UNSWEETENED MILK AND VITAMIN C SUPPLEMENTS AMELIORATE LEAD ACETATE INDUCED HEPATIC INJURIES THROUGH IMPROVEMENT IN LIVER FUNCTIONS AND HEMATOLOGY

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Abstract

Lead poisoning poses one of the major health challenges affecting all organ systems but mostly the nervous, renal, haematopoietic, liver and cardiovascular systems.This study investigated the effects of unsweetened milk and vitamin C supplements on markers of hepatic function and hematological parameters of lead acetate exposed albino rats.Twenty male albino rats were randomized into four groups of five rats each. Normal control received feed and water only. Lead group (Pb) received 80mg/kg body weight lead acetate. Standard control (Pb+Vit C) was given 80mg/kg lead acetate daily plus 100mg/kg of vitamin C, while treatment group (Pb+Milk) was given 80mg/kg lead acetate plus 400mg/kg milk. Animals were allowed access to feed and water. All administration was done once daily by oral gavage for 42days.Biochemical analyses was done using standard procedures.Rats exposed to lead acetate showed a significant ($p<0.05$) increase in the activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin level, indicating liver dysfunction with a significant ($p<0.05$) decrease in plasma albumin when compared with the control groups. The results also showed significant ($p<0.05$) decrease in red blood cell count, packed cell volume, hemoglobin, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration with significant elevations in the mean corpuscular volume and platelet concentrations when compared with the control.Unsweetened milk and vitamin C supplements ameliorated the negative effect of lead on the liver and improved hematological parameters.
Keywords: lead acetate, unsweetened milk, liver function and hematological parameters

Introduction

Lead exposure is a prevalent public health issue facing the populace as a result of its hazard effects on various physiological systems. Lead toxicity has posed a health threatening situation shown by nature of their environmental decadence (Alisha et al. 2017), especially since its

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manufacturing transformations evolved. Almost all organ systems are associated with toxicity of heavy metals. However, the most susceptible systems include the cardiovascular, renal, haematopoietic, and nervous system (Abhay et al. 2024). Lead (Pb), one of the venomous heavy metals, has been implicated in various aspects of environmental and biological systems, especially in industrialized localities (Srivastava et al. 2024). Lead is one of the oldest deleterious agents known to mankind as its toxicity in both human and experimental animals can be traced back to the ancient times of the Romans, Arabs, Egyptians and Greeks (Jasbir et al. 2023). Lead exists in three isoforms: metallic lead, inorganic lead and organic lead, each with distinct properties and implications for health and its environment (Shafia 2022). Commonly encountered form of lead can enter the body through multiple routes including; ingestion, inhalation and dermal contact of their presence in food, air and tobacco leaves (Ashkan 2023). On absorption into systemic circulation, some of its components complexes with erythrocytes, and the remaining stays in plasma to be transported to other tissues (Elayat and Bakheelf 2010).

Lead-induced liver toxicity has been associated with oxidative stress, inflammation, and impairment of liver function parameters, which can have profound health implications (Ambreen 2024). The liver performs a critical role in detoxification, metabolism, and maintaining overall homeostasis in the body (Wu et al. 2023). As researchers seek innovative strategies to mitigate the adverse effects of lead exposure, the potential of unsweetened milk as a dietary intervention in alleviating liver and hematological damage requires thorough investigation.

Milk is an integral part of human and animal diets, recognized for its nutritional constituents. It’s rich in essential nutrients, proteins, vitamins, minerals, and bioactive compounds with potential health-promoting properties (Felicito 2024). These components suggest that milk consumption could influence liver health by modulating oxidative stress, inflammation, and enzymatic activities that impact liver function and hematological parameters (Giovanna et al. 2023). Some studies have explored the potential hepatoprotective effects of milk and the specific impact of milk consumption on liver function parameters in the context of lead exposure remains under-investigated (Yasmin et al. 2023). Elucidating the potential benefits of unsweetened milk in mitigating lead-induced liver and hematological damage could offer a novel and practical dietary approach to reducing the adverse effects of lead toxicity. Therefore, this study investigated the effects of unsweetened milk and vitamin C supplements on markers of hepatic function and hematological parameters of lead acetate exposed albino rats.

Materials and Methods

Equipment, Chemicals and Reagents. All equipment used were optimally functional and chemicals/reagents were of high analytical quality. Lead acetate (Merck, Germany) was procured from Cjay Enterprise Ogoja Road, Abakaliki, Ebonyi State Nigeria while Peak® instant full cream unsweetened milk was purchased from St. Margret Umahi International Market, Lot 1, Abakaliki, Ebonyi State Nigeria.

Animal Collection. Adult albino Wistar rats with body weights of >100 g were used for this study. These rats were purchased from Pharmacy Department, University of Nigeria Nsukka, Enugu State, Nigeria. The rats were kept in steel cages in a conventional laboratory setting for a week of acclimatization, with free access to commercial basic diet (Top Feed Growers Mash) and water *ad libitum*. They were kept in the Animal House at the Presco Campus of Ebonyi State University, Nigeria.

Experimental Design. Twenty male albino rats were randomized into four groups of five rats each. Normal control (NC) received feed and water only. Lead group (Pb) received 80mg/kg body weight lead acetate (Okediran et al. 2017, Newairy and Abdou 2009). Standard control

(Pb+Vit C) was given 80mg/kg lead acetate daily plus 100mg/kg of vitamin C (Ait Hamadouche et al. 2012), while treatment group (Pb+Milk) was given 80mg/kg lead acetate plus 400mg/kg milk (Chuang et al. 2004). They were fed with commercial top feed growers mash and water. All administration was done once daily by oral gavage for 42days. Biochemical analyses was done using standard procedures.

Blood Sample collection. The experiment was carried out for 42days and blood samples collected via ocular puncture with the animals anesthetized with chloroform after 24hrs fasting. Blood samples were collected using lithium heparin bottles for other tests and EDTA bottles for hematology after which samples were centrifuged at 2000 x g for 5 minutes and the plasma was isolated into plain bottles and stored in refrigerator with temperature of -4°C for analysis.

Liver Function Test. The following parameters were measured: total bilirubin (TB) (Doumas et al. 1973), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel 1957), alkaline phosphatase (ALP) (Babson et al. 1966) and plasma albumin (ALB) (Doumas and Peters 1997).

Hematology Analysis. Hematological parameters were measured using the CELL-DYN Ruby Auto analyzer (Abbott, Abbott Park, IL, USA). The following hematological parameters were examined: red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (HbC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT).

Statistical Analysis. Data generated were analyzed using the statistical package for social sciences software (Version 25) and thereafter subjected to one way analysis of variance (ANOVA).

Results and discussions

The results of this study showed a significant elevation ($P < 0.05$) in the activities of liver markers ALT, AST and ALP of lead acetate exposed rats relative to the control (Figure 1) with similar observation in Total Bilirubin level (Figure 2). The result in Figure 3 indicated a significant decrease ($P < 0.05$) in plasma albumin when compared with the control groups.

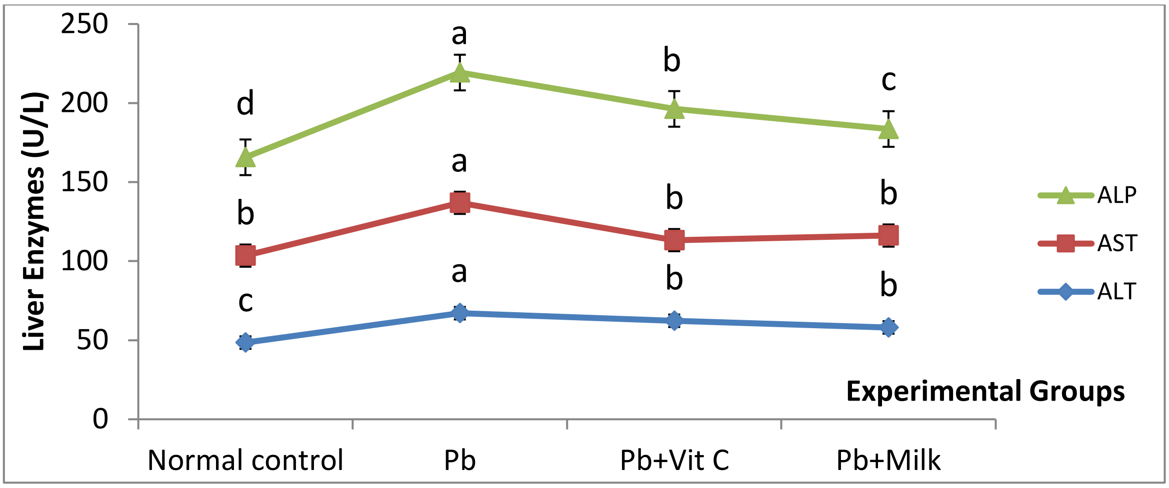

Figure 1. Effect of unsweetened milk and vitamin c supplements on liver enzymes in lead acetate exposed rats

Data are shown as mean ± SEM (n=5). **SEM:** Standard error of means. Coordinates with different alphabets are significantly different ($p<0.05$).

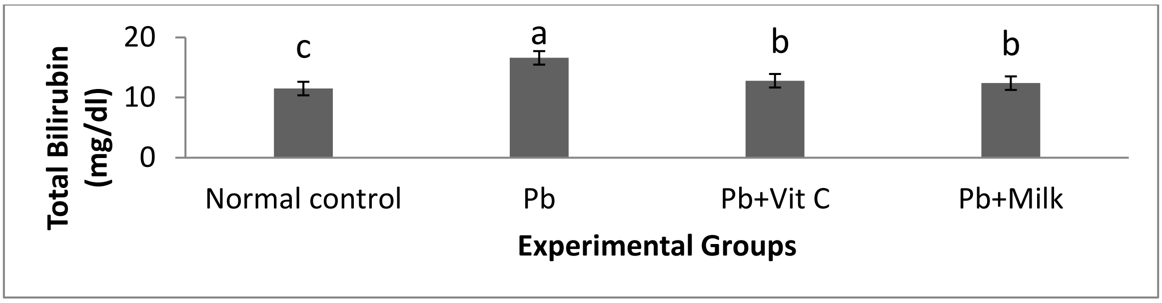

Figure 2. Effect of unsweetened milk and vitamin c supplements on plasma bilirubin level in lead acetate exposed rats
Data are shown as mean ± SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly different (p<0.05)

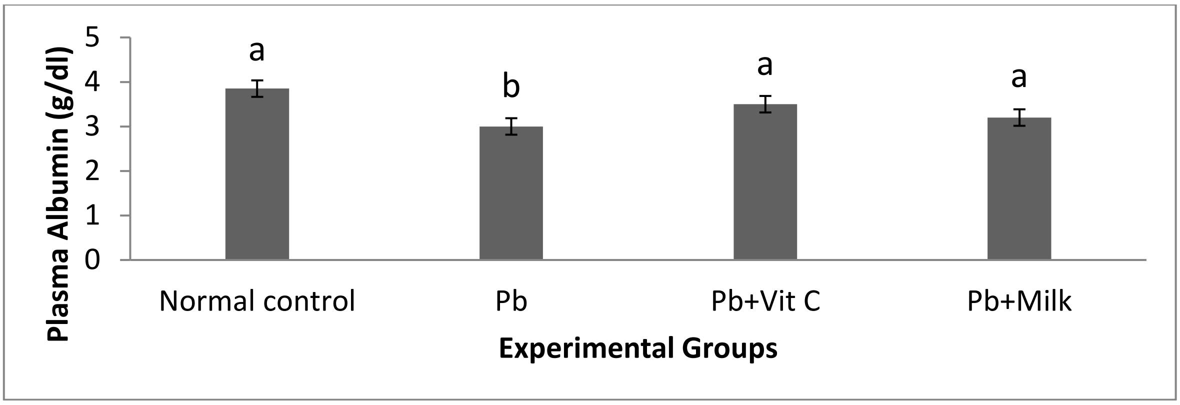

Figure 3. Effect of unsweetened milk and vitamin c supplements on plasma albumin level in lead acetate exposed rats
Data are shown as mean ± SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly different (p<0.05)

The administration of lead acetate to rats led to a significant (P<0.05) elevation in plasma AST, ALT and ALP activities in the group treated without intervention when compared with the normal control, indicating that exposure to lead acetate may induce a detectable damage to the liver. The elevation in plasma ALT, AST and ALP could be as a result of an increase in cell membrane permeability due to damage exerted on the plasma membrane of hepatocytes associated with lead acetate hepatotoxicity. The result is in accordance with the works of Mehta et al. (2002) and Patil et al. (2007) who reported a significant increase in AST and ALT concentrations after treatment with lead acetate. The hepatotoxicity induced by lead acetate tends to damage the liver cells with a concomitant increase in serum concentrations of AST and ALT (Abdou et al. 2007). The increased level of total bilirubin observed in the results after induction of lead acetate could be initiation of hemeoxygenases that play an important role in heme catabolism or damage to the hepatocytes as discussed above or both (Alya et al. 2007).
The groups that received 400mg/kg of milk and 100mg/kg of vitamin C revealed a significant (P<0.05) decrease in Total bilirubin concentration, ALT, AST and ALP activities when compared to the group that received lead acetate only. This finding is in accordance with the works of Abdel-Mobdy et al. (2023), who reported that liver function parameters and bilirubin level in serum significantly decreased in all administered groups with the inclusion of the milk-induced groups relative to normal control. Our findings is also in agreement with the works of Magjeed (2005), Khan and Zohair (2011), and Abdel-Mobdy et al. (2021); who reported the hepatoprotective influence of camel milk on poisons; similarly, camel milk ameliorate the liver functions of lead acetate induced rats; they equally reported that camel milk's beneficial

impacts could be due to the incorporation of mineral compositions in it, which plays vital roles in decontamination and as nutraceuticals in the liver.

There was a significant ($P<0.05$) reduction in the plasma albumin level of the rats exposed to lead acetate when compared with the normal control. The observed variations in plasma albumin is an indication of liver malfunction since it's responsible for proteins synthesis, mostly albumin (Mariam et al. 2023).

The results in Figure 4 and 5 indicated significant reduction ($P < 0.05$) in packed cell volume (PCV), hemoglobin (HB), red blood cell count (RBC), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) with a significant ($P < 0.05$) elevation in the mean corpuscular volume (MCV) and platelets count (Figure 6) when compared with normal control groups of lead acetate exposed rats.

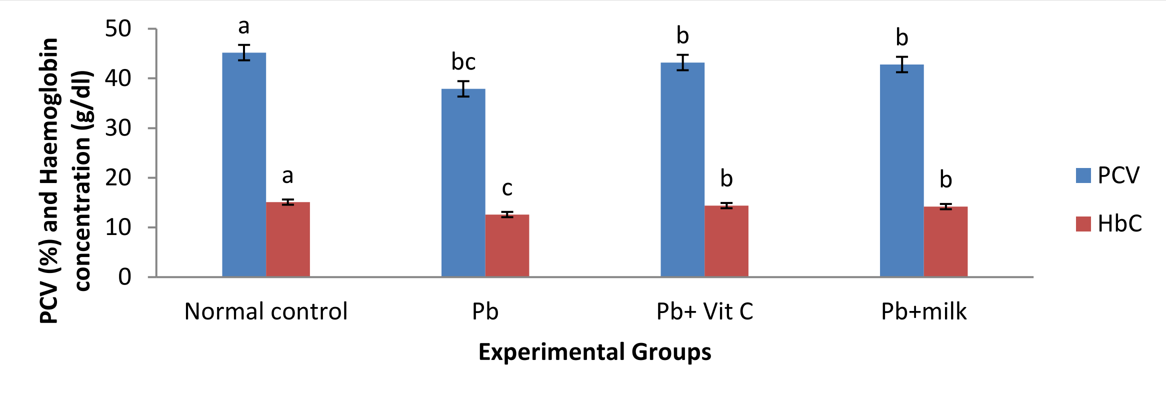

Figure 4. Effect of unsweetened milk and vitamin c supplements on packed cell volume (PCV) and haemoglobin concentration (HbC) of lead acetate exposed rats

Data are shown as mean ± SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly ($p<0.05$) different.

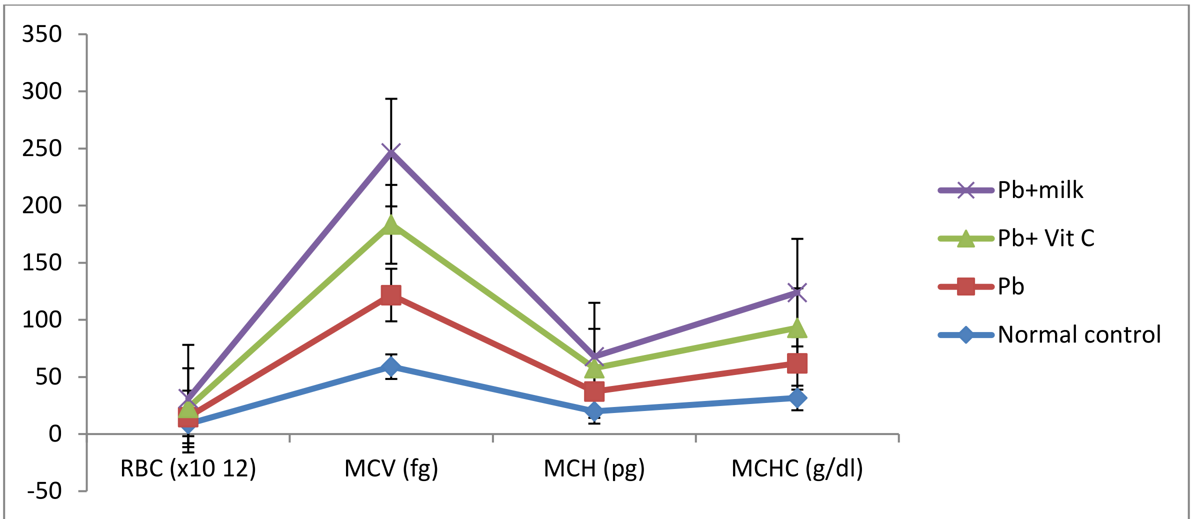

Figure 5. Effect of unsweetened milk and vitamin c supplements on red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and indices of lead acetate exposed rats

Data are shown as mean ± SEM (n=5). **SEM:** Standard error of means.

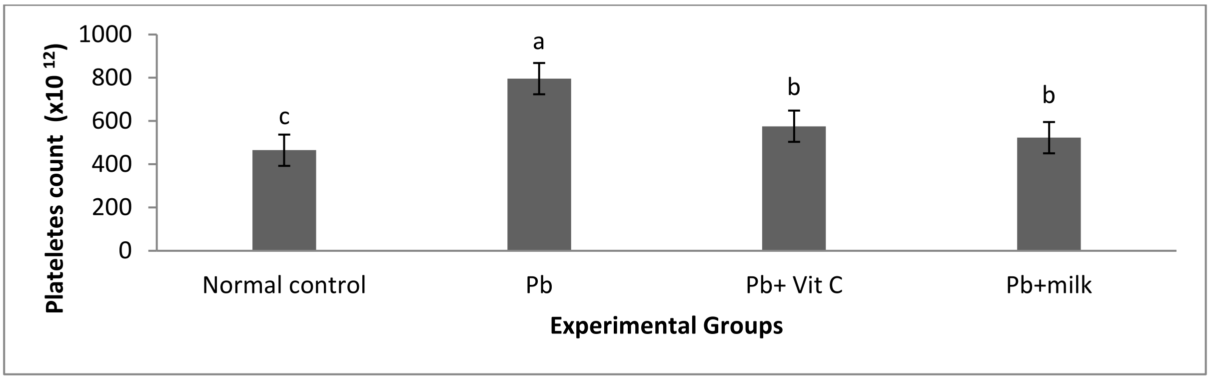

Figure 6. Effect of unsweetened milk and vitamin c supplements on platelets count of lead acetate exposed rats
Data are shown as mean ± SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly ($p<0.05$) different.

The findings from this study suggest that unsweetened milk and vitamin c supplements may have a positive influence on haematological parameters in lead acetate exposed rats as shown in figure 4. There was a significant ($P<0.05$) reduction in PCV level in lead acetate group when compared to the normal control. Groups that received unsweetened milk and vitamin c supplements showed significant ($P<0.05$) elevation in PCV level in relation to lead acetate group. This indicates that milk consumption may help maintain healthy red blood cell volume in the presence of lead exposure. The decrease in PCV and HbC in the lead acetate exposed group aligns with the known hematotoxin effects of lead, which can impair erythropoiesis and haemoglobin synthesis (Katarina et al. 2024, Wahab et al. 2010). The improvement observed in the lead acetate + vitamin C group implies a potential ameliorative outcomes of vitamin C on lead-induced haematological changes. Notably, the lead acetate + milk group displayed results approaching those of the normal control, indicating that milk consumption might have a positive influence on blood parameters of animals exposed to lead acetate (Doris and Jorge, 2020).The protective effect of milk could be attributed to its nutritional composition, including proteins, vitamins, and minerals. Calcium and other components in milk might counteract the hematotoxin effects of lead, contributing to the observed improvements (Christian et al. 2021). These findings suggest that incorporating milk into the diet may have haematological benefits for individuals exposed to lead. However, it is pertinent to conduct further studies, including human trials, to validate these results and understand the underlying mechanisms.
The results in figure 5, indicates a significant reduction in the red blood cell (RBC) count in the group that received lead acetate. This aligns with the general understanding of adverse effects of lead on haematopoiesis. The increase in MCV as shown in the present study indicates changes in the size of red blood cells, potentially suggestive of alterations in their maturation. The decrease in MCH and MCHC in the same group further supports the notion of lead-induced anaemia, an indication of a decrease in haemoglobin content per red blood cell and its concentration.
Conversely, the incorporation of 100mg/kg body weight of vitamin C and 400mg/kg body weight of milk seemed to have a positive effect on red blood cell count and related indices, suggesting a potential protective role. The effects of vitamin C were more pronounced in comparison to milk, whereas milk appeared to play a role in enhancing red blood cell count, the sharp decrease in MCH and MCHC suggests that it might have a unique influence on the haemoglobin content and concentration within each red blood cell (Scholz-Ahrens et al. 2003, Torres et al. 1995).

Understanding the mechanisms behind these observed effects, especially the unexpected changes in MCH and MCHC in the milk-supplemented group, calls for further investigation. It's essential to explore how milk components interact with lead and influence erythropoiesis, haemoglobin synthesis, and red blood cell characteristics

In figure 6, the platelet count of animals given lead acetate increased significantly when compared to the normal control. This elevation in platelet count with lead acetate exposure aligns with the known association between lead exposure and inflammatory processes. Meanwhile, vitamin C and milk intervention tends to mitigate this effect. This suggests potential protective effects of these substances against lead acetate induced platelet alterations. An elevated platelet count (thrombocytosis) can have clinical implications, including an increased risk of thrombosis. On the other hand, excessively low platelet counts (thrombocytopenia) can lead to bleeding disorders. Therefore, understanding the factors that influence platelet count, including dietary components like milk, is essential for assessing their potential impact on overall health and homeostasis, especially in the context of lead exposure (Wati et al. 2023).

Conclusions

Our findings suggest protective effects of unsweetened milk and vitamin C supplements on hepatotoxicity and hematological damages associated with exposure to lead acetate in rats. Hence, unsweetened milk and vitamin C possess hepatoprotective potentials that deserve to be further evaluated towards understanding the underlying mechanisms of their hepatoprotective potentials in lead toxicity.

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