ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com *INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

RESEARCH ARTICLE

Hemato-biochemical, Behavioral and Neurological effects of Vitamin C administration against lead exposure in mice

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Manuscript Info

Manuscript History:

Received: 22 October 2014
Final Accepted: 15 November 2014
Published Online: December 2014

Key words:
Vitamin C, Lead toxicity, hemato-biochemical changes, brain antioxidant status, anxiety, learning, aggression.

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Abstract

Lead (Pb) is widely used in industry and has a great environmental health problem of both human and animal. Effects of reactive oxygen species (ROS) generation have been postulated to be major contributors to lead-exposure related disease. Therefore, the present experiment was carried out to determine the effectiveness of vitamin C in alleviating the toxicity of lead on certain behavioral, hemato-biochemical parameters and antioxidant status in brain of mice. Ten mice (20-25gm) per group were assigned to 1 of 3 treatment groups for 3 months: Group 1 served as control; Group 2 received lead acetate (50 mg/kg) orally; Group 3 received lead acetate with vitamin C (100 mg/l) in drinking water. Evaluations were made for hemato-biochemical, behavioral parameters and lipid peroxidation in brain tissue. Results obtained showed that lead acetate intoxication reduced hemoglobin concentration, hematocrit value and mean corpuscular hemoglobin concentration. Furthermore, it increased serum levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase; increased brain malondaldehyde level and decreased brain superoxide activities. Also, lead (pb) exposure resulted in increased anxiety and fear related behavior in both elevated plus maze and light dark box tests, impaired learning ability and memories, increased aggression and reduced body weight and weight gain. However, vitamin C kept the studied hematological and biochemical parameters within normal ranges. In addition, it prevented lipid peroxidation and oxidative stress induced by lead intoxication. Vit C administration also, resulted in reduced anxiety and aggression and improved learning ability and memories, body weight and weight gain to the level of control animals. Therefore, these results indicated that vitamin C ameliorating the toxic effects of lead and it appeared to be a promising protective agent against lead-induced toxicity.

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Introduction

Lead (Pb) is one of the most toxic metals that induce a wide range of behavioral, biochemical and physiological dysfunctions in humans and experimental animals (Pokras and Kneeland, 2009). Both occupational and environmental exposures remain a serious public health problem in many developing and industrializing countries (Yücebilgic et al., 2003). Lead induced oxidative stress in blood and other soft tissues have been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande et al., 2001). Lead is reported to cause

oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides (El-Nekeety et al., 2009).

Free radicals were generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Flora et al., 2003). Lead is a toxic agent with multiple target organs such as hematopoietic system, immune system, kidneys, and nervous system (A.T.S.D.R., 1993). Lead enters the brain and selectively deposited in the hippocampus and cortex, as well as in non-neuronal elements that are important in the maintenance of the blood brain barrier function. Studies have demonstrated that lead impairs learning, memory processes and cognitive functions both in animal models and human beings (Canfield et al., 2003). The neuropathlogical effects of lead include nervousness, anxiety and symptomatic encephalopathy at the end (Kadhim and Mona, 1999). Lead exposure at low concentrations, causes alterations in the nervous functions, which leads to a reduction of brain ability to use serotonin and dopamine, neurotransmitters directly associated with conduct disorders and aggressiveness in mice (Liliana et al., 2010). Studies have reported a correlation between lead and various aspects of human behavior such as violent and criminal behavior, homicide, "delinquent, antisocial behavior and aggression (Pokras and Kneeland, 2009).

Lead was shown to induce microcytic hypochromic anemia that was due to interference with iron and copper metabolism. Furthermore, decreased hematocrit and hemoglobin levels might arise from reduction in serum copper as well as reduced iron metabolism and consumption induced by lead (Klauder and Petering, 1977). Lead suppresses bone marrow hematopoiesis, probably through its interaction with the enteric iron absorption (Chmielnika et al., 1994). Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids (Chaurasia and Kar, 1997).

Vitamin C is an important water-soluble chain-breaking antioxidant and enzyme co-factor (Wilson, 2002). It has many diverse biological functions since it influences numerous enzyme activities, in particular, those involved in the biosynthesis of collagen, carnitine, and neurotransmitters. In addition, Vitamin C is used as a co-factor for catecholamine biosynthesis, in particular the conversion of dopamine to norepinephrine catalysed by dopamine β-monooxygenase (Burri and Jacob, 1997).

Development of anemia is common in animals with ascorbic acid deficiency because there is reduction in the absorption and redistribution of iron and consequently a reduction in the synthesis of hemoglobin (Shiau and Jan, 1992). Importantly, Ascorbic acid is an antioxidant which is essential for numerous cellular functions. It is considerably accumulated in endocrine tissues and cells including leukocytes where it acts as free radical scavenger (Schwager and Schulze, 1997). Even small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids, carbohydrates, and nucleic acids from damage by free radicals and ROS that can be generated during normal metabolism as well as through exposure to toxins and pollutants. Vitamin C may also be able to regenerate other antioxidants such as vitamin E (Carr and Frei, 1999).

Oral supplementation with vitamins which free radical scavengers (e.g. ascorbic acid) may protect the animals from the harmful effect of lead. The role of ascorbic acid against lead-induced changes in neurobehavioral, blood hematology, lipid peroxidation in brain of mice has not fully studied. Therefore, the aim of this study was to investigate the role of vitamin C in alleviating the negative effects of lead on hemato-biochemical parameters, lipid peroxidation in the brain, anxiety like behavior, learning ability and memory and aggression in mice.

2. Materials and methods

2.1. Animals

Thirty male mice weighting approximately 20-25 gm were used in this experiment. Animals were obtained from the laboratory unit, Department of Husbandry and Animal Wealth Development, University of Sadat City, Egypt. They were maintained in polyethylene cages, with stainless steel wire lids (bedded with wood shavings), on a standard laboratory feed diet. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature and humidity-controlled room. Water and feed were supplied ad libitum. Animals were quarantined and allowed to acclimate for a week prior to experimen. All animal-handling procedures were carried out following the regulations of Institutional Animal Ethics Committee and with their prior approval for using the animals.

2.2. Experimental conditions

Mice were randomly distributed according to the treatment into three groups of ten animals each (n=10). The experiment lasts 3 months. The first group (G1) served as the control and received water and feed ad libitum through out the experiment. Group 2 received lead acetate (50mg/kg) orally for 3 months (Gupta et al., 1995) obtained from Mid Egypt Chemical Company. Group 3 received lead acetate (50mg/kg) with Vitamin C (100 mg/l) in drinking water (Weyers et al., 2008) obtained from Middle East Company, Egypt.

2.3. Hematological and Biochemical Investigations

At the end of the test period, the mice were sacrificed by decapitation after diethyl ether anesthesia, and blood samples were drawn into dry tubes (for obtaining serum) and heparinized tubes (for obtaining whole blood). Serum were separated after centrifuging the blood sample and stored at -20°C for subsequent analysis.
Hematological parameters were determined by standard methods. Hemoglobin concentration was determined by the Cyanomethemoglobin Method (Pilaski, 1972). Packed cell volume (PCV) was determined by microhematocrit method as described by Feldman et al. (2000) using microhematocrit centrifuge. Mean corpuscular hemoglobin concentration (MCHC) was calculated according to the following equations (Feldman et al., 2000):

$$\text{MCHC \%} = \frac{\text{Hb}}{\text{PCV}} \times 100$$

The activities of serum AST, ALT and ALP were estimated by the method of Young (2001).

2.4. Lipid peroxidation and Superoxide Dismutase estimation

The brain was removed and quickly excised, minced with ice cold saline, blotted on filter paper and homogenized in 50 mmol/l phosphate buffer (pH7.4). The supernatant were frozen at -20 C for further determination of antioxidant enzymes activities and MDA level. Brain homogenate was prepared according to Combs et al. (1987).

Measurement of brain Malondialdehyde (MDA Concentration):

Brain lipid peroxidation product such as malondialdehyde (MDA) was determined by the method of Yashkochi and Masters (1979). MDA reacts with thiobarbituric acid (TBA) in an acid medium giving a colored TBA-complex measured colorimetrically at 520-535nm against blank and MDA values were expressed as n moles MDA/g protein

Measurement of brain Superoxide Dismutase Activity:

Superoxide dismutase (SOD) activity was estimated according to Giannopolitis and Ries (1977). The optical absorbance was measured at wave length 560 nm against blank reagent. SOD= Reading (absorbance) of (SOD)/ mg protein.
Protein Determination: The total protein concentration of supernatant was determined by the method of Lowry et al. (1951).

2.5 Behavioral measurements:

At the last two weeks of the experiment, behavioral tests were performed in the first half of light phase of the light/dark cycle. A video-camera was used to record the behavior of each mouse in different behavioral testes. All behaviors were scored by a single trained observer unfamiliar with treated animals. Hand operated counters and stop watches were used to score animals' behavior. Behavioral tests were separated by at least 48 h from each other.

2.5.1 Elevated plus maze test (EPM):

Anxiety-related responses were evaluated using EPM. The elevated plus maze apparatus consisted of two open arms, 50X10 cm (length- width) and two closed arms, 50X10 X30 cm (length- width- height) with an open roof arranged such that the two arms of each type were opposite to each other. The maze was elevated 50 cm from the floor. For the test, each animal was placed in the center of the maze, facing one of the closed arms and the number of entries into, and the time spent in the open and the closed arms were registered for five min. (Walf and Frye, 2007). The degree of avoidance of the open arms of the maze has been considered as a measure of strength of fear drive. Arm entries were counted when more than half of the rat body entered the arm. After each trial the maze was wiped with a cloth dipped in 70% ethyl alcohol and allowed to dry.

2.5.2 Light-Dark transition task

The light dark exploration task represents a naturalistic conflict between the tendencies of mice to explore a novel environment versus the tendency of mice to avoid a brightly lit open field. The light-dark box apparatus consists of a rectangular box (44 cm × 8.5 cm × 25 cm) divided equally into a light, open compartment connected by a door (17 cm in height) leading to a dark closed compartment in which the animal is placed. Each animal was placed facing the side away from the door and then released. During three min. (Costall et al., 1988), the time spent in dark and light compartments, respectively, was measured to determine degrees of anxiety.

2.5.3 Classic maze test

Associative learning was assessed using classic maze test. The base of the maze measured (100x 100cm) with walls height of 25cm. The entire maze was made of plywood with a glass cover in order to prevent escape of animals and allow observation. Testing was carried out between 09:00 and 15:00 h, where all groups were randomly allowed for testing at the same day. Mice were deprived from feed for a 23 hours period before start of testing. Mice were given their daily feed amount as a reward at the end of the maze. Animals were given one trial per day for five consecutive days. Time elapsed to locate the feed at the end and numbers of entries of blind alleys were recorded (Staddon, 1983; Kamel et al., 2010).

2.5.4 Resident-intruder test of territorial aggression:

A rectangular observation cage (45 x 27 x 40 cm: length x breadth x height) was used for evaluation of aggressive behavior in mice. A stud male mouse was placed in the testing arena for 10 days and served as the resident. The tested male mouse (intruder) of no previous contact with the resident was then placed into the test arena, confronted with the resident male for 5-min test period (Bataineh et al., 1998). The patterns of recording were : 1-Boxing: Refers to the subject rearing up and pushing each other with their fore paws. 2- Bite: Refers to subjects making a piercing contact with the teeth that scratch or breaks the skin of the other. 3- Wrestling: Refers to wrapping around each other into a tight ball, rolling around together and biting, frequently screaming. 4- Dominance posture: Refers to subjects standing on their hind legs, with their back slightly arch, and with forelimbs thrown forward facing another mouse (Balogun, 2012).

2.6 Body weight

Mice were weighed at the onset of treatment, and then individual body weight of all mice per group was recorded weekly throughout the study using equilibrated scales (Sartorius, AG, Gottingen, Germany), and body weight gain was calculated as the difference between final and initial weight.

3. **Statistics:** Data are reported as mean ± SE and were subjected to a one-way analysis of variance (ANOVA). Post hoc differences between groups means were tested with the Duncan multiple tests. Values of ($p < 0.05$) was considered significant. Statistical analyses were performed using the computer software SPSS 10.0 for Windows (Alan and Duncan, 2001).

Results and Discussion

1- Hematological and Biochemical parameters

Data obtained as a result of comparing hematological parameters in control and treated groups are shown in Table 1. Compared to the control mice, those after treatment with lead acetate had significantly lower Hb content ($P<0.05$) and MCHC. Vitamin C supplementation to lead acetate treated mice reversed these effects and attained the hematological parameter changes to normal levels. The reduction of Hb in lead acetate group might be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocyte destruction in hemopoietic organs. The observed decrease of Hb in mice exposed to lead was a manifestation of swollen erythrocytes and macrocytic anemia. This result is in agree with that reported by Klauder and Petering (1977) who reported that administration of lead resulted in a decreased hemoglobin level they attributed that to reduction in serum copper as well as reduced iron metabolism. In addition, Lead suppresses bone marrow hematopoiesis, probably through its interaction with the enteric iron absorption (Chmielnika et al., 1994). Data obtained in this study referred that Vitamin C supplementation to lead acetate treated mice reversed these effects and attained the hematological parameter changes to normal levels. This may be attributed to an increase the absorption and redistribution of iron and consequently an increase in the synthesis of hemoglobin. As well as,

Ambali et al (2010) reported that vitamins C and E pretreatment ameliorated cholinergic toxic signs and changes in PCV, Hb, RBC and WBC count induced by chlorpyrifos toxicity.

Hepatic enzymes levels in control and treated groups are shown in Table 2. Lead acetate had significantly increased ALT, AST and ALP As well as, Vitamin C supplementation to lead acetate treated mice attenuate these effects.

Liver injury following lead exposure is well characterized by elevated levels of serum hepatic marker enzymes which indicate cellular leakage and loss of functional integrity of hepatic membrane architecture. High levels of aminotransaminases (ALT and AST) and ALP are crucial parameters in detecting liver damage. Haleagrahara et al. (2010) reported increased activities of ALT, AST and ALP in rats treated with lead acetate. These findings are consistent with our results which showed increased activities of ALT, AST and ALP in serum of lead-treated mice.

Vitamin C has hepato-protective property linked to its antioxidative property. Vitamin C was reported to attenuate hepatic damage induced by some chemical agents especially in animals. This is supported by the work of Bashandy and Alwasel (2011).

Results indicated that malondialdehyde level was significantly increased, while superoxide dismutase activity were significantly ($P < 0.05$) decreased in brain of mice treated with lead acetate (Table 3). Administration of vitamin C with lead acetate caused reduction in the elevation of brain malondialdehyde, and maintained the activity superoxide dismutase to the normal values as compared to the control group. This means that the presence of vitamin C minimized the hazardous effect of lead.

These observations confirm the findings of several studies, which reported alterations in antioxidant enzyme activities in lead exposed animals (El-Nekeety et al., 2009). Lead is known to produce oxidative damage in various organs by increasing lipid peroxidation (El-Missiry, 2000). The observed increased lipid peroxidation in the current study in lead-treated group may be due to the formation of free radicals or through exhaustion of antioxidants, leading to oxidative stress. Intense lipid peroxidation caused by lead exposure may affect the mitochondrial and cytoplasmic membrane causing more severe oxidative damage in the tissues and consequently releasing lipid hydroperoxides into circulation (Abdel-Wahhab et al., 2008).

Treatment with lead acetate significantly decreased the activities of superoxide dismutase. These results are in agreement with previous reports (Newairy and Abdou, 2009). Lead acetate is known to cause free radical damage in tissues by two mechanisms: Increased generation of ROS, including hydroperoxides, singlet oxygen and hydrogen peroxides, and by causing direct depletion of antioxidant reserves (Upasani et al., 2001). Superoxide dismutase enzyme takes part in maintaining glutathione homeostasis in the tissues. This antioxidant enzymes are involved in the defense system against free radical mediated tissue or cellular damage after lead exposure (Ercal et al., 2001).

Vitamin C is an effective antioxidant owing to its high electron-donating power and ready conversion back to the active reduced form.

Regarding the effect of vit C supplementation. The data presented in table (3) demonstrated that, vit C ameliorate the effect of lead on malondaldehyde and superoxide dismutase. These results are in accordance with the finding of Yousef et al. (2003). Also, Huang et al. (2002) reported that Vitamin C supplementation in nonsmokers reduced lipid peroxidation. Additionally, Vitamin C can protect biomembranes against peroxidative damage. Two major properties of Vitamin C make it an ideal antioxidant. First is the low one-electron reduction potentials of both ascorbate and its one-electron oxidation product, the ascorbyl radical. These low reduction potentials enable ascorbate and the ascorbyl radical to react with and reduce basically all physiologically relevant radicals and oxidants. The second major property that makes Vitamin C such an effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species (Tsao, 1997).

2- Behavioral parameters

Anxiety like behavior

In this study, the effects of exposure to chronic lead administered and its amelioration with Vitamin C on parameters of anxiety and related fear behaviors were investigated in male mouse.

The effect of lead on measurement of (EPM) was demonstrated in Table 4. Mice under lead effects (G2) were significantly diminished the numbers of entries in the open arms of the maze ($p<0.05$), accompanied with non significant increase ($p>0.05$) of this measure in the closed arms compared to the other groups. Concerning time

spent in the open arms, the control mice (G1) and mice treated with Lead+Vit C (G3) were spent significantly longest time ($p<0.05$), and mice received lead only (G2) spent shortest time ($p<0.05$). Conversely, the time spent in closed arm recorded was significantly higher in G2 compared to G1 ($p<0.05$), with the group G3 being intermediary between the G1 and G2 but not different from them. This result indicated that, chronic lead exposure caused a significant increase in the anxiety levels of mice. Interestingly, a significant improvement in the indicated measurement was observed when mice were treated with both lead and Vit C (G3). Our result is in agreement with Kahloula, et al. (2013) who found that, lead administration reduced time spent exploring the open arms, the time spent in open arms and percentage of entries into the open arms compared to control rat. In addition, in female Swiss mice, exposure to lead at 500 ppm, provokes an anxiogenic effect demonstrated by the elevated plus Maze (Soeiro et al., 2007). Similar results were indicated by Seddik et al. (2010). On the other hand, the data obtained was not the same as that obtained by Trombini et al. (2001) who reported that exposure to 750 ppm of lead acetate in drinking water during pregnancy and lactation had no effect on the behavior determined by EPM.

Results from dark-light box test (Table 5), showed that time spent in the light box was significantly increased in lead-treated mice (G2) comparing with groups 1 and 3 ($p< 0.05$). Conversely, time spent in dark compartment was reduced significantly in G2 compared to other groups ($p< 0.05$). Administration of Vit C (G3) significantly reverses the effects of lead. Our result is in agreement with Benammi et al. (2014) who reported in the dark-light box test showed in lead-treated male rats that time spent in the dark compartment is reduced significantly ($p<0.05$) compared to control animals. Similarly, Sansar et al. (2012) reported an anxiogenic effect of lead using dark light box test, when male adult Wistar rats are chronically exposed for 3 months to 0.5% lead acetate in drinking water as lead-treated rats spent significantly more time in the light chamber compared with controls ($P=0.001$). Moreover, Kahloula et al. (2013) reported that mother rats during gestation and lactation ingested the Pb showed an increase of residence time in the compartments with light compared to control rats in light dark test ($P<0.001$).

The data obtained herein in EPM and dark/light box tests showed that, an obvious anxiety like behavior of chronic exposure to Pb in the male mice. This finding indicates that Pb treated mice showed abnormal behavior when compared to control mice. Vit C administration has beneficial effects with respect to state of anxiety. Studies have been reported role of oxidative stress in anxiety-like behavior in rodents, and increased anxiety has been found to be positively correlated with increases in reactive oxygen species in granulocytes (Bouayed et al., 2007). Hence, increased anxiety like behaviors in the current study in Pb exposed mice (G2) may be attributed to oxidative stress which indicated by significantly increased of malondialdehyde level and significantly decreased of superoxide dismutase activity ($P<0.05$) in brain of lead treated mice (G2). Administration of Vit C ameliorates the adverse effect of effect of lead acetate (Table 3) and (Table 4). Our results are in accordance with Hassan and Jassim (2010) who found that, administration of 600 mg/kg diet of vitamin E, 100 mg/kg, of vitamin C orally reverse the adverse effects of lead acetate (10 mg/kg) on neurobehavioral of rats. In the same trend, Hughes et al. (2011) reported that, vitamin C and E separately and together would lead to lower anxiety related open-field behavior and acoustic startle in hooded rats. They suggested that decreases in anxiety produced by the vitamins may have arisen from their antioxidant properties, attenuation of cortisol activity or some as yet undetermined effects on anxiety-related brain structures and neurotransmitters. In addition, Pretreatment with vitamin C has also been shown to affect indices of fear in poultry such as attenuated tonic immobility and decreased neophobia in Japanese quail and broiler chickens (Satterlee et al. 1993).

Associative learning

The associative learning and memories are based on the acquisition of a predictive link between a specific event and a stimulus. The animal must build a cohesive spatial representation of the maze to end with the food. On repeating the maze experiment several times, the changes in latency, and errors made to reach the food are indicators for learning and memory abilities of the mice (Kamel et al., 2010). The data presented in table (6) showed that, Mice administrated with lead, demonstrated a significant higher latency ($P<0.05$) with increased numbers of errors ($P<0.05$) in the maze reflecting poorer memory retention comparing with control mice (G1) and mice treated with Lead+Vit C (G3). Our results are in accordance with Soodi et al. (2008) who reported that lead exposure may have the ability to induce memory loss and impair spatial learning. Also, Chandra et al. (1981) observed that, rates exposed to different concentration of lead acetate (5.0, 8.0 and 12.0 mg/kg daily for 14 days) showed a significant deficit in learning at 7 and 14 days. Similarly, Li et al. (2006) demonstrated that, lead may inhibit the expression of $Hoxa^{9}$ mRNA and induce atrophy and necrosis of brain, which gives rise to a damage of learning and memory in rats. Moreover, Adonaylo and Oteiza (1999) reported that, mice given lead (1 g/l) in drinking water for 8 weeks developed learning deficit, memory loss and increased activity of the cell death marker enzyme caspase-^{3} was

observed. The increased activity of the cell death marker enzyme caspase-^{3} observed in the brain of mice treated with lead suggested that the memory loss could be caused by lead-induced loss of neurons in the brain. In addition, Molina et al (2011) suggested that Pb exposure results in decreased iron incorporation into red blood cells. Even with sufficient dietary iron, Pb excess causes anemia. This concomitant anemia would result in decreased oxygenation of vital organs, especially the brain, and may exacerbate Pb toxicity. Taken together, Mn and Pb2 exposure each interferes with iron homeostasis that may exacerbate their neurobehavioral effects. This notation confirm our results in the current study (Table 1) as pb exposure resulted in a significant reduction in both blood hemoglobin (Hb) and mean corpuscular hemoglobin concentration (MCHC) ($P<0.05$.

Regarding the effect of vit C supplementation. The data presented in table (6) demonstrated that, vit C ameliorate the effect of lead on associative learning. This finding is in accordance with that of Fan et al. (2009) who reported optimum combinations of nutrient antagonize learning and memory impairment induced by lead in rats appear to be methionine, taurine, zinc, ascorbic acid and glycine. Similarly, Harrison et al. (2009) found reasonable amount of evidence from animal research of improved learning and memory following treatment with vitamin C. The memory and learning deficient of lead treated animals may result from harmful and damage effects of lead on brain tissues. Lead acetate induced cellular damage in the cerebellum of adult Wistar rats and it was observed that ascorbic acid prevents or minimize lead induced cellular damage in the cerebellum of adult Wistar rats (Musa et al., 2012). Moreover, Bhattacharjee et al. (2003) reported that, Ascorbic acid administration offered protection to the cell from expansion or abnormalities in their structural features and they concluded that ascorbic acid not only confers protection against lead toxicity but it can also perform therapeutic role against toxicity.

Territorial Aggression

The effect of lead and vit C supplementation on territorial aggression was summarized in Table (7). The data presented indicated that chronic lead exposure resulted in significant increases in all aggressive parameters measured ($P<0.05$) as well as the number of total aggression ($P<0.05$) in G2 compared to other groups. Our result is in consistent with Balogun (2012); Ebuehi and Ayinde (2012) who reported that, lead intoxication resulted in an increase in aggressive behavior in rats, also, Mphele et al. (2013) demonstrated significant negative effects of chronic lead administration on social behavior of rats, and lack of emotive reactivity which supported the hypothesis about lead (Pb) influence on the development of antisocial and impulsive aggressive conducts (Liliana et al., 2010). Conversily, Bataineh et al. (1998) reported that adults male rat ingested solutions of industrial metal salts (manganese sulfate, aluminum chloride, lead acetate and copper chloride) along with drinking water at a concentration of 1000 ppm for 12 weeks showed markedly suppressed lateralizations, boxing bouts, fight with stud male and ventral presenting postures. Male rat aggression was also abolished after the ingestion of manganese sulfate, aluminum chloride, lead acetate and copper chloride. Similarly, Ogilvie and Martin (1982) reported decreased aggression in mice exposed to lead.

Concerning the effect of administration of Vit C to lead resulted in significantly reduction of aggressive behavior parameters measured in treated mice (G3) compared to mice treated with lead only (G2). This result was in accordance with Ebuehi and Ayinde (2012) who found that rats exposed to lead 75 mg/kg body weight with 40 mg/kg body weight Vit C showed significantly less aggressive behavior compared to rats exposed to lead. They also observed a significant increases in brain serotonin levels of the vitamin C treated rats when compared to the lead only group. Brain serotonin (5-HT) more than any other neurotransmitters, has been implicated in the neural control of expressing aggressive behavior.

3- Body weight

The data presented in table (8) elucidated that, mice exposed to Pb (G2) showed significant reduction in body weight and body weight gain compared to mice in groups 1 and 2 ($P<.05$). However, there was no significant difference ($P>0.05$) in both body weight and weight gain between the (Pb+Vit C) and control groups. Our results are in agreement with Ibrahim et al. (2012) who reported that mice ingested 1/20 LD_{50} of lead acetate showed significant reduction in weight gain and food efficiency to 56% and 50% respectively compared to control mice. In the same trend, Khan et al. (2008) reported significant reduction ($p<0.01$) of mean body weight of mice exposed to lead acetate 100 mg/kg body weight in 5 ml distilled water. Also, Seddik et al. (2010) found that chronic Pb exposure for 90 days was associated with a decrease in body weight gains when compared to control rats. Moreover, Molina et al. (2011) found the body weight of pups exposed to Pb from their dam rats during gestation and lactation was significantly lower than (33%) that of the control pups at weaning. The reduction of body weight might be due to reduced food consumption through the contact of lead with appetite-depressant receptors in the gastrointestinal tract

and the interruption in absorption and metabolism of feed nutrients essential for health (Marchlewicz et al., 2007). On the other hand, Molina et al. (2011) found that, the body weights of Pb-exposed adult mother rats (2.84 mg/ml) during gestation and lactation were not different from controls.

Results in this study indicated that Vit C ameliorate the effect of lead on body weight and weight gain. This effect may be attributed to the positive effect of Vit C on absorptions and metabolism. Reports have found that vitamin C might act as a potential chelator of lead (Flora et al., 2003).

In conclusion, the present study demonstrates ameliorating effects of Vitamin C administered in combination with lead acetate to minimize its hazardous effects in the mentioned parameters (neurobehavioral, hematological and biochemical). Consequently, we have to reduce our exposure to lead and pay attention to sources of lead in our foods, water, ambient air and personal products. Also, using diet-rich in Vitamin C could be a beneficial way to overcome the toxicity of lead.

Table 1: **Effect of lead toxicity and its amelioration by Vit-C on blood hemoglobin (Hb), packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC)**

Experimental group	Hb (g/dl)	PCV (%)	MCHC (g/dl)
G1	13.09±0.86[a]	36.60±1.21 [a]	35.81±2.11 [a]
G2 (lead)	10.25±0.83[b]	36.80±0.66 [a]	27.86±2.22 [b]
G3 (lead+vit C)	13.18±0.66[a]	38.60±0.40 [a]	33.23±1.42 [a b]

Different letters in the same column show significant difference at the level of $p<0.05$

Table 2: Hepatic enzymes level in the serum of control and treated groups

Experimental group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
G1	132.3±2.46[b]	52.4±1.6 [b]	55.4±1.8 [c]
G2 (lead)	179.0±5.9[a]	64.2±1.7 [a]	155±4.2 [a]
G3 (lead+vit C)	142.3±2.02[b]	60.9±1.1 [a]	116±3.7 [b]

Different letters in the same column show significant difference at the level of $p<0.05$.

Table 3: Effect of lead toxicity and its amelioration by Vit-C on Malondialdehyde, Superoxide dismutase activity in brain of male mice

Experimental group	Malondialdehyde	Superoxide dismutase
G1	0.62±0.04[b]	0.060±0.006 [a]
G2 (lead)	0.84±0.04[a]	0.043±0.002 [b]
G3 (lead+vit C)	0.57±0.02[b]	0.056±0.006 [a]

- Different letters in the same column show significant difference at the level of $p<0.05$.
- MDA is expressed as nmol/g tissue protein.
- SOD is expressed as IU/mg tissue protein.

Table 4: Effect of lead toxicity and its amelioration by Vit-C on the behavior of mice during the elevated plus maze test (EPM).

	G1(control)	G2 (lead)	G3 (lead+Vit C)
No. of entries(open arm)	8.20±0.37 [a]	4.80± 0.58 [b]	6.80±0 .51 [a]
No. of entries(closed arm)	7.20±0.58 [a]	9.20±0.66 [a]	7.60±0.68 [a]
Time spent (open arm)(s)	154.63±6.50 [a]	76.84±14.23 [b]	134.84±5.67 [a]
Time spent (closed arm)(s)	119.21±6.60 [b]	188.76±16.03 [a]	154.32±9.81 [ab]

- Different letters in the same row show significant difference at the level of $p<0.05$.

Table 5: Effect of lead acetate and its amelioration by Vit-C on the behavior of mice during the Dark-light transition test.

	G1(control)	G2 (lead)	G3 (lead+ Vit C)
Time spent (light compartment) (s)	90.39±3.56 [b]	123.88±5.67 [a]	94.27±5.23 [b]
Time spent (dark compartment) (s)	89.61±2.11[a]	56.12±8.09[b]	85.73±5.61[a]

- Different letters in the same row show significant difference at the level of $p<0.05$.

Table 6: Effect of exposure to lead and its amelioration by Vit-C on measurements of maze test

	G1(control)	G2 (lead)	G3 (lead+ Vit C)
Latency (s)	68.86±12.73 [b]	189.56±14.40 [a]	113.97±14.94[b]
No. of entries of blind alleys	2.33 ±0.42[b]	5.33 ±0.49 [a]	3.50 ±0.43[b]

- Different letters in the same row show significant difference at the level of $p<0.05$.

Table 7: Effect of lead exposure and its amelioration by Vit-C on territorial aggression in adult male mice

	G1(control)	G2 (lead)	G3 (lead+Vit C)
Boxing	1.60±0.24[b]	2.80±0.20[a]	1.80±0.37[b]
Bite	1.20±0.20[b]	2.40±0.25[a]	1.40±0.40[b]
Wrestling	1.40±0.24[b]	2.60±0.25[a]	1.80±0.20[b]
Dominant Posture	2.20±0.20[b]	3.40±0.24[a]	2.60±0.24[b]
Total aggression	6.40±0.24 [b]	11.20±0.58[a]	7.60±0.40[b]

- Different letters in the same row show significant difference at the level of $p<0.05$.

Table 8: Effect of chronic lead toxicity and its amelioration by Vitamin C on the body weight and weight gain.

	G1(control)	G2 (lead)	G3 (lead+Vit C)
Initial weight	23.44±0.40[a]	24.25±0.31[a]	23.74±0.26[a]
Body weight	32.86±0.22[a]	28.37±0.49[b]	31.60±0.40[a]
Weight gain	9.44±0.33[a]	4.12±0.71[b]	7.95±0.39[a]

Different letters in the same row show significant difference at the level of $p<0.05$.

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