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Evaluation of Petrol fumes on Hepatic Enzymes activities in albino rats

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Abstract

The study was carried out by exposing 35 albino rats divided into seven (7) groups of five (5) rats each to petrol fumes respectively at concentrations (Parts per Minute) of 0.00, 16,737 for 5 minutes daily, 20,240 for 10 minutes daily, 23,077 for 15 minutes daily, 27,344 for 20 minutes daily, 30,920 for 25 minutes daily and 34,458 for30 minutes daily for 21 days using a modified nose-inhalation exposure method. Alanine aminotransferase (ALT) and Aspartate aminotransferase (ALT) activities were determined by Reitman-Frankel method while Alkaline Phosphatase (ALK PHOS) activity was determined by Phenolphthalein Monophosphate Method. The data were subjected to statistical analysis using statistical package for social sciences (SPSS) version 21. There was significant difference ($P<0.05$) in Aspartate amino transferase (U/L) of 12.8±1.83, 14.6±2.16, 15.6±2.16, 19.0±2.35, 20.2±2.13, 23.2±3.35 and 25.8±3.68 respectively and Alanine amino transferase (U/L) was 13.2±0.58, 20.0±1.41, 24.2±1.43, 28.8±2.42, 32.2±1.56, 31.6±2.79 and 34.8±2.15 at petrol concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively while there was no significant difference ($P>0.05$)in Alkaline phosphatase (U/L) was 38.2±0.92, 47.4±4.64, 50.4±6.04, 62.0±11.02, 55.8±1.85, 54.8±1.59 and 59.6±3.06 at petrol concentrations (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively. Overall there was significant difference ($P<0.05$) in Alkaline Phosphate (U/L) activities of 38.2±0.92 in control groups and 55.0±2.31 in petrol fumes and Aspartate amino transferase (U/L) activities of 12.8±1.83 in control groups and 19.7±1.24 in petrol fumes while Alanine amino transferase (U/L) activity of 13.2±0.58 was significantly different ($P<0.05$) from 28.6±1.20 in petrol fumes group. The result of this study indicated that petrol fumes may impair liver functions in rats suggestive that petrol fumes can cause damage to the Liver.

Keywords: Hepatic, Enzymes, Petrol, fumes.

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1. Introduction

Petrol or Gasoline is a transparent, petroleum derived liquid that is used primarily as a fuel in spark-ignited internal combusting engines. It is a petroleum derived mixture of over 500 hydrocarbons that may have between 3-12 carbon atoms [1]. It is derived during fractional distillation process and has a translucent liquid form. It's not used in its crude form. Different additives are added like ethanol to use it as fuel for passenger vehicles. The major sources of exposure to petrol are oil refineries, oil fields, petrol filling stations, petrochemical industries, motor mechanical workshops and machines powered by two stroke engines [2-4]. Occupationally exposed Individuals as well as those residing in a heavy traffic area constitute the population at greater risk of frequent and long term exposure to constituents of petrol [4]. It has been estimated that about 110 million individuals are exposed to petrol during the process of refueling at self-service filling stations [5]. Although the composition and characteristics

of gasoline depend on the origin of the crude oil, differences in the processing methods and blends, seasonal variations as well as additives such as oxygenates that are required to meet a specific engine performance [6].

Some of the major components of gasoline of health concern are Benzene, Toluene, and Xylene and are abbreviated as BTX [7]. These compounds are also known as volatile organic compounds (VOC) [8]. Several studies have reported that significant exposure to benzene exists during self-refueling of cars at service stations [9, 10]. It has been estimated that for every 30 litres of gasoline containing 5% benzene that is pumped during the process of refueling, about 700mg of benzene is vaporized [11]. Significant levels of other VOCs such as toluene and xylene have been found in the blood of both petrol station attendants and individuals who self-refuel their vehicles [8, 10, 12]. The risk of exposure to these VOCs among petrol station attendants is significantly related to the duration and the volume of petrol pumped in a given period [7, 8].

The liver is the largest gland in the body and has a wide variety of functions. Weight is: 1/50 of body weight in adult & 1/20 of body weight in infant. It is exocrine (bile) & endocrine organ (Albumen, prothrombin & fibrinogen). It is situated in the right upper quadrant of the abdomen. It occupies the right hypochondrium and epigastrium and also extends to the left hypochondrium. It is covered by Glisson's capsule, a visceral continuation of the peritoneum. The greater part of the liver is situated under cover of the right costal margin. The Diaphragm separates it from the pleura, lungs, pericardium, and heart. The lobes of the liver include; right and left lobe, and 2 accessory lobes; quadrate and caudate lobe. The right lobe is six times larger than left lobe [13].

The aim of this study is to determine the effect of petrol fumes on hepatic function in male albino wistar rats using hepatic enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) as indicators.

2. Materials and Method

2.1 Animals

Fifty-five (55) male albino wistar rats weighing about 177.20-198.80g were obtained from animal house of Pharmacology Department of University of Port Harcourt. The rats were housed in a wire meshed cage *ad libitum* (12 hours light and 12 hours darkness cycles) at standard temperature of 35-37^{0}C and were fed rat pellets and water.

2.2 Reagents

Commercially prepared reagents for Alanine amino transferase and Aspartate amino transferase were purchased from Randox Diagnostics, London while Alkaline phosphatase reagent was purchased from Quimica Chinica Applicada, Spain.

2.3 Petrol

The petrol sample was obtained from the Nigerian National Petroleum Corporation (N.N.P.C) zonal office at Moscow road, Port Harcourt.

2.4 Method for LC_{100}

Preliminary study was done to determine the LC_{100} and LC_{50} of petrol fumes on the rats. Twenty albino rats were divided into four groups of 5 rats each and exposed to 500ml of Petrol fumes for 0hour, 4hours, 6hours and 8hours for 14 days. Physical changes (weakness, abnormal movement, and diarrhea), Behavioral changes (aggressiveness, rotational movement, biting and hyperactivity) and death were observed. The LC_{50} was calculated based on Arithmetic method of Karber [14].

2.5 Animal studies

The 35 albino rats used were divided into 7 groups of five rats each. The animals were acclimatized for six weeks and fed *ad libitum* with normal rat food and water at 12 hours daylight and 12 hours of darkness before commencement of studies.

2.5.1 Experimental design

An animal model consisting of 35 rats was used; they were divided into seven (7) groups containing five (5) rats each and were exposed to petrol fumes at petrol concentrations (Parts per Minute) 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 ppm for groups 1, 2, 3, 4, 5, 6 and 7 respectively. Signs of toxicity due to petrol fumes were observed in the rats such as grooming, sniffing around the cage, and standing on their hind legs also hyperactivity and weakness. The Group 1 served as the control and was not exposed to petrol fumes, Group 2 were exposed to16,737ppm of petrol fume for 5 minutes daily, Group 3 were exposed to 20,240ppm of petrol fume for 10 minutes daily, Group 4 were exposed to 23,077ppm of petrol fume for 15 minutes daily, Group 5 were exposed to 27,344ppm of petrol fume for 20 minutes daily, Group 6 were exposed to 32,047ppm of petrol fume for 25 minutes daily and Group 7 were exposed to 34,458ppm of petrol fume for30 minutes daily for 21 days. The Exposure to petrol fumes was carried out using a modified nose-inhalation exposure method [15]. According to this modification, the cages housing the animals in the test groups were placed in respective exposure chambers (1 cage per chamber), each with one open calibrated beaker of 1000mls containing 500mls of petrol. The petrol was allowed to evaporate freely within the respective exposure chambers at ambient humidity and temperature for an hour and test animals in cages were exposed to fumes generated from direct evaporation of petrol. The animals were exposed at 5 minutes time interval for 30 minutes/day after saturation of chamber with fumes, 7 day/week to fumes for 21 days. At the end of each exposure day, the animals were transferred to petrol fumes-free section of the experimental

animal house. During the exposure period, the initial and final volumes of petrol were respectively recorded before and after daily exposure. The daily differences in volume were used to estimate relative concentrations of fumes used in this exposure method.

2.5.2 Sample collection

After three weeks of exposure, all the rats were sacrificed by chloroform sedation. Each of the animals was slaughtered and blood samples from each animal were collected into plain containers; Blood samples collected into plain containers were allowed to stand for about 15 minutes to clot and further spun in a centrifuge. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurement of selected hepatic enzyme activities.

2.5.3 Biochemical analysis

Alanine aminotransferase and Aspartate aminotransferase activities were determined by Reitman-Frankel method. Alanine aminotransferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, dinitrophenylhydrazine (α-oxoglutarate + L-alanine $\xrightarrow{GPT}$ L-glutamate + Pyruvate) while Aspartate aminotransferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, dinitrophenylhydrazine (α-oxoglutarate + L-aspartate $\xrightarrow{GOT}$ L-glutamate + oxaloacetate) [16].

The test tubes were respectively labeled blank, sample, control blank and control. 0.5ml of buffer solution was dispensed into all the test tubes, 0.1ml of sample and control into test tubes labeled sample and control respectively. All the tubes were incubated at 37ºC for 30minutes. 0.5ml of 2, 4 dinitrophenylhydrazine was dispensed into all test tubes. 0.1ml of sample was dispensed into test tube labeled blank, while 0.1ml of control was dispensed into control blank tube. The contents of each test tube was mixed and allowed to stand for 20minutes at 25ºC. 5ml of 0.4N sodium hydroxide was added to each tube, mixed and read at 550nm against the sample blank prepared. The value of the unknown was extrapolated from the calibration curve already prepared.

Alkaline Phosphatase activity was determined by Phenolphthalein Monophosphate Method. Serum alkaline phosphatase hydrolyses a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline PH values turns into pink colour which can be photometrically determined at 550nm [17]. The test tubes were respectively labeled sample, standard and control. 1.0ml of distilled water was pipetted into each tube followed by a drop of the substrate into each test tube. All the test tubes were incubated at 37ºC for 5minutes. 0.1ml of sample, standard and control were dispensed into their respective labeled test tubes. The test tubes were incubated at 37ºC for 20minutes. 5ml of colour developer was added to each test tube, mixed, and read at 550nm using water as blank. The concentration of Alkaline Phosphatase (U/L) was determined by multiplying the absorbance of test with concentration of standard and dividing by absorbance of standard.

2.5.4 Quality control

A Randox normal quality control serum was assayed with all analyses to determine the precision.

2.5.5 Statistical Analysis

The biochemical data were subjected to statistical analysis using tools such as analysis of variance (ANOVA) and student's t-test using Statistical Package for Social Sciences (SPSS) version 21.0 for windows 8.1. Probability values less than 0.05 was taken to be significant.

3. Result

Table 1 shows the response of the rats during the toxicity study of petrol fumes in wistar rats. Group A presented no changes in physical and behavioral responses. Group B presented slight changes in physical and behavioral responses after 4 hours daily exposure for two weeks. Group C presented moderate physical and behavioral changes after 6 hours daily exposure for two weeks. Group D presented severe physical and behavioral changes observed after 8 hours of daily exposure. No death was recorded in any group for the time span of the toxicity study.

Table 1: Toxicity study of different durations of petrol fume

Toxicity study	Groups	Time (hrs.)	Action
Physical changes (weakness, abnormal movements, diarrhea)	A	-	- - -
	B	4	- + -
	C	6	++-
	D	8	+++
Behavioral changes (aggressiveness, rotational movements, biting, hyperactivity)	A	-	- - - -
	B	4	+ -+ -
	C	6	+ + -+
	D	8	+ + + +
Death	A	-	-
	B	4	-
	C	6	-
	D	8	-

500ml of Petrol fumes were used but for different durations

Table 2 below shows the effect of different concentrations of petrol fumes on liver enzymes.Alkaline phosphatase (U/L) was 38.2±0.92, 47.4±4.64, 50.4±6.04, 62.0±11.02, 55.8±1.85, 54.8±1.59 and 59.6±3.06 at petrol concentrations (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively. Aspartate amino transferase (U/L) was 12.8±1.83, 14.6±2.16, 15.6±2.16, 19.0±2.35, 20.2±2.13, 23.2±3.35 and 25.8±3.68 at petrol concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively. Alanine amino transferase (U/L) was 13.2±0.58, 20.0±1.41, 24.2±1.43, 28.8±2.42, 32.2±1.56, 31.6±2.79 and 34.8±2.15 at gasoline concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively.

Table 2: Effect of different concentrations of petrol fumes on Liver Parameters

Concentrations (ppm)		Alkaline Phosphatase (u/l)	Aspartate amino transferase (u/l)	Alanine amino transferase (u/l)
0.00		38.2±0.92	12.8±1.83	13.2±0.58
16,737		47.4±4.64	14.6±2.16	20.0±1.41
20,240		50.4±6.04	15.6±2.16	24.2±1.43
23,077		62.0±11.0	19.0±2.35	28.8±2.42
27,344		55.8±1.85	20.2±2.13	32.2±1.56
30,920		54.8±1.59	23.2±3.35	31.6±2.79
34,458		59.6±3.06	25.8±3.68	34.8±2.15
F		2.335	3.298	16.622
P		0.059	0.014	0.000
Post Hoc				
0.00	16,737	0.677	1.000	.064
	20,240	0.652	0.996	0.008
	23,077	0.584	0.593	0.023
	27,344	0.002	0.324	0.001
	30,920	0.001	0.319	0.022
	34,458	0.015	0.205	0.003
16,737	0.00	0.677	1.000	0.064
	20,240	1.000	1.000	0.588
	23,077	0.964	0.937	0.196
	27,344	0.803	0.727	0.007
	30,920	0.875	0.558	0.113
	34,458	0.537	0.349	0.010
20,240	0.00	0.652	0.996	0.008
	16,737	1.000	1.000	0.588
	23,077	0.997	0.991	0.829
	27,344	0.998	0.890	0.074
	30,920	1.000	0.693	0.465
	34,458	0.933	0.446	0.060
23,077	0.00	0.584	0.593	0.023
	16,737	0.964	0.937	0.196
	20,240	0.997	0.991	0.829
	27,344	1.000	1.000	0.976
	30,920	1.000	0.993	1.000
	34,458	1.000	0.867	0.723
27,344	0.00	0.002	0.324	0.001
	16,737	0.803	0.727	0.007
	20,240	0.998	0.890	0.074
	23,077	1.000	1.000	0.976
	30,920	1.000	1.000	1.000
	34,458	0.990	0.947	0.996
30,920	0.00	0.001	0.319	0.022
	16,737	0.875	0.558	0.113
	20,240	1.000	0.693	0.465
	23,077	1.000	0.993	1.000
	27,344	1.000	1.000	1.000
	34,458	0.924	1.000	0.998
34,458	0.00	0.015	0.205	0.003
	16,737	0.537	0.349	0.010
	20,240	0.933	0.446	0.060
	23,077	1.000	0.867	0.723
	27,344	0.990	0.947	0.996
	30,920	0.924	1.000	0.998

Alkaline Phosphate (U/L) was 38.2±0.92 and 55.0±2.31 in control groups and groups exposed to petrol fumes respectively. Aspartate amino transferase (U/L) was 12.8±1.83 and 19.7±1.24 in control groups and groups exposed to petrol fumes respectively. Alanine amino transferase (U/L) was 13.2±0.58 and 28.6±1.20 in control groups and groups exposed to petrol fumes respectively as shown below in table 3.

Table 3: **Effect of petrol fumes on liver enzymes Parameters**

Parameter	Control	Petrol	t	P
Alkaline Phosphate (u/l)	38.2±0.92	55.0±2.31	8.561	0.006
Aspartate amino transferase(u/l)	12.8±1.83	19.7±1.24	4.806	0.036
Alanine amino transferase(u/l)	13.2±0.58	28.6±1.20	26.41	0.000

4. Discussion

This research evaluates the effect of petrol fumes on liver function parameters with emphasis on the duration of exposure. The result of the study showed Lc_{50} of petrol to be 137,832ppm. The studied concentration is a quarter of the $LC_{50.}$ This concentration has been shown to be tolerable to rats [18].

The result of the study showed dose dependent increase in alkaline phosphatase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT) activities in albino rats treated with petrol fumes compared to their respective controls. This is similar to the study by Adegoke *et al* [18].This increase maybe due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in petrol[19].This increase shows hepatic toxicity and possible damage caused by the exposure to the petrol fumes. Petrol is used for various reasons by humans at home, petrochemical and manufacturing industries. Exposure to petrol has been reported to have genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestation [20]. The ratio of AST/ALT is also an important index for the measurement of toxicity.

A significant elevation was seen in ALT level in all test groups indicating toxic influence of petroleum fumes on liver cells. The activity of AST was also significantly higher ($P<0.05$) in subjects exposed occupationally to petroleum fumes in comparison to level observed in control group subjects. This damage is attributed to reactive free radical species generated from the metabolism of aromatic and aliphatic hydrocarbons present in petroleum fumes [21, 22]. The ratio of ALT/AST is also an important index for measurement of hepatotoxicity.

There was significant difference ($p<0.05$) in aspartate amino transferase and alanine amino transferase activities in albino rats treated with petrol fumes. This is similar to study by Deepa *et al.,* [23]. Liver enzymes are markers of hepatocellular injury and their levels correlates with extent of tissue damage. Such elevation is indicative of liver injury, especially the rise in L-ALT level [24]. The level of serum ALT activity has been reported to be increased as a result of liver injury in patients developing severe hepatotoxicity [25]. ALT might have leaked from damaged cells due to necrosis, indicating or gandys function [26]. Salie *et al*.,[27] 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 ketoacids. This increase maybe due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in 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 [21, 22].

However, the remarkable increase in the level of ALP may simply that damages occurred more in the liver cells of rats exposed to petrol fumes, since the activity of this enzyme in the serum is reported to be increased in the liver damage [28]. Alkaline phosphatase is involved in the transport of metabolites across the cell membranes, protein synthesis, glycogen metabolism, synthesis of certain enzymes and secretory activities. 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 [29].

This result also showed significant increase ($p<0.05$) in alkaline phosphatase, aspartate amino transferase and alanine amino transferase of albino rats exposed to petrol fumes compared with their respective controls. This is similar to previous studies by Patrick-Iwuanyanwu *et al.,* [30] and Uboh *et al.,*[19].These marker enzymes are cytoplasmic in origin and are released into the circulation after cellular damage [31]. This is as a result of damage to the liver architecture causing seepages of alkaline phosphatase, aspartate amino transferase and alanine amino transferase from the liver cells into the bloodstream. Elevated liver enzymes point towards hepatic injury. Alanine amino transferase (ALT) and Aspartate Amino Transferase (AST) are markers of hepatocellular injury whereas alkaline phosphatase is marker of

cholestasis [32]. Serum aminotransferases are the sensitive markers of acute hepatocellular injury. ALT is a cytosolic enzyme while AST is both cytosolic and mitochondrial. Normally, aminotransferases are present in serum at a low level. When necrosis or death of cells containing these enzymes occurs, aminotransferases are released into blood and their concentration in blood increases. The level correlates with extent of tissue damage [33]. Alkaline phosphates level is a marker of cholestasis.

5. Conclusion

The result of this study indicated that petrol fumes may impair liver functions as shown by changes in liver enzymes in rats is suggestive that petrol fumes can cause damage to the Liver.

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