ABSTRACT

Lead a free-radical generating agent is a multi-systemic toxicant which affects major body systems especially the hepatic axis. Several natural products rich in antioxidant agents have been used to ameliorate lead toxicity. Vitamin C present abundantly in palm wine has been noted for its ability to modulate oxidative stress. This study investigated the ameliorative effects of palm wine in lead-induced hepatotoxicity in Wistar rats. Adults Wistar rats randomly divided into Groups A-H, consisting of 7 rats per group were used for the study. Groups A and B were administered with distilled water and palm wine respectively. Groups C, E, and G were dosed daily with lead nitrate at dosage levels of 50 (low dose), 150 (intermediate dose) and 600 (high dose) mg/kg body weight (BW). On the other hand, Groups D, F, and H were administered daily with lead nitrate at dosage levels of 50, 150 and 600 mg/kg body weight (BW) as well as palm wine (10 mL/kg BW). All experimental animals were allowed access to standard feed and water without any form of restriction. Estimation of biochemical parameters i.e. total protein, albumin, alkaline phosphatase and aminotransferases (ALT; AST) took place using standard biochemical methods. The liver was harvested and processed for histological study using haematoxylin and eosin staining techniques.

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Statistical analysis was done using one-way analysis of variance (ANOVA) and Student’s t-test. P<0.05 was considered significant. While albumin concentrations were not significantly different, both total protein and globulin concentrations in lead administered rats were significantly reduced compared with control. Periportal and interstitial hepatitis and necrosis occurred from lead exposure at different levels suggesting hepatotoxicity. Meanwhile, lead and palm wine-administered rats featured similar histologic results. In conclusion, the results of the study, therefore, indicate that palm wine does not possess an ameliorative effect on lead-induced hepatotoxicity.

Keywords: Palm wine; lead; hepatic dysfunction.

1. INTRODUCTION

Lead is a cumulative, multi-systemic toxicant which affects major body systems; it is associated with several changes that include impairment of liver function [1]. Lead is known to cause histological liver damage and possibly disturb the normal biochemical processes, resulting in increased liver enzyme levels. Mechanisms of lead-induced liver injury include increased production of reactive oxygen species (ROS), and induced oxidative stress which results in DNA damage as well as distortion to the structures of other cellular biomolecules [2].

Occupational, as well as environmental exposures, remain a global health problem. Given the oxidative stress induction by Pb, a therapeutic strategy to elevate the antioxidant defences of the body may be of assistance in protecting from Pb toxicity, especially among those who are occupationally exposed, to which complete or absolute eradication of lead exposure cannot be achieved. This includes occupations such as auto-repairing, battery manufacturing, painting, solid waste management etc. National Institute for Occupational Safety and Health (NIOSH) revealed that elevated blood lead levels are common in individuals in these occupations especially those that do not use required personal protective equipment, in which not only regular blood monitoring of lead is required but various integrative measures are encouraged. Therefore, some naturally occurring agents (e.g. garlic, vitamins C and E) have been suggested for integrative intervention for combating high blood lead level.

Palm wine is a sweet, effervescent and alcoholic beverage obtained by the natural fermentation of the sap from various palm trees [3]. Palm wine has also been reported to have some nutritional components and mineral elements such as vitamins A and C, iron, copper, magnesium, calcium, and zinc [4,5]. Vitamin C is known to be a good antioxidant which could be of help in inhibiting the oxidative stress in hepatotoxicity induced by lead. Hence, this study is designed to investigate the ameliorative potential of palm wine in lead-induced hepatotoxicity.

2. MATERIALS AND METHODS

2.1 Materials

Chemical: Lead nitrate Pb(NO\textsubscript{3})\textsubscript{2} was procured from KADLAD NIGERIA LIMITED, Beside Capital Hotel, along Iwo-Ibadan Road, Osogbo.

Palm wine: Palm wine was gotten freshly from oil palm (Elaeis guineensis) after tap.

2.2 Methods

Study site: The animal house of the Department of Medical Laboratory Sciences, Ladoke Akintola University of Technology, Osogbo, Osun State.

Study design: The study is an experimental study.

Animal handling/animal model: Fifty-six adult Wistar rats with an average weight of 175 g, were purchased from the animal house of Ladoke Akintola University of Technology, Mercyland campus, Osogbo, Osun state. The rats were kept in cages and made to acclimatize for 7 days and maintained under standard conditions of temperature and humidity. The rats were randomly divided into eight (8) groups of seven (7) rats each. They were allowed access to standard feed and tap water ad libitum.

Lead administration: Lead nitrate was given through oral gavage daily at doses50 mg/kg, 150 mg/kg and 600 mg/kg body weight to induce hepatotoxicity in rats [6-8].

Administration of palm wine: Daily administration of palm wine took place orally using gavage needles, for 2 weeks. The dosage level =10 mL/kg body weight [9].
### Table 1. Experimental protocol table

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No Lead + No palm wine</td>
<td>Control</td>
</tr>
<tr>
<td>B</td>
<td>No Lead + Palm wine</td>
<td>Palm wine control</td>
</tr>
<tr>
<td>C</td>
<td>Low Lead dose (50 mg/kg) + No palm wine</td>
<td>Test</td>
</tr>
<tr>
<td>D</td>
<td>Low Lead dose (50 mg/kg) + Palm wine</td>
<td>Test</td>
</tr>
<tr>
<td>E</td>
<td>Intermediate Lead dose (150 mg/kg) + No palm wine</td>
<td>Test</td>
</tr>
<tr>
<td>F</td>
<td>Intermediate Lead dose (150 mg/kg) + Palm wine</td>
<td>Test</td>
</tr>
<tr>
<td>G</td>
<td>High Lead dose (600 mg/kg) + No palm wine</td>
<td>Test</td>
</tr>
<tr>
<td>H</td>
<td>High Lead dose (600 mg/kg) + Palm wine</td>
<td>Test</td>
</tr>
</tbody>
</table>

**Clinical observations and body weight measurement:** Every rat in each of the treatment group was observed twice daily (before and after exposure) for signs of clinical toxicity in the appearances of the skin and fur, eyes and mucous membrane, behavioural pattern, respiratory system and morbidity /mortality. The body weight of each animal in the treatment and control groups was measured at the beginning of the experiment and the end of the exposure.

**Protocol for blood collection and organ weight measurement:** At the end of the exposure period, after an overnight fast, animals were weighed before blood collection and then euthanized by cervical dislocation. Blood was collected through cardiac puncture. It was dispensed into lithium heparin bottle, centrifuged at 3000 rpm for 10 minutes to obtain plasma and stored at -20°C before biochemical analyses. The liver of the animals was surgically removed, rinsed with ice-cold physiological saline, blotted dry and weighed.

**Biochemical analysis:** Estimations of serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were as described by Reitman and Frankel [10] whereas estimation of serum activity of alkaline phosphatase (ALP) was as described by Babson et al. [11]. Meanwhile, the determinations of total protein and albumin were by Biuret (Gomall et al. [12]) and Bromocresol green (Doumas et al. [13]) methods respectively. Globulin levels were obtained by subtracting albumin concentration from that of total protein.

**Histopathological study:** The liver tissues from the experimental animals were fixed in 10% neutral buffered formalin. Representative samples were placed in cassettes and processed using the automatic tissue processor. After processing, the tissues were embedded in molten paraffin wax which formed solid support for microtome after cooling. The tissue blocks were cut into thin sections of 4 microns using a rotary microtome. They were stained with Haematoxylin and Eosin (H&E) staining technique for general tissue morphology.

**Statistical analysis:** Data obtained were expressed as mean ± standard deviation. Data will be subjected to one-way analysis of variance (ANOVA) and Student’s t-test. Significance differences were at p<0.05.

### 3. RESULTS

The results of the study are presented in Tables 2 and 3 and Figs. 1 and 2.

The study groups consisted of control; palm wine, low lead dose (50 mg/kg); low lead dose (50 mg/kg) + palm wine; intermediate lead dose (150 mg/kg); intermediate lead dose (150 mg/kg) + palm wine; high lead dose (600 mg/kg); and high lead dose (600 mg/kg) + palm wine administered groups. Some of the clinical manifestations of lead toxicity exhibited by the rats in the high lead dosage level included very dark coloured faeces; the mortality rate of ~30% and respiratory distress e.g. uncontrollable sneezing. Also, weight loss was observed in high lead dose group and its palm wine counterpart as demonstrated with percentage weight loss of 7.71% and 7.81% respectively. Meanwhile, with the cessation of contact to lead, percentage weight gain of 10.32% and 8.62% were observed in the lead and lead + palm wine treated groups respectively.

As shown in Table 2, both intermediate and high lead dosage levels as well as their palm wine administered counterparts presented with significantly reduced total protein levels compared with control (p= 0.001). In the same vein, the plasma globulin levels of the above four groups also were significantly decreased compared with the control group (p= 0.001). Albumin plasma concentration was not significantly different in palm wine and low lead
dose + palm wine administered rats compared with control as shown in Table 2. Similarly as shown in Table 2, it was observed that intermediate lead dose; high lead dose, and high lead dose + palm wine featured a non-significant difference in plasma albumin level compared with control (p>0.05).

In Table 3, all lead-exposed groups (low, intermediate, high) and their palm wine administered counterparts presented with non-significantly differences in their serum activity of alkaline phosphatase (ALP) compared with control (p>0.05). Contrary to this, the activities of ALP showed a significant decrease in the palm wine group when compared with control. At variance with other groups, the AST result of high dose lead group was significantly increased (p= 0.001) compared with control. The plasma activity of alanine aminotransferase (ALT) in low lead dose, intermediate lead dose and their palm wine counterparts presented with no significant differences compared with the control (p> 0.05). The plasma activity of ALT in palm wine group was significantly reduced compared with the control (p= 0.001). The histology results of the study areas presented in Figs. 1 and 2.

4. DISCUSSION

The results of histology revealed the hepatotoxic nature of lead nitrate at dosage levels of 50, 150, & 600 mg/kg. This is in agreement with the observations of Nakhaee et al. [14] as well as Jarrar and Taib [15] that identified alterations in hepatocytes and Kupffer cells as well as the sinusoids. Lead alters liver architecture through free radical generation. Free radicals are highly reactive entities that interact with cellular biomolecules leading to distortion of cell morphology. Of all organs susceptible to Pb toxicity, the liver is the most common depository of Pb [16]. It is the first organ exposed to enterally absorbed Pb via the portal system. This no doubt is reflected by the histologic results of the study in which features of lead exposure included dilated and congested vessels as well as mild periporal and interstitial hepatitis (50 mg/kg) and appearance of focal necrosis (150 & 600 mg/kg).

**Table 2. Serum concentrations of protein, albumin and globulins of experimental animals**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.71±1.44</td>
<td>34.86±0.63</td>
<td>42.86±1.03</td>
</tr>
<tr>
<td>Palm Wine</td>
<td>75.00±1.48</td>
<td>35.00±0.62</td>
<td>40.67±1.28</td>
</tr>
<tr>
<td>Lead (50 mg/kg)</td>
<td>78.00±1.59</td>
<td>37.33±0.84*</td>
<td>40.00±1.07</td>
</tr>
<tr>
<td>Lead (50 mg/kg) + Palm Wine</td>
<td>74.43±0.87</td>
<td>35.43±0.37</td>
<td>39.00±0.87</td>
</tr>
<tr>
<td>Lead (150 mg/kg)</td>
<td>71.43±1.04**</td>
<td>35.71±0.36</td>
<td>35.71±1.17*</td>
</tr>
<tr>
<td>Lead (150 mg/kg) + Palm Wine</td>
<td>68.00±0.62**</td>
<td>36.83±0.31*</td>
<td>31.17±2.59**</td>
</tr>
<tr>
<td>Lead (600 mg/kg)</td>
<td>71.33±3.71</td>
<td>32.33±1.20</td>
<td>39.00±2.65</td>
</tr>
<tr>
<td>Lead (600 mg/kg) + Palm Wine</td>
<td>68.80±0.80**</td>
<td>35.60±0.87</td>
<td>33.20±1.16**</td>
</tr>
</tbody>
</table>

*p-value:* 0.001 0.002 0.001

Data are expressed as Mean ± SEM (n = 7); ** Significantly different from control group at p<0.01; * Significantly different from control group at p<0.05

**Table 3. Activities of serum alkaline phosphatase, alanine and aspartate aminotransferases among test groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>ALP</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47.43±0.72</td>
<td>39.43±1.36</td>
<td>91.43±4.12</td>
</tr>
<tr>
<td>Palm Wine</td>
<td>41.00±1.20**</td>
<td>32.86±0.99</td>
<td>70.29±5.09**</td>
</tr>
<tr>
<td>Lead (50 mg/kg)</td>
<td>48.83±1.58</td>
<td>43.67±2.16</td>
<td>95.33±5.09</td>
</tr>
<tr>
<td>Lead (50 mg/kg) + Palm Wine</td>
<td>47.43±0.61</td>
<td>34.14±2.21</td>
<td>77.29±7.91</td>
</tr>
<tr>
<td>Lead (150 mg/kg)</td>
<td>46.80±2.48</td>
<td>36.29±3.04</td>
<td>96.43±2.50</td>
</tr>
<tr>
<td>Lead (150 mg/kg) + Palm Wine</td>
<td>51.33±3.13</td>
<td>33.67±3.54</td>
<td>95.00±1.84</td>
</tr>
<tr>
<td>Lead (600 mg/kg)</td>
<td>49.00±0.00</td>
<td>30.67±4.18*</td>
<td>103.67±2.60</td>
</tr>
<tr>
<td>Lead (600 mg/kg) + Palm Wine</td>
<td>46.20±2.92</td>
<td>24.00±2.07**</td>
<td>93.60±3.11*</td>
</tr>
</tbody>
</table>

*p-value:* 0.025 0.001 0.001

Data are expressed as Mean ± SEM (n = 7); *Significantly different from control at P < 0.05; **Significantly different from control at P < 0.01; Abbreviations: ALP- alkaline phosphatase; ALT- alanine aminotransferase; AST- aspartate aminotransferase
Normally, AST and ALT are present in high concentration in the liver. Due to hepatocyte necrosis, these enzymes are released from the cells and their levels are increased. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic disease is unusual. ALT is more selectively a liver parenchyma enzyme than AST [17]. When there is hepatopathy, liver enzymes leak into the bloodstream in conformity with the extent of damage [18,19].

According to Jalali et al. [19], upon lead administration, various markers of the liver are altered. Jalali et al. [19] reported a significant elevation in levels of hepatic enzymes AST and ALT upon lead exposure. In the study, elevation in activity of hepatic enzyme AST was observed as a result of lead exposure (at 600 mg/kg). The elevation in the activity of AST does not suggest that the liver is the only source especially as AST is widely recognized as a multi-organ enzyme. AST is found in liver, skeletal muscle, heart, kidneys, brain, and red blood cells. Interestingly, the toxic effects of lead occur in various organs as well. For example, lead adversely affects the nervous, hematopoietic, renal, cardiovascular and hepatic systems [20]. A significant amount of AST is found in RBCs. While lead through its inhibition of the enzymes; δ-aminolevulinic acid
dehydratase (ALAD), aminolevulinic acid synthetase (ALAS), and ferrochelatase prevents the process of erythropoiesis; it has also been reported that Pb decreases the life span of circulating red blood cells by increasing the fragility of cell membranes [21]. The later effect is capable of leading to an increase in AST activity in the blood. The fact that extra-hepatic sources of AST were likely in the present study is corroborated by the result of ALT. Although ALT was increased in 50 and 150 mg/kg, it was not significant.

On the other hand, in clinical enzymology it has been recognized that cell death leads to a short-lived increase in enzyme levels; raised enzyme levels are not synonymous with organ failure - highest levels are often seen in acute injury to previously healthy tissue. Conversely, organ failure (occurring from persistent exposure to a toxic agent) is associated with unimpressive enzyme increases. This may be the basis of a slight but non-significant increase in the levels of ALT at 50 and 150 mg/kg levels of exposure.

Alkaline Phosphatase (ALP) is a marker used to assess obstruction to bile duct among other diagnostic functions. Usually, elevated activity of serum ALP is due to the increased synthesis in the presence of increasing biliary pressure and is linked to regurgitation of bile. In all categories of lead-exposed rats, the activities of ALP were not different when compared with the control group. This is an indication that although lead nitrate altered hepatic cells morphologically, it did not result in significant biliary obstruction in the Wistar rats. Meanwhile, the possibility of lead-induced biliary obstruction has been raised by Can et al. [22] who reported increased ALP activity in lead-exposed battery and muffler repair workers compared with controls; although the values were within the normal range.

The present observation is in accord with the report of Andjelkovic et al. [23], in which the administration of 150 mg/kg of lead citrate did not result in a significant difference in albumin level. Unlike Andjelkovic et al. [23] who observed non-significant difference in total protein levels, a significant decrease was observed for total protein levels at 150 and 600 mg/kg levels of lead exposure for the present study, an indication that Pb exposure leads to a compromise of globulin synthesis as confirmed by the significant decrease in globulin levels. This may be fundamental for the initiation and progression of hepatotoxicity, which is evident in different histopathologic features in photomicrographs of lead-exposed rats. The ability of lead to altering globulin level has also been reported but Offor et al. [24] did not observe any significant change.

Of all the naturally occurring antioxidants, glutathione (GSH) is the most physiologically important. Glutathione exists in both reduced (GSH) and oxidized form (GSSG). GSH can be regenerated from GSSG by the enzyme glutathione reductase, this requires ascorbic acid. Vitamin C is found in palm wine of different sources [4]. It is the most common antioxidant found in palm wine. The persistent distortion in the liver architecture of lead/palm wine exposed groups suggests that vitamin C (or any other antioxidant present in palm wine) did not elevate the antioxidant potential of the groups sufficient enough to modulate the hepatotoxic effect of lead exposure. Meanwhile, large doses of vitamin C have been noted for their ameliorative effects on lead-induced toxicity [25]. Lack of amelioration in this study may be ascribed to a low level of vitamin C found in palm wine.

The liver that has rightly been described as the metabolic factory of the body metabolizes a wide range of naturally occurring products. It is assumed to be altered by alcoholic products. Yet the results of the present study revealed that palm wine administration caused a decline in hepatic enzymes, this is not in agreement with the observation of Oyewo et al. [26] who reported a significant increase in ALP level in the post-palm wine-exposed state. The differences in result outcome of various studies may be linked with sources, types and storage conditions of different palm wine used. The significant decrease in AST and ALT levels in rats administered with palm wine may be ascribed to increased clearance from the body. This naturally occurring product has been described to have diuretic properties. Moreover, whether it is capable of selectively altering the genetic machinery responsible for ALT and AST synthesis has not been elucidated.

The significant decrease in weight as a result of Pb exposure can be linked to declining in feed consumption; although the quantity of feed consumed was not monitored yet such possibility has been raised in an earlier study. Decreases in body weight were observed in high dose lead exposure group (600 mg/kg) as well as rats administered with both lead (600 mg/kg) and palm wine (with values of 7.71% and 7.81% respectively) compared with control. Hammond
et al. [27] reported that lead administration results in reduced feed consumption since Pb interacts with the appetite centre of the brain. That such effect may be reversible is supported by the fact that on cessation of lead administration there was a percentage weight gain of 10.32% and 8.62% in lead and lead cum palm wine treated groups respectively. The present observation of a reduction in body weight is in accord with the studies of Shakoor et al. [28]; Annabi Berrahal et al. [29]; Allouche et al. [30]; and Ibrahim et al. [31] also.

5. RECOMMENDATION

While the results of the study do not suggest possible ameliorative effects of palm wine on lead-induced hepatotoxicity, it raises the possibility of palm wine as a natural beverage capable of modulating hepatic markers. Some past studies also suggest such possibility, it is being recommended that the molecular mechanism by which this is achieved deserves further investigation to identify if it is due to increase clearance from the body or decrease in the transcription/biosynthetic process.

6. CONCLUSION

The histological study revealed periportal and interstitial hepatitis and necrosis of some areas which indicated that hepatotoxicity was achieved with lead administration. However, the study does not suggest possible ameliorative effects of fresh palm wine (Elaeis guineensis) on lead-induced hepatotoxicity. Meanwhile, palm wine is capable of altering biochemical markers of liver function.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animals received humane care following the principle of Laboratory Animal care of the National Society of Medical Research and Guide for the care and use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health Publication no. 80-23).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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