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Histological Effect of Coconut Water on Lead Induced Hepato-Toxicity in the Liver Sinusoids of Adult Male Wistar Rats

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Abstract

Lead is among the naturally occurring toxic metals in the earth's crust and has been known to pose a very great health hazard to young children and adults, with the liver being among the principal organs of assault. The work, therefore, was designed to study the histological effects of tender coconut water on lead-induced liver toxicity in adult male Wistar rats. Twenty-eight male Wistar rats obtained from the Pharmacological Department of Niger Delta University were divided into five groups and allowed to acclimatize for two weeks. The treatment groups received 2 mg/kg of lead acetate intraperitoneally and were followed by the administration of various concentrations of coconut water. The animals were maintained on a 12-hour cycle of water and growers' marsh. After four weeks of administration, the rats were sacrificed and their livers were processed and stained with H&E. Histopathological examination through liver staining revealed that the lead-only group developed liver damage represented by central vein congestion, focal necrosis, occluded sinusoidal space, and the presence of a number of Kupffer cells. The lead-treated groups given coconut water had developed similar liver damage. Rats in the coconut water-only treated group showed normal liver histology. In conclusion, the given dosages of coconut water did not protect against the toxic effect of lead on liver toxicity in adult male Wistar rats. A higher dose or variant treatment regimen could be researched for possible protection.

Key words: Coconut water, lead, Sinusoids, Hepato-toxicity.

Introduction

Coconut is a peculiar plant grown in tropical and subtropical regions [12]. This plant is essential in domestic, commercial, and industrial fields. It is regarded as a fruit that supplies nutrition to several populations because of its high-water content. Immature fruits are called tender or jelly nuts, which provide a refreshing drink when plucked. In addition to its significance as a primary component in cooking and frying, coconut water and milk are also key raw materials in the production of soap and cosmetics due to their beneficial characteristics. Various uses of coconuts include traditional medicine due to their potential health benefits from bioactive compounds such as saponins and flavonoids, among others [2]. The coconut water of Cocos nucifera is a natural liquid inside the core of the immature green coconuts, usually collected at 6 to 7 months of maturation; some liquid, however, remains in the more mature coconuts [13]. In general, this liquid is consumed as a beverage and possesses several health therapeutic benefits. Cocos nucifera is rich in essential nutrients, consisting of minerals, vitamins, and antioxidants, which are important in reducing oxidative stress and liver health [15]. It has also been researched for its effectiveness against hepatotoxic agents, including heavy metals such as lead. Lead is a naturally occurring toxic metal in

the Earth's crust, recognized for its widespread uses and persistence in the environment, making it a major public health concern [17]. Lead poisoning can target several physiological systems, especially in young children and females of reproductive age [11]. Target organs include the brain, kidneys, and skeletal system. The mobilization of lead from bones into the blood circulation system, particularly during pregnancy, is yet another route through which lead can pose risks to the developing fetus. The most common sources of lead poisoning include industrial processes of mining, smelting, and recycling, and lead-containing products such as paints, pipes, and some traditional medicines. These exposures cause severe hepatic injury, manifested mainly by oxidative stress, inflammation, and degeneration of hepatocytes. The liver is one of the main organs that is involved in detoxification processes; therefore, it is highly susceptible to lead poisoning, which may cause chronic health disorders [10]. With this background, an extensive investigation has been carried out into its medicinal oral usage, although there is minimal attention to its anti-inflammatory and hepatoprotective capabilities on lead-induced toxicity. Histological effects are, therefore, assessed regarding the outcomes of coconut water on lead-induced hepatic toxicity in adult male Wistar rats. This study, thus, evaluates the potential therapeutic advantages of coconut water by mitigating the toxic effects of lead exposure through the maintenance of liver cytoarchitecture and reduction of oxidative damage.

Materials and Methods

Location of Study/study area

The study was carried out in Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island Amasomma, Bayelsa state.

Procurement Of *Coco Nucifera* Extract and *Lead acetate*

The seeds of the plant *Coco Nucifera* were obtained from Amasomma, Bayelsa State.

Animal Housing

Twenty-eight albino rats weighing 130.58g+ 179.6g- were used for this study. These rats were obtained from the Animal House of the Pharmacology Department of Niger Delta University, Bayelsa State, Nigeria. They were housed under standard temperature (27- 20C) with twelve hours of light and dark periodicity. These animals were housed in clean gated groups and fed on standard feed pellets (Guinea Feed Nigeria Plc) and clean water ad libitum throughout the study. ACC limitation was for two weeks. Animals were handled in the study according to institutions' guidelines for experiments involving the use of animals.

Experimental Design

The rats were weighed and divided into five groups. The duration of this study was for four weeks the animals were allowed to acclimatize for two weeks. After the acclimatization period 28 rats were randomly divided into 5 groups.

Lead Acetate

Lead acetate amehydrate, Molychem (India) was purchased from K-prime medical and drug store, Yenagoa, Bayelsa state.

GROUPS	Group 1 5	Group 2 6	Group 3 6	Group 4 6	Group 5 5
Treatment Regime	Control (No administration)	Lead acetate only (2ml/kg)	Lead acetate (2ml/kg) and Coconut water (1.3ml)	Lead acetate (2ml/kg) and Coconut water (0.9ml)	Coconut water only (1.1ml)

Table 3.1: Experiment designs showing groups administration of extract and Acetic acid.

Statistical Analysis

This study investigates the effects of lead acetate and coconut water on the weights of male Wistar over two weeks. Twenty-eight albino rats were divided into seven groups with specific treatments, including control, lead acetate, lead acetate with varying doses of coconut water, and only coconut water. Initial weights were recorded, and treatments administered accordingly. Descriptive statistics, including mean initial weights and standard deviations, were calculated for each group. A one-way ANOVA revealed significant differences in weight changes among the groups ($p < 0.05$). Post-hoc analysis indicated that the group treated with only lead acetate had significantly different weight changes compared to the control and coconut water groups. The results suggest that lead acetate and coconut water significantly affect rat weight changes, warranting further investigation into the mechanisms involved.

Results

4.0 Histology Photomicrograph Plates

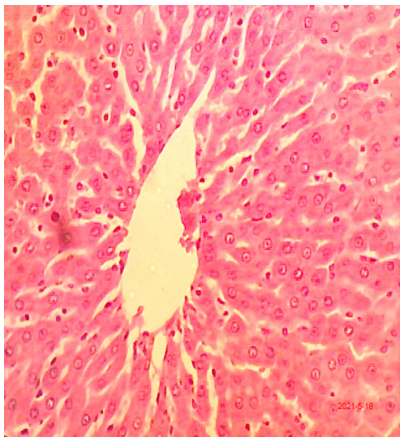

PLATE 4.1: Shows the morphology of the liver after the administration of the various treatments for 14 days. Slide shows normal morphology of the liver,central vein (yellow arrow),hepatocytes with intact sinusoidal space (black arrow) (X400) H&E.

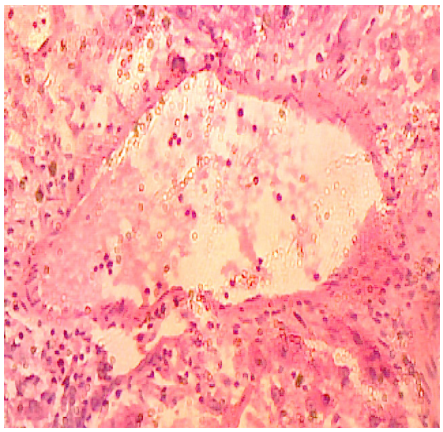

PLATE 4.2: Shows the morphology of the liver after the administration of the various treatments for 14 days. Slide shows congestion of the central vein (yellow arrow),areas of focal necrosis (black arrow) ,occluded sinusoidal space with marked presence of Kupffer cells (X400) H&E.

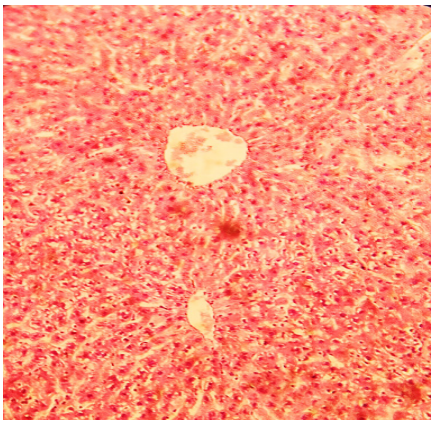

PLATE 4.3: Shows the morphology of the liver after the administration of the various treatments for 14 days. Slide shows congestion of the central vein (yellow arrow),occluded sinusoidal space (black arrow),balloon degeneration of hepatocytes with marked presence of Kupffer cells (X400) H&E.

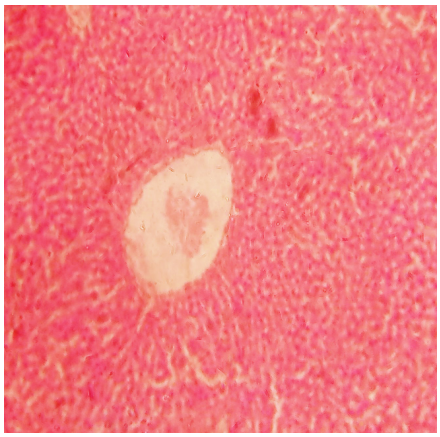

PLATE 4.4: Shows the morphology of the liver after the administration of the various treatments for 14 days. Slide shows congestion of the central vein with degeneration of it lining wall (yellow arrow) ,occluded sinusoidal space (black arrow),balloon degeneration of hepatocytes with marked presence of Kupffer cells (X400) H&E.

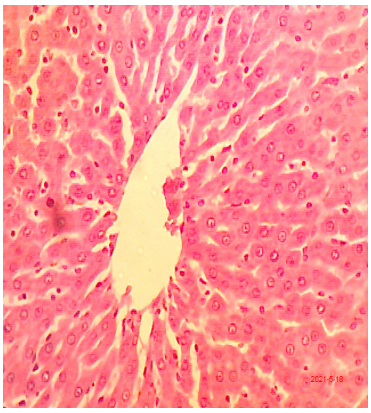

PLATE 4.5: Shows the morphology of the liver after the administration of the various treatments. Slide shows normal morphology of the liver, central vein (yellow arrow), hepatocytes with intact sinusoidal spaces (X400) H&E.

Discussion

Plate 4.1 -4.5 shows the photomicrograph of the liver after coconut water administration in lead-induced toxicity.

The control group showed the normal morphology of the liver, central vein, and hepatocytes with intact sinusoidal space.

The lead group showed the morphology of the liver, the slide shows congestion of the central vein, areas of focal necrosis, and occluded sinusoidal space with marked presence of Kupffer cells this is in agreement with findings from the work carried out by [7] in which Adult male Wistar albino rats (Rattus norvegicus) were exposed to lead acetate trihydrate in drinking water (0.0%, 0.25%, 0.5%, 1% and 2% for 1–12 months) to investigate histological and histochemical alterations induced by lead intoxication in the liver. Chronic exposure to subtoxic lead levels caused alterations in the hepatocytes, portal triads, and sinusoids. The alterations in the hepatocytes were mainly anisokaryosis, nuclear vesiculation, binucleation, cytoplasmic inclusions, cytoplasmic swelling, hydropic degeneration, necrosis, and reduction in glycogen content [6].

In addition, portal triads mild chronic inflammation, Kupffer cells hyperplasia, and occasional fatty change were seen together with hemosiderosis. No portal fibrosis or cirrhosis was detected due to chronic sub-toxic doses of lead exposure in the liver of any member of the dose groups over the entire period of the study. Chronic lead exposure also increased the activities of alkaline phosphatase and α-glycerophosphate-dehydrogenase which might be an adaptation to the metabolic, structural, and functional changes in the organelles of hepatic cells due to lead intoxication. The findings revealed that chronic exposure to lead produced significant histological and histochemical changes in the liver of the Wistar albino rats [7]. Also, another study was carried out by [9] in which the effect of sodium selenite on lead-induced toxicity was studied in Wistar rats. There were statistically significant changes in liver function tests, antioxidant enzyme activities, and lipid peroxidation levels in lead nitrate and sodium selenite and lead nitrate treated groups, as well as in diabetic and non-diabetic groups. Furthermore, histopathological alterations were demonstrated in the same groups the study found that sodium selenite treatment did not show a completely protective effect on diabetes mellitus-caused damages, but diabetic rats are more susceptible to lead toxicity than non-diabetic rats [9].

Another study in agreement with our work is the study carried out by [14] to evaluate the effect of different doses of lead acetate (1/20, 1/40, and 1/60 of LD50) on body weight gain, blood picture, plasma protein profile and the function of liver, kidney and thyroid gland. The results showed that the ingestion of Pb2+ induced significant stimulation in glutamic-pyruvic transaminase (ALT) and glutamic-oxalacetic transaminase (AST) activity. Also, total soluble protein and albumin contents of plasma were significantly decreased, while the content of globulin was changed by the Pb2+ treatments. The cholinesterase activity was inhibited, but the activities of alkaline and acid phosphates, and lactate dehydrogenase were stimulated, while plasma glucose levels were enhanced as a result of lead acetate intoxication. In the case of the blood picture, Pb2+ ingestion reduced the contents of hemoglobin and RBC count of intoxicated rat's blood, and the plasma levels of T3, T4, and blood WBC count were decreased. It can be concluded that lead acetate hurts experimental male albino rats. Therefore, the present work advises people to prevent exposure to lead compounds to avoid injurious hazard risks [14].

The morphology of the liver of the experimental group shows congestion of the central vein, occluded sinusoidal space, and balloon degeneration of hepatocytes with a marked presence of Kupffer cells. It also indicates congestion of the central vein with degeneration of its lining walls. In contrast to our study, a study was carried out by [5] to determine the ameliorative effect of coconut water on hematobiochemical changes due to lead poisoning in Wistar albino rats for six weeks. The mean values of red blood cells, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, and platelets reduced in lead-treated rats when compared with control mean values and these values increased in75ml coconut water only group, and the group of 0.10g/l lead + 75ml coconut water except mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red blood cell distribution width of the 75ml coconut water only. The mean values of white blood cells, lymphocytes, total cholesterol, triglyceride, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol/high-density lipoprotein cholesterol, and total cholesterol/high-density lipoprotein-cholesterol increased in the lead group when compared with mean values of the control group but the mean values decreased when compared with the mean values of a group of 75ml coconut water only and a group of 0.10g/l lead + 75ml coconut water, except the mean values of high-density lipoprotein-cholesterol. These results indicate that coconut water could ameliorate the effects of lead toxicity [8]. Another research in contrast to our work is by [3] to investigate the effect of tender coconut water to prevent anemia in lead-induced rats. The study revealed decreased levels of hematocrit, hemoglobin, and erythrocytes in the lead group compared with the control group. The tender coconut group had considerably higher levels of hematocrit, hemoglobin, and erythrocyte compared to the lead group. Another study by [4] examined the ameliorative potentials of coconut water on carbon tetrachloride (CCl4) induced toxicity in rats. Treatment with coconut water significantly ($p<0.05$) increased red blood cell, packed cell volume, hemoglobin, high-density lipoprotein, glutathione, superoxide dismutase, catalase, total protein, and albumin compared to the negative control in both sexes of the rats.

Furthermore, platelets, white blood cells, urea, low-density lipoprotein, triglyceride, total cholesterol, malondialdehyde, bilirubin, alkaline phosphatase, alanine, and aspartate transaminases decreased significantly ($p<0.05$) compared to the negative control in both male and female rats. Thus, coconut water supplementation may reverse CCl4-induced toxicity and distortions on hematological parameters, lipid profile and antioxidant enzymes, and liver and kidney biomarkers in rats [4]. Another study by [18] evaluated the hepatoprotective activity of hydroalcoholic leaf extract of Leucas aspera on male albino Wistar rats. The hepatoprotective activity of hydroalcoholic leaf extract of Leucas aspera on male albino Wistar rats at 400 mg/kg was evaluated during exposure to lead acetate at an oral dose of 50 mg/kg. A dose of 400 mg/kg hydroalcoholic leaf extract of Leucas aspera showed a significant reduction in the liver enzymes ($P< 0.05$) in the dose-dependent manner. Hence the hydroalcoholic leaf extract of Leucas aspera has shown hepatoprotective activity [19].

The group administered with coconut only shows normal morphology of the liver, central vein, and hepatocytes with intact sinusoidal spaces. Studies have shown that Fresh coconut water has traditionally been used for oral rehydration. It provides several nutritional, physiological, and medicinal benefits including anti-fungal, antioxidant, antibacterial, hypoglycemic, anti-cancer, anti-tumor, anti-dermatophyte, antiviral, anti-parasitic and hepatoprotective properties [4]. These biological activities have been attributed to their high concentration of bioactive chemicals. Researchers have also uncovered that virgin coconut oil has anti-inflammatory, analgesic, antipyretic, immune-stimulatory, and anti-cancer [5]. A study by [5] revealed that in the histology of the liver and kidney, pathological changes were evident; however, there was slight enlargement of the central veins and tubules, respectively, compared to the negative control. Coconut water administration revealed a dose-dependent protection compared to the untreated group. Injuries in the liver included deformed hepatocytes, dilated sinusoids, microvascular steatosis, and an enlarged central vein, while congested blood vessels and dilated tubules were detected in the kidney of the negative control. Coconut water (CW), a natural drink exorbitantly available in many tropical countries is used as an isotonic beverage as CW contains electrolyte levels similar to human blood. The richness of macro and micronutrients in CW is reported to possess many medicinal properties including hypolipidemic, cardioprotective, antihypertensive, and hepatoprotective effects [16]. [16] in their studies on the Oral administration of Mature Coconut Water in diabetic rats showed a significant reduction in blood glucose and glycated hemoglobin levels with an improvement in plasma insulin levels. Activities of carbohydrate metabolizing enzymes were higher in Mature Coconut Water treated diabetic rats along with increased concentration of liver glycogen. Histopathological analysis of the pancreas revealed that treatment with Mature Coconut Water reduced the pancreatic damage induced by alloxan and stimulated β-cell regeneration in diabetic rats. The overall results show that MCW exerts significant antihyperglycemic potential and could be developed as a potent drug candidate or nutraceutical for the management of diabetes and associated complications [16]. [1] evaluated the anti-oxidative and anti-inflammatory effects of coconut water (CCW) and milk (CCM) in phenylhydrazine (PHZ)-induced hemolysis and hepatotoxicity in the rat with possible adjunctive effects with dexamethasone (DXM).

PHZ caused a significant reduction in concentration of all the hematological parameters, with elevated serum aminotransferases. However, CCW and CCM alone, or in combination with DXM significantly attenuated the PHZ-induced hematotoxicity and hepatoxicity. CCW and CCM effectively restored PHZ's elevated LDL and TG with decreased HDL. Elevated tissue Malondialdehyde (MDA), Myeloperoxidase (MPO), Tumor Necrosis Factor (TNF-α), Interleukin 1beta (IL-1β) and nuclear factor-kappa B (NF-kB) in response to PHZ were all mitigated by CCM and CCW, in favor of glutathione peroxidase (GPX), total superoxide dismutase (T-SOD) activities, total antioxidant capacity (TAOC), Interleukin 10 (IL-10) and nuclear factor erythroid 2-related factor 2 (NrF2). Histopathology revealed that only a combination of CCM or CCW with DXM could distinctively reverse

PHZ-induced alterations like macrovesicular steatosis and periportal inflammation. Phenylhydrazine-induced toxicity is mitigated by either coconut water and milk or in combination with dexamethasone via oxidative stress reduction and inflammation suppression. [3] also documented in their study aimed to evaluate the anti-diabetic effect of mature coconut water on some biochemical parameters of liver and kidney functions, and histological architecture of the pancreas in alloxan-induced diabetic rats, that mature coconut water can be included in the treatment regimen for diabetes mellitus due to its hepatorenal protective functions.

Conclusion

Central vein congestion, occluded sinusoidal space, ballooning degeneration of hepatocyte, and presence of Kupffer cells in the liver of the treated animals with coconut showed that coconut water at the used dose in this study could not reduce lead toxicity in Wistar rats. Further studies could be done using higher doses of coconut water.

Conflict of Interest

There is no conflict of interest whatsoever in this research work.

Acknowledgement

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