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Effect of Acute Kerosene Toxicity on the Histology of the Small Intestine, Intestinal Enzyme Amylase and Malondialdehyde (MDA) on Adults Male Wistar Rats

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Abstract
This study was carried out to determine the acute toxicological effect of ingesting kerosene on malondialdehyde, intestinal enzyme amylase and histology of the small intestine. The study was carried out among 20 rats, with an average weight of 110-180grams, 10 were used for control while the other 10 for experimental study. 13mls of kerosene was added to300ml of water to give a concentration of 10mg/kg. Kerosene component was detected in blood serum for MDA and amylase determination, and fragment of the small intestine was sent for histological study. The result showed a decrease in the weight of the experimental rats when compared to control rat. There was also a significant increase ($p<0.5$) in amylase level in the treated rats (157$\pm$18.17Units/L) when compared with the control (195.54$\pm$28.68Units/L) in experimental Wistar rats. Furthermore Malondialdehyde concentration showed significant increase from (1.139 ± 0.58)µmol/L in control group to (2.49$\pm$ 0.65)µmol/L in the intoxicated group. From the histological studies, the small intestine of the intoxicated rat showed mild mucosal devitalization and separation. These findings suggest that kerosene when ingested can cause toxicological effects; hence all measures should be taken to minimize the ingestion of kerosene contaminated water.
Keywords: kerosene, amylase, malondialdehyde, wistar rats

1. Introduction

1.1 Background of the Study

In Nigeria today, contamination as a result of oil spillage has become one of the severest forms of environmental pollution. Generally, petroleum and its refined products reaching the terrestrial and aquatic ecosystems arrive as a consequence of spillage which may result from natural sepages, offshore exploration, leakages from oil wells or from oil tankers, accident from oil tankers, land based discharges and sabotage (Awobajo, 1981; Wardley-Smith, 1983; Jackson et al., 1989). The toxic effect of petroleum hydrocarbons in an organism is manifested subsequent to bioaccumulation of the offending xenobiotic.

The phenomenon of bioaccumulation is associated with direct transfer of compounds through body surface into the circulatory fluids in a process known as bioconcentration, or they are transferred through food across the gastrointestinal tract (GIT) into the circulatory fluids in a process called biomagnifications (Connell, 1980). Kerosene inhalation is associated with dysfunctions that range in severity from subtle cognitive impairment to encephalopathy and death (Cairney et al., 2002). Individual components of kerosene are known to undergo dermal absorption (Singh and Singh, 2003; Mattorano et al., 2004) and kerosene vapour is absorbed following pulmonary exposure (Risher et.al., 1995). The extent of dermal and pulmonary absorption is dose and time-dependent (Bebarta and Dewitt 2004). Many of these substances are considered to have the potential to affect human health if consumed directly or via food (Pollock et al., 1991). Environmental and physiological factors are known to affect many parameters in the blood.

Gastrointestinal tract studies are of ecological and physiological interest. Such studies help in understanding the relationship of absorption mechanisms. Several toxic components of petroleum products have been documented (Cairney et al., 2002). However, Berepubo*et al.,* (1994) observed that a relatively short exposure to petroleum products led to the inhibition of growth in weaner rabbits. Wang *et al.;* 1994) reported similar observation in juvenile pink salmon (Oncorhyncusgorbuscha). The metabolism of a great variety of pollutants, including hydrocarbons, produces reactive oxygen species (ROS) (Regoli et al., 2002). Oxidative stress can be understood as a situation derived either from an enhanced rate of generation of oxygen radicals or from a diminished level of antioxidant (enzymatic or non-enzymatic) defenses.

Moreover, the biological situation associated with oxidative stress may be estimated by the physico-chemical condition in which an increase in the steady-state levels of oxidative species (i.e., O_2 _, H_2O_2, HO _, R_ and ROO_) occurs. This increased steady-state level of oxidants may consequently lead to reversible or irreversible cell damage and eventually to cell death. Oxygen free radicals are produced in a series of biochemical reactions that will normally occur within the cellular compartments (i.e., mitochondria and the endoplasmic reticulum are the most important sources of oxygen free radicals). It is well known that a number of

different enzymes and non-enzymatic compounds participate in the antioxidant chain in biological systems. Among them, super oxide dismutase (SOD) converts H_2O_2 to water, and malondialdehyde (MDA) detoxifies both H_2O_2 and organic hydroperoxides (ROOH) using malondialdehyde (MDA) as a cofactor. Intracellular redox homeostasis is superoxide anion ($O_{2\_}$) to hydrogen peroxide (H_2O_2), catalase (CAT) reduces regulated by antioxidants, particularly by thiol-containing molecules such as glutathione. This hydrosoluble non-enzymatic antioxidant has been shown to protect cell membranes against oxidative stress.

Kerosene is distilled from crude petroleum. This fraction of crude petroleum contain aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons (Henderson et al., 1993; Kato et al., 1993; Anderson et al., 1995).

Kerosene is a liquid mixture of chemicals produced from the distillation of crude oil. In the UK, kerosene is also known as paraffin and home heating oil. The word kerosene comes from the Greek word keros, meaning wax. Kerosene is a major component (>60%) of aviation (jet) fuels. It is used for central heating systems and can be used as a cleaning agent or solvent. Approximately 7½ millions tons of kerosene was used in the UK in 2005 (Chilcott, 2007). Swallowing fuel oil such as kerosene can cause vomiting, diarrhea, swelling of the stomach, cramps, coughing, drowsiness, restlessness, irritability and breathing difficulties. Drinking more than an ounce can result in coma or death. Most accidental poisonings involve children. Many of these children drink kerosene that was being kept in old soft drink bottles. Despite the important and use of kerosene in our contemporary society, it is pertinent to note that petroleum products (kerosene) is often attributed to toxic effect on both human and animal subjects. Moreover, kerosene vapors may be mildly irritating to the respiratory system, Signs of oral kerosene poisoning include diarrhea, nausea and vomiting as well as causing mild, transient ocular irritant that may produce conjunctivitis, cardiac arrhythmia, ventricular fibrillation hyperemia and lacrimation. However, in extreme cases, problems of exposure of kerosene is associated with a variety of CNS effects, including irritability, restlessness, ataxia, drowsiness, convulsions, coma and death

The present study seeks to explore the effect and toxicological impacts of kerosene toxicity on Malondialdeyde, amylase and histology of the small intestine using male wistar rats due to invasive nature of the work in order to provide knowledge on the impact of kerosene contamination on human.

2.Animal Grouping

The rats were divided into two groups. Each rat was weighted using manual weighing scale at the beginning of the experiment (i.e. initial weight) and at the end of each week after feeding with water contaminated with kerosene.

2.1 Experimental Design

Group A (Control Group). The rats in this group received only feed and distilled water.

Group B (Experimental Group). The rats in this group received feed and 300ml of water mixed with 13ml of kerosene.

2.2 Sample Collection

After 28 days the rats were anesthetized in a chamber saturated with di-ethyl ether. A laparoscopy was carried out to expose blood vessels and internal organs. 5ml of blood was collected with the aid of a syringe by cardio puncture from each rat into well labeled plain collecting specimen bottles (Edta anticoagulant). And also the small intestine was harvested for microscopic examinations.

2.3 Estimation of Serum plasma

2.5ml of buffer was added to the test and blank test tubesand incubated at37^0C for 5mins. 0.1ml of the serum was the added to the solution and incubated again at 370C for 7.5mins, 2.5ml of working iodine solution was then added to the test solution and the blank. While 0.1ml of the serum was also added to the blank solution . 20mls of distilled water was then added to both the test solution and the blank. Both test tubes were mixed thoroughly and read at an absorbance of 660nm. Serum amylase was the n calculated.

2.4 Malondialdehyde (MDA) Assay

The Assay method of Hunter,et al., (1963), modified by Gutteridge and Wilkins (1980) was adopted.3ml each of glacial acid and 1% TBA solutions were added to test tubes appropriately labeled blank and tests. 0.6ml of the appropriate intestinal tissue extract was added to each of the test tubes. There were thoroughly mixed, incubated in a boiling water Bath for 15minutes, and the cooled to 25^0c, after which they are centrifuged and their supernatants collected. The supernatant from the blank was used to zero the spectrophotometer (preset At 532nm) before reading the absorbance of the supernatants from the test solution

2.5 Statistical Analysis

The results were expressed as mean $\pm$ seem (standard error of the mean) and statistical significance of the treatment effect was analyzed using the student's t-test statistics and significance at p. values <0.05 while ^p. value >0.05 were considered to be statistically non-significant.

3.Results

The results obtained from the acute effect of kerosene toxicity on the histology of small intestine, amylase and MDA using animal model of male wister rats. Meanwhile, the results were presented in Mean ± S.D where; *P <0.05 was considered significant and ^P >0.05 was considered not significant when control groups was compared with the experimental groups.

4. Discussion

The present study evaluated the effects of acute kerosene toxicity on the histology of small intestine, using animal model of male wister rats. Results showed thattrats intoxicatedwith kerosene contaminated water (experimental) rats had a significant decrease in body weight when compared with the control group that was not toxicated at the end of the four weeks of toxication . This is in agreement with the findings of Anon, (2006) that decrease in body weight could be due to toxicity from administration of kerosene on the Wister rats.

Also, this alteration in weight could be an indication of impairment in the normal functioning of the organ of the small intestine. Consequently, the small intestine weight of the group treated with kerosene (119.96±36.17) showed a significant decrease ($p<0.5$) when compared with the control group (143.12±36.24).

Results obtained on the statistical analysis on the effect of kerosene contaminant on amylase, it was observed that the amylase level progressively increased from (157±18.17) Unit/L in the control wistar rats to (195.54±28.68) Unit/L experimental wister rats, this could also be due to a damage in the pancrease in agreement with the findings ofDreiling, (1956), who found out that experimental animals exposed to kerosene contamination alters intestinal secretion. Furthermore, the MDA concentration showed significant increase in the treated group(2.49±0.65µmol/L) when compared with a the control group(1.139±0.58 µmol/L)). Elevated levels of reactive oxygen species initiate lipid peroxidation that is increase in MDA levels (Frei, 1994; Val and Almeida-Val, 1999) that culminates in oxidative stress in tissues (Halliwell, 1989; 1992; Liu and Mori, 1994). During the lipid peroxidation process the activities of different membrane – bound enzymes are altered (Kukreja*et al*.,1988; Thomas and Poznasky, 1990; Thomas and Reed, 1990), resulting in the degeneration of cell membrane, (Nohl, 1993; Reiter, 1995), which predisposes the affected organisms to a wide array of disease processes (Morris *et al*., 1995). This could be the cause of acute pancreatitis observed in the intoxicated rats.

Histopathological studies carried out on the intoxicated rats was in agreement with the results obtained in Table 1 and 2 confirming that there have been some form of damage to the small intestine. conducted this is in agreement with the work of Jollow*et al*., (2003) that the epithelial lining of the small intestine of digesting pythons exposed to petroleum product which showed the typical features of a functional mucosal epithelium which is a single-layered columnar epithelium.

5. Conclusion

The acute health risks involved in handling and using kerosene have been proved to possess increased acute toxicological effects on the organs of animals hence much care should be taken to prevent contamination of drinking water taken by animals and man by kerosene

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Table 1: **Effects of kerosene on the body weight of experimental group compared to control group of male wistar rats,**

Groups	**Control (g)**	**Experiment** (***Kerosene***) (g)
Initial weight	135±20.21	124.6±28.9
Week 1	136.4±20.4	124.8±23.4
Week 2	142.7±21.7	115.2±17.3
Week 3	147.3±23.2	133.9±21.1
Final weight	152.9±25. 9	110.2±18.1

Statistical results are presented in Mean ± S.D.

Table 2: Effects of kerosene on the Amylase and serum MDA of male wistar rats

Parameter	**Control Group**	**Experimental Group**
Serum Amylase (Unit/L)	157.7±18.17	195.54±28.68
Serum MDA (µmol/L)	1.139±0.58	2.49±0.65

Statistical results are presented in Mean ± S.D.

HISTOPATHOGICAL STUDIES

This study shows the histological appearance and effect in H&E stain and magnification of X400 of the small intestine organ of the control group and kerosene treated group

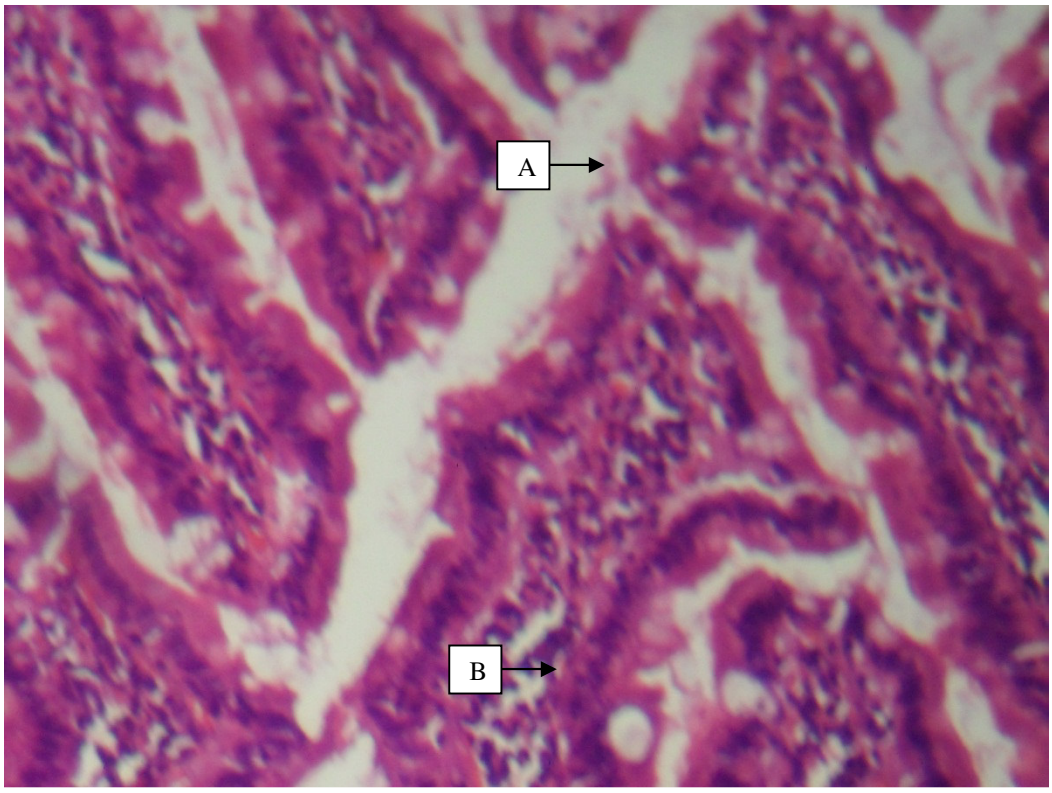

Fig 1: Control plate showing normal small intestine mucosa showing epithelia lining A and lamina propria B (H&E x 10)

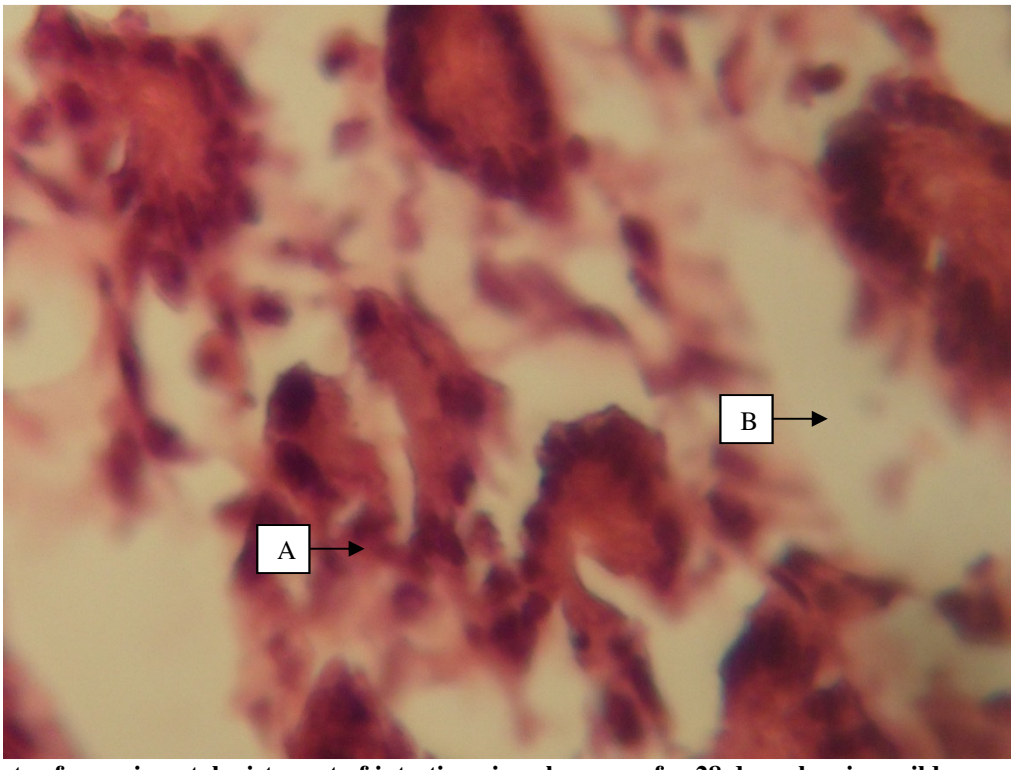

Fig 2: Plate of experimental wister rat of intestine given kerosene for 28 days showing mild mucosal tissue devitalisation A and separation B (H&E x 40)

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