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Research Article

Hepatoprotective effects of palm oil and coconut water in the serum of Wistar rats exposed to cadmium chloride contaminated diet.

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ABSTRACT: To study the hepatoprotective effects of palm oil and coconut liquor in the liver, twenty four Wistar rats were exposed to moderate level of cadmium chloride for 6 weeks. The rats were divided into four Groups of 6 rats each. Group I animals received distilled water and a diet free of CdCl₂ while Group II animals were given CdCl₂ contaminated diet of 50mg/kg diet. Also, Group III and IV animals were administered 3ml palm oil and 3ml coconut water respectively along side fed with a CdCl₂ contaminated diet of 50mg/kg diet for the same duration. The palm oil and coconut water were given orally once daily by gavage. CdCl₂ caused an elevated increase in the levels of ALT, AST and ALP which was significantly ($p < 0.05$) different from the control and other Groups. On the contrary, palm oil and coconut water decreased ALT, AST and ALP to a significantly ($p < 0.05$) level. There was also a drop in the protein level of the CdCl₂ administered Group alone when compared with the palm oil and coconut water Groups, indicating loss of hepatocytes. In conclusion, this study reveals that palm oil and coconut liquor possess properties that can be able to ameliorate CdCl₂- induced toxicity (which is dose dependent) and this supports its usage in local treatment as an antidote upon the accidental consumption of some xenobiotics.

KEYWORDS: Cadmium chloride (CdCl₂), coconut water, hepatoprotective, palm oil.

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INTRODUCTION

Food contamination has become a big problem in the world today (WHO, 1992). It refers to the presence of harmful chemicals and microorganisms which can cause consumer illness. The impact of chemical contaminants on consumer health and well-being is often apparent only after many years of processing prolonged exposure at low levels (Okorie *et al.*, 2014). Chemical contaminants present in foods are often unaffected by thermal processing unlike most microbiological agents (Valdes and Ziobro, 2000).

One common chemical contaminant of food is cadmium chloride. Cadmium chloride is a metal salts that is widely used in industries. It is a toxicologically important contaminant which accumulates in the body and inhibits a number of enzyme containing sulfhydryl Groups (Rhman *et al.* 2011; Latinwo, 1997). It is made up of cadmium and chloride with the formula, $CdCl_2$. Cadmium is a chemical element with the symbol Cd and atomic number 48. This soft, bluish-white metal is chemically similar to the two other stable metals in Group 12, zinc and mercury. Like zinc, it prefers oxidation state +2 in most of its compounds and like mercury it shows a low melting point compared to transition metals. Cadmium is also an environmental hazard. There have been a few instances of general population toxicity as the result of long-term exposure to cadmium in contaminated food and water, and research is ongoing regarding the estrogen mimicry that may induce breast cancer (Rhman *et al.*, 2011; Mann, 2012). It was reported that the maximum tolerable dietary $CdCl_2$ level for domestic animals was 0.5ppm. Dietary concentrations of 1ppm result in undesirable effects while 5ppm caused adverse health effects (Manca *et al.*, 1991; McDowell, 1992).

Most natural substances like herbs and fruits have been and are still being tested in experiments to discover their effects in xenobiotic management in the biological system (Sirajudeen *et al.*, 2006). Some examples of these substances are coconut water and palm oil.

Coconut water, the clear liquid endosperm obtained from immature coconuts, is a refreshing and nutritious beverage, widely consumed around the world due to its beneficial health properties. (Pummer *et al.*, 2001; Paniappan, 2002). In addition to protect against induction of myocardial infarction (Anurag and Rajamohan, 2003), coconuts water plays an important role for oral rehydration and even for intravenous hydration of patients in remote regions (Campkell-Falck *et al.*, 2000; Barclay, 2011). Results obtained from one study suggest that tender coconut water (TCW) treatment could prevent and reverse high blood pressure induced by high fructose diet probably by inhibition of lipid peroxidation, upregulation of antioxidant status and improved insulin sensitivity (Bhagya *et al.*, 2010).

Palm oil (also known as dendê oil, from Portuguese) is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palms, primarily the African oil palm

Elaeis guineensis (Reeves *et al.*, 1979), and to a lesser extent from the American oil palm *Elaeis oleifera* and the Maripa palm *Attalea maripa*. The benefit of palm oil in both the industry and in medicine cannot be overemphasized. The presence of high amount of Vitamin E (α tocopherol) in palm oil has shown positive health benefits. Tocotrienols from red oil is a potent inhibitor of lipid peroxidation and protein oxidation (Kamat and Devasagayam, 1995).

Locally in some part of Delta State, Nigeria (*Uhrobo* land); palm oil and coconut water are used as first line treatment when poisonous, toxic substances or xenobiotics (such as kerosene or crude oil products) are accidentally consumed by individuals. It is on the bases of this folk assumption that this present study attempts to evaluate the hepatoprotective effect of palm oil and coconut liquor on some serum biomarkers of liver damage in rats fed on a moderate dose of cadmium chloride contaminated diet.

MATERIALS AND METHODS

Collection Plant Material

Palm Oil: In other to avoid adulteration, fresh palm oil used was purchased from the Okitipupa Oil Palm Mill Ltd, Ondo State, Nigeria.

Coconut liquor: Coconut water from mature coconuts (*Cocosnucifera* L., Arecaceae) of 10-12 months of age, (West Coast Tall variety) grown in the University campus were used for this study. The coconuts were dehusked and liquid endosperm was collected filtered and pooled. The pooled mature coconut water was stored at 0- 4 °C and used for the experiment. It was freshly reconstituted with distilled water prior to administration to rats.

Experimental Animal

Twenty-four white albino rats (Wistar strain, weighing 160-200 g) used for this study was purchased from the Animal Unit, College of Medicine, Ambrose Ali University, Ekpoma, Edo state, Nigeria. They were divided into four experimental Groups of six rats per Group. Member of each Group were housed in standard rat cages (Griffin and George Modular Cage System; model YSM 580 cage base and YSM 600-540 cage top) and allowed to acclimatize to laboratory conditions for 7days before the commencement of the experiment. All rats were allowed access to drinking water and chow (feed)-product of Edo Feeds and Flour Mill (EFFM), EWU, Edo State, Nigeria. Permission and approval for animal studies were obtained from the College of Health Sciences Animal ethics committee, Delta State University, Abraka. This study was carried out between the periods of October – December, 2014.

Animal Treatment

Animals in Group I which served as the positive control were fed normal diet and distilled water only ($CdCl_2$ free) for 6 weeks. Rats in Group II received cadmium contaminated diet

(50 mg CdCl₂/Kg diet) alone for 6 weeks. Group III animals were given orally by gavage, 3ml Palm oil/ Kg body weight once daily for same period (at the early hours of the day), before the commencement of feeding with cadmium contaminated diet (50 mg CdCl₂/Kg diet). For the same duration and in the same manner, rats in IV received 3ml Coconut water/ Kg body weight and 50 mg CdCl₂/Kg diet. All animals were allowed access to drinking water.

Average body weight and body weight gain was measured weekly for each Group. Also, the amount of feed left over, were collected and measured. The administration of moderate dose of 50 mg CdCl₂/Kg diet was adopted as described by Noël *et al.*, 2004.

Preparation of Serum and tissues homogenate:

Each rat was anaesthetized in di-ethyl ether saturated chamber and under such condition; the thoracic and abdominal regions were opened to expose the liver and kidney. Blood was obtained via heart puncture by means of a 5ml disposable hypodermic syringe and needle and placed in plain to clot in ice-cold bottles. The blood samples were centrifuged at 1000x g (Uniscop Model SM 902B Bench Centrifuge) for 10 minutes at room temperature in order to obtain serum samples. They were collected and stored frozen until required for assay which was performed within 48 hours.

Biochemical Assay

The biochemical investigation of the samples was carried out with the following commercially available kits as supplied by TECO Diagnostic, Anaheim, USA. Alkaline phosphatase (ALP) as described by Tietz and Shuey (1986), aspartate aminotransferase (AST) (Bergmeyer *et al.*, 1985) and alanine aminotransferase (ALT) (Klauke *et al.*, 1993) were adopted. Also, serum protein determination was examined by following the method of Lowry *et al.*, (1951) using bovine serum albumin (BSA), at 660nm.

Statistical analysis

One-way ANOVA with Dunnett's post test was performed using GraphPad Prism version 4.00 for Windows, GraphPad software, San Diego California USA. Difference were considered to be statistically significant at $p < 0.05$.

RESULTS

After 6 weeks of administration, there was no death or physical sign of toxicity in all the animals throughout the period of study, except for the reduced activity in some animals in Group II (CdCl₂ alone). A significant ($p < 0.05$) reduction in food intake was observed for the cadmium chloride treated Group alone when compared with the control and other Groups (Table 1). Although a significant reduction in food intake was observed for the palm oil and coconut treated Groups respectively when compared with the control,

this was significantly higher than that observed for the Cd administered Group alone (Table 1). Result from table 1 also revealed a significant ($p < 0.05$) decrease in body weight again for all cadmium treated Groups when compared to the control.

The result from Table 2 showed that the administration of cadmium chloride caused a significant ($p < 0.05$) increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) when compared with the control rats. However, animals pre-treated with palm oil and coconut liquor significantly reduced these enzymes below levels that can cause hepatic damage. Similarly, serum albumin in CdCl₂ treated Group alone was significantly ($p < 0.05$) reduced compared with the control and animals that were pre-treated with palm oil and coconut water (Table 2).

DISCUSSION

Cadmium toxicity is directly related to cadmium absorption and retention in some tissues such as liver, kidney and gastrointestinal tracts (Rhman *et al.*, 2011). Following oral administration, the most common effects induced by short-term exposure to cadmium chloride are reduced growth, alterations in organ weights or histopathology (particularly of the kidney, testes, liver, and intestine), and effects on the immune system (Borzelleca *et al.*, 1989).

The liver is a major site for xenobiotic metabolism and is thus prone to oxidative damage by toxicants, pro-drugs or pro-carcinogens. The highest concentrations of cadmium in the human body accumulate in the kidney (particularly the cortex) and the liver, although the metal can be detected in virtually all tissues (Elinder, 1985). The result from this study (Table 1) indicated that, after 6 weeks of administration, a reduced feed intake as well as reduced weight gain in the cadmium chloride treated Group alone was observed when compared with the control and other Groups. Although palm oil and coconut liquor showed a significant ($p < 0.05$) increase in feed intake per week and body weight gain when compared with the CdCl₂ Group, the value obtained was significantly ($p < 0.05$) lower from the control Group. Previous studies has shown that high dietary levels of Cd results in suppressed feed intake and weight gain, reduction in bone mineralization and anaemia (Vallec and Ulmer, 1972; WHO, 1992). The reason for this decrease in weight gain and feed intake by CdCl₂ may not be clear but previous studies have suggested that CdCl₂ might interfere with the absorption and metabolism of some dietary nutrient because of its solubility (Vallec and Ulmer, 1972). Also, the biochemical alteration which occurs prior to morphological changes in the organs and the changes in certain enzyme levels in extracellular fluids may reflect the extent of Cd-induced damage in target organs (Khandelwal *et al.*, 1991, Saskia *et al.*, 2014). Palm oil and coconut liquor showed positive effects in ameliorating the toxicity of Cd by improving weight loss (Table 1).

Table 1: Mean intake and change in body weight and body weight gain in rats fed with cadmium chloride contaminated diet.

Treatment (g/kg)	Food intake (g/weeks/rat)	Body Weight (g)	Body Weight gain (g)
Group I (Control)	212.3 ± 4.11	199 ± 5.1	50 ± 1.6
Group II (CdCl ₂ /kg diet) only	159.4 ± 1.11 ^{***}	147 ± 3.3 ^{***}	17 ± 3.5 ^{***}
Group III (CdCl ₂ /kg + 3ml Palm oil/kg)	188.5 ± 0.94 [*]	166 ± 1.8 [*]	29 ± 3.1 [*]
Group IV (CdCl ₂ /kg + 3ml Coconut water/kg)	179.7 ± 1.20 [*]	162 ± 2.5 [*]	31 ± 2.9 [*]

Values are expressed as Mean ± SD of 6 rats per group (n=6). p < 0.05 values are considered to be statistically significant. Values with the superscript ^{***} are significantly different from the control and other groups. Values with ^{*} are significantly different from control.

Table 2: The effect of Palm oil and Coconut liquor on Cadmium Chloride contaminated diet.

Treatment (g/kg)	ALT (IUL ⁻¹)	AST (IUL ⁻¹)	ALP (IUL ⁻¹)	Albumin (mg/ml)
Group I (Control)	52.5 ± 3.5	15.7 ± 0.9	133.8 ± 5.5	4.02 ± 1.2
Group II (CdCl ₂ /Kg diet) only	111.3 ± 2.3 ^{***}	30.2 ± 1.2 ^{***}	222.3 ± 11.7 ^{***}	2.03 ± 3.1 ^{***}
Group III (CdCl ₂ /Kg + 3ml Palm oil/kg)	89.0 ± 2.7 [*]	23.5 ± 2.3 [*]	135.1 ± 6.0	3.86 ± 2.9
Group IV (CdCl ₂ /Kg + 3ml Coconut water/kg)	88.8 ± 1.9 [*]	21.1 ± 1.9 [*]	137.4 ± 5.7	4.49 ± 1.6

Values are expressed as Mean ± SD of 6 rats per group (n=6). p < 0.05 values are considered to be statistically significant. Values with the superscript ^{***} are significantly different from the control and other groups. Values with ^{*} are significantly different from control.

In the assessment of liver damage by cadmium chloride, the determination of enzyme marker levels such as ALT and AST is often used. In necrosis or membrane damage, the enzymes are released into circulation and it can therefore be measured in serum as markers of hepatic damage. High levels of AST and ALT indicates liver damage, such as in viral hepatitis as well as cardiac infarction and muscles injury (Moss and Butterworth, 1974). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978, Saskia *et al.*, 2014). Similarly, serum ALP level on the other hand is related to the function of hepatic cell.

In this study, we demonstrated the effectiveness of palm oil and coconut liquor by the administration of CdCl₂ contaminated diet in rats, which is a known model for hepatic injury. Although the liver has a good number of defence mechanisms against oxidative stress caused by toxicants, long term exposure to these toxicants as well as increased concentration might deplete the level of protection hence leading to liver damage (Oyabemi and Odetola, 2010).

Results from Table 2 revealed significant increases in the levels of AST, ALT, and ALP in the CdCl₂ treated Group alone when compared with the control and other Groups. The elevated serum liver enzymes can be attributed to the damage in the histostructural integrity of the liver hepatocytes (Kaplowitz, 2001). Furthermore, the palm oil and coconut water Groups showed significantly reduced level in AST, ALT and ALP when compared with the CdCl₂ Group alone. The abnormal high level of serum ALT, AST and ALP observed in this study (Table 2) are the consequences of CdCl₂ induced liver damage to the hepatocytes. Treatment with palm oil and coconut water respectively reduced the enhanced level of serum ALT, AST and ALP, which seems to offer protection and maintain the functional integrity of hepatic cells.

Previous research has shown that palm oil and coconut water prevents lipid peroxidation of cells especially in the liver because they possess antioxidant (Prathapan and Rajamohan, 2011; Preetha *et al.*, 2012; Preetha *et al.*, 2013; Kamat and Devasagayam, 1995). Also, glutathione peroxidase activity has being shown to significantly increase in the liver of rats with fed palm oil supplemented diet (Oluba

et al., 2008). Beyond the unique lipid profile of palm oil, there is growing scientific interest in the lipophilic palm oil-associated tocopherol members of the vitamin E family that possess potent nutritional and therapeutic value (Kamat and Devasagayam, 1995; Sen et al., 2007, Sen et al., 2010). Palm oil derived from *Elaeis guineensis* represents the richest source of the lesser characterized vitamin E, α -tocotrienol. One of 8 naturally occurring and chemically distinct vitamin E analogs, α -tocotrienol possesses unique biological activity that is independent of its potent antioxidant capacity (Sen et al., 2007, Sen et al., 2010). Current developments in α -tocotrienol research demonstrate neuroprotective properties for the lipid-soluble vitamin in brain tissue rich in polyunsaturated fatty acids (PUFAs). Arachidonic acid (AA), one of the most abundant PUFAs of the central nervous system, is highly susceptible to oxidative metabolism under pathologic conditions. Cleaved from the membrane phospholipid bilayer by cytosolic phospholipase A₂, AA is metabolized by both enzymatic and non-enzymatic pathways. A number of neurodegenerative conditions in the human brain are associated with disturbed PUFA metabolism of AA, including acute ischemic stroke (Sen et al., 2010). Palm oil-derived α -tocotrienol at nanomolar concentrations has been shown to attenuate both enzymatic and nonenzymatic mediators of AA metabolism and neurodegeneration (Sen et al., 2010).

Studies have revealed that coconut water treatment could prevent and reverse high blood pressure induced by high fructose diet probably by inhibition of lipid peroxidation, upregulation of antioxidant status and improved insulin sensitivity (Bhagya, et al., 2010; Preetha et al., 2013).

The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water, was shown to contain phytohormone; auxin; cytokinin; gibberellin; inorganic ion; and vitamin (Yong et al, 2009). Phytohormones are a Group of naturally occurring organic compounds that play crucial roles in regulating plant growth in a wide range of developmental processes. Initially, the term phytohormone was synonymous with auxin. Later on, the other plant growth regulators such as gibberellins (GAs), ethylene, cytokinins, and abscisic acid (ABA) were categorized together with auxins as the “classical five” hormones (Kende and Zeevaart, 1997). Coconut water contains auxin, various cytokinins, GAs and ABA (Yong et al, 2009; Kobayashi et al., 1997; Wu and Hu, 2009; Ma et al., 2008).

Vitamins and inorganic ions (such as calcium, sodium potassium and magnesium) which are essential for the normal functioning of the human body are also found in coconut water (IOM, 2000). Greater consumption of fruits and vegetables is associated with the reduced risk of cardiovascular disease, stroke, and cancers of the mouth, pharynx, esophagus, lungs, stomach, and colon (Riboli and Norat, 2003; Bazzano, 2002) because they contain vitamins and minerals vital for normal physiological functions (Tucker and Roberts, 2000). Coconut water contains vitamins B1, B2, B3, B5, B6, B7 and B9 (Yong et al, 2009). The B vitamins are

water-soluble and are required as coenzymes for enzymatic reactions essential for cellular function (Depeint, 2006). In addition to vitamin B, coconut water also contains vitamin C (Yong et al, 2009), which is an important dietary antioxidant (Shenkin, 2006). The presence of these ions and vitamins might have contributed to the therapeutic value inherent in coconut water.

A reduction in serum protein (albumin) observed in the CdCl₂ treated rats (Table 2), may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and consequently decrease in the liver weight. However, the administration of palm oil and coconut water revealed an increase in the level of albumin (Table 2), an indication of reduced hepatocyte degeneration or probably hepatic cell regeneration.

Conclusion

The outcome of this study however demonstrates that palm oil and coconut water has some protective effect against cell oxidation. This also corroborates its use in folk medicine as one of the first line treatment methods upon consumption of toxic or poisonous substances. However, since the data reported from this study were generated for short-term treatment with palm oil and coconut water, it is recommended that long term animal studies be carried out to evaluate the effects of palm oil and coconut liquor on these biomarkers as well as the biochemical mechanisms involving xenobiotic enzymes.

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