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Kerosene: A Study of Serum Vitamin Levels of Female Wistar Rats Chronically Exposed

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Author's contribution

The corresponding author is solely responsible for this research work.

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ABSTRACT

Background: Kerosene is a commonly available product used for cooking and lighting purposes in many parts of Asia and Africa where it is sold in beverage bottles and jerry cans in both commercial and residential places because of inadequate filling stations. Therefore excessive exposure through both dermal and oral routes is common.
Objective: This study is embarked upon to determine the impact of trace amount of kerosene on serum vitamin levels in female Wistar rats.
Methods: Kerosene (0.4 ml/kg body weight) was administered to rats either through the oral or dermal route daily for a period of 30 days and the levels of vitamins were estimated using the high performance liquid chromatography technique.
Results: Using Student t test only pantothenic acid was not significantly ($p>0.05$) different when oral or dermal group was compared with control, all other vitamins were significantly decreased ($p<0.05$), Moreover, using ANOVA, riboflavin, folic, niacin and vitamins A and D were more depleted in rats in oral route of administration than those in dermal group.
Conclusion: The results of this study suggest that exposure to this product either through the oral or dermal route may be detrimental to health as it induced vitamin depletion.

Keywords: Kerosene; vitamin; wistar rat; chronic exposure.

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1. INTRODUCTION

Kerosene, a product obtained through fractional distillation of petroleum crude and widely used all over the world for a variety of purposes, has been recognized as a source of occupational hazard. Specifically, its hazardous effect has been documented in fuel attendants working in filling stations as well as workers who come in contact with JP-8 jet fuel, a kerosene-based product, known to contain both gasoline and kerosene. In non-electrified parts of Asia and Africa especially, kerosene is used for lightening and cooking purposes. It is widely used in both rural and urban Nigeria and since filling stations are not commonly found in some parts of these regions, this has created the need for the hawking of this product. These hawkers sell this product in open containers and beverages bottles, a situation which can lead to constant exposure to this product, most especially through the dermal route. Moreover, since its sale is not restricted to commercial centers (e.g. market places), but is also sold in homes, the possibility of this product contaminating food items can also not be ruled out, which raises the likelihood of further exposure through the oral route.

Data obtained from the study of Rao et al. [1] have established the toxic effects of subcutaneously administered kerosene in albino rats. Moreover, the nephrotoxic and hepatotoxic effects of kerosene have also been studied; but the aim of that study has been to determine its LD_{50} [2] rather than the impact of trace quantity as it is commonly experienced by these hawkers. Furthermore, because a number of agents and products that have been recognized as sources of occupational hazard have been linked to abnormality of serum vitamin levels as well as clinical manifestations of such deficiency [3] it becomes imperative to investigate if exposure to trace quantity of kerosene is capable of such changes.

Vitamins are organic compounds that play important physiologic roles in the body (e.g. vitamin D in bone formation). Shenkin et al. [4] have recognized that they are required in trace quantities from the diet, usually in micrograms to milligrams level per day, to maintain health, sustain growth and reproduction. Some of these important roles are as follows; L-ascorbic acid is an important co-factor for hydroxylases and monooxygenases; it prevents the oxidation of low density lipoprotein (LDL) and is essential in wound repair and healing/regeneration process as it stimulates collagen synthesis. Vitamin A plays an important role not only as retinal in vision but also in growth, reproduction, immune response and embryonic development [4]. Vitamin E on the other hand is essential in reproduction, as well as inhibition of free-radical chain reactions of lipid peroxidation. Folate acts as a coenzyme in several single carbon transfers involved in biosynthesis of purine nucleotides and deoxythymidylic acid essential for DNA and RNA synthesis and its deficiency has been associated with increase risk of genome instability and birth defects, particularly neural tube defects (NTDs) [5,6].

This study is designed to identify the impact of trace amount of kerosene on serum vitamins in Wistar rats which Rao et al. [1] have used as an ideal model for the study of kerosene exposure. This study will investigate the impact of this agent in these animals not only through the oral route but dermal one as well.

2. MATERIALS AND METHODS

2.1 Petroleum Product

Petroleum products namely kerosene was purchased from Mobil filling station located in Osogbo, Osun State, Nigeria in December, 2011.

2.2 Experimental Animals

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). Matured female albino rats of average weight of 250 g were obtained from the Animal House attached to 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 housed in cages at ambient temperature of 23±3ºC and a 12 h light, 12 h dark cycle. All the animals were fed with their specific diets and water *ad libitum*.

2.3 Experimental Animals

Eighteen rats were separated into 3 groups comprising of 6 rats per group. The first group was treated with kerosene through dermal route while the second group was administered through the oral route (as contaminant of feed). Six rats served as the control. The treatment groups were exposed to this product for a period of 30 days and 0.4 ml of kerosene/kg body weight was adopted as quantity sufficient to study the toxic effect of trace amount of kerosene after the study of Rao et al. [1]. Dermal exposure was carried out by discharging kerosene directly on the skin of each rat. Due to the volatility of the components of this product, kerosene was mixed thoroughly daily with the feed. To prevent cage mates from grooming and ingesting the fuel, rats in the dermal route of exposure were held individually in their cages for the entire duration of the experiment and kerosene was administered high on the back of each rat immediately behind the head to prevent self grooming.

2.4 Preparation of Serum Samples & Vitamin Estimation

On the 31st day, blood was withdrawn through retro-orbital bleeding and introduced into an anticoagulant free bottle. Serum was separated by centrifugation at 3000 rpm and stored in a refrigerator at -20ºC. The measurement of serum concentrations of folic acid, thiamine, niacin, riboflavin, pantothenic acid, and vitamins A, B_6, C, D and E was performed using the High Performance Liquid Chromatographic technique. The HPLC equipment supplied by Waters® Corporation Milford, Massachusetts USA was used for this purpose. Established methods were used for the determination of these vitamins. For example, vitamins D and E were determined as described by Piccione et al. [7]

2.5 Statistical Analysis

The mean values of the serum levels of the vitamins for control and each of the treatment groups were compared using the Student's t-test. Analysis of variance was used to test inter-group comparison. Value of $p \leq 0.05$ was considered significant.

3. RESULTS

The results of serum vitamin levels are presented in Tables 1 & 2. Treatment of rats with kerosene through the dermal or oral route resulted in significant decreases ($p<0.05$) in the serum levels of vitamins A, C and E using both Student's t test and analysis of variance (ANOVA) as shown in Table 1. The serum levels of riboflavin, folic acid, niacin, thiamine, pyridoxine, vitamin D and pantothenic acid are presented in Table 2. Using Student's t test all the vitamins in Table 2 were significantly reduced ($p<0.05$) in rats in both dermal and oral routes compared with control except pantothenic acid that was not significantly different ($p>0.05$). Moreover, in Table 2 statistical analysis using ANOVA revealed significant differences ($p<0.05$) when control, dermal and oral groups were compared for riboflavin, folic acid, niacin, thiamine, pyridoxine and vitamin D, pantothenic acid though was not significantly different.

Table 1. Serum levels of antioxidant vitamins

	Vitamin A (µmol/L)	**Vitamin C (mmol/L)**	**Vitamin E (µmol/L)**
X ± SD (control)	2.70±0.11	44.09±3.01	20.69±0.93
Oral			
X ± SD	1.80±0.11	35.00±0.74	14.90±0.36
P	0.008*	0.015*	0.003*
Dermal			
X ± SD	2.23±0.14	22.22±2.04	14.78±2.49
P	0.010*	0.002*	0.039*
F-value	132.81	200.08	222.01
p-value	0.001§	0.006§	0.006§

*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 using ANOVA.*

Table 2. 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)
X ± SD (control)	1093.94±28.59	7.86±0.72	70.01±3.07	137.63±7.68	74.50±2.31	2.09±0.29	134.36±6.01
Oral							
X ± SD	612.38±43.66	13.44±0.23	40.41±1.08	94.89±2.50	69.73±1.64	2.10±0.19	90.12±12.50
P	0.001*	0.004*	0.001*	0.003*	0.005*	0.893	0.003*
Dermal							
X ± SD	743.46±30.07	15.48±0.44	48.59±1.11	114.75±7.28	62.01±1.47	1.95±0.11	109.97±27.06
P	0.003*	0.013*	0.007*	0.009*	0.002*	0.158	0.039*
F-value	17.35	150.25	219.09	104.71	16.55	1.41	8.43
p-value	0.005§	0.002§	0.001§	0.003§	0.004§	0.283	0.010§

*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 using ANOVA.*

4. DISCUSSION

This study revealed significant alterations in the levels of many of the serum vitamins compared with the control, specifically riboflavin, thiamine, pyridoxine, folic acid and the antioxidant vitamins A, C and E were significantly decreased. Moreover, these decreases were more pronounced for rats in the oral group than dermal route. These significant differences might have arisen through a modification in a number of physiologic processes. For instance, most of these vitamins are absolved in upper small intestine and for the rats in the oral route of exposure, the possibility of this product affecting the functional integrity of this section of the gastro-intestinal tract can not be ruled out. That as well as interaction between kerosene and the vitamins in the feed might have resulted in decrease digestion and delayed or hindered absorption, but the fact that both water and lipid soluble vitamins were significantly decreased suggests generalized vitamin malabsorption. Rats in the dermal route also might have experienced such changes; that kerosene can be extensively distributed in different organs has been confirmed by the observation of Rostami et al. [8]. The results of their study revealed that abrogated traditional treatment of hemorrhoids with local kerosene injection was probably a cause of death through heart attack one month after initial exposure.

Kerosene is one of the toxic hydrocarbons to which lower-level exposures through dermal absorption, pulmonary inhalation, or oral ingestion routes may occur. According to Ritchie et al. [9]; Baynes et al. [10]; Koschier et al. [11] as well as Ritchie [12], some of the consequences of such exposure may include any of the following; damaging effects on the nervous system, asthma, allergies, infertility, miscarriage, and child behavior disorders especially learning disabilities, mental retardation, hyperactivity, and attention deficit disorders. These were described after prolonged and chronic kerosene exposure, although there is little epidemiological evidence for fuel-induced death, cancer, or other serious organic diseases. The results of this study of significant alteration in many of the serum vitamin levels raises the possibility that many of these diseases associated with kerosene exposure may also be related to serum vitamin abnormality. To postulate a possible role for altered vitamin levels in many of the related disorders of kerosene exposure may rest on the fact that the beneficial effects of vitamins cut across all or nearly all tissues.

One of the notable kerosene-exposure related disorders that have also been closely associated with vitamins is the abnormal immune response. Vitamins, such as vitamins A, C and E which have been implicated in the processes of immune response were significantly altered. Specifically 1,25-dihydroxyvitamin D(3) (1,25(OH)(2)D(3)), the active form of vitamin D, apart from its role in regulating calcium and phosphorus metabolism and therefore being a key-player in bone-formation is also an immunomodulator targeting different immune cells, especially monocytes, macrophages, dendritic cells (DCs), as well as T-lymphocytes and B-lymphocytes. This means it therefore modulates both innate and adaptive immune responses [13,14] and abnormality in vitamin levels may be the mean by which kerosene-induced abnormal immune response occurs.

Maggini et al. [15] have also indicated that deficiency of vitamin C and zinc adversely affects the physical and mental growth of children and can impair their immune defenses. For over a century, vitamin A has been implicated as an essential dietary component in host resistance to infectious disease although it is only recently that studies have elucidated the cellular and molecular mechanisms of how vitamin A regulates cell-mediated and humoral-mediated immunity, most especially as retinoic acid impacts on leukocyte growth and differentiation [16].

Vitamin E on the other hand, though the most important chain-breaking, lipid-soluble antioxidant present in body tissues of all cells and considered the first line of defense against lipid peroxidation also plays a significant role in normal functioning of the immune cells. Such that impairment of both T- and B-cell functions occurs in vitamin E deficient states.

The results of this study seem to suggest that alteration in serum vitamin levels will accompany exposure to trace quantity of kerosene, and since micronutrients (e.g. vitamins and trace elements) are components of the antioxidant system, cofactors of enzymes, components of transcription factors, and epigenetic modulators, and there are also indications that they influence various metabolic processes that are directly associated with immune functions there seems to be a need to enact laws that will prevent such exposure. To support such postulation i.e. that many of the manifestations of kerosene exposure may be vitamin depletion-induced is the fact that there seems to be an overlap in immune manifestations of vitamin deficiency and kerosene exposure. Ullrich & Lyons [17] have linked dermal application of JP-8 (kerosene-based) jet fuel to immune suppression; classic delayed-type hypersensitivity as well as the induction of contact hypersensitivity to allergens applied to the shaved skin of JP-8-treated mice was suppressed. Apart from this, there was inability of T cells isolated from JP-8-treated mice to proliferate in vitro. The mechanism by which application of JP-8 through the dermal route suppresses cell-mediated immune reactions appears to be through the production of immune biological-response modifiers, some of which have been linked with vitamins.

What may make continuous exposure to this product a dangerous thing in human subjects in these regions is that malnutrition (which is also a common feature in many parts of Africa) has also been documented to adversely affect the thymus gland, where T lymphocyte development takes place. Malnutrition has been confirmed to cause severe thymic atrophy, resulting from massive thymocyte apoptosis especially of the immature CD4+CD8+ cell subset as well as decline in cell proliferation [18]. This is because the thymic microenvironment especially the non-lymphoid compartment that drives intrathymic T-cell development is also affected in malnutrition: this is more so for morphology of thymic epithelial cells, in which definitive changes have been reported.

5. CONCLUSION

In conclusion, data obtained from this study suggest that exposure to 0.4 ml kerosene per kg body weight through either the dermal or oral route is associated with lower serum levels of vitamins.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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