

Hepatotoxicity of House Hold Kerosene (HHK) on Liver Enzyme Markers and Its Effect on Hematological and Oxidative Stress Parameters on Wistar Albino Rats

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ABSTRACT

The exposure of Nigerians to House Hold Kerosene (HHK) is on the increase following carelessness from handling the product and proliferation of sales outlet. Against this backdrop, hepatotoxicity of HHK on liver enzyme markers and its effect on hematological and oxidative stress parameters on wistar albino rats were investigated. Preliminary toxicity study to determine the volume of HHK that could cause toxicity was carried out using 30 healthy albino rats. Another set of 20 albino rats were grouped into two groups and used for the biochemical analysis: Group I animals were the control group and Group II animals were administered with 1ml/kg body weight of HHK. The results of this study shows that HGB, RBC and HCT values were significantly reduced ($P<0.05$) in the group administered with kerosene compared to the healthy group. WBC, lymphocyte# count, MCV and MCH values were significantly increased ($P<0.05$) in the treated group compared to the control group. All the liver enzyme markers: AST, ALT, ALP and GGT were significantly increased ($P<0.05$) in group II compared to group I. This is an indication of impaired liver function. The total bilirubin (TB) value increased in group II while their total protein (TP) values significantly reduced ($P<0.05$) when compared to group I. HHK administration in group II rats caused significant reduction ($P<0.05$) in catalase, superoxide dismutase and reduced glutathione activities of liver homogenate. The MDA values were significantly high in group II compared to group I.

KEYWORDS: Hepatotoxicity, liver enzyme markers, hematological parameters, house hold kerosene and oxidative stress parameters.

INTRODUCTION

Various environmental pollutants, particularly those associated with crude oil cause many biochemical and toxic effects in terrestrial and marine animals (Berepubo *et al.*, 1994, Ngodigha *et al.*, 1999 and Carls *et al.*, 1999). The chemical composition of crude petroleum varies between geologic formations. (Coppock *et al.*, 1995). Crude petroleum is a mixture of different hydrocarbons and metals. (Edwards., 1989). It may be refined into fractions of kerosene, petrol, diesel, heavy gas oils, lubricating oils, as well as residual and heavy fuels, among others. However, kerosene, petrol, and diesel are the most commonly used fractionated crude petroleum products. Studies have shown that kerosene contains aliphatic, aromatic, and a variety of branched saturated and unsaturated hydrocarbons

(Henderson *et al.*, 1993 and Kato *et al.* 1993) The application of kerosene as cooking and lighting fuels in homes have resulted to direct contact of these products to a good percentage of the populace. Studies have shown that petroleum products like kerosene have great effects on the hematological parameters and the different organs in the body. Some of the biomarkers used to determine pollution levels include: alkaline and aspartate aminotransferase, Carboxylesterase, lactate dehydrogenase; as well as alkaline and acid phosphatase (Baron *et al.*, 1999 and Basagalila, 2000).

METHODOLOGY

Analysis of House Hold Kerosene (HHK)

The house hold kerosene (HHK) samples were bought from Nigeria National Petroleum Corporation (NNPC) and analysed in SGS Inspection Service Nigeria Limited to ascertain it to be House Hold Kerosene with the SON/DPR Specification using ASTM Methods (American Society for Testing and Material).

Experimental animals

Ten (10) weeks old albino rats weighing 140- 160g were obtained from Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria. They were housed in plastic cages with saw dust as beddings; food and water were given *ad libitum*. The rats were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

Preliminary Toxicity Study

Preliminary toxicity study to determine the volume of house hold kerosene (HHK) that could cause toxicity was carried out using 30 healthy albino rats. The rats were divided into six groups of five rats per group and were treated orally with 1, 2, 3, 4,5 and 6 ml/kg body weight of HHK respectively. The rats were observed over 24 hours period for nervousness, salivation, stretching, dullness, in-coordination and death. From the range of doses used, 1ml/kg was chosen for this study.

Grouping of animals

Another set of animals were grouped into two groups of ten rats per group as shown below:

Group I - control (Healthy group)

Group II- test group (1ml/kg body weight of HHK).

Collection of blood samples for hematological parameters and plasma preparation

The albino rats were sacrificed after 24 hours fast on the eighth day. They were anaesthetized by dropping into a jar containing cotton wool soaked in chloroform. Blood was collected by cardiac puncture for hematological analysis and the plasma was separated from whole blood by centrifugation at 3000 rpm for 10 min using a centrifuge and stored at 4 °C. The clear supernatant was used for the estimation of total protein, total bilirubin and liver function tests.

Determination of hematological parameters.

The total red blood cell (RBC), hemoglobin concentration (HGB) ,white blood cell count (WBC), platelet count and other hematological parameters were determined in the blood using BC-3200 Auto Hematology Analyzer in University of Lagos Teaching Hospitals (LUTH) in Idi-araba, Lagos, Nigeria.

Estimation Total Protein (TP) and Total Bilirubin (TB)

The plasma albumin and total protein were determined using Randox diagnostic kits.

Estimation of liver enzyme markers

Plasma enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) were determined

using Randox diagnostic kits.

Estimation of oxidative stress markers

Preparation of Liver Homogenate

The Liver tissues of the sacrificed albino rats were excised and some part homogenized in potassium chloride (10 mM) phosphate buffer (1.15 %) with Ethylenediamine tetra - acetic acid (EDTA; pH 7.4) and centrifuged at 12,000 x g for 30 minutes. The supernatant was used to assay for oxidative stress parameter.

Estimation of Lipid peroxidative (LPO) indices

Lipid peroxidation as evidenced by the formation of TBARS was measured in the homogenate by the method of Niehaus and Sameulsson, 1968 .

Estimation of superoxide dismutase (SOD)

The homogenate was assayed for the presence of SOD by utilizing the technique of magwere *et al* ,1997 with slight modification.

Estimation of catalase (CAT)

The liver homogenate was assayed for catalase colorimetric ally at 620nm and expressed as μ moles of H₂O₂ consumed/min/mg protein as described by sinha, 1972.

Estimation of Reduced glutathione (GSH)

Reduced glutathione (GSH) was determined in the liver homogenate using the method of Ellma,1959.

Data Analysis

Data analysis was done using the GraphPad prism computer software. Students 't'-test and one-way analysis of variance (ANOVA) were used for comparison. A P-value < 0.05 was considered significant.

RESULTS

Table I. Analysis of House hold Kerosene

Test	Method	Result	Units	SON/DPR Specification
APPEARANCE	VISUAL	C&B		Clear & Bright
COLOUR	ASTMD 156-07a	18		+20 Min
DENSITY@15°C	ASTMD 4052-11	0.8325	g/ml	
SPECIFIC GRAVITY @ 15°C	ASTMD 4052-11	0.8333		0.775-0.825
FLASH POINT(ABEL)	IP 170	49	°C	45 Min
CU. CORR. @100°C FOR 2HRS	ASTMD 130-10	1a		1b Max
TOTAL ACIDITY	ASTMD 3242-11	0.004	mg/KOH	0.01Max
TOTAL ACIDITY	ASTMD 1322-08	20	Mm	22 Min
TOTAL SULPHUR	ASTMD 4294-10	0.022	%wt	0.14 Max
DISTILLATION	ASTMD 86-12			
IBP		160.0	°C	
5% recovery		177.0		

10% recovery		184.0		
20% recovery		194.0		
50% recovery		219.0		
90% recovery		263.0		
95% recovery		274.0		
FBP		285.0		300 Max
RECOVERY		98.5	%vol	
RESIDUE		1.0		
LOSS		0.5		

The above table shows that the HHK used in this research work meet SON/DPR specification.

Table II. Effect of House Hold Kerosene on hematological parameters of albino rats.

Hematological Parameters	GROUP I	GROUP II
WBC ($\times 10^9/L$)	9.1 \pm 1.1	18.2 \pm 1.6*
Lymph# ($\times 10^9/L$)	6.2 \pm 2.6	11.7 \pm 2.5*
HGB (g/dl)	12.4 \pm 1.5	8.5 \pm 1.2*
RBC ($\times 10^{12}/L$)	7.9 \pm 1.5	5.06 \pm 0.1*
HCT (%)	40.1 \pm 3.6	30.1 \pm 5.2*
MCV(fl)	56.3 \pm 1.1	63.8 \pm 0.9*
MCH (pg)	17.8 \pm 0.3	19.7 \pm 0.4*
MCHC (g/dl)	31.8 \pm 0.2	30.8 \pm 0.4
RDW-CV(%)	16.2 \pm 0.1	16.6 \pm 0.4
RDW-SD (fl)	32.1 \pm 1.0	34.1 \pm 1.2
PLT ($\times 10^9/L$)	634.1 \pm 59.3	824.0 \pm 69.6*
MPV (fl)	6.2 \pm 0.2	6.3 \pm 0.1
PDW	16.1 \pm 0.2	15.8 \pm 0.4
PCT %	0.434 \pm 0.023	0.429 \pm 0.082

The values are the Means \pm SD for ten rats in each group.. *Significantly different from the control at P<0.05. White blood count (WBC), Lymphocyte number (Lymph#), Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT), Mean cell volume(MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration(MCHC), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV), Red Blood Cell Distribution Width Standard Deviation (RDW-SD), Platelet count (PLT), Mean platelet volume (MPV), platelet Distribution Width (PDW) and Plateletcrit (PCT).

Table II above shows the haematological parameters for the control group (group I) and the animals administered kerosene (group II).

Table III. Effects of House hold kerosene (HHK) on liver of albino wistar rats.

Parameters	AST (U/l)	ALT (U/l)	ALP (U/l)	GGT (U/l)	TB (mg/dl)	TP (g/l)
GROUP I	5.2 \pm 3.1	8.3 \pm 4.1	51.2 \pm 10.5	18.3 \pm 4.6	0.43 \pm 0.2	31.3 \pm 6.1
GROUP II	23.2 \pm 10.2 *	25.3 \pm 9.6 *	154.3 \pm 72.6 *	59.4 \pm 5.8 *	0.71 \pm 0.3*	19.8 \pm 1.1 *

The values are the Means \pm SD for ten rats in each group. *Significantly different from the control at P<0.05.

The above table shows the effect of HHK on the liver function of animals in group II.

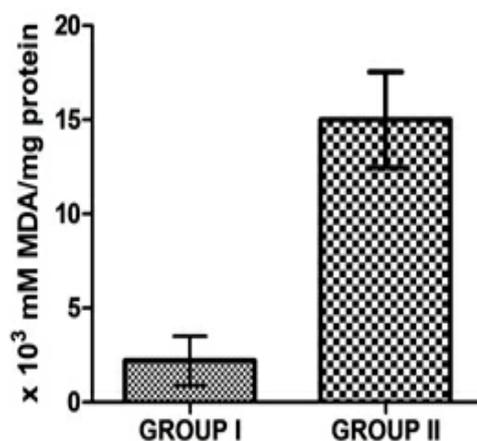


Figure I. Mean value of TBARS in liver homogenate of control group and group administered with 1ml/kg body weight of kerosene.

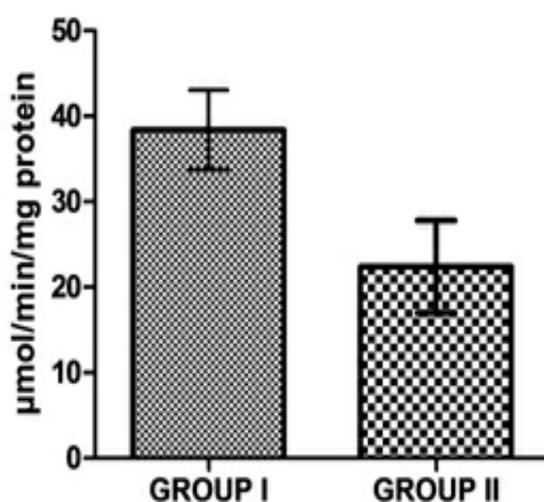


Figure II. Activities of Catalase in liver homogenate of control group and group administered with 1ml/kg body weight of kerosene.

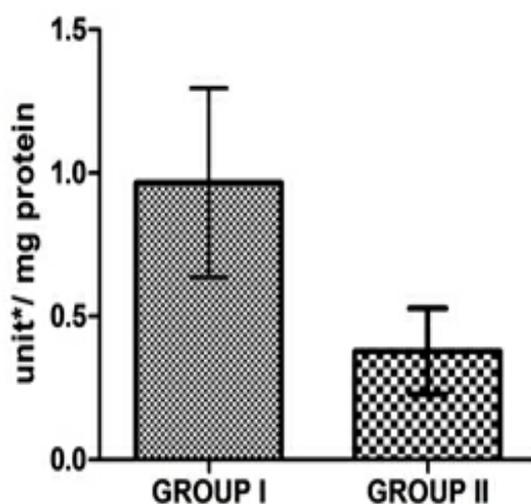


Figure III. Activities of Superoxide dismutase in liver homogenate of control group and group administered with 1ml/kg body weight of kerosene.

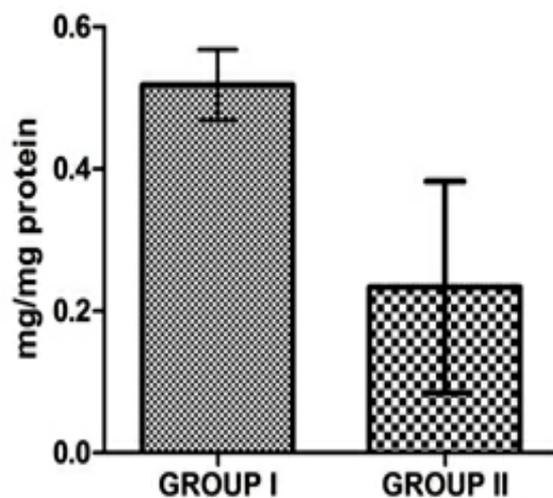


Figure IV. Mean value of Reduced glutathione in liver homogenate of control group and group administered with 1ml/kg body weight of kerosene.

Figure I-IV explain the effect of HHK on oxidative stress indices of animals administered with kerosene. The rats administered with kerosene have high MDA values, low catalase, superoxide dismutase and reduced glutathione activities when compared to the healthy animals.

DISCUSSION

Kerosene is an essential constituent of human life due to their domestic and industrial use. Table I shows that the house hold kerosene (HHK) used in this study met the SON/DPR Specification. Preliminary toxicity study to determine the volume of HHK that could cause toxicity shows that the animals exhibited changes in behavioural pattern such as salivation respiratory distress, coma, sedation and death. Hematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals [Sexena *et al* (2011) and Ohaeri *et al* (2011)].

The result as presented in Table I shows that there is a significant decrease ($P < 0.05$) in RBC, HGB and HCT in the group treated with kerosene compared to the control group. The primary reasons for assessing the RBC is to check anemia and to evaluate normal erythropoiesis. HGB level indicates the amount of intracellular iron, while HCT, representing the volume of RBC in 100ml of blood helps to determine the degree of anemia or polycythaemia. The significant reduction of RBC may be attributed to the cytotoxic effect of compounds present in kerosene.

These compounds cause oxidative damage on red cell membrane leading to hemolysis. Shakirov and Farkhutdinov (2000) showed that exposure of chemicals in oil-refining industry caused changes in the red adenyl and blood monooxygenase system. They suggested that such effect could alter the integrity of the red cell membrane to cause cellular hemolysis. Therefore, the result of this study agreed with their claim. Reduction in the values of RBC, HGB and HCT content as recorded in this study is suggestive of anemic condition which agrees with the work reported by Eyong *et al* (2004) on the hematotoxicity of crude oil. The MCV and MCH served to indicate variation in erythrocyte shape, size and hemoglobin content. There is a significant increase ($p < 0.05$)

in MCV and MCH values in group II compared to group I (Table II).

This indicate that group II animals have macrocytic anemia since increased in MCV and MCH values are known to be indicative of macrocytic anaemia. The increase in MCV values obtained in this study could have been due to the present of reticulocytes (even though reticulocytes count was not estimated in this study) in the circulating blood than the mature red blood cells. The number of circulating reticulocytes increased in order to carry sufficient oxygen to meet cellular demand where most of the mature red blood cells have been destroyed.

This perhaps may have accounted for the increased in MCV values recorded in this study. The WBC and the lymphocyte number were significantly increased ($P < 0.05$) in group administered with HHK compared to the control group. Leukocytosis observed in group II may be due to luekemia, bone marrow infection and inflammatory disease of the animals administered with kerosene. White blood cells function primarily in body defense against foreign bodies and this is often achieved through leucocytosis and antibody production (Marieb, 1995).

The mean platelet values was very high in the group administered with HHK compared to the healthy rats. Other hematological parameters (MCHC, RDW-CV, RDW-SD, MPV, PDW and PCT) show no significant difference ($P < 0.05$) both in group I and II. It is therefore concluded that kerosene is highly toxic and are potential damaging agents to the hematopoietic system.

AST is an enzyme found mostly in the heart muscle, liver cells, skeletal muscles and kidneys. Injury to these tissue result in the release of the enzyme into the blood stream. Elevated levels are found in myocardial infarction, cirrhosis and hepatitis (Sood, 2009). The result from this study showed significant increases ($P < 0.05$) in activities of liver damage marker enzymes- AST and ALT (Table II1).

This increase in activities of these enzymes indicated cellular leakage and failure of functional integrity of liver cell

membranes (Mukherjere, 2003). Increase in the ALP and GGT values of group II animals compared to the control group animals may imply that damage occur in the liver cells of the rats administered with kerosene (Table III), since the activities of these enzymes are reported to be increased in liver damage (Halim *et al* 1997).

ALP is involved in the transport of metabolites across membrane, synthesis of *certain* enzymes, protein synthesis, 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). The increase in these liver marker enzymes (AST, ALT, ALP and GGT) in the liver homogenate is responsible for the hepatotoxicity of the liver in the group administered with kerosene.

Total bilirubin increased significantly ($P<0.05$) in group II rats compared to control rats. A rise in plasma level of bilirubin suggests liver cell damage, since liver cells are responsible for removing bilirubin from serum (Nelson and Cox, 2005).

Another possible reason for an increase in bilirubin may be a metabolic disturbance in liver involving defective conjugation and/or excretion of bilirubin (Mankani *et al.*, 2005). The bilirubin route of elimination is perhaps the most important contributing source to the excretion of xenobiotic substances, but is of primary importance for the excretion of the animal's metabolites. Since the liver encounters nutrients, environmental toxicants and waste products, within this framework, it extracts the environmental toxicants and waste products to prevent their circulation to other parts of the body. The total protein values of group II rats reduced drastically compared to group I.

Oxidative stress is the presence of reactive oxygen species (ROS) in excess of the available antioxidant-buffering capacity (Adly., 2010). ROS can damage molecular targets—lipids, proteins, and DNA, thus altering the structure and function of the cell, tissue, organ, or system (Roberts and Hubel 2004).

It has been reported that metabolism of aliphatic and aromatic hydrocarbons, the major constituents of petroleum products (e.g. kerosene) as well as other xenobiotics substances significantly increase the productions of free radical species in various tissues (Lam *et al.*, 1994; Bondy *et al.*, 1995).

In this study there is a significant reduction ($P<0.05$) in activities of SOD, CAT and reduced glutathione (GSH) and malondialdehyde (MDA) values significantly increase ($P<0.05$) in the liver homogenate of group II compared to group I (Figure I-IV). This is a result of hepatic injury from oxidative stress caused by the administration of kerosene. In system, organ and tissue damage, GSH makes up the first line of defense against free radicals resulting from xenobiotic ingestion.

The drop in the concentration of liver GSH and corresponding increase in concentration of malondialdehyde indicates hepatocytes damage. Increased

MDA indicates increased lipid peroxidation which could have resulted from depletion of GSH concentration.

CONCLUSION

The results of this work shows that administration of house hold kerosene affects hematological parameters, causes impairment of the liver function and results in oxidative stress in wistar albino rats.

RECOMMENDATIONS FOR FURTHER STUDIES

Further studies are needed to determine the effect of house hold kerosene on the genetic makeup of animals.

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