Exposure To Gasoline And Kerosene Vapours: A Risk Factor For Nephrotoxicity In Rats

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Abstract
This study assessed the nephrotoxic effect associated with exposure to gasoline and kerosene vapours in rats, using the levels of urea, creatinine, uric acid, BUN, glucose, Na$^+$ and Cl$^-$ in the serum. The rats were wholly exposed to vapours generated from direct evaporation of liquid kerosene and unleaded premium motor spirit (PMS) blend of gasoline at the levels of 20.7±5.8cm$^3$ hr$^{-1}$ m$^{-3}$ day$^{-1}$, 6hr/day for 20 weeks. The results showed that the levels of serum urea, creatinine, uric acid, BUN, glucose and K$^+$ were significantly higher, while Na$^+$ and Cl$^-$ were significantly lower (p<.05) among and within the rats exposed to gasoline and kerosene vapours, compared to the control. However the percentage increase in serum urea, creatinine, uric acid, BUN, glucose and K$^+$, as well as percentage decrease in serum Na$^+$ and Cl$^-$ obtained for rats exposed to kerosene vapours were observed to be significantly higher (p<.05) compared to those obtained for rats exposed to gasoline vapours. The observations made in this study define azotaemia, hyperkalaemia, hyponatraemia and hypochloraemia, indications of nephrotoxicity and renal function impairment, to be associated with exposure to gasoline and kerosene vapours in rats, and that exposure to kerosene vapour produces a more nephrotoxic effect than gasoline vapour.

INTRODUCTION
Petroleum, either in its crude or refined form, is one of the most widely utilized natural resources in most oil-producing economies. It forms the mainstay of the economy and the major determinant of national finance and industry in such Societies. The oil exploration, production and utilization activities have brought with them an alarming increase in industrial activities, which have contributed immensely to the unnecessary disruption of the natural ecological setting of the oil producing areas. This implies that despite the financial benefits accruing from oil exploration, production and utilization activities, they have serious attendant environmental consequences. One of such attending environmental consequences is the pollution of the surrounding environment. The intensity of these consequences vary with the quantity and fractions of the petroleum products released into the environment.

Crude petroleum may be distilled into such fractions as gasoline, kerosene, diesel, heavy gas oils, lubricating oils, as well as residual and heavy fuels among others [1]. Diesel, gasoline and kerosene are among the commonly used fractionated products of crude petroleum. These fractions contain aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons at variable proportions [1-4]. According to EHC20 [1], gasoline, kerosene and diesel contain predominantly, hydrocarbons with carbon atoms 4-10, 11-13 and 14-18, respectively. The volatility of these fractions vary with the predominant hydrocarbon species. Unleaded gasoline for instance, is reported to contain about 300 different hydrocarbon species, most of which are highly volatile and may evaporate if left exposed, to constitute ubiquitous chemical pollutants in the environment [5]. In the course of day to day activities, a greater percentage of the populace are directly or indirectly exposed to pollutants of petroleum origin in their environments. However, those that are occupationally exposed tend to be at a greater risk of exposure [6,7].

Moreover, it has been documented that exposure of rat to gasoline exhaust and organic extracts of the exhaust particulate caused a dose- and time- dependent increase in oxygenases and glutathione-s-transferase in the liver, kidney and lung microsomes; as well as pulmonary dysfunction and parenchymal damage among dogs [8,9]. Other adverse effects associated with exposure to petroleum vapours have been reported in both the experimental animals and humans [6,10,11]. In our previous studies, adverse effects of exposure to gasoline vapour on haematological indices, weight changes; liver and reproductive functions in rats were
Observed and reported [12 - 15].

Like other known xenobiotics, the chemical pollutants from gasoline vapours may be metabolically transformed into various metabolites in the body [16]. Some of these metabolites may be very reactive, interacting in various ways with the metabolizing, transporting and excreting tissues to elicit toxic effects [17]. The interaction of these metabolites with the renal tissues may cause cellular injury, hence, damage to the tissues. Once the renal tissues are damaged, the overall functionality of the kidneys may be compromised.

The kidney functions may be assessed from the level of some electrolytes (such as K\(^+\), Na\(^+\), Cl\(^-\)) and metabolites (such as creatinine, urea and blood urea nitrogen) in the plasma [18 - 20]. Renal dysfunction may be caused by several diseased conditions and exposure to certain reactive or toxic metabolites [20-22]. Renal dysfunction of any kind affects all parts of the nephron to some extent, although sometimes, either glomerular or tubular dysfunction is predominant. The net effect of renal disease on plasma and urine depends on the proportion of glomeruli or tubules affected, and on the number of nephrons involved. In this study, comparative changes in some renal function indices associated with exposure of male rats to gasoline and kerosene vapours were assessed.

**MATERIALS AND METHODS**

**ANIMALS AND ANIMAL HANDLING**

Twenty one Wistar albino rats weighing 180-200g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar, Nigeria and used for this study. The animals were allowed one week of acclimatization to laboratory conditions and handling, after which they were distributed, according to weight into three groups as outlined in Table 1. The animals were housed individually in cages with plastic bottom and wire mesh top (North Kent Co. Ltd) and fed with normal rat chow (Guinea Feeds Product) purchased from the High Quality Livestock Feeds stores, Calabar, Nigeria. They were supplied with tap water ad libitum throughout the experimental period. The control group (Group I) was maintained in the animal room adequately ventilated under standard conditions (ambient temperature, 28\(^\circ\)C, and relative humidity, 46%, with a light/dark cycle of 12/12h). The test groups (Groups II and III) were kept in the exposure chambers (vapours cupboards) previously saturated respectively with premium motor spirit (PMS) blend of gasoline and kerosene vapours. The liquid gasoline (PMS blend) and kerosene were obtained from the Mobil Refueling station, Marian Road, Calabar, Nigeria.

All animal experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

**EXPOSURE TO GASOLINE VAPOURS**

A modified nose-inhalation exposure method previously described [12,14, 23], was used in this study. According to this modification, the cages housing the animals in the test groups were placed in respective exposure chambers (2 cages per one chamber) of 2.835 m\(^3\), each with two open calibrated beakers of 1000cm\(^3\) containing 500cm\(^3\) of liquid gasoline and kerosene, respectively. The gasoline and kerosene were allowed to evaporate freely within the respective exposure chambers at ambient humidity and temperature, and all animals in cages were exposed to vapours (20.7±5.8cm\(^3\) hr\(^-1\) Kg\(^-1\) m\(^-3\) day\(^-1\)) generated from direct evaporation of the liquid gasoline and kerosene. The animals were exposed to the vapours, 6h/day (9.00a.m to 3.00p.m) for 20 weeks. At the end of each exposure day, the animals were transferred to gasoline vapours-free section of the experimental animal house.

During the exposure period, the initial and final volumes of liquid gasoline and kerosene were respectively recorded before and after daily exposure. The daily differences in volume were used to estimate relative concentrations of vapours used in this exposure method.

**COLLECTION AND HANDLING OF BLOOD SERUM FOR ANALYSES**

Twenty-four hours after last exposure, the animals were anaesthetized with chloroform vapour and dissected. Whole blood from each animal was collected by cardiac puncture into well-labelled non-heparinized sample tubes and allowed to clot for 3 hrs in iced water. The serum was separated from the clots after centrifuging at 10,000 rpm for 5 min into well-labelled plain sample bottles, and used for assays.

**BIOCHEMICAL ASSAYS**

Serum Urea and Blood urea nitrogen: Urea in serum was estimated by the end point colorimetric method using Dialab reagent kits [24]. In this method, urease enzyme hydrolyses
urea to ammonia and carbon dioxide. The ammonia so formed reacts with alkaline hypochloride and sodium salicylate in the presence of sodium nitroprusside to form a coloured chromophore whose absorbance was measured with DREL 3000 HACH (England) model spectrophotometer.

Serum Creatinine: The concentration of serum creatinine was assayed based on the reaction of creatinine with an alkaline solution of sodium picrate to form a red complex [25]. The red coloured complex which is proportional to the concentration of creatinine in the sample was measured spectrophotometrically.

Serum Glucose: Serum glucose level was estimated, using Dialab reagent kits, by the principle of glucose oxidase reaction [26]. In this principle, glucose oxidase oxidizes glucose to gluconic acid, and hydrogen peroxide, formed as a byproduct. The peroxides whose concentration is in proportion to glucose in sample develops quantifiable colour via 4-aminophenazone in the presence of a peroxidase.

Serum Potassium: Potassium in serum was determined by photometric turbidimetric test, using TECO analytical reagent kits [27]. Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium tetraphenylboron, whose turbidity is in proportion to the potassium concentration originally in the sample.

Serum Sodium: Serum sodium concentration was estimated using Mg-uranylacetate reaction method described in Dialab diagnostic kits [28]. Sodium in serum is precipitated with Mg-uranylacetate, the remaining uranyl ions form a yellow-brown complex with thioglycolic acid. The difference between reagent blank analysis is proportional to the sodium chloride.

Serum Chloride: Chloride in serum was determined using mercuric thiocyanate reaction method described in Dialab diagnostic kits (27). Chloride ions in the sample react with mercuric thiocyanate displacing the thiocyanate ions. The displaced thiocyanate ions react with ferric ions producing a coloured complex.

**STATISTICAL ANALYSIS**

All data are expressed as mean±SEM. The results were analyzed by one-way analysis of variance (ANOVA), followed by pair wise comparison between test and control groups using Student’s t-test. Differences between control and respective test groups were considered significant at P<0.05.

**RESULTS**

The results of this study on the effect of gasoline and kerosene vapours on some serum renal function indices in male rats are shown in Tables 1 and 2.

The results showed that the levels of serum urea, BUN, uric acid and creatinine increased significantly (P<0.05) following exposure to gasoline and kerosene vapours (Table 1). However, the increase in the level of these parameters were observed to be specific-fraction vapour-dependent. The levels of serum urea, BUN, uric acid and creatinine obtained for the group exposed to kerosene vapour were significantly higher (P<0.05) compared to the levels obtained for the group exposed to gasoline vapour (Table 1). Hence the percentage increase in the levels of serum urea, BUN, uric acid and creatinine following expose to kerosene vapour (36.5±1.4, 36.5±0.7, 36.9±0.07 and 93.2±0.04 percents respectively) were observed to be significantly higher (P<0.05) compared to the percentage increase in the level of the respective indices following exposure to gasoline vapour (22.0±2.3, 22.8±1.2, 20.2±0.08 and 69.5±0.05 percents respectively).

**Table 1. Effect of Gasoline and Kerosene vapours on the levels of some serum catabolites commonly used in the assessment of renal functions.**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Urea (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Uric (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>42.1±1.26</td>
<td>19.6±0.59</td>
<td>19.9±0.08</td>
<td>1.1±0.03</td>
</tr>
<tr>
<td>II</td>
<td>51.7±3.00</td>
<td>26.1±1.78</td>
<td>3.3±0.06</td>
<td>3.0±0.05</td>
</tr>
<tr>
<td>III</td>
<td>57.4±1.54</td>
<td>36.8±0.72</td>
<td>5.7±0.05</td>
<td>5.2±0.04</td>
</tr>
</tbody>
</table>

Value are presented as means ± SD; n=7; *P<0.05 compared with group I; **P<0.05 compared with group II. Group I = Control; Group II = Group exposed to Gasoline vapours; Group III = Group exposed to kerosene vapours.

Also the serum glucose and potassium levels were observed to increase, while the levels of sodium and chloride ions were observed to decrease significantly (P<0.05) within and among the rats in the groups exposed to gasoline and kerosene vapours, compared with the control group. The results also indicated that the increase in the levels of serum glucose and potassium ion, as well as the decrease in the levels of serum sodium and chloride ions varied with the specific vapour fraction (Table 2). The levels of serum glucose and potassium ion obtained for the group exposed to kerosene vapour were however significantly higher (P<0.05).
compared respectively with the levels obtained for the group exposed to gasoline vapour. On the other hand, the levels of serum sodium and chloride ions obtained for rats exposed to kerosene vapour were observed to be significantly lower (P<0.05) compared to the levels obtained for the rats exposed to gasoline vapour. Also, the percentage increase in serum glucose and potassium ion (73.5\%/3.3 and 43.8\%/0.2 percents respectively) and percentage decrease in serum sodium and chloride ions (29.2\%/3.9 and 6.0\%/0.9 percents respectively) obtained for rats exposed to kerosene vapours were observed to be significantly higher (P<0.05) compared with the percentage increase in serum glucose and potassium ion (59.7\%/3.7 and 23.0\%/0.1 percents respectively), and percentage decrease in serum sodium and chloride ions (22.9\%/3.7 and 3.0\%/0.6 percents respectively) obtained for rats exposed to gasoline vapour.

Figure 3

Table 2. Effect of Gasoline and kerosene vapours on the levels of serum Glucose and some Electrolytes commonly used in the assessment of renal functions.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Glucose (mg/dl)</th>
<th>Na+ (mEq/L)</th>
<th>K+ (mEq/L)</th>
<th>Cl- (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>69.03±3.95</td>
<td>119.22±7.61</td>
<td>4.61±0.16</td>
<td>106.07±0.75</td>
</tr>
<tr>
<td>II</td>
<td>110.24±3.36</td>
<td>91.85±0.65</td>
<td>5.67±0.09</td>
<td>97.10±0.40</td>
</tr>
<tr>
<td>III</td>
<td>159.77±2.56</td>
<td>84.37±1.06</td>
<td>7.38±0.22</td>
<td>98.54±0.97</td>
</tr>
</tbody>
</table>

Values are presented as means ± SD; n=7; *P<0.05 compared with group I; †P<0.05 compared with group II; Group abbreviations as in Table I.

The observations made from the results of this study indicate that the nephrotoxic effect associated with kerosene vapour is significantly higher (P<0.05) compared to the nephrotoxic effect associated with gasoline vapours in male rats.

DISCUSSION

The kidney participates in the maintenance of the constant extracellular environment that is required for adequate functioning of the cells. This is achieved by excretion of waste products of metabolism (such as urea, creatinine and uric acid) and by specifically adjusting the urinary excretion of water and electrolytes to match the net intake and endogenous production. Alteration from the normal levels of these waste products and electrolytes in blood, which may be caused by several factors, is an indication of renal impairment [18,29,30].

Subjects with kidney dysfunction may have a variety of different clinical presentations. Some of these presentations may be asymptomatic, only detected on routine laboratory examinations from abnormal serum catabolites (such as creatinine, urea, uric acid, blood urea nitrogen) and electrolytes (such as Na+, K+, Cl-, HCO3-, etc). One of the clinical manifestations of the renal disorders is azotaemia, a biochemical abnormality characterized by elevation of serum urea, NUN and creatinine levels [31]. A persistently elevated serum creatinine is reported to be a risk factor for progression of chronic kidney disease to kidney failure [32, 33].

In this study, exposure to gasoline and kerosene vapours is reported to be among the predisposing factors to the impairment of kidney function in rats. From the result of this study, increase in the level of serum creatinine, urea, blood urea nitrogen, uric acid, glucose and potassium ion, as well as decrease in the levels of serum sodium and chloride ions are reported in rats following exposure to gasoline and kerosene vapours. These results indicate that the absorbed constituents of these vapours and / or their metabolites might have reacted and interacted with the renal tissues to impair the kidney functions. However, the degree of alteration in the levels of the assayed renal function indices was observed to be higher in the rats exposed to kerosene than those exposed to gasoline vapours. This gives an indication that the chemical constituents of kerosene vapours and their metabolites are more nephrotoxic than those from gasoline vapours. This agrees with our earlier report that kerosene fumes are more haematotoxic and hepatotoxic than gasoline fumes[12,13].

According to Ernest et al. [34], the development and standardization of biotest protocols has enabled environmental regulators and managers to make better environmental decisions and generate environmental regulations and guidelines for the protection of ecosystems and human health. The measurements of risk of toxic substance to the environment and human health as obtained in this study may be useful for the environmental resource managers to determine the levels of remediation operations required for the protection of the environment. A toxicity study is very important because exposure of humans and other species to so-called “environmental” levels of hazardous compounds may perturb certain fundamental pathways of intermediary metabolism in ways that may lead to cumulative toxicity [35].

In conclusion, the results obtained for this present study confirm that exposure to gasoline and kerosene vapours is also a predisposing factor to renal function impairment in rats; and that kerosene vapour is more nephrotoxic than gasoline vapour. The observations made here may have
important implication(s) on the effects of chronic exposure to kerosene and gasoline vapours exposure on human health.

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