same blood parameters in rats (Zaman *et al.*, 1993; Chmielnicka *et al.*, 1994; Osman *et al.*, 2012) [13, 14, 15].

4. Conclusion

The effect of the ethanolic extracts of *B. linariifolia* on haematological parameters was compared to that of control group. Uptake of ethanolic extract of *B. linariifolia* alone or co-administered with AlCl₃ had mitigated aluminum chloride – induce anemia and raised the values of blood indices of anemia, almost, to their normal levels. The findings of this study could provide satisfactory pre-clinical evidence of safety to launch clinical trial on standardized formulation of plant extracts. Bioassay-guided fractionation and purification lead to isolation of active ingredient which can help in production of compounds with improved characteristics.

5. References

1. Al-Hashem FH. Camel's milk alleviates oxidative stress and lipid peroxidation induced by chronic aluminum chloride exposure in rat's testes. *Am. J. Applied Sci.* 2009; 6:1868-1875. DOI: 10.3844 /ajassp. 2009. 1868.1875.
2. Cannata J, Gomez A, Ferandez M, Ferandez S, MC- Gregor S, Menendez F, Brock J. Iron uptake in aluminum overload: In vivo and in vitro studies. *Nephrol Dial Trasplant.* 1991; 6(9):637-642.
3. Chmielnicka J, Nasiadek M, Pinkowski R, Paradowski M. Disturbances of morphological parameters in blood of rats

- orally exposed to aluminum chloride. *Biol. Trace Elem. Res.* 1994; 42:191-199.
4. Coles EH. *Veterinary clinical pathology*. WB. Saunders, Philadelphia, USA, 1986, 10-42.
 5. De Gruchy GC. *Clinical haematology in medical practice*. Blackwell Scientific Publication. Oxford, London, 1976, 130-155.
 6. Ganchev T, Dyankov E, Zacharieva R, Pachalieva I, Velikeva M, Kavaldjieva B. Influence of aluminum on erythropoiesis, iron metabolism and some functional characteristics of erythrocytes in rats. *Acta Physiol Pharmacol Bulg. J.* 1998; 23(1):27-31.
 7. Guo CH, Hsu GS, Lin LY, Wang YH, Lin CY, Yeh MS. Distribution patterns of trace metals and of lipid peroxidation in plasma and erythrocytes of rat exposed to aluminum. *Biol Trace Elem. Res.* 2004; 101(1):61-71.
 8. Han J, Han J, Dum MA. Effect of dietary aluminum on tissue non-heme iron and ferritin levels in the chick. *Toxicol J.* 2000; 142(2):97-109.
 9. Jung-Hoon J, Masroor F, Kang J. Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquaculture Research.* 2005; 36:898-905.
 10. Kaiser L, Schwartz KA. Aluminum induced anemia. *Am J Kidney Dis.* 1985; 6(5):348-352.
 11. Khanna AK, Chandar R, Kapoor NK, Singh C, Srivastava AK. Hypoglycaemic activity of *T. chebulain* in rats. *Fitoterapia LXIV.* 1992; 4:315-356.
 12. Newairy AA, Salama AF, Hussien HM, Yousef MI. Propolis alleviates aluminum induce lipid peroxidation and biochemical parameters in male rats. *Food. Chem. Toxicol.* 2009; 47:1093-1098. PMID: 19425229.
 13. Osman HM, Shayoub ME, Babiker EM, Osman B, EL-Hassan AM. Effect of ethanolic leaf extract of *Moringa oleifera* on aluminum – induced anemia in white albino rats. *Jordan Journal of Biological Sciences.* 2012; 5(4):255-260.
 14. Polenakovic M, Sikole A. Is erythroprotein a survival factor for red blood cells? *J. Am. Soc. Nephrol.* 1996; 7(8):1178-1182.
 15. Sanchez – Elsner T, Ramirez JR, Rodriguez–Sanz F, Varela E, Bernabew C, Botella LM. A cross talk between hypoxia and TGF-beta orchestrates erythroprotein gene regulation through SPI and smads. *J. Mol. Biol.* 2004; 36(1):9-24.
 16. Zaman KW, Zaman, Siddique H. Haematological and enzymatic results of aluminum intoxication in rats. *Compar. Biochem. Physiology J.* 1993; 105:73-76.
 17. Acknowledgement. Special thanks for Dr. Reem Hassan, Bahry University for their kind help and assistance in laboratory work, 1993.