Toxicological Effects of Exposure to Gasoline Vapour in Male and Female Rats

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Citation

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Abstract

Toxicological effects of premium motor spirit (PMS) blend of gasoline vapours was assessed from the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyltransferase activities, total serum bilirubin level and the relative percentage liver weight in male and female Wistar rats after 6 hrs daily exposure to gasoline vapours for 20 weeks. The results showed that gasoline vapours produced significant increase ($P<0.05$) in the enzymes activities, total bilirubin levels and relative percentage liver weight in male and female rats. However, the percentage increments in these parameters were significantly higher ($P<0.05$) in females compared to the male rats. The increase in liver enzyme activities could be due to hepatocellular damage. These observations indicate that exposure to PMS blend of gasoline vapours has a significant adverse effect on the liver functional integrity in both male and female rats, and that the female rats are more vulnerable than the males.

INTRODUCTION

The use of gasoline in the industries and homes has rapidly increased in the recent times. In the course of usage, individuals are frequently exposed to pollutants from gasoline fuel in both outdoor and indoor environments. However, the major route of exposure is inhalation by workers during production and distribution of the fuel, and by the general public during refueling at service stations [1]. From the report, it has been estimated that more than 3.6 billion gallons of unleaded gasoline (UG) are released into the air as gasoline vapours annually, with about 40% of this amount occurring during refueling of vehicles at service stations.

Generally, the overall constituents of gasoline vapours depend on the composition of the liquid gasoline, which varies with the brand and storage period. Among the brands commonly used in the United States include the blend of unleaded gasoline (UG) designated PS-6, API 91 – 01UG and the methyl tertiary butyl ether (MBTE) blended gasoline [2]. The API 91 – 01 blend of UG has been reported to contain a slightly higher percentage of saturated hydrocarbons, compared with the PS-6 blend. An estimate of 25% or more of the gasoline supply in the United State in 1995 has been reported to have been supplemented or blended with MBTE [3]. MBTE is an octane enhancer and oxygenate added to some formulations of UG to decrease air pollution in accordance with the US clean Air Act Amendment of 1990. Oxygenated gasoline typically contains about 15% of MBTE by volume, while premium gasoline generally contains 2 to 9% MBTE [3]. In Nigeria, the unleaded gasoline designated PMS (Premium Motor Spirit) is commonly used.

Human health risks from intermetent, low-dose exposure to gasoline vapour is not quite consistent. To identify the potential health risk of chronic exposure to UG, American Petroleum Institute sponsored a cancer bioassay, in which B6C3F1 mice and F-344 rats were exposed to UG vapour for 6 hrs/day, 5days/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, ?$_{2?}$- globulin [4, 5]. The accumulation of this protein in proximal tubule cells may lead to cytolethality, regenerative cell proliferation and ultimately, renal cancer [6]. Other reports also indicate that UG vapours stimulate the growth of diethyl nitrosamine-induced hepatic preneoplastic lesions in mice, and induce an enzyme activity associated with cytochrome P_{450} 2B [7, 8, 9]. 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. However, our recent studies shows that the female rats are more vulnerable to the adverse effect of exposure to gasoline fumes on sex hormones profile [10]. Also in our previous studies we discovered that repeated exposure to kerosene and petrol fumes causes degenerative changes in the ultrastructural integrity of the hepatic cells which may impair the normal liver function [11]. This study is therefore designed to investigate the sex-dependent effect of exposure to gasoline vapours on the liver functions' integrity in rats.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Thirty two male and female growing Wistar albino rats weighing 123-129g were obtained from the animal house of the College of Medical Sciences, University of Calabar, Calabar, Nigeria and used for this study. The animals were divided according to sex into four groups (i.e. male test group, male control group, female test group and female control group) of eight rats each, and were allowed to acclimatize in the experimental animal house for seven days before the commencement of the experiment. The animals, housed in stainless steel cages, were fed with the normal rat pellets. All the rats in both test and control groups were allowed free access to food and water throughout the experimental period. All animal experiments were carried out in accordance to the guidelines of the Institutional Animals Ethics Committee.

EXPOSURE TO GASOLINE VAPOUR

The animals in the test groups were exposed to gasoline vapours in the exposure chambers, while those in the control group were kept in gasoline vapours-free section of the experimental animal house. A modified nose inhalation exposure method, previously described [10, 11, 12] was used to expose the animals to the vapours liberated from direct evaporation of liquid gasoline. In this exposure method, the cages housing the test animlas were kept in an exposure chamber (100x75x200cm) saturated with gasoline vapours for 6 hours after which they were transferred to vapours-free section of the animal house. Saturation of the exposure chamber was done by allowing liquid gasoline in plastic containers, highly perforated at the upper end, to evaporate and fill the chambers at ambient temperature and humidity. The liquid gasoline was obtained from National filling station, Calabar, Nigeria. The test animals were wholly exposed to the vapours evaporating from the containers during the exposure period. An exposure period of 6 hours (9.00am to 3.00pm) daily was adopted for 20 weeks. The animals were sedated with chloroform vapours after their respective weights have been taken, at the end of the experimental period. The animals were then dissected and blood specimens were collected and prepared for biochemical analyses.

COLLECTION AND PREPARATION OF BLOOD SPECIMEN FOR ANALYSES

Blood samples were collected by cardiac puncture into plain screw-cap sample bottles. The blood samples collected were allowed to clot, and the serum extracted with Pasteur pipette after spinning with MSE model (England) table-top centrifuge at 2000 rpm for 5 minutes. The serum collected was used for biochemical analyses. All biochemical analyses were carried out within 24hours of serum separation.

BIOCHEMICAL ANALYSES

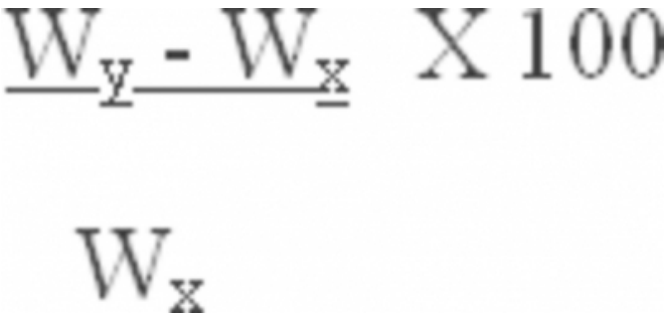
Biochemical analyses carried out included measurement of the concentration of alanine transaminase (ALT), aspartate transminase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and bilirubin in the serum. The measurements of the concentrations of these biochemical parameters were done by spectrophotometric determination of their absorbances, using analytical grade laboratory reagent kits. The laboratory reagent kits from Biosystems Laboratories (S. A. Costa Brava, Barcelonia, Spain) were used to assess the concentration of ALT, AST and ALP in the serum. While reagent kits from Randox Laboratories (United Kingdom) were used to assess the concentration of GGT, and bilirubin in the serum. All absorbance readings were taken with DREL3000 HACH model spectrophotometer.

DETERMINATION OF TOTAL BODY AND LIVER WEIGHTS

The total body weight of each rat was determined using digital chemical balance before and after the experimental period (as initial and final body weights, respectively), and the mean body weight for each group calculated. Weight changes were expressed as percentage weight increase and percentage growth rate where:

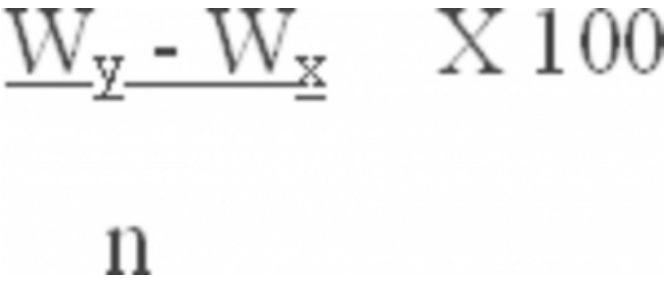
(i) Percentage weight increase was calculated from the formula:

Figure 1



(ii) Percentage growth rate from the formula:

Figure 2



Where W_x = Initial mean total body weight

W_y = Final mean total body weight

n = Number of days in exposure period

The weight of the liver of respective rats was measured with digital chemical balance and presented as percentage liver weight per total body weight (PLW/TBW). The PLW/TBW was calculated from the formula:

Figure 3

STATISTICAL ANALYSIS

Students' t-test was used to evaluate the significance of the differences between the mean values of the measured parameters in the respective test groups, compared with the corresponding control groups. A significant difference was accepted at $P<0.05$

RESULTS AND DISCUSSION

The results obtained in this study show that the serum levels of ALT, AST, ALP, GGT and bilirubin in male rats exposed to the gasoline vapour, i.e., male test rats were significantly higher ($P<0.05$) compared with the levels obtained for the male control rats. Also, the serum levels of these parameters in female test rats were significantly higher ($P<0.05$) compared with the levels obtained for female control rats (Table 1). Comparatively, these results showed that the percentage increase in the levels of ALT, AST, ALP, GGT, total bilirubin and relative liver weights in female rats (101.9±3.8, 106.4±5.3, 16.3±2.5, 192.7±6.5, 134.3±6.4 and 70.8±5.3 percents respectively) were significantly higher ($P<0.05$) than the corresponding percentage increase in male rats (82.7±4.5, 93.4±5.2, 9.8±1.5, 146.4±6.7, 88.3±3.8 and 29.6±2.7 percents respectively) following exposure to gasoline vapours.

From the result, the final body weight and percentage weight increase of the male and female test rats were significantly lower ($P<0.05$) compared respectively with the final body weight and percentage weight increase of of the male and female control rats. However, the percentage growth rate and liver weight per total body weight obtained for the male and female test rats were significantly higher ($P<0.05$) compared respectively with the values obtained for the male and female control rats, respectively (Table 2). The percentage decrease in the final total body weight and percentage weight increase (23.6±2.3 and 72.7±5.2 percent respectively) as well as the percentage increase in the percentage growth rate (34.7±4.2 percent) obtained for the female rats were significantly higher ($P<0.05$), compared respectively to the percentage decrease in the final total body weight and percentage weight increase (11.0±1.9 and 26.5±2.8 percent respectively) and percentage increase in the percentage growth rate (16.4±2.4 percent) obtained for the male rats.

Various pollutants are introduced into the environment from the increasing industrial activities in most societies of the world. Petroleum vapours generated from increasing activities of petroleum and the related industries contribute an appreciable percentage of pollutants in the environment. And since petroleum vapours are ubiquitous in the environment a greater percentage of the entire populace in the urban areas are directly or indirectly exposed to the petroleum pollutants. The threat posed by environmental pollutants to health has been a subject of great concern in the recent times. Hence, it becomes necessary that investigations on the health effect of exposure to various environmental pollutants should be on the increase.

It has been demonstrated that after inhalation of equal

concentrations of petroleum vapour through chronic exposure, lower concentrations of saturated hydrocarbons than unsaturated aromatics are detected in human and animal blood [13]. Also biological monitoring of exposured to bitumen vapours during road-paving operations indicated urinary excretion of 1-hydroxypyrene and thioethers in the exposed workers [14]. This indicates that the constituents of the vapours are metabolized before urinary excretion. Hydrocarbons and other constituents of petroleum and the related products, like other xenobiotics, are metabolized primarily in the liver [15]. Overloading of the liver tissues with reactive metabolites may cause the liver functions to be compromised.

In this study, changes in the levels of serum ALT, AST, ALP, GGT and total bilirubin were determined in male and female rats, following exposure to PMS blend of gasoline vapours, to assess the effect of vapours' constituents on the functional integrity of the liver. These indices are useful markers for the assessment of tissue damage associated with toxicity effects of xenobiotics in the body. A rise in plasma or serum level of these indices is a sensitive indicator of hepatocellular damage [10, 11, 16, 17, 18]. This is due to the fact that once the liver cells are damaged, the enzymes and other metabolites leak out of the cellular compartments into the extracellular fluid, thereby increasing their concentrations in the blood.

We observed that the levels of serum ALT, AST, ALP, GGT and total bilirubin in male and female rats were increased, following frequent exposure to PMS blend of gasoline vapours. Also, an increase in the relative liver weight (hepatomegaly), and reduction in the percentage weight increase following exposure to gasoline vapours, is in consistence with our previous report [10, 11]. Moreover, the observed increased adverse effect on the female rats agrees with our recent laboratory findings [12]. These results indicate that the vapours introduce some chemical substances into the body which may biochemically be converted to reactive intermediates that interact and cause damages to the liver tissues. It has been reported that metabolism of aliphatic and aromatic hydrocarbons, the major constituents of petroleum and petroleum-derivatives, as well as other xenobiotics generates a significant increase in the level of reactive free radical species in various tissues [19, 20]. The generated reactive intermediates can interact and disrupt the cell membranes of the affected tissues; thereby causing the tissue enzymes and other metabolites to leak out and increase the plasma concentrations as observed in this study.

Various health hazards associated with exposure to vapours from different blends of gasoline used in the US have been reported [2, 7, 21]. For instance, APT 91-01 blend of UG vapour has been reported to increase the incidence of liver tumours in a chronic bioassay, only in female mice [21]. Also, inhalation of PS- 6 UG and MTBE blends has been reported to be hepatocarcinogenic in female mice, only at high dosage [2, 8, 9, 21]. From the results obtained in this study, it is clear that frequent exposure to PMS blend of gasoline vapours, commonly used in Nigeria, may cause hepatotoxicity in both male and female rats, although the females tend to be the more vulnerable sex than the males. Hence, it may be concluded that the hepatotoxic effects associated with exposures to PMS blend of gasoline vapours is sex-dependent in rats, with the female rats being more adversely affected. Further investigation on the specific mechanism of sex-dependent hepatotoxic effects of gasoline vapours in rats is in progress.

Figure 4

Table 1: Effect of exposure to PMS blend of gasoline vapours on serum ALT, AST, ALP, GGT and total bilirubin in male and female albino rats.

Group	ALT(u/l)	ALP(u/l)	ALP(u/l)	GGT(u/l)	Total Bilirubin(µmol/l)
Mc	6.87±2.00	19.82±1.70	283.15±12.84	26.30±3.44	2.65±0.47
Mt	12.55±1.01*	38.34±2.74*	310.79±14.33*	64.81±9.95*	4.99±0.28*
Fc	5.85±1.64	17.63±8.73	250.24±8.73	17.97±1.49	2.07±0.40
Ft	11.81±0.77*	36.38±1.95*	291.15±18.01*	52.60±4.94*	4.85±0.61*

Values are presented as mean ± SEM, n=8, P<0.05 compared to the control, Mc = male control rats, Fc = female control rats, Mt = male test exposed to gasoline vapours, Ft = female test rats exposed to gasoline vapours.

Figure 5

Table 2: Effect of exposure to PMS blend of gasoline vapours on the total body and liver weights of male and female albino rats

Group	IBW (g)	FBW(g)	PWI (%)	PGR (%)	PLW/FBW (%)
Mc	124.83±7.67	243.88±17.45	95.4±4.3	6.63±0.40	2.7±0.3
Mt	125.33±14.56	219.78±19.49*	75.4±4.8*	7.72±0.48*	3.5±0.4*
Fc	127.08±5.62	237.08±10.72	91.7±5.7	5.80±0.28	2.4±0.1
Ft	128.00±11.99	191.75±15.53*	53.1±2.9*	7.81±0.66*	4.1±0.2*

Values are presented as mean ± SEM, n=8, P<0.05 compared to the control, IBW = initial body weight, FBW= final body weight, PWI/FBW = percentage liver weight per final body growth, Mc = male control rats, Fc = female control rats, Mt = male test exposed to gasoline vapours, Ft = female test rats exposed to gasoline vapours.

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