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Hepatotoxic and Nephrotoxic Effects of Kerosene and Petrol-Contaminated Diets in Wistar Albino Rats

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ABSTRACT

The purpose of this study was to investigate the hepatotoxic and nephrotoxic effects of kerosene and petrol-contaminated diets on male and female wistar albino rats after 12 weeks of chronic exposure. The LD_{50} values of kerosene and petrol were obtained as 212 and 128 mL kg^{-1} b.wt. of rats, respectively. The animals exhibited changes in behavioural pattern such as respiratory distress, sedation, coma and death with symptom being more severe with petrol when compared to kerosene. Serum L-alanine amino transferase (L-ALT), L-aspartate amino transferase (L-AST) and alkaline phosphatase (ALP) levels, 12 weeks after feeding male and female wistar albino rats with contaminated diets increased significantly ($p \leq 0.05$). Feeding rats with diets contaminated with kerosene and petrol for 12 weeks resulted in remarkable decrease in Final Body Weights (FBW) and Percentage Weight Increase (PWI) of test animals compared to control. There was, however, a significant ($p \leq 0.05$) increase in the relative weights of liver and kidney of male rats fed kerosene-contaminated diets when compared to the control. Histological examination of the liver and kidney indicated that kerosene and petrol-contaminated diets induced significant degenerative changes in the structural integrity of both the hepatic and renal cells. The result obtained in this study suggest that long term ingestion of kerosene and petrol indicated stress on animals which could possibly cause adverse effect on the kidney and impaired liver function.

Key words: Contaminated diet, hepatotoxicity, histological examination, nephrotoxicity, petrol and kerosene toxicity

INTRODUCTION

Petroleum samples are complex mixtures of different hydrocarbons and related substances, some of which are known to be highly toxic or carcinogenic to consumers of marine products (EHC 20, 1982).

Kerosene and petrol are distilled from crude petroleum. These fractions of crude petroleum contain aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbon (Anderson *et al*., 1974; Henderson *et al*., 1993; Kato *et al*., 1993). Petroleum fumes are ubiquitous in our environment and the common sources of contact or exposure are petrochemical industries (refineries, oil fields, filling stations) and homes. The applications of kerosene as cooking and lighting fuels in the home have resulted in direct exposure of these products to a good percentage of the populace. Moreover, the day-to-day use of petrol outside the

industrial settings is likely to have the same effect on the users as kerosene since they have been reported to contain most of the same hydrocarbons. However, the most affected are those who are occupationally or domestically exposed to petroleum products (Smith *et al*., 1993; Carballo *et al*., 1994; Rothman *et al*., 1996).

Petroleum production began in Nigeria in 1958 and the industry has, over the years become a vast operation covering both offshore and onshore oilfields (Odu, 1977; Awobajo, 1981). Through these years however, cases of petroleum and refined petroleum spills onto agricultural lands through petroleum production operation have been reported (Imevbore and Adeyemi, 1981; Gravy, 1995; Moffat and Linden, 1995). Pollution of farmlands by burst overlaid 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).

Pollution by petroleum is a widespread and common problem that can arise either accidentally or operationally wherever oil is produced, transported, stored, processed, or used at sea or on land. On land, petroleum products can account for a large proportion of the chemicals at contaminated sites (Kostecki *et al*., 1995). Contamination of groundwater and soil by specific hydrocarbon components has led many countries to enact regulatory requirements for action and clean up of these compounds (Wilson and LeBlanc, 2000). Due to its complicated composition, petroleum has the potential to elicit multiple types of toxic effects. It can cause acute lethal toxicity, sub-lethal chronic toxicity, or both depending on the exposure, dosage and the organism exposed. Some components of petroleum have the potential to bio accumulate within susceptible aquatic organisms and can be passed by trophic transfer to other levels of the food chain (Eisler, 1987; Gardner *et al*., 1991).

Knowledge of human responses to acute exposures to petroleum components comes from studies with several solvents containing benzene and petroleum (Zedeck, 1980). Recognized human biochemical and physiological responses associated with acute exposures to natural petroleum products are mainly transient and short lived unless the concentrations of the components are unusually high (Campbell *et al*., 1993).

There is a concern that workers and other individuals exposed to petroleum products might have an increased incident of organ damage especially the fuel attendants and auto mechanics who are constantly exposed to the harmful effects of petrol and kerosene because of the nature of their job. Unfortunately, they handle these samples without cognizance for proper protection against possible effects of the petroleum samples (such as the mask, gloves etc.). There is therefore possibility of exposure to light ends of these samples (Dede *et al*., 2002). There are other reported cases of the acclaimed folkloric and therapeutic abuse of petroleum samples. These include the use of petroleum products as an antidote to snake bites, as an anticonvulsant and in the treatment of arthritis, gastrointestinal disorders, burns, foot rot and leg ulcers, poisoning and witchcraft (Orisakwe *et al*., 2000; Dede *et al*., 2002). Also exposed are those individuals who use petrol or kerosene in the treatment of skin and eye infections (conjunctivitis), Eczema and scabies. After absorption via pulmonary or gastrointestinal routes, petroleum samples are transported in plasma initially bound to albumin and other larger proteins to the liver. The toxicity of a petroleum fraction is related to its hydrophobicity (Freedman, 1989) because lipid solubility is an important factor in the passage of petroleum components through the cell plasma membrane, as well as the degree of membrane disruption.

Despite the high utilization of kerosene and petrol, our knowledge is sparse on the toxicological effects of diets contaminated with these petroleum samples.

This study was designed to investigate the biochemical and toxicological implications of chronic ingestion of diets contaminated with refined petroleum products namely kerosene and petrol in wistar albino rats.

MATERIALS AND METHODS

Petroleum products: Petroleum products namely kerosene and petrol were purchased from African Petroleum (AP) filling station located in Choba, Rivers State, Nigeria in November, 2007.

Matured male and female albino rats weighing between 160-170 g were obtained from the Animal House attached to the Department of Biochemistry, University of Port Harcourt, Nigeria. The animals were acclimatized for one week prior to commencement of the experiment. Animals were housed singly at ambient temperature of 23±3°C and a 12 h light, 12 h dark cycle. All the animals were fed with their specific diets and water *ad libitum*.

Mammalian acute toxicity tests (determination of LD_{50}): LD_{50} for each of the toxicants were determined. Animals were injected intraperitoneally with different doses of the samples as calculated from the range finding tests. The animals were then monitored for 24 h. A group of control rats were given equal volume of 0.9% normal saline. Behavioural patterns of the injectorates were monitored and particular attention was paid to the onset of toxic symptoms to death. After 24 h, the experiment was terminated. LD_{50} values were then calculated using the arithmetic method of Karber (Dede and Igbigbi, 1997).

Mammalian chronic toxicity tests: Forty eight rats consisting of equal numbers of male and female rats were used for this study. The rats were separated into 6 groups (comprising of 3 male groups and 3 female groups) of 8 rats per group each in a cage and were acclimatized for two weeks before actual test. A set of 8 male rats per group and 8 female rats per group were fed with diets contaminated with kerosene and petrol, respectively. Another set of 8 male rats per group and 8 female rats per group were fed with normal diets and these served as the general control.

Preparation of feed: The LD_{50} values of the petroleum products-kerosene and petrol, as calculated were multiplied by a factor of +2.5 to make up for loss of the volatile components of these products. Calculated quantities (volumes) of the sample were then mixed with measured amounts of animals feed and the mixture was compacted with water to form a constant mass. The feed for the control groups was compacted with water only. The feed was then molded into small balls, air-dried and stored in well-labeled sacks to last for the duration of the test period.

Preparation of samples: Twelve weeks after feeding with diets contaminated with kerosene and petrol, the rats were anaesthetized in a chloroform-saturated chamber. The animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture using a 5 mL hypodermic syringe and needle and introduced into an anticoagulant free bottle. Serum was separated by centrifugation and stored in a refrigerator. The measurement of different biochemical parameters (L-AST, L-ALT and ALP) was performed using the reflotron system. The liver and kidney of both male and female rats were excised and fixed in formalin for histological examinations.

Statistical analysis: The mean values of the control and test serum activities of a given enzyme were compared using the student's t-test (Zar, 1984). The significance level was set at $p \leq 0.05$.

RESULTS

Table 1 shows the effect of kerosene and petrol-contaminated diet on body weight, percentage weight increase, absolute and relative weights of liver and kidney respectively after 12 weeks of chronic exposure to male and female rats. The animals demonstrated a significant decrease ($p \leq 0.05$) in the final body weight and percentage weight increase of the male and female rats exposed to kerosene and petrol when compared to the male and female control rats. From the result, male rats fed diets contaminated with kerosene showed a significant increase ($p \leq 0.05$) in relative liver and kidney when compared to the control rats. Male rats fed diets contaminated with petrol also showed a marked decrease ($p \leq 0.05$) in the absolute liver when compared to the control.

The results of the biochemical parameters investigated are as shown in Table 2, the result shows a significant increase ($p \leq 0.05$) in the activity levels of the enzymes: (AST, ALT and ALP) in serum of the male and female rats fed diets contaminated with kerosene and petrol when compared to the controls. Histopathological examination (Fig. 1a-c) of male and female rat liver exposed to kerosene and petrol-contaminated diets revealed defects ranging from dense eosinophilic cytoplasm with sinusoidal expansion with red blood cells to larger and more numerous kupfer cells. The histological changes of kidney in male and female rats fed kerosene and petrol-contaminated diet are as shown in Fig. 2a-c. The result revealed changes ranging from congested capillaries, patchy degeneration of the convoluted tubules marked by pyknosis of nuclei and focal collapse of glomeruli and segmented sclerosis of lobules (Fig. 2a-c). However, the control group showed normal histological structure.

Table 1: Body and organ weights of male and female rats

Diets	FBW	PWI (%)	Absolute liver	Absolute kidney	Relative liver	Relative kidney
Male						
Control	225±22.8 (165±15.0)	36.4±6.6^{a}	9.52±2.0^{a}	1.28±0.2^{a}	4.23±0.1^{a}	0.57±0.1^{a}
Kerosene	175±13.2 (170±14.4)	3.0±1.1^{b}	8.29±1.5^{a}	1.21±0.1^{a}	4.74±0.1^{b}	0.70±1.1^{b}
Petrol	170±6.0 (160±28.3)	6.3±4.7^{b}	7.18±1.3^{b}	1.13±0.1^{a}	4.22±0.2^{a}	0.66±2.2^{a}
Female						
Control	190±10.6 (165±6.6)	15.2±1.6^{a}	8.20±1.8^{a}	1.00±0.1^{a}	4.32±0.2^{a}	0.53±1.5^{a}
Kerosene	167±4.3 (160±15.2)	4.4±3.7^{b}	7.34±1.2^{a}	0.95±0.1^{a}	4.40±0.3^{a}	0.57±2.1^{a}
Petrol	167±10.9 (165±15.0)	1.2±1.4^{b}	7.15±1.1^{a}	0.89±0.1^{a}	4.30±0.1^{a}	0.53±1.0^{a}

Values are Means±SEM. n = 8 rats in each group. Means with different superscript letter(s) (a, b,) in the same column are significantly different at the 0.05 level, FBW: Final body weight, PWI: Percentage weight increase. $(W_x\text{-}W_y)/(W_y)\times100$, where, W_y is the initial mean total body weight, W_x is the final mean total body weight. Numbers in parenthesis indicate initial body weight of rats: Relative weights = (Absolute weight)/(Final body weight)×100

Table 2: Effects of kerosene and petrol-contaminated diets on serum AST, ALT and ALP in wistar albino rats

Diets	AST	ALT	ALP
Male			
Control	47.25±6.49^{a}	45.00±2.35^{a}	60.75±5.58^{a}
Kerosene	90.50±6.50^{b}	87.50±8.73^{b}	64.00±1.40^{b}
Petrol	68.00±6.71^{c}	66.00±8.25^{c}	67.50±2.50^{c}
Female			
Control	48.50±9.96^{a}	45.75±9.04^{a}	54.00±4.18^{a}
Kerosene	78.50±11.41^{b}	73.50±12.50^{b}	66.50±4.61^{b}
Petrol	66.50±4.61^{c}	64.50±4.61^{c}	72.50±13.09^{c}

Values are means±SEM. n = 8 rats in each group. Means with different superscript letter(s) (a, b, c) in the same column are significantly different at the 0.05 level

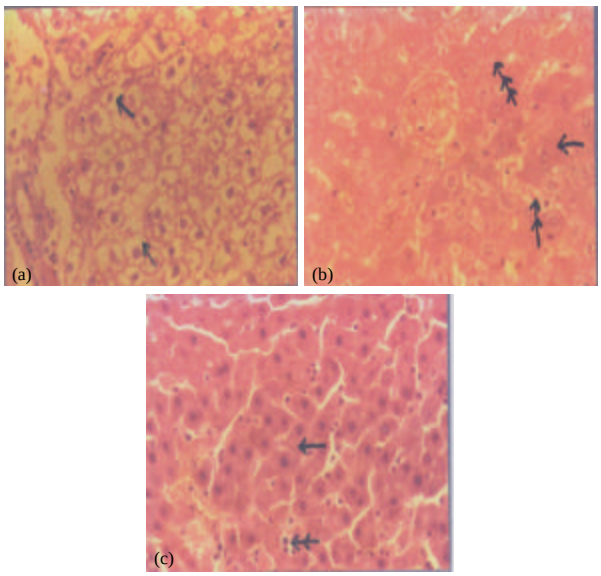

Fig. 1: (a) A section of control rats showing normal architecture, (b) a section of rat liver exposed to kerosene showing a more granular, eosinophilic cytoplasm with large and numerous kupper cells. Nuclei are more vesicular and sinusoids are expanded with RBCs and (c) a section of rat liver exposed to petrol showing a dense eosinophilic cytoplasm with sinusoids inflamed with neutrophils

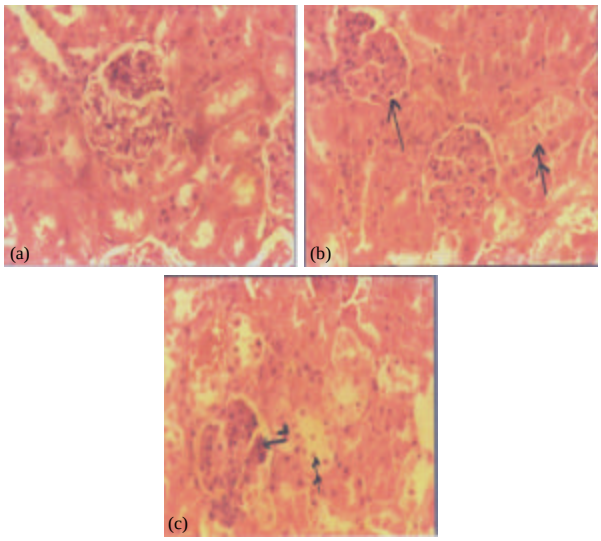

Fig. 2: (a) A section of control rat kidney showing normal architecture with intact glomeruli, (b) a section of rat kidney exposed to kerosene showing lobulated glomerulus with congested capillaries and patchy degeneration of the convoluted tubules marked by pyknosis of nuclei and (c) a section of rat kidney exposed to petrol showing focal collapse of glomerulus and segmented clerosis of lobules and pyknosis of nuclei in tubules

DISCUSSION

Exposure of humans and animals to petrol and kerosene, which is increasing in terms of the environmental levels and different usage of these petroleum products, may be toxic. Petrol and kerosene are used in various homes to run many types of engines, lamps and heaters. Most fuel oil entering the environment comes from spills or leaking storage tanks.

The biochemical parameters investigated showed a significant increase ($p \leq 0.05$) when compared to the control. Treatment of rats with kerosene and petrol-contaminated diets resulted to a significant hepatic damage as elicited by the elevated levels of serum marker enzymes: AST, ALT and ALP. These marker enzymes are cytoplasmic in origin and are released into the circulation after cellular damage (Lin *et al*., 2000). The activity of AST was significantly higher in male and female rats exposed to kerosene and petrol-contaminated diets when compared to the control. Increase activity of AST has also been reported in CCl_4-induced toxicity in rats (Halim *et al*., 1997; Patrick-Iwuanyanwu *et al*., 2007a, b; Patrick-Iwuanyanwu and Wegwu, 2008). This increase may be due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in kerosene and petrol (Uboh *et al*., 2005).

The activity of ALT was also significantly higher in male and female rats exposed to kerosene and petrol-contaminated diets when compared to the control. Such elevation is indicative of liver injury, especially the rise in L-ALT level (Lin and Wang, 1986). The level of serum ALT activity has been reported to be increased as a result of liver injury in patients developing severe hepatotoxicity (Beckett *et al*., 1989). ALT might have leaked from damaged cells, due to necrosis, indicating organ dysfunction (McIntyre and Rosalki, 1992). The result in this study is also in agreement with the findings of Salie *et al*. (1999), who discovered that the rise in the enzyme AST is usually accompanied by an elevation in the levels of ALT, which plays a vital role in the conversion of amino acids to keto acids. This increase may be due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in kerosene and petrol. The changes in the cell membrane may have been as a result of the reactive free radical species from the metabolism of aliphatic and aromatic hydrocarbons which are the major constituents of petroleum products as well as other xenobitics (Leighton *et al*., 1985; Bondy *et al*., 1995).

Alkaline phosphatase activity in the animals exposed to kerosene and petrol-contaminated diet were significantly higher ($p \leq 0.05$) when compared to the control animals. However, the remarkable increase in the level of ALP in male and female rats fed diets contaminated with petrol may imply that damages occurred more in the liver cells of rats fed petrol-contaminated diets than those fed kerosene-contaminated diet, since the activity of this enzyme in the serum is reported to be increased in the liver damage (Halim *et al*., 1997). Alkaline phosphatase is involved in the transport of metabolites across the cell membranes, protein synthesis, synthesis of certain enzymes, secretory activities and glycogen metabolism. However, the increase in this enzyme activity may not be unconnected with a disturbance in the transport of metabolites or alteration in the synthesis of certain enzymes as in other hepatotoxic conditions (Sharma *et al*., 1995).

Result observed in animals fed diets contaminated with kerosene and petrol showed a marked decrease ($p \leq 0.05$) in the final body weight and percentage weight increase. This result is in agreement with the findings of Uboh *et al*. (2008). Also, the increase in relative weights of liver (Hepatomegaly) and kidney observed in male rats fed diets contaminated with kerosene is similar to the findings of Uboh *et al*. (2008), who reported an increase in the relative liver weight and a reduction in the percentage weight increase in rats following exposure to gasoline vapours. It has been reported that metabolism of aliphatic and aromatic hydrocarbons, the major constituents of

petroleum and petroleum-derivatives, as well as other xenobiotics generates a significant increase in the level of free radical species in various tissues (Lam *et al*., 1994; Bondy *et al*., 1995). The generated reactive intermediates can interact and disrupt the cell membranes of the affected tissues thereby causing the tissue enzymes and other metabolites to leak out and increase the plasma concentrations as observed in this study. This may be an indication that the animals could not convert the feed consumed into useful nutrients required by the body, thus accounting for the reduced final body weight and percentage weight increase when compared to the control (Table 1). However, the decrease in overall body weight and organ weight may probably be an adaptive feature or a pathological response to the toxic compounds (hydrocarbon) present in the petroleum products.

In conclusion, the result generated from this study is suggestive of the fact that kerosene and petrol are environmental stressors. Thus, long term ingestion of these petroleum products could possibly cause adverse effect on the kidney and impaired liver functions.

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