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Hepatotoxic hyperplasia, cellular degeneration, and biochemical alterations associated with gasoline vapour-induced liver injury in rats

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ABSTRACT

The aim of this study was to assess the biochemical and structural changes associated with exposure to gasoline vapour in rat. Twenty-four female rats were divided into test and control groups. After the exposure period, all animals were sacrificed, blood was obtained for biochemical analysis, and the liver was removed, and processed for histopathological examination. Abnormal liver architecture, liver enzymes, bilirubin, and malondialdehyde were significantly higher while serum protein were significantly lower in the exposed group than in the unexposed group ($p < 0.05$). Exposure to GV may represent a significant risk factor for a wide spectrum of liver disorders.

Introduction

Gasoline is a fractionated product of crude petroleum. It contains over 500 saturated and unsaturated hydrocarbons, each containing 3–12 carbon atoms. Various additives are blended into gasoline that may affect its properties and toxicity. It is a colourless to pale brown or pink, volatile, flammable liquid. GV is heavier than air and may collect in low-lying areas. Many gasoline constituent hydrocarbons vapourize readily at room temperature and may travel to a source of ignition and flash back.

Gasoline is a major environmental pollutant, and gasoline exposure is widespread across all ages, especially in developing countries. Occupational exposure is a particular problem. Exposure can occur through inhalation, dermal contact, or ingestion; however, inhalation is the most common route of exposure in humans. When inhaled, gasoline is readily absorbed and distributed throughout the body. After being metabolized in the liver, a small fraction is eliminated either unchanged in exhaled air or as an inactive metabolite in the urine. The remaining active metabolites undergo further toxicokinetic processes, including generation of reactive oxygen species (ROS), which causes oxidative damage to tissues and organs, leading to altered structures and functions, and multi-system effects. (Figures 1 & 2).

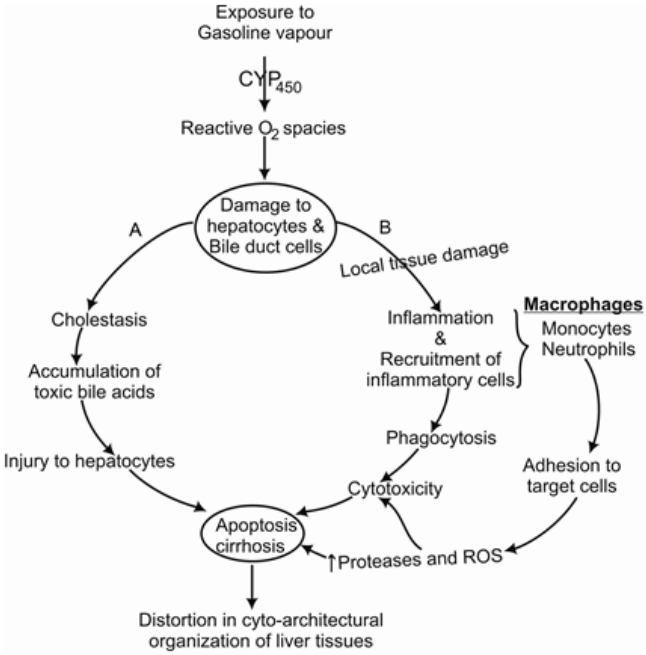

Figure 1. Schematic illustration of the general mechanism of GV-induced cholestasis and inflammation dependent hepatotoxicity. (A) Gasoline metabolites induced injury to hepatocytes and bile duct cells lead to cholestasis. This is followed by intrahepatic accumulation of toxic bile acids leading to hepatic injury, apoptosis, cirrhosis and changes in the cyto-architectural organization of the normal liver tissues. (B) GV-induced liver inflammation causes recruitment of macrophages and neutrophils into liver vasculature. The detrimental effects of their phagocytotic activities result in injury to healthy liver cells (cytotoxicity), apoptosis, cirrhosis and distortion in the cyto-architectural organization of the normal liver tissues.

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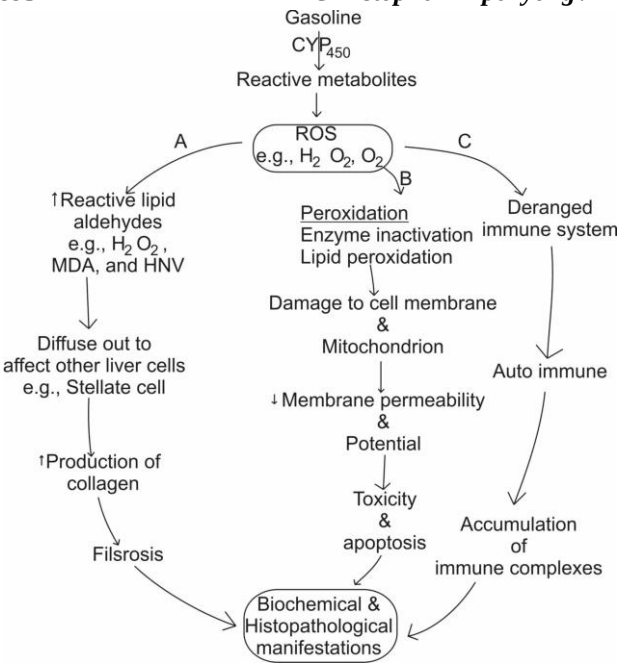


Figure 2. Schematic illustration of the mechanism of GV-induced CYP2EI- dependent hepatotoxicity. (A) ROS can cause increase production of reactive lipid aldehyde molecules which diffuse out to affect other liver cells such as the stellate cells. This is associated with increase production of collagen, fibrosis and liver injury and ensuing biochemical and histopathological manifestations. (B) GV compounds are metabolized into reactive metabolites by CYP2EI generation of ROS leads to lipid peroxidation, mitochondrial and cell membrane damage, a reduction in membrane permeability and potential leading to hepatotoxicity and apoptosis. (C) ROS can derange the immune system leading to auto-immunity and accumulation of immune complexes causing damage to the liver tissues and associated clinical and histopathological manifestations.

Previous studies showed variations in the geographic distribution of gasoline exposure and its effects; however, studies of the biochemical changes and histopathological effects following exposure to GV are sparse. Therefore, this correlative study was aimed at evaluating and comparing the changes in the biochemical markers of hepatic function and the histomorphological alterations in liver tissues following exposure to GV.

The findings of this study may enhance our understanding of the pathophysiology of gasoline-induced hepatic disorders, which may lead to improved therapy and prevention.

Materials and Methods

The experimental animals were 24 mature, healthy female Wistar albino rats weighing 180–200 g. The animals were obtained from the animal house at the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. They were randomly divided into two groups (n = 12 per group): rats in group 1 (G1) served as the control group, which was unexposed, while rats in group 2 (G2) served as the test group, which was exposed to GV.

All animals were housed in ventilated wooden cages with wood shavings as their bedding. Adequate ventilation, ambient temperature (27 ± 2°C), 44% relative humidity, and a 12-h light-dark cycle were ensured throughout the study period. The animals were acclimatized for one week before the experiment. The animals were fed normal animal chow and allowed free access to water.

The research protocols were approved by Institutional Animal Care and Use Committee, and performed in accordance with the rules governing the internationally acceptable use of laboratory animals. Only animals in G2 were exposed to unleaded GV from gasoline purchased from a Nigerian National Petroleum Cooperation (NNPC) refuelling station on Itam Ikot Ekpene Road in Uyo, Nigeria. The cages of the exposed animals were placed in exposure chambers (80 × 60 × 100 cm) containing two calibrated 500-mL beakers containing petrol. The exposure duration was 6 h (9 am–3 pm) daily for 35 consecutive days. Differences in volume at the onset and end of the exposure period were calculated and were used to estimate the daily GV exposure. The average exposure was approximately 80ml/day

At the end of the exposure period (35 days), the animals were sacrificed by chloroform anaesthesia. Blood samples were collected by cardiac puncture, and the liver were removed, weighed, and fixed with 10% buffered formalin for tissue processing and histological examination.

Serum indices of hepatic function including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP) and bilirubin (total and conjugated) were determined using a multi-channel automated analyzer (Reflotron (R) plus system, Roche, Germany). Serum glucose level and lipid sub-fractions including T-chol, LDL-C, HDL-C and TG were determined using a multichannel automated measuring system (Lipid pro TM, Model KM-001A; Info Pia Co., Ltd., South Korea).

Statistical Analysis

Data obtained were analyzed using descriptive statistics and reported as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to detect differences among groups. Differences in means between groups were examined using the independent t-test. Differences were considered statistically significant at $p < 0.05$. All analysis were performed using the Statistical Package for the Social Sciences (SPSS 20.0). The assumption of normality and homogeneity of distribution of the data were tested using Shapiro-Wilk's test and Levene's test respectively. Results showed that these assumptions were not violated.

Results and Discussion

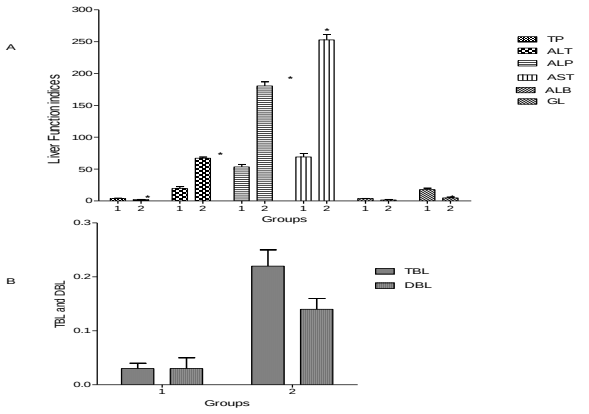
This study demonstrated significant changes in the markers of hepatic function in rats exposed to GV for 35 days. Histological sections of the liver showed significant distortion of the normal liver architecture in exposed animals compared to that of unexposed animals. Significant increases in plasma levels of malondialdehyde (MDA) and a reduction in post-exposure body weight were also observed.

Specifically, plasma levels of the liver enzymes ALT, ALP, and AST were more than 3-fold higher in exposed animals than in control animals. Interestingly, the highest increased was observed in plasma AST levels. In addition, in exposed animals, plasma total bilirubin (TBL) and direct bilirubin were 7 times and approximately 5 times higher, respectively, than the corresponding levels in control animals. Total plasma protein (TPP) and protein sub-fractions (albumin [AL] and globulin [GL]) were significantly lower in the exposed group than in the control group.

Significant increases in the aforementioned biochemical parameters, in particular plasma AST and ALT, are common in most acute and sub-acute hepatocellular disorders.

In fact, increased plasma levels of AST and ALT are usually used as surrogate markers of acute hepatic cell injury [1].

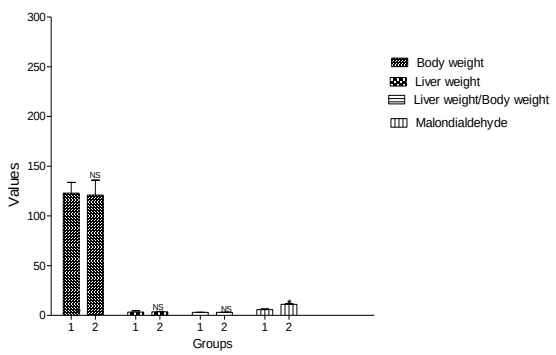
Similarly, elevated serum levels of ALP, particularly along with a concomitant increase in bilirubin, usually indicate impaired flow and excretion of bile, as is observed in hepatobiliary tract obstruction [2,3]. Collectively, these results suggest GV-induced hepatic injury, and are consistent with the results of earlier studies. For instance, Nosko and Mikhailova [4] showed that animals exposed to GV had a significant increase in serum levels of ALT, AST, ALP, TG and cholesterol compared to animals in the unexposed group. In 2005, Uboh and colleagues found a significant higher serum level of liver enzyme activities (AST, ALT and ALP), and extensive degenerative changes in the hepatocytes of animals exposed to GV compared to those in the unexposed group. Momoh and Oshin [3] reported a significant increase in serum liver enzyme activities (AST, ALT, ALP and GGT), oxidative stress biomarker (MDA), and a significant decrease in serum antioxidant enzyme levels (SOD, CAT, and GSH), as well as evidence of liver inflammation and cirrhosis, in the exposed compared to unexposed animals (figures 3A&B and 4).



Values of GL plotted are multiplied by 10.

Figure 3A.Exposure of the experimental animals caused a significant (p<0.05) increase in Serum Liver enzyme activities (AST, ATL, ALP) while Serum total protein sub-fractions (GL and Alb) significantly (p<0.05) decreased.

Figure 3B. Serum total bilirubin (TB) and direct bilirubin significantly (p<0.05) increased in the exposed compared with unexposed.

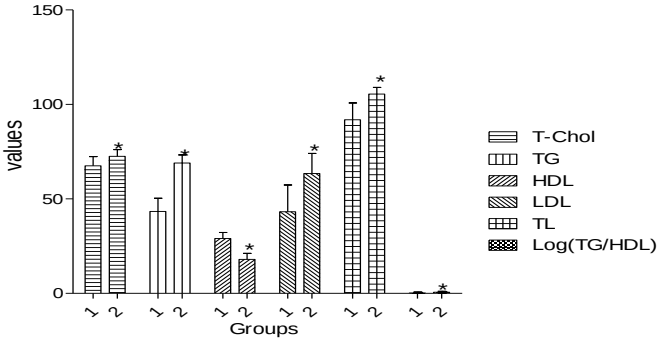


of Liver weight/ Body weight plotted are multiplied by 100.

Figure 4. Shows a significant increase in level of Serum malondialdehyde (MDA) in the exposed compared to unexposed group. Body weight, liver weight, and Liver to body weight ratio were not significantly different between the exposed and unexposed groups.

Likewise a recent study carried out by EL-Ghazaly et al [5] found that animals exposed to GV had a significant increase in serum ALT, AST, ALP levels and marked histomorphological changes including cellular infiltration, dilation of blood sinusoids and cytoplasmic vacuolation in the liver tissues of the animals exposed to GV compared to the unexposed group. Previous and present study findings corroborate an earlier report by Sirdah et al [6] who observed a significant higher serum AST, ALT and ALP levels among liquefied petroleum workers in Gaza governorate. Indeed, numerous established studies have confirmed that exposure to GV is a significant risk factor for hepatotoxicity.

In the present study, I also observed a significant alteration in serum levels of lipid sub-fractions. Specifically, significant increases in serum total cholesterol (T-chol), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), and a significant decrease in serum high density lipoprotein cholesterol (HDL-C), as well as increase in atherogenic index of plasma (ALP) (a biomarker of atherosclerotic cardiovascular disease) (figure 5).



These observations confirmed earlier reports by other investigators with similar research findings [7, 8, 9, 10] and could be due in part, to the direct effect of gasoline metabolites on lipid metabolism, and/or GV-induced perturbation in liver lipid metabolism [7]. The liver plays a central role in lipid metabolism by modulating synthesis, circulation and storage of a significant quantity of excess lipid, It oxidizes free fatty acids (FFAS) and converts them into ketone bodies to be used as fuel by extra hepatic tissues [11].

The liver also contains several enzymes and receptors that contribute to lipid homeostasis, for instance, peroxisome proliferative activated receptor alpha (PPAR-α) is a liver enzyme that functions in regulation of FFA system and ensuring the production of energy, reduction in toxicity of FFA, and has anti-inflammatory effect.

Gasoline metabolites and other xenobiotics can impair fatty acid influx into the liver, denovo synthesis, and conversion to TG.

They can also impair mitochondrial β-oxidation which is responsible for the shortening of fatty acids into acetyl CoA subunits due to their ability to induce oxidative stress. These could lead to perturbation in hepatic lipid homeostasis, leading to abnormal serum lipid profile, (dyslipidemia), and increase risk of atherogenic cardiovascular diseases as observed in the present and previous studies.

When inhaled, gasoline is readily absorbed in the respiratory and gastro intestinal tracts (GIT) due to its lipophilic property. Absorption is followed by uptake and metabolism by liver cytochrom p450 (CYP450) enzyme system, and leading to increase generation of reactive metabolites, ROS

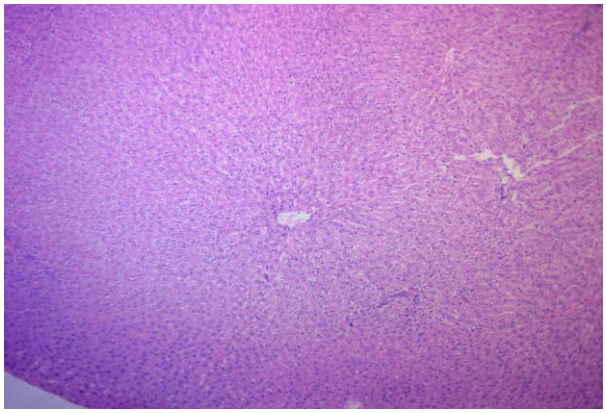
and subsequently causing lipid peroxidation and damage to hepatic membrane causing leakage of hepatic enzymes and deranged bilirubin metabolism and tissues. Therefore, induction of oxidative stress appears to play a central etiopathogenic role in the initiation and progression of gasoline induced hepatobiliary toxicity [12] (Figure 1).

In the present study, lipid peroxidation was assessed by measuring serum malondialdehyde (MDA) levels, and increased lipid peroxidation was shown in the GV-exposed rats by the significant increase in serum MDA. MDA is an end product generated by peroxidation that has been used to demonstrate oxidative stress in diseases affecting several organs/systems, including the heart, liver and kidney [13,14].

Leakage of hepatic enzymes leads to increased serum levels, as was observed in the present study. In addition, a previous study [15] showed that ROS could impair CYP450 activity, leading to a cascade of altered biochemical processes, such as impaired hepatocellular function, phase II detoxification, bilirubin diglucuronidation, and impaired mRNA expression of GSH synthetase. These could lead to decreased bile acid-independent bile flow and accumulation of the cholestatic reaction, which subsequently increases serum levels of bilirubin and ALP, as were observed in the present study.

The observed biochemical findings of gasoline-induced hepatotoxicity correlate with the histopathological findings from the liver sections of the exposed animals, which showed areas with irreversible distortion of cytoarchitectural organization, cellular degeneration, vascular congestion, sinusoidal dilation, and oedema as well as hepatocytic hyperplasia, pyknotic nuclei, and inflammatory changes compared to the liver sections of control animals (figures 6 and 7).

6A



6B

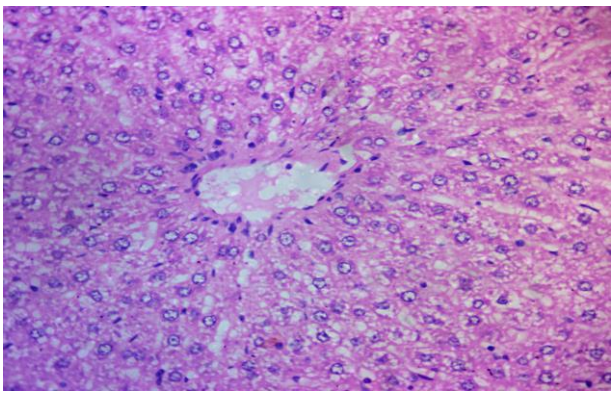
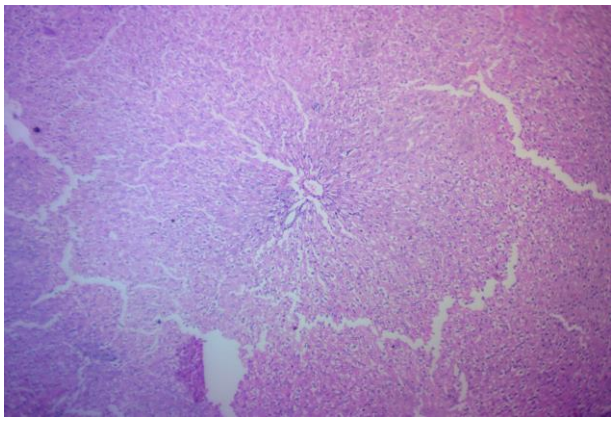


Figure 6: Histologic section of the liver without exposure to GV at magnification of A(x100) and B(x400) showing normal liver cyto-architecture with intact hepatocytes, central vein and sinusoidal lining.

7A



7B

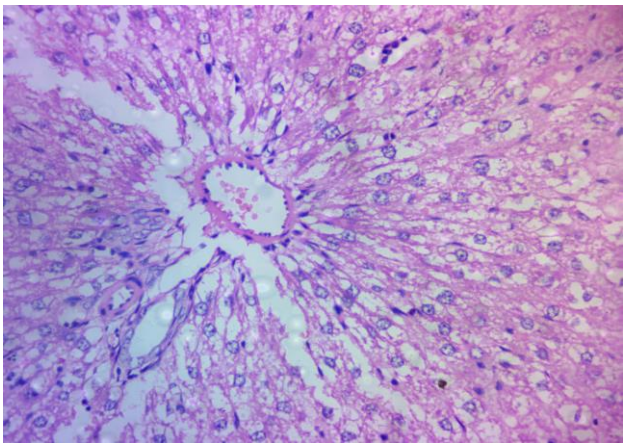


Figure 7. A & B: Histologic section of the liver of animals exposed to GV (8ml/day) for 35 days at magnification C(X100) and D(X400) showing areas with Cellular Degeneration (CD), Hepatocytic Hyperplasia (HH), Vascular Degeneration (GD), Vacuolation and Portal trial affection.

The pathophysiological mechanisms underlying gasoline/other solvents induced liver injury and leading to abnormal histopathological features as observed in the present study have been postulated to include bile acid-induced liver cell injury during cholestetasis, pathophysiological effects of mitochondrial dysfunction and cell damage by ROS ([16], Figures 1 and 2).

Indeed, exposure to GV may be associated with biochemical and histopathological changes in the liver. These changes could lead to a wide spectrum of liver disorders and constitute a significant human health hazard, especially to those who are occupationally exposed, particularly in developing and some developed countries where GV recovery and self-refuelling systems are not used.

In addition to occupationally exposed individuals, the available data indicate that approximately 100 million people are exposed to gasoline constituents for a few minutes per week and approximately 100 minutes per year when refuelling at self-service gasoline stations [17]. In children younger than 6 years, hydrocarbon exposure is posited to account for 1–2% of non-pharmacologic exposures and 10% of all single fatalities [18]. Therefore, efforts should be directed at limiting exposure to GV to prevent irreversible liver damage.

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