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Total antioxidant status of zinc, manganese, copper and selenium levels in rats exposed to premium motor spirit fumes

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Abstract

Background: Frequent exposure to premium motor spirit (PMS) is common and could be a risk factor for liver dysfunction in those occupationally exposed. A possible association between PMS fumes and plasma total antioxidant status as well as plasma levels of zinc, manganese, copper and selenium using a rodent model could provide new insights into the pathology of the liver where cellular dysfunction is an established risk factor. **Aim:** This study aimed to determine the total antioxidant status and plasma levels of zinc, copper, selenium and manganese in those occupationally exposed using rodent model. **Materials and Methods:** 25 albino Wistar rats of both sexes were used for this study. The animals were divided into five groups of five rats in each group. Group 1 rats were not exposed to PMS 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 determination of plasma total antioxidant status as well as plasma levels of zinc, manganese, copper and selenium. **Results:** Results showed significant increases in means of plasma copper (69.70±0.99 for test and 69.20±1.02 for control, $P < 0.05$) and selenium (72.70±1.58 for test and 68.20±0.86 for control, $P < 0.05$) in the exposed rats when respective mean values were compared with those of corresponding controls. Mean body weight index (BWI) and percentage weight increase (PWI) were significantly lower ($P < 0.05$) in exposed rats when compared with the unexposed group. The mean plasma levels of zinc (137.40±4.06 for test and 147.80±2.52 for control) and manganese (65.75±1.02 for test and 70.00±0.71 for control) showed significant decrease ($P < 0.05$) when compared with control. Plasma level of total antioxidant status (TAS) did not differ significantly in exposed rats when compared with the control group. **Conclusion:** This study showed that frequent exposure to PMS fumes may lead to increase plasma levels of copper and selenium probably due to liver dysfunction and decrease in plasma levels of zinc and manganese probably as a result of interference in their metabolic pathway of the exposed groups.

Keywords: Premium motor spirit, trace elements (copper, zinc, manganese and selenium), total antioxidant status.

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Introduction

Premium motor spirit (PMS) also known as "Petrol" or "Gasoline", is a petroleum-derived liquid mixture, obtained by fractional distillation. It is primarily used as fuel in internal combustion engines. It consists mostly of aliphatic hydrocarbons enhanced with iso-octane or aromatic hydrocarbons; toluene and benzene to improve its quality and prevent engine knock (octane rating). Unleaded gasoline for instance, is reported to contain

about 300 different hydrocarbon fractions, most of which are volatile and may evaporate if left exposed, to constitute ubiquitous chemical pollutants in the immediate environment [1].

A greater percentage of the automobile users, those residing at or around refueling stations and traffic congested areas as well as hydrocarbon addicts may directly or indirectly be exposed to these pollutants in their environment [2, 3]. The most popular additive was tetra-ethyl lead. However, with the discovery of the environmental and health damage caused by the lead, this practice began to wane in the 1980s. Many of the non-aliphatic hydrocarbons naturally present in gasoline (especially aromatic ones like benzene) as well as additives are carcinogenic due to free radicals they generate (which are of two types; reactive oxygen species (ROS) e.g. superoxide and hydroxyl radical and reactive nitrogen species (RNS) e.g. nitrous oxide). For instance, benzene may affect the liver and kidney, but usually mild, temporary impairments occur; chronic effects may arise and persist long after an acute exposure [4]. Chronic inhalation studies resulted in liver tumours in female mice and kidney tumors in male rats [5]. Chronic exposure to ethyl alcohol may result in weight loss, with anorexia and diarrhea, and cirrhosis of the liver [4]. It has been reported that radicals have the capacity to react in an erratic manner leading to damage to almost all cellular component. An extensive range of antioxidant defenses both endogenous are present to protect cellular components from free radical induced damage.

Antioxidants are referred to as any substances which when present in low concentration compared to the oxidisable substrate, significantly delay or inhibit the oxidation of that substrate [6]. The physiological role of antioxidants is to prevent damage to cellular components arising from the activity of chemical reactions involving free radicals. Antioxidants can be divided into three main groups: Antioxidant enzymes which include: catalase, glutathione peroxidase and glutathione reductase, superoxide dismutase. Chain breaking antioxidants which are aqueous phase (Vitamin C) lipid phase (Vitamin E). The transition metal binding proteins are ferritin, transferrin, lactoferrin and cerulospamin [6]. Enzymes that perform antioxidant activities are dependent on certain metalloenzymes. These metalloenzymes depend on trace elements to function effectively. Trace elements are micro-nutrients present at very low concentrations (μg/dL) in body fluids [7]. Examples of trace elements based on the scope of this study include zinc, manganese, copper and selenium.

Substantial evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases including chronic renal failure and diabetes. Previous works have shown that constituent of the fumes can be carcinogenic [5], by producing free radicals which reacts with trace elements and in turn affects the total antioxidant status of the individual. This study is therefore designed to examine the possible effect of PMS fumes on trace elements (Selenium, Copper, Manganese and Zinc) and total antioxidant status of those occupationally exposed using rodent models.

Materials and Methods

Study Area

This study was carried out from June 2nd to 30th 2010 at the animal house of the Department of the Chemical pathology, University of Benin Teaching Hospital, Benin City, Nigeria.

Experimental Animals

Twenty-five albino Wistar rats weighing 35-97g were obtained from the Animal holdings of the University of Benin, Benin City, acclimatized for 7days 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 groups, with group 1 as control, while 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 3 hours, group 4 for 5 hours and group 5 for 7 hours. 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 Petroleum Motor Spirit (PMS)

At the end of one week acclimatization, PMS purchased from one of the NNPC mega filling stations in Benin City 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 1 hr
Group 3	5	Exposed for 3 hr
Group 4	5	Exposed for 5 hr
Group 5	5	Exposed for 7 hr

Protocol for Exposure to PMS Fumes

An improvised modified nose-inhalation method as described by Uboh *et al.* [8, 9] was used in this study. According to this method, the cages housing the test animals were placed in an isolated room. Calibrated beakers of 1000 Cm^3 containing 500 Cm^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 28 days. At the end of each day's exposure, they were transferred to the PMS vapour-free experimental rooms.

Blood Collection and Storage
Two milliliters of 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 3500 rpm for 15 minutes. The plasma was collected into a plain tube and stored at -20^{0}C until analysis.

Biochemical analysis
The plasma concentration of manganese, copper, selenium and zinc were determined using atomic absorption spectrophotometry method [10]; while total antioxidant status (TAS) was measured spectrophotometrically [11].

Statistical Analysis
Statistical analysis was done on this study using SPSS v16.0 and a $p < 0.05$ was considered statistically significant (n=25).

Result

After biochemical analysis, the values (of the control and the test) obtained were evaluated statistically to check for their significance, they are summarized in the table below (Table 2 and 3).

Table 2 Means (±SE)**,** F-value and P-value of plasma manganese, selenium, copper, zinc, TAS and body weight of control and test groups

Parameters	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	F	P
Manganese (µg/dL)	70.0 ±0.71	64.2 ±0.80	64.8 ±0.86	66.0 ±1.70	68.0 ±0.71	10.57	.001
Selenium (µg/L)	68.2 ±0.86	71.4 ±1.70	71.2 ±0.58	75.6 ±0.81	72.6 ±3.23	4.489	.003
Copper (µg/dL)	69.2 ±1.02	69.6 ±1.12	69.4 ±1.21	70.0 ±1.00	69.8 ±0.66	4.489	.003
Zinc (µg/mL)	147.8 ±2.52	137.6 ±3.94	130.0 ±0.45	140.6 ±1.66	142.0±10.2	2.928	.027

Group I: Unexposed rats, Group II: Rats exposed for 1hr daily, Group III: Rats exposed for 3hrs daily, Group IV: Rats exposed for 5hrs daily, Group V: Rats exposed for 7hrs daily.

Table 3 Means (±SE)**,** F-value and P-value of plasma TAS and body weight of control and test groups

Parameters	Grp1	Grp2	Grp3	Grp4	Grp5	F	P
Status(TAS) (mmol/L)	1.123 ±0.09	2.122 ±0.37	1.32 ±0.21	1.402 ±0.38	1.432 ±0.43	2.388	NS
Body weight (g)	94.0 ±3.12	63.2 ±1.53	45.8 ±1.02	31.8 ±0.97	37.6 ±1.03	664.78	.001

Group I: Unexposed rats, Group II: Rats exposed for 1hr daily, Group III: Rats exposed for 3hrs daily, Group IV: Rats exposed for 5hrs daily, Group V: Rats exposed for 7hrs daily, NS: Not significant.

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

Discussion

The toxicological effects of premium motor spirit (PMS) fumes have been studied at different levels and have shown to be a predisposing factor to various metabolic defects and haematotoxicity in those occupationally exposed [12, 13]. This can be seen from the mechanism of damage ensued by exposure to PMS fumes as described by Robertson *et al*. [14].

One of the main hypotheses is that the mechanism of hepatocytes injury due to PMS fumes 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.

This study showed a significant increase in plasma selenium level when compared with the control group. Normally, selenium has a high affinity for substituting sulphur in the body, especially in cysteine where sulphur is substituted by selenium to form selenocysteine which is now regarded as the 21st amino acid with the codon UGA [16, 17]. Hence it is suggestive that when sulphur is inhaled, selenium quickly replaces it in the extracellular compartment. This might lead to an increase in selenium extracellular concentration.

During this study, significant increase in plasma concentration of copper was also observed when compared to that of control; in the normal physiology of the body, copper is metabolized and excreted in the liver. Hence liver dysfunction (which was also reported during this experiment) might lead to accumulation of copper in the plasma [18].

Manganese in contrast to both selenium and copper showed decrease in plasma concentration which was significant when compared with the control group. This may be due to the interaction between manganese and some constituents of PMS fumes which might lead to inhibition of manganese activity such as enzyme activation and induction of binding proteins or interfere with manganese metabolic pathway. Reduction of plasma manganese concentrations leads to prolidase deficiency [19] which is characterized by splenomegaly and recurrent infections as seen in some of the rats which had an unexplained abscess on their neck.

This study also showed significant decrease in plasma zinc concentration when compared with the control group. This finding can be explained by the reduced growth rate of the rats, behavioural disturbances exhibited by the rats. In addition, zinc interferes with the bioavailability of copper by inducing metallothionein which sequester dietary copper and prevents its absorption [20, 21], hence increased plasma copper concentration might indicate decrease in zinc plasma concentration. Also, poor zinc absorption might be due to interaction by calcium in diet. The total antioxidant status (TAS) is used to determine the level of oxidative stress [22] an increased formation of reactive oxygen species leads to increased oxidative stress and decreased antioxidant capacity.

This study showed no significant difference in plasma TAS when compared with the control group; this indicates low oxidative stress in the rats, possibly due to increase in both selenium and copper levels in plasma of the rats. The enzymes which selenium and copper serves as metalloenzymes such as glutathione peroxidase and superoxide dismutase are still functional and help prevent oxidative stress by maintaining the total antioxidant status. The final total body weight of the test group showed significant decrease when compared with the control, this is in concordance with the work done by Uboh *et al.* [23], who exposed rats to petrol and kerosene fumes, and observed a significant decrease in body weight.

Conclusion

Although no precise work has been done on this topic, results obtained from this study suggests that prolong exposure to PMS fumes might lead to hepatotoxicity, increase in plasma levels of copper and selenium as well as decrease in plasma levels of zinc and manganese. With these results, persons occupationally exposed to PMS fumes are advised to go for regular medical check-up to ascertain their health status. It is recommended that this study be continued subsequently using appropriate human subjects that are occupationally exposed.

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