Insights into the potential mechanism underlying liver dysfunction in male albino rat exposed to gasoline fumes

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ABSTRACT
The mechanism underlying the reported hepatotoxicity of gasoline fumes in experimental studies still remains unclear. In this study, we present insights into the mechanism underlying hepatotoxicity of gasoline fumes in 72 male albino rats. The rats were randomized into six groups. Group I (control) was exposed to distilled water while groups II, III, IV, V and VI were daily exposed to gasoline fumes for 1, 3, 5, 7 and 9 hours respectively for 10 weeks. Serum hepatic and oxidative stress markers, activity of the mixed function oxygenase (MFO) and membrane-bound ATPase enzymes in the liver as well as histopathological examination of the organ were monitored. Gasoline compositions and its residues in the liver were quantified with Gas Chromatography Mass Spectrometry. Significant (p < 0.05) alterations in the activities of all the enzymes, oxidative stress marker and MFO were observed in the exposed rats compared to control. A total of seventeen gasoline residues and metabolites were detected in the liver of exposed rats. Severe degenerative changes in the hepatic cells were seen in the exposed rats. Accumulation of gasoline residues and metabolites in the liver of exposed rats could potentially result in ROS production capable of inducing liver injury.

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**Introduction**

Gasoline is one of the refined liquid products of crude oil and contains more than 2000 hydrocarbons with some additives and blending agents. These hydrocarbon contents are highly volatile if left exposed and the vapor released into the atmosphere may be considered as gasoline fumes. Gasoline is mainly used as vehicle fuel. It is also used to power electricity generator at homes and industries [1]. This increasing daily consumption of gasoline has resulted in the increase in frequency with which humans are exposed to its fumes. An assessment from the California Office of the Environmental Health Hazard [2] noted that gasoline and its fumes contain some toxic substances including benzene, toluene, ethylbenzene and xylene (BTEX), which are capable of causing various health hazards.

Of all the possible routes and pathways (including dermal contact, sniffing) through which an individual can be exposed to gasoline fumes, occupational inhalation exposure during automobile refueling at the filling station, refining process and bulk transfer serve as the principal exposure routes of gasoline fumes to humans [3]. Exposure to BTEX has been shown to increase workers’ likelihood of increased petroleum product-related health risks [4,5]. The normal physiologic and immune responses have been reported to be modulated by BTEX components [6], and exposure may lead to hepatic dysfunction [7,8].

Liver is the chief organ that chemically alters all compounds entering the body. Hydrocarbons and other constituents of petroleum and petrochemical products have been reported to be metabolized in the liver to a greater extent [9]. Benzene has to be metabolized to some hepatic metabolites including phenol, catechol and hydroquinone before it can induce its toxic effect [10]. Cytochrome-P450-mediated biotransformation of BTEX increases its toxicity, especially by hepatic CYP2E1 [11] resulting in increase in reactive oxygen species (ROS) production [12].

An
imbalance between ROS and antioxidant defenses can lead to oxidative stress, which can alter hepatic function. Oxidative stress-associated pro-inflammatory factors, including xanthine oxidase (XO) may outstrip endogenous protective capability [6,13], further enhancing cellular damage.

Occupational exposure to gasoline components has been reported to alter some liver function markers in humans [14,15] [16], then concluded that hydrocarbon-induced occupational liver injury should be considered as a cause when evaluating a patient with liver injury with possible exposure in relevant occupations. Experimental studies on animals have also reported hepatotoxicity of gasoline vapor [17,18]. We have also reported hepatotoxicity of gasoline fumes in our pilot study [19]. Up till now, the mechanism underlying the reported hepatotoxicity of gasoline vapor in animal and human has not been clearly stated.

Considering the increasing rate of human exposure to gasoline fumes worldwide, addressing and fully understanding the hepatotoxic potential of gasoline fumes and the mechanism involved are of utmost importance. In view of this, the findings of our previous study [19] therefore serve as a step toward the need to further carry out a mechanism-based approach study which will eventually provide the basis for the detail understanding of the hepatotoxicity status of gasoline fumes in male albino rat.

Materials and methods

Experimental animal and gasoline

A total of seventy two (72) adult male albino rats (183 ± 10 g) obtained from the breeding section of the animal house of the Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago-Iwoye were used for this study. The rats were initially acclimatized for one week under the laboratory conditions of 25 ± 5°C and 65 ± 5% Relative Humidity in a well-ventilated experimental section of the animal house before the commencement of the study. The rats were individually allotted into wooden cages with free access to standard laboratory rat chow and clean drinking water. Gasoline, premium motor spirit (PMS) blend, was purchased at once from the Nigerian National Petroleum Corporation (NNPC) filling station located at Ijebu North Local Government Area in Ogun State, Nigeria.

Experimental design

The method of exposure earlier described by [18], and in our previous studies [19,20] was adopted for this present study with little modifications. The rats were randomized into six (06) groups (a control group and five experimental groups) of twelve (12) rats per group as follows;

Group I (control): Rats exposed to distilled water
Group II: Rats exposed to gasoline fumes for 1 hour per day
Group III: Rats exposed to gasoline fumes for 3 hours per day
Group IV: Rats exposed to gasoline fumes for 5 hours per day
Group V: Rats exposed to gasoline fumes for 7 hours per day
Group VI: Rats exposed to gasoline fumes for 9 hours per day

The cages housing the exposed groups were placed in exposure chambers. Three calibrated 500 ml cans containing 300 ml of gasoline were initially placed in the chamber one hour prior to the commencement of the exposure to ensure that the chamber is saturated with gasoline fumes. The rats were later placed in the chamber and allowed to inhale the fumes generated from the direct evaporation of the liquid gasoline from the cans at ambient humidity and temperature for a specific time. At this point of exposure, individual rat was maintained to a single can containing 300 mL of gasoline throughout the exposure period of 10 weeks. At the end of the daily exposure scenario for
a particular group, the rats were moved from the exposure chamber to a gasoline fume-free section of the animal house. The rats were handled in accordance with the regulations of the local ethics committee in the Animal Care Unit (ACU) of Olabisi Onabanjo University, Agolwoye and animal experiment was performed according to ethical guidelines of animal experimentation (regulation CEE 86/609).

**Sample collection and preparation**

Blood sample was collected into plain sample tubes by retro orbital sinus with micro hemato-crit tube. The blood sample was centrifuged at 2500 × g for 10 min at 4°C and the serum obtained was stored at −20°C until used. The rats were sacrificed and liver was excised after dissection and washed in saline solution. A portion of the liver excised was homogenized (10%, w/v) in 50 mM tris-HCl (pH 7.4) and the obtained homogenate was centrifuged at 3,000 × g for 10 min at 4°C. The resulting supernatant was aliquoted and used for some enzymatic and non enzymatic assays while the remaining portion of the liver was subjected to histopathological examination.

**Serum hepatic biochemical analysis**

Serum sample was analyzed for the estimation of serum alanine aminotransferases (ALT), aspartate aminotransferases (AST) and Alkaline phosphatase (ALP) activities using Chemistry Hitachi Model 917 multichannel analyzer (Roche Diagnostics, Indianapolis, IN). Accuracy and precision were checked with Bio-Rad Quality controls.

**Determination of reactive oxygen species in the liver**

Production of ROS in the liver was estimated according to an established method (Pérez-Severiano et al. 21), which is based on the incubation of 100 μL of the liver aliquot in the presence of 5 μL of 2’,7’-dichlorodihydrofluorescindiacetate (DCFH-DA) at 37°C for 60 min. Fluorescence emission of DCF resulting from DCFH-DA oxidation was recorded at an excitation wavelength of 488 nm and an emission wavelength of 525 with a SpectraMax fluorescence plate reader. The result was expressed in nmoles/DCF/g tissue/min.

**Antioxidant parameters analysis in the liver**

Lipid peroxidation was evaluated by measuring its end product, malondialdehyde (MDA), as thiobarbituric acid reactive substance [TBARS] in TBARS assay described by [22, 23]. Concentration of reduced glutathione (GSH) was determined by employing an established protocol (Hassan and Barakat, 24), which is based on the formation of 2-nitro-S-mercaptopbenzoic per mole of glutathione through the reduction of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) by SH groups of glutathione. The concentration of GSH was quantified spectrophotometrically at 412 nm using the extinction coefficient of 13.7 mM⁻¹ cm⁻¹. Activity of catalase [CAT] was measured spectrophotometrically according to [25]. Superoxide dismutase [SOD] activity was according to [26], while glutathione peroxidase [GPx] activity was determined according to [27].

**Mixed function oxygenase enzyme analysis**

Cytochrome P-450 activity was estimated in liver microsomal fraction according to standard method of [28], with little modifications. The liver homogenate was centrifuged at 15,000 g for 20 min, and the supernatant was re-centrifuged at the same speed and the pellet was discarded. The supernatant obtained was re-centrifuged at 100, 000 g for 1 h, and the pellet of microsomes was suspended in 2.0 ml sodium phosphate buffer (0.05 M, pH 7.6) containing 1 mM EDTA. The microsomal preparations containing 2 mg protein per ml was placed in sample
reference cells and the base line was recorded from 420 to 510 nm after which a pinch of sodium dithionite was added in the cuvettes and vigorously mixed, carbon monoxide was bubbled gently into the sample cell for 20 sec, and scanned again from 420 to 510 nm in spectrophotometer with 1.0 cm optical path. The quality of the enzyme was calculated from the absorbance difference (450–480 nm) taking molar extinction coefficient of 90 nM⁻¹ cm⁻¹. Protein in the microsomal preparation was estimated by the method of [29], using bovine serum albumin as standard.

Xanthine oxidase (XO) activity was evaluated by spectrophotometric quantification of uric acid formation by measuring the absorbance at 290 nm (A290), an absorption maximum of uric acid according to Shintani (30). The time-dependent increases in the absorbance at 290 nm during incubation of the reaction mixture at 25 or 37°C were recorded. XD activity in the sample was determined by adding NAD⁺ to the reaction mixture.

**Estimation of the liver membrane-bound ATPase enzyme**

Activities of membrane-bound ATPases enzymes including Na+/K⁺ -ATPase, Ca²⁺ -ATPase and Mg²⁺ -ATPase, in the liver homogenate of the rats were determined according to the method of [31–33], and as modified [34]. The assay was carried out by estimating the amount of phosphorus liberated from the incubation mixture containing the tissue homogenate, 5 mM of ATP, 2 Mm of CaCl₂, 2 mM of MgCl₂, 60 mM of NaCl, 20 mM of KCl and protein enzyme. Incubation of the tubes was at 37°C and enzyme inactivation was done by the addition of 1 mL of cold 10% (w/v) trichloroacetic acid (TCA) after 25 min. We kept the tubes in ice cold for 20 min and removed the precipitated proteins by centrifugation. A control was simultaneously set up by adding enzyme after TCA at the end of the incubation period. Protein concentration was estimated by the method of [29], with bovine serum albumin as standard.

**Extraction, identification and quantitative determination of hydrocarbons in testis**

The method of extraction, identification and determination of hydrocarbon compounds in the liver of rats was in accordance with [35–37], with little modifications. Briefly, the extraction was carried out by dissolving 2 g of minced liver tissue, which have been prewashed with diethyl ether in 10mls dichloromethane/ n-pentane in a well corked reagent bottle. This was thoroughly mixed using an ultra sonicator and eluted on a glass column packed with (from bottom to top) a 2 cm layer of non activated silica gel followed by an 6 cm layer of anhydrous sodium sulfate. The mixture was rewash with 20 ml dichloromethane/n-pentane for two more consecutive times. The combined aliquot was evaporated on a steam berth to 5 ml and filtered through a pasture pipette stocked with glass wool (membrane) with packed anhydrous sodium sulfate silica gel to remove the left over moisture and other impurities. The filtrate was concentrated to 1 ml under reduced pressure on a rotary evaporator at 30°C and transferred to a 2 ml graduated vial. Clean up was done using a column chromatography technique with 2 g activated silica gel. The extract was transferred to the column and eluted with 30 ml of a 1:1 mixture of diethyl ether and pentane. The eluate was collected in a graduated flask, followed by the addition of small quantity of isooctane and finally concentrated to 1 mL on a rotary evaporator. The concentrated extracts were screened for their compound composition on a GC-MS. The procedure for the analysis followed US EPA Good Laboratory Practice Standards (40 CFR Part 79.60, 1994). The gas chromatographic (Model: 7890A GC) analysis was performed on an Agilent Technologies interfaced with Mass Selective Detector (Model: 5975 C MSD). The oven temperature was programmed at initial temperature of 40°C to hold for 2 min at 4°C per min to the temperature of 240°C at 5°C per minute to the temperature of 300°C to hold for 11 min. The electron ionization was at a 70 v with an ion
source temperature at 250°C. The volume of sample injected was 1 µl and the compounds were separated on Agilent Technologies HP5MS column (30 m x 0.25 mm x 0.320 µm). The carrier gas used was Helium gas at 65 psi. Each separated compound was identified at the end of each run by comparing its total ion chromatogram (TIC) and mass spectrum with 2004 NIST e-library.

**Histopathological examinations**

The processing of the excised liver sample for histopathological examination was in accordance with the method previously used in our study [19]. The liver sample was routinely processed and sectioned at 4–5 µm thick. The obtained liver sections were stained with Hematoxylin-Eosin (H&E) before mounting in neutral DPX medium. Prepared slides were examined at 400× magnifications with the Celestron LCD Digital microscope II (model no. 44,341, Torrance CA 90503 USA).

**Statistical analysis**

Data obtained were subjected to statistical analyses using the IBM Statistical Package (SPSS) version 20.0 [38]. Mean values were compared using Analysis of Variance (ANOVA). Results were presented as Mean ± Standard Error of Mean (SEM). Post hoc test was done using the Student-Newman-Keuls (SNK). Probability value (p value) less than 0.05 was considered to be statistically significant.

**Results**

**Activity of liver enzyme marker in the serum of the experimental rat**

The activities of serum AST, ALP and ALT in rats subjected to varying periods of daily gasoline fumes exposure are represented in (Figure 1). The enzyme activities were observed to significantly (p < 0.05) increase in the experimental rats with increase in the daily gasoline fumes exposure. However, activities of ALT and AST were not significantly (p > 0.05) different in groups IV, V and VI.

**Level of reactive oxygen species in the liver of experimental rat**

(Figure 2) presents the level of ROS produced in the liver of rats subjected to varying periods of daily gasoline fumes exposure. Level of ROS was highest and significant (p < 0.05) in group VI and not significantly difference (p > 0.05) from the values recorded in groups IV and V. Levels of ROS recorded for groups I and II were also observed not to be significantly difference.

**Level of oxidative stress parameter in the liver of experimental rat**

Activities of SOD, CAT, GPx as well as concentration of GSH recorded in the liver of rats subjected to varying periods of daily gasoline fumes exposure are shown in (Table 1). These parameters were significantly (p < 0.05) highest in group II and lowest in group VI compared to other groups.

**Level of lipid peroxidation in the experimental rat**

Results showed that MDA was lower (p > 0.05) in the liver of rats in groups I and II compared to other groups (Figure 3). This was however observed to significantly (p < 0.05) increase in the liver of the experimental rats with increase in the daily gasoline fumes exposure period.

**Activities of Xanthine oxidase and CYP450**

Activity of CYP-450 was observed to significantly (p < 0.05) increase in the liver of experimental rats with increase in the daily gasoline fumes exposure period (Figure 4). Similarly, activity of XO was significantly (p < 0.05) reduced in the liver of rats in groups I and II compared to other groups. This
Figure 1. Activity of liver enzyme marker (U/L) in rats subjected to varying hours of daily gasoline fumes exposure. Group I (control); rats exposed to distilled water, Group II; rats exposed to gasoline fumes for 1 h/day, Group III; rats exposed to gasoline fumes for 3 h/day, Group IV; rats exposed to gasoline fumes for 5 h/day, Group V; rats exposed to gasoline fumes for 7 h/day, Group VI; rats exposed to gasoline fumes for 9 h/day.

Figure 2. Level of ROS (nmoles/DCF/g tissue/min) in the liver of rats subjected to varying hours of daily gasoline fumes exposure. Group I (control); rats exposed to distilled water, Group II; rats exposed to gasoline fumes for 1 h/day, Group III; rats exposed to gasoline fumes for 3 h/day, Group IV; rats exposed to gasoline fumes for 5 h/day, Group V; rats exposed to gasoline fumes for 7 h/day, Group VI; rats exposed to gasoline fumes for 9 h/day. was also observed to increase significantly in the experimental rats with increase in the daily gasoline fumes exposure period. However, there was no significant (p > 0.05) difference in the activity of XO recorded in groups IV and VI.

Activity of membrane bound ATPase enzyme in the liver of experimental rat

There were no significant differences (p > 0.05) in the activities of Mg ATPase, Ca ATPase, Na/K ATPase and total ATPase observed in groups I, II and III, although the activities were observed to be highest in group I (Table 2). Activities of these enzymes were observed to significantly reduce (p < 0.05) in group VI.

Composition of hydrocarbon in gasoline and its residues in the liver of experimental rat

A total of 23 hydrocarbon components were detected in the gasoline used for this study (Supplementary Table S1). Toluene has the highest percentage composition in the
Table 1. Oxidative stress parameter in the liver of rats subjected to varying hours of daily gasoline fumes exposure.

| Group | SOD (U/mg Protein) | GSH (U/g Tissue) | CAT (U/mg Protein) | GPx (U/mg Protein) |
|-------|---------------------|------------------|--------------------|-------------------|
| Group I | 20.78 ± 1.50b | 33.65 ± 2.72b | 35.95 ± 2.13b | 45.70 ± 2.04b |
| Group II | 25.93 ± 2.81a | 40.72 ± 2.55a | 40.63 ± 1.92a | 51.09 ± 1.79a |
| Group III | 18.16 ± 1.96c | 26.83 ± 1.73c | 28.54 ± 1.85c | 35.66 ± 2.00c |
| Group IV | 13.13 ± 1.86d | 17.91 ± 1.88d | 27.12 ± 0.97c | 26.51 ± 2.74d |
| Group V | 13.30 ± 1.55d | 20.12 ± 1.32d | 21.60 ± 1.35d | 26.88 ± 1.64d |
| Group VI | 8.85 ± 0.68e | 14.31 ± 1.68e | 15.97 ± 1.45e | 22.14 ± 2.02e |

Means with similar superscript in the same column are not significantly different (p > 0.05).

**Group I** (control); rats exposed to distilled water, **Group II**; rats exposed to gasoline fumes for 1 h/day, **Group III**; rats exposed to gasoline fumes for 3 h/day, **Group IV**; rats exposed to gasoline fumes for 5 h/day, **Group V**; rats exposed to gasoline fumes for 7 h/day, **Group VI**; rats exposed to gasoline fumes for 9 h/day.

**Figure 3.** Level of MDA (nmol/g tissue) in the liver of rats subjected to varying hours of daily gasoline fumes exposure. **Group I** (control); rats exposed to distilled water, **Group II**; rats exposed to gasoline fumes for 1 h/day, **Group III**; rats exposed to gasoline fumes for 3 h/day, **Group IV**; rats exposed to gasoline fumes for 5 h/day, **Group V**; rats exposed to gasoline fumes for 7 h/day, **Group VI**; rats exposed to gasoline fumes for 9 h/day.

Gasoline sample. This was followed by o-xylene, naphthalene, undecane, ethylbenzene and p-Xylene. A total of seventeen (17) hydrocarbon components, including gasoline metabolites, were detected in the liver of the experimental rats (Table 3). Benzene was detected in the liver of rats in all the groups. However, benzene level was significantly reduced in group I. Paracyclophane and ethylbenzene were only detected in the liver of rats in group III. Similarly, 4,7-Methano-1 H-indene, Azulene, Cyclobutane, 3-Phenylthiane, Quinoline and 1-benzylindole were only detected in the liver of rats in group V.

**Histopathological examination of the liver of experimental rat**

The liver histopathological evaluations of rats exposed to gasoline fumes at varying hours are presented in (Figure 5). The sections revealed normal liver cell architecture in group I while progressive degenerated hepatocytes, dilated sinusoids, vacuolations, were observed in the liver of rats in other groups. Irregular distortion and dilated sinusoids were the major lesions observed in the liver of rats in group III. Hepatic degenerations characterized by hepatocytes vacuolation, hepatocytes loss, cytoplasmic vacuolations with eosinophilic substances were
Figure 4. Activities of Xanthine oxidase (U/mg protein) and CYP-450 (nmol cp450/mg microsomal protein) in the liver of rats subjected to varying hours of daily gasoline fumes exposure. **Group I (control)**; rats exposed to distilled water, **Group II**; rats exposed to gasoline fumes for 1 h/day, **Group III**; rats exposed to gasoline fumes for 3 h/day, **Group IV**; rats exposed to gasoline fumes for 5 h/day, **Group V**; rats exposed to gasoline fumes for 7 h/day, **Group VI**; rats exposed to gasoline fumes for 9 h/day.

| Group     | Mg ATPase     | Ca ATPase   | Na/K ATPase | Total ATPase |
|-----------|---------------|-------------|-------------|--------------|
| Group I   | 0.73 ± 0.03^a | 0.60 ± 0.02 | 0.53 ± 0.04^a | 1.81 ± 0.03^a |
| Group II  | 0.72 ± 0.03^a | 0.56 ± 0.03 | 0.48 ± 0.03^a | 1.76 ± 0.02^a |
| Group III | 0.63 ± 0.04^a | 0.52 ± 0.02 | 0.45 ± 0.04^a | 1.58 ± 0.01^a |
| Group IV  | 0.38 ± 0.06^b | 0.38 ± 0.03 | 0.35 ± 0.02^b | 1.11 ± 0.05^b |
| Group V   | 0.42 ± 0.07^b | 0.36 ± 0.05 | 0.37 ± 0.02^b | 1.15 ± 0.02^b |
| Group VI  | 0.26 ± 0.03^b | 0.18 ± 0.03 | 0.14 ± 0.01^c | 0.60 ± 0.05^c |

Means with similar superscript in the same column are not significantly different (p > 0.05).

**Group I (control)**; rats exposed to distilled water, **Group II**; rats exposed to gasoline fumes for 1 h/day, **Group III**; rats exposed to gasoline fumes for 3 h/day, **Group IV**; rats exposed to gasoline fumes for 5 h/day, **Group V**; rats exposed to gasoline fumes for 7 h/day, **Group VI**; rats exposed to gasoline fumes for 9 h/day.

seen in the liver of rats in groups IV and VI. Apart from the loss of hepatocytes, inflammatory cells were observed to surround the bile duct in the liver of rats in group V.

**Discussion**

The hepatic enzymes AST, ALT and ALP are often used as biomarker for early acute hepatic damage. AST and ALT are cytosolic marker enzymes, which reflect hepatocellular necrosis. Meanwhile, ALP is known to be a good indicator of biliary function and cholestasis. Therefore, elevated activities of these enzymes in the circulation could serve as a good indicator of hepatic damage. Thus the higher activities of AST, ALT and ALP observed in the rats exposed to gasoline fumes in this study signify cellular leakage and failure of the functional integrity of liver cell membrane. This may possibly result from an abnormal dynamic property of cellular membrane induced by benzene, toluene and xylens (BTX), known to be the most dangerous hydrocarbon components in gasoline [39]. Meanwhile, an increased activity of the serum hepatic marker observed among gasoline station workers was associated with occupational exposure to BTX [40].

Generally, antioxidant defense system including both enzymatic (SOD, CAT GPx) and non-enzymatic (GSH) components protects the body cells against the damaging effects of ROS [41]. Oxidative stress occurs when the production of ROS overwhelmed the activity of cellular antioxidant defense system. Our findings reveal that gasoline fumes exposure has induced
Table 3. Hydrocarbon components (ppm) in liver of rats subjected to varying periods of daily gasoline exposure.

| Compound                        | Group I | Group II | Group III | Group IV | Group V | Group VI |
|---------------------------------|---------|----------|-----------|----------|---------|----------|
| 1 Benzene                        | 0.002d  | 0.023c   | 0.038b    | 0.054a   | 0.048a  | 0.020c   |
| 2 Ethylbenzene                   | ND      | ND       | 0.003a    | ND       | ND      | ND       |
| 3 p-Xylene                       | ND      | ND       | 0.004a    | ND       | ND      | ND       |
| 4 Mesitylene                     | ND      | ND       | 0.008a    | ND       | 0.004a  | 0.009a   |
| 5 Indane                         | ND      | ND       | 0.002a    | ND       | 0.002a  | ND       |
| 6 4,7-Methano-1 H-indene         | ND      | ND       | ND        | ND       | 0.005a  | ND       |
| 7 Naphthalene                    | ND      | 0.002a   | ND        | ND       | ND      | ND       |
| 8 1 H-Indene                     | ND      | ND       | 0.018a    | ND       | 0.024a  | ND       |
| 9 1 H-indole                     | ND      | ND       | 0.013a    | ND       | ND      | 0.018a   |
| 10 Methylene chloride            | ND      | ND       | 0.013a    | 0.010a   | ND      | ND       |
| 11 Azulene                       | ND      | ND       | ND        | ND       | 0.020a  | ND       |
| 12 Cyclobutane                   | ND      | ND       | ND        | ND       | 0.004a  | ND       |
| 13 3-Phenythiane                 | ND      | ND       | ND        | ND       | 0.005a  | ND       |
| 14 Quinoline                     | ND      | ND       | ND        | ND       | 0.010a  | ND       |
| 15 1-benzylindole                | ND      | ND       | ND        | ND       | 0.018a  | ND       |
| 16 2.2 Paracyclophane            | ND      | ND       | 0.002a    | ND       | ND      | ND       |
| 17 2,3-diphenylcyclopropylmethyl phenyl sulfoxide | ND     | ND       | ND        | 0.036a   | 0.043a  | ND       |

Means with similar superscript in the same row are not significantly different (p > 0.05). ND- Not Detected.

**Group I (control)**: rats exposed to distilled water, **Group II**: rats exposed to gasoline fumes for 1 h/day, **Group III**: rats exposed to gasoline fumes for 3 h/day, **Group IV**: rats exposed to gasoline fumes for 5 h/day, **Group V**: rats exposed to gasoline fumes for 7 h/day, **Group VI**: rats exposed to gasoline fumes for 9 h/day.

Figure 5. Liver sections of rats H&E X400: (a) group I showing normal histo-architecture of liver, central vein (blue arrow), hepatocyte (green arrow) and sinusoids (yellow arrow). (b) Group II showing mild dilated sinusoid (yellow arrow). (c) Group III showing severe dilated sinusoid (yellow arrow). (d) Group IV showing severe degenerated hepatocytes (blue star), vacuolation (black arrow) and cytoplasmic vacuolations with eosinophilic substances (red arrow). (e) Group V showing severe degenerated hepatocytes (blue star), inflammatory cells surrounding bile duct (purple arrow). (f) Group VI showing severe degenerated hepatocytes (blue star), vacuolation (black arrow) and cytoplasmic vacuolations with eosinophilic substances (red arrow), dilated sinusoid (yellow arrow).

Redox imbalance and oxidative stress in the liver of the exposed rats. This is evidenced in this study by the progressive increases in hepatic MDA and ROS levels accompanied by the reduction in the activities of SOD, CAT, GPx and concentration of GSH. Lipid peroxidation is widely accepted as the specific consequence of the oxidative action of ROS on membrane
lipids and as such, used as accurate marker of oxidative stress. In addition, ROS formation is widely known to represent a general index of oxidative damage since the formation is more related to the potential risk of oxidative toxicity in the body cells as well as the tendency of ROS to exert their effects on different biomolecules [42, 43]. It is clearly seen in this study that the excessive production of ROS overwhelmed the protective roles of the antioxidant molecules in the liver of the exposed rats and this resulted in peroxidation of the hepatic membrane polyunsaturated fatty acids. However, the main gasoline component responsible for the observed oxidative stress in this study remains unknown. But benzene is a possibility, because occupational exposure to benzene has been documented to induce oxidative stress among workers in gasoline filling station [44]. In addition, metabolic activation of benzene to metabolites, such as hydroquinone and 1, 2, 4-benzenetriol has been reported to generate ROS, impair antioxidant defense system and consequently induce oxidative stress [44]. Therefore, it is reasonable to attribute the oxidative stress observed in the liver of the exposed rats to the presence of benzene therein.

XO is a molybdenum-containing enzyme, which catalyses a two-step oxidation reaction of hypoxanthine to xanthine as well as xanthine to uric acid [45,46]. The reoxidation of XO led to reduction of molecular oxygen, which acts as an electron acceptor and production of ROS [47]. Thus, XO is one of the endogenous sources of ROS in the body and its over activity has been implicated in the pathogenesis of several diseases, including acute inflammatory arthritis [48]. We have shown, for the first time, that gasoline fume is one of the potential inducers of XO activity in the liver tissue and this could explain the higher level of ROS in the liver of the exposed rats and the observed physiopathological manifestations.

It has been reported that many xenobiotics may increase or decrease the activity of CYP isozyme either by inducing the biosynthesis of an isozyme or by directly inhibiting its activity (Meunier et al. 49). This study shows that gasoline fume is one of the xenobiotics capable of increasing the activity of CYP-450 in the liver of the exposed animal. CYP-450 is a member of the superfamily of heme containing monooxygenases, which are important in the metabolism and detoxification of many environmental pollutants. The induction so noted may suggest metabolic detoxification of some hydrocarbon residues in the liver tissue. This is evidenced by the occurrence of some metabolites, including 4,7-Methano-1 H-indene, 2,3-diphenylcyclopropyl)methyl phenyl sulf oxide as well as Quinoline in the liver of the exposed rats. Meanwhile metabolism of gasoline and its components has been documented to produce reactive metabolites, such as 1,2,4-benzenetriol and benzoquinone, which are capable of interacting with the hepatocytes membrane lipids to generate ROS and lipid peroxide (lipid peroxidation) [50]. However, it remains unknown if the metabolites noted in this study contribute to the observed pathologies of the liver of gasoline fumes-exposed rats.

It has been reported that ATPases associated with membrane indicate alterations to the membrane under the condition of oxidative stress [51]. By hydrolysis, ATPases supply energy to metabolic processes, regulate membrane permeability and transportation of ions (including Na+, Ca2+ and K+) across the membrane at the expense of ATP [52]. In this study, gasoline fumes was observed to reduce the activities of membrane bound Ca2+-ATPase, Mg2+-ATPase, Na+/K+-ATPase and Total ATPase in the liver of the exposed rats. Na+/K+-ATPase is lipid dependent and has high affinity for SH group and Mg2+-ATPase activity is sensitive to lipid peroxidation [53]. Hence, we can attribute the reduction of these enzyme activities to the observed increased in lipid peroxidation by ROS in the liver of the exposed rats. It is also possible for some gasoline components in the tissue to have bonded with several SH-rich enzyme proteins and consequently inactivate them [34]. Reductions in the activities of these ion dependents ATPases could impair signal transduction
due to a disturbance in ion homeostasis in the liver, an alteration in cellular metabolism, change cell membrane integrity and permeability as well as induce a rise in membrane fluidity and disturbances in vital functions [54]. In addition [53], noted that Na+/K+-ATPase inhibition could lead to a decrease in sodium efflux, which disrupts membrane permeability, a condition that leads to the leakage of Ca2+ ions into the cytoplasm. This will consequently decrease the activity of Ca2 + -ATPase in the membrane, thereby potentiating irreversible cell destruction. This mechanism could also be a possibility in the liver of the gasoline fumes exposed rats in this study.

This study shows, for the first time, that some gasoline compositions and metabolites such as 2,3-diphenylcyclopropyl)methyl phenyl sulfoxide, 1-Buten-3-yne, 2,2 Paracyclophane, 1-benzylindole, 3-Phenythiane, Methylene chloride, 4,7-Methano-1 H-indene, 3-Hepten-2-one and N-Butylbenzylamine could be retained in the hepatic tissue of animals exposed to gasoline fumes for some hours. This implies that gasoline fume has the potential to condense and settle in the tissue of an exposed animal. Although some compounds were metabolized in the hepatic tissue, but the metabolic processes vary. Report has shown that phase 1 reaction of xenobiotic metabolism sometimes converts a toxic compound into a more toxic and reactive metabolite, which are capable of interacting with vital intracellular macromolecules resulting in toxic effects [55,56,57]. Reactive metabolites may be free radicals or act as radical generators that can interact with oxygen to produce ROS, which are capable of inflicting damage to membranes and other macromolecules [58,59]. It therefore remains unclear if the gasoline residues or the metabolites are the main compounds responsible for the ROS generation and hepatotoxicity observed in this study.

Exposure to gasoline fumes hydrocarbons may have disrupted the liver tissue and consequently resulted in severe degenerated hepatocytes, dilated sinusoids and hepatic necrosis. This is evidenced by the histopathological lesions observed in the liver of gasoline fumes exposed rats. This clearly shows that liver is one of the main target organs of gasoline fumes-induced injury.

Conclusions

Our findings suggest that exposure to gasoline fumes could result in accumulation of gasoline residues and metabolites in the hepatic tissue of the exposed animal. This in turn, may generate ROS and induction of oxidative stress, which can induce hepatic degenerative changes, increase the activities of MFO, damage the membrane and reduce the activities of the membrane-bound ATPase enzymes. It will be of interest to know the major hydrocarbon compound in gasoline fumes that is responsible for the observed liver dysfunction in the exposed animal. In addition, further study on clinical trials in a population exposed to gasoline fumes is recommended. Meanwhile, cell inflammation has been noted to play a significant role in abnormal hepatic response to an organophosphorus exposure in rats [60] and positive correlation between inflammations, oxidative stress and apoptosis in animals exposed to hepatotoxin has been observed [61–64]. This strongly advocates the need to further investigate the role of pro-inflammatory cytokines and apoptotic proteins in gasoline fumes-induced hepatotoxicity.

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Disclosure statement

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