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Antioxidative Effect of Zinc on Gasoline Induced Hepatotoxicity

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ABSTRACT

Gasoline, an environmental pollutant has demonstrated some hepato-toxicity in excessive acute exposure. This study investigates the protective effect of zinc supplementation on the liver of gasoline poisoned rats. The rats were divided into two experimental groups and each experimental group is further subdivided into 3 sub-groups of six rats per group. Rats in experiment 1 were exposed to varying duration of gasoline vapour alone while in experiment 2, in addition to the varying duration of exposure, were administered with zinc supplement. Histopathological study, liver enzymes, malonildialdehyde determination of treated and untreated rats were among investigations carried out. Malonildialdehyde concentration and hepatic enzymes were significantly high ($p < 0.05$) in exposed groups. The histology showed a moderate to severe derangement in the hepatic cytoarchitecture at longer duration of exposure to gasoline. There was an improvement in all the parameters used in groups which zinc was concomitantly administered. The results suggest that zinc supplement has some protection on the hepato-toxicity induced by gasoline.

Key words: Gasoline, antioxidant, zinc, liver.

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INTRODUCTION

Gasoline, one of the refined products of crude oil, continued to pose health hazards in animal and man in Nigeria and many other parts of the world. Many people who are exposed to gasoline in the form of exhaust fumes from automobile through inhalation, oral or dermal route have suffered from lots of health problems [1]. The fear of toxicity of gasoline is treated in part by the other additives or components of gasoline, some of which are classified by the US Environmental Protection Agency(EPA) as known or probable human carcinogens e.g. benzene and 1, 3-butadiene [2].

Inhalation exposure to gasoline has been measured for service stations and self-service customers, for truck drivers and distribution workers and for workers removing leaking underground storage tanks [3]. Like some other solvents, gasoline can sensitize the heart to catecholamines, defat the skin upon repeated contact, and induce hepatic UDP – glucuronyltransferase activities [4].The issue of whether there is a “fetal gasoline syndrome” has been raised, although case reports offered in support of the affirmative are confounded by tetraethyl lead, alcohol abuse, and the possibility that an aberrant gene is distributed within the small Amerindian population, where the case reside [5].

There is a paucity of data on the reproductive toxicity of gasoline, but reports of enhanced estrogen metabolism and uterine atrophy among unleaded gasoline-treated mice suggest that this endpoint warrants investigation.

Chronic inhalation of gasoline has also resulted in increased hepatocellular adenomas and carcinomas in female mice [6]. This increase may have been due to the promotion of spontaneously initiated cells that occur with unusually high frequency in this mouse strain. Gasoline has been shown to induce cytochrome P450 activity and to produce hepatomegaly and a transient increase in hepatocyte proliferation, all of which are considered relevant to tumour - promoting activity [7].

Mark *et al.*,[8]noted that although the antioxidant properties of Zinc were first demonstrated in-vitro, there is also clear evidence that zinc functions as an antioxidant in the body. One area of growing interest is the role, of zinc as an antioxidant in the central nervous system (CNS), particularly in the brain. Compared to other soft tissues, the human brain contains significant amount of zinc. Among the essential trace elements, Zinc is second only to iron in total concentration in the brain, thus zinc deficiency has been proposed to lead to nervous system disorder including mental disturbances, loss of sensory acuity and impaired cognitive and psychological function [9].

The liver is the major organ responsible for metabolism, detoxification and plays a vital role in secretory functions in the body. Hence, it regulates various important metabolic functions in mammalian systems. Hepatic damage is associated with the distortion of these metabolic functions. Different chemical agents, including gasoline vapor constituents, are known to be hepatotoxic [10].

With the increasing awareness of its hepato toxicity, it has become a major concern of environmental and biochemical toxicologists who are motivated to making antidotes to

its toxic effects especially the oxidative effects of gasoline. Several antioxidants have been used in a bid to establish a variety of vitamins, microelements and other substances with antioxidant effect that may in the long run provide for useful therapy. This study is therefore, designed to investigate the antioxidant properties of zinc on the hepatotoxic effects of gasoline.

MATERIALS AND METHODS

Gasoline sample:

The gasoline sample used was obtained from Total filling station, Mbiama, Ahoada West Local Government Area of Rivers State and preserved in a freshly acquired four (4) litre gallon and covered with black polythene bag. This method was to avoid contamination or escape of volatile and light fractions of gasoline. The density of gasoline was measured and put at 0.727g/ml which was within range of average density.

Rats and treatment

A total of fortytwo (42) Albino rats were acquired from the animal farm of Department of Pharmacology and Toxicology of the University of Port Harcourt. The average weight of the rats was 200g (0.2kg) \pm 2.09. The rats were kept in the Anatomy laboratory for two weeks in rat cages for acclimatization. A modified whole body inhalation exposure method, previously described by Ubohet *al.*, [11] was used to expose the animals in test groups to ungraded concentrations of the vapor generated from direct evaporation of liquid gasoline. A varying exposure period of between 4 to 6hrs (from 0800 to 1400 hours) daily for 6 weeks was applied. The rats were divided into two major experimental groups. The first group, (Experiment. 1) containing 18 rats were further subdivided into three groups with six rats per group which was randomly selected as slated below:

Group A. Exposed to gasoline vapour for fours daily for six weeks.

Group B. Exposed to gasoline vapour for five hours daily for six weeks.

Group C. Exposed to gasoline for six hours daily for six hours.

The second group (Experiment 2), in addition to the varying exposure period of gasoline, had zinc supplement (0.02gm/1ml of water) given through gastric route. The sub-groups are as follow:

A. Exposed to gasoline vapour for fours daily and treated with zinc supplement consecutively for six weeks.

B. Exposed to gasoline vapour for five hours daily and treated with zinc supplement consecutively for six weeks.

C. Exposed to gasoline vapour for six hours daily and treated zinc supplement consecutively for six weeks.

Control group: In addition, there was also a control group comprising of a total of six rats.

Alanine and Aspartate Aminotranferases Determination

Plasma assays for tests on the function of liver vis-a-viz serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated with the Randox reagent kit using 2,4-dinitrophenylhydrazine as substrate according to the method described by Reitman and Frankel (1957)¹² in the Department of Chemical Pathology, University of Port-Harcourt Teaching Hospital Port-Harcourt..

Malonildiahyde Concentration (MDA)

Determination of malonildiahyde (MDA): MDA level was determined in the supernatant of the liver homogenates by the modified method of Buege and Aust [13]. Concentration was calculated using the molar absorptivity of malonildialdehyde which is 1.56×100000 M. It is an index of the degree of oxidative damage in biological tissues.

Tissue Preparation for Histology

The liver issues were fixed in 10% formalin for 48 h and then removed from the solution. It was then dehydrated through ascending grades of alcohol (70%, 80%, 90%, absolute). When dehydration was completed the tissues were cleared in xylene, infiltrated and embedded in paraffin wax for light microscopic studies, then sections of 5 micron thickness were cut on Reichert ultra microtome, mounted on slides and stained with Haematoxylin and Eosin (H and E) according to routine procedures for light microscopy. Tissue prepared was examined for qualitative differences in comparison with group B by an anatomical pathologist who does not know the nature of the experiment.

Ethical considerations

The research proposal was submitted to the Research Ethic Committee of the College of Health Sciences of the University of port-Harcourt for consideration and approval before commencement of this research work

STATISTICAL ANALYSIS

Data are expressed as mean \pm SEM and the test of significance analyzed by the student's *t*-test. The differences were considered significant at $p < 0.05$.

RESULTS

Aspartate and Alanine Transferases

Results presented in Table 1 and 2 respectively showed that there was a significant increase in both Aspartate and Alanine aminotransferases levels in all except group B of experiment 1 in comparison to the control group ($p < 0.05$). Administration of zinc reduced both Aspartate and Alanin aminotransferase of experiment 2 significantly when compared to the non-treated groups except group D in which high doses of gasoline was administered as illustrated in table 2.

TABLE 1. The Effect of Zinc on Serum Levels of AST in Varying Doses of Gasoline Treated Rats.

GROUPS	Exp.1. AST(UI/L) UNTREATED	Exp.2. AST(UI/L) TREATED
A	273.0±2.14*	217.2±2.37
B	293.5±3.71*	220.1±1.09
C	337.3±3.12#	331.0±2.92

All values are expressed as Mean±SEM (n=6) the groups are: A; exposure to 4hrs of gasoline vapour daily, B; exposure to five hours of gasoline vapour daily, C; exposure to six hours of gasoline vapour daily. Groups in exp.2 are concomitantly treated with zinc supplement # = P>0.05; * =P<0.05.

TABLE 2. The Effect of Zinc on Serum Levels of ALT in Varing Doses of Gasoline Treated Rats.

GROUPS	Exp.1. AST(UI/L) UNTREATED	Exp.2. AST(UI/L) TREATED
A	119.4±1.09*	81.7±2.40
B	122.3±2.02*	87.3±2.90
C	143.5±1.92*	109.4±3.40

All values are expressed as Mean±SEM (n=6) the groups are: A; exposure to 4hrs of gasoline vapour daily, B; exposure to five hours of gasoline vapour daily, C; exposure to six hours of gasoline vapour daily. Groups in exp.2 are concomitantly treated with zinc supplement # = P>0.05; * =P<0.05.

Malonidiahide(MDA) Concentration of Zinc Treated and Untreated Rats.

Malonildialdehyde concentration, an index of lipid peroxidation was significantly increased in all groups of experiment 1 compared to the control. The level of peroxidation was significantly reduced in groups A and B of experiment 1(p<0.05) in comparison to the untreated group as shown in table 3. The rate of inhibition of peroxidation was highest in group C with 33.9%.

TABLE 3. Malonildiahidehyde Concentrationin Zinc Treated and Untreated Rats.

GROUPS	MDA(umol/ mg)UNTREAT ED	MDA(umol/mg) INHIBITION RATE TREATED	(%)
A	0.95±0.07	0.50±0.21 *	30.3
B	0.82±0.09	0.76±0.05 *	27.4
C	0.15±0.03	0.80±0.05 *	33.9

All values are expressed as Mean±SEM (n=6) the groups are: A; exposure to 4hrs of gasoline vapour daily, B; exposure to five hours of gasoline vapour daily, C; exposure to six hours of gasoline vapour daily. Groups in exp.2 are concomitantly treated with zinc supplement # = P>0.05; * =P<0.05.

Histopathology

The liver architecture was well preserved with few infiltrating mono nuclear cells in the control group as shown in fig. 1.

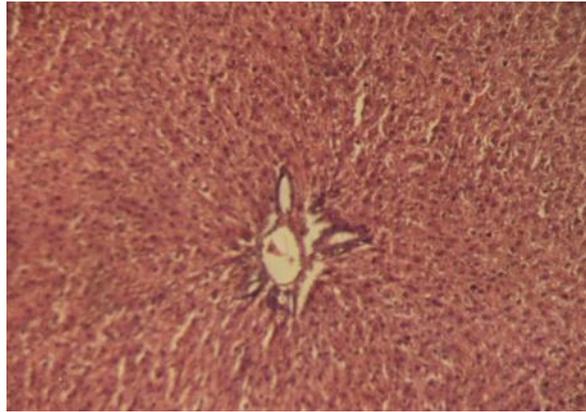


Fig.1.A micrograph of rat's liver from control group showing normal architecture

In group A of experiment 1, the hepatocytes were slightly enlarged and the sinusoids were compressed at the exposure duration of four hours to gasoline vapour while that of group A of experiment 2, the architecture was preserved and infiltrated by mononuclear cells as shown in fig. 2 and 3 respectively.

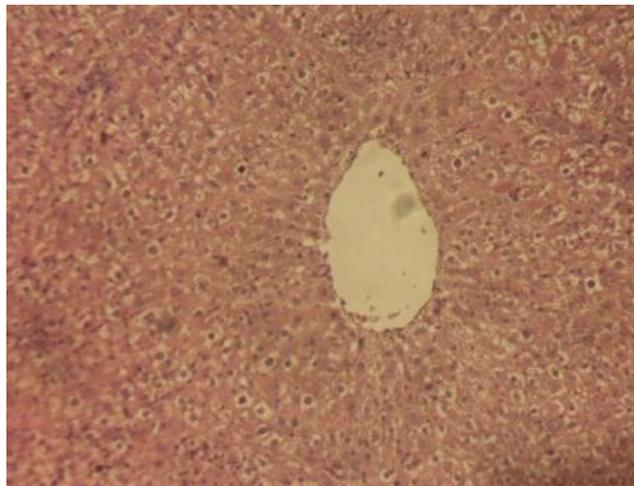


Fig. 2. A micrograph of rat from group A of exp. 1 exposed to 4hrs of gasoline showed slight enlargement of the hepatocytes and sinusoidal compression.

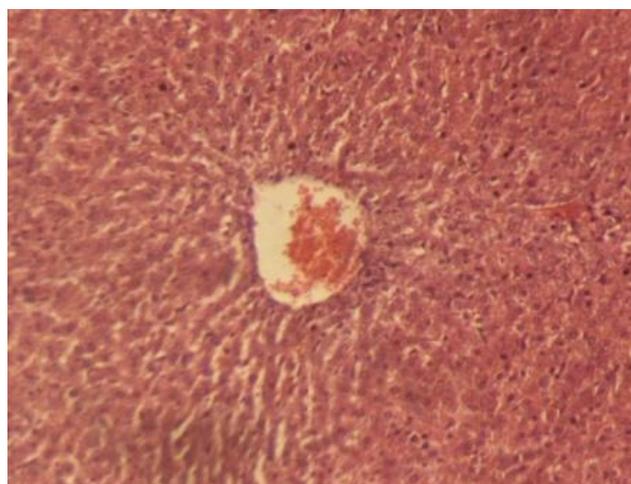


Fig. 3. A micrograph of rat from group A of exp.2 exposed to 4hrs of gasoline and zinc supplement showing preserved architecture, infiltration with mononuclear cells and venous haemorrhage.

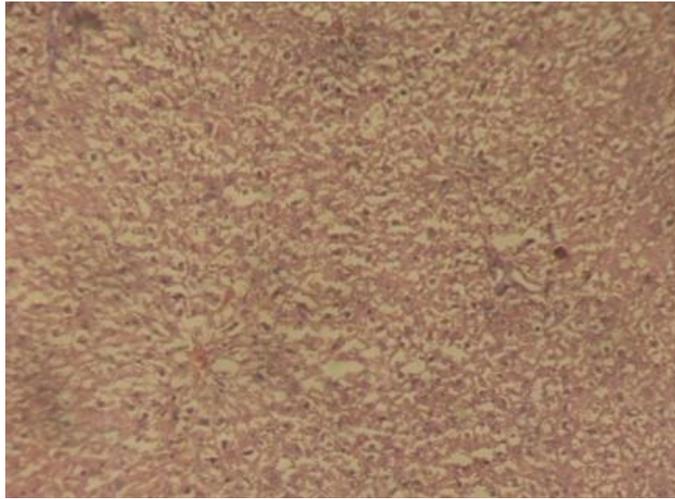


Fig. 4. A micrograph of rat from group A of exp. 1 exposed for 5hrs showed loss of the normal architecture, cytoplasmic vacuolation and fatty infiltration

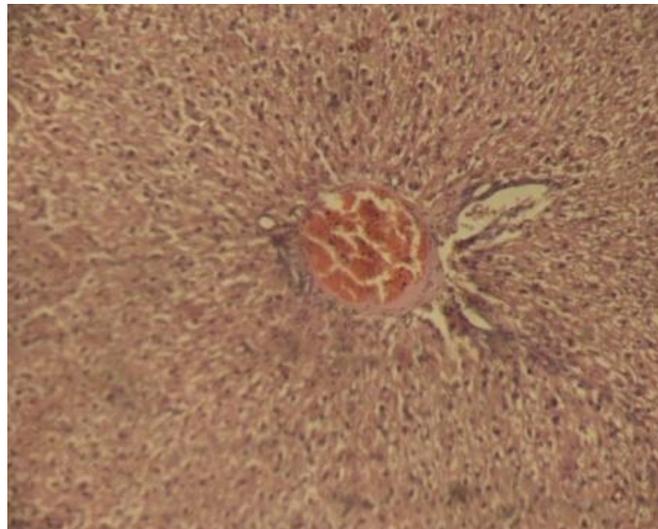


Fig. 5. A micrograph of rat from group A of exp. 2 exposed to gasoline and zinc supplement showed vascular congestion and fatty changes

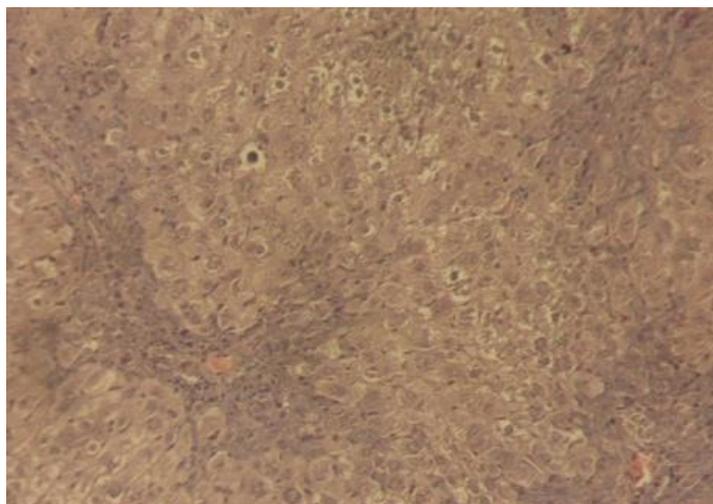


Fig. 6. A micrograph of rat from group A of exp. 1 exposed to 6hrs of gasoline vapour showed showed fatty changes, tissue necrosis, ballooning of the hepatocytes and interlobular fibrosis



Fig. 7. A micrograph of rat from group A of exp 2. Exposed to 6hrs gasoline vapour showed fatty changes and loss of architecture but normal sized hepatocytes and no fibrotic changes

At the exposure duration of five hours to gasoline vapour, there was a loss of the normal architecture, cytoplasmic vacuolation and fatty infiltration. However at the same duration of exposure of gasoline vapour in the presence of zinc, the liver only showed vascular congestion and fatty changes as illustrated in fig. 4 and 5.

Finally at the exposure duration of six hours daily for six weeks, the hepatocytes showed fatty changes, tissue necrosis, ballooning of the hepatocytes and interlobular fibrosis as shown in fig. 6. In the experiment 2 group, there were still fatty changes and loss of architecture but normal sized hepatocytes and no fibrotic changes as shown in fig. 7.

DISCUSSION

The results of this study indicate that exposure of rats to gasoline caused significant alterations in the biochemical parameters of liver function. Liver enzymes assayed; ALT and AST are known enzyme markers for the assessment of the functional integrity of the liver cells [14, 15]. These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication due to damage to the liver [14]. The present available data indicate that the constituents of gasoline exert possible hepatotoxic effects, as the increase in the concentration of serum ALT and AST is indicative of liver damage. The findings in this study are in conformity with previous studies, which indicated that exposure to gasoline vapor induced severe adverse biochemical disturbances that affect the functional and structural integrity of the liver and kidney tissues in experimental animals [10,16]. In the present study, it was revealed that zinc supplement administration to rats exposed to gasoline resulted to an appreciable improvement in the hepatotoxic effect associated with its exposure. We also found that Zn had an inhibitory effect on the spontaneous lipid peroxidation in rat liver whole homogenates. This is evidenced by the reduction in the malondialdehyde concentration which is an index of lipid peroxidation in rats concomitantly treated with zinc supplement. These results support the antioxidant properties of Zn, which may be potentially relevant to the protection of hepatic constituents, competing with the transition metals for redox reactions.

It may be therefore, right to say that zinc supplement counteracted the hepatotoxic effect associated with gasoline associated free radical generation and enhances the antioxidant capacity of the several endogenous antioxidant factors. This may be because it is being found that when Super Oxide Dismutase, (SOD) an endogenous antioxidant enzyme loses its zinc atom, it becomes neurotoxic. However, its functions as an antioxidant might be through the prevention of free radical formation by other metals such as iron and copper. Unlike highly reactive iron and copper, zinc does not readily undergo oxidation and reduction or redox reaction. In redox reaction electrons are transferred to and from different compounds, sometimes, resulting in the generation of free radicals [17]. When zinc, instead of iron and copper, is incorporated into proteins, free radical generating reactions that may otherwise occur are inhibited.

The observations made from histological section indicated liver injury, as compared to the section from the control group. This suggests that the cellular integrity of the liver tissues was altered by the constituents of gasoline, and hence, the derangement of their cellular functions which tallies with earlier work done by Ayalogue *et al.*, [18]. The histopathological observations of a typical liver section from experimental test rats treated with zinc supplement improves the histo-architecture of the liver in high dose gasoline treatment and depict normal to near normal levels in low dose gasoline treated groups. The effects of liver damage caused by the exposure of gasoline is in agreement with [11]. These sections suggest that concomitant administration of zinc supplement restored possible histological damage associated with gasoline exposure.

In conclusion, gasoline which has been known to be environmental pollutant has demonstrated some toxicity associated with some health hazards in body tissues such as the liver. Thus, the ameliorating effects of zinc supplement on gasoline induced hepatotoxicity is likely to be mediated via the inhibition of free radical generation or its free radical scavenging activity.

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