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Research Article

Effect of Aluminium Chloride and Protective Effect of Ginger Extract on Hematological Profiles in Male Wistar Rats

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Abstract

The present study was planned to investigate the effect of aluminium chloride and the protective effect of ginger extract on hematological profiles in male rats. One group of rats was given aluminium chloride orally (100mg/kg bw) for 60 days, another group with aluminium chloride and ginger extract (50mg/kg bw) simultaneously and the third group with ginger extract alone. All the three groups were compared with one control group. The hematological parameters were: total erythrocytes count (RBC), White blood cells (WBC), hemoglobin(HB), hematocrit (HCT), and red blood indices: mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHV) was investigated. The obtained results showed significant increase in WBC count and decrease in HCT, HB and RBC. No change was noted in (MCV) and there was reduction in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHV) in $AlCl_3$ administered group (E1). However, no appreciable changes were noted in $AlCl_3$ and ginger extract treated (E2) and Ginger alone treated group (E3). The changes in the values of blood indices may be a defensive mechanism against aluminum toxicity through stimulation of erythropoisis. It may be concluded that Ginger rich in antioxidant and also spicy content which is safety food for human consumption. This study showed that hematologic effects of $AlCl_3$ are reversible by aqueous ginger extract.

1. INTRODUCTION

The increase in pollution is a major global problem and this is due to the use of toxic chemicals or xenobiotic substances or by certain synthetic compounds such as heavy metallic compounds[1-2]. Aluminum (Al) is the third most prevalent and the most abundant metal in the earth's crust, representing approximately 8% of total mineral components[3]. Al components are primarily found in corn, yellow cheese, salt, herbs, spices, tea. It is widely used in cosmetics, cookware utensils and containers, food additives and toothpaste[4] and used in medicines, e.g., antacids, phosphate binders, buffered aspirins, vaccines and injectable allergens[5-7] and also added to drinking water for purification purposes[8]. Recently, increased attention is being paid to aluminum due to its serious effects on the central nervous system, energy metabolism and hematology. The different forms of Al have been shown to be systemic toxicants[9] and there is now considerable evidence demonstrating that the accumulation of Al in body tissues is associated with damage to the target organs[10].

Blood, which rapidly and constantly flows through the brain and other body organs and play an important role in the transportation of nutrients, antioxidants, hormones and some other chemicals. Once in the blood circulation, Al is mainly transported by plasma transferrin in its sites left vacant by iron, and to a much lesser extent by albumin[11]. Hematological and biochemical profiles of blood can provide important information about the internal environment of the organism[12]. Hematological parameters as red blood cells counting (RBC), hemoglobin concentration (HB), hematocrit(HCT), leukocytes, neutrophils and mononuclear cells counting and Mean Corpuscular Volume (MCV) are largely applied to diseases diagnostics, including effects due to exposure to toxic substances.

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Now a day's lot of research has been conducted on the use of herbal products as natural antioxidants because of their fewer side effects, easy and cheap availability. Medicinal plants have been used in various traditional systems, as they have immune potential against numerous diseases[13]. Ginger (*Zingiber officinale*) is commonly used as food spice since in ancient times[14]. It has long been used as a remedy for common ailments like digestive problems, cold, fever, morning sickness. All major active ingredients of ginger such as zingerone, gingerdiol, zingibrene, gingerols and shogaols have antioxidant activity[15]. Studies have revealed a wide range of pharmacological effects demonstrated are anti-platelet, antioxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxicity and anti-arthritic effect[16-20].

Ginger can be successfully used as a component of curry powder, sauces, ginger bread and ginger flavoured carbonated drinks and in preparation of dietaries for its aroma and flavor. With regard to antioxidant properties of ginger, it can be selected for this study to evaluate the ameliorative effects. Therefore, the present study was carried out to investigate the effect of aluminium chloride in blood hematological profile and the possible protective effect of ginger extracts on it.

2. MATERIALS AND METHODS

2.1 Animals

Adult albino rats (Wistar strain) weighing 250-260g were used in this study. Animals were maintained as per the guidelines of the Institutional Animal Ethical Committee (IAEC). All the animals were housed in polypropylene cages at standard husbandry conditions (Temperature: 23 ± 2ºC, Relative humidity: 60-70%, 12h: 12h light /dark cycle) and were provided with standard pellets and water ad libitum. They were initially acclimatized for the study. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) Approval No 140/PHARMA/SCRI, 2013. After 15 days adaptation period the rats were divided into four groups of 6 animals each.

2.1 Experimental groups

Group I – Control group

Rats given water as vehicle orally, daily for 60 days.

Group II – Experimental group I (E1)

Rats given aluminium chloride (100 mg/kg body weight) orally, daily for 60 days.

Group III – Experimental group II (E2)

Rats given aluminium chloride 100 mg/kg body weight simultaneously with ginger extract (50mg/kg body weight) orally, daily for 60 days.

Group IV – Experimental group III (E3)

Rats given aqueous ginger extract (50mg/kg body weight) orally, daily for 60 days.

During the treatment period, the feed consumed every day was recorded and the body weight was taken on every day and the percent changes were calculated. The animals were treated in the morning. 24 hours after the last treatment, blood samples were collected from the retro-orbital sinus of all animals at the end of the experimental period and EDTA was used as an anti-coagulant. Noncoagulated blood was tested, shortly after collection, for hemoglobin (HB), White blood cells (WBC), Red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHV) using Hematoanalyzer (Siemens).

3. RESULTS

Table 1 shows the results of total WBC, RBC, hemoglobin (HB), hematocrit (HCT), and red blood cell indices (MCV, MCH and MCHC) in all groups of rat. The present study reveals that, aluminium chloride administration showed significant elevation in WBC ($p<0.01$) and decline in RBC ($p<0.01$), HB ($p<0.01$), HCT ($p<0.05$) (Fig- 1 and 2). No change was observed in MCV and non significant decrease was noted in MHC and MCHC (Fig-3). On the other hand, administration of ginger extract simultaneously with Al treated animals showed the normal levels similar to control animals. Furthermore, the animal treated with aqueous ginger extract alone does not produce any alteration in all these parameter when compared to control animal.

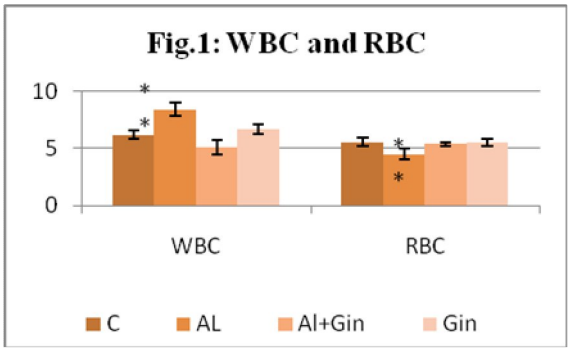
Table 1: Changes in Hematology and RBC'S indices (MCV, MCH, and MCHC) of the aluminium and ginger treated groups compared to control group.

Parameter	Control	$AlCl_3$	$AlCl_3$ + Ginger	Ginger alone
WBC ($\times10^9$ /L)	6.2 ± 0.326	8.4 ± 0.559 **	5.1 ± 0.664	6.7 ± 0.393
RBC ($\times10^{12}$/L)	5.57 ± 0.383	4.49 ± 0.483 **	5.39 ± 0.178	5.5 ± 0.327
HB (g/dL)	9.08 ± 0.660	7.69 ± 0.753**	8.86 ± 0.308	9.2 ± 0.632
HCT (%)	27.0 ± 1.796	23.23 ± 1.334*	25.84 ± 0.861	27.4 ± 1.704
MCV (fL)	48.6 ± 1.684	46.21 ± 0.736	47.23 ± 0.547	49.0 ± 0.280
MCH (pg)	16.2 ± 0.333	15.6 ± 0.736	16.6 ± 0.231	16.4 ± 0.225
MCHC (g/dL)	33.4 ± 0.254	32.7 ± 0.266	34.2 ± 0.330	33.5 ± 0.369

The results are expressed as Mean ± SEM (n = 6) per treatment and respective control groups.

*Levels of significance values are *p<0.05, **p<0.01, ***p<0.001 compared with control group.*

P <0.05 considered to be statistically significant.



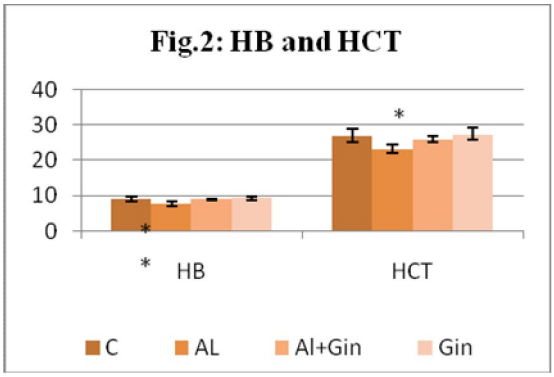


Fig.1 and 2 : demonstrates the significant changes in the Hematology parameter (WBC, RBC, HB and HCT) of Aluminium administered and ginger treated group compared to control animals.

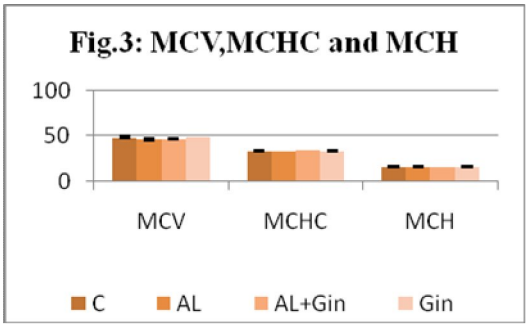


Fig. 3 : Illustrates the significant change in the RBCs indices (MCV, MHCH and MCH) of Aluminium administered and ginger treated group compared to control animals.

4. DISCUSSION

Among toxic environmental metals, Al has a remarkable toxic potential for humans. After long term exposure of Al may cause deleterious effect on the morphology and physiology of blood cells. Hematological parameter namely PCV, HB, RBC, WBC and differentials were monitored during the chronic toxicity study in rats because of their role in providing reliable information concerning hematological changes toxicants could cause. The evaluation of hematology and biochemical characteristics has become an important health indicators and means of understanding normal and pathological processes and toxicological impacts[21].

Our study results indicated that Al administration showed significant decline in the concentration of HB, RBC, HCT, etc. It is suggestive that altered peripheral blood composition is a reflection of disrupted hematopoietic process and interfering with different stages of red-cell synthesis and mature red blood cells. However, Al-induced hematological alterations leading to microcytic hypochromic anemia in albino rats on the same blood parameters in rats[22-24]. There was a significant reduction was observed in MCV and RBCs

deformability and significant increments in whole blood viscosity where the animals were treated with 5mg $Al_2(SO4)_3$/kg b.w. for 2 weeks[25].

The white blood cells are the regulators of the immune system and the increase in WBCs count may be due to generalized immune responses and a protective response to metals stress[26]. In general, Stimulation of lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissue under toxic stress may lead to an increase in WBCs number[27]. The increased level of WBC in the blood of *C. gariepinus* collected from El-Rahawy drain and this attributed to expose to chronic sewage[28], and increased WBCs count in fish exposed to copper indicates leucocytosis[29].

The primary function of erythrocytes is to carry oxygen bound to HB from the lungs to tissues. Furthermore, HB in erythrocytes is an excellent acid-base buffer (for the majority of proteins), thus erythrocytes are mostly responsible for the buffer capacity of whole blood[30]. Significantly decreased HB levels were seen in the Swiss Albino mice after long-term groundwater exposure (180 days) due to heavy metal toxicity [31]. Decreased HB% as well as PCV was reported in wild Libyan Jird, *Merioneslibycus* treated with mixture of lead, copper, mercury via drinking water in Riyadh[32].

Hematocrit (HCT) is used to measure the blood carrying capacity and it is directly associated with the HB% and RBC ability to conduct tissue oxygenation activity. Therefore, HCT shares the reasons for cause of fall in its level with HB% declination in this study. Decrease in HCT may be due to suppresses of bone marrow hematopoietic system, Causing iron deficiency in synthesis of Haem protein of Hemoglobin[33-34]. The low HCT would indicate anaemia or oligohaemia[35]. The changes in the blood HCT value has often been shown to be a good indicator of aluminum toxicity.

Short term exposures to low concentrations of heavy metals mostly induce an increase in the hematological indices[36]. Oliveira Ribeiro *et al*., observed increase in red blood cells counts in *H. malabaricus* exposed to Methylmercury (MeHg) and attributed this to an increase in the blood oxygen carrying capacity[37]. The RBCs may also be affected in *O. mykiss* after an acute exposure to Al[38]. Further, significant decrease in RBC count and HB were seen in rats and human with high blood lead levels[39-42].

Significantly decreased RBC, HB levels and HCT values were observed in rats exposed to lead[43-44] and in *Salmo gairdneri*[45]. It was also reported that the decrease in the HCT value of *Salmo gairdneri* blood exposed to cadmium [46]. Moreover, exposure of Zinc to *Oncorhyn chuskisutsh* caused decrease in HB and HCT values[47].

The reduction in RBCs, HB and HCT might be due to an inhibition of erythropoiesis and hemoglobin synthesis and to an increase in the rate of erythrocytes destruction[48]. Several mechanisms have been proposed for the Al-induced anemia, and the proposed mechanisms appear to involve inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization [49-51].

Jadhav *et al*.(2007), have reported decreased RBC count after exposing a mixture of heavy metals including (Pb, Cd, Fe, Zn, Cu) in wistar rats[52]. Heavy metal toxicity suppresses the hematopoietic system. Another reason may be decline in erythropoietin synthesis and secretion. In the absence of this hormone, RBC productions were decline considerably[53-54].

The MCV gives an indication of the status or size of the red blood cells and reflects an abnormal or normal cell division during erythropoiesis. In spite of the increase in the RBC count, a decrease in MCV may show the extent of the shrinking cell size due to Al intoxication[55]. But it was interesting fact in our results that no appreciable changes were seen in MCV in all the experimental groups even though decrease was observed in RBC. The decrease in MCV may indicates that RBC have shrunk , therefore , it is suggestive that the increase in RBC value but the reduced erythrocyte size indicate a high percentage of immature red blood cells in circulation after long term exposure to Al. These can be a marker of anemia with subsequent result of inhibition of erythropoesis in the hemopoietic system[56].

It is quite alarming that the use of some medicinal plants seems to pose some health hazards despite their overwhelming health benefits either due to their inherent toxic effect or abuse resulting from over-dosage or prolong usage. Concerns over their safety were raised after incidents of toxicity were reported following consumption of some herbal preparations but the underlying pathogenesis remained cryptic[57-58].

The rhizome of ginger (*Zingiber officinale*) has been reported to possess a broad-spectrum of prophylactic and therapeutic activities[59]. Ginger is effective in the control of a range of bacterial, viral, fungal and parasitic diseases[60]. It has been extensively studied for the ginger extracts which have a broad range of biological activities; especially antioxidant activities found that ginger significantly lowered lipid peroxidation [61-64].

Tatfeng and Enitan, [who reported significant increases in total leucocyte count, absolute count of lymphocytes, neutrophils and summation of monocytes, eosinophils and basophils in their work on effects of onion and garlic extracts on some immunologic cells[65]. Yin *et al*., reported that oral administration of ginger (*Zingiber oficinale*) extract increases the phagocytic capability of cells in rainbow trout[66].

Thus increasing lymphocytes count in the blood following increase in white blood cells count can play an important role in stimulating the immune system of animals. The increase in white blood cells count represents stimulation of the host's immune system. In other words, anti-viral, anti-bacterial and antifungal properties of medicinal plants and symbiotics can be effective in improving immune function and may make the environment unfavorable for invading foreign agents[67]. Herbal immuno-stimulants are substances which activate white blood cells (WBC) and may render fishes more resistant to infectious diseases, by the stimulating phagocytic cells as well as complement lysozyme and antibody responses[68]. Thus, the present study has, however, demonstrated that Al induced anemia can be reversed by the supplementation of ginger extract.

5. CONCLUSION

Considering the importance of white blood cells and role of red blood cells in daily human life, the present study was carried out. It was evident in the study that the long term exposure of Al was able to induce the changes in hematological parameters. Any changes made in their structure and number can cause very large physiological changes for animals. On the other hand, supplement administration of ginger extract reversed the Al toxicity effects to the normal level similar to that of control animals. Thus, the present study explores the possibility Al induced anemia can be reversed by the supplementation of ginger extract in rats.

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