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A Study of Serum Levels of Select Heavy Metals and Histology of Brain, Lung, Heart and Ileum of Rats Chronically Exposed to Kerosene

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Author's contribution

The corresponding author AAI is solely responsible this research work.

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ABSTRACT

In rural Africa, a number of alternative treatment options are explored because of poverty and unawareness. Kerosene is one such agent used for the treatment of a myriad of diseases. The aim of the study was to investigate the effects of trace amounts of kerosene on lung, heart, brain and intestine in rats. Eighteen rats were used consisting of 6 rats per group. The first and second groups were administered kerosene (0.4 ml of kerosene/kg body weight) through oral and dermal route respectively. Six other rats served as the control. Results showed significant damage to all the tissues examined; with pathologic presentation comprising of pulmonary congestion, severely stunted villi, congestion of coronary vessels, and diffuse spongiosis of the cerebral cortex in kerosene administered group while control group featured no visible lesion. Although As, Al and Cd were not significantly different in kerosene exposed groups compared with control, Si was significantly lower (oral), and significantly increased (dermal) ($p<0.05$) compared with control. Results of this study suggest that exposure to even small quantities of kerosene may damage a variety of organs/tissues in the body and its exposure to human subjects for whatever reason should be discouraged.

Keywords: Heavy metals; kerosene; female rats; histology of tissue.

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1. INTRODUCTION

World Health Organization Reports have identified that the use of traditional medicine is common in most of the developing countries of the world [1]. This is especially so, among not only the rural Africans but among urban dwellers as well. Treatment obtained from orthodox medical practitioners is usually supplemented with traditional medicine [2]. The use of crude oil for medical reasons though common only among Nigerians dwelling in rural South-south region of the country [3], its refined products are used as traditional medicine all over the six geo-political zones.

The use of kerosene and all other petroleum products is widespread because many people hold tenaciously to the erroneous belief that they are effective in the treatment of a number of ailments [1]. Apart from this, some other reasons for such widespread use include poverty [4], inability to access quality orthodox medicine, illiteracy and ignorance. Crude oil or its refined products are used in a variety of ways and for a number of reasons. Its application through the skin or being served as drink mixed with alcohol or water has been reported [3]. Exposure to the body through the nostrils, ears, anus, vagina and urethra is equally common. Many of the medical conditions with which kerosene- as a therapeutic agent -has been linked include gastrointestinal disorders, burns, 'foot rot' and leg ulcers, and poisoning; it is also used in cases of witchcraft [5]. Its use for the treatment of skin and eye infections such as conjunctivitis, eczema and scabies has also been described [6].

Even with all the possibility of the effectiveness of kerosene as traditional medicine in the treatment of these conditions, reports are available that suggest that ingestion of kerosene either accidentally or intentionally is toxic. For example Prasad et al. [7] have raised the possibility of bilateral hemorrhagic pleural effusion due to kerosene aspiration occurring in human subjects subsequent to exposure to large quantities of kerosene. The liver, the most abundant source of the enzymes cytochrome P450s had earlier been studied in relation to its response to kerosene exposure with the results obtained showing that this product is both hepato and nephrotoxic [6]. The goal of the current study was to determine the impact of trace element of quantity on the lung, heart, brain and ileum of female rats exposed to trace quantities of kerosene. Moreover, since heavy metal-induced oxidative stress is a common phenomenon, the levels of select heavy metals (aluminum, silicon, cadmium, arsenic, lead, nickel) will also be assessed, so as to establish if kerosene-induced tissue damage is associated with abnormal heavy metal presentation.

2. MATERIALS AND METHODS

2.1 Petroleum Product

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). Adult female albino rats of average weight of 230 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±3°C and a 12 h light, 12 h dark cycle. All the animals were fed with their specific diets and water *ad libitum*.

Eighteen rats were divided into 3 groups comprising of 6 rats per group. The first group was treated with kerosene oral route (as contaminant of feed), the second through the dermal route while the third group 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. [8]. Due to the volatility of the components of this product, kerosene was mixed thoroughly with the feed daily while for the rats in dermal group application was directly to the skin.

2.3 Blood Sampling and Heavy Metal Estimation

At the end of the experimental period, blood sample was drawn from each rat by retro-orbital bleeding and introduced into an anticoagulant free tube. The blood was centrifuged at 3000 g after which serum was separated and stored at -20ºC. Serum levels of aluminum, silicon, cadmium, lead, arsenic, nickel were carried out using the atomic absorption spectrometric method [9]. Buck Scientific 205 Atomic Absorption supplied by Buck Scientific (East Norwalk, Connecticut, USA) was utilized for this purpose.

2.4 Histopathology

Approximately 1 g of tissue (lung, heart, brain, and ileum) section was cut and fixed in 10% neutral buffered formalin. The tissue was embedded in paraffin block and cut in 5 µm sections using motorized rotary microtome. After which they were stained with haematoxylin and eosin (H&E), then slides were examined under compound light microscope and photographed and histopathological changes were assessed. Mag. x 400.

2.5 Statistical Analysis

The mean values of the serum levels of the heavy metals 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

While the serum levels of heavy metals are shown in Table 1, the histology results of tissues of the brain, lung, heart and ileum are presented in Figs 1-3. In Table 1: Serum levels of As, Cd and Al were not significantly different ($p>0.05$) in oral or dermal group when compared with control. On the other hand, while Si was significantly reduced in the oral group, it was significantly elevated in the dermal group ($p<0.05$). Results of other two heavy metals; Ni and Pb- revealed significant decreases for Ni (dermal) and Pb (oral) compared with control.

Histology results showed that oral administration caused moderate pulmonary congestion; the interalveolar septum appeared thickened (lung); severely stunted villi (intestine); congestion of coronary vessels (heart) and severe diffuse spongiosis of the cerebral cortex (brain) as shown in Fig. 1. On the other hand, administration through the dermal route resulted in expanded bronchial associated lymphoid compartment (lung); mildly stunted; villi (intestine); congestion of coronary vessels (heart) and diffuse spongiosis of the cerebral

cortex (brain) (Fig. 2). Control rats featured no visible lesion for all tissues as presented in Fig. 3.

Table 1. Serum levels of select heavy metals in control and kerosene administered rats

	As	Cd (mg/dl)	Si (µg/L) **	Ni(µg/L) **	Pb (µg/L) **	Al (µg/dl)
Controls	0.003±0.001	0.003±0.001	0.009±0.003	0.012±0.004	0.020±0.005	0.009±0.002
Oral	0.004±0.001	0.002±0.001	0.007±0.002*	0.011±0.003	0.010±0.003*	0.009±0.003
Dermal	0.004±0.001	0.004±0.001	0.015±0.004*	0.009±0.002*	0.021±0.005	0.008±0.002

*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.*

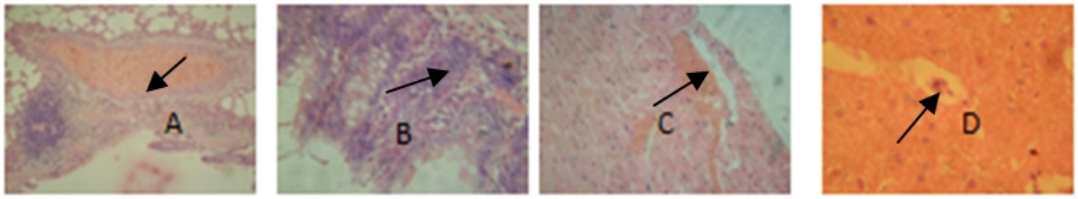

Fig. 1. A-(lung) There is moderate pulmonary congestion, the interalveolar septum appears thickened; B-(intestine) The villi are severely stunted; C-(heart) The coronary vessels are congested; D-(brain)- There is severe diffuse spongiosis of the cerebral cortex in oral kerosene exposed rats .

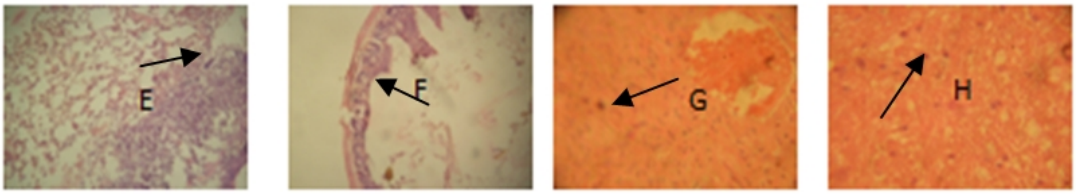

Fig. 2. E-(lung) The bronchial associated lymphoid compartment appears expended; F-(intestine) The villi are mildly stunted; G-(heart) The coronary vessels are congested; H-(brain). There is diffuse spongiosis of the cerebral cortex in dermal kerosene exposed rats.

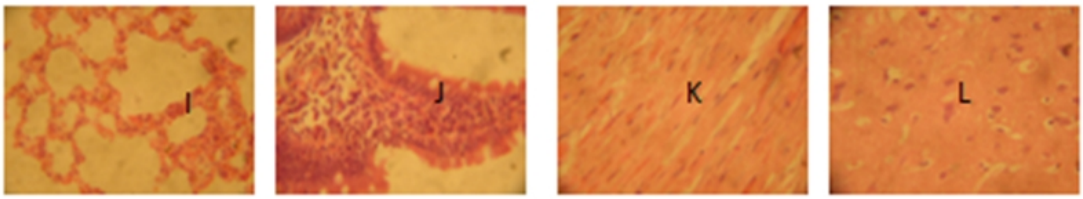

Fig. 3. I-(lung). J-(intestine). K-(heart) and L-(brain) of control rats showed no visible lesion.

4. DISCUSSION

An earlier observation has indicated that kerosene is toxic to the hepatic and renal cells [6]. Kerosene, a product consisting of different individual hydrocarbon compounds in different isomeric forms is believed to be metabolized through the CYP pathways. That earlier observation was linked to the rich source of different isoforms of CYP found in the liver and kidney. It is known that while for a number of xenobiotics, their toxicities are determined entirely by their metabolic processing through the CYP system, for some others both CYP processing as well as direct interaction between xenobiotics and target organs play significant role in toxic response e.g. acetaminophen and some carcinogens [10,11].). Although the intestine and the lung also possess appreciable amount of CYP activities, the brain and the heart possess small activities compared with the liver [12]. But all the tissues studied showed significant degree of damage as revealed by the histology results of these organs. This raises two possibilities that either a pathway of kerosene toxicity is possible apart from the CYP pathway, especially that of direct binding of any of the component compounds to these organs or that this product is profoundly toxic at such low doses.

The histologic presentation of different sections of brain of these rats administered with small amount of kerosene repeatedly, also suggests abnormality of the brain section, which may be the basis of mental retardation which has been linked with kerosene exposure in many subjects. For example, section of the brain showed severe diffuse spongiosis of the cerebral cortex in oral kerosene exposed rats. Vitamins which a previous similar study [13] has shown to be significantly lower in kerosene administered rats compared with control might have played a role in many of these abnormal presentations. This is because vitamins are known to play significant roles in the normal functioning of the brain.

Vitamin A is known to maintain higher function in the central nervous system [14] as well as protects neurons from the oxidant damage associated with neurodegenerative diseases [15] while data are available to support that vitamin D affect brain development [16,17]. Although oral and dermal routes were sites of kerosene administration to the rats used for this study, some other specific signs of kerosene neurotoxicity that have been identified in humans include ataxia, hypoactivity, and prostration of central nervous system which have been observed to occur through high inhalation exposures [18-21].

The histology results of this study showed significant level of damage to these organs as a result of kerosene exposure, but the implication of such damage may be compounded by the fact that many xenobiotics metabolize through the cytochrome pathway have either inhibitory or inductory action on the enzyme system. For example, alcohol is known to induce the activity of CYP2E1, the enzyme that metabolizes it as well as paracetamol, which makes chronic alcohol usage the basis of enhanced paracetamol toxicity in alcoholics. The possibility of such alteration in the CYP enzyme system post-kerosene exposure, especially if those enzymes take part in metabolic processing of therapeutic drug will suggest that apart from these damages, concurrent administration of orthodox and traditional medicine (kerosene) may affect the efficacy of some agents.

In all mammals, the role of the enzymes that metabolize kerosene components cannot be over emphasized since they play vital functions in the metabolism of not only exogenous compounds but endogenous ones as well. Studies abound to suggest that the activities of these enzymes can either be inhibited or induced by a number of agents. To raise the possibility of the constituents of kerosene modulating this system is the fact that not only naturally occurring but artificial compounds present in food such as fruits, herbs, vegetables

and alcoholic or soft beverages have been implicated to alter the activities of these vital enzymes [22-25]. These enzymes have great medical importance because of the role in the therapeutic effectiveness of many agents. It is therefore essential that both naturally occurring and artificial compounds do not cause failure of therapies leading to serious health alterations.

The small intestine another organ with substantial quantities of CYP enzymes is one of those organs responsible for cytochrome P450-dependent metabolism of various food-derived substances, drugs and toxic agents and has been confirmed to be rich in CYP enzymes that metabolize various components of kerosene [26]. Benet et al. [27]; Zhang and Benet [28] and Watkins [29] have highlighted that the metabolic processes which take place in the gut mucosa may affect not only the uptake but also efflux and transport of orally delivered pharmaceuticals and dietary xenobiotics by cells, tissues and organs.

The heavy metals As, Al and Cd [30] did not show significant difference in treated rats compared with control, which means that tissue damage and other possible abnormalities that have been noted to accompany kerosene toxicity will not be compounded by abnormal high levels of these metals. Therefore, these results raises the possibility that tissue damage always linked with kerosene toxicity is not As, Al and Cd/oxidative stress- induced. On the other hand, Si that is present in kerosene was understandably significantly higher in dermal group but the significant low level in the oral group can be associated with the abnormal histology of the intestine and may also be the basis of significantly low Pd level in the oral group compared with control.

Although it had earlier been established in acute study that large dose of kerosene are required to achieve both renal and hepatic toxicity in rodents [6], this study has shown that administration of small doses repeatedly in a chronic setting is equally capable of inducing significant toxicity. This kind of observation has been noted for other xenobiotics. For example, while ingestion of 200 mg/kg acetaminophen over 24 hours will cause toxicity in a child, chronic administration of this drug at a dose level as low as 4 g/day has also been reported to significantly elevate hepatic enzymes [31]. Another reason that can be adduced for such a wide variation in response to kerosene is that there exist not only inter-species but intra-species differences to xenobiotic administration even at the same level of exposure.

5. CONCLUSION

Results of this study raise the possibility of tissue damage (brain, lung heart, intestine) occurring as a result of kerosene exposure. Further studies are required to determine if these changes are permanent since recovery of tissue from toxic effects of kerosene is possible after kerosene administration has stopped.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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