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RESEARCH ARTICLE

LEAD TOXICITY ON HEMATOLOGICAL CHANGES AND AMELIORATION WITH GINGER (*Zingiber officinale*) EXTRACT IN MALE ALBINO RATS

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Abstract

Lead toxicity is a worldwide health problem due to continuous exposure of the population to lead in the environment especially workers in industries. It affects many body organs especially the liver and kidneys. The present investigation aimed to study the protective role of *Zingiber officinlae* against lead acetate toxicity on various haematological parameters in male albino wistar rats. Adult 42 healthy rats were randomly divided into seven groups viz. normal control (group I), lower dose of ginger extract @200mg/kg b wt (group II), higher dose of ginger extract @300mg/kg b wt (group III), lead acetate control @200mg/kg b wt (group IV), lower dose of ginger + Lead acetate (group V), higher dose of ginger + Lead acetate (group VI) and lead + silymarin @100mg/kg b wt (group VII) orally for 8 weeks. The mean RBC and Hb values were reduced significantly ($P<0.05$) in lead treated rats but the mean WBC values were increased. Whereas significant improvement was noticed in ginger treated rats increase RBC and Hb values and WBC levels were recovered to normal as dose dependent manner. The alteration in haematological parameters in the present study indicates decreased lifespan and fragility of RBC and damage to liver and kidney in lead poisoned male wistar albino rats.

Introduction:

Lead is the most common heavy toxic and its toxic effect has been recognized for more than 2,000 years. Lead is a heavy metal that is present in petrol and octane to produce toxic effects on the various biological systems. Lead may be deposited in the red blood corpuscles, soft tissues of children mainly in the kidney region, but the greater concerning matter is that 70 to 90% of this lead is deposited in bones. This is the most hazardous because the half-life of lead in the bones is 28 years, whereas lead in the blood and kidneys remains only up to two to four weeks (Haque, 2005). Environmental sources of lead include inhalation of automobile exhaust from gasoline containing alkyl lead additives from ingestion of dust contaminated with lead and from drinking water that had passed through lead piping (Benowitz, 2001).

Exposure to low levels of lead has been associated with functional and structural impairments in both human and experimental animals (Reza et al., 2008). The main targets of lead are the hematopoietic, nervous and renal tissues. Moreover, it hinders the efficacy of the hepatic, reproductive and immune function (Durgut et al., 2008). Furthermore, accumulation of lead produces damaging effects in the hematopoetical, hematic, renal and gastrointestinal system (Sansar et al., 2010 and Ibrahim et al., 2012).

About 99% of the lead present in the blood is bound to erythrocytes. They have a high affinity for lead and contain the majority of the lead found in the blood stream, which makes them more vulnerable to oxidative damage than many other cells. Moreover, erythrocytes can spread lead to different organs of the body (Sivaprasad et al., 2003).

Recent studies have shown that lead toxicity facilitates conversion of Hb into met-Hb. During Hb oxidation in the presence of lead, H_2O_2 is generated, which may induce lipid peroxidation in the erythrocyte cell membrane

(Vargas et al., 2003). As a result, lead might induce generation of ROS by interacting with oxy-Hb, leading to peroxidative damage of erythrocyte membranes (Ribarov et al, 1981).

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. Ginger (*Zingiber officinale*) is commonly used as food spice in this region since ancient times (Morakinyo et al., 2008). It has long been used as a remedy for common ailments like digestive problems, cold, fever, morning sickness. Studies have revealed a wide range of biological activities like anticancer, antioxidant, anti-inflammatory and antimicrobial (Chrubasik et al, 2005). All major active ingredients of ginger such as zingerone, gingerdiol, zingibrene, gingerols and shogaols have antioxidant activity (Min-Ji Bak et al., 2012).

Materials and Methods:

Chemicals:

Lead acetate was purchased from MERK India Ltd., Silymarin suspension purchased from Micro labs, Bangalore. All other chemicals used were of technical grade.

Animal Ethical Clearance:

Local Institutional Animal Ethical Committee of our University, obtained ethical clearance for conducting experiments on animals from committee for the purpose of control and supervision of experiments on Animals (CPCSEA) (REGD.No.470/01/a/CPCSEA, DT.24th Aug 2001).

Procurement of Animals and maintenance

Adult male albino rats wistar strain (Rattus norvegicus) weighing 150±30gms obtained from Sri Raghavendra Animal Supplier, Bangalore, K.A. They were kept in cages under standard laboratory conditions (25 ± 2^0C, 12 hrs dark/light) and were fed with commercial rat feed supplied by Sai Durga Feeds and Foods, Bangalore and water adlibitum. They were allowed to laboratory conditions for seven days after arrival before use.

Preparation of ethanolic extract of rhizome of *Zingiber officinale*:

The ginger was collected from local market and cut into small pieces and dried under ceiling fan for 5 to 6 days. The dried ginger was ground in an electronic grinder and powder was collected. 50g of powder was extracted in 250ml ethanol for 18hrs in soxhlet apparatus. The extract was dried at reduced pressure, stored at 0-4^{o}C and used for the experimentation.

Treatment:

The animals were divided into 7 groups of 6 rats each and treated as follows:

Group- I: Normal control (Nc): This group of rats received vehicle solution (5% Tween 80).

Group-II: Ginger treatment (Gt1): Rats received ethanolic extract of ginger (200mg/Kg body weight) orally for 8 weeks.

Group-III: Ginger treatment (Gt2): Rats received ethanolic extract of ginger (300mg/Kg body weight) orally for 8 weeks.

Group-IV: Lead treatment (Lt): Rats received lead acetate orally at a dose of (200mg/Kg body weight) orally for 8 weeks.

Group-V: Lead treatment + Ginger treatment (Lt+Gt1): This group of rats received both lead acetate and ginger as described in group II and group IV for 8 weeks.

Group-VI: Lead treatment + Ginger treatment (Lt+Gt2): This group of rats received both lead acetate and ginger as described in group III and group IV for 8 weeks.

Group-VII: Lead treatment + Silymarin treatment (Lt+St): This group of rats received both lead acetate and silymarin. Lead as described in group IV and silymarin (100mg/Kg body weight) orally for 8 weeks.

Lead acetate was dissolved in distilled water before administration. Food was withdrawn 12hr before Lead acetate administration. Ginger was suspended in 5% Tween 80.

Hematological parameters:

Total Red blood corpuscle (RBC), White blood corpuscles (WBC) and Hemoglobin content (Hb %) were studied for hematological investigation.

Blood collection:

The animals were sacrificed 24hr after last treatment. Blood was collected from the heart (cardiac puncture) of each animal in an eppendorf tubes with anticoagulant containing EDTA (1mg/ml) was used for estimation of total RBC, WBC and hemoglobin.

Red blood corpuscle (RBC) count:

RBC count was made with a Neubauer crystalline counting chamber as described by Davidson and Henry (1969). The blood was collected in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. The blood was drawn up to 0.5 marks in RBC pipette and immediately the diluting fluid was drawn

up to the mark 101 (thus the dilution is 1:200). The solution was mixed well by shaking gently. It was allowed to stand for 2 or 3 minutes. The counting chamber and cover glass were cleansed and the cover glass was placed over the ruled area. Again the solution was mixed gently and stem full of solution was expelled and a drop of fluid was allowed to flow under the cover slip holding the pipette at an angle of 400, it was allowed to stand for 2 to 3 minutes to allow RBC to settle. Afterwards the ruled area of the counting chamber was focused under the microscope and the number of RBC's were counted in five small squares of the RBC column under high power and the number of RBC per cu mm were calculated accordingly.

$$\frac{\text{Number of cells X dilution factor X depth factor}}{\text{Area counted}}$$

White blood corpuscles (WBC) count:

Blood is drawn from the vial into WBC pipette up to 0.5 marks and immediately the diluting fluid is drawn up to 11 marks. The solution is mixed thoroughly by shaking gently. The rest of the procedure is the same as described by Davidson and Henry(1969) for RBC count. In case of WBC, count was made in bigger squares of the chamber. The WBC count was expressed in cu mm.

Estimation of hemoglobin (Hb) concentration:

The hemoglobin concentration was estimated by Acid - haematin method (Sahli, 1966). N/10 hydrochloric acid was taken up to 20 marks in a graduated tube. Blood was collected directly from the eyeball up to 20 cu mm in the Hb pipette and the outer side was wiped out and this was transferred into the graduated tube containing N/10 hydrochloric acid.

Pipette was rinsed two or three times with dilute hydrochloric acid. It was allowed to stand for 10 to 20 minutes after thorough mixing. Then N/10 HCl was added drop by drop, mixing between each addition until the blood color matched with the standard color. And then the results were read from the scale on the graduated tube and the Hb concentration was expressed in grams percent.

Results:

In the present investigation the effect of lead toxicity, protective effect of ginger and silymarin treatment against lead toxicity on the blood cell count (RBC & WBC) and hemoglobin content were carried for 8 weeks. Table: 1. shows the levels of RBC, WBC and Hb in all experimental groups.

Table: 1. Hematological parameters in animals of different experimental groups

Sl. No.	Parameter		Group-I (Normal control)	Group-II (Ginger control-I)	Group-III (Ginger control-II)	Group-IV (Lead control)	Group-V (Lead+ Ginger-I)	Group-VI (Lead+ Ginger-II)	Group-VII (Lead+ Silymarin)
1.	RBC (Million/ cu.mm)	*Mean*	5.7000^{a}	6.0333^{a}	5.8833^{a}	3.3833^{d}	4.0833^{c}	4.6833^{b}	5.7500^{b}
		S.D	±0.3916	±0.1491	±0.3337	±0.3131	±0.1344	±0.3532	±0.4193
2.	WBC (Thousand /cu.mm)	*Mean*	3.6833^{c}	3.8167^{c}	3.7500^{c}	5.5833^{a}	4.8000^{b}	3.9333^{c}	3.7333^{c}
		S.D	±0.1863	±0.2115	±0.2500	±0.4220	±0.2769	±0.2134	±0.2357
3	Hb (gr/dL)	*Mean*	15.1000^{a}	15.2167^{a}	14.7500^{a}	10.1500^{d}	11.9167^{c}	12.9667^{b}	13.1667^{b}
		S.D	±0.3467	±0.4058	±0.5439	±0.3686	±0.3715	±0.1247	±0.3636

Values are mean ± S.E.M

Values with different superscripts with in the column are significantly different at $P<0.05$ (Duncan's Multiple Range Test)

Oral administration of lead acetate toxicity on RBC, WBC count and Hb content were studied in all experimental groups. RBC and WBC and Hb levels in normal control rats (group-I) were found to be 5.7cu.mm, 3.6833cu.mm and 15.10g/dL respectively. Group IV (lead acetate treated alone) showed significant ($p<0.05$) decrease in the count of RBC and Hb levels were found to be 3.3833cu.mm and 10.15g/dL respectively but significant ($p<0.05$) increase in the count of WBC was found to be 5.5833cu.mm when compared to normal control group.

Discussion:

In the present study there was significant decrease in levels of RBC, hemoglobin but increase in the level of WBC. In Group IV (lead acetate treated alone) shows decreased levels of RBC, hemoglobin. Exposure to lead significantly decreased red blood cell counts, hemoglobin levels and hematocrit values in rats (Terayama, 1993 and Al-Saleh, 1994). Lead can cause harmful effect to certain types of blood cells, including reduced hemoglobin production and reduced life span and function of RBC, has been associated with low level exposure to inorganic lead in the work place (Manser et al., 1990). Erythrocytes have a high affinity for lead, binding 99% of the lead in the bloodstream. Lead has a destabilizing effect on cellular membranes, and in red blood cells (RBC). It decreases cell membrane fluidity and increases the rate of erythrocyte hemolysis. Hemolysis appears to be the end result of ROS-generated and lipid peroxidation in the RBC membrane (Lawton and Donaldson, 1991). Hypochromic or normochromic anemia is a hallmark of lead exposure; it results from ROS generation and subsequent erythrocyte haemolysis (Patrick, 2006). Significant decrease in RBC count and hemoglobin (Hb) were seen in rats and human with high blood lead levels (Alexa et al 2002; Othman et al., 2004; Toplan et al., 2004; Noori et al., 2003). Similar results were reported by Szymezak et al (1983) observed that Hb level was reduced after intoxication with lead acetate in dose of 400mg/kg of the fodder. Berney et al (1994) observed significant reduction on Hb, but increase on the TLC following lead acetate administration at different doses.

Group V (lead+ginger-I), group-VI (lead+ginger-II) shows recovered levels of RBC, Hb and decreased levels of WBC when compared to lead controlled rats. . In support of these authors Miller et al (1993) and Ahmed et al (2000) reported previously ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities found that ginger significantly lowered lipid peroxidation. Group VII also showed recovered levels of RBC, WBC and Hb when treated with standard drug silymarin over lead control which are very close to normal control and ginger treated ones. The protective action of silymarin is associated with its antioxidant properties, as it possibly acts as a free radical scavenger, an inhibitor of lipid peroxidation and a plasma membrane stabilizer (Ramadan et al., 2002).

In support of our work Hudson et al (1977) reported the exposure of lead at a concentration as low as 0.013mg/litre, led to increase in WBC numbers, decrease in RBC volume, decrease in blood cells iron content and decrease of RBC amino levulinic acid dehydratase activity (Delta-ALAD). Schutz and Skerfuring (1976) reported inhibition of several enzymatic steps in the synthesis of hemoglobin. Similarly, Rice et al (1997) evaluated the long-term effect of lead exposure on haematology and blood biochemistry. However, Klauder et al (1977) reported anemia due to lead intoxication. Christensen et al (1977) also reported that there were significant decrease in blood Hb levels after exposure to lead at 59ug/litre for eight weeks. But at the same condition and duration lead acetate treated animals showed significant increase in number of WBC in blood of albino rats, because whenever any toxicity occurs in body, the body immune system enhances the production of WBC. However, Hogan et al (1979) reported lead induced leukocytosis in female mice.

Conclusion:

The results in the present study suggest that the hematological RBC and Hb values were significantly decreased and increased WBC values in lead induced Wistar male albino rats. In giner treated groups hematological parameters were improved when compare to its lead treated group. This might be due to its hemoprotective immune stimulatory, anti inflammatory effect and antioxidant property.

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