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Does A Singular Exposure Of Male Albino Rats To Kerosene Affect The Liver?

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ABSTRACT

This study evaluated the toxic effect of kerosene on the liver of male rats within 24 h of acute exposure. The kerosene samples total hydrocarbon analysis by gas chromatography revealed that kerosene contained C12 to C16 carbons and a total hydrocarbon content of 65,332 mg/L. The rats were intraperitioneally injected with kerosene at different dose levels (22.2, 44.2, 88.9 and 177.8g/kg kerosene) following the determination of LD50 of kerosene as 86.7g/kg. The rats were observed within 24 h for signs and symptoms of toxicity, and approximate time in minutes of occurrence of each symptom noted. The appearance and severity of symptoms were dose dependent. The onset of symptom which was fast (within 1 h of injection) occurred only at fairly high doses. The results indicated that exposure to kerosene is potentially toxic and the degree of toxicity and onset of symptoms were influenced by the dose to which the rats were exposed. The biochemical parameters Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP) studied showed significant dose dependent increases in levels, $P < 0.001$, indicating hepatic damage. This study has shown that exposure to kerosene is toxic to the liver and its severity is dependent on the dose of exposure.

Key words: Biochemical parameters, Intraperitoneal, Kerosene, Liver, Rats and Toxicity.

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INTRODUCTION

Kerosene is a complex mixture of hydrocarbons with carbon numbers predominantly in the range of C_9 toC_{16} and boiling point between 145 to 300^{o}C (CONCAWE, 1991). Kerosene is mainly used as domestic cooking and lighting oil in Nigeria in spite of introduction of cooking gas and electricity over the years, kerosene based cooking and lighting, still is, and will remain in use for some time to come. This is because of the level of impoverishment and inadequacy of gas and electricity supply in Nigeria.

Exposure to kerosene, which occurs frequently in Nigeria, can result in toxicity. The sources of exposure include accidental ingestion by children, motor mechanics and non-accredited vendors, occupational inhalation by storage tank workers, transport tank workers, service station workers and petroleum refinery workers; environmental pollution of land, water or air resulting from spillage as a results of kerosene pipeline leakages as a result of vandalization or ageing; and common practices such as use of kerosene for washing of hands by motor mechanics or drinking of kerosene as antidote to snake bite (Igboh et al., 2001).

The acute toxicological effects of exposure to kerosene which are related to dose and exposure time have been reported to include burning in the mouth, throat and chest, stomach irritation, nausea, vomiting and cyanosis

Table 1a. Acute toxicity study of kerosene on male rats.

Group	Dose (g/kg)	Number dead	Number alive	Ave. time of death (h)
1	0.00	0	5	-
2	22.20	0	5	-
3	44.40	1	4	32.25
4	88.90	3	2	18.15
5	117.80	5	0	6.10

n = 5 rats.

(bluish discoloration of extremities), restlessness, excitement, confusion, disorientation, ataxia and coma (IPCS, 1982; Beck, 1984; Grant, 1986; Klaasen, 1996). The role of the liver in detoxification of exogenous compounds such as kerosene has made it susceptible to damage. The liver contains enzymes which are present in serum in low concentration. Elevated serum levels of these enzymes occur when there is liver injury, damage or necrosis of liver cells.

There have been reports on damage to the liver from inhalation and ingestion of kerosene, resulting in elevations of the aminotransferases ALT, AST, and ALP (IPCS, 1982; Beck, 1984; Grant, 1986; Klaasen, 1996; Ayalogu, 2001; Dede et al., 2002; Obianime, 2001). Despite these reports, human exposure to kerosene has continued to increase in Nigeria, especially in the Niger Delta region, as a result of the irresponsible activities of the petroleum industry and frequent episodes of kerosene scarcity which has lead to hoarding of kerosene around the house and frequent episodes of kerosene pipeline sabotage. The aim of the present study was therefore to investigate the extent to which kerosene is hazardous to man by carrying out acute toxicity tests and evaluating the changes that occur in the levels of serum enzymes ALT, AST, and ALP.

MATERIALS AND METHODS

Kerosene

The kerosene with density 0.8g/ml(kg/l) used for the study was purchased from AP filling station, Rumuokwuta junction, Port Harcourt, Rivers State. It was stored in a 1 litre industrial bottle, well corked and kept in the dark to avoid loss of any volatile components and reaction with light.

Test Animals

Male Wister albino rats (*Rattus ratus*) were bred for this study at the animal house of the Department of Medical Laboratory Science, Rivers State University of Science and Technology, Port Harcourt. The rats were fed with rat pre-mix rat feed and water *ad libitum.* Twenty well ventilated iron/plastic cages (standard rat cages) with plastic water cans and feed troughs were used for rat breeding and the study.

Reagents

Commercially prepared AST, ALT and ALP kits (Randox diagnostics) were purchased in Port Harcourt and used for biochemical study.

Equipment

Jenway spectrophotometer, model 6301 was used for biochemical analysis. Gas chromatography (GC - FID, HP 6890 series) with GC recorder interfaced to a HP pentium 111 mmx computer was used for gas chromatography analysis.

Total Hydrocarbon Analysis of Kerosene

This was done by gas chromatographic analysis (courtesy Technology Partners International, Nigeria, Ltd.). Diluted samples were rapidly injected by means of hypodermic syringe through a rubber septum in the column. Separation occurred as the vapour constituent was partitioned between the gas and liquid phases. The sample was automatically detected as it emerged from the column (at a constant flow rate) with FID detector whose response was dependent upon the composition of the vapour, by measuring the retention time (that is, the minutes between the time the sample was injected and the time the chromatographic peak was recorded). Run time for the analysis was 38.3 min.

Toxicity Testing

LD_{100} value (minimum dose of kerosene that caused 100% death of the animal population) was obtained from a pilot study. This was 177.8 g/kg, Table 1a (Dede et al., 1997; Dede and Iyanuwura, 2003; Matsumura, 1975). Dose ranges for the acute toxicity study were determined as 22.2, 44.4, 88.9 and 177.8 g/kg. Five cages arranged and grouped as A, B, C, D and E with five male rats per group, each weighting 180 ± 10 kg were used for the study. The rats were administered 22.2, 44.4, 88.9 and

Table 1b. LD_{50} calculation of kerosene treated male rats.

Group	Dose (g/kg)	Dose diff.	Number dead	Mean dead	Dose diff. x mean dead
X 1	0.0	-	0	-	-
2	22.2	22.2	0	-	-
3	44.4	22.2	1	0.5	11.1
4	88.9	44.4	3	2.0	88.9
5	117.8	88.9	5	4.0	355.6
Total					455.6

177.8 g/kg intraperitoneally. Behavioural pattern of injected rats were monitored and particular attention paid to the onset of each symptom (pallor, sedation, respiratory, distress, coma and death). The measure of acute toxicity, LD_{50} (which is the dose that killed 50% of the animals under investigation was determined as 86.7g/kg in Table 1 using the arithmetic method of Karber and was finally rated using Matsumura toxicity rating method (Matsumura, 1975). At termination, the animals were anaesthetized with inhaled chloroform. Blood was withdrawn from the cardiac aorta for biochemical analysis.

Histopathological Assays

The liver tissues were excised and fixed in 10% neutral buffered formalin at room temperature and the tissues were processed using Automated Vacuum Tissue Processor Leica ASP6025 (Biosystems, Barcelona, Spain). They were embedded using paraffin wax and the blocks were sectioned into 5 mm thick slices, and stained with Hematoxylin and Eosin. Specimens were examined for morphological changes under light microscope (Olympus 6V20WHAL) (Olympus Imaging America Inc., PA, USA) and images were captured using a Smartphone digital camera obtained by its autofocus and automatic exposure control.

Statistics

Statistical analysis was carried out using window SPSS package (SPSS 22.0.0.0 version). Data were analysed using one way analysis of variance (ANOVA), results obtained were further subjected to test for least significant difference (LSD). Data were expressed as the mean ± standard error of mean and values of $P \leq 0.001$ were considered significant.

Determination of LD_{50} of Kerosene Treated Male Albino Rats

LD_{50} = Minimum dose that caused 100% Death - (Dose Difference x mean dead) ÷ Number of rats, from Table 1(b) Minimum dose that caused 100% Death = 177-8, Dose difference x Mean dead = 455.6, Number of rats per group = 5.

RESULTS AND DISCUSSION

The total hydrocarbon content of kerosene (Table 2) showed that the hydrocarbons in the kerosene sample had between 12 to 16 carbons and the total hydrocarbon content was 65,332 mg/L. In Figure 1, peak areas (PA) are presented as a function of retention time within the gas chromatographic column. The different molecular species that were present, that is, each peak represents a particular molecular specie or set of species with a common molecular mass, with many smaller molecular mass species corresponding to smaller retention times. The PA values correspond to the abundance of species. Dose-specific deteriorative changes in behavioural pattern with respect to onset of symptoms (Pallor, Sedation, Respiratory distress, Coma and Death) were observed for kerosene treated rats (Table 3). Severities of symptoms of toxicity (Table 4) were quantified, by using the following score; $pallor^{+,}$ $sedation^{2+}$, respiratory $distress^{3+}$, $coma^{4+}$ and $death^{5+}$, then multiplying by the number of rats affected.

Severities of symptoms were observed to be increasing with increasing dose of kerosene. At 22.2g/kg, only pallor and sedation occurred while at higher doses (44.4, 88.9 and 177.8g/kg) respiratory distress, coma and death occurred. Biochemical indices ALT, AST and ALP used in assessing the acute toxicity effect of gasoline on blood samples are shown on Table 5. Statistically significant dose dependent increases in ALT, AST and ALP from control values were observed at $P < 0.001$.

$$= 177.8 - \left(\frac{455.6}{5}\right) = 177.8 - 91.1 = 86.7$$

LD_{50} = 86.7 g/kg. Based on the International Classification of Chemical Toxicity, Kerosene with an LD_{50} of 86.7g/kg can be classified as relatively harmless.

Table 2: Total hydrocarbon content of Kerosene.

Ret Time(Min)	Type	Area(pA*s	Amt/Area	Amount(mg/l)	Grp	Name
4.386		-	-	-	1	C6
5.221		-	-	-	1	C7
6.212		-	-	-	1	C8
7.166		-	-	-	1	C9
8.283		-	-	-	1	C10
9.203		-	-	-	1	C11
10.238	MM	3.972274e4	7.66899e-1	3.04669e4	1	C12
12.272	MM	4.42225e4	7.87071e-1	3.48062e4	1	C14
14.073	VV X	3.86854	15.13422	58.54736	1	C16
15.589		-	-	-	1	C18
16.740	VV T	49.28559	0.00000	0.00000	1	C20
19.228		-	-	-	1	C24
21.254	VV T	20.09703	0.00000	0.00000	1	C28
23.717		-	-	-	1	C32
27.964		-	-	-	1	C36
36.123		-	-	-	1	C40
Totals				6.53317e4		

Results obtained with enhanced integrator!

Group summary:

Group ID	Use	Area (pA*s)	Amount(mg/l)	Group Name
1		8.40231e4	6.53317e4	Aliphatic Hydrocarbon

PA: Peak area, Ret Time:Retention Time.

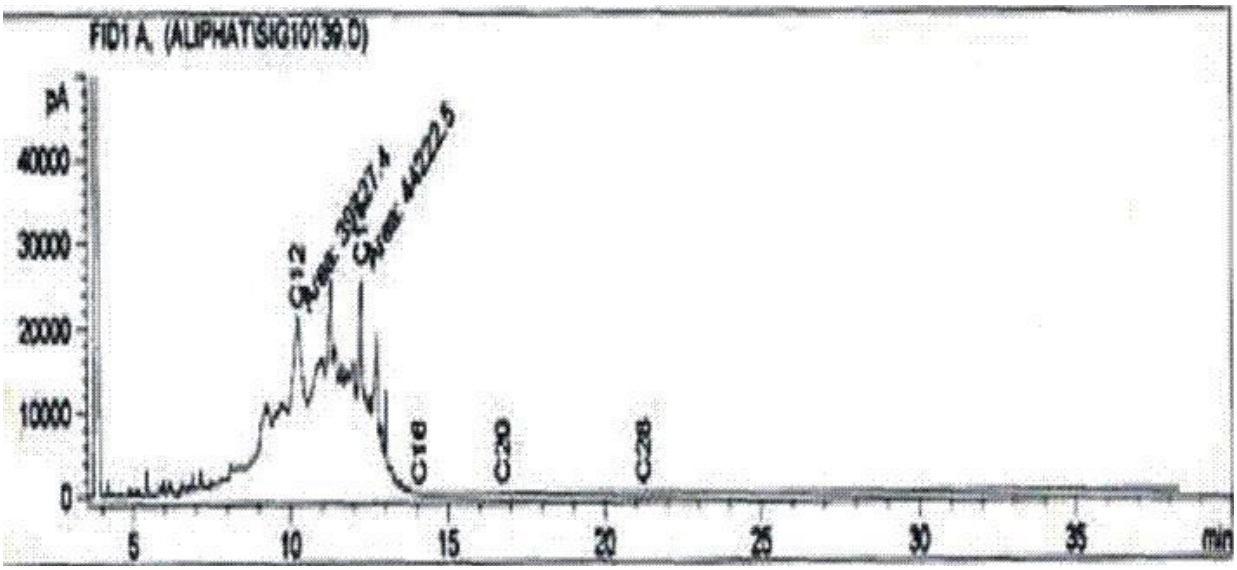

Figure 1. Total hydrocarbon content of kerosene.

$LD_{50} = LD_{100}$ - $\frac{\text{Dose difference} \times \text{mean dead}}{\text{Number of rats per group}}$ = 177.8 – $\frac{455.6}{5}$ = 86.7g/kg

The red arrow indicates congestion. The black asterisk indicates disorganization of hepatic cords. The red asterisk indicates fusion of the portal triad. CV = central vein; HA = hepatic artery; PV = portal vein; BD = bile duct and eosinophilic infiltrations of the lobular cells. The minimum dose that caused 100% death (LD_{100}) was determined as 177.8 g/kg from a pilot study. Furthermore, LD_{50} of kerosene was calculated to be 86.7 g/kg (Table 4). Based on Matsumura toxicity rating of chemicals (Matsumura, 1975), the kerosene sample could be rated as relatively harmless. Changes in behavioural pattern with respect to onset of symptoms (pallor, sedation,

Table 3. Dose-specific changes in behavioral patterns of rats under kerosene toxicity.

Dose (g/kg)	Pallor (mins)	Sedation (mins)	Resp. distress (mins)	Coma (mins)	Death (mins)
0.00	ND	ND	ND	ND	ND
22.2	ND	ND	ND	ND	ND
44.4	60.8 ± 4.0	75.2 ± 3.4	306.8 ± 9.0	1625 ± 38.2	19352 ± 3.9
88.9	38.0 ± 1.4	37.4 ± 4.3	152.0 ± 3.7	549.0 ± 4.4	111.0 ± 4.4
177.8	22.6 ± 3.1	21.6 ± 3.2	86.4 ± 8.4	191.2 ± 2.2	370.4 ± 18.9

ND = Not detected, n = 5, X ± SEM.

Table 4. Severity of symptoms of toxicity versus dose of kerosene.

Dose (g/kg)	Pallor	Sedation	Resp. distress	Coma	Death	Total
0.0	0	0	0	0	0	0
22.2	5^+	10^+	0	0	0	0
44.4	5^+	10^+	3^+	4^+	5^+	27
88.9	5^+	10^+	9^+	12^+	15^+	51
177.8	5^+	10^+	15^+	20^+	25^+	75

Key Symptoms = (Pallor, Sedation, Resp. distress, Coma and Death) X^+ = no. of rat.

Table 5. Dose specific changes in biochemical parameters in rats injected kerosene.

Groups	Doses (g/kg)	ALT (iu/L)	AST (iu/L)	ALP (iu/L)
1	0.0	23.0 ± 5.1	27.0 ± 4.3	37.1 ± 5.5
2	22.2	27.3 ± 3.1	34.3 ± 4.0	60.0 ± 5.0
3	44.4	28.7 ± 1.5	37.3 ± 2.5	96.3 ± 6.0
4	88.9	36.3 ± 4.0	50.0 ± 3.6	355.0 ± 42.7
5	177.8	46.7 ± 5.5	74.0 ± 10.2	636.0 ± 39.0

n=3; X ± SEM (Where n = no. of rats, SEM = Standard Error of Mean, X = mean).

respiratory distress, coma and death) were observed for kerosene treated rats (Table 2). The onset of pallor, sedation and respiratory distress occurred at 22.2 g/kg while the onset of coma and death occurred at 44.4 g/kg. It was however observed that the onset of symptoms at the various doses occurred slowly (between 21 to 1935 min after injection at all doses).

The severity of symptoms (Table 3) increased from the onset of symptoms with increasing dose concentration. These findings were in agreement with Igboh et al. (2001). The slow onset of symptoms of toxicity observed could be explained by the molecular properties of kerosene. The large molecular size of kerosene (Carbon atoms between 12 and 16), accounted for the inability of kerosene to penetrate cellular membranes quickly, this finding agrees with the reports of Overbeck et al. (1954), Kuhnhold et al. (1980) and Igboh et al. (2001). The biochemical parameters monitored were ALT, AST and ALP, often used in studies as markers of liver damage. These enzymes are usually present in the blood in low levels but when liver hepatocytes are diseased or its integrity disturbed, the activity of the enzymes increase in blood. Table 3, shows dose dependent significant increases of the liver enzymes ALT, AST, ALP at $P < 0.001$ level of significance.

The increases in AST and ALT activity may be as a result of injury or damage to liver cells following exposure to hydrocarbon fraction present in Kerosene. Metabolism of aliphatic and aromatic hydrocarbons which are the major constituent of Kerosene may have given rise to reactive free radical groups, which resulted in injury to the cells. Alkaline phosphatase is involved in the transport of metabolites across the cell membranes, protein synthesis and synthesis of some enzymes secretors activities and glycogen metabolism. This increase in the enzyme activity may be due to reactive intermediates which may have disrupted the cell membranes leading to release of the enzyme. These findings were corroborated by Uboh et al. (2005), Patrick-Iwuanyanwu et al. (2011) and Sharma et al. (1995), were they collectively agreed that kerosene inhalation or contaminated diets led to degenerative changes in the structural integrity of the hepatic cells, which led to the highly significant increases in the liver enzymes. The analysis of the liver tissues

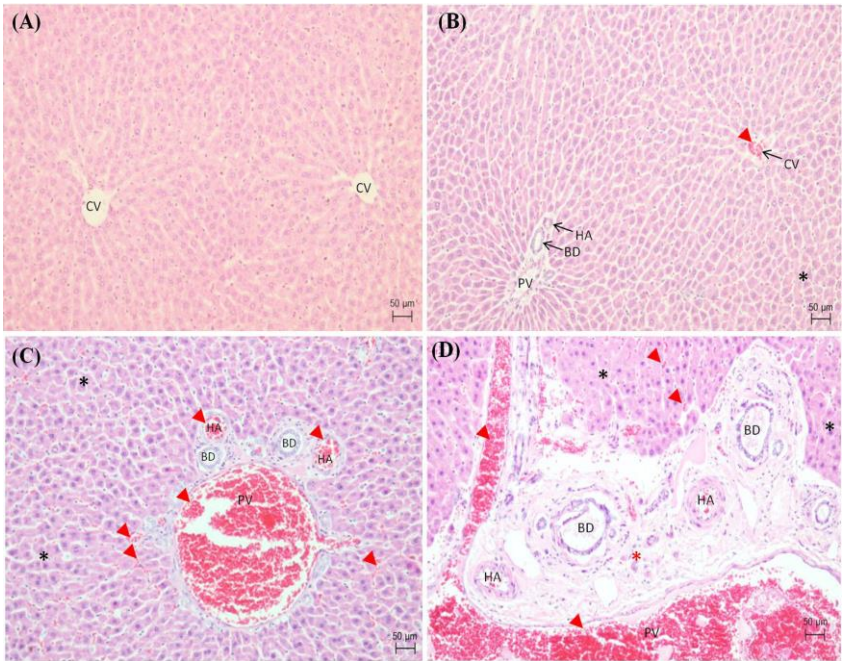

Figure 2. Histological alterations in the liver cells of rats within 24 h, after singular (i.p.) administration of kerosene (H and E staining, 100×). (A for 0 and 22.2 g/kg photomicrographs of liver in the control group (1) and test group (2). (C, D and E) represents photomicrographs of liver cells after singular (i.p.) administration of kerosene at 44.40, 88.90 and 117.80 g/kg, for test groups 3, 4 and 5, respectively.

revealed defective changes in the structural integrity of the hepatic cells (Figure 2 A, B, C and D). Changes like cytoplasmic granularity and congestion of the central vein, focal sinusoidal dilatation and vascular congestion with eosinophilic infiltrations of the lobal cells and necrosis which were dosed dependent were observed. This agreed with the reports of Mclntyre and Rosalki (1992) and Abdel et al. (1997).

CONCLUSION

The results of this work suggests that repeated exposure to kerosene may elicit hepatotoxicity, thereby impairing the normal liver function, and in so doing, the health status of the individual. Petroleum workers should therefore have regular medical check-up to ascertain their health condition early enough. To avoid serious health implication that could impair the individual affected. Further in-depth chronic study should be conducted to ascertain the biochemistry of kerosene toxicity.

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