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## Comparative Effect of Gasoline Vapours on Renal Functions in Male and Female Albino Wistar Rats

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**Abstract:** The effect of gasoline vapours ( $17.8 \pm 2.6 \text{ cm}^3/\text{h}/\text{m}^3/\text{day}$ ) on renal functions was assessed from the total kidney weights and the levels of serum creatinine, urea and blood urea nitrogen (BUN) in male and female rats, following 64 days of exposure. The results showed an insignificant ( $p > 0.05$ ) increase in percentage kidney weight per total body weight (PKW/BW), decrease in total serum protein and a significant increase ( $p < 0.05$ ) in serum creatinine, urea and BUN levels in both male and female test rats, compared respectively with the control. However, the percentage changes in the PKW/BW, serum creatinine and urea levels obtained for female rats were observed to be significantly higher ( $p < 0.05$ ), compared to the respective percentage changes obtained for male rats. This observation indicates that frequent exposure to gasoline vapours may cause renal dysfunction in rats, with females at greater risk.

**Key words:** Gasoline vapours, creatinine, urea, BUN, kidney weights

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### INTRODUCTION

Gasoline is one of the fractionated products of crude oil. The indispensability of this product in our daily life cannot be over emphasized. It is widely used as fuels for automobiles and some electricity generating machines. Liquid gasoline is known to be very volatile, with several organic and inorganic constituents. Unleaded gasoline for instance, is reported to contain about 300 different hydrocarbon fractions, most of which are volatile and may evaporate if left exposed, to constitute ubiquitous chemical pollutants in the immediate environment (Zahlsen and Tri-Tugaswati, 1993). A greater percentage of the automobile users and those residing at/and around refueling stations and traffic-congested areas may directly or indirectly be exposed to these pollutants 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).

Most often, much consideration is not given to possible health hazards that might be associated with exposure to the constituents of gasoline vapours released into the environment. It has been reported that a higher concentration of unsaturated aromatic hydrocarbons and a lower concentration of the saturated fractions accumulate in the blood of humans and animals equally exposed to petroleum vapour (Zahlsen *et al.*, 1993). Based on this report, the potential harmful effects associated with chronic or sub-chronic exposure to gasoline vapour should be the concern of the general public and the scientific community.

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 (Ueng *et al.*, 1998; Lewis *et al.*, 1974). Other adverse effects associated with exposure to petroleum vapours have been reported in both the experimental animals and humans (Wixton and Brown, 1992; Smith *et al.*, 1993; Tilbury *et al.*, 1993). In the earlier studies, adverse effects of exposure to gasoline vapour on haematological indices, weight changes, liver and reproductive functions in rats were observed and reported by Uboh *et al.* (2005a, b, 2007a, b).

Like other known xenobiotics, the chemical pollutants from gasoline vapours 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 (Macfarland *et al.*, 1984; 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<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>) 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 (Chatterjea and Shinde, 2002; Jimoh and Odutuga, 2004; Crook, 2007). 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 associated with exposure of male and female rats to gasoline vapours were assessed.

## MATERIALS AND METHODS

### Animals and Animal Handling

Twenty to eighty Wistar albino rats [fourteen males and fourteen females] 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 which lasted from March to May, 2008. The animals were allowed one week of acclimatization to laboratory conditions and handling, after which they were distributed, according to weight and gender, to 4 groups (two male and two female groups) (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 groups (Mc and Fc) were 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 (Mt and Ft) were kept in the exposure chamber (Vapours cupboard) previously saturated with Premium Motor Spirit (PMS) blend of gasoline vapours. The liquid gasoline (PMS blend) was 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.

Table 1: Distribution of experimental groups

Groups	No. of rats	Treatment
Male control (Mc)	7	Vapours-free
Female control (Fc)	7	Vapours-free
Male test (Mt)	7	Exposed to vapours
Female test (Ft)	7	Exposed to vapours

### **Exposure to Gasoline Vapours**

A modified nose-inhalation exposure method earlier described by Uboh *et al.* (2005a, 2007a, 2008), was used in this study. According to this modification, the cages housing the animals in the test groups were placed in exposure chambers (2 cages per one chamber) of 2.835 m<sup>3</sup>, each with two open calibrated beakers of 1000 cm<sup>3</sup> containing 500 cm<sup>3</sup> of liquid gasoline. The gasoline was allowed to evaporate freely within the exposure chambers at ambient humidity and temperature and all animals in cages were exposed to vapours (17.8±2.6 cm<sup>3</sup>/h/kg/m<sup>3</sup>/day) generated from direct evaporation of the liquid gasoline. The animals were exposed 6 h/day (9:00 am to 3:00 pm) to vapours for 64 days. At the end of each exposure day, the animal were transferred to gasoline vapours-free section of the experimental animal house.

During the exposure period, the initial and final volumes of liquid gasoline 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 Analysis**

Twenty-four hours after last exposure, the animals were anaesthetized in 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 Dialab Kits Endpoint colorimetric method, based on the principles of 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 assay is based on the reaction of creatinine with an alkaline solution of sodium picrate to form a red complex (Newman and Price, 1999). The red colour is proportional to the concentration of creatinine in the sample.

#### **Serum Protein**

The concentration of protein in the serum was measured spectrophotometrically by Biuret method, using Randox reagent kits (Randox Laboratory Ltd., Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

### **Statistical Analysis**

All data are expressed as Mean±SEM. The results were analysed 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**

The final total body weight and percentage weight increase of male and female rats exposed to gasoline vapours were observed to be significantly lower ( $p < 0.05$ ) compared respectively to the total body weight and percentage weight increase of male and female rats in the control group. The whole kidney weight of both male and female rats exposed to gasoline vapours were observed to be

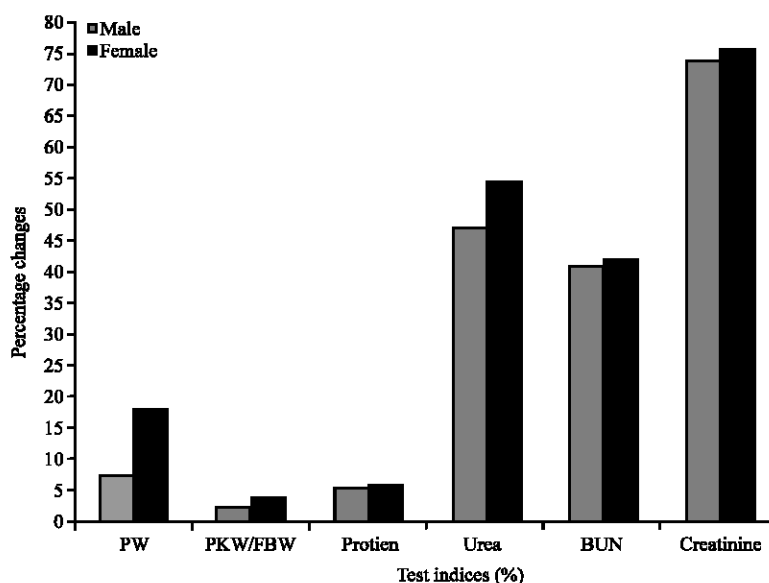


Fig. 1: Gender differences in the effect of gasoline vapours on percentage changes in the levels of the test indices

Table 2: Effect of gasoline vapours on total body and kidney weight of Wistar rats

Groups	IBW (g)	FBW (g)	PWI (%)	KW (g)	PKW/FBW (%)
I (M <sub>c</sub> )	124.83±7.670	243.83±17.45	48.80±6.34	1.21±0.08	0.05±0.02
II (M <sub>e</sub> )	125.56±14.81	229.90±19.49*	45.39±5.13*	1.17±0.05	0.51±0.01
III (F <sub>c</sub> )	127.08±5.620	187.18±10.72	32.11±4.87	1.03±0.04	0.55±0.02
IV (F <sub>e</sub> )	128.81±10.11	175.04±11.23 <sup>+</sup>	26.41±3.98 <sup>+</sup>	1.00±0.06	0.57±0.03

Values are presented as Means±SEM; n = 7; \*p<0.05 compared with group I; <sup>+</sup>p<0.05 compared with group III. IBW = Initial Body Weight; FBW = Final Body Weight; PWI = Percentage Weight Increase; KW = Kidney Weight; PKW = Percentage Kidney Weight

Table 3: Effect of gasoline vapours on total serum protein and some renal function indices in Wistar rats

Groups	Protein (mg L <sup>-1</sup> )	Urea (mmol L <sup>-1</sup> )	BUN (mmol L <sup>-1</sup> )	Creatinine (mmol L <sup>-1</sup> )
I (M <sub>c</sub> )	6.88±2.255	4.22±0.22	6.52±0.84	33.74
II (M <sub>e</sub> )	6.62±2.62	7.97±0.14*	11.01±1.05*	128.37±5.42*
III (F <sub>c</sub> )	6.63±2.56	4.32±0.029	6.56±1.07	30.23±5.23
IV (F <sub>e</sub> )	6.26±2.94	9.45±0.21 <sup>+</sup>	11.26±0.72 <sup>+</sup>	122.80±7.79 <sup>+</sup>

Value are presented as Means±SEM; n = 7; \*p<0.05 compared with group I; <sup>+</sup>p<0.05 compared with group III

insignificantly lower (p>0.05), while the percentage kidney weight per total body weight of male and female rats exposed to gasoline vapours were observed to be insignificantly higher (p>0.05) compared respectively values obtained for the male and female control rats (Table 2). However, the percentage decrease in the percentage weight increase and the percentage increase in the percentage kidney weight per total body weight of female rats (17.75±3.28 and 3.51±0.73%, respectively) were significantly to the higher (p<0.05) compared respectively to the values obtained for the male rats (6.99±1.56 and 1.96±0.32%, respectively) following exposure to gasoline vapours (Fig. 1).

The total serum protein obtained for both male and female rats exposed to the vapours were insignificantly lower (p<0.05) compared respectively to the values obtained for male and female rats in the control group (Table 3). This result showed that the percentage decrease in total serum protein of female rats (5.58±1.96%) was insignificantly higher (p>0.05) compared to the percentage decrease in total serum protein of male rats (5.16±1.89%) following exposure to gasoline vapours

(Fig. 1). From the results of this study, it was also observed that the levels of serum urea, BUN and creatinine in both male and female test rats were significantly higher ( $p < 0.05$ ) compared respectively to the levels obtained for male and female control rats (Table 3). However, the percentage increase in serum urea and creatinine in female rats ( $54.29 \pm 6.97$  and  $75.38 \pm 6.98\%$ , respectively) was observed to be significantly higher ( $p < 0.05$ ) compared respectively with the percentage increase in serum urea and creatinine levels obtained for male rats ( $47.05 \pm 6.84$  and  $73.72 \pm 7.32\%$ , respectively) following exposure to gasoline vapours (Fig. 1).

The observations made from the results of this study showed that the effect of gasoline vapours on renal functions is comparatively more significant ( $p < 0.05$ ) in female rats, compared to the male gender.

## DISCUSSION

Subjects with kidney dysfunction may have a variety of different clinical presentations. Some manifest such symptoms as hematuria, edema, hypertension and signs of uremia. However, some of the subjects may be asymptomatic, only detected on routine laboratory examinations from elevated serum creatinine, urea and BUN levels, among others. A persistently elevated serum creatinine is reported to be a risk factor for progression of chronic kidney disease and independent factor for progression of chronic kidney disease to kidney failure (Appel *et al.*, 2003). The clinical manifestations of renal disease can be grouped into reasonably well defined syndromes, some of which are peculiar to glomerular diseases, while others affects any of the renal components. One of such group of clinical manifestations is azotaemia a biochemical abnormality characterized by elevation of serum urea, BUN and creatinine levels (Cotran *et al.*, 1999). This condition is produced by many renal disorders may also be implicated.

Exposure to toxic environmental pollutants (such as lead), among other factors, have been reported to be associated with the development of hypertension and kidney disease (Nwankwo *et al.*, 2006; Johnson *et al.*, 2005). A perceived health concern from inhalation of gasoline vapours is the potentials for carcinogenicity, based on the induction of kidney tumors in male rats and liver tumors in female rats exposed to wholly-vaporized gasoline (Macfarland *et al.*, 1984). According to Page and Mehlman (1989), branched-chain alkanes component of gasoline bind to a globulin to form a complex cannot be degraded in the usual manner such that protein accumulation occurs in renal cells, leading to cytotoxicity, death, proliferation and with prolonged exposure, kidney cancer.

In this study, the levels of serum creatinine, urea and BUN were observed to be significantly higher, while the total serum protein was significantly lower in rats exposed to gasoline vapours. According to Cotran *et al.* (1999), this observation indicate that exposure to gasoline vapours may cause such renal clinical manifestation as azotaemia in both male and female rats. However, the female rats tend to be adversely affected than the males. This observed sex-dependent effect agrees with our earlier reports on the effect of gasoline vapours on sex-hormones and haematological indices (Uboh *et al.*, 2007a, 2008). These observations indicate that the absorbed constituents of the vapour and/or their metabolites might have reacted and interacted with the renal tissues in a sex-dependent pattern to impair the renal excretion of these metabolites.

While Page and Mehlman (1989) reported adverse effect of gasoline vapours on the kidneys of male rats only, we observed in this study that the renal function of female rats are more affected by frequent exposure to gasoline vapours than males. From this observation, it is suspected that the sex-, or gender-differences in the endocrine functions among other factors, may influence the pattern of interactions between the renal tissues and the metabolites of gasoline vapours constituents. However, further studies to elucidate the specific mechanism(s) of the sex-dependent renal function impairment observed in this study are in progress.

In conclusion, the result of this study shows that exposure to gasoline vapour is also a predisposing factor to renal function impairment in rats and that the females are at greater risk than males.

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