Hepatotoxicity and genotoxicity of gasoline fumes in albino rats

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ABSTRACT

Toxic effects of gasoline fumes have been reported, but evidence of its hepatotoxicity and genotoxicity are rare. Therefore, this study assesses hepatotoxicity and genotoxicity of gasoline fumes on forty Albino rats randomly assigned to five experimental treatments (T) with eight rats per treatment (T1, T2, T3, T4 and T5). T1(Control) was housed in a section of experimental animal house free from gasoline fumes while T2, T3, T4 and T5 were exposed to gasoline fumes in exposure chambers for one, three, five and nine hours daily respectively for twelve weeks. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and histopathological examination of the liver tissues were used as diagnostic markers to assess liver dysfunction. Genotoxicity test was conducted on the lung tissues using randomly amplified polymorphic DNA fingerprinting polymerase chain reaction (RAPD PCR) technique. Significant increase (p < 0.05) in the level of ALT, AST and ALP for T2, T3, T4 and T5 compared to T1 were recorded. Photomicrograph examination of the liver sections of T1 showed hepatic tissue with normal liver cell architecture while that of T2, T3, T4 and T5 revealed degenerative changes in the ultrastructural integrity of the hepatic cells. Genotoxicity test revealed DNA bands at a reducing intensity from T1 to T5. Dendrogram showed DNA damage in the lungs of T3, T4 and T5 were closely similar and the genotoxic impact was more in T3. Frequent exposure to gasoline fumes was observed to induce hepatotoxicity and genotoxicity, hence impairing the normal liver function and gene structure.

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1. Introduction

It has been reported that normal physiological state of an organism can be altered by substances called xenobiotics, which can exist in gaseous, liquid, semi-solid and solid states and can easily enter into organisms through inhalation, ingestion, dermal contact and diffusion (Murray, 2003). Gasoline in Nigeria, which is one of the fractionated products of crude oil, is an example of a xenobiotic. Gasoline contains very complex and inflammable substances which are xenobiotic and endocrine disrupting chemicals (EDC). Gasoline and its components are also known to be very volatile if left exposed and its fume constitutes chemical pollutants in the environment (Zahlsen and Tri-Tugaswati, 1993). It comprises of more than 500 saturated or unsaturated hydrocarbons having 3–12 carbons such as n-pentane, n-hexane, toluene and benzene.

Some adverse effects of xenobiotic have been reported. It has been found to reduce seminal parameters (De Jager, 2006), impaired semen quality (Aneck-Hahn, 2007), causes male genital anomalies (Bhatia, 2005) and thus fertility status. It has also been found to cause a wide range of biochemical and physiological dysfunctions by generating reactive oxygen species and various free radicals. It also inhibits antioxidant enzymes activities such as superoxide dismutase (SOD), catalase (CAT) and decreases the level of glutathione (Rahman and Sultana, 2006).

To satisfy the need of the growing industrial establishments over the years in Nigeria, there has been a high increase in the daily demand for gasoline and other petroleum products (Isa et al., 2013). This increasing daily use has increased the frequency at which individuals are exposed to its fume. Some of the uses of gasoline include fuel for vehicles, cooking and lightning fuel in homes and outside homes, as chemical feedstock for industries, therapeutic reasons (Huckabay et al., 1995) and as fuel for electricity generators at homes, offices and industries. The diverse use of gasoline has greatly increased the establishment of petrochemical industry and petroleum exploration which has been reported as one of the main contributors of environmental and global problems (Carlos and Donna, 2008).
Exposures to petroleum products both in and outside petroleum industries have been reported to have some effects on the users, with those who are occupationally exposed being more likely to be affected than their counterparts (Rothman et al., 1996). Recent research from the national institute for occupational safety and health (NIOSH) and the occupational safety and health administration (OSHA) showed that oil and gas workers could be exposed to hydrocarbon gases and vapors while working on or near production and flowback tanks and the exposure can have immediate health effects, including loss of consciousness and death (NIOSH, 2016). This means workers can face significant health and safety risks when they manually gauge or sample tanks (Esswein et al., 2014; Jordan, 2015). Nine worker fatalities had been recorded among workers who manually gauged or sampled production tanks from year 2010 to 2014 in USA (NIOSH, 2015) and exposure to the petroleum vapors are believed to be the primary or contributory factors to the oil and gas extraction workers’ deaths (Harrison et al., 2016). In addition, sudden death due to gasoline inhalation had been reported (Martinez et al., 2012). As reported in Chi (2015), oil and gas workers exposed to chemicals produced and used in oil and gas industry may suffer occupational diseases of lungs, skin and other organs at levels depending on the length of exposure time and the effects from these occupational health hazards comprise of dizziness, drowsiness, headaches and nausea (commonly associated with hydrocarbon exposure), dermatitis and irritation and inflammation of respiratory system. However, significant hematopoietic changes were observed among 146 out of 292 workers sampled at filling stations in Baghdad city (Ali and Sahb, 2011). Following a similar trend in Nigeria, Odewabi et al. (2014) observed marked reductions in plasma antioxidant defense system among gas station attendants. Also, Atif and Riffar (2012) had reported hematotoxicity among workers of automobile repair workshops in Pakistan.

As reported by Health Protection Agency (HPA, 2007), the major route of exposure is inhalation by workers during production and distribution of the fuel and general public refueling at the gas station and consequently brings concentrated hydrocarbons into the lungs and bloodstream. The vaporization or presence of gasoline fume in our environment should therefore necessitate accurate identification of its potential hazards to human and animal health.

Exposure to gasoline has been reported to cause haematotoxicity, nephrotoxicity and alters lipids metabolisms and some biochemical activities (Uboh et al., 2008, 2010). HPA (2007) has reported that exposure to the gasoline vapour may affect the central nervous system and consequently produce effects such as staggered gait, slurred speech, confusion, rapid unconsciousness and death due to respiratory failure. Owagboriaye et al. (2016) have recently reported that inhalation exposure to gasoline fume may be harmful to the normal body physiology by increasing serum lipid peroxidation, corticosterone and aldosterone level. But little is known on the hepatotoxic effect of gasoline fume exposure in animals. Liver is the main organ that chemically alters all components entering the body and this implies that liver could be one of the major target organ by environmental pollutant, thereby, disrupting the metabolic action of this organ.

Genotoxicity is developed to identify the elements or compounds presents in the environment having the potential to cause mutation by damaging the DNA of animals (Khlood et al., 2011). It has been reported that acrylamide, a xenobiotic, can be absorbed into the circulation and distributed to various organs, and reacts with DNA, neurons, haemoglobin and essential enzymes (Rayburn and Friedman, 2010) causing several toxic effects as animal carcinogen and germ cell mutagen (Ghanayem et al., 2005), but there is paucity of information on the genotoxic effects associated with gasoline fume exposure in animals. It is on this basis this study was therefore designed to clarify the possible hepatotoxic and genotoxic effects associated with inhalation exposure to gasoline fumes in albino rat.

2. Materials and methods

2.1. Experimental animal

We used forty apparently healthy adult male albino aged 8–9 weeks (220±10 g) obtained from the breeding section of the animal house of the Department of Zoology, Olabisi Onabanjo University, Ago-Iwoye Nigeria for this study. Prior to the commencement of the experiment, the rats were acclimatized under the laboratory conditions of 25 ± 5 °C and 65 ± 5% Relative Humidity in the experimental animal house for one week. The rats were randomized into five experimental treatments (T) with eight rats per treatment. The rats were individually kept in wooden cages (65 cm × 35 cm × 50 cm) in a well-ventilated animal house and were allowed free access to clean drinking water and food.

2.2. Exposure to gasoline fume

The method of exposure earlier described by Uboh et al. (2008) and Owagboriaye et al. (2016) was adopted for this study. The animal cages housing the test groups were placed in an exposure chamber (165 cm × 95 cm × 220 cm). Two calibrated 1000 ml cans containing 500 ml of gasoline were placed in the chamber one hour prior to the commencement of the exposure to ensure that the exposure chamber was saturated with gasoline fume. The exposed animals were later placed in the chamber and allowed to inhale the fumes generated from the direct evaporation of liquid gasoline from the cans at ambient humidity and temperature. At the end of each daily exposure period, the rats were removed from the exposure chamber. T2, T3, T4 and T5 were exposed to gasoline fume for one, three, five and nine hours respectively at a room temperature for a period of twelve weeks while the control treatment, T1 was housed separately in a section of the experimental animal house free from gasoline fume and exposed to distilled water. The experimental protocol was conducted in accordance with the regulations of the local ethics committee in animal care unit of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. Animal experiment was performed according to ethical guidelines of animal experimentation (regulation CEE 86/609). The care of the animals was done in accordance with the U.S. public health service guidelines (National Research Council, 2011).

2.3. Sample collection

Blood samples were collected from the rats 24 h after the last exposure by retro orbital sinus with micro haematocrit tube. Serum samples were separated within 1 h after collection of blood and centrifuged at 3000g for 5 min and stored in a freezer till assayed. Biochemical analyses on the serum samples were carried out after the sample collection. The rats were sacrificed and liver excised through dissection were fixed in 10% neutral-buffered formalin and processed for histopathological examinations. Lung samples was also collected and subjected to genotoxicity test.

2.4. Histopathological examinations

The liver specimens were routinely processed and sectioned at 4–5 μm thick. The obtained liver sections were stained with Hematoxylin-Eosin (H&E) dye before mounting in neutral DPX medium. Prepared slides were examined at 100 X and 400 X magnifications.
2.5. Biochemical analysis

Serum samples were analysed for estimation of serum alanine aminotransferases (ALT), aspartate aminotransferases (AST) using the standard colorimetric method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was estimated using Randox commercial enzyme kit as described by German Society for Clinical Chemistry (1972).

2.6. Extraction of DNA from lungs

Extraction of DNA from the animal lung tissues was carried out using the Qiagen DNA extraction kit which uses the spin column method. 25 mg of lung was cut into small pieces, placed in a micro-centrifuge tube and 180 μL of ATL Buffer and 20 μL of proteinase K was added. It was mixed thoroughly by vortexing and incubated at 56 °C overnight until the tissue is completely lysed. After complete lysis of the tissue, 200 μL of ATL buffer was added, mixed thoroughly by vortexing then 200 μL of ethanol (96–100%) was added. The whole mixture (including any precipitate) was pipetted into the DNeasy mini spin column placed in a 2 mL collection tube and centrifuged at 6000 g for 1 min. The flow-through was discarded while the spin column was placed in a new 2 mL collection tube, 500 μL of AW1 buffer was added and centrifuged at 6000g for 1 min. The flow-through was discarded while the spin column was placed in a new 2 mL collection tube, 500 μL of buffer AW2 was added, centrifuged at 20,000g for 3 minutes to dry the DNeasy membrane and flow through was discarded. The DNeasy mini spin column was then placed in a clean 2 mL microcentrifuge tube and 200 μL of buffer AE was pipetted directly onto the DNeasy membrane. It was incubated at room temperature for 1 min and then centrifuged at 6000g for 1 min to elute the DNA. The eluted DNA was quantified using Fisher Scientific nanodrop spectrophotometer and kept at -20 °C until used (Ausubel et al., 2002).

2.7. Electrophoresis of extracted DNA

100 mL of 1% agarose gel was prepared in 1X TAE (Tris Acetate EDTA) buffer, boiled in a microwave to dissolve the agarose, allowed to cool to about 60 °C and 5 μL of ethidium bromide was added and mixed thoroughly. The agarose was then poured into the gel tray after the comb has been fixed in the tray. The agarose was allowed to solidify and the comb was removed to create the wells where the DNA was loaded. 2 μL of loading buffer was added to 18 μL of DNA extracted and this mixture was loaded along with DNA marker into the wells of the gel. After loading, electrophoresis was carried out at 70 Volts for 45 min in 1X TAE buffer and DNA was viewed using UVP gel documentation system (Thompson et al., 2012).

For genetic distance analysis, PyElph was used to convert the gel images into matrices, which were fed into the clustering program of Fingerprint Analysis with Missing Data (FAMD version 1.31). Based on similarity matrices using the unweighted pair group method analysis, FigTree version 1.4.0 was used to generate UPGMA dendrogram.

2.10. Data analysis

Data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0. Data were presented as Mean ± Standard error of mean (SEM). One way Analysis of Variance (ANOVA) was conducted to determine significant difference between parameters. Post hoc test was done using the Student-Newman-Keuls (SNK). P value less than 0.05 (P < 0.05) was considered statistically significant.

3. Results

Table 2 shows the level of AST, ALT and ALP in the blood of the experimental rats exposed to gasoline fume for varying periods of time. Serum concentrations of AST, ALT and ALP in the experimental rats were significantly different (p < 0.05). T1 recorded the lowest concentrations of AST, ALT and ALP compared to T2, T3, T4 and T5. The T5 rats had significantly higher (p < 0.05) level of AST, ALT and ALP compared to T2, T3, T4 and T5. The T5 rats were significantly different (p < 0.05) in T5 compared to other treatments. The liver histopathology of the control and experimental rats are shown in Plates 3-7. Photomicrograph examination of the liver sections of T1 showed a hepatic tissue with normal liver cell architecture. Photomicrograph examination of the liver sections of T2, T3, T4 and T5 revealed increasing level of distorted histoarchitecture,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Showing the programs for PCR analysis.</th>
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<tbody>
<tr>
<td>Stages</td>
<td>EZ</td>
</tr>
<tr>
<td>1</td>
<td>94 °C, 1:00 min</td>
</tr>
<tr>
<td>2</td>
<td>54.6 °C, 2:00 min</td>
</tr>
<tr>
<td>3</td>
<td>72 °C, 2:00 min</td>
</tr>
<tr>
<td>4</td>
<td>Goto 1, 5 °C</td>
</tr>
<tr>
<td>5</td>
<td>94 °C, 0:15 min</td>
</tr>
<tr>
<td>6</td>
<td>59.6 °C, 0:30 min</td>
</tr>
<tr>
<td>7</td>
<td>72 °C, 1:00 min</td>
</tr>
<tr>
<td>8</td>
<td>Goto 5, 45 °C</td>
</tr>
<tr>
<td>9</td>
<td>72 °C, 15:00 min</td>
</tr>
<tr>
<td>10</td>
<td>4 °C, ∞</td>
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swelling/degenerated hepatocytes, degenerated endothelium, chromatolytic hepatocytes and pyknosis, patchy inflammation with remarkable sinusoidal space and large central vein.

The DNA banding profile using EZ and OPA2 primer of the lungs of the rats exposed to varying hours of daily gasoline fume (1 h, 3 h, 5 h, 9 h and 0 h [Control]) for 12 weeks are presented in Fig. 1a and bb respectively. DNA banding pattern using EZ primer (5'-GC ATCACAGACCTGTTATTGCCTC-3') revealed more DNA bands in the control rats than those exposed to the varying hours of gasoline fume (Fig. 1a). The DNA bands ranged from 400 kb and below 100 kb which was the lowest band on the marker lane. The number of DNA bands observed was however lowest in the lungs of rats exposed to 3 h of gasoline fume daily with bands of 100 kb and 300 kb missing. The DNA bands observed in the experimental rats using OPA2 primer (5'-TGCCGAGCTG-3') ranged from 850 kb to 200 kb (Fig. 1b). More number of DNA bands was observed in the lungs of the control rats than those subjected to the varying hours of gasoline fume exposure. However, a similar DNA banding pattern was observed in all the groups of rats subjected to the varying hours of gasoline fume exposures and the bands were observed to show a reducing intensity from the control to the rats exposed to gasoline fumes.

Coefficient of similarity of the DNA bands of the lungs of rats daily exposed to 1 h, 3 h, 5 h, 9 h and 0 h (Control) of gasoline fume is presented in a dendrogram shown in Fig. 2. Compared to the control, the similarity between the DNA bands of the experimental rats was followed by rats exposed to 1 h, 5 and 9 h and 3 h respectively.

### 4. Discussion

The serum levels of ALT, AST and ALP of rats exposed to gasoline fume for varying periods of time in this study were shown to be significantly higher and time-dependent, compared to the control rats. This could be indicative of liver cell damage and injury since these enzymes have been shown by Lin et al. (2000) and Charles (2014) to be released greatly into circulation after cellular damage. In this study, the cellular damage to the liver as signified by the increased serum enzyme activities could be due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon components of the gasoline fumes. Masoud et al. (2015) reported increased in activities of the serum enzymes among gasoline station workers occupationally exposed to benzene, toluene and xylene (BTX) in gasoline as it has also been documented that BTX are the most dangerous elements of gasoline (Adami et al., 2006). This finding confirms Uboh et al. (2009) and Mehdi-Araghi and Ahmadi (2013) who had noted increased in the activities of serum ALT, AST and ALP in rats hourly exposed to gasoline fume due to liver injury.

Histological analysis of the liver tissues of the exposed rats revealed that frequent exposure to gasoline fume affects the structural integrity and architecture of the liver cells. This implies that the liver is one of the major target organs of gasoline fume-induced injury. Uboh et al. (2009) had earlier reported that the cumulative oxidative damage is likely to be one of the underlying mechanisms responsible for the hepatotoxic effects of gasoline fumes, as observed in this study.

Genotoxicity is developed to identify the elements or compounds presents in the environment having the potential to cause mutation by damaging the DNA. RAPD-PCR technique has been shown to be a powerful tool for gene mapping, population, pedigree analysis, phylogenetic studies and strain identification (Grayson et al., 2000). Also, its use in surveying genomic DNA for evidence of various types of damage and mutation suggests that they may potentially form the basis of novel genotoxicological assays for the detection of DNA damage and mutation (Shimada and Shima, 1998). Previous study has shown that changes in band patterns observed in DNA fingerprint analysis reflect DNA alterations from single base changes (point mutations) to complex chromosomal rearrangements (White et al., 1990). DNA adducts and strand breakages with reduced intensity are indicators of genotoxic materials (Landis and Yu, 2003). In this study, DNA damage induced by gasoline fume was reflected by changes in RAPD profiles and disappearance of bands which occurred in the profiles generated by exposed rats to the gasoline fume. The present data showed that the RAPD-PCR method is useful for the screening and characterization of genomic regions that have undergone alterations as the result of gasoline fume exposure. Similar findings have been reported by Castano and Becerril (2004) and Liu et al. (2005) that used RAPD-PCR to analyze the induced DNA damage. The observed reduction in the intensity of the DNA strand of the exposed rats in this work could be adduced to the impact of the hydrocarbon fractions of the gasoline by inhibiting the polymerization of the DNA tag polymerase in the PCR reaction, which led to

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>AST (μ/L)</th>
<th>ALT (μ/L)</th>
<th>ALP (μ/L)</th>
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<tbody>
<tr>
<td>T1</td>
<td>126.07 ± 12.25a</td>
<td>15.47 ± 1.04a</td>
<td>142.83 ± 12.86a</td>
</tr>
<tr>
<td>T2</td>
<td>166.53 ± 3.59a</td>
<td>16.07 ± 1.08b</td>
<td>205.53 ± 8.13a</td>
</tr>
<tr>
<td>T3</td>
<td>187.33 ± 7.92a</td>
<td>18.27 ± 1.33a</td>
<td>205.67 ± 14.84a</td>
</tr>
<tr>
<td>T4</td>
<td>189.43 ± 2.07a</td>
<td>20.63 ± 0.93a</td>
<td>207.50 ± 5.83a</td>
</tr>
<tr>
<td>T5</td>
<td>415.63 ± 188.23a</td>
<td>28.00 ± 3.36a</td>
<td>222.30 ± 22.84a</td>
</tr>
</tbody>
</table>

a Mean values (±Standard deviation) in the same column having the same superscript are not significantly different (p > 0.05).
Fig. 2. UPGMA dendrogram showing clustering of lungs of rats exposed to 1 h, 3 h, 5 h, 9 h and 0 h (Control) of gasoline fume for 12 weeks.

Plate 1. (a) Photomicrograph of T1. H&E X100: Showing normal histoarchitecture and central vein (Red Arrow). (b): Photomicrograph of T1. H&E X400: Showing normal hepatocytes (yellow arrows); sinusoids (blue arrows) and central vein (Red Arrow).

Plate 2. (a) Photomicrograph of liver exposed to gasoline fume for 1 hr (T2). H&E X100: Showing slightly distorted histoarchitecture and dilated central vein (Red Arrow) (b): Photomicrograph of liver exposed to gasoline fume for 1hr (T2). H&E X400: Showing slightly distorted histoarchitecture, swelling/degenerating/hepatocytes and pyknotism (purple arrow) and dilated central vein (Red Arrow).

Plate 3. (a) Photomicrograph of liver exposed to gasoline fume for 3 h (T3). H&E X100: Showing slightly distorted histoarchitecture, degenerating endothelium and dilated sinusoids and central vein (Red Arrow) (b): Photomicrograph of liver exposed to gasoline fume for 3 h (T3). H&E X400: Showing extensive distorted histoarchitecture, shrinkage/degenerating/hepatocytes (yellow arrows), pyknotism (purple arrow) and dilated central vein (Red Arrow).
decrease RAPD band intensity of the exposed rats. Also, band intensity decreasing had been previously reported to be as a result of the loss of some alleles (Weinberg, 1991). However, this finding is in contrary to the report by American Petroleum Institute (1980) who had earlier exposed groups of male mice to diesel fuel vapour (6 h/day, 5 days/week, for 8 weeks) and reported no genotoxicity effects.

5. Conclusion

The results of this work suggested that frequent exposure to gasoline fume may induce hepatotoxicity and genotoxicity, hence impairing the normal liver function and gene structure. This assumes significance and public health concern considering the increasing use of gasoline and consequent exposure to its fumes in Nigeria. Direct exposure to gasoline fume by the populace should be discouraged and frequent and timely medical check-up by the petroleum industries workers should therefore be encouraged to ascertain their health condition. However, more researches are needed on the genotoxic effect of gasoline fumes on animals.

Conflict of interest

Authors declare no competing interest in this research.

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Roles of author

FOO conceived the experiment. FOO and GAD designed the experiments. FOO, JSA and WEO performed the experiment. FOO and AAA analyzed and interpreted the data. FOO drafted the manuscript which was critically revised by GAD, JSA, WEO and AAA and gave final approval. All authors agree to be accountable for integrity and accuracy in all aspects of the work.

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