GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

COMPARATIVE EFFECTS OF PROPOLIS AND MALIC ACID ON HEMATOLOGICAL PARAMETERS OF ALUMINUM EXPOSED MALE RATS

Majida A.J. Al-Qayim[1], Laith Sadiq Ghali[1] & Tahani S. Al-Azwai[2]
[1]Department of physiology and pharmacology /Coll.Vet. Med. Univ. Baghdad/Iraq
[2]Departmant of Physiology, Veterinary Public Health, University of Baghdad, Iraq

ABSTRACT
A study was conducted to compare the effect of propolis and malic acid on hematological parameters of Aluminum exposed adult male rates. Forty adult male rats were divided randomly into four equal groups; group (C) : control group daily orally administered with distil water; group (AL) : daily orally administered with $AlCl_3$ (50 mg /kg body weight), group (ALP) : daily orally administered with $AlCl_3$ (50 mg /kg body weight)+ propolis (50 mg /kg body weight) ;group (ALM) :daily orally administered with AlCl3 (50 mg /kg body weight) + Malic acid (45 mg /kg body weight). The experiment lasted for 60 days. Results of the present study showed that $AlCl_3$ administration for 60 days significantly decreased total RBC_S, Hb concentration, PCV% , erythrocytes indices (MCV, MCH, MCHC) and increased reticulocytes indicating a microcytic – hypochromic anemia. At the same time, propolis or malic acid maintained the normal limits of these parameters against Aluminum chloride effects. On the other hand there were no differences between propolis and malic acid in brought back the decrease in RBCs osmotic fragility, serum catalase activity, SI, TIBc, serum Ferritin, and total bilirubin where they were decreased by $AlCl_3$, reflecting their ameliorative effects.

KEY WORDS: Aluminum chloride. propolis, malic acid, hematologic, osmotic fragility, catalase

INTRODUCTION

Aluminum (Al) is a widely distributed metal in the environment and is extensively used in modern daily life. Al enters into the body from the environment and from diet. It presents in many manufactured foods and medicines and is also added to drinking water for purification purposes[1]. Al is widely used in antacid drugs, as well as in food additives and tooth paste[2]. Environmental pollution with different Aluminum containing compounds, especially those in industrial waste expose people to higher than normal levels of Al[3]. However, there is no known physiological role for aluminum within the body and hence this metal may produce adverse physiological effects[4]. Aluminum (Al) is a ubiquitous element and has been proposed as an environmental factor that may contribute to some neurodegenerative diseases and affects several enzymes and other biomolecules relevant to Alzheimer's disease [5]. It is present in many manufactured foods, medicines, cheese, tea, cosmetics and is also added to drinking water during purification purposes [6,1]. Different forms of Al are environmental xenobiotics that induce free radical mediated cytotoxicity and neurotoxicity[7]. In recent researches, aluminum have been reported to accelerate oxidative damage to biomolecules like lipid, protein and nucleic acids[8], Ingestion of aluminum chloride in humans & animals causes an anemia that is usually microcytic – hypochromic anemia and there is a mild rise in reticulocytes, but Jaundice is rare[9] and there is reduction in the MCV, MCH and MCHC[10]. The cause of the anemia appears to be multifactor: hemolysis, damage to mitochondria with defective hemoglobin production due to inhibition of the enzymes of haem synthesis and Red cell lifespan is shortened, are the major factors [11]. Red blood cell destruction (hemolysis) and loss are causes of regenerative anemia, microcytic anemia's are associated with inadequate iron use [12]. Aluminum seems to influence the metabolism of iron-related proteins (Han *et al*., 1997, 2000; Mahieu *et al*., 2000; Gonzalez-Revalderia *et al*., 2000). Ferritin is the major cellular iron storage protein and, by sequestering iron, protects cells from iron-induced oxidative damage. Of particular importance for aluminum toxicity, liver, kidney and intestinal ferritin levels are reduced by aluminum intake in chicks (Han *et al*., 1997, 2000). Transferrin is known primarily for its role in the transport and cellular uptake of iron but is also the major serum binding protein for Al (Trapp, 1983; Golub *et al*., 1999). The aluminum– transferrin complex binds to the transferring receptor, and receptor-mediated cellular uptake appears to be an important factor in the uptake of aluminum by the tissues (Harris, 1996). Studies have showed that chronic aluminum exposure diminishes the percentage of transferrin saturation (Mahieu et al., 2000; Gonzalez-Revalderia *et al*., 2000) and that iron–transferrin saturation influences the transferrin binding of aluminum not only by occupying binding sites otherwise available for aluminum, but also by lowering the affinity of transferrin for aluminum (VanLandeghem *et al*., 1997). In contrast, Vittori *et al*. (1999) did not observe alterations in plasma iron concentrations, total iron binding capacity (TIBC) or transferrin saturation in rats chronically exposed to aluminum, although anemia signs were observedin these animals. Several studies have shown that bee propolis (a flavonoid-rich, resinous substance that bees collect from tree buds) can counteract the damaging effects of aluminum, rats given aluminum plus propolis, or propolis alone, demonstrated an elevation in antioxidant enzymes and a return to normal blood lipid profiles

processes [13,14,15]. Malic Acid is one of the most potent chelators of aluminum and was the most effective of several chelators tested at reducing aluminum levels in the brain[16]. Treatment with Malic Acid has been shown to greatly increase the fecal and urinary excretion of aluminum and reduce the concentration of aluminum found in various organs and tissues [17, 18]. Malic acid provedto be one of the most effective chelating agent for reducing aluminum effects [19].

MATERIAL AND METHODS

Experimental Animals

A total of forty Male Albino Wister rats were at age 8 – 9 weeks and their body weight ranged between 100 – 120 grams, were kept in the same suitable environmental conditions of 22 – 27 °C, and photoperiod of 12 hours daily. After two weeks of adaptation, rats were divided randomly into four groups and each group contained ten animals; Group (C): Control group orally administered distil water, Group (AL): Aluminum group orally administered $AlCl_3$ (50 mg /kg body weight), dissolved in distil water daily Group (ALP): Aluminum + Propolis group orally administered $AlCl_3$ (50 mg /kg body weight) dissolved in distil water and propolis (50 mg /kg body weight), daily. Group (ALM): Aluminum + Malic group acid orally administered $AlCl_3$ (50 mg /kg body weight) dissolved in distil water and Malic acid (45 mg /kg body weight) dissolved in distil water. All applications were administered daily for 60 consecutive days. At The end of the experiment blood sample (4-5 ml) was collected from the rat obtained via cardiac puncture technique from each anesthetized animal using disposable syringe (5 ml) and blood was withdrawn into EDTA tube, for immediate hematological measurements; total RBCs count, PCV, Hb, osmotic fragility test and reticulocytes count, and plastic test tubes (gel tube) for serum isolation for other tests.

Hematological and biochemical changes

Total RBCs count, Hb concentration, PCV %, MCV, MCH, MCHC, by using heamatological analyzer (Hycel Hematology analyzer, version B, ver2.5x. Reticulocytes were counted in 1000 cells of the total RBCS and expressed as a percentage Serum iron concentration was enzymatically measured using enzymatic assay kit (Biolabo SA, Maizy-France)

Serum Ferritin was measured by Ferritin enzyme immunoassay test Kit (Linear chemicals, Barcelona-Spain)using DANA 3200 ELISA Reader.

Serum Total Iron Binding Capacity TIBC (µg/dl) measured by colorimetric method at 600 nm using a commercial kit

Serum Total Bilirubin Concentration (mg/dl) was enzymatically measured using standard assay (SB-Kit)

Catalase activity was determined by spectrophotometric method depending on measuring the concentration of stable yellow complex produce from the reaction of substrate for catalase, hydrogen peroxide and ammonium molybdate at 405 nm (Goth, 1991).

Statistical analysis

Data are shown as the Mean± SE (stander error) when a significant interaction between major factors was identified by ANOVA SPSS version 11.5. Duncan's new multiple range test was used post-ANOVA to identify significant differences between mean values at probability level of (p<0.05) was taken as significant

RESULTS

Results represented in table (1) showed the changes in hematological parameters related to erythrocytes. The present results reveled that AlCl3 administration for 60 days significantly($P< 0.05$) decreased total red blood cell, PCV %, Hb gm/dl (6.318 ± 0.1 , 10.83 ± MCV (FL), MCH (Pg), MCHC (g/dl), 0.13, 34.22 ± 0.4 , 55.02 ± 0.2 , 17.54 ± 0.2 31.81 ± 0.2 ,) in comparism with control (6.983 ± 0.02, 12.70 ± 0.28, 39.21 ± 0.8, 56.11 ± 0.32,18.04 ± 0.05 , 32.09 ± 0.13), respectively.

TABLE 1: Comparative effects of Propolis and Malic acid on RBCS (X106 / ml), (Hb g/dl) , PCV (%), MCV (FL), (MCH / Pg), MCHC (g/dl), Reticulocyte number (%) of Aluminium exposed male Rats. N= 6 for 60 days.

Experimental groups	*RBCS (X106 /* ml	(Hb g/dl)	PCV (%)	MCV (FL	), (MCH / Pg	MCHC (g/dl	Reticulocyte number (%)
Control group (C)* (Distil Water)	6.983 ± 0.02 [a]	12.70 ± 0.28 [a]	39.21 ± 0.8 [a]	56.11 ± 0.32 [a]	18.04 ± 0.05 [a]	32.09 ± 0.13 [a]	2.88 ± 0.1 [b]
Aluminum chloride (AL)* (50 mg /kg B.W.)	6.318 ± 0.1 [b]	10.83 ± 0.13 [b]	34.22 ± 0.4 [b]	55.02 ± 0.2 [b]	17.54 ± 0.2 [b]	31.81 ± 0.2 [b]	5.40 ± 0.3 [a]
Aluminum chloride* & propolis (AP) (50 mg/kg B.W.)	7.126 ± 0.03 [a]	13.10 ± 0.2 [a]	40.16 ± 0.7 [a]	56.55 ± 0.2 [a]	18.38 ± 0.1 [a]	32.62 ± 0.02 [a]	2.50 ± 0.1 [b]
Aluminum chloride* & Malic acid (AM) (45 mg/kg B.W.)	7.077 ± 0.1 [a]	12.73 ± 0.2 [a]	39.36 ± 0.8 [a]	56.24 ± 0.2 [a]	18.13 ± 0.1 [a]	32.36 ± 0.2 [a]	2.68 ± 0.1 [b]

- Small superscript denote significant ($p<0.05$) difference between groups (Column).

⋆ Orally administration

Reticulocytes were elevated significantly in AlCl adiministered group (5.40 ± 0.3) when compare with control, AlCl+P, and AlCl+M groups (2.88 ± 0.1, 2.50 ± 0.1, 2.68 ± 0.1). RBCs osmotic fragility test (fig-1) reveled that $AlCl_3$ caused increased of NaCl conc. that cause starting and complete erythrocytes hemolysis (0.65±0.02,

0.44±0.01) indicating an increased osmotic fragility of RBCs. Propolis and malic acid administration succeeded to decrease the osmotic fragility either in starting hemolysis (0.56±0.01, 0.59±0.02) or complete hemolysis (0.34±0.01, 0.38±0.02) in compare with control (0.59±0.01, 0.39±0.01), respectively. Fig-2 represents the catalase activity (ku/I) of different experimental groups. Catalase were decreased significantly in Al group(95.36 ± 87), mean while both propolis and malic acid succeeded in correcting catalase activity to semi normal values (126.04 ± 2, 120.43 ± 3.2) in compare with control(122.53 ± 2.6)

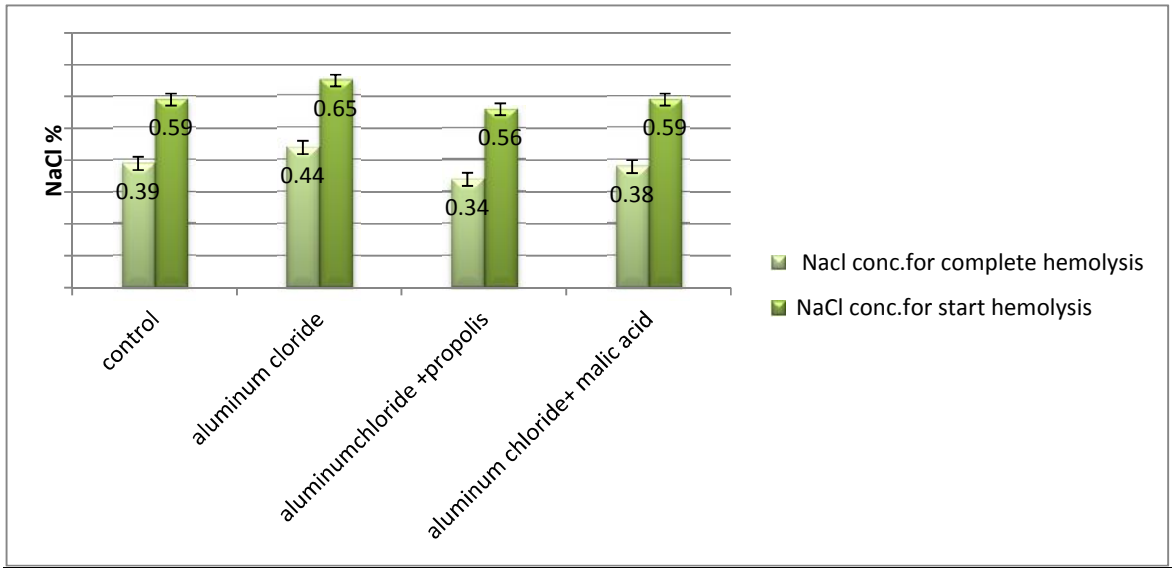

FIGURE 1: Comparative effect of Propolis and Malic acid on Osmotic Fragility of erythrocyte of Aluminum exposed male Rats.

TABLE 2: Comparative effect of Propolis and Malic acid on Serum Iron concentration SI (µg/dl) Total Iron Binding Capacity TIBC (µg/dl), Ferritin Concentration (ng/ml) Bilirubin Concentration (mg/dl), of Aluminum exposed male Rats

Groups	Iron (µg/dl)	TIBC(µg/dl),	Ferritin (ng/ml)	Bilirubi(mg/dl)
Control group (C)* (Distil Water)	206.36 ± 4.9 ^{B}	534.76 ± 11.2 ^{B}	3.153 ± 0.16 ^{B}	0.29 ± 0.01 ^{a}
Aluminum chloride (AL)* (50 mg /kg B.W.)	227.16 ± 6 ^{a}	596.74 ± 53 ^{a}	4.271 ± 0.1 ^{a}	0.38 ± 0.01 ^{a}
Aluminum chloride* & propolis (AP) (50 mg/kg B.W.)	201.09 ± 5.3 ^{a}	535.55 ± 24 ^{a}	2.850 ± 0.2 ^{a}	0.23 ± 0.01 ^{a}
Aluminum chloride* & Malic acid (AM) (45 mg/kg B.W.)	210.09 ± 4.7 $^{B\,a}$	552.23 ± 14 ^{a}	3.062 ± 0.1 ^{a}	0.30 ± 0.01 ^{a}

- Small superscript denote significant ($p<0.05$) difference between groups (Column).

⋆ Orally administration

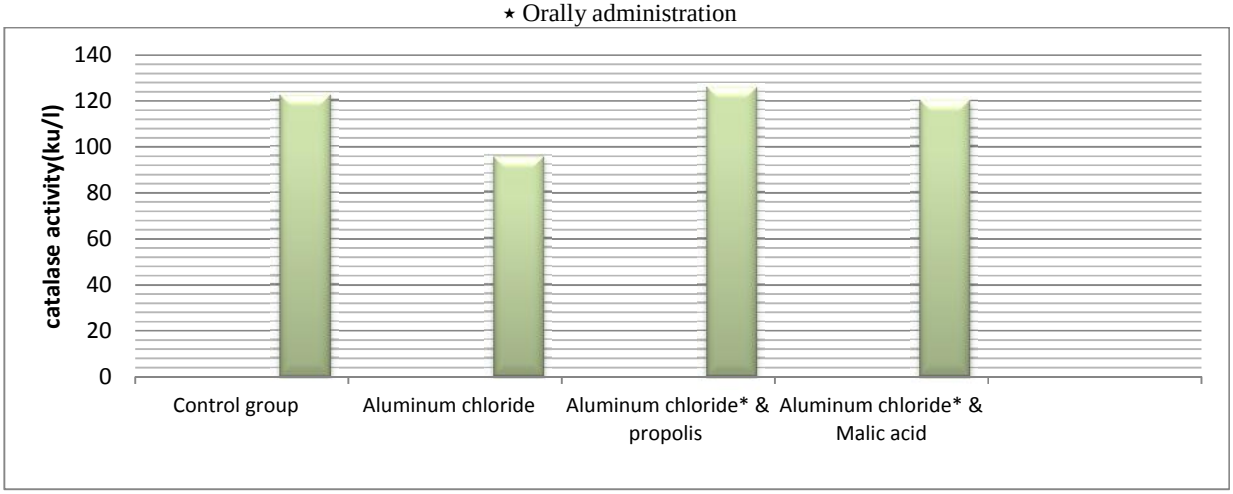

FIGURE 2: Comparative effect of Propolis and Malic acid on Serum Catalase activity (ku/I) of Aluminum exposed male Rats.

DISCUSSION

The results obtained from the present study (table-1), referred to the type of anemia caused by $AlCl_3$ 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[20,1,17]. 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 $AlCl_3$ can be associated with RBCs hemolysis which confirmed by reduced RBCs count in the present study, or disturbances in heme biosynthesis. Al interferes with several enzymes taking part in heme biosynthesis pathway including aminolevulinic acid (ALAs) synthetase, δ-aminolevulinic dehydratase (ALA-D), ferrochelatas, as a result of inhibit linking of iron with heme and drop in activity of these enzymes[19]. The competition of aluminum with iron, since aluminum is similar to some other ions with two positive charge, aluminum also can take the place of iron, but aluminum cannot perform the functions that iron can perform for instance resulting in impaired biosynthesis of hem in bone marrow, Lead and mercury also inhibited ALAD and ALAS[21,22].

Erythrocytes indices; MCV, MCH and MCHC, which are important tools for description of RBCS changes, differentiates the erythropoietic disorders, differentiation and detection of anemia[23]. In the present study the decrease MCV, MCH and MCHC, in rats administered $AlCl_3$ alone refer to the type of anemia (microcytic–hypochromic anemia). The significant reticulocytosis documented in the present study in rats administered $AlCl_3$ reflecting the bone marrow hyperplasia as response to the anemia that induce hypoxia at the level of the kidney to stimulate erythropoietin release. Increased reticulocytes may indicate that bone marrow could overcome the subchronic exposure to $AlCl_3$, that there has been time for erythroid hyperplasia in the marrow indicated a regenerative anemia. If the reticulocyte count is not raised in an anemic patient this suggests impaired marrow function or lack of erythropoietin stimulus [24]. As in the present study increased reticulocyte count is reported by some other researchers like[24] who used rats in there experiment, treatment with aluminum sulphat $Al_2(SO_4)$ at dose 67.5 mg/kg b.w., I.M. for about 7 days, they recorded that increased reticulocytes in absolute & relative counts. Elevated iron levels may temporarily occur in healthy subjects, but the rise is installed in this study may be the result of either increased RBCs hemolysis caused by the harmful oxidative stress induced by $AlCl_3$ on cell membrane, or defect in the mechanism of iron transport across the cell membrane. The present study in agreement with Cannata JB. *et al.* (1996) in that the serum iron concentration & total iron binding capacity TIBC was increased in case of $AlCl_3$ poisoning. Chmielnicka *et al.* (1996) (TIBC) is the amount of iron that serum transferrin can bind when all iron - binding sites are saturated in other words it is the transferrin saturation reflect a stimulated state of low iron within cells because of disruption of transferrin phagocytosis process caused by the aluminum chloride, For the purpose of comparing the preventive effects of both propolis and malic acid against aluminum chloride effects, the present results indicate convergence of the effects of each of the propolis and malic acid. Administration of propolis and malic returned the values of the RBCs indices near or above normal values, after 60 days of the experiment, which confirm their protective role in reducing the deleterious effect of aluminum chloride. The significant decrease in reticulocytes count found in rats administered the Propolis or Malic acid against aluminum compared with rats administered $AlCl_3$, reflecting the protective role of them in reducing severity of anemia produce by $AlCl_3$ through modulating of abnormal erythrocytes production & release from bone marrow to the peripheral blood. The protective mechanism of propolis against side effect of AlCl3 on hematological parameters could be attributed to many properties.The antioxidants effects of propolis have been instrumental in avoiding oxidative effects of $AlCl_3$ on RBCs[26] membrane and thus maintain the other hematological parameters within normal limits. The present results was agree with results of[7] who reported that propolis important antioxidant & propolis has beneficial influence and could be able to antagonize the side effect of AlCl3.Propolis is heterogeneous substance with a large number of elements and many vitamins includes; B1 , B2 , B6 , E and vitamins C and several amino acid[27]. Several recent studies have pointed to the protective role of each of vitamins E&C in reducing the effects of AL on blood [28].

The results of the present study, clearly indicate that providing the rats with propolis found to be effective as improving the antioxidant level and decreasing the oxidative stress, in comparison with group that was administration $AlCl_3$ alone (AL group), increased catalase activity and erythrocytes osmotic fragility observed with oral administration of propolis with $AlCl_3$ ALP group, suggested that propolis consumption may be reducing or suppressing the release of free radicals. Alterations in the oxidant–antioxidant status during the administration of propolis have been reported by different studies [29] showed that propolis increases CAT activity, [30]who determined effects of propolis on the resistance of the erythrocyte to the osmotic effect of hypotonic solution, reported decreased the osmotic fragility of erythrocyte in vitro through added the same volume of propolis to RBC_S samples.

Malic acid is one of the most potent chelators of aluminum and was the most effective of several chelators tested at reducing aluminum levels in the various organs specially brain & tissue. Treatment with malic acid has been shown to greatly increase the fecal & urinary excretion of aluminum and reduce the concentration of aluminum in tissue and organs[16]. Malic acid can act as chelating agent, the mechanism responsible for the observed malic acid as chelating agent by reduce the concentration of $AlCl_3$ levels in the brain, various organs and tissues during the increase fecal & urinary excretion of aluminum[16,17]. And in addition to chelating properties, malic acid act as powerful

antioxidant[19], by decreasing the oxidative stress in comparison with group that was received $AlCl_3$ alone.

In conclusion, treatment with propolis or malic acid could improve cellular membrane & organ functioning more profoundly and brought all these variables in catalase activity and osmotic fragility to ward normal values, and thus maintain all hematological parameters in its normal values and limiting the impact bad effects of aluminum.

REFERENCES

[1]. Newairy, A. A., Salama, A.F., Hussien, H.M. and Yousef, M.I. (2009) propolis alleviates aluminum induce lipid peroxidation and biochemical parameters in male rats. Food. Chem. Toxicol., 47: 1093-1098. PMID: 19425229.

[2]. Abbasali Karimpour Malekshah, Zhila Torabizadeh and Farshad Naghshwar (2005) Development toxicity of Aluminum from High Doses of AlCl3 in Mice. Medical Sciences, Sari, Iran .Vol.5, No.4, 2005.

[3]. Beceria, A., Campbell, A. and Bondy, S.C. (2002) Aluminum as a toxicant. Toxicol. Ind. Health, 18: 309-320.

[4]. Turgut, S., Bor-Kucukatay, M., Emmungil, G., Atsak, P. ve Turgut, G. (2007) The Effects of Low Dose Aluminum on Hemorheological and Hematological Parameters in Rats, Arch. Toxicol., 81, 11-17.

[5]. Frisardi, V., Solfrizzi, V., Capurso, C., Kehoe, P.G., Imbimbo, B.P., Santamato, A., Dellegrazie, F., Seripa, D., Pilotto, A., Capurso, A. ve Panza, F. (2010) Aluminum in the Diet and Alzheimer's Disease: From Current Epidemiology to Possible Disease- Modifying Treatment, J Alzheimers Dis., in press.

[6]. Al-Hashem, F.H. (2009a) Camel's milk protects against aluminum chloride-induced toxicity in the liver and kidney of white albino rats. Am. J. Biochem. Biotechnol., 5: 98-108. DOI: 10.3844/ajbbsp.2009.98.108

[7]. Yousef, M. I. and Salama, A. F. (2009) Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxicol., 47: 1168-1175. PMID: 1942523.

[8]. Azadeh Mohammadirad and Mohammad Abdollahi (2011) A systemic Review on Oxidant/ Antioxidant Imbalance in Aluminum Toxicity. International Journal of pharmacology 7(1): 12 – 12.

[9]. Karmakar, R., Bhattacharya, R. & Chatterjee, M. (2000) Biochemical, haematological and histopathological study in relation to time-related cadmium-induced hepatotoxicity in mice. Biometals 13(3): 231-239.

[10]. Chmielnicka, J., Nasiadek, M., Pinkowski, R., and Paradowski, M. (1994) Disturbances of morphological parameters in blood of rats orally exposed to aluminum chloride. Biol. Trace Elem. Res. 42:191-199.

[11]. Hoffbrand, A. V.;, Catovesky, D., and Tuddenham, E.G.D. (2005) Postgraduate Haematology. Fifth edition, Blackwell Publishing Ltd. Massachusetts. Pp 27, 34-35, 40.

[12]. Barger, A. M. (2003) The complete blood cell count: a powerful diagnostic tool .*Vet. Clin. Small Anim.* 33: 1207–1222.

[13]. Cole, N., Sou, P.W., Ngo, A., Tsang, K.H., Severino, J.A., Arun, S.J., Duke, C.C. ve Reeve, V.E. (2010) Topical 'Sydney' Propolis Protects against UV-Radiation-Induced Inflammation, Lipid Peroxidation and Immune Suppression in Mouse Skin, Int. Arch. Allergy. Immunol., 152, 87-97.

[14]. Tatli Seven, P., Yilmaz, S., Seven, I., Cerci, I. H., Azman, M. A. and Yilmaz, M. (2009) The effect of propolis on selected blood indicators and antioxidant enzyme activities in broilers under heat stress. Acta Vet. Brno 78:75-83.

[15]. Volpi, N., Bergonzini, G. (2006) Analysis of flavonoids from propolis by on-line HPLC–electrospray mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 42:354–361.

[16]. Domingo, J. L., Gomez, M., Liobet, J. M., Corbella J. (2011) Comparative effects of several chelating agents on the toxicity, distribution, & excretion of aluminum. Am. J. Bioch. Biotech. 7(2): 74-83.

[17]. Rim Kabouj (2007) aluminum leaching using chelating agent as a composition food. Food and Chemical toxicology. Vol. 45, Issue 9, pages 1688-1693.

[18]. Crisponi, G., Nurchi, V. M., Bertolasi V., Remelli M., faa G. (2012) chelating agents for human diseases related to aluminium overload. Coordination chemistry Reviews 256, 89-104

[19]. Sadhana Shrivastava (2011) S-Ally-Cysteines Reduce Amelioration of Aluminum Induced Toxicity in Rats. Toxicol. Lab. 7 (2): 74-83, 2011.

[20]. Al-Hashem, F.H. (2009b) Camel's milk alleviates oxidative stress and lipid peroxidation induced by chronic aluminum chloride exposure in rat's testes. Am. J. Applied Sci., 6: 1868-1875. DOI: 10.3844 /ajassp. 2009. 1868.1875.

[21]. Blake, S. (2008) Vitamins and Minerals Demystified. The McGraw-Hill Companies, Inc. New York. pp 111-121, 275.

[22]. Rose, S., Melnyk, S., Savenka, A., Hubanks, A. and Jernigan, S. (2008) The frequency of polymorphisms affecting lead and mercury toxicity among children with autism. Am. J. Biochem. Biotechnol., 4: 85-94. DOI: 10.3844/ ajbbsp. 2008. 85.94

[23]. Simmons, A. and Statland, B. E. (1997)Hematology a combined theoretical and technical approach. Second edition. Batterworth- Heinemann, Johannesburg. pp 1-45, 273.

[24]. Hoffbrand, A. V., Moss, P. A. H. and Pettit, J. E. (2006) Essential hematology .fifth edition. Blackwell publishing, Oxford. pp 23-24 .265-276.

[25]. Ganchev, T., Dyankov, E., Zacharieva, R., Pachalieva, I., Velikova, M., Kavaldjieva, B. (1998) Influence of aluminium on erythropoiesis, iron metabolism and some functional characteristics of erythrocytes in rats._Medical Univesity of Varna, Department of Physiology, Bulgaria. Acta Physiol Pharmacol Bulg, 23(1):27-31

[26]. Farooqui, T., Farooqui, A. (2010) Molecular Mechanism Underlying the Therapeutic Activities of Propolis: A Critical Review. Curr Nutr Food Sci 6: 188-199.

[27]. Taylor, J.S. (2002) A look at the energizing properties of the royal jelly, bee pollen, propolis and honey. Health Media publishing. Landon.

[28]. Ismael, I.S., Abdel Aziz1 & Baker M Zabut (2011) Determination of blood indices of albino rats treated with aluminum chloride and investigation of antioxidant effects of vitamin E and C. Egyptian Journal of Biology, 2011, Vol. 13, pp 1-7.

[29]. Sobocanec, V. Sverko, T. Balog, A. Saric, G. Rusak, S. Likic (2006). Oxidant/antioxidant properties of Croatian native propolis. J Agric Food Chem, 54, pp. 8018–8026

[30]. Leandro, L. Moreira Teresa Dias, Luís G. Dias, Mónica Rogao, Jose P. Da Silva, Letícia M. Estevinho[a]. Propolis influence on erythrocyte membrane disorder (hereditary spherocytosis): A first approach. Food and Chemical Toxicology. Volume 49, Issue 2, February 2011, Pages 520–526.