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Full Length Research Paper

Thirty days exposure to kerosene induces alteration in select serum trace element levels in rats

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Trace elements are essential micronutrients required in minute amount by a number of metabolic processes. In many parts of Africa, kerosene is widely available since it is the most commonly used fuel for cooking purposes. As a result of this common availability, excessive exposure to this product has been described. The aim of this study is to identify if exposure to trace amount of kerosene is capable of causing depletion in magnesium and trace element levels. Eighteen Wistar rats divided equally into three groups were used for the study. Administration of trace amount of kerosene (0.4 ml/kg body weight) by oral or dermal route resulted in significant increase ($p<0.05$) in the level of Cr and significant decreases ($p<0.05$) in the levels of Fe, Mo, Co, Mn and Mg in rats in both dermal and orally administered routes except Mo and Mn which were not significantly different ($p>0.05$) in rats in dermal route. Magnesium and trace elements play significant physiologic roles and their depletion has been identified as an etiologic factor of many disease conditions. The results of this study therefore suggest that kerosene exposure even at trace quantity is capable of altering magnesium and trace element status in a mammalian species. A situation which may predispose an individual to many of the clinical conditions associated with essential element deficiencies.

Keywords: kerosene; magnesium, trace elements

INTRODUCTION

Kerosene is a colorless, thin liquid generated through fractional distillation of petroleum (Collins (2007). It is a combustible hydrocarbon liquid also known as paraffin or paraffin oil in the United Kingdom, Hong Kong, Ireland and South Africa. Although in Nigeria only one type is predominantly available, in the United Kingdom, two different grades are found, while BS2869 Class C1, the lightest grade, is usually used for lanterns, wick heaters, and combustion engines; the other type BS2869 Class C2, a heavier distillate, is used as domestic heating oil. In many parts of the developing World, it is chiefly used for domestic purposes but in the developed World, it has mainly industrial uses. An example of such industrial use is in the area of transportation where it is a component of fuel for jet engines i.e. Jet A and Jet A-1, Jet B, e.t.c. (Banse, 2010).

Kerosene (paraffin) is the most commonly used fuel in non-electrified dwellings worldwide; this is especially so in Africa and South Asia, because of its relatively low cost and easy availability unlike electricity and gas. This hydrocarbon fuel produced through distillation of petroleum is invaluable for many reasons. Compared with other fuel types it is relatively inexpensive to produce and consume, in addition it is readily available, such that it provides heat, light, and cooking opportunities to millions of individuals especially in the rural areas who would

otherwise be without a domestic source of energy (Schwebel and Swart, 2009), but it poses two major risks of injury, especially to children. The first is poisoning, either through ingestion or through inhalation of smoke and fumes. Poisoning through ingestion is possible because it has the appearance and viscosity of water, which makes thirsty toddlers drink it mistakenly.

In Nigeria and some other African countries (e.g. South Africa and Kenya) there are no regulations enforcing safe packaging, this has resulted in paraffin being transported and stored or sold in empty beverage bottles and other items used for water storage. Many sellers of this product are deceived into doing this because trace amount of exposure does not produce instant signs or manifestations of toxicity (Schwebel and Swart, 2009). Various paraffin-safety initiatives have been put forth by many countries worldwide, e.g. Australia has mandated that paraffin be dyed blue to prevent confusion with water. Since exposure to this agent in most cases is deliberate, some of the measures that have been put in place in other parts of the world may not be helpful. This measure may only be helpful in preventing accidental ingestion by children.

Kerosene like most other xenobiotics may be metabolized by the hepatocytes to yield reactive species and because many of these trace elements possess antioxidant activities, it is highly probable that exposure to this product may modulate serum trace element presentation. The aim of this study therefore is to determine if exposure to trace amount of kerosene is capable of inducing alteration in trace element levels in female Wistar rats, which may help to suggest the likely consequences of such exposure in human subjects.

MATERIALS AND METHODS

The kerosene used for the study was purchased from Mobil filling station located in Osogbo, Osun State, Nigeria in December, 2011. The experimental animals used for the study consisted of female Wistar rats weighing averagely (250 g), supplied by the Animal House of the Department of Veterinary Physiology, University of Ibadan, Nigeria. The animals were left to acclimatize for two week prior to commencement of the experiment. Animals were kept in cages at ambient temperature of 23±3°C and a 12 h light, 12 h dark cycle. All the animals were fed with their specific diets and water *ad libitum*. This study was carried out in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; as promulgated and adopted by United States Institutes of Health (1985).

Experimental Animal Treatment

The experimental animals consisted of eighteen rats, six served as the control while the remaining twelve rats were separated into 2 groups of 6 rats per group. Each group of the treated rats was exposed to this product either through dermal or oral route (as contaminant of feed). According to the study of Rao et al. (1984) 0.4ml of kerosene/kg body weight was adopted as quantity sufficient to study the toxic effect of trace amount of kerosene and the treatment period lasted for 30 days. Since some of the components of this product are slightly volatile, kerosene was mixed thoroughly with feed each day for the animals in the oral group while exposure through the dermal route was by direct application to the skin of each rat

Preparation of serum samples and elements estimation

After 30 days of daily application, blood was drawn from each animal through retro-orbital bleeding and introduced into an anticoagulant free bottle. Serum was separated from cells by centrifugation at 3000 g for ten minutes and stored at -20 ºC. Estimation of serum levels of the following elements; Mg, Co, Cr, Fe, Mo was carried out using the Atomic Absorption Spectrometric method. Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, Connecticut, USA) was used for this purpose. Some of the materials used for atomic absorption spectrometric method included high-purity analytical grade reagents supplied by Merck, Darmstadt, BDH, Chemicals Ltd. Deionized, doubly distilled water and re-deionized shortly before use (Millipore Co., Bedford, MA), with specific resistance of > 3 MΩ was utilized for the preparation of reagents and working standard. Working standard on the other hand, was prepared from the Spectrosol stock standard 1g/L (Buck Scientific). Samples and standards were diluted with a 2mM/L of aqueous solution of Triton X-100 (BDH Chemicals, Ltd). Test accuracy was carried out using Bovine Reference Material and human serum Standard Reference Material. All dilutions were carried out with the aid of automatic pipetting system (PD 100; Crony Instruments s. r. l., Rome, Italy). All disposable apparatus was vigorously washed before use by immersing in concentrated nitric acid and thoroughly rinsed with the same deionized, doubly distilled and redeionized water.

Table 1. The operating characteristics of AAS for iron, magnesium, chromium, molybdenum, cobalt and manganese.

Slit Width	Fe (0.2), Mg (0.7), Cr (0.7), Mo (0.7), Co (0.2), Mn (0.2)
Wave lengths(nm)	. Fe (248), Mg (285), Cr (357), Mo (313), Co (240), Mn (279)
Burner height	Low
Gas mixture	Air – acetylene
Acetylene	12 psi
Air pressure	50 psi
Temperature	2300 ºC
Analytical mode	Concentration
Measurement mode	Peak area
Linear range (mg/L)	Fe (5.00), Mg (1.50), Cr (5.00), Mo (20.00), Co (5.00), Mn (25.0)
Sensitivity (mg/L)	Fe (2.5), Mg (0.015), Cr (2), Mo (0.15), Co (3.5), Mn (1.25)
Detection limit (mg/L)	Fe (0.05), Mg (0.005), Cr (0.04), Mo (0.80), Co (0.05), Mn (0.03)
Atomizing air flow	83µl / second
Lamp current	18mA
Scale expansion	3
Noise suppression	2

Table 2. Serum magnesium and trace element concentrations

	Fe (µg/dl)	Mg (mg/dl)	Cr (µg/L)	Mo (µg/L)	Co (µg/L)	Mn (µg/dl)
Controls	137.67±6.28	0.74±0.04	0.19±0.02	1.01±0.06	0.60±0.01	10.98±0.39
Group 1	104.79±7.32*	0.63±0.07*	0.37±0.04*	0.99±0.07*	0.52±0.09*	9.46±0.18*
Group 2	96.29±7.62*	0.67±0.04*	0.26±0.01*	1.03±0.02	0.54±0.05*	11.11±0.24
F-value	8.326	10.767	106.580	0.269	4.106	98.239
P-value	0.009**	0.006**	0.002**	0.843	0.029**	0.008**

Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student't' test. **p <0.05 is significant using ANOVA.

Statistical analysis

The data obtained were subjected to statistical analysis using SPSS version 15 (SPSS Inc., Chicago, USA). Results were expressed as mean ±SEM. Student 't' test was used to test the level of significance difference in the test groups compared to the controls while ANOVA (analysis of variance) was employed to establish inter-group comparison. Pearson's correlation coefficient was used to assess the level of correlation among the elements. Value of P≤0.05 was considered as significant.

RESULTS

The serum concentrations of magnesium and select trace elements of rats administered with trace quantity of kerosene (oral/dermal routes) are shown in Tables 2 and 3.

Table 3. Intra-group correlation among magnesium and trace element levels in control, oral and dermal kerosene administered rats

	Mg	Fe	Cr	Mo	Co	Mn
Mg		CONT 0.718	CONT 0.681	CONT 0.453	CONT 0.226	CONT <u>0.947</u>
	-	GP1 0.293	GP1 0.335	GP1 0.546	GP1 - 0.354	GP1 763
		GP2 -0.432	GP2 0.723	GP2 -0.449	GP2 0.438	GP2 542
Fe			CONT 0.480	CONT - 0.248	CONT -0.321	CONT 509
			GP1 <u>0.902</u>	GP1 0.675	GP1 0.538	GP1 364
		-	GP2 0.401	GP2 0.217	GP2 0.079	GP2 611
Cr				CONT 0.436	CONT <u>-0.957</u>	CONT 103
				GP1 -0.321	GP1 -0.602	GP1 365
			-	GP2 0.599	GP2 0.292	GP2 287
Mo				-	CONT -0.076	CONT <u>905</u>
					GP1 -0.289	GP1 701
					GP2 <u>0.911</u>	GP2 480
Co					-	CONT 356
						GP1 329
						GP2 117
Mn						-

*The underlined numbers are statistically significant at the $p < 0.05$ level. CONT -control; Gp 1- oral route of exposure; Gp2-dermal route of exposure.

DISCUSSION

Many reports exist to suggest the toxic nature of kerosene using hepatic and renal indices. The significant alterations in the levels of many of these elements are a further confirmation of the toxic property of this product. Many other studies have identified that not only household products like kerosene, other agents even some with therapeutic usefulness have been recognized to be capable of altering physiologic processes that may cumulate in abnormality of body biochemistry or micronutrient alteration, cholestyramine induces Fe, Mg and Zn depletion (Shenkin et al., 2006).

Both rats and man have been reported to have somewhat similar level of toxicity/susceptibility to many drugs (e.g. paracetamol) and xenobiotics (e.g. aflatoxin). Since the response of rats to xenobiotics in some cases are identical to that of man because of close similarity in their physiology, the findings of this study raises the possibility that trace element alterations may be a likely findings in human subjects. What will make significant decreases in the serum levels of these elements (e.g. Mg and Fe) in mammals (e.g. human subjects such as kerosene hawkers/retailers) of great concern is that many of them may be low-income earners. Since it is only in households of the low-income earners of low- and middle-income countries that depend on biomass and fossil fuels (e.g. kerosene) to heat their dwellings, cook their food, and light their environment (Peck et al., 2008). It will seem then that poverty is one of the predominant factors responsible for its choice as fuel, but another associated feature of poverty is malnutrition. Malnutrition is a condition defined as faulty nutrition resulting from poor diet.

That poverty and malnutrition occur together in Africa has been revealed by Atinmo et al. (2009). Apart from their observation, findings of different studies are also available to suggest that this twin-evil cut across different sub-groups across many nations of Africa. According to Daboné et al. (2011), malnutrition is highly prevalent in developing countries and the result of their study revealed that malnutrition and micronutrient deficiencies are particularly widely prevalent in schoolchildren. Petrou and Kupek (2010) concluded from the results of their study that childhood under-nutrition in developing countries is strongly rooted in poverty. Many of the hawkers are either children or are within the reproductive stage where there is tendency that their offspring will also be exposed to this product. Oldewage-Theron et al (2010) have also identified that poor dietary intake and high prevalence of micronutrient deficiency is common among low- income South African elderly population. These are indications that most of the hawkers of this product may already be in a state of micronutrient deficiency.

The rats used for this study were fed standard laboratory diet yet exposure to this product resulted in significant decrease in the levels of many of the essential elements. This may suggest that kerosene is capable of inducing micronutrient depletion as well as modulating the nutritional state of an organism and by inference exaggerate the nutritional insufficiency of an already

malnourished individual. The significant differences observed for many of the indices of antioxidant stress may be in consonance with the findings of Rao et al. (1984) who revealed that by treating male Wistar rats repeatedly through the subcutaneous route with commercial kerosene (0.5 ml/kg body wt, 6 days a week) for a period of 35 days there were correspondingly changes in DNA, RNA, protein, and lipid contents of liver and spleen.

The alteration in DNA observed by these workers was probably as a result of an increase in oxidative stress due to significant decrease in the serum activities of the antioxidant enzymes or other components of the antioxidant system; many of the trace elements possess antioxidant property (Mg) or are cofactors for these enzymes, e.g. Mn and Fe are catalytic components for superoxide dismutase and glutathione reductase respectively. The significant increase in the levels of elements such as Cr, which studies have indicated that though it plays vital roles, may become oxidant at elevated level show a probable involvement of oxidative stress in many of the pathological presentations of kerosene toxicity of chronic exposure.

To confirm the possibility of such effects is the fact that data are available to suggest that used gasoline engine oils (UGEO) are carcinogenic and/or mutagenic in long-term studies, they increased the number of mutagen- or carcinogen-DNA adducts when applied dermally to mice. Although Lee and Talaska (1999) have attributed the carcinogenic or mutagenic risk of UGEO to the concentration of polycyclic aromatic compounds (PAC) that accumulate in the lubricating system during combustion of gasoline especially when dermal exposure to UGEO takes place, many of the refined petroleum contain the same hydrocarbon components. This risk became more pronounced in subjects who use kerosene, as solvent-based cleanser, to remove UGEO following dermal exposure. By employing a 32P post labeling technique, the results of their findings confirmed that the total levels of DNA adducts in skin and lung were significantly increased in kerosene-treated mice whereas the application of UGEO followed by kerosene washing significantly depressed skin DNA adduct levels but elevated lung adduct levels as early as the 8^{th} hour after exposure.

Results of this study seem to suggest that many Asians and Africans who misuse this product by constantly being exposed to it may manifest some of these presentations, but our results may have wider application than that. This is because as many as over 2 million military and civilian personnel per year (over 1 million in the United States) are occupationally exposed, respectively, to jet propulsion fuel-8 (JP-8), JP-8 +100 or JP-5, or to the civil aviation equivalents Jet A or Jet A-1, with the quantity of these kerosene-based jet fuels being as much as 60 billion gallons annually worldwide to over 2 million military and civilian personnel. The magnitude of this problem may be evident in the fact that JP-8, represents the largest single chemical exposure in the U.S. military, while Jet A and A-1 are among the most common sources of nonmilitary occupational chemical exposure.

Furthermore, according to International Agency for Research on Cancer (1992), in 1990 alone approximately 4.06 billion gallons of kerosene were consumed in the United States. Even though exposure to this product in that country is not exclusively through dermal and oral routes, but repeated exposure to raw fuel, vapor phase, aerosol phase, or fuel combustion exhaust by dermal absorption, pulmonary inhalation, or oral ingestion routes have also been confirmed to occur. Apart from exposure to military and civilian personnel, Ritchie et al (2003) have raised the possibility of the public being repeatedly exposed to lower levels of jet fuel vapor/aerosol or to fuel combustion products through atmospheric contamination, or to raw fuel constituents by contact with contaminated groundwater or soil even in the developed World.

To compound this problem is the fact that kerosene-based hydrocarbon fuels are complex mixtures of up to over 260 aliphatic and aromatic hydrocarbon compounds (with as many as over 2000 isomeric forms), including different levels of potential toxicants such as benzene, n-hexane, toluene, xylenes, trimethylpentane, methoxyethanol, naphthalenes (including polycyclic aromatic hydrocarbons [PAHs], and certain other C 9 -C 12 fractions (i.e., n-propylbenzene, trimethylbenzene isomers). Though it should also be noted that there is little epidemiological evidence for fuel-induced death, cancer, or other serious organic disease in fuel-exposed workers, but significant numbers of self-reported health complaints from these personnel have been reported.

The ability of the body to maintain successfully, accurate DNA replication and repair is essential to human health and once this homeostatic balance is derailed, genomic instability events occur which usually compromises the integrity of the genome, and may initiate fundamental events leading to human diseases. Several micronutrients (e.g. trace elements) have been found to play effective role in reducing and/or protecting against DNA damage. Moreover, studies have shown that the micronucleus (MN) index (a biomarker of DNA damage), are increased in developmental and degenerative diseases, an index which has been shown to be predictive of increased cancer risk and cardiovascular disease. This may be because trace elements act as co-factors for enzymes required in DNA repair or maintenance of methylation patterns essential for optimal gene expression (Thomas et al., 2011). Damage to DNA damage occurs spontaneously as a result of exposure to ubiquitous environmental agents but fortunately living organisms are capable of repairing their damaged DNA (Czupryn et al., 1993; Stano et al., 2006; Dion et al., 2007). Magnesium, the trace element found to be significantly decreased in all categories of treated rats is an important cofactor for many ATP requiring enzymes,

some of which may be involved in many of the repair steps

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