Original Article

Triglycerides, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol in rats exposed to premium motor spirit fumes

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Abstract

Background: Deliberate and regular exposure to premium motor spirit fumes is common and could be a risk factor for liver disease in those who are occupationally exposed. A possible association between premium motor spirit fumes and plasma levels of triglyceride, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol using a rodent model could provide new insights in the pathology of diseases where cellular dysfunction is an established risk factor. **Aim:** The aim of this study was to evaluate the possible effect of premium motor spirit fumes on lipids and lipoproteins in workers occupationally exposed to premium motor spirit fumes using rodent model. **Materials and Methods:** Twenty-five Wister albino rats (of both sexes) were used for this study between the 4th of August and 7th of September, 2010. The rats were divided into five groups of five rats each. Group 1 rats were not exposed to premium motor spirit fumes (control group), group 2 rats were exposed for 1 hour daily, group 3 for 3 hours daily, group 4 for 5 hours daily and group 5 for 7 hours daily. The experiment lasted for a period of 4 weeks. Blood samples obtained from all the groups after 4 weeks of exposure were used for the estimation of plasma levels of triglyceride, total cholesterol, high density lipoprotein- cholesterol and low density lipoprotein- cholesterol. **Result:** Results showed significant increase in means of plasma total cholesterol and low density lipoprotein levels ($P<0.05$). The mean triglyceride and total body weight were significantly lower ($P<0.05$) in the exposed group when compared with the unexposed. The plasma level of high density lipoprotein, the ratio of low density lipoprotein to high density lipoprotein and the ratio of total cholesterol to high density lipoprotein did not differ significantly in exposed subjects when compared with the control group. **Conclusion:** These results showed that frequent exposure to petrol fumes may be highly deleterious to the liver cells.

Keywords: Premium motor spirit, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol, total cholesterol, triglyceride.

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Introduction

Premium motor spirit (PMS), otherwise known as gasoline, being a volatile liquid may evaporate if left exposed to constitute ubiquitous chemical pollutants in the immediate environment [1]. However, those occupationally exposed tend to be at greater risk [2,3]. PMS is a petroleum-derived volatile liquid mixture, gotten from the fractionation of petroleum. It is used primarily as fuel in the internal combustion engines and some electricity generating machines [4, 5]. Gasoline consists mostly of aliphatic hydrocarbons, cyclic hydrocarbons and aromatic hydrocarbons. Additives such as tetraethyl lead, tetra methyl lead, methylcyclopentadienyl Manganese carbonyl (MMT), and ethanol are added to petrol to improve its quality (octane rating) or to prevent engine knocking.

Some constituents of PMS are toluene, pentane, octane, xylene, hexane, heptane, 2, 2, 4–trimehtylbenzene and others. Some constituents of gasoline such as benzene, toluene (which are non-aliphatic hydrocarbons) have been reported to be carcinogenic, which may be due to the free radicals they generate. It has been reported that a higher concentration of unsaturated hydrocarbons and a lower concentration of the saturated fractions accumulate in the blood of humans and animals equally exposed to petroleum vapour [1]. Acute-duration inhalations of gasoline have been associated with irritation, headache, dizziness, nausea, euphoria and drowsiness [4]. Exposure to toxic environmental pollutants, such as lead, among other factors have been reported to be associated with hypertension and kidney disease [6]. Studies with rats and mice with chronic inhalation exposure to gasoline vapours have found hepatocellular tumors in female mice, and nephropathy and related renal tumors in male rats [7]. Based on these report, the potential harmful effects associated with chronic or sub-chronic exposure to gasoline vapour should be the concern of the general public and scientific community.

The liver has been shown to be one of the target organs of petrol fumes - induced injury [8]. The expression of toxicity on the liver is usually determined biochemically by the monitoring of some plasma enzymes and lipids. A rise in serum aspirate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatises (ALP), triglyceride (TG) and cholesterol are commonly measured as indices of the damage of the liver cells [9, 10]. There is likelihood that bouts of petrol fumes can predispose the subject to atherosclerotic condition. In this study we attempt to assess the toxic effects of petrol fumes on albino Wistar rats by measuring parameters like total cholesterol (TC), triglycerides, low density lipoproteins cholesterol (LDL), high density lipoproteins cholesterol (HDL).

Materials and Methods

Study Area

This study was carried out at the University of Benin Teaching Hospital, Benin City, Nigeria between the 4th of August and 7th of September 2010 at the Department of Chemical Pathology.

Experimental Animals

Twenty-five (25) albino Wister rats of both sexes weighing 35-97g were obtained from the Animal holdings of the University of Benin, Benin City, acclimatized for 7 days and fed with feed from Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria and water was provided *ad libitum.*

Experimental Design

The females were separated from the males and divided into five (5) groups, with group 1 as control, groups 2-5 served as test. The groups from 2-5 were based on the time of exposure, with group 2 exposed for 1 hour, group 3 for 3hours, group 4 for 5hours and group 5 for 7hours.

Four groups (2-5) were placed in the experimental room to get acclimatized for a period of one week and the first group (the control) was kept in the main animal house away from the experimental room and the rats had free access to water and food (rats pellets).

Exposure to PMS fumes

At the end of one week acclimatization, PMS purchased from one of the NNPC mega filling stations in Benin City, and was measured into highly perforated containers and placed in each test group cages for their respective hours of exposure, while the control group was left unexposed. Distribution and duration of exposure of animals are shown in Table 1.

Table 1 Distribution and duration of exposure of animals

Groups	*No. of Rats*	*Treatment*
Group 1	5	Control
Group 2	5	Exposed for 1hr
Group 3	5	Exposed for 3hr
Group 4	5	Exposed for 5hr
Group 5	5	Exposed for 7hr

Protocol for Exposure to PMS Fumes

An improvised modified nose-inhalation method as described by Uboh *et al.,* [12, 11, 8] was used in this study. According to this method, the cages housing the test animals were placed in an isolated room. Calibrated beakers of 1000cm^3 containing 500cm^3 of liquid gasoline were placed in each of the cages and allowed to freely evaporate for the different periods of exposure. The animals were exposed for period of 28days. At the end of each day's exposure, they were transferred to the gasoline vapour-free experimental rooms.

During the period of exposure, initial and final volumes of gasoline were recorded before and after daily exposure respectively. The differences in volumes were used to ascertain the relative evaporated PMS used for this study.

Blood Collection and Storage

Two milliliters of fasting blood samples was obtained from each animal in the control and test group via cardiac puncture after each of the animal had been anaesthetized with chloroform at the end of the exposure. Each blood sample was collected into a tube containing lithium heparin as anticoagulant. Plasma was obtained after centrifugation at 3500rpm for 15minutes. The plasma was collected into a plain tube and stored at -20^{0}C until the analysis.

Biochemical Analysis

The plasma concentration of TC, TG, HDL-cholesterol and LDL-cholesterol were measured using spectrophotometric methods. Laboratory kit reagents (Randox Laboratory Ltd, UK) were used for all biochemical analysis and their absorbance were read using a UV-Vis spectrophotometer (DREL 3000 HACH).

Table 2 Mean ±S.E, F-value and P-value of plasma lipid profile for control and test groups

Parameters	Controls	1 hour	3 hours	5 hours	7 hours	F-value	P-value
Total cholesterol	1.26 ± 0.18	1.71 ± 0.08	2.00 ± 0.08	2.26 ± 0.12	2.24 ± 0.23	14.22	.001
HDL	0.92 ± 0.15	1.30 ± 0.21	1.32 ± 0.10	1.34 ± 0.07	1.32 ± 0.23	1.69	NS
LDL	0.41 ± 0.09	0.47 ± 0.09	0.61 ± 0.11	0.94 ±0.04	0.56 ±0.16	10.82	.001
Triglyceride	0.61 ± 0.06	0.53 ± 0.02	0.39 ±0.07	0.45 ± 0.03	0.50 ± 0.04	8.26	.001
LDL/HDL	0.41 ± 0.11	0.46 ± 0.07	0.71 ± 0.06	0.53 ± 0.27	0.51 ± 0.15	1.23	NS
TC/HDL	1.43 ± 0.19	1.55 ± 0.13	1.70 ± 0.02	1.80 ± 0.24	1.50 ± 0.26	1.91	NS
Total Body weight (g)	94.00±3.12	63.20±1.53	45.80±1.02	31.80±0.97	37.60 ±1.03	664.80	.001

Each value is an average of five determinations ± SEM. Values are significantly different in comparison with control at $p<0.05$. Control=unexposed rats, 1 hour=rats exposed for 1hour, 3hours=rats exposed for 3hours, 5hours=rats exposed for 5hours, 7hours=rats exposed for 7hours, NS=non significant.

Results

After biochemical analysis, the values (of the control and the test) gotten were evaluated statistically to check for their significance (at the level of $p<0.05$) as shown in Table 2.

Discussion

In this study, lipid parameters in rats exposed to PMS was evaluated. A significant increase in the mean LDL-cholesterol level was observed in the test group when compared against the control in the study. Mean HDL-cholesterol was statistically insignificant, while mean triglyceride level was significantly decreased. Also, the mean ratio of TC to HDL-cholesterol and the mean ratio of LDL-cholesterol to HDL- cholesterol were increased but statistically insignificant. Also, significant increase was also observed in the mean TC level. This result is similar to the work of Uboh *et al*., [8] who discovered an increase in TG and TC in rats exposed to petrol and kerosene fumes. But the decrease in the mean TG level was in contrast to the work of Uboh *et al*., [8] whose mean TG was increased. This could be as a result of a number of factors which include prolonged fasting prior to blood collection [13].

This significant increase in lipid profile is an indication that inhalation exposure to petrol fumes may affect lipid metabolism. On one hand, lipid metabolism is affected once there is liver damage since the disturbance of cell membrane integrity is likely to cause some membrane lipids to be released into circulation; while on the other hand, it causes the tissue to compromise its effectiveness in regulating lipid metabolism. One of the main hypotheses of mechanism of hepatocyte injury from petrol fume metabolism is associated with oxidative stress and lipid peroxidation resulting from the imbalance between pro-oxidant and antioxidant chemical species [14]. Such an imbalance is associated with increased β-oxidation of fatty acids by mitochondria, peroxisomes and cytochrome P450 2E1 (CYP2E1) pathways. These oxidative processes produce free electrons, H_2O_2, and reactive oxygen species (ROS) while depleting the potent antioxidants, glutathione and vitamin E [15]. The increased levels of free fatty acids present in the fatty liver provide a perpetuating and propagating mechanism for oxidative stress via lipid peroxidation, with secondary damage to cellular membranes and key organelles such as mitochondria [14]. Lipid peroxidation usually leads to the formation of peroxyl radicals, which are central species in the peroxidation chain reaction.

Increased lipid peroxidation and oxidative stress in hepatocytes of male and female rats have been reported to be associated with exposure to gasoline vapours [11]. Such reactive oxygen species as hydroxyl and superoxide radicals are known to provoke severe cellular alterations resulting in cell damage or death, due to their high reactivity. These species attack such important cell constituents as proteins, lipids and nucleic acids, and the lipid peroxides that accumulate due to lipid peroxidation are known to be very harmful to cells and tissues [16]. The liver has been shown to be one of the target organs of petrol fumes - induced injury [8]. There is likelihood that petrol fume predisposes the subject to atherosclerotic condition.

Conclusion

From the results of this study, it can be deduced that frequent exposure to petrol fumes may be highly deleterious to the liver cells; frequent exposure to PMS fumes should be discouraged and those occupationally exposed should always go to hospital for regular check-up.

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