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Research Article

Cardiotoxicity induced by inhalation of petroleum products

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ABSTRACT

Exposure to petroleum products has been associated with high blood pressure. This study was designed to investigate the effect of petroleum products on cardiac tissue architecture and creatine kinase (CK- MB). Forty male Sprague Dawley rats were exposed to diesel, kerosene and petrol by inhalation for eight weeks. At the end of the study, blood samples were collected, blood pressure was measured and animal heart was harvested for histological study. Blood pressure and serum creatine kinase (CK-MB) was significantly higher in the exposed compared with control rats. Degeneration of myocardial tissue was also observed in the exposed rats. The findings from the study revealed that the cardio-toxic effect of petroleum products is via creatine kinase-dependent mechanism.

INTRODUCTION

Human exposure to petroleum hydrocarbon can occur through ingestion of contaminated food, drinking contaminated water, contact with contaminants (dermal exposure) or inhalation of vapour and air-borne soil (Azeez et al, 2012; Streicher et al, 1981). Inhalation of petroleum products can be accidental or intentional. Inhalant abuse, the deliberate inhalation of hydrocarbon as a form of recreational drug use has become a significant issue affecting children and adolescents(Steffe et al., 1996). Their low cost, ready availability and ease of use contribute to this problem. Inhalation is most commonly achieved by sniffing, huffing and bagging (Azeez et al, 20012; McHugh 1987). Sudden death of undiagnosed cause is becoming common in our environment; sudden death was recognised in misuse of volatile substances (Azeez et al 2012; Chalmer 1988). Pipeline breaks have resulted in exposure of livestock to crude oil or refined petroleum hydrocarbons. Ingestion of petroleum hydrocarbons have resulted in sudden death from per-acute bloat (Edwards WC 1989).

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Creatine is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to all cells in the body, primarily muscle. Creatine kinase (CK), also known as phosphokinase is a "leakage" enzyme present in high concentration in the cytoplasm of myocytes and is the most widely used enzyme for evaluation of neuromuscular disease (Gasper et al., 2005), unlike other enzymes found in skeletal muscle (e.g. lactate dehydrogenase, aldolase and transaminase). CK has relative predominance in skeletal muscle, is not falsely elevated by hemolysis, and being unbound in cell cytoplasm is readily released in cellular injury (Gasper et al,. Coodley 1970; Katriji et al,.2002). Creatine kinase was indicated to be a constituent of the integral proteins of erythrocyte membrane or to be tightly bound to the membrane, and was contrasted to the results obtained with adenylate kinase (Shiro Mawatari and Nobue Shinnoh, 1981)

CK catalyses the formation of phosphocreatine (PCr) and adenosine phosphate (ADP) from creatine and Adenosine triphosphate (ATP) (Bong et al., 2002). This CK enzyme reaction is reversible such that ATP can be generated from PCr and ADP (Goldblatt 1969). There are three isoenzymes: CK-MM, CKBB and CKMB. The gene for the subunits are located on different chromosomes: B on 14q32 and M on 19q13 (Schlattner et al., 2006). In addition to the three cytosolic CK isoforms, there are two mitochondrial creatine kinase

isoenzymes, the ubiquitous and sarcometric (Schlattner et al, 2006). In normal conditions, there is very little creatine kinase (CK) circulating in the blood of the average, healthy human being (Wallimann and Hemmer 1994; Gasper et al., 2005). Elevation of CK is indicative of damage to muscle and heart. It is therefore indicative of injury such as myocardial infarction, myositis, myocarditis or any cardiac or muscular disease.

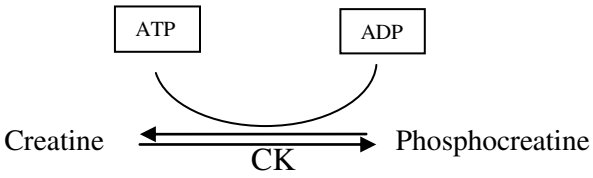

Fig. 1:
conversion of creatine to phosphocreatine by creatine kinase

The present study sought to investigate whether or not the cardiotoxic effect of petroleum hydrocarbon is associated with creatine kinase. In general terms, high serum CK in some ethnic groups may reflect a genetic condition of naturally increased levels of CK muscle tissue activity, which is not related to exercise frequency or muscle disturbance (Brewster et al, 2006). It has been proposed that higher than normal levels of tissue CK activity may augment the availability of cellular energy and improve myofibril contraction responses (Brewster et al, 2006). Therefore, high levels of serum CK, in the absence of muscle damage or other pathological conditions, may reflect the level of enzyme tissue activity of the individual.

MATERIALS AND METHODS

Forty Sprague Dawley rats of comparable weight were obtained from the animal house of Department of Vet Physiology and Biochemistry, University of Ilorin, Ilorin, Nigeria. They were housed in well ventilated cages. They were on standard rat chow and tap water ad-libitum. They were acclimatized for 2 weeks before the experiment began (Azeez et al. 2012).

Procedure involving animals and their care were performed in accordance with the National Institute of Health (NIH) guideline for the care and use of animals. Rats were assigned to one of the four experimental groups with ten rats in a group; control, diesel-exposed, kerosene-exposed and petrol-exposed. The control group was not exposed to any petroleum product. Diesel-exposed (DH), Kerosene-exposed (KH) and petrol-exposed (PH) groups were exposed to diesel, kerosene and petrol respectively; by placing them in exposure chamber and enclosed with the appropriate petroleum product. A modified nose-inhalation method used by Azeez et al., 2012 was used. The animals were exposed for five minutes daily for eight weeks. At the end of exposure, the animals were transferred to petroleum free area of the animal house. At the end of the exposure period, rats were anaesthetized with 1% chloralose and 25% urethane (BDH chemicals limited, Poole, England) given intra-peritoneally at a standard dose of 5ml/ kg body weight. Blood pressure was then measured by cannulating femoral artery using grass polygraph 7D. Blood samples were collected via the retro-orbital sinus. The samples were collected into plain sample bottles centrifuged at 3000 rpm for 15 minutes, and the serum was obtained within 1 hour of collection. Analysis of creatine kinase (CK-MB) was done by Elecsys CK-MB. The rats were later sacrificed by cervical dislocation, the heart removed and dropped into formol-saline and prepared for histology.

Statistical analysis

Data are expressed as Means ± standard error of Mean (SEM); statistical analysis was done using ANOVA of 10 rats per group. Unpaired t- test was used to analyse level of significance between control and treated groups. $P<0.05$ was considered to be significant. All analysis was done using Graph Pad Prism5.

RESULTS

Table1: Cardiovascular variables following inhalation of diesel, kerosene and petrol

variables	control	diesel	kerosene	petrol
SP (mmHg)	116.0 ± 1.79	139.0±1.18*	160.0± 3.54*	196.2± 1.16*
DP (mmHg)	81.60 ± 0.98	112.6±0.81*	106.8± 1.54*	135.8±1.11*
MAP (mmHg)	93.07 ± 1.22	121.4± 0.73*	124.5± 2.18*	155.9± 1.10*
HR (beats/min)	382.0 ± 0.89	453.4± 5.91*	460.0± 4.30*	461.6± 3.33*

Data are expressed as mean± SEM. Significant increase (*$P<0.05$) when compared with corresponding control. Key: SP=systolic pressure, DP= diastolic pressure, MAP= mean arterial pressure, HR= heart rate.

DISCUSSION

This study showed that inhalation of petroleum products led to significant rise in arterial blood pressure, and heart rate. This is consistent with our previous study (Azeez et al., 2012) and coroborates previous studies that documented the pressor effect of petroleum hydrocarbon (Steffe et al., 1996; Chalmers, 1991; Levecchio and Fulton, 2001; Mills, 2005). This is due to the ability of petroleum hydrocarbon to enhance the sensitization of myocardium to catecholamines,

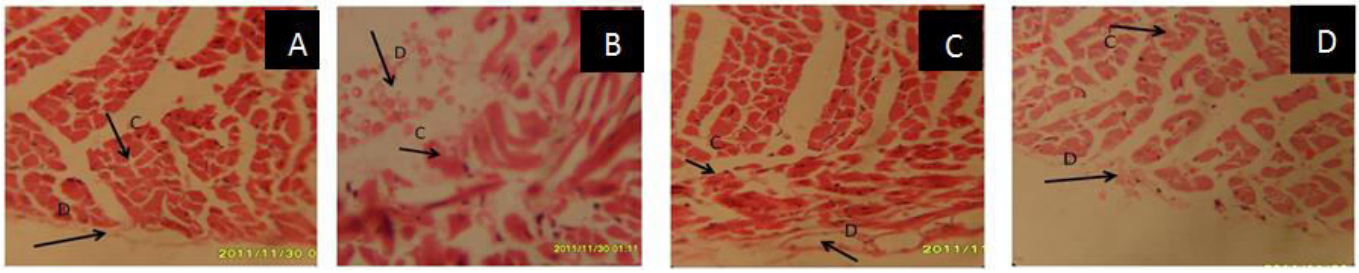

Plate 1
A: Photomicrograph of cardiac tissue in control rats showing normal myocytes (C) and normal apex (D).
B: Photomicrograph of cardiac tissue in rats exposed diesel showing ranging degree of cellular degeneration (C) that appeared to have originated from the apex (D).
C: Photomicrograph of cardiac tissue in rats exposed to kerosene showing focal loss of myocyte /degeneration.
D: Photomicrograph of cardiac tissue in rats exposed to petrol showing area of mild degeneration (C) and some degree of apical degeneration (D)

impair vagal activity (Steffe et al., 1996; Chalmers, 1991; Levecchio and Fulton, 2001; Mills, 2005), and increased baroreceptor activity (Azeez et al., 2012) with resultant vasoconstriction and increased arterial blood pressure.

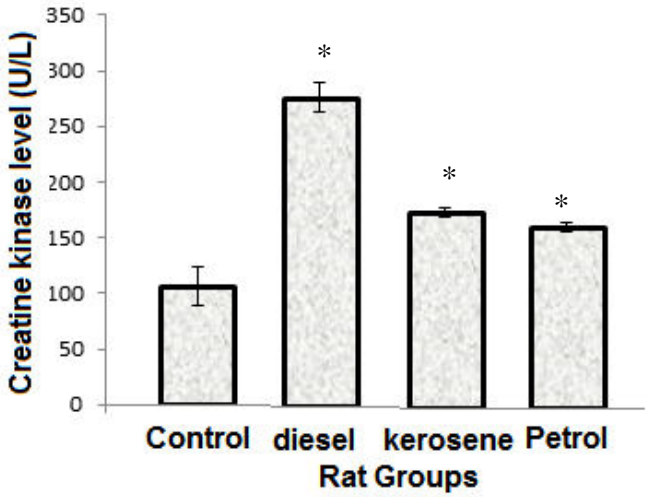

Figure 2: Effect of inhalation of diesel, kerosene and petrol on creatine kinase of Sprague Dawley rats. creatine kinase level increased significantly (*P<0.05) in the diesel, kerosene and petrol exposed groups,
the effect was highest in the diesel group

The present study is the first to determine the temporal profile of CK-MB mass after exposure to petroleum products. CK-MB mass assay is more sensitive and specific than the activity assay for myocardial injury.
Elevated CK values are found physiologically in children, pregnant women, and after exercise (FrancobVassella et al 1965). The exact mechanism is uncertain, but it is thought to be due to increased muscle catabolism or membrane permeability. The elevation of creatine kinase seen in this study was highest in diesel exposed group followed by kerosene the petrol exposed group. This observation has similar picture to the result of the effect of these substances on blood pressure and baroreflex sensitivity (Azeez et al, 2012). Koskelo et al, 1964 and Connor 1968; found out that Stroke-related myocardial injury is pathologically characterized by scattered foci of microlesions, similarly in this study the histological finding revealed ranging degree of cellular degeneration that appeared to originated from the apex (plate1).
I conclusion elevated CKMB level has been found with exposure to diesel, kerosene and petrol and suspected to be cardiac in origin. The highest increase as found in the diesel group showed that exposure to diesel caused more damage to the heart compared with other petroleum products used in the study. The molecular mechanisms that result in CK release from muscle after mild exercise are unclear. A key regulator in this event would be the energy sensor enzyme AMPK, which can phosphorylate CK and is sensitive to Cr/PCr ratios. *This* control involves expulsion of CK from the cytosol. Heled et al. (2007) explored the possibility of a genetic association between CK MM, angiotensin-converting enzyme (ACE) genotypes, and CK response to exercise. A genetic association was found between a specific CK-MM genotype of the Ncol polymorphism with an augmented response to exercise. Yamin et al. (2007) found an association between type of ACE genotype and CK levels. ACE genotypes may be involved in the excitation coupling process and influence the risk for developing rhabdomyolysis and,

conversely, protection against exercise-induced muscle injury.

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