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Gasoline-induced haematological changes and the associated hepatotoxicity in albino rats

N.M. IGBOH[1], D. ONWUBIKO[1], E.N. AGOMUO[2], B.A. AMADI[2], I. ONYESOM[3] and U.E. UZUEGBU

[1]College of Medicine and Health Sciences, Abia State University Uturu (Nigeria).
[2]Department of Biochemistry, Faculty of Sciences. Imo State University , Owerri (Nigeria).
[3]Department of Medical Biochemistry, Delta State University, Abraka (Nigeria).

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ABSTRACT

Haematological and biochemical indices were used to monitor the toxicity of gasoline in *albino* rats.The rats were placed in four groups and were intraperitoneally administered 0.0, 2.0, 4.0 and 10.0g/kg of gasoline, respectively, for 2 phase periods of 1 and 2 months. At the end of each period, rats were withdrawn from each group for analysis. The control rats were similarly treated with normal saline. Blood samples were taken for analysis of hemoglobin (Hb), packed cell volume (PCV), and white blood cell counts (WBC). Serum enzymes such as *Aspartate transaminase* (AST), *Alanine transaminase* (ALT), Alkaline Phosphatase (ALP). *Glutathione-S- transferase* (GST) and Glutathione (GSH) were equally monitored. There was significant reduction in Hb and PCV, particularly in group four rats, which received. $10gkg^{-1}$gasoline compared with control and other ($P < 0.05$). A significant decrease in the number of white blood cells in the first month exposure to gasoline was recorded, especially in rats administered $10gkg^{-1}$gasoline. Substantial increase in the activities of liver enzymes - ALT, AST and ALP were observed in all the groups. However, GST increased marginally from first to second month in all the groups. Furthermore, there was consistent reduction in the level of GSH after the first dose in all the groups compared with control ($P < 0.05$). The study demonstrates that long term exposure of rats to gasoline could induce anaemia and liver damage.

Key words: Gasoline, Serum enzymes, glutathione, anaemia, liver damage.

INTRODUCTION

In Nigeria, the oil industry is the most important sector of the economy. Unfortunately the incidence of recorded environmental pollution, due to high rate of petroleum-related activities have been associated with frequent oil spills, especially through oil well blowouts, tankers accidents, rupture of pipelines, and sabotage. These mishaps result in the release of Crude Oil and refined petroleum products into the terrestrial and aquatic environment (Wemedo, *et. al.,* 2002). Incidentally aquatic lives are the major target of the toxicity of the petroleum pollution usually from those of heavy metals to those of hydrocarbons (Moor and Dwyer, 1974).These compounds are rapidly absorbed by most aquatic organisms because of their high lipid solubility (Krishana and Veena, 1980).

Considering that fish constitute our major proteins source, when fishes that survive the deleterious effects of the petroleum pollution are consumed, the polycyclic aromatic compounds are also ingested along.

Apart from the use of gasoline in sterilizing equipment use in salons against HIV transmission, of much worry however, is the indiscriminate

therapeutic use of gasoline (which is one of the refined products of crude petroleum) in treating various ailments such as insect bite, arthritis, abdominal discomfort, and conjunctivitis. It is also used as antidotes to snake poisoning and as anticonvulsant. This observed therapeutic use of gasoline, makes it indeed necessary for information to be available on the possible effect of gasoline on rats .Though the study of Kuhnhold *et. al*., (1980) indicated the deleterious effects of low hydrocarbons on embryonic larvae. In Nigeria not much information is available on the effect of gasoline on animals.

In recognition of this, we decided to monitor any significant hematological and biochemical changes in rats expose to gasoline in order to provide some information for possible extrapolation.

MATERIAL AND METHODS

Animals: Thirty six male albino rats of 0.2kg average body weight obtained from the Department of Biochemistry and Pharmacology Animal houses, University of Port Harcourt, Rivers State, Nigeria, were used for the study. The animals were acclimatized in the Pharmacology Laboratory for six weeks, on rat chow and portable drinking water.

The gasoline used in the study was obtained from the Port Harcourt Refinery Company (PHRC), Alesa Eleme. Port Harcourt, Rivers State, Nigeria. These were stored in four litre industrial bottles, well corked, and kept in the dark to avoid lost of any volatile components and reaction with light.

Treatment

The rats were randomly divided into four groups and were intraperitoneally administered 0.0, 2.0, 4.0 and 10.0g/kg of gasoline respectively, for first and second month. At the end of 30 days, three rats were withdrawn from each group for analysis. The control rats were given normal saline. The animals were fed ad libitum with normal feed and given water freely. The doses used were based on LD_{50} determined by Igboh *et al.,* (2001) which indicated LD_{50} for gasoline as 9.6g/kg.

Analyses

Hematological parameters such as haemoglobin (Hb), packed cell volume (PCV) and white blood cell count (WBC) as well as aspartate transaminase (AST), alanine transaminase (AST), alkaline phosphatase (ALP), glutathione – S - transferase (GST) and Glutathione (GSH) were monitored. The haematological parameters were determined from the cardiac blood collected with sample bottles containing anticoagulants. For Hb, the cyanomethaemoglobin method of Fairbanks (1982) was used. While, the methods of Dacie and Lewis (1991) were used for PCV and WBC counts. ALP, ALT and AST were determined from cardiac blood collected with sample bottles without anticoagulant. The activities of AST and ALT were determined using Reitman and Frankel (1957) method. ALP activity was determined by employing Bower and McComb (1970) method. Five grams of the liver of the sacrificed rats were collected for GST and GSH evaluation. GST and GSH were determined using Anosike *et al*. (1991) and Reev, *et al*., (1980) methods respectively.

Statistics

The statistical analysis used was the one-way analysis of variance (ANOVA). This was used for haematological parameters, while the Student's T-test was used in evaluating the activity of the enzymes. $P < 0.05$ was regarded as significance (Obi, 1986).

RESULTS

The results obtained are shown on Table 1 and 2. Table 1 shows the effect of gasoline exposure on some haematological parameters (haemoglobin, Hb, packed cell volume, PCV, and white blood cell count, WBCC).

Table 2, shows the effect of gasoline on some serum liver enzymes, hepatic activity of glutathione-s-transferase, GST, and the tissue (liver) level of glutathione, GSH.

The result indicated a significant reduction in Hb and PCV, particularly in group four rats which were administered10.0gkg^{-1}gasoline compared with control and other groups ($P < 0.05$). Also observed

Table 1: Changes in some haematological parameters induced by gasoline in albino rats

	Changes in some haematological parameters after exposure to gasoline					
	After one month exposure			After two months exposure		
	Hb (g/dL)	PCV (%)	WBCC ($x10^9L$)	Hb (g/dL)	PCV (%)	WBCC ($x10^9L$)
Group 1	13.0±1.9	40.8±5.7	4.4±1.2	13.4±2.5	40.2±7.5	4.2±0.6
Group 2	12.2±1.5	36.6±4.5	3.7±0.8*	10.0±1.9*	30.0±5.7*	3.8±0.5*
Group 3	9.0±1.3*	27.0±3.8*	2.9±0.6*	7.1±1.4*	21.3±2.1*	3.6±0.4*
Group 4	5.0±0.5*	15.0±1.5*	2.3±0.4*	3.3±0.5*	9.9±1.6*	3.4±0.3*

Values are expressed as Mean±SD

*Significantly different ($p<0.05$) from control (Group 1) value.

Hb – Haemoglobin, PCV – Packed Cell Volume, WBCC – White Blood Cell Count

Group 1 (control): 0.00g gasoline/kg body weight

Group 2: 2.0g gasoline/kg body weight

Group 3: 4.0g gasoline/kg body weight

Group 4: 10.0g gasoline/kg body weight

was a significant decrease in the number of white blood cells in the first month when the rats were exposed to gasoline. This was very obvious in rats administered $10gkg^{-1}$gasoline. Noted equally was a substantial increase in the activities of liver enzymes - ALT, AST and ALP in all the groups. However GST increased marginally from first to second month in all the groups. Furthermore, there was consistent reduction in the level of GSH after the first dose in all the groups compared with control ($P < 0.05$).

DISCUSSION

From the results obtained, the study has demonstrated that long term exposure to gasoline could induce anaemia. This was shown through the low Hb and PCV which decreased significantly ($P<0.05$), from the first month to the second month. Also there was a remarkable reduction in WBC counts which was dose dependent. The low Hb, PCV and WBC could be due to excessive destruction of the blood cells (hemolysis). Low WBC is associated with weak immune system and this could render the organism susceptible to infections.

Again, it was observed that ALT was elevated. Elevation of ALT appears to reflect hepatic disease and it is more specific for hepatic disease than AST because of the subcellular location of the enzymes. Though, the activity of any of the enzymes (ALT or AST) may be elevated in extra hepatic disease. However, the elevation of AST and ALT along with the elevation of ALP activity may reflect some inflammatory disease or injury to the liver. In this study the maximum activity of ALP obtained was high. Thus, suggesting the possibility of hepatocellular damage. Some investigators have illustrated that enzyme patterns in the serum, reflect the physiological state of the organ, for instance increase in serum levels of AST, ALT and ALP was observed in serum of fish exposed to 2, 3, 4 triaminazo benzene resulting to the hepatocellular damage (Krishan and Veena (1980). Other studies indicated an increase in the activities of the hepatic enzymes following liver damage in fish and albino mouse exposed to toxic substances (Dheer *et al*., 1987; Mohssen 1997; Sharp *et al*., 1996). The result of this study is in conformity with these findings.
Inactivation of GST may be responsible for the low activity of GST. In the study of Chiapotto *et al*. (1995) have reported inactivation of GST by different concentrations of acetaldehyde and the result of this study seems similar.

The marked reduction in level of GSH observed in the first and second months, may not necessary be as a result of its utilization in the conjugation of reactive metabolites generated from

Table 2: Effect of gasoline on some liver enzymes and hepatic GSH level in albino rats

Changes in some liver enzymes and hepatic GSH level following exposure to gasoline in albino rats

	After one month exposure					After two months exposure				
	ALT(U/L)	**AST(U/L)**	**ALP(U/L)**	**GST(U/L tissue)**	**GSH (mmol /L/gtissue)**	**ALT(U/L)**	**AST(U/L)**	**ALP(U/L)**	**GST(U/L tissue)**	**GSH (mmol/ L/g tissue)**
G1	8.0±1.4	10.5±2.8	156.3±1.4	12.5±1.4	0.8±0.4	7.5±1.2	11.0±2.3	161±2 3.4	13.0±1.5	0.8±0.4
G2	14.0±2.4*	21.6±3.0*	183.2±2.1*	16.5±2.4*	0.4±0.3*	15.0±2.5*	25.0±2.8*	182±5 3.9*	16.8±1.9*	0.6±0.3
G3	19.0±2.8*	25.0±3.6*	185.3±2.8*	24.0±3.1*	0.3±0.2*	20.5±3.7*	30.0±3.5*	186.6±4.1*	25.5±2.5*	0.5±0.2*
G4	40.0±3.8*	85.0±5.5*	220.0±3.5*	18.5±2.6*	0.2±0.1*	62.0±5.0*	97.0±6.0*	295.8±13.0*	27.0±2.7*	0.1±0.1*

Values are expressed as Mean±SD

*Significantly different ($p<0.05$) from control (G1) value.

Hb – Haemoglobin, PCV – Packed Cell Volume, WBCC – White Blood Cell Count

G1: 0.00g gasoline/kg body weight

Group 2: 2.0g gasoline/kg body weight

Group 3: 4.0g gasoline/kg body weight

Group 4: 10.0g gasoline/kg body weight

GST – Glutathione-s-transferase, GSH – Glutathione (reduced)

ALT – Alamine transaminase, AST – Aspartate transaminase, ALP – Alkaline phosphatase.

gasoline as observed in the studies of Jollow *et al*. (1973); Igboh and Braide (2003); Guerri and Grisolia (1980), but rather due to decreased synthesis of GSH caused by functional disturbances associated with inflammation and injury to the liver.

Incidentally, the liver is the primary site for the synthesis of many substances including plasma proteins and short peptides like glutathione (Arias, 2002).Therefore, under severe or long standing hepatic disease the synthesis of some of these substances could be impaired. Exposure to gasoline could induce anaemia and hepatic dysfunction/ damage.

If animal to human extrapolation is permissible, then it is important that one avoid any exposure to gasoline and its therapeutic use for any reason should be highly discouraged.

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