Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2013; 5(1): 22-25

ISSN: 0975-5160

Research Article

Altered Serum Micronutrient Levels Following Kerosene Exposure

*Iyanda AA

Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.

ABSTRACT

The impact of oral or dermal kerosene administration on serum micronutrients in Female Wistar rats has previously been determined but because concurrent exposure through many different routes is common in human subjects in Nigeria. The effect of exposure through more than one route at the same time is being investigated through this study. Twenty-four male Wistar rats were divided into four groups (n=6) and administered with 0.4 ml/kg body weight of kerosene through oral, dermal or combined routes with 6 serving as control. Three weeks after daily administration, blood was collected through retro-orbital bleeding and serum levels of vitamins (niacin, folic acid, riboflavin, thiamine, pyridoxine, pantothenic acid, vitamins A, C, D, E) and elements (Zn, Cu, Se, Fe, Mn, Mg, Mo, Cr, Co) were determined.All estimated vitamins were found to be significantly decreased ($p<0.05$) irrespective of the route of exposure except thiamine and pyridoxine compared with control. Inter-group comparison using ANOVA confirmed significant differences for all vitamins. All the elements were significantly different except Cr that was not changed in oral and dermal group and Fe in the dermal group. The greater depletion in the levels of all micronutrients observed in the combined group compared with either oral or dermal suggest that exposure through more than one route is capable of aggravating micronutrients depletion in these rats. In addition, Cr that was not significantly changed in rats in oral or dermal route was significantly decreased in the combined group.

INTRODUCTION

Kerosene, a combustible hydrocarbon liquid, is the most commonly used fuel in non-electrified dwellings in Africa and South Asia. Because it has a number of other uses in Nigeria, exposure to this product through many different routes at the same time is common. This thin oil, distilled from petroleum is also an alcohol denaturant. In both industrialized and developing countries, this liquid has additional uses such as an aircraft gas turbine and jet fuel by both commercial airlines and the military service[1], and as a spray oil to combat insects on citrus plants.

Major constituents of kerosene are alkanes and cycloalkanes (68.6%); benzene and substituted benzene (13.7%); and naphthalene and substituted naphthalenes, although its average chemical composition by weight has been described to be 35% paraffins, 60% naphthenes, and 15% aromatics. The short-term potential hazards of the lighter, more volatile and water soluble compounds (e.g. benzenes, toluene, xylenes) in kerosene include contamination of groundwater, the long-term effects that have been linked with polycyclic aromatic hydrocarbons (PAHs), alkyl PAHs, and alkyl benzene (such as xylene) - constituents of kerosene include alteration in the morphology of the hepatocytes and harmful effects on the kidneys, heart, lungs, and nervous system. Increased risk of cancer as well as immunological, reproductive, fetotoxic and genotoxic effects has also been documented to occur from oral or dermal route.

Since many of these kerosene constituents have been found to be capable of generating reactive oxygen and nitrogen species, the aim of this study that is carried out on male rats is to investigate not only the effects of kerosene administration through oral or dermal route as was determined for female rats in a past study but the combine impact of these two routes of exposure.

MATERIALS AND METHODS

Experimental Animals & Treatment**:** Twenty-four female albino rats of 14 weeks of age, (230 g) obtained from the Animal House attached to the Department of Veterinary Physiology, University of Ibadan (Nigeria) were used for the study. Prior to the commencement of the kerosene exposure to the experimental animals, they were left to acclimatize for two week. These rats 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 without any form of restriction. This study was carried out in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985).

Eighteen of these rats were randomly selected and divided into 3 groups comprising of 6 rats per group. Each group was treated with kerosene either through dermal, oral, or combined routes. Exposure through the oral route was as contaminant of feed while dermal exposure was carried out by discharging kerosene directly on the skin of each rat. The remaining six rats served as the control. The treatment groups were exposed to kerosene (purchased at Mobil filling station, Osogbo, Nigeria) for a period of 21 days. Unlike the previous study[2] carried out on female rats in which 0.4 ml/kg BW was used, 0.3 ml of kerosene/kg body weight of rat was adopted for the present study as quantity sufficient to

*Author for correspondence: E-mail lapeiyanda@yahoo.com

Table 1: Serum element levels in rats administered with trace quantity of kerosene

	Zn (µmol/L)	Cu (µmol/L)	Se (µmol/L)
Control	14.49±0.89	19.48±1.89	1.35±0.09
Combined routes	9.54±0.47*	15.45±0.64*	0.95±0.08*
Oral route	10.73±0.73*§	15.78±1.42*§	1.04±0.08§*
Dermal route	13.32±1.0*§§	18.79±1.23§§	1.16±0.06*§§

*Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student's t test. §p <0.05 is significant when control, oral and combined routes were compared and §§ p < 0.05 is significant when control, dermal and combined were compared using ANOVA, n = 6.*

Table 2: Serum magnesium and trace element concentrations

	Fe (µg/dl)	Mg (mmol/L)	Cr (nmol/L)	Mo (nmol/L)	Co (nmol/L)	Mn (nmol/L)
Controls	113.01±5.94	0.89±0.09	200.00±10.11	9.94±0.48	6.78±0.52	120.67±12.02
Combined routes	87.04±9.00*	0.57±0.08*	170.53±12.01*	7.26±0.61*	5.13±0.44*	84.04±10.38*
oral	99.44±6.98*§	0.76±0.05*§	202.64±20.04	8.75±0.54*§	5.29±0.63*§	90.47±11.00*§
Dermal	109.83±7.10§§	0.83±0.03§§	190.77±21.05	8.95±0.59*§§	6.10±0.71*§§	100.16±9.68*§§

*Results are expressed as mean ± standard error of mean. *p < 0.05 is significant when compared with control using Student's t test. §p <0.05 is significant when control, oral and combined routes were compared and §§ p < 0.05 is significant when control, dermal and combined were compared using ANOVA, n = 6.*

Table 3: Serum levels of antioxidant vitamins

	Vitamin A (µmol/L)	Vitamin C (mmol/L)	Vitamin E (µmol/L)
control	2.61±0.08	50.64±7.92	18.09±1.09
Combined routes	1.99±0.03*	41.56±4.31*	12.60±1.21*
Oral	2.06±0.05*§	44.76±6.02*§	13.07±2.15*§
Dermal	2.25±0.06*§§	46.04±7.99*§§	14.56±3.06*§§

*Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student's t test. §p <0.05 is significant when control, oral and combined routes were compared and §§ p < 0.05 is significant when control, dermal and combined were compared using ANOVA, n = 6.*

Table 4: Serum vitamin levels in rats administered with trace quantity of kerosene

	Riboflavin (nmol/L)	Folic (nmol/L)	Niacin (nmol/L)	Thiamine (nmol/L)	Pyridoxine (nmol/L)	Pantothenic acid (µmol/L)	Vitamin D (nmol/L)
control	984.63±35.81	20.79±1.06	65.85±5.75	124.09±9.04	90.11±6.90	1.99±0.23	138.99±9.82
Combined routes	865.30±29.00*	15.16±2.51*	49.49±5.64*	91.32±6.00*	79.19±5.30*	1.61±0.21*	97.33±7.34*
Oral	900.87±25.94*§	16.06±1.11*§	55.17±4.49*§	102.04±8.07*§	82.45±5.08*§	1.61±0.14*§	106.03±6.20*§
Dermal	906.60±36.82*§§	18.22±0.98*§§	59.05±7.06*§§	120.74±6.54§§	87.04±7.52§§	1.80±0.19*§§	124.60±9.55*§§

*Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student's t test. §p <0.05 is significant when control, oral and combined routes were compared and §§ p < 0.05 when control, dermal and combined were compared using ANOVA, n = 6.*

study the toxic effect of trace amount of kerosene and the application was carried out daily between the hour of 10:00 and 12:00. Due to the volatility of the components of this product, kerosene was mixed thoroughly with the feed daily.

Serum vitamins and elements estimation: At 10: 00 hr of the day after the 21st kerosene exposure, blood was withdrawn through retro-orbital bleeding and discharged into an anticoagulant free bottle. Serum was separated by centrifugation at 3000 g and stored in a refrigerator at -20 °C. The following vitamins: riboflavin, folic acid, thiamine, niacin, pantothenic acid, and vitamins A, B_6, C, D and E were estimated in the serum using the High Performance Liquid Chromatographic technique (HPLC). The HPLC equipment supplied by Waters® Corporation Milford, Massachusetts USA was employed for this purpose. Serum levels of Zn, Cu, Se, Mn, Mg, Co, Cr, Fe and Mo were determined using the Atomic Absorption Spectrometric method. Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, Connecticut, USA) was used for the estimation.

STATISTICAL ANALYSIS

A p value of ≤ 0.05 was considered significant, after the results obtained were subjected to statistical analysis using Student's t test and analysis of variance (ANOVA). Student's t-test was used to determine the level of significant difference between the serum levels of the vitamins and serum elements of control and each of the treatment groups whereas inter-group comparison among the three different routes of exposure and the control was carried out by using analysis of variance (ANOVA).

RESULTS

The results of the study are presented in Tables 1-4 below.

DISCUSSION AND CONCLUSION

The fact that many of these micronutrients that are known to play significant role in maintaining the genome were significantly altered, has once again raised the danger of constant exposure to kerosene even at its trace amount. Studies exist that have linked many of the elements with enzymes of the repair system and conditions resulting from oxidative stress. A study by Ferguson et al[3] revealed that blood selenium levels that were lower than 100 ng/ml was a cause of impaired DNA repair as well as surveillance of oxidative (and other) DNA damage in middle-aged New Zealand men. To support that various depletions recorded for many of these micronutrients may have grave consequences for exposed rats is the fact that even marginal deficiencies in folate, vitamin B12, niacin and zinc impact significantly on spontaneous chromosome damage rate[4]. Moreover, dietary zinc level has been identified to influence both transplanted tumor growth and the carcinogenicity of several organic compounds[5].

In most cases spontaneous chromosome damage may not result in serious pathology, since the body is equipped to repair DNA molecule, but in oxidative stress- states in which great depletions in the levels of micronutrients that are essential for the replication and repair processes occur, derailment in homeostatic balance may result leading eventually to genomic instability. Genomic instability is known to compromise the integrity of the genome, an event capable of initiating fundamental events leading to human diseases[6-8].

This may be one of the reasons why cancer and related disorders are commonly found in experimental animals exposed to kerosene. For instance, chronic treatment of animals with some of the constituents in kerosene (benzene, toluene, xylene, naphthalenes, alkyl benzenes, and various alkyl PAHs) caused changes in the liver as well as harmful effects on the kidneys, heart, lungs, and nervous system. That DNA damage is probable as a result of micronutrient depletion can be deduced from earlier observation in which increased rates of cancer, immunological, reproductive, fetotoxic, and genotoxic effects have also been associated with some of the compounds found in kerosene. This can also be substantiated by the fact that when PAHs do degrade through metabolism, they often break down into even more toxic, carcinogenic, and mutagenic compounds.

It has also been postulated that micronucleus (MN) index that is a biomarker of DNA damage and is usually elevated in developmental and degenerative diseases and is a well established index that is predictive of increased cancer risk and cardiovascular disease has been extensively linked with alterations in micronutrient levels[4]. The problem with many African countries to which Nigeria is a member is that micronutrient deficiency (malnutrition) is a common occurrence even without xenobiotic exposure, therefore continuous exposure of human subjects to this product may be more dangerous than observed in these well nourished rats. Apart from many of above postulated medical conditions that have been suggested to probably manifest in kerosene exposed individuals, micronutrient deficiency (malnutrition) has also been documented to adversely affect the thymus gland, this means kerosene-induced immune comprise is also a possibility.

Major constituents of kerosene such as alkanes and cycloalkanes; benzene and substituted benzene and naphthalene and substituted naphthalenes are metabolized to yield reactive species that may be deleterious to a living system. Like many of the other endogenously derived free radicals that are detoxified in a healthy state by the endogenous antioxidant enzymes such as SOD, CAT, GS peroxidase (GPX) and GST which are the first-line cellular defenses against oxidative stress. These are enzymes that are involved in decomposing oxygen and H2O2 before they interact to form the more reactive hydroxyl radical (OH). Many of these enzymes have micronutrients as their co-enzyme or co-factor[9].

The implications of these significant decreases are diverse, apart from their antioxidant roles and the fact that their depletions may increase the risk of oxidative stress-induced diseases in exposed individuals; other physiologic functions of these micronutrients may be adversely affected. For example, deficiency of vitamins A, C and E is associated with abnormal immune response. While the active form of vitamin D is known to modulate both innate and adaptive immune responses[10,11] through its role as an immunomodulator that targets different immune cells, especially monocytes, macrophages, dendritic cells (DCs), as well as T-lymphocytes and B-lymphocytes, vitamin C in association with Zn is known to play a role in immune defenses. On the other hand, vitamin A regulates cell-mediated and humoral-mediated immunity, and when it occurs as retinoic acid, it impacts on leukocyte growth and differentiation[12] and vitamin E Supplementation is known to stimulate both cell mediated and humoral immune functions in humans ad experimental animals[13].

REFERENCES

1. Banse T (2010) Kerosene Outboards: An Alternative Fuel?. Marine Engine Digest. http://marineenginedigest.com/specialreports/kerosene-outboards.htm.
2. Iyanda AA (2012) Changes in serum markers of oxidative status in Wistar rats following exposure to kerosene. J Exp & Integ Med (in press)

3. Ferguson LR. Philpott M, Karunasinghe N (2004) Dietary cancer and prevention using antimutagens. Toxicology 198(1-3): 147-59.
4. Fenech M (2002) Micronutrients and genomic stability: a new paradigm for recommended dietary allowances (RDAs). Food Chem Toxicol 40(8), 1113-7.
5. Nordberg GF, Andersen O (1981) Metal interactions in carcinogenesis: enhancement, inhibition. Environ. Health Perspect, 40: 65-81.
6. Czupryn M, Falchuk Kh, Stankiewicz A, Valle BL (1993) A Euglena gracilis zinc endonuclease. Biochemistry 32: 1204-11.
7. Stano NM, Chen J, Mc Henry CS (2006) A coproofreading Zn(2+)-dependent exonuclease within a bacterial replicase. Nat Struct Mol Biol 13(5): 458-9
8. Dion S, Demattéi MV, Renault S (2007) Zinc finger proteins: tools for site-specific correction or modification of the genome. Med Sci (Paris), 23(10): 834-9.
9. Yim MB, Chock PB, Stadtman ER (1990) Copper, zinc superoxide dismutase catalyzes hydroxy radical production from hydrogen peroxide. Proc Natl Acad Sci U S A 87: 5006–10.
10. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C (2010) Vitamin D: modulator of the immune system. Curr Opin Pharmacol 10 (4): 482-96
11. Chambers ES, Hawrylowicz CM (2011) The impact of vitamin D on regulatory T cells. Curr Allergy and Asthma Reports 11(1): 29-36
12. Pino-Lagos K, Guo Y, Noelle RJ (2010) Retinoic acid: a key player in immunity. Biofactors 36 (6): 430-6
13. Pekmezci D (2011) Vitamin E and immunity. Vitamins and Hormones 86: 179-215