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Research Article

Histopathologic manifestations of wistar rats exposed to virgin engine oil

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Abstract

Constant contact with used-engine oil has been identified as a cause of many biochemical derailments which eventually results in histopathological changes, yet only slight differences in chemical composition exists between used and virgin engine oil. In parts of Africa, virgin engine oil is widely applied by human subjects for both cosmetics and therapeutically purposes and hence, the present study was undertaken to evaluate the repeated daily exposure to virgin engine oil and its capability of causing tissue damage. Two groups of albino rats consisting of six rats per group were used for the study. The first group was administered with 1.0 ml/kg body weight of virgin engine oil through the oral route as contaminant of feed while the second group served as the control. The study was for a period of 30 days after which the rats were sacrificed and organs harvested. Sections of investigated tissues were fixed in 10% neutral buffered formalin. Haematoxylin and Eosin staining technique was used for histopathological study. Results of the study revealed that exposure to engine oil caused significant histological alterations to cells of liver, kidney, lung and intestine. Results of the study suggest that incessant exposure to virgin engine oil can lead to tissue damage.

Key words: Virgin engine oil, oral route, dermal route, histopathological changes.

Introduction

In Africa, engine oil has multipurpose utilization especially for cosmetics and therapeutics for human as well as farm animals. A survey conducted with the aid of structured questionnaires and general conversation among 59 resource-limited cattle farmers revealed wide disparity in methods used on frequency of the types of tick control methods employed. While frequency of usage of methods such as manual removal, and pouricides ranged between 1-5 % and farmers that utilize plants, mainly the leaf of *Aloe ferox* and the bark of *Ptaeroxylon obliquum* constituted 6.8%, as many as 10.2% of the farmers employed used engine oil as a mean of tick

control methods employed. While frequency of usage of methods such as manual removal, and pouricides ranged between 1-5 % and farmers that utilize plants, mainly the leaf of *Aloe ferox* and the bark of *Ptaeroxylon obliquum* constituted 6.8%, as many as 10.2% of the farmers employed used engine oil as a mean of tick control in cattle (1,2). The fact that the efficacy of the used engine oil on the treated group was found to be as high as 64.8% -in a study (3) performed in a peri-urban agricultural system at Botshabelo, a city in the south-eastern Free State has encouraged a more extensive utilization of this agent for tick control method.

A few studies have demonstrated toxic effects of exposure to used engine oil is extensive as shown through hematological markers and histologic features of sections of heart, kidney and spleen of the male rats (4). Since usually there are slight differences in the constituents of both used and virgin engine oil, it seems expedient that this study which focuses on the effects of virgin oil on different tissues of wistar rats be carried out. Personal communication with different categories of human subjects by the author revealed that, virgin oil is sometimes applied to the scalp to treat dandruff by some local hairdressers. Although there are indications that while it may eliminate dandruff, through personal observation of many hairdressers and their clients it seems it also discourages hair growth. This can be ascribed to possible poisoning of the hair follicles or that application of engine oil hinders hair growth in some other unidentified process. The objective of this study is to identify whether exposure to virgin engine oil is capable of causing damage to some other cell types/tissues (e.g. lung, brain, intestine, etc) in wistar rats.

Materials and Methods

Adult female albino rats weighing between 200-240 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 weeks 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 supplied with water without any form of restriction. Virgin engine oil (AP engine oil) used for the study was purchased from a filling station located in Osogbo, Osun State, Nigeria in October, 2011. The study was conducted according to laid down national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985).

Eighteen wistar rats used for the study were divided equally into 3 groups. The first group was administered with virgin engine oil through the oral route as contaminant of feed. On the other hand, engine oil was applied to the skin of the rats and this group served for dermal exposure. The rats in the control group were not exposed to engine oil. Dosage of exposure was 1.0 ml/kg body weight. The study lasted 30 days after which the rats were sacrificed and organs harvested.

From the brain, heart, lung, ileum, liver and kidney, approximately 1.0 g of tissue 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), and slides were examined under compound light microscope histopathological changes were determined.

Results and discussion

Many xenobiotics including therapeutic agents are associated with profound beneficial effects which they exert when consumed at tolerable levels. A great number of foreign substances though, are known to invoke serious pathological processes consequent to exposure. This is especially common with agents meant for other uses (e.g. industrial/mechanical) which have been adapted to human exposure to serve one purpose or the other. Petroleum products are examples of such agents may result in such pathological manifestations upon their direct and constant contact with human. Several mechanisms exist in mammals to prevent initiation of pathological changes after exposure to chemicals. The most common mechanism is that opposing the distribution of xenobiotic to a target tissue, by processes involved in binding to plasma proteins, presence of specialized barriers, and distribution to storage sites such as adipose tissue, association with intracellular binding proteins, and export from cells.

While agents like dichlorodiphenyltrichloroethane (DDT) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) that bind to high molecular weight proteins or lipoproteins in plasma, cannot leave the capillaries by diffusion, and in some other instances dissociation from proteins may be required for other xenobiotics to leave the blood and enter cells to cause toxicity, it seems that components of orally administered engine oil did not encounter such limitations as their harmful effects were felt in organs such as ileum, lung, liver and kidney. As shown in Fig 1, treatment of rats with 1.0 ml/kg of engine oil caused diffused vacuolar degeneration and necrosis of hepatocytes; mild interstitial thickening that might have resulted from proliferation of alveolar pneumocytes in the lung; stunted villi in the intestine; and protein casts in the renal lumen. The section of heart of engine oil-treated rats (Fig. 1) and all examined tissues of the rats in the control group showed no visible lesion (Fig. 2).

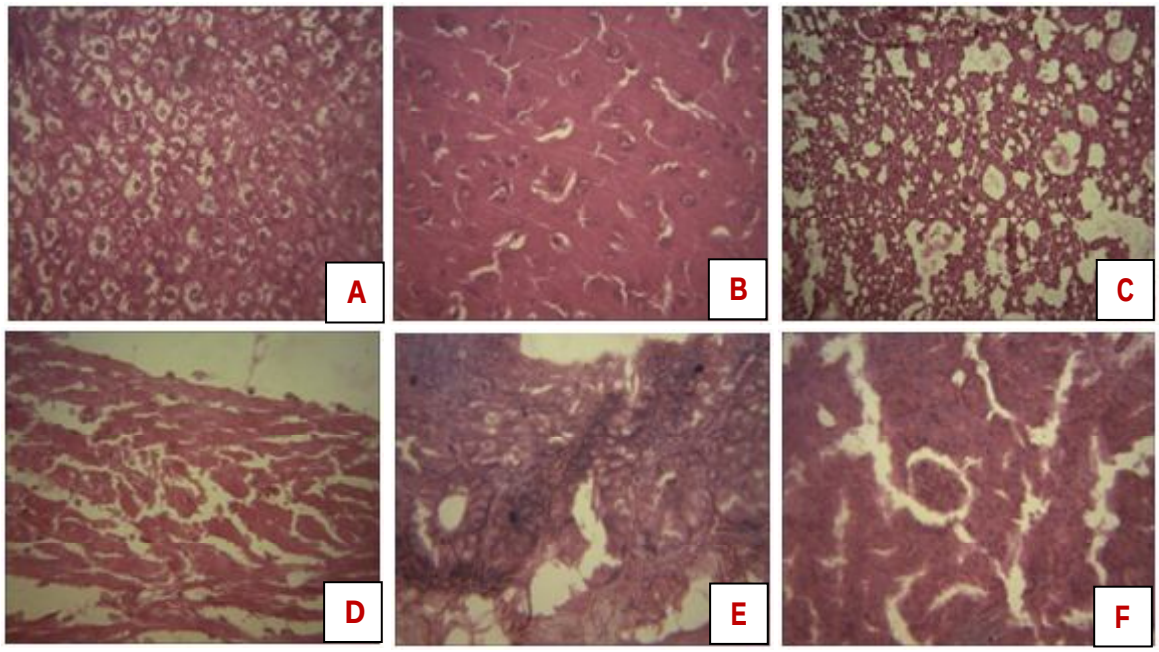

Fig. 1: Histologic presentations of different tissues of rats treated (oral route) with 1 ml/kg of engine oil. (A) liver (diffuse vacuolar degeneration of hepatocytes and necrosis), (B) brain (no visible lesion), (C) lung (mild interstitial thickening probably caused by proliferation of alveolar pneumocytes), (D) heart (no visible lesion), (E) intestine (the villi are slightly stunted), (F) kidney (tubules have protein casts in the lumen).

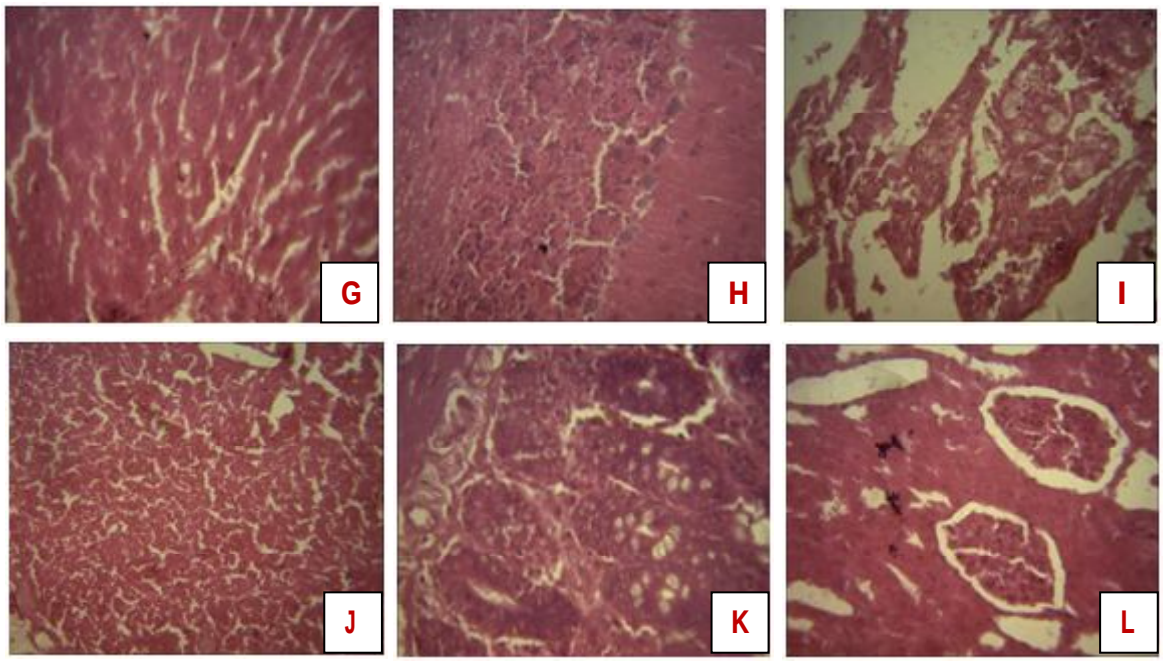

Fig. 2: Histologic presentations of different tissues of control rats (G) liver (no visible lesions seen), (H) brain (no visible lesions seen), (I) lung (no visible lesions seen), (J) heart (no visible lesion), (K) intestine (no visible lesions seen), (L) kidney (no visible lesions seen).

The brain, an organ with specialized barriers also did not feature any damage as revealed by results of histology in which no visible lesion was observed (Fig. 1). Brain capillaries are known for their very low aqueous porosity; which cannot be unassociated with the fact that their endothelial cells lack fenestrae and are linked by extremely tight junctions. Blood–brain barrier disallows the access of hydrophilic chemicals to the brain; this is with the exception of agents or their metabolites that can be actively transported. Components of engine oil are predominantly lipid in nature but their metabolism cannot rule out generation of water soluble intermediates or end-products. As a result of tight junctions that limit paracellular diffusion as well as influence of drug efflux transporters and drug-metabolizing enzymes, the blood brain barrier has been viewed by some as a metabolic and somewhat physical barrier that selectively controls brain penetration of xenobiotics (5).

While the results of this study showed that there were no significant alterations to the brain cells, results of another study (6) showed that kerosene-another petroleum product, induced alterations to brain cells. The differences in the result outcomes of both studies can be linked to differences in composition of kerosene and engine oil, degree of exposure, and route of administration. Differences in composition have been implicated as being a cause of variations in many of their chemical properties e.g. density (7). Moreover, it has also been indicated that the composition of petroleum product of even the same type (e.g. engine oil) varies widely and depends on the original crude oil, the process used during refining, and differences in the types of additives used (8).

The toxic effects of virgin engine oil on the ileum, lung, kidney and lungs may not be unassociated with the biological fate of the chemical components of this petroleum product. The response of an organism to a substance usually depends on the individual properties of the specific chemicals in the oil. For example, it has been revealed that chronic effect of naphthalene, a component of engine oil includes alterations in the liver and harmful effects on the kidneys, heart, lungs and nervous system (9). Aside these tissues, information obtained from a case report involving a human subject showed that alteration in the morphology or functions of many organs can occur. Virich *et al.* (10) reported that a 64-year-old male with longstanding occupational exposure to used engine oil - manifested extra mammary Paget's disease of the left scrotum and groin. In addition, animals that have been exposed to polyaromatic hydrocarbons (PAHs) contained in petroleum products are known to exhibit substantial risk of skin cancer.

In many instances, the moment the repair system is over-whelmed tissue damage occurs which in at few cases have led to cell death e.g. necrosis or apoptosis. Although both processes have some common features, these results which showed necrotic cell death in hepatic and renal cells have implication for surrounding tissue. As it has been reported by Kim *et al.* (11); Kroemer *et al.*(12); and Quian *et al.*(13) that both of them are types of cell death caused by cytotoxic agents that may involve similar metabolic disturbances and mitochondrial permeability transition. It cannot be ruled out that exposure to engine oil at a lower dosage than the one employed for this study will not induce apoptosis in the brain of engine oil-treated rats using appropriate tests, since it is an established fact that the severity of the insult determines the type of cell death.

In addition, it is understood that many toxicants induce other manifestations at low exposure levels or early after exposure at high levels, although they cause necrosis later or at high exposure levels. This result portrays that 1.0 ml/kg level of exposure is high enough to cause cellular damage especially necrosis. This seems to be in agreement with the observations made by many workers in past studies (11,14,15). According to Kim *et al.* (11); and Rodriguez- Enriquez *et al.* (14), large dose exposure causes necrotic cell death rather than apoptosis; this is because large dose exposure incapacitates cells from undergoing apoptosis. Necrosis occur when any of the following cellular events is present as a result of chemical insult; elevation in number of mitochondria undergoing mitochondrial permeability transition, reduction of ATP, and failed activation of caspases or their inactivation (15).

This position has been corroborated through many studies in which a model was proposed that indicates that result outcome of toxicity depends on the number of mitochondria undergoing mitochondrial permeability transition and this also varies with quantity of chemical exposure. According to them when only a few mitochondria develop mitochondrial permeability transition, they are eliminated by lysosomal autophagy and the cell survives whereas when more mitochondria suffer mitochondrial permeability transition, the autophagic mechanism becomes exhausted, and the released proapoptotic factors such as cyt *c*, Smac, AIF, initiate caspase activation and apoptosis, a situation in which nearly all mitochondria are involved, usually lead to cytolysis. The histology results of this study, which presented damage- in tissues such as liver, kidney, lung, etc cannot be disassociated from the fact that vitamin E may also be significantly decreased in such rats. According to Kang (16) no lipid fragmentation in microsomal membranes takes place as a result of toxicant exposure until alpha-tocopherol is depleted in those membranes.

Conclusion

It is therefore -speculated that the levels of vitamin E and other antioxidant vitamins needs to be evaluated to determine the relationship between these histological presentations and antioxidant vitamins. Engine oil is commonly being used for both cosmetic and therapeutic purposes In Africa, the vicious cycle of poverty and inability to access orthodox medicine have resulted in usage of virgin engine oil for - therapeutic purposes. The findings of this study have clearly demonstrated, the tissue damage - in the experimental animals indicating concerns of serious threat to human and animal health. In essence, this study envisages the fact that the use of engine oil for such cosmetic and therapeutic purposes has to be completely discouraged which may be detrimental to human and animal health.

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Author contributions

A. A. Iyanda designed the study, performed the experiments and wrote the manuscript. C. I. Iheakanwa planned the study and performed the histology. O. O. Aina performed the histology. All authors approved the final manuscript.

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