

A Comparative Evaluation of Acute Toxicities of Gasoline and Kerosene in Rats Using Haematological Parameters

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Abstract: *Acute toxicity studies were done to determine the effect of gasoline and kerosene treatment on male Wister albino rats using haematological parameters. Total hydrocarbon analysis was done to determine the total hydrocarbon content of gasoline and kerosene samples used in the study. The gasoline contained C₆ – C₁₂ carbon and total hydrocarbon content of 103,741mg/L while kerosene content of C₁₂ – C₁₆ carbons and total hydrocarbon content of 65,332mg/L. The male albino rats were intraperitoneally injected with gasoline and kerosene at different dose levels (14.6, 29.2, 58.3 and 116.17g/kg gasoline, and 22.2, 44.2, 88.9 and 177.8g/kg kerosene; after determination of LD₁₀₀ from a pilot study as 116.7g/kg for gasoline and 177.8g/kg for kerosene). The LD₅₀ of gasoline was determined as 56.8g/kg and kerosene as 86.7g/kg. Result of haematological parameters Haemoglobin (Hb) and Packed Cell Volume (PCV) for kerosene and gasoline treated rats which were dose dependent showed significantly decreased mean values while White Blood Cell Count (WBC) values increased significantly (P < 0.05, 0.01 and 0.001). The major finding from this study was that both kerosene and gasoline were toxic to blood cells and the effect was dose dependent. Also the effects tended to be more pronounced for gasoline than for kerosene, suggesting that gasoline had a higher toxicity effect than kerosene.*

Keywords: Acute toxicity, Gasoline, Haematological parameters, Kerosene, Wister Rat..

1. Introduction

Petroleum products are very important to man as sources of energy, second only to food and shelter as essential commodities [1]. In Nigeria, petroleum industry is the lifeline of the economy contributing immensely to the physical development of infrastructures and revenue for the nation.

Due to oil prospecting activities, especially in the Niger Delta area of Nigeria, petroleum products have become a major source of pollution of soil and water over the years. The rate of occurrence of pollution has been rising with the increase of exploration, exploitation, production and distribution of petroleum products. This has created tremendous concern about its effects on living organisms.

Petroleum products such as gasoline and kerosene used in this study are very useful to man. Kerosene is mainly used as domestic cooking and lighting oil in Nigeria. In spite of the 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. Gasoline is mainly used as fuel for motor vehicles, motorcycles and generators in Nigeria. Both gasoline and kerosene are also used as components of many pesticides, cleaning agents, paint thinners and solvents.

Gasoline contamination of kerosene which occurs frequently in Nigeria, especially during periods of gasoline and kerosene scarcity, has been implicated in accidental fires associated with the use of kerosene illumination lamps and stoves. Sources of contamination of kerosene by gasoline include switch loading of transport trucks (fuel tankers) i.e. delivering a load of gasoline from a tanker on one delivery and a load of kerosene from the same tanker on the next delivery; and the use of the same unmarked container by vendors and consumers for storage of gasoline and kerosene at different times.

Exposure to gasoline and kerosene can result in toxicity. The sources of exposure include accidental ingestion by children, motor mechanics and fuel 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 result

of gasoline pipeline leakages due to age and sabotage; and common practices such as use of gasoline and kerosene for washing of hands by motor mechanics or drinking of kerosene as antidote for snake bite [2].

The acute toxicological effect of exposure to gasoline and kerosene which are related to dose and duration of exposure have been reported to include burning in the mouth, throat and chest, stomach irritation, nausea, vomiting and cyanosis (bluish discoloration of extremities) restlessness, excitement, confusion, disorientation, ataxia and coma [3] - [9].

Haematological parameters have been used in studies over the years as important indices of health change. These include Hb, PCV and WBC which were monitored in this study to determine the effect of kerosene pre-treated with gasoline in rats induced by chronic exposure [8]. In this study these indices were used to do a comparative evaluation of the acute toxicities of kerosene and gasoline in rats.

2. Materials and Method

2.1 Kerosene and Gasoline

The kerosene and gasoline used for the study were 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.

2.2 Test Animals

Male Wister albino rats (*Ratus rattus*) 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. They were fed with 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.

2.3 Equipment

Erma Inc full automatic blood cell counter, model PCE – 210N was used to analyze all samples for haematological study at the University of Port Harcourt Teaching Hospital.

2.4 Total Hydrocarbon Analysis of Kerosene and Gasoline

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 samples were automatically detected as they emerged from the column (at a constant flow rate) with FID detector whose response was dependent the composition of the vapour, by measuring the retention time (i.e. the minutes between the time the sample was injected and the time chromatographic peak was recorded). Run time for the analysis was 38.3 minutes.

2.5 Toxicity Testing

A pilot study was done to obtain LD₁₀₀ value (minimum dose of gasoline and kerosene that caused 100% death of the animal population). This was determined as 116.7g/kg for gasoline and 177.8g/kg for kerosene (Table 1 and 2). Dose ranges for the

acute toxicity study were subsequently determined as 14.6g/kg, 29.2 g/kg, 58.3 g/kg and 116.7g/kg of gasoline and 22.2 g/kg, 44.4 g/kg, 88.9 g/kg and 117.8 g/kg of kerosene.

Ten groups of five male rats per group, of mean weight 180.0 + 10.0g were intraperitoneally injected with gasoline and kerosene at different dose levels (0.0, 14.6, 29.2, 58.3, 116.7g/kg; and 0.0, 22.2, 44.4, 88.9, 117.8g/kg respectively).

At termination, the animals were anaesthetized with inhaled chloroform. Blood was withdrawn from the heart using cardiac puncture procedures for haematological study.

3. Result

The total hydrocarbon content of gasoline (Table 3) and kerosene (Table 4) showed that the hydrocarbons in the gasoline sample had between 6 to 12 carbons and total hydrocarbon content of 103, 741mg/L while kerosene had between 12 to 16 carbons and total hydrocarbon content of 65,332mg/L. In Fig 1 and 2, peak areas (PA) were presented as a function of retention time with the gas chromatographic column. The different peaks represent different molecular species that are present i.e. each peak presents a particular molecular species or set of species with a common molecular mass, with mainly smaller molecular mass species corresponding to smaller retention times. The PA values correspond to the abundance of species.

Haematological indices Hb, PCV and WBC were used in the assessing the acute toxicity effect of gasoline and kerosene on blood samples (Tables 5 and 6). Gasoline produced statistically significant dose dependent decreases in the Hb and PCV from control at 58.3 g/kg and 116.7 g/kg at $P \leq 0.05$. WBC increased significantly from control at 58.3 g/kg and 116.7 g/kg $P \leq 0.01$. For the kerosene sample, significantly decreased values in PCV from control were found only at dose 177.8 g/kg $P \leq 0.05$. Hb values decreased significantly from control at 22.2 g/kg ($P \leq 0.005$) 44.4 g/kg, 88.9 g/kg and 177.8 g/kg $P \leq 0.01$. WBC values increased significantly from control at only 177.8 g/kg $P \leq 0.01$.

Table 1(a): Determination of Median Lethal Dose LD₅₀ of gasoline treated male rats.

S/N	DOSE g/Kg	No of Death	No Alive	Average Time of Death (hr)
1	0.00	0	5	--
2	14.6	0	5	--
3	29.2	1	4	7.00
4	58.3	3	2	2.40
5	116.7	5	0	1.20

$n = 5$ rats; where $n =$ no of rats, SEM = standard error of mean, $X =$ mean

Table 1(b): LD₅₀ determination of gasoline treated male albino rats.

Group	DOSE (g/Kg)	Dose Diff.	No Dead	Mean Dead	D. Diff x Mean Dead
1	0.00	0.0	0	--	--
2	14.6	14.6	0	--	--
3	29.2	14.6	1	0.5	7.3
4	58.3	29.1	3	2.0	58.2
5	116.7	58.9	5	4.0	233.6
				Total	299.1

$$LD_{50} = \text{Minimum dose that caused 100\% Death} - \frac{\text{Dose diff.} \times \text{mean dead}}{\text{No of rats per group}}$$

From Table 1(b)

Minimum dose that caused 100% Death = 116.7

Dose difference x Mean dead = 299.1

Number of rats per group = 5

$$= 116.7 - \frac{299.1}{5}$$

$$= 116.67 - 59.82$$

$$LD_{50} = 56.85 \text{ g/kg}$$

Based on the international classification of chemical toxicity (App), Gasoline with an LD₅₀ of 56.85g/kg can be classified as a relatively harmless substance [9].

Table 2(a): Determination of Median Lethal Dose LD₅₀ of Kerosene treated male albino rats.

S/N	DOSE g/Kg	No of Death	No Alive	Average Time of Death (hr)
1	0.00	0	5	--
2	14.6	0	5	--
3	29.2	1	4	32.25
4	58.3	3	2	18.15
5	116.7	5	0	6.10

n = 5 rats; where n = no of rats, SEM = standard error of mean, X = mean

Table 2(a): LD₅₀ determination of Kerosene treated male albino rats.

Group	DOSE (g/Kg)	Dose Diff.	No Dead	Mean Dead	D. Diff x Mean Dead
1	0.00	0.0	0	--	--
2	22.20	22.2	0	--	--
3	44.40	22.2	1	0.5	11.1
4	88.90	44.4	3	2.0	88.9
5	117.80	88.9	5	4.0	355.6
				Total	455.6

$$LD_{50} = \text{Minimum dose that caused 100\% Death} - \frac{\text{Dose diff} \times \text{mean dead}}{\text{No of rats per group}}$$

From Table 2(b)

Minimum dose that caused 100% Death = 177.8

Dose difference x Mean dead = 455.6

Number of rats per group = 5

$$= 177.8 - \frac{455.6}{5}$$

$$= 177.8 - 91.1 = 86.7$$

$$LD_{50} = 86.7 \text{ g/kg.}$$

Based on the international classification of chemical toxicity Kerosene with an LD₅₀ of 86.7g/kg can be classified as relatively harmless.

TABLE 3: Total Hydrocarbon content of Gasoline

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/l]	Grp	Name
4.318	MM	1.15912e4	9.10958e-1	1.05591e4	1	C6
5.177	MM	2.16506e4	8.98609e-1	1.94555e4	1	C7
6.223	MM	1.79682e4	8.48185e-1	1.52404e4	1	C8
7.222	MM	3.26924e4	8.11288e-1	2.65230e4	1	C9
8.186	MM	2.44600e4	7.73578e-1	1.89217e4	1	C10
9.230	VV S	1.12322e4	7.89047e-1	8862.75110	1	C11
10.202	VV S	5329.62061	7.83975e-1	4178.28880	1	C12
12.202		-	-	-		C14
14.036		-	-	-		C16
15.589		-	-	-		C18
16.895	VV X	57.62395	0.00000	0.00000		
19.231	VV X	11.29666	0.00000	0.00000		
21.192	VV T	7.24655	0.00000	0.00000		C28
23.717		-	-	-		C32
27.964		-	-	-		C36
36.123		-	-	-		C40

Totals : 1.03741e5

Results obtained with enhanced integrator!
Group summary :

Group ID	Use	Area [pA*s]	Amount [mg/l]	Group Name
1		1.25000e5	1.03741e5	Aliphatic Hydrocarbon

PA= Peak area Ret Time = Retention time

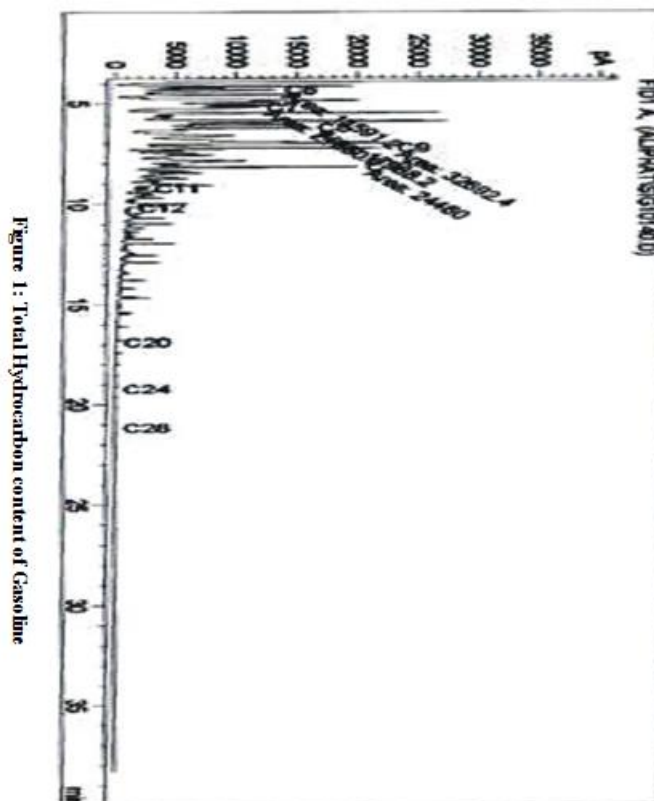


Figure 1: Total Hydrocarbon content of Gasoline

Table 4: Total Hydrocarbon content of Kerosene

RetTime [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	3.97274e4	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

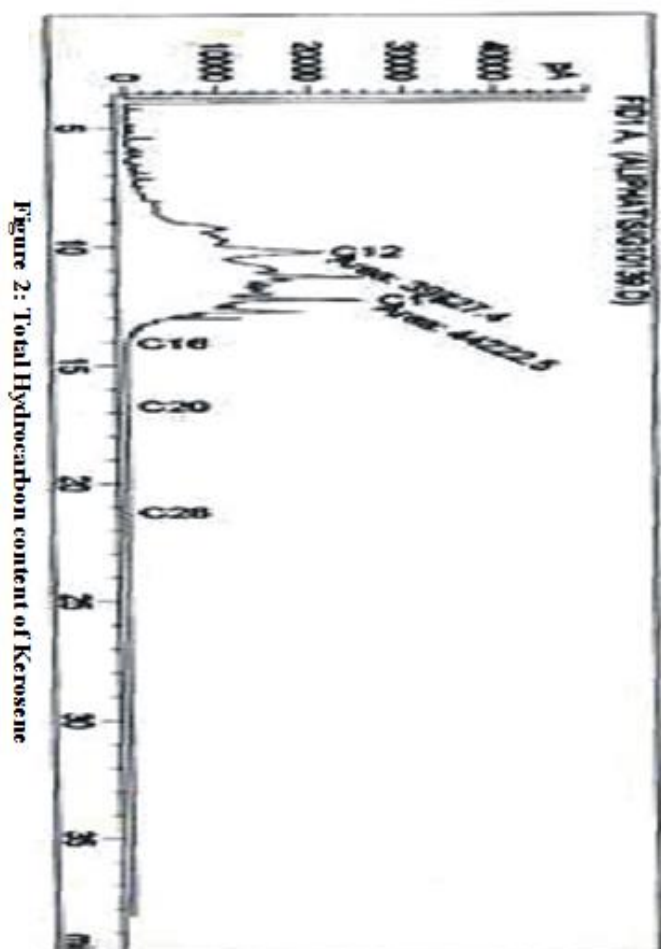


Table 5: Toxic effect of Gasoline on Haematological parameters

Groups	1	2	3	4	5
Dose (g/kg)	0	14.6	29.2	58.3	116.7
Hb (g/dl)	14.2 ± 2.2	13.0 ± 2.0	12.2 ± 1.9	11.9 ± 0.7	10.1 ± 0.7
PCV (%)	42.7 ± 6.4	39.0 ± 6.1	36.7 ± 5.5	35.7 ± 2.1	30.3 ± 2.1
WBC x 10 ⁹ /L	5.3 ± 0.61	4.9 ± 0.3	5.7 ± 0.8	7.8 ± 0.0	8.1 ± 0.4

n = 3, X ± SEM

Where n = no. of rats, SEM = standard error of mean,

X = mean

Table 6: Toxic effects of Kerosene on Haematological Parameters

Groups	1	2	3	4	5
Dose (g/Kg)	0	22.2	44.2	88.9	177.8
Hb (g/dl)	12.9 ± 0.3	12.0 ± 0.4	11.0 ± 0.3	10.7 ± 0.4	10.0 ± 0.3
PCV (%)	39.3 ± 2.1	38.3 ± 3.8	37.7 ± 2.5	35.0 ± 4.0	33.3 ± 4.2
WBC (X 10 ⁹ /L)	5.1 ± 0.6	5.5 ± 0.4	5.9 ± 0.3	6.0 ± 0.4	6.3 ± 0.9

n = 3, X ± SEM

Where n = no. of rats, SEM = standard error of mean, X = mean

4. Discussion

Haematological parameters have been used in studies over the years as important indices of health change. These include Hb, PCV and WBC which were monitored in this study. The results obtained (Table 5 and Figure 3) for gasoline and kerosene (Table 6 and Figure 4) showed statistically significant dose dependent decreases in the levels of Hb and PCV for both gasoline and kerosene as doses increased. This was indicative of development of the state of anaemia, which could have been caused by excessive destruction of erythrocytes. The decrease in Hb and PCV values occurred at lower doses for gasoline than for kerosene. This can be accounted for by the fact that gasoline molecules are smaller than kerosene molecules and they are able to penetrate cellular membranes more easily than kerosene (Tables 3 and 4, Figures 1 and 2).

The results obtained also showed a significant increase in the total WBC count which increased with the doses of treatment of the rats. The increase in WBC was probably a defensive mechanism by the immune system. When an antigen is introduced into an organism, antibodies are produced in response to the stimulus. The Hb and PCV and WBC results corroborated with the studies of Cowell [10], Christenssen, et al, [11], Connel and Miller [12], Krishan and Veena [13], Dede and kagbo [14], Wachukwu, et al [15] and Momoh and Oshin [16].

5. Conclusion

The results from this study show dose dependent acute toxicities for both gasoline and kerosene as indicated by decreases in PCV and Hb and increases in WBC. These changes were more so for gasoline than for kerosene. The significance of this study therefore is that care should be taken to avoid acute exposure to gasoline and kerosene, but particularly gasoline because of its higher toxicity effect.

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