

ARTICLE

Evaluation of toxicological implications of inhalation exposure to kerosene fumes and petrol fumes in rats

Friday E Uboh, Monday I Akpanabiatu*, Eyong U Eyong, Patrick E Ebong, Offiong O Eka

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria

ABSTRACT Toxicological implications of exposure to ungraded concentrations of kerosene and petrol fumes in albino Wistar rats were investigated after two weeks of 4 hours daily inhalation. Serum aminotransferases (AST and ALT), alkaline phosphatase (ALP), total cholesterol (Chol), triglyceride (TG) levels and histological analysis of the liver tissues were used as diagnostic markers to assess liver dysfunction. The mean levels of these markers determined for the group of rats exposed to kerosene and petrol fumes (test groups), as compared with the levels for the control group were significantly ($p < 0.05$) higher. ALT, AST and ALP levels of the kerosene exposed group were raised by 191%, 161% and 204% while serum total cholesterol and TG levels increased by 129% and 118%, respectively. The increases in the serum levels of AST, ALT, ALP, Chol, TG in the petrol exposed group were 177%, 140%, 191%, 100% and 97%, respectively, when compared with the controls. Histological analysis of the liver tissues of the experimental test groups indicated degenerative changes in the ultrastructural integrity of the hepatic cells. These results showed that frequent exposure to kerosene and petrol fumes may be highly deleterious to the liver cells.

Acta Biol Szeged 49(3-4):19-22 (2005)

KEY WORDS

kerosene
petrol
fumes
liver enzymes
histopathology
serum lipids

Kerosene and petrol are distilled from crude petroleum and vapours obtained from their evaporation may be considered as kerosene and petrol fumes. These fractions of crude petroleum contain aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons (Henderson et al. 1993; Kato et al. 1993; Anderson et al. 1995). It has been demonstrated that after inhalation of equal concentrations of petroleum vapour through chronic exposure, lower concentrations of saturated hydrocarbons are detected in human and animal blood than that of the unsaturated aromatic hydrocarbons (Zahlsen et al. 1993). Biological monitoring of exposure to bitumen fumes during road-paving operations indicated urinary excretion of 1-hydroxypyrene and thioethers in the exposed workers (Burgaz et al. 1992).

Petroleum fumes are ubiquitous in our environment and the common sources of contact or exposure are petrochemical industries (refineries, oils fields, filling stations) and homes. The applications of kerosene as cooking and lighting fuels in the home have resulted in direct exposure of these products to a good percentage of the populace. Moreover, the day-to-day use of petrol outside the industrial settings is likely to have the same effect on the users as kerosene since they have been reported to contain most of the same hydrocarbons. However, the most affected are those who occupationally exposed to the fumes (Smith et al. 1993; Carballo et al. 1994; Rothman et al. 1996).

Despite the high utilization of kerosene and petrol, our knowledge is sparse on the toxicological effects of inhaling the composite fumes evaporating from kerosene and petrol. However, mutagenic, genotoxic, carcinogenic, neurotoxic, immunotoxic and haemotoxic effects of some petroleum and petrochemical products' constituents have been reported in experimental studies on humans and animals (Sim 1980; Hu and Wells 1994; Hallier et al. 1995). Hydrocarbons and other constituents of petroleum and petrochemical products, like other xenobiotics, are metabolized in the liver to a greater extent (Sims 1980; Nelson et al. 1993). Ueng et al. (1998) reported that exposure of rats to motorcycle exhaust and organic extracts of the exhaust particulate caused a dose- and time-dependent increase in cytochrome P-450-dependent monooxygenases and glutathione-S-transferase in the liver, kidney and lung microsomes. Since kerosene and petrol contain some of these constituents, chronic or frequent exposure to their fumes may affect the normal liver functions.

The expression of toxicity of xenobiotics is usually determined biochemically by the monitoring of some plasma enzymes and lipids. A rise in AST, ALT, ALP, TG and cholesterol are commonly measured as indices of the damage of the liver cells (Abdel-Baset et al. 1997; Owu et al. 1998). In this study we attempt to place on record the biotoxicity effects of kerosene and petrol fumes on albino Wistar rats by measuring some of these parameters. Histopathological changes in the liver tissues of both the control and experimental animals were also examined to support the biochemical findings.

Accepted April 15, 2005

*Corresponding author. E-mail: akpanabiatu@yahoo.com

Table 1. Effects of exposure to kerosene and petrol fumes on serum ALT, AST, ALP, Chol and TG levels of Wistar albino rats.

Group	ALT (U/L)	AST (UL)	AST/ALT	ALP (UL)	Chol (mmol/l)	TG (mmol/l)
Control	10.37±0.86	11.40±1.50	1.10	90.95±5.55	1.54±0.26	1.02±0.05
Kerosene fumes	30.12±0.58	29.72±1.37	0.99	276.24±3.36	3.53±0.33	2.22±0.05
Petrol fumes	28.70±1.06	27.33±3.06	0.95	264.67±0.36	3.08±0.36	2.01±0.09

Materials and Methods

Experimental animals

Young adult Wistar albino rats obtained from the animal house of the College of Medical Science, University of Calabar, Nigeria, were used for this study. Rats weighing 80-113 g were randomly selected into 3 groups (control, kerosene and petrol, respectively) of eight animals each. Kerosene and petrol were obtained from the Mobil Filling station, Calabar, Nigeria. All experimental animals were housed in stainless steel cages (60 cm x 30 x 45 cm) in a well-ventilated animal house and had free access to water, and normal rat chow obtained from Livestock Feeds, Calabar, Nigeria. The test groups were exposed to kerosene and petrol fumes, respectively, while the control group was kept in a section of the experimental animal house free from petroleum fumes.

Exposure to kerosene and petrol fumes

The method of exposure employed in this study was by inhalation. The animal cages housing the test groups were placed in exposure chambers measuring 150 cm x 90 cm x 210 cm. Two highly perforated 1000 ml cans containing 500 ml of kerosene were placed in the exposure chamber and the animals were allowed to inhale the fumes evaporating from the cans. The same procedure was adopted for the petrol fumes. In both cases, exposure lasted for 4 h daily for a period of 2 weeks. The time of exposure was 9.00 am to 1.00 pm, after which the animals were transferred to fumes-free section of the experimental animal house.

Collection of blood samples and liver tissues for analysis

The animals were anesthetized with chloroform 24 h after the last exposure and blood samples collected by cardiac puncture into plain sample tubes. Serum samples were separated 1 h after extraction of blood by centrifugation at 3000 g for 5 min and stored in a refrigerator. Biochemical analyses on the serum samples were done 24 h after sample collection. The liver tissues were collected and processed according to the method reported by Akpanabiatu et al. (2003).

Biochemical analyses were carried out for the measurement of serum alanine (ALT) and aspartate aminotransferases (AST), alkaline phosphatase (ALP), cholesterol (Chol) and triglyceride (TG) levels. Laboratory kit reagents (Randox laboratory Ltd, UK) were used for all biochemical analyses

and the absorbances were read using a UV-Vis spectrophotometer (DREL 3000 HACH). Statistical analysis was carried out by employing Student's *t*-test to compare the mean values of the test groups with the controls ($p < 0.05$ was used as a level of significance).

Results and Discussion

The serum enzymes activities of the controls and the animals exposed to kerosene and petrol fumes are shown in Table 1. The activity of ALT was significantly higher in animals exposed to the kerosene and petrol fumes when compared to the controls. The level of serum ALT activity has been reported to be increased as a result of liver injury in patients developing severe hepatotoxicity (Beckett et al. 1989). ALT might have leaked from damaged cells, due to increased permeability of the hepatocellular membrane, or due to necrosis, indicating organ dysfunction (McIntyre and Rosalki 1992).

The activity of AST was also significantly higher in the animals exposed to kerosene and petrol fumes when compared to the control animals. Increased activity of AST has been reported in CCl_4 -intoxicated experimental animals (Abdel-Baset et al. 1997). This increase may be due to the abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in kerosene and petrol fumes. Metabolism of aliphatic and aromatic hydrocarbons which are the major constituents of petroleum fumes as well as other xenobiotics have resulted in changes in the cell membrane due to reactive free radical species (Leighton et al. 1985; Bondy et al. 1995). The ratio of AST/ALT is also an important index for the measurement of toxicity. The decrease in the ratios in the animals exposed to kerosene and petrol fumes showed that the liver is likely to be most affected tissue.

Alkaline phosphatase activity in the animals exposed to kerosene and petrol fumes were significantly higher ($p < 0.05$) as compared to the control animals. This implies that damages may have occurred in the liver cells, since the activity of this enzyme in the serum is reported to be increased in liver damage (Abdel-Baset et al. 1997). Alkaline phosphatase is involved in the transport of metabolites across the cell membranes, protein synthesis, synthesis of certain enzymes, secretory activities and glycogen metabolism. The increase in this enzyme activity may not be unconnected with a disturbance in the transport of metabolites or alteration in the synthesis of certain enzymes as in other hepatotoxic conditions (Sharma et al. 1995).

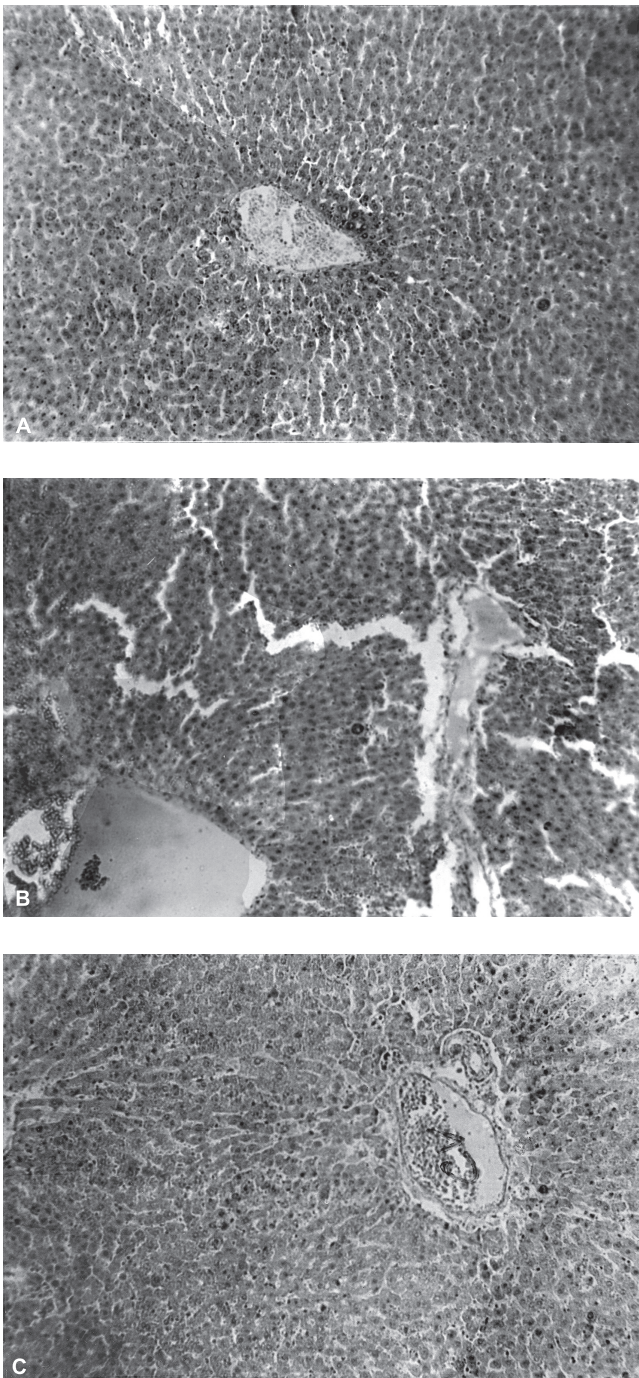


Figure 1. Photomicrograph of a typical liver section. (A) Control animal showing normal liver cell architecture. (B) Kerosene fumes-exposed animals show massive tissue degeneration. (C) Petrol fumes-exposed animals show liver necrosis. Magnification: 50x

The attendant effect of the exposure of experimental animals to kerosene and petrol fumes is that the reactive intermediates generated may have disrupted the cell membranes leading to enzyme leakage and tissue damage. Histological

analysis of the liver tissues of the experimental animals (Fig. 1) indicates that frequent exposure to kerosene and petrol fumes affects the structural integrity of the liver cells. This implies that the liver is one of the major target organs of kerosene and petrol fumes-induced injury. The cumulative oxidative damage is therefore likely to be one of the underlying mechanisms responsible for the hepatotoxic effects of kerosene and petrol fumes, as observed in this study.

The significant increase ($p < 0.05$) in the levels of serum total cholesterol and triacylglycerol observed in this work is an indication that inhalation exposure to kerosene and petrol fumes also affect lipid metabolism. On one hand, lipid metabolism is affected once there is liver damage since the disturbance of cell membrane integrity is likely to cause some membrane lipids to be released into circulation, while on the other hand, it causes the tissue to compromise its effectiveness in regulating lipid metabolism. There is a likelihood that exposure to kerosene and petrol fumes predisposes the subject to atherosclerotic condition.

In conclusion, the results of this work suggest that repeated exposure to kerosene and petrol fumes may elicit hepatotoxicity, thereby impairing the normal liver function. Petroleum workers therefore should have regular medical check-up to ascertain their health condition. Further work is ongoing on the influence of kerosene and petrol fumes on oxidative metabolism.

References

- Abdel-Baset Halim, Omar El-Ahmady, Samar Hassab Allah, Fathy Abdel Galil, Yosir and Hafez, AmrDarwish (1997) Biochemical effect of antioxidants on lipids and liver function in experimentally induced liver damage. *Ann Clin Biochem* 34:656-663.
- Akpanabiatu MI, Igiri AO, Eyong EO, Eteng MU (2003) Biochemical and histological effects of *Eleophorbia drupifera* leaf extract in Wistar albino rats. *Pharmaceut Biol* 41:96-99.
- Anderson D, Yu TW, Schmezer P (1995) An investigation of DNA-damaging ability of benzene and its metabolites in human lymphocytes using the comet assay. *Environ Mol Mutat* 26:305-314.
- Beckett GJ, Foster GR, Hussey AD, Oliveira DBG, Donovan JW, Prescott LF, Proudfoot AT (1989) Plasma glutathione s-transferase and F-protein are more sensitive than alanine aminotransferase as markers of paracetamol (acetaminophen)- induced liver damage. *Clin Chem* 35:2186-2189.
- Bondy SC, Lam HR, Ostergard G, Guo SX, Ladefoged O (1995) Changes in markers of oxidative status in brain, liver and kidney of young and aged rats following exposure to aromatic white spirit. *Arch Toxicol* 69:410-414.
- Burgaz S, Borm PJ, Jongeneelen FJ (1992) Evaluation of urinary excretion of 1-hydroxy-pyrene and thioethers in workers exposed to bitumen fumes. *Int Arc Occup Environ Health* 63:397-401.
- Carballo M, Nigro ML, Fraga I, Gadano A (1994) Ethylene oxide: Cytogenetic and biochemical studies in persons occupationally exposed. *Environ Mol Mutagenesis* 23:7.
- Hallier E, Geogergens HW, Karels H, Golk K (1995) A note on individual differences in urinary excretion of optical enantiomers of styrene metabolites and of styrene-derived mercapturic acids in humans. *Arch Toxicol* 69:300-305.
- Henderson RF, Sabourin PJ, Bechtold WE, Steinberg B, Chang IY (1993) Isobutene (2-methylpropene). *Toxicol Appl Pharmacol* 120:50-61.
- Hu Z, Wells PG (1994) Modulation of benzo α -pyrene bioactivation by

- glucuronidation in lymphocytes and hepatic microsome from rats with a hereditary deficiency in bilirubin UDP-glucuronosyltransferase. *Toxicol Appl Pharmacol* 127:306-311.
- Kato M, Rocha ML, Carvallio AB, Chaves ME, Rana MC, Oliveira FC (1993) Occupational exposure to neurotoxicants; preliminary survey in five industries of Camacari petrochemical complex. *Brazil Environ Res* 61:133-139.
- Lighton FA, Lee YZ, Kahimula AD, O'Brien PJ, Peakall DB (1985) Biochemical and functional disturbance of RBCs of herring gull ingesting Prudhoe Bay crude oil. *Toxicol Appl Pharmacol* 81:25-31.
- McIntyre N, Rosalki (1992) Biochemical investigation in the management of liver disease. In *Hepatobiliary diseases* ed., J Prieto, J Rodes and DA Shafritz pp 39-71. Springer Verlag, Berlin.
- Owu DU, Osim EE, Ebong PE (1998) Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. *Acta Tropica* 69:65-73.
- Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, Titenko-Holland N, Zhang LP, Blot W, Yin SN, Hayes RB (1996) Haematotoxicity among Chinese workers heavily exposed to benzene. *Am J Ind Med* 29:236-246.
- Sharma A, Mathur R, Skukla S (1995) Hepatoprotective action of a proprietary herbal preparation against carbon tetrachloride intoxication. *Indian Drugs* 32:120-124.
- Sims P (1980) The metabolic activation of chemical carcinogens. *British Med Bulletin* 36:11-18.
- Smith TJ, Hammand SK, Wond O (1993) Health effects of gasoline exposure, 1: Exposure assessment for US. Distributions workers. *Environ Health Perspectives* 101:13-21.
- Ueng TH, Hwang WP, Chen RM, Wang HW, Kuo ML, Park SS, Guengerich FP (1998) Effects of motorcycle exhaust on cytochrome P-450 dependent monooxygenases and glutathione S-Transferase in rat tissues. *J Toxicol Environ Health* 54:509- 527.
- Zahlsen K, Eide I, Nilsen AM, Nilsen OG (1993) Inhalation kinetics of C8 to C10 1-alkanes and iso-alkanes in the rat after repeated exposures. *Pharmacol Toxicol* 73:163-168.