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Original Research

Possible protective role of palm oil and beef liver on the kidney and liver of wistar albino rats fed diesel-contaminated diet.

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ABSTRACT: We investigated the effects of eight weeks of a diesel-contaminated diet on liver and kidney of Wistar Albino rats, as well as the possible protective role of palm oil and beef liver. There was a significant increase in serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of rats fed with diesel-contaminated diet compared with the controls, indicating damage to the liver. Rats fed with diesel-contaminated diets also exhibited a significant increase in serum urea and creatinine, indicating kidney damage. While there was a significant reduction in the activities of serum ALT and AST, as well as serum urea and creatinine in rats fed palm oil-pretreated diesel-contaminated diet, rats fed with beef liver-pretreated diesel-contaminated diet showed no significant decrease in serum ALT, AST, urea and creatinine as compared to those fed with diesel-contaminated diets. Histological examination of the kidney and liver revealed severe degenerative changes in the structural integrity of both hepatic and renal cells of rats fed with diesel-contaminated diets as compared to those of the controls and palm oil-pretreated diets. However, pretreatment of the diesel-contaminated diet with palm oil conferred some protection on both renal and hepatic cells. These observations may therefore suggest that palm oil may play a protective role against diesel-induced nephrotoxicity and hepatotoxicity.

KEYWORDS: Contaminated-diet, nephrotoxicity and hepatotoxicity

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INTRODUCTION

Nigeria is one of the major crude oil producing countries of the world. However, this came alongside some drawbacks. One drastic effect associated with crude oil exploration is the contamination of the immediate environment with petroleum hydrocarbons (Amadi *et al.* 1993) The major route of contamination of both terrestrial and aquatic ecosystems by petroleum hydrocarbons is spillage which may result from natural seepages, offshore exploration, leakages from oil wells or accident from oil tankers, land based discharges and sabotage (Awobajo 1981.). The pollution of farmlands by burst over laid pipeline conveying crude or refined petroleum products has been known to expose livestock, games/wildlife and poultry to varying degrees of toxicological effects (Lolomari 1983).

In Nigeria, the common sources of human exposures to petroleum fumes include petrochemical industries (refineries oil fields, filling stations) and homes (Patrick- Iwuanyanwu *et al.* 2011). The use of kerosene for cooking and lighting and the everyday use of diesel and petrol for electricity certainly expose a large percentage of Nigerians to petroleum hydrocarbons. Moreover, crude oil is used in folkloric medicine in the Niger-delta region of Nigeria for the treatment of various ailments such like stomach upset, wound, and burns (Orisakwe *et al.* 2000) According to Ita and Udofia (2011), roadside automobile mechanics have formed wrong habits such as sucking of petrol with their mouth to wash their hands after work.

Petroleum and its refined products such as kerosene, gasoline and diesel have the potential to elicit multiple types of toxic effects due to their complicated composition (Patrick-Iwuanyanwu *et al.* 2011). Several studies have shown that crude oil and its refined products including diesel can cause acute lethal toxicity, sub-lethal chronic toxicity, or both on a wide range of organisms depending on the dosage, time of exposure and the organism involved (Patrick-Iwuanyanwu *et al.* 2011; Adegoke *et al.* 2012; Dede and Kagbo 2001; Sunmonu and Oloyede 2006; Uboh *et al.* 2011; 2012). Diesel is a mixture of hydrocarbons with appropriately 30% n-paraffin, 45% cycloalkanes and 25% aromatics (Frankenberger and Jahanson 1982; Speight 1992). The metabolism of aliphatic and aromatic hydrocarbons results in generation of free radical species (Achuba and Osakwe 2003) and hence oxidative stress (Shakirov Farkhutdinov 2000; Nwaogwu and Onyeze 2014).

Previous studies have shown antioxidant vitamins C and E to confer protection against petroleum hydrocarbon-induced toxicity in experimental animals (Achuba 2005; Uboh *et al.* 2009). Palm oil is a rich source of vitamin E and it is the richest source of super-powerful forms of vitamin E called tocotrienols which have about sixty times the antioxidant efficiency of ordinary vitamin E (Fife, 2007). Moreover, Palm oil is used as an anti-poison among the Niger Delta people; children who accidentally drink kerosene, or eat soap are made to drink large quantity of red palm oil either to regurgitate the poison or neutralize its effects (Agbogun 2012). Beef liver is a rich source of haem iron, which is needed for the synthesis of red blood cells (Harper 2012). This research, therefore, was designed to establish a possible protective role of red palm oil and beef liver against diesel contaminated diet induced nephrotoxicity and hepatotoxicity.

MATERIALS AND METHODS

Chemicals: The diesel used in this study was obtained from NNPC Mega station, Agbor, Delta State, Nigeria. Other reagents used were of high quality analytical grade

Experimental Animals

Thirty (30) mature male albino Wistar rats were obtained from the animal house of Department of Anatomy, Delta State University, Abraka. The experimental rats were housed in clean wooden cages and left to acclimatize for two weeks on growers mash. After the acclimatization period, the rats were weighed and their weights range between 110 to 150 g.

Experimental Design And Treatment Of Animals

The thirty (30) adult male Wistar Albino rats were randomly assigned to six (6) groups with five in each group. Rats in the control group (group 1) were fed with grower's mash only. Rats in group 2 were fed with grower's mash contaminated

with diesel (4ml per 100g of feed). Group 3 were fed with grower's mash- contaminated with diesel (4ml per 100g of feed) plus 4ml of red palm oil. Group 4 were fed with grower's mash contaminated with diesel (4ml per 100g of feed) plus 8ml of red palm oil. Rats in groups 5 and 6 were fed with grower's mash contaminated with diesel (4ml per 100g of feed) plus 3.5g and 7.0g of ground beef liver respectively. The rats in each group were allowed free access to clean drinking water while the experiment lasted. The feeds for the test groups (groups 2-6) were prepared fresh daily and stale feed remnants were regularly discarded.

Collection of Samples.

At the end of the experiment, the rats were anaesthetized with chloroform soaked in swab of cotton wool in desiccators. They were then sacrificed and 5ml sterile syringes with needle were used for collection of blood from the vena cava into properly labeled plain sample bottles. After the rats were anaesthetized with chloroform, they were dissected and the liver and the kidneys were collected and preserved in 10% formaldehyde.

Determination Of Serum Metabolite Concentrations

Serum creatinine was determined by Jaffe-Slot alkaline Picrate Colorimetric method as described by Cheersbrough (2006). The urea was determined using Randox kits. The Bicarbonate ion (HO_3^-) was determined by titration method as described by Ochei and Kalhatkar (2005). The sodium ion (Na^+) and Potassium ion (K^+) were determined by the use of 410 Clinical flame photometer made by Sherwood Scientific. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the Colorimetric method of Reitman and Frankel as described by Ochei and Kolhatkar (2005).

Statistical Analysis

All the results were expressed as means \pm SD and all data were analyzed using Analysis of variance (ANOVA). Significant difference between the control and treatment means were determined at 5% ($P < 0.05$) confidence level using Duncan's Multiple Range Test (Duncan, 1955)

RESULTS

The results (Table 1) indicated a significant ($P < 0.05$) increase in the activities of ALT and AST in rats fed with diesel-contaminated diet as compared to the controls and those fed with palm oil pretreated diets. There was no significant increase in the activity of ALT in rats fed with diesel –contaminated diet relative to those fed with diesel-contaminated diet plus beef liver. However, there was a significant decrease in the activity of AST in rats fed diesel –contaminated diet and those exposed to diet pretreated with palm oil and beef liver

Table 1: Effect of palm oil and beef liver on Serum creatinine and bicarbonate ion (HCO₃⁻) of albino wistar rats fed diesel-contaminated diets

GROUPS	CREATININE	UREA	Na ⁺ (mM/l)	K ⁺ (mM/l)	HCO ₃ ⁻ (mM/l)
Control	70.20 ±7.04 ^a	7.94 ±0.59 ^a	136.28 ±4.00 ^a	42.88±2.22 ^a	4.56 ±1.55 ^a
Diesel diet	104.40 ±8.90 ^b	11.56 ±0.70 ^b	119.94 ±5.89 ^b	59.36±6.21 ^b	2.18 ±0.54 ^b
Diesel diet + 4ml palm oil	81.00 ±3.16 ^a	8.66 ±0.74 ^a	129.84 ±4.37 ^a	48.99±5.25 ^a	3.32 ±0.76 ^a
Diesel diet + 8ml palm oil	80.60±12.19 ^a	8.36 ±0.73 ^a	131.22 ±3.08 ^a	46.54±8.52 ^a	3.34 ±1.35 ^a
Diesel diet + 3.5g beef liver	99.00 ±6.97 ^b	9.58 ±0.68 ^b	121.64 ±5.00 ^b	56.36±4.22 ^b	2.46 ±0.49 ^b
Diesel diet + 7g beef liver	100.20 ±6.97 ^b	10.35 ±1.08 ^b	122.56 ±2.62 ^b	55.74±4.22 ^b	2.48 ±0.78 ^b

Values are means ±SD for 5 rats. Means with different superscript letters in the same column are significantly at the 0.05 (P<0.05) level.

Table 2: Effect of palm oil and beef liver on Serum ALT and AST of Wistar albino rats fed diesel-contaminated diets. Activities of ALT and AST are expressed as units /g tissue

GROUPS	ALT	AST
Control	26.6 ^a ±3.64	124.6 ^a ±2.65
Diesel diet	34.8 ^b ±3.83	148.2 ^c ±6.09
Diesel diet + 4ml palm oil	31.2 ^a ±2.58	130.0 ^a ±5.09
Diesel diet + 8ml palm oil	28.6 ^a ±2.30	125.8 ^a ±5.26
Diesel diet + 3.5g beef liver	34.0 ^b ±2.73	140.8 ^b ±7.65
Diesel diet + 7g beef liver	33.2 ^b ±6.87	141.2 ^b ±4.16

Values are means ±SD for 5 rats. Means with different superscript letters (a,b,c) in the same column are significantly different at P <0.05.

Table 2 shows a significant increase in serum creatinine and urea levels of rats fed diesel contaminated diet compared to the controls and those fed with palm oil pretreated diets. However, there was no significant increase in serum K⁺ of rats fed with diesel diet compared to other groups. Serum Na⁺ and HCO₃⁻ decreased significantly in rats fed with diesel contaminated diets relative to the controls and the palm oil groups.

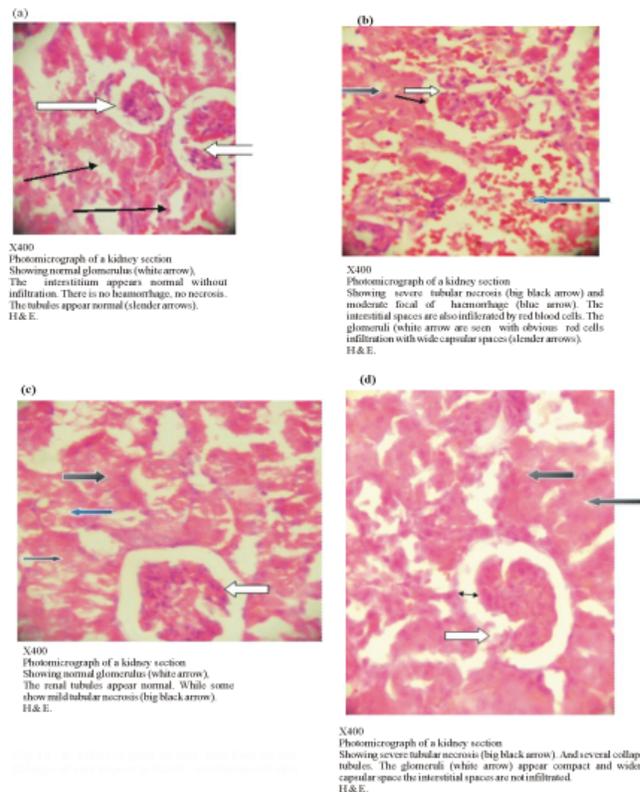


Figure 1: Effect of palm oil and beef liver on the kidney of rats exposed to diesel contaminated diet. (a) Control (b) Diesel contaminated diet (c) Diesel contaminated diet plus 4ml of palm oil (d) Diesel contaminated diet plus 3.5g of beef liver

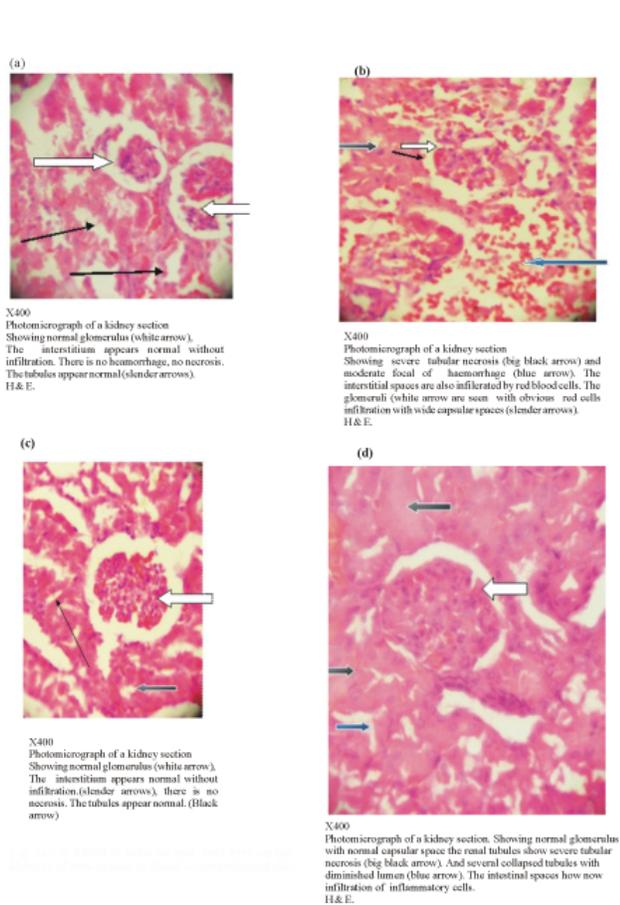


Figure 2: Effect of palm oil and beef liver on the kidneys of rats exposed to diesel contaminated diet. (a) Control (b) Diesel contaminated diet (c) Diesel contaminated diet plus 8 ml of palm oil (d) Diesel contaminated diet plus 7.0 g of beef liver

The analysis of the light micrographs (figures 1a-d and 4a-d) revealed degenerative changes in both the kidney and liver of rats fed with diesel diet compared to the controls. There were severe tubular necroses (black arrow) and moderate focal area of haemorrhage (blue area) of the kidney of rats fed with diesel diet. The glomeruli (white arrow) and the interstitial spaces were infiltrated by red blood cells. Moreover, there were severe hepatic necroses (back arrows) and destruction of liver plates. The sinusoids were severely dilated (white arrows) with occasional attendance of lymphocytes. There was presence of haemorrhage in some sinusoids. These alterations were reversed in rats fed palm oil pre treated diet but not in rats fed beef liver pre treated diets.

DISCUSSION

The present investigation indicated that exposure of rats to diesel-contaminated diet resulted nephrotoxicity. This is indicated by the significant increases in serum levels of creatinine and urea as well as a significant decrease in serum

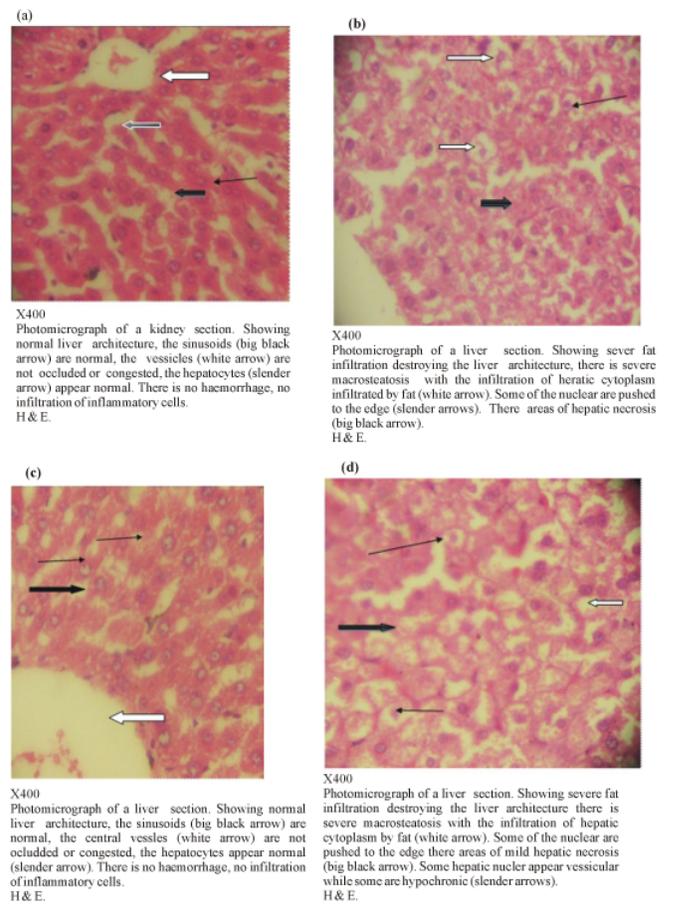


Figure 3: Effect of palm oil and beef liver on the liver of rats exposed to diesel contaminated diet. (a) Control (b) Diesel contaminated diet (c) Diesel contaminated diet plus 4ml of palm oil (d) Diesel contaminated diet plus 3.5g of beef liver

levels of sodium ions (Na^+) and bicarbonate ions (HCO_3^-) in rats fed with diesel-contaminated diet compared to the controls was observed in this study (Table 1). This indicates kidney damage in rats exposed to diesel-contaminated diet as elevated serum levels of creatinine and Urea, and decreased levels of Na^+ and HCO_3^- are markers of impaired kidney function (Ochei and Kohatkar 2000, Cheersbrough 2006, Uboh *et al.*, 2009). Moreso, ingestion of diesel contaminated diet resulted in tissue damage (Figure 1a). This agrees with the work of Patrick-Iwuanyanwu *et al.* (2011). However, pretreatment of diesel-contaminant diesel with palm oil resulted in protection from the deleterious effects of the diesel. Serum creatinine and urea levels in rats fed palm oil pre-treated diets were not different ($P < 0.05$) from those of the controls. However, serum Na^+ and HCO_3^- were significantly different in palm oil diets compared to the control. Pretreatment with beef liver did not produce any significant difference compared to the diesel diet (Table 1). Except in serum urea level, rats fed with beef liver pretreated diet did not differ significantly from those in diesel-contaminated diets.

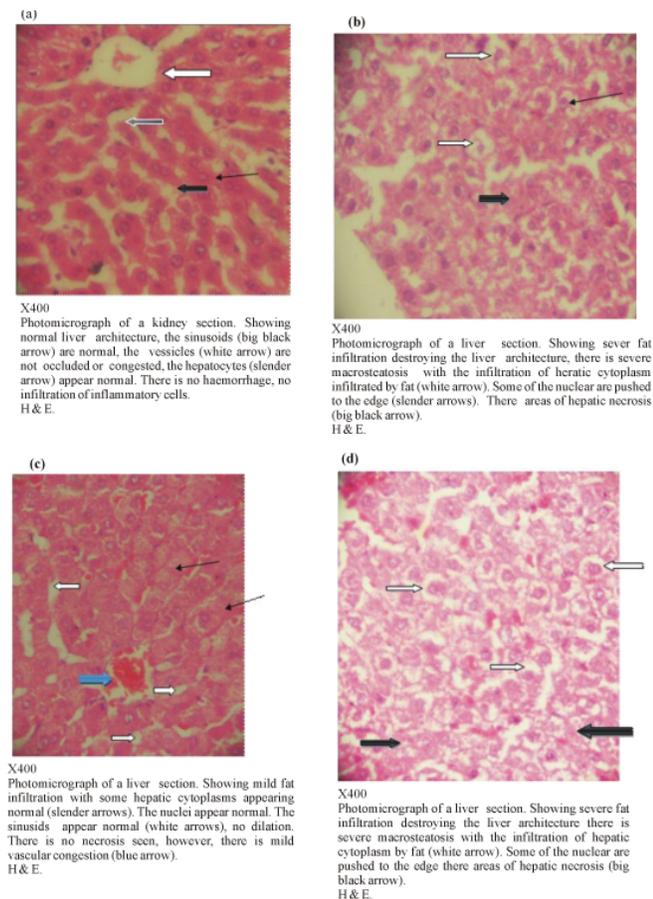


Figure 4: Effect of palm oil and beef liver on the liver of rats exposed to diesel contaminated diet. (a) Control (b) Diesel contaminated diet (c) Diesel contaminated diet plus 8 ml of palm oil (d) Diesel contaminated diet plus 7.0 g of beef liver

The ameliorative effect of antioxidant vitamins on petroleum-induced toxicity was reported by Achuba (2005), while the antioxidant efficiency of palm oil has been documented (Fife, 2007).

It has been shown that crude oil and its refined products including diesel to cause adverse alterations in biochemical parameters (Achuba 2005; Patrick-Iwuanyawu *et al.* 2011; Nwaogu and Onyeze 2014) and Histology (Dede and Kagbo 2001; Patrick-Iwuanyawu *et al.* 2011; Nwaogu and Onyeze 2014) in experimental animals. Results shown in Table 2 shows a significant increase in serum levels of ALT and AST in rats fed diesel-contaminated diets compared to the controls. This agrees with the findings of Patrick-Iwuanyawu *et al.* (2011). The increased levels of ALT and AST indicate hepatic damage as these cytoplasmic marker enzymes are released into circulation after cellular damage (Lin *et al.* 2000; Patrick-Iwuanyawu *et al.* 2011). Histological studies indicated that exposure of the rats to diesel contaminated diet elicited adverse effect on the hepatic cells (figures 3a -d to 4a-d). Petroleum hydrocarbon induced histological damage was earlier reported (Nwaogu and Onyeze 2014). Moreover,

pretreatment of the diets with palm oil conferred some protection on the hepatocytes cells. Fig 3c shows a moderately preserved liver architecture with some normal hepatocytes and without any congestion.

Generally, pretreatment of diets with beef liver did not confer any protection on both the kidney and liver as evidenced by moderate tubular necrosis with compacted glomerulus, haemorrhage (slender arrow) and mild fat deposits (fig 1d) and severe macro steatosis, moderate hepatic necrosis (big black arrow) (fig 3d). This is against the protection exhibited by palm oil. Palm oil being rich in the antioxidant, vitamin E, may induce its protection by reducing the susceptibility of liver and kidney cells to the damaging effects of free radicals generated from the metabolism of aliphatic and aromatic hydrocarbons present in diesel. Protective effect of vitamin E was previously reported (Achuba 2005, Uboh *et al.* 2009a; 2009b; 2009c).

Conclusion

The results of this present studies showed that the consumption of diesel-contaminated diet may result in extensive anatomical damage to both the liver and the kidney. However, the consumption of palm oil may offer some protection against diesel induced hepatotoxicity and nephrotoxicity.

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