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Effects of Nominal Exposure to Petrol on Organ Pathogenesis and Histopathology of Rats

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Abstract
Ten (10) male Wistar rats of average body weight 160 g were nominally exposed to petrol for seven (7) days and the effect of this on the organs:- brain, lungs, spleen, kidneys and liver were investigated. Another set of 10 male Wistar rats of similar weight which were not exposed to petrol served as the control. All the rats were maintained *ad libitum* on chick's mash and water and were sacrificed after 7 days. The results show that rats exposed to petrol gained significantly ($p < 0.05$) lower weights than the control rats after 7 days. There were no significant differences ($p > 0.05$) between the relative weights of the visceral organs of the test and control animals. Histologic changes were however observed in the liver, kidneys, lungs and brain of animals exposed to petrol vapours. These changes were characteristically vascular, degenerative/necrotic and inflammatory. These results suggest that nominal exposure to petrol may precipitate organ damage.

Keywords: petrol, rats, organ pathogenesis.

Introduction

Petrol, premium motor spirit (PMS) or gasoline is a complex mixture of hydrocarbon blends derived from catalytic reforming, polymerization, isomerization and hydrolytic cracking plus small amounts of additives designed to further improve the overall efficiency and reliability of the internal combustion engines (Considine, 1976). Non-hydrocarbon compounds such as tetraethyl lead and methyl – t-isobutyl to control knock and a variety of other additives to control rusting, deposits, icing, etc are also needed in blending petrol (Bartok and Sarofin, 1991).

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Gasolines vary in their compositions and are blended to meet local requirements from a wide variety of components. There are about five hundred brands of premium motor spirit (PMS) or petrol and a direct relationship has been reported to exist between the composition, time of exposure and level of health effects (Hoekman, 1992 and Kaiser *et al.,* 1992). Emissions of petroleum product vapours have been reported to increase in the environment during refining, transportation and refuelling (Romieu *et al,* 1999). Fuel station attendants are exposed to petrol for long hours.

This work was designed to elucidate the effects of nominal exposure to petrol vapour on organ and histopathology of rats.

Materials and Methods

Animals

The animals used for this work were adult male Wistar rats weighing between 100 – 180g bought from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The petrol was purchased from Tobechukwu Petrol Service Station, Nsukka, Nigeria.

Chemicals

All the chemicals used in this work were of analytical grade and were products of reputable companies based in Europe.

Experimental Design

The rats were divided into test and control groups of ten (10) each. Each group had an average body weight of 160g. The test rats were exposed to petrol from a beaker covered with a piece of chiffon cloth and fastened to the corner of the cage to avoid toppling. The rats were maintained *ad libitum* on tap water and chick's mash bought from Bendel Feeds Ltd, Ewu, Edo State, Nigeria. Both control and test animals were sacrificed after 7 days.

Body weight gains

Both the test and control animals were weighed on day zero and the last day of the experiment.

Weight of Visceral Organs

The wet weight of the organs – brain, lungs, spleens, kidneys and liver of each of test and control animals were taken and calculated as a percentage of their body weights.

Histopathology

The specimens viz: liver, kidneys, brain and lungs were obtained gross and thoroughly fixed in 10% formaldehyde buffered saline. This was allowed to stay for 3 – 4 days in 10 times (10x) the volume of the specimen. Processing started with the dehydration in which graded levels of ethanol 70 – 100 % (in ascending order) were

used. The alcohols were changed after steeping the tissues in them for 1½ -2 hours. The tissues were cleared in chloroform and infiltrated or impregnated with paraffin wax and sectioned at 5 microns thickness using rotary microtome. The sections were floated on a water bath maintained at 2 -3°C below the melting point of paraffin wax and dried on a hot plate thermostatically maintained at a temperature of 2 -3°C above the mid point of the paraffin wax used. When properly dried (15 – 30 mins), they were stained with haematoxylin and eosin (H.E), dehydrated, cleared and mounted (D.C.M) in D.P.X mountant, avoiding air bubbles

Statistical Analysis

Data were analysed with a computer using the SPSS version 7.5 software package. Difference between groups were assessed by a student's t-test. The acceptance level of significance was $p < 0.05$ using a 2 – tailed distribution.

Results

Table 1 shows the effect of nominal exposure to petrol on body weight gain and relative weight of visceral organs of rats after 7 days. Rats exposed to petrol vapour gained significantly lower weights ($p < 0.05$) than the controls after 7 days. The relative liver weight of the test animals were insignificantly higher than the control. There were no significant differences between the relative weights of the lungs, kidney, heart and spleen of the test and control rats. The brain had a slightly decreased weight relative to the control.

Table 1: Effect of Nominal exposure to petrol on body weight gain and relative weight of visceral organs of rats after 7 days.

Treatment	Period of exposure	Body weight gain (%)	Organs (% of body weight)					
			Liver	Lungs	Kidneys	Spleen	Heart	Brain
Test	7 days	9.5 ± 1.35	4.05 ± 0.28	0.76 ± 0.13	0.58 ± 0.06	0.29 ± 0.07	0.36 ± 0.07	0.58 ± 0.11
Control	7 days	21.86 ± 2.49	3.79 ± 0.15	0.67 ± 0.08	0.56 ± 0.05	0.49 ± 0.07	0.46 ± 0.08	0.76 ± 0.11

n = 10, mean = ± S.E.M

Histopathological Changes

Histologic changes were observed in the liver, kidneys, lungs and brain of animals exposed to petrol vapours for seven (7) days. These changes were characteristically vascular, degenerative/necrotic and inflammatory reactions.

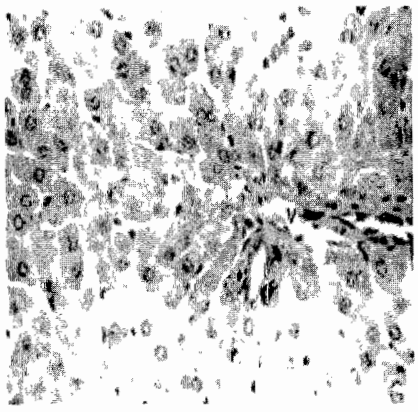

Plate 1: Liver section of control rat showing normal bile duct closed arrow head), vein (V) and artery (closed arrow) in the portal area, H & E stain X 400

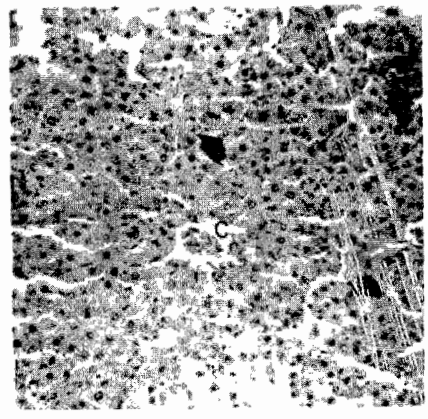

Plate 2: Liver section of rat exposed to petrol vapour (Day 7) (showing congested central vein (C) and hepatocytes in necrosis further away (closed arrow head). H & E stain X 400

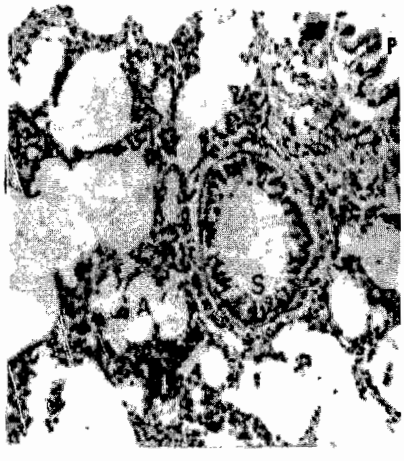

Plate 3: Lung section of control rat showing primary (P) and Secondary (S) bronchioles; and Alveoli ducts (A) H & E stain X 400

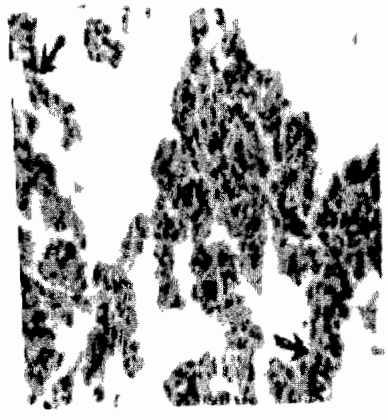

Plate 4: Lung section of rat exposed to petrol vapour (Day 7) showing thickening of alveoli walls and interstices by fibrous Exudation and mononuclear cells (closed arrow) H & E stain x 400

Liver

Liver sections of rats exposed to petrol vapour for seven (7) days were mildly hyperaemic/haemorrhagic. There was mild to moderate degeneration/necrosis of hepatocytes, which was mostly located in the periportal areas of the hepatic lobule (plate 2). There was also degeneration/necrosis of epithelia/cells lining the bile ductules; with mild infiltration of mononuclear cells into the portal areas.

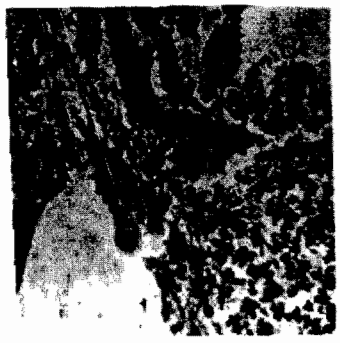

Plate 5: Lung section of rat exposed to petrol vapour showing Hyperplasia nodule (N) surrounding a bronchiole (8) H & E stain X 400

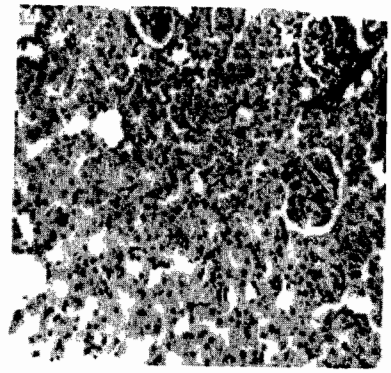

Plate 6: Kidney section of control rat showing renal corpuscles (Glomeruli) and tubules in the cortical area H & E stain X 400

Plate 7: Kidney section of rat exposed to petrol vapour (7 Days) showing congested blood vessel (b) and severe degeneration/necrosis of epithelial cells lining the tubules (t) H & E stain X 400

Plate 8: Section of the cerebellum showing hyperaemic meninges (H) and Glial cells (dark spots) proliferation in the outer molecular layer, H & E stain X 400

Lungs

Lung sections of rats exposed to petrol vapour were moderately hyperaemic. The alveolar walls in particular, and the interstices in general had become thickened by infiltrating mononuclear cells and fibrous deposits (plate 4). In addition, there was mild to moderate hyperplasia of the peribronchiolar lymphoid tissue, and peribronchiolitis (plate 5).

Kidneys

Sections collected from kidneys of rats exposed to petrol vapour were mildly to moderately hyperaemic. There was also moderate tubular degeneration (Plate 7).

Brains

Brain sections from rats exposed to the petrol vapour showed mild hyperaemia/haemorrhage in the parenchyma throughout the period of study (plate 8). This was accompanied with mild distension of perivascular spaces. Sections of the liver (plate 1), lung (plate 3), kidney (plate 6) and brain collected from control rats were devoid of the histologic changes described above for the test rats.

Discussion

Whole body exposure of rats to petrol in this study resulted in significant ($P< 0.05$) suppression of body weight gain relative to control rats which were not exposed to petrol. This suppression in body weight may be due to the strong offensive smell of petrol, which caused listlessness and loss in appetite observed in the animals at the beginning of the exposure. Suppression in body weight gain in animals exposed to various organic solvents have been reported (Tatrai *et al.*, 1981, Ungvary *et al.*, 1981 and Uemura *et al.*, 1995).

None of the visceral organs showed any remarkable change in weight when compared with their controls. Cramption *et al.*, (1977), suggested that liver enlargement unaccompanied by sustained induction of drug metabolising enzyme activity may be an index of hepatotoxicity. But enlargement of the liver accompanied by induction of microsomal enzymes is an adaptive response and beneficial to the body. In this study, even though there was an increase in relative liver weight, it was not significant. The insignificant weight increase observed in this study, may indeed be an adaptive response. Induction of Cyt p_{450} upon whole body exposure to petrol has been reported (Obidoa *et al*, 2001). This induction has several implications including formation of highly reactive epoxides.

Histologic changes were observed in the liver, kidneys, lungs and brain after whole body exposure to petrol vapours for seven (7) days. Inflammation and necrotic changes observed in the liver after whole body exposure to this vapour may be due to free radical metabolites. Eirewele (2001), reported increased lipid peroxidation in the blood and liver of rats exposed to petrol vapour. Several authors (Slater, 1984; Halliwell and Gutteridge, 1986; Borg, 1993 and Comporti, 1993) have implicated free radicals in tissue injury. Histologic changes in the lungs were characterised by necrosis, oedema and atelectasis. In an experiment with chloroform, Plaa, (1981), suggested that increased biotransformation of this compound possibly potentiated the observed nephrotoxicity. Some metabolites of the enhanced induction of petrol may be responsible for the nephrosis observed in the kidney sections. Rao and Pandya, (1978) in their study of toxicity of petroleum products reported that primary attack on membrane lipid (lipid peroxidation) by petrol may allow pathological effects like centrolobular necrosis to emerge. The histological changes observed in this work further support the report of Zahlsen and Eide, (1996) that after whole body exposure to petroleum products, some saturated hydrocarbons are rapidly distributed to organs and tissues particularly those rich in lipids like the brain.

The results of this work suggest that nominal exposure to petrol may

precipitate organ damage.

References

Bartok, W. and Sarofin, A.F. (1991). Composition and properties of major fuels. In: Fossil fuels combustion. Wiley Inter science publication. New York. Pp. 17 – 48.

Borg, D.C. (1993). Oxygen free radicals and tissue injury, In: Oxygen free radicals in tissue damage. Birkhauser Boston. Pp 12 – 53.

Comporti, M. (1993). Lipid peroxidation: An overview In: Free radicals from Basic science to medicine. G. Poli, E. Albano and M.U. Dianzani (eds). Birkhatser-Verlag Basel, Switzerland. Pp 65 – 79.

Considine, D.M (1976). Van Nostraind's Scientific encyclopaedia. Van Nostrand's Reinhold Company, New York. Pp 1736 – 1754.

Crampton, R.E., Gray, T. J.B., Grasso, P. and Parke, D.V. (1977). Long term studies on chemically induced liver enlargement in the rat. Sustained induction of microsomal enzymes with absence of liver damage on feeding phenobarbitone or butylated hydroxytoluene. *Toxicol.* **7**: 289 – 306.

Eirewele E. (2001). Toxicity studies in male albino rats (*Rattus rattus*) exposed to petrol and kerosene vapours. M.Sc. dissertation. Department of Biochemistry, University of Nigeria, Nsukka.

Halliwel, B. and Gutteridge, J.M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine. Some problems and concepts. *Arch. Biochem Biophys.* **246**: 501 -514.

Hoekman, S.K. (1992). Speciated measurement and calculated reactivity of vehicle exhaust emissions from conventional and reformulated gasolines. *Environ. Sci, Technol.* **26**: 1206 – 1216.

Kaiser, E. Siegel, W., Cotton, D. and Anderson, R., (1992) Effect of fuel structure on emission from spark-ignited engine; naphthalene and aromatic fuels. *Environ. Sci. Technol.* **26**: 1581 -1586.

Obidoa, O., Eirwele, E. And Ezeanyika, L.U.S. (2001). Effects of whole body exposure to petrol and kerosene vapours on cytochrome P_{450}, glutathione S-transferase and DNA concentration on albino rats. *Nig. J. Biochem. & Mol. Biol.* **16(3)**, 142 – 144.

Plaa, G.L (1981). Potentiation of haloalkane –induced hepatotoxicity and nephrotoxicity. Role of biotransformation. In: Industrial and environmental xenobiotics. I. Cirkt and G.L. Plaa (eds). Springer-Verlag, Berlin. Pp 96 – 110.

Rao, G.S. and Pandya, K.P. (1978). Toxicity of petroleum products. Effects on alkaline phosphatase and lipid peroxidation. *Environ. Res.* **16**: 174 – 178.

Romieu, I., Ramirez, M., Meneses, F. Ashley, D., Lambe, S., Colome, S., Fung, K. and Hernandez-Avila, M. (1999). Environmental exposure to volatile organic compounds among workers in Mexico City, as assessed by personal monitors and blood concentrations. *Envion. Health. Perspec.* **107**: 511 -515

Slater, T. F. (1984). Free radical Mechanisms in tissue injury. *Biochem. J.* **212**: 1 – 15.

Tatrai, E., Ungavry G., Cseh, I.R., Manyai, S., Szeberenir, S., Molnar, J. and Morvai, W.

(1981). The effect of long term whole body exposure to orthoxylene on the liver. In: Industrial and environmental xenobiotics. I Cirkt and G.L. Plaa (eds). Springer –verlag, Berlin pp. 161 – 168.

Uemura, T., Omae, k., Nakashima, H., Sakurai, H., Kazuto, Y., Toshikatsu, S., Mori, k., Kudum M., Kanoh, H. and Tati, M. (1995). Acute and sub-acute exposure toxicity of diborane in male ICR mice. *Arch. Toxicol.* **69**: 397 – 404

Ungavry, G. Szeberenyi, S., and Tatrai, E. (1981). Effects of benzene and its methylderivatives on mixed oxidase system. In: Industrial and environmental xenobiotics. I. Cirkt and G. L. Plaa (eds). Springer – Verlag, Berlin PP 285 – 292.

Zahlsen, K. and Edie, I. (1996). Exposure experiments with mixtures of hydrocarbons *Arch. Toxicol.* **70**: 394 -403