Journal of Toxicology and Environmental Health Sciences Vol. 1(4), pp. 068-074, December 2009
Available online at http://www.academicjournals.org/JTEHS
ISSN 2006-9820 © 2009 Academic Journals

Full Length Research Paper

Comparative nephrotoxic effect associated with exposure to diesel and gasoline vapours in rats

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Accepted 1 December, 2009

Comparative effect of exposure to 20.7 ± 5.8 cm^3 $h^{-1}kg^{-1}m^{-3}$ day^{-1} of diesel and gasoline vapours on the kidney functions was assessed in rats. It was observed that exposure to diesel and gasoline vapours produced a significant increase ($P < 0.05$) in serum creatinine, urea, BUN, uric acid, glucose and K^+; and a significant decrease ($P < 0.05$) in serum Na^+ and Cl^- levels. However, the percentage increase in serum creatinine, urea, BUN, uric acid, glucose, K^+; and decrease in serum Na^+ and Cl^- levels recorded for the rats exposed to diesel vapour were significantly higher ($P < 0.05$) compared to the percentages recorded for rats exposed to gasoline vapour. The result of this study indicates that exposure to diesel and gasoline vapours may be a risk factor for nephrotoxicity in rats; and that diesel vapour tends to contain chemical substance(s) that are more nephrotoxic than gasoline vapour.

Key words: Diesel, gasoline, creatinine, urea, electrolytes, nephrotoxicity.

INTRODUCTION

Domestic and industrial use of petroleum, either in its crude or refined forms, has increased tremendously in recent times. Crude petroleum may be refined into such fractions as gasoline, kerosene, diesel, heavy gas oils, lubricating oils, as well as residual and heavy fuels among others (EHC 20, 1982). 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 concentrations (EHC 20, 1982; Henderson et al., 1993; Kato et al., 1996; Anderson et al., 1995). The constituents of the vapours from these fractions, to a greater extent, depend on the composition of their liquid forms, which varies with the brand and storage period.

The blend of unleaded gasoline (UG) designated PS-6, API-0I UG and the methyl tertiary butyl ether (MBTE) blended gasoline are among the brands of gasoline commonly used in the United States (Moser et al., 1996). Gasoline, kerosene and diesel are reported to contain predominantly, hydrocarbons with carbon atoms 4 - 10, 11 - 13 and 14 - 18, respectively (EHC 20, 1982). The volatility of these fractions varies 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 (Zahlsen and Tri-Tugaswati, 1993). Reports also indicate that API 91-0I UG contains slightly higher percentage of saturated hydrocarbons than PS-6 blend, and that an estimate of 25% or more of the gasoline supplied in the United States in 1995 were supplemented or blended with MBTE (Lorenzetti, 1994). In the course of usage of these products, and other day to day activities, individuals are frequently 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 (Smith et al., 1993; Carballo et al., 1995). Human health hazards arising from intermittent, low-dose exposure to petroleum vapours are not quite consistent. The potential harmful effects associated with chronic or sub-chronic inhalation of the petroleum pollutants in the atmosphere constitute the concern of the general public and the scientific community. To identify the potential health risk of chronic exposure to UG, it is

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Table 1. Distribution of experimental groups.

Group	No. of rats	Treatment
Control (I)	5	Vapours-free
Gasoline (II)	5	Exposed to Gasoline vapour
Diesel (III)	5	Exposed to Diesel vapours

reported that American Petroleum Institute sponsored a cancer bioassay, in which B6C3F1 mice and F-344 rats were exposed to UG vapour for 6 hrs/day, 5 day/week for 2 years. The results indicated that the carcinogenic effects detected were the induction of male rat kidney tumours and female mouse liver tumours. The kidney tumours were believed to result from the interaction of the metabolites of certain isoparaffinic components of UG with a male rat-specific renal protein, µ-globulin (Chun et al., 1992; Hard et al., 1993). The accumulation of this protein in proximal tubule cells may lead to cytolethality, regenerative cell proliferation and ultimately, renal cancer (Borghoff et al., 1992). UG vapour is also reported to stimulate the growth of diethyl nitrosamine-induced hepatic preneoplastic lesions in mice, and induce an enzyme activity associated with cytochrome $P_{450}2B$ (Standeven et al., 1993; Standeven et al., 1994; Standeven et al., 1993). These reports indicate that mice are more vulnerable to the toxicity effects associated with gasoline vapours inhalation than rats, and that the male rats are affected than the females when exposed to gasoline vapours. In our previous studies, adverse effects of exposure to gasoline and kerosene fumes/vapours on haematological indices, weight changes, liver and reproductive functions in rats were observed and reported (Uboh et al., 2005a, b; Uboh et al., 2007a, b; Uboh et al., 2008a, b). The chemical pollutants from gasoline vapours, like other known xenobiotics, may be metabolically transformed into various metabolites in the body (Hu and Wells, 1994). Some of these metabolites may be very reactive, interacting in various ways with the metabolizing and excreting tissues (mainly the liver and kidneys) to elicit toxic effects (Page and Mehlman, 1989; Nygren et al., 1994). 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 (Nwankwo et al., 2006; Atangwho et al., 2007; Crook, 2007). Renal dysfunction may be caused by several diseased conditions and exposure to certain reactive or toxic metabolites (Crook, 2007; Chatterjea and Shinde, 2002; Jimoh and Odutuga, 2004). 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 to tubules affected, and on the number of nephrons involved. In this study, comparative changes in some renal function indices, nephrotoxicity, hence associated with exposure of male rats to gasoline and diesel vapours were assessed.

MATERIALS AND METHODS

Animals and animal handling

Fifteen male Wistar albino rats weighing 180 - 200 g 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 ± 2°C and relative humidity, 46% with a light/dark cycle of 12/12 h). 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 diesel vapours. The liquid gasoline (PMS blend) and diesel 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 and diesel vapours

A modified nose-inhalation exposure method previously described (Uboh et al., 2005a, b; Uboh et al., 2007a, b;), 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 1000 cm^3 containing 500 cm^3 of liquid gasoline and diesel, respectively. The gasoline and diesel 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 ± 5.8 cm^3 h^{-1} $Kg^{-1}m^{-3}$ day^{-1}) generated from direct evaporation of the liquid gasoline and diesel. The animals were exposed 6 h/day (9.00 a.m - 3.00 p.m), 6 day/week, to vapours for 64 days. At the end of each exposure day, the animals were transferred to gasoline and diesel vapours-free section of the experimental animal house.

During the exposure period, the initial and final volumes of liquid gasoline and diesel 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.

Table 2. Comparative effect of diesel and gasoline vapours on the levels of some serum catabolites commonly used in the assessment of renal functions.

Group	Urea (mg/dl)	BUN (mg/dl)	Uric (mg/dl)	Creatinine (mg/dl)
I	42.10 ± 1.26	19.66 ± 0.59	1.98 ± 0.08	1.18 ± 0.03
II	51.72 ± 3.40*	24.15 ± 1.78*	2.38 ± 0.08*	2.00 ± 0.06*
III	72.96 ± 1.86*+	34.07 ± 0.87*+	3.73 ± 0.17*+	3.17 ± 0.24*+

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

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 h 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 endpoint colorimetric method using Dialab reagent kits (Searcy et al., 1967). 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 which 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 pirate to form a red complex (Newman and Price, 1999). 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 (Barham and Trinder, 1972). 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 turbidemetric test using TECO analytical reagent kits (Tietz, 1976). Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium tetraphenyboron, 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 (Trinder, 1957). 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 analyses is proportional to the sodium chloride.

Serum chloride

Chloride in serum was determined using mercuric thiocyanate reaction method described in Dialab diagnostic kits (Tietz, 1976). 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 (that is, standard error of the mean).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 groups were considered significant at $p < 0.05$.

RESULTS

Changes in the levels of serum creatinine, urea, blood urea nitrogen (BUN), uric acid, glucose, as well as sodium, potassium and chloride ions (that is Na^+, K^+ and Cl^- respectively) were used to assess the renal function impairment effect of gasoline and diesel vapours in rats. The results of this study are shown in Tables 2 and 3 as well as Figures 1 and 2.

The results showed that the levels of serum creatinine, urea, BUN and uric acid increased significantly ($P < 0.05$) within and among the groups of rats exposed to gasoline and diesel vapours, compared with the rats in the control group (Table 1). However, the levels of serum creatinine, urea, BUN and uric acid obtained for the group of rats exposed to diesel vapour (3.17 ± 0.24, 72.96 ± 1.86, 34.07 ± 0.87 and 3.73 ± 0.17mg/dl respectively) were observed to be significantly higher ($P < 0.05$) compared to the levels obtained for the group of rats exposed to

Table 3. Comparative effect of diesel and gasoline vapours on the levels of serum glucose and some electrolytes commonly used in the assessment of renal functions.

Group	Glucose (mg/dl)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)
I	69.01 ± 3.95	119.20 ± 6.71	4.61 ± 0.16	100.76 ± 0.75
II	110.24 ± 3.36*	91.85 ± 0.65*	5.67 ± 0.09*	97.71 ± 0.40*
III	126.10 ± 5.13*+	74.67 ± 3.53*+	8.03 ± 0.15*+	89.90 ± 1.62*+

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.

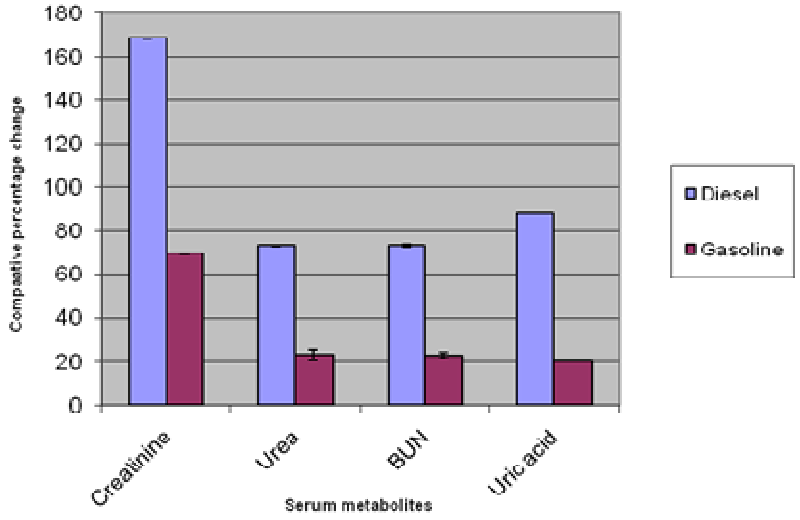

Figure 1. Comparative percentage increase in some serum renal function assessment metabolites in rats exposed to diesel and gasoline vapours.

gasoline vapour (2.00 ± 0.06, 51.72 ± 3.40, 24.15 ± 1.78 and 2.38 ± 0.08 mg/dl respectively). Consequently, the percentage increase in the levels of serum creatinine, urea, BUN and uric acid resulting from exposure to diesel vapour (168.6 ± 0.14, 73.3 ± 1.56, 73.3 ± 0.73, 88.4 ± 0.13% respectively) were significantly higher ($P < 0.05$) compared respectively with the percentage increase resulting from exposure to gasoline vapour (69.5 ± 0.05, 22.9 ± 2.30, 22.8 ± 1.20 and 20.2 ± 0.08% respectively) (Figure 1).

Moreover, the levels of serum glucose and K^+ were increased, while the levels of Na^+ and Cl^- were decreased significantly ($P < 0.05$) sequel to exposure to gasoline and diesel vapours, in comparison with the levels obtained for the control group (Table 2). The results showed that the levels of serum glucose and K^+ for the rats exposed to diesel vapour (126.1 0 ± 5.13 mg/dl and 8.03 ± 0.15 mEq/l respectively) were significantly higher ($P < 0.05$) compared with the respective levels obtained for the rats in the group exposed to gasoline vapour (110.24 ± 3.36 mg/dl and 5.67 ± 0.09 mEq/l respectively); while the levels of serum Na^+ and Cl^- obtained for the group exposed to diesel vapour (74.67 ± 3.53 and 89.90 ± 1.62 mEq/l respectively) were significantly lower ($P < 0.05$) compared with the respective levels for the group exposed to gasoline vapour (91.85 ± 0.65 and 97.71 ± 0.40 mEq/l respectively). On the basis of this, the percentage increase in the levels of glucose and K^+ and percentage decrease in the levels of serum Na^+ and Cl^- for the rats exposed to diesel vapour were observed to be significantly different ($P < 0.05$) from the respective percentage changes obtained for the rats exposed to gasoline vapour, as illustrated in Figure 2.

The results obtained from this study indicated that exposure to diesel and gasoline vapours may cause impairment of the renal functions, with diesel vapour being more adverse than gasoline. The impairment of the renal function reported in this study implies that diesel vapour contains more nephrotoxic constituents than gasoline vapour.

DISCUSSION

The kidney maintains constant extracellular environment by its involvement in the excretion of such catabolites as urea, creatinine and uric acid; and regulation of water and

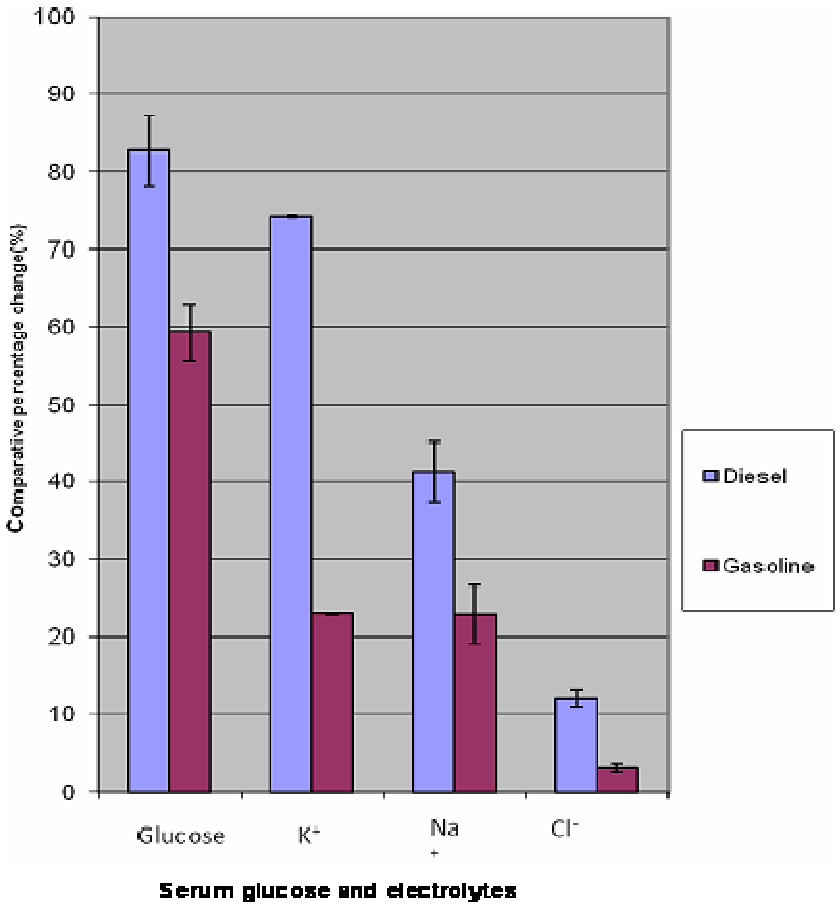

Figure 2. Comparative percentage changes in serum glucose and some electrolytes level in rats exposed to diesel and gasoline vapours.

electrolyte balance. Abnormal concentration of these catabolites and some electrolytes in the plasma or serum is a clear indication of renal function impairment (Nwankwo et al., 2006; Crook, 2007; Gidado et al., 2001; Zanna et al., 2008). Impairment of the renal functions may be caused by exposure to different nephrotoxic substances, in addition to certain diseased conditions. For instance, exposure to lead from automobile exhaust is reported to be a risk factor for nephrotoxicity among traffic policemen (Mortada et al., 2001).

Renal function impairment manifests in a variety of different clinical presentations, some of which may be asymptomatic. The renal function impairment with asymptomatic presentations can only be detected by routine laboratory examinations. Azotaemia, a clinical condition associated with renal function impairment, is one of such presentations that can rightly be detected by laboratory findings. The condition is characterized by elevated levels of serum creatinine, urea and BUN (Cotran et al., 1999). A persistently increased serum creatinine is reported to be one of the risk factors for chronic kidney disease, which may results in renal failure (Mortada et al., 2001; Appel et al., 2003).

In this study, elevated levels of serum creatinine, urea, BUN, uric acid, glucose, and K^+, as well as decreased levels of serum Na^+ and Cl^- are reported for rats exposed to diesel and gasoline vapours. However, the derangement in the levels of these serum parameters recorded for rats exposed to diesel vapour was observed to be greater than that recorded for rats exposed to gasoline vapours.

The results of this study showed that exposure to diesel and gasoline vapours may be a predisposing factor for renal functions impairment in rats. This result agrees with our recent routine laboratory findings that exposure to gasoline vapour may cause elevation of serum urea, BUN and creatinine; an indication of renal function impairment in rats. In our previous studies, we have also observed that exposure to gasoline and/or kerosene vapours causes hepatotoxicity in rats (Uboh et al., 2005a, b; 2007a, b; 2008a, b).

The nephrotoxicity observed in this study suggests the presence of some nephrotoxic chemical substances in diesel and gasoline vapours. For instance Mortada et al. (2001) reported the presence of lead in automobile exhaust as a risk for nephrototoxicity among traffic

policemen. While Halder et al. (1985) reported that lead is the component of gasoline responsible for nephrotoxicity observed to be associated with exposure to leaded gasoline. However, the specific chemical constituent(s) and mechanism(s) responsible for the nephrotoxic effect reported in the study is not very clear; but it is believed that the reactive metabolites of the hydrocarbons and other constituents of the vapours must have interacted with the renal tissues to cause derangements in glomerular function. Further study to elucidate the specific nephrotoxic constituent(s) and mechanism(s) responsible for nephrotoxicity reported in this study is in progress.

In conclusion, the result of this study indicates that diesel and gasoline vapours contain chemical constituents whose metabolites may interact with the renal tissues to impair renal functions in rats; and that the nephrotoxicity risk, as judged by changes in serum glucose and electrolytes, associated with diesel vapour is greater than that associated with gasoline vapour. Hence stringent regulation of the amount of the liquid and vapour forms of diesel and gasoline released inadvertently into the environments is highly recommended to the various environmental protection agencies.

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