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Evaluation of gasoline fumes on Bilirubin and Protein concentrations 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. The Bilirubin, Total protein and albumin estimations was done by Spectrophotometric, Biuret and Bromocresol green methods respectively. The data were subjected to statistical analysis using statistical package for social sciences (SPSS) version 21. There was significant difference ($P<0.05$) in Albumin (g/L) concentrations of 40.6±2.32, 38.4±0.75, 34.0±2.12, 37.0±0.77, 37.6±0.93, 39.4±0.87, 43.2±0.37 and Total bilirubin (Umol/L) concentrations of 9.79±1.36, 8.95±1.04, 10.1±0.42, 14.0±1.23, 13.2±0.96, 14.0±1.08 and 13.4±1.33 at gasoline 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 Total protein (g/L) of 48.4±1.66, 47.8±0.73, 50.0±3.66, 49.4±2.06, 51.6±1.54, 50.6±1.91 and 50.6±0.40 and Direct bilirubin (Umol/L) of 0.72±0.11, 0.69±0.11, 0.81±0.11, 0.70±0.13, 0.60±0.13, 0.60±0.13 and 0.65±0.12 at gasoline concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively. There was no significant difference ($P>0.05$) in Total Protein (g/L), Albumin (g/L), Total Bilirubin (Umol/L) and Direct Bilirubin (Umol/L) of 48.4±1.66, 40.6±2.32, 9.79±1.36 and0.72±0.11 in control groups compared with 50.0±0.78, 38.3±0.66, 12.3±0.54 and 0.68±0.05 in petrol exposed groups respectively. The result of the study showed that petrol fumes caused changes in bilirubin and protein suggesting of impaired liver function.

Keywords: Petrol, Bilirubin, Proteins, Liver.

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1. Introduction

The liver is the largest gland in the body and has a wide variety of functions. The liver is a reddish-brown, wedge-shaped organ with four lobes of unequal size and shape. A human liver weighs 1.44-1.66 kg (3.2-3.7 lb), [1] and has a width of about 15cm. It is both the heaviest internal organ and the largest gland in the human body. Located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlies the gallbladder [2]. The liver is connected to two large blood vessels: the hepatic artery and the portal vein. The hepatic artery carries oxygen rich blood from the aorta via the celiac plexus, whereas the portal vein carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into small capillaries known as liver sinusoids, which then lead to lobules. Lobules are the functional units of the liver. Each lobule is made up of millions of hepatic cell (hepatocytes), which are the basic

metabolic cells. The lobules are held together by a fine, dense, irregular, fibroelastic connective tissue layer which extends from the fibrous capsule covering the entire liver known as Glisson's capsule [3]. This extends into the structure of the liver, by accompanying the blood vessels (veins and arteries), ducts, and nerves at the hepatic hilum. The whole surface of the liver except for the bare area is covered in a serous coat derived from the peritoneum, and this firmly adheres to the inner Glisson's capsule. The liver has many functions which include; Metabolic function of the liver which includes carbohydrate, protein, amino acid and ammonia, bilirubin and bile sail metabolism. Detoxication functions which includes metabolism of many drugs, metabolism of alcohol by oxidation to acetaldehydes and acetic acid and metabolism of hormones. Storage functions which include storage of vitamins, iron as ferritin, glycogen and blood. Excretory and secretory functions which include the secretion and excretion of bile into the intestine to aid in digestion and secretion of erythropoietin [4].

There are different types of fuel; the premium unleaded petrol and is the standard petrol sold in Nigeria. 95 RON refers to the octane level of the petrol. This is a measure of how easily the fuel will ignite inside an engine. Higher octane levels mean that the fuel will not ignite easily and are required for some high performance engines [5]. Petroleum hydrocarbons and other related carbon containing compounds are converted into free radicals or activated metabolites during their oxidation in the cells [6], especially mammalian liver and kidney cells. It is these activated metabolites that react with some cellular components such as membrane lipids to produce lipid peroxidation products [7] which may lead to membrane change. They may also react with enzymes and cause inactivation through protein oxidation [8] and/ or DNA strand breaks [6,9]. Exposure to petroleum and its products therefore constitute health hazards. These manifest as nervous system damage, blood disorders (including anaemia, leukaemia), renal damage, hepatic dysfunction and intoxication leading to serious psychotic problems, anaesthetic effects, dermatitis etc. [6,10]. Possible sources of exposure to petroleum products include: Accidental ingestion of petroleum products, Touching petroleum products, soil or water contaminated by petroleum products; for example, children may inadvertently swallow petroleum-based fuels when they are stored in a familiar container such as a soda bottle, Swimming in bodies of water contaminated by petroleum products, Using fuel oils to wash paint or grease from skin or equipment, Fixing gasoline or diesel engines, Cleaning up fuel spills without proper protective equipment [11], Working in the oil and gas industry, Some people also inhale gasoline or diesel fumes in order to become intoxicated. This is known as "huffing" or "sniffing" and is done more frequently by children and adolescents than adult [12]. According to a 2004 study, gasoline was the most commonly abused volatile substance in the United States.

The study is aimed to determine the effect of petrol fumes on hepatic proteins and Bilirubin in male albino wistar rats.

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 Total Protein, Albumin, and bilirubin were purchased from Randox Diagnostics, London.

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 [13].

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.

2.6 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 to 16,737 ppm 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 [14] diagram as shown in fig 1 in the appendix. 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.

2.7 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 bilirubin and proteins parameters.

The Serum Total Bilirubin Concentration was determined using Jendrassik-Grof method [15]. The serum total bilirubin concentration is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction which diazotized sulphanillic acid [15, 16]. 200ml of reagent 1 (sulphanillic acid) was dispensed each into two different test tubes labeled sample blank ‘and sample‘ followed by the addition of 1 drop (50µl) of reagent 2 (nitrite) and 1000 µl of reagent 3 (caffeine). 200 µl of the test serum was the dispensed into each of the test tubes and the mixtures incubated in a water bath for 10 minutes at 25^0C. This was followed by the addition of 100 µl of reagent 4 (tartrate) and the mixture incubated again at 25^0C for 10 minutes. The absorbance of the sample (ATB) was then read against the sample blank using a colorimeter at 578nm wavelength. The total bilirubin concentration (umol/l) was then calculated by multiplying 185 × absorbance of total bilirubin (578nm).

The Serum direct Bilirubin Concentration was determined using Jendrassik-Grof method. The serum Direct/indirect bilirubins react with diazotized sulphanillic acid in alkaline medium to form a blue coloured complex**.** [15,16]. Exactly, 200ml of reagent 1 (sulphanillic acid) was dispensed each into two different test tubes labeled sample blank and sample followed by the addition of 1 drop (50µl) of reagent 2 (nitrite) and 2000 µl of 0.9 % physiological saline. 200 µl of the test serum was the dispensed into each of the test tubes and the mixtures incubated in a water bath for 10 minutes at 25^0C. The absorbance of the sample (ATB) was then read against the sample blank using a colorimeter at 546 nm wavelength. The direct bilirubin (umol/l) concentration was then calculated by multiplying 246 × absorbance of Indirect Bilirubin (546nm)

Serum Total Protein concentration was determined using Biuret method based on Cupric ions, in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex [17]. The test tubes were respectively labeled blank, standard, test, and control. 5.0ml of Biuret reagent was pipetted into each tube. 0.1ml of distilled water, standard, sample and control were pipetted into their respective tubes, mixed, incubated for 30minutes at 25ºC. The absorbances were measured against the reagent blank at wavelength of 546nm. The concentration of total protein (g/l) was determined by multiplying the absorbance of test with concentration of standard and dividing by absorbance of standard.

The Albumin Concentration was determined using Bromocresol Green (BCG) method. The measurement of serum albumin is based on its quantitative binding to the indicator 33'55'tetrabromo-m cresol sulphonephthalein (bromocresol green BCG). The albumin BCG – Complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample [18].

The test tubes were labeled blank, standard, sample and control. 3ml of Bromocresol green reagent was pipetted into each tube. 0.01ml of distilled water, standard, sample and control was pipetted into their respective tube mixed, and incubated at 25ºC for 5minutes. The absorbances were measured at 578nm against the reagent blank. The concentration of Albumin (g/l) was determined by multiplying the absorbance of test with concentration of standard and dividing by absorbance of standard

2.8 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

Total protein (g/L) was 48.4±1.66, 47.8±0.73, 50.0±3.66, 49.4±2.06, 51.6±1.54, 50.6±1.91 and 50.6±0.40 at gasoline concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively. Albumin (g/L) was 40.6±2.32, 38.4±0.75, 34.0±2.12, 37.0±0.77, 37.6±0.93, 39.4±0.87, 43.2±0.37 at gasoline concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively. Total bilirubin (Umol/L) was 9.79±1.36, 8.95±1.04, 10.1±0.42, 14.0±1.23, 13.2±0.96, 14.0±1.08 and 13.4±1.33 at gasoline concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively. Direct bilirubin (Umol/L) was 0.72±0.11, 0.69±0.11, 0.81±0.11, 0.70±0.13, 0.60±0.13, 0.60±0.13 and 0.65±0.12 at gasoline concentration (ppm) of 0.00, 16,737, 20,240, 23,077, 27,344, 30,920 and 34,458 respectively.

Table 1: Effect of different gasoline fumes on Proteins and Bilirubin

	Concentrations (ppm)	Total Protein (g/l)	Albumin (g/l)	Total Bilirubin(µmol/l)	Direct Bilirubin (µmol/l)
	0.00	48.4±1.66	40.6±2.32	9.79±1.36	0.72±0.11
	16,737	47.8±0.73	38.4±0.75	8.95±1.04	0.69±0.11
	20,240	50.0±3.66	34.0±2.12	10.1±0.42	0.81±0.11
	23,077	49.4±2.06	37.0±0.77	14.0±1.23	0.70±0.13
	27,344	51.6±1.54	37.6±0.93	13.2±0.96	0.60±0,13
	30,920	50.6±1.91	39.4±0.87	14.0±1.08	0.60±0.13
	34,458	50.6±0.40	43.2±0.37	13.4±1.33	0.65±0.12
	F	0.459	4.616	3.959	0.390
	P	0.833	0.002	0.005	0.879
0.00	16,737	1.000	.996	1.000	1.000
	20,240	1.000	.582	1.000	1.000
	23,077	1.000	.887	.488	1.000
	27,344	0.926	0.966	0.626	1.000
	30,920	0.999	1.000	0.421	1.000
	34,458	0.943	0.977	0.691	1.000
16,737	0.00	1.000	0.996	1.000	1.000
	20,240	1.000	0.669	0.987	1.000
	23,077	1.000	0.958	0.181	1.000
	27,344	0.529	1.000	0.212	1.000
	30,920	0.926	0.999	0.129	1.000
	34,458	0.162	0.017	0.324	1.000
20,240	0.00	1.000	0.582	1.000	1.000
	16,737	1.000	0.669	0.987	1.000
	23,077	1.000	0.936	0.284	1.000
	27,344	1.000	0.860	0.282	0.964
	30,920	1.000	0.478	0.192	0.964
	34,458	1.000	0.103	0.486	0.996
23,077	0.00	1.000	0.887	0.488	1.000
	16,737	1.000	0.958	0.181	1.000
	20,240	1.000	0.936	0.284	1.000
	27,344	0.999	1.000	1.000	1.000
	30,920	1.000	0.607	1.000	1.000
	34,458	1.000	0.006	1.000	1.000
27,344	0.00	0.926	0.966	0.626	1.000
	16,737	0.529	1.000	0.212	1.000
	20,240	1.000	0.860	0.282	0.964
	23,077	0.999	1.000	1.000	1.000
	30,920	1.000	0.927	1.000	1.000
	34,458	1.000	0.025	1.000	1.000
30,920	0.00	0.999	1.000	0.421	1.000
	16,737	0.926	0.999	0.129	1.000
	20,240	1.000	0.478	0.192	0.964
	23,077	1.000	0.607	1.000	1.000
	27,344	1.000	0.927	1.000	1.000
	34,458	1.000	0.094	1.000	1.000
34,458	0.00	0.943	0.977	0.691	1.000
	16,737	0.162	0.017	0.324	1.000
	20,240	1.000	0.103	0.486	0.996
	23,077	1.000	0.006	1.000	1.000
	27,344	1.000	0.025	1.000	1.000
	30,920	1.000	0.094	1.000	1.000

Total Protein (g/L) was 48.4±1.66 and 50.0±0.78 in control groups and groups exposed to petrol fumes respectively. Albumin (g/L) was 40.6±2.32 and 38.3±0.66 in control groups and groups exposed to petrol fumes respectively. Total Bilirubin (Umol/L) was 9.79±1.36 and 12.3±0.54 in control groups and groups exposed to petrol fumes respectively. Direct Bilirubin (Umol/L) was 0.72±0.11 and 0.68±0.05 in control groups and groups exposed to petrol fumes respectively as shown below in table 2.

Table 2: **Effect of gasoline fumes on Proteins and Bilirubin**

Parameter	Control	Petrol	t	P
Total Protein (g/l)	48.4±1.66	50.0±0.78	0.619	0.437
Albumin (g/l)	40.6±2.32	38.3±0.66	1.587	0.217
Total Bilirubin (umol/l)	9.79±1.36	12.3±0.54	3.031	0.091
Direct Bilirubin (umol/l)	0.72±0.11	0.68±0.05	0.125	0.726

4. Discussion

The result of the study showed dose dependent increase in total protein, albumin, total bilirubin concentrations in albino rats treated with petrol fumes compared to their respective controls. This is similar to the study by Adegoke *et al* [19]. This increase maybe due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in petrol [20]. 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 [21]. The liver is the sole site for synthesis of most of the plasma proteins except gamma globulins therefore, increases in the level of protein and albumin indicates liver cell injury.

The study also showed that there was no significant difference ($p>0.05$) in Total protein and Direct bilirubin concentrations in the rats exposed to petrol fumes compared with their respective controls. The result of the study showed significant difference in albumin concentrations of rats exposed to petrol fumes compared to the control while there was no significant difference in the total protein among the rats. This is similar to study by Deepa *et al* [22]. Measurement of serum protein is a test to assess the synthetic function of liver. Liver is the sole site for synthesis of most of the plasma protein except gamma globulins which are synthesized by plasma cells.

The study also showed that there was no significant difference in Total protein, albumin, Total bilirubin and Direct bilirubin, concentrations in the rats exposed to petrol fumes compared with their respective controls. This is suggestive that exposure to petrol fumes may not have effect on bilirubin metabolism. Bilirubin is the excretory product formed by the catabolism of haeme. It is conjugated by the liver to form bilirubin diglucuronide and excreted through bile. In hemolytic jaundice, unconjugated bilirubin is increased where as in obstructive jaundice, conjugated bilirubin is increased. In hepatocellular injury, both conjugated and unconjugated bilirubin is increased. Unconjugated bilirubin is increased due to reduced ability of liver cells to conjugate bilirubin. Conjugated bilirubin is raised from cholestasis due to hepatocyte swelling [23]. As the direct as well as indirect bilirubin was increased in our study group, it suggests possible hepatocellular damage due to toxins from petroleum fumes.

5. Conclusion

The result of the study showed that petrol fumes caused changes in bilirubin and protein suggesting of impaired liver function.

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