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MALE WISTAR RATS RESPONSE TO KEROSENE EXPOSURE THROUGH DIFFERENT ROUTES: FOCUS ON ANTIOXIDANT INDICES

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ABSTRACT

Due to poverty, kerosene is widely applied for the treatment of a number of ailments in Africa. The impact of kerosene on antioxidant indices is therefore determined in Wistar rats. The experimental animals were divided into four groups (n=6). Trace quantity of kerosene (0.4 ml/kg body weight) was administered through oral, dermal or combined routes. At the end of 3 weeks of daily administration, activities of the antioxidant enzymes, levels of malondialdehyde and reduced and oxidized glutathione were estimated. Levels of oxidized glutathione and malondialdehyde were significantly elevated ($p<0.05$); while the levels or activities of reduced glutathione and reduced glutathione/oxidized glutathione ratio ($p<0.05$) as well as all the antioxidant enzymes were significantly decreased. The results of this suggest that trace administration of kerosene to male Wistar rats is capable of inducing significant oxidative stress and also support the same kind of observation earlier observed in female rats.

Keywords: male rats; kerosene; antioxidant indices.

INTRODUCTION

Excessive free radical generation has been linked with numerous pathological processes.[1] Reports are also available to indicate that gene expression can be regulated by oxidants and antioxidants as well as cellular redox status. Kerosene administration has been demonstrated to induce alteration in antioxidant levels in female Wistar rats.[2] Kerosene like most xenobiotics is metabolized through the different isoform of cytochrome P450 and because there is sex bias in the distribution of these enzymes, in most cases there are sex differences in experimental animal response to many xenobiotics.[3] For instance, aflatoxin B1, a hepatotoxic agent is known to be highly toxic to many male animals than female ones even at the same level of exposure.[4]

Cellular free radical scavenging antioxidants (enzymes, non-enzymes) protect cells against toxic oxygen derived radicals. The enzymes especially, mediate reactions in which oxygen free radicals yield non-radical products. The aim of this study is to determine the serum levels of the markers of oxidative stress; glutathione reductase, glutathione S transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase, reduced glutathione (GSH), oxidized glutathione (GSSG), and malondialdehyde (MDA) in male Wistar rats and observe if their response will be comparable to that observed for the female rats in an earlier study.

MATERIALS AND METHODS

Experimental Animals: The experimental animals used for this study were treated in compliance with laid regulations as contained in national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; especially as promulgated and adopted by United States Institutes of Health (1985). Mature male Wistar rats of between 12 and 14 weeks of age, housed in cages at ambient temperature of 23±3°C and a 12 h light, 12 h dark cycle in the animal house of the Department of Veterinary Physiology, University of Ibadan were used for the study. The

animals were left to acclimatize for two weeks prior to the commencement of the experiment. All the animals were fed with their specific diets and water without any form of restriction.

Materials: All the reagents used for the determination of levels of glutathione and malondialdehyde and the activities of antioxidant enzymes; glutathione peroxidase, catalase, superoxide dismutase, glutathione reductase, glutathione S transferase were of analytical grade. Kerosene was obtained from Mobil filling station located in Osogbo (Nigeria).

Treatment: Rats were divided into 4 groups, with each group consisting of 6 rats. Rats in Group 1 were exposed to 0.4ml of kerosene/kg body weight (BW) of rats through the oral route as contaminant of feed; rats in Group 2 on the other hand were treated with the same dosage but the route of administration was dermal; because kerosene in known to be volatile, contamination of feed with kerosene was carried out daily. Group 3 rats were administered with kerosene through two routes of exposure; dermal & oral while rats in Group 4 served as the control and were not exposed to kerosene. This study lasted for a period of 21 days. Rats in the dermal route of exposure were held individually in their cages for the entire duration of the experiment, to prevent cage mates from grooming and ingesting the fuel.

***Assessment of levels and activities of antioxidant parameters*:** On the 22nd day, whole blood was obtained through retro-orbital bleeding. Blood was centrifuged for 10 minutes at 3000 g using a table centrifuge. The serum samples obtained were used for the estimation of indices of oxidative stress. These estimations were carried out using Hitachi 902 Automated machines (Roche Diagnostic®, Germany). Reduced and oxidized glutathione were determined using the methods of Prins and Loos,[5] Owen Joshua and Butterfield.[6] Estimation of the serum activities of superoxide dismutase, glutathione peroxidase, catalase and MDA were by the methods of Kakkar et al.,[7] Rotruck et al.,[8] Sinha,[9] and Ohkawa et al.[10] respectively. The GR activity was assessed by using the method of Zhou & Freed[11] while that of GST was by the method of Habig et al. [12]

***Statistical analysis*:** Data obtained were expressed as mean ± SEM (standard error of mean) of six observations. Using SPSS version 15, results were statistically assessed by Student's t test to determine the degree of difference between each of the treated group and the control. Analysis of variance (ANOVA) was employed to assess inter-group differences. The level of significance of $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Estimation of lipid peroxidation in terms of activities of enzymes of antioxidant defense system glutathione peroxidase, glutathione-S-transferase, superoxide dismutase (SOD) and catalase (CAT) and levels of malondialdehyde (MDA) and reduced glutathione (GSH) is widely used to investigate oxidative stress-induced states.[1] In the present study, sub-chronic exposure to kerosene caused a significant increase in MDA levels and reduction in GSH levels as well as inhibition in the activities of antioxidant enzymes; glutathione peroxidase, GST, SOD and CAT in serum of kerosene-exposed rats. Lipid peroxidation is a well-recognized mechanism of cellular injury and is usually employed as an indicator of oxidative stress in cells and tissues. Both the products and byproducts of lipid peroxidation e.g. lipid hydroperoxides (LOOH) are increased in oxidative stress-induced conditions.[13,14]

Whereas Luqman et al. (2006) have identified that lipid peroxidation is known to cause cellular injury by inactivation of membrane enzymes and receptors, depolymerizaton of polysaccharide, as well as protein cross linking and fragmentation. Parodi et al.[15] and Sener et al.[16] have also highlighted that oxidative stress, which causes lipid peroxidation is not only involved in cellular injury but is critical in the process of cellular toxicity and has also been implicated through data obtained from many studies as a major factor in the pathogenesis of several diseases. Usually when free radical generation overwhelms the antioxidant defense, these entities interact with endogenous macromolecules and change cellular functions.

Data obtained from this study suggest that administration of kerosene has resulted in diminution of antioxidant enzymes activities in the serum of these rats. Among many of the antioxidant molecules assessed are SOD and CAT, these two mutually function as important enzymes in the elimination of ROS. Decrease in SOD and CAT activities in these rats may be due to the over-generation of superoxide radical anions. Two other antioxidant indices; glutathione peroxidase and GST are enzymes which prevent the generation of hydrogen peroxide and alkyl hydroperoxides in association with GSH and glutathione reductase, as well as the generation of more harmful metabolites such as the hydroxyl radical,[15] both were also significantly reduced as

revealed by the results of this study in all categories of rats treated with kerosene with most significant decrease seen in rats with combined routes of exposure, a confirmation of the possible oxidative potential of kerosene.

The results of our study of significant decreases in the level of GSH and activities of antioxidant enzymes revealed a probable involvement of cytotoxic free radical activity generated from kerosene exposure; a situation that is capable of causing loss of membrane integrity, as well as disintegration of polyunsaturated fatty acids in the membrane bilayer, which can also exert unfavorable effects on the susceptible organ structure and their functions. In biologic system endogenous antioxidant enzymes (e.g. SOD, CAT, GST, glutathione peroxidase) are the first-line cellular defense against oxidative stress, these decompose singlet oxygen and H2O2 before they interact to form the more reactive hydroxyl radical ($OH^{\cdot}$). Usually the activities of these enzymes should be for the effective removal of oxygen stress in intracellular organelles. It is an established fact that both SOD and CAT are essential antioxidant enzymes in mitigating free radical-induced cell injury. A decline in SOD and CAT activities as observed in these rats, post-kerosene exposure can lead to decreased removal of superoxide ion and H2O2 radicals that brings about a number of reactions, which are deleterious to susceptible tissues.

The implication of these results is that the process of lipid peroxidation, i.e. the oxidative deterioration of polyunsaturated fatty acids, which usually result in the formation of hydroperoxides, short-chain aldehydes, ketones, and other oxygenated molecules may be a common occurrence in many human subjects who are constantly exposed to even trace quantities of kerosene. That is a process that has been highlighted through results obtained from numerous studies as being responsible for the development of a number of diseases; examples being atherosclerosis,[18] diabetes,[19] cancer[20] and may be one of the main contributing factors in aging.[20] Moreover, free radical-mediated lipid peroxidation has been identified as being a critical event that is involved in disease states such as brain dysfunction, cardiovascular disease, and cancer as well as in the degenerative processes associated with aging. Enzymatic antioxidants such as superoxide dismutase and glutathione peroxidase protect cell membranes from lipid peroxidation.[21] Furthermore, Voss and Siems[22] have linked an imbalance between free radical generation and the defense system in the pathogenesis of not only atherosclerosis and diabetes mellitus but of cancer and Parkinson's disease as well. This is because lipid peroxidation commences when hydroxyl radicals attack fatty acid side chains of membrane phospholipids, which leads to certain chromosomal aberrations as well as carcinogenesis.[23] These are pathological manifestations which are probable with continuous exposure to kerosene in not only this mammal but other mammalian species as well. As Esterbauer and Cheeseman[24] have observed MDA is a product of lipid peroxidation, and thiobarbituric acid reactive substances (TBARS), as indicated by the MDA concentration, serves as an ideal oxidative damage index. Estimation of SOD and glutathione peroxidase activities and MDA levels is a reflection of the degree of oxygen free radical metabolism and the extent of oxidative stress, as SOD and glutathione peroxidase are the major antioxidant enzymes that eliminate free radicals and possess antioxidative stress functions. MDA is a lipid peroxidation product formed after free radical attack cell membranes.

Because kerosene is made up of different constituents, metabolic transformation of these constituents is a possible mechanism leading to the formation of reactive oxygen species (ROS), such as superoxide anion (O2-•), hydrogen peroxide (H2O2) and hydroxyl radical (HO•), reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite (ONOO-), and peroxidation reaction products. Direct interaction between any of the constituents and cellular components though cannot also be discounted. It seems that this may be the cause of kerosene-associated depletion of serum GSH, alterations in glutathione disulfide (GSSG) contents as well as reductions in the activities of the antioxidant. These features have been identified to occur with another chemical-induced toxicity i.e. acetaminophen.[25,26]

In kerosene exposed rats, the decrease in the activity of glutathione reductase, an enzyme that plays a critical role in oxidative stress is capable of interrupting the cycling between GSSG and GSH and may be another possible cause of significantly low reduced glutathione level. The observed differences in inhibitory action by kerosene on glutathione-related enzymes between dermal and oral routes of exposure are in close agreement with the results of an earlier study in female Wistar rats. In addition, glutathione peroxidase, a SH-requiring enzyme participating in peroxide elimination, is known to be greatly susceptibility to inhibition by peroxides. Although it is always assumed that GSH depletion is a result of increase in free radical generation, possible interaction between any of kerosene constituents and

GSH cannot be excluded leading eventually to increase in free radical generation. These results in which male rats featured considerable oxidative stress as revealed by significant alterations in the levels or activities of the antioxidant indices suggest that kerosene is also toxic in male rats, this raises the possibility that the use of kerosene for therapeutic reasons is dangerous. Moreover, it can also be deduced that exposure through multiple routes can aggravate a rat's toxic response to kerosene. Conclusion: The results of this study suggest that exposure of male rats to kerosene is capable of inducing significant alteration in the activities or levels of indices of oxidative stress after 3 weeks of daily exposure.

Table 1: Serum levels of reduced glutathione, oxidized glutathione, reduced/oxidized glutathione ratio and malondialdehyde in rats administered with trace quantity of kerosene

	GSH (mol/ml)	GSSG (mol/ml)	GSH/GSSG Ratio	MDA (nmol/ml)
Control	1.83±0.06	0.09±0.005	20.33±1.99	16.11±2.90
Combined	1.19±0.03*	0.29±0.003*	4.10±0.09*	25.07±3.58*
Oral route	1.48±0.04*ǂ	0.17±0.006*ǂ	8.71±1.01*ǂ	22.05±3.69*ǂ
Dermal route	1.63±0.04*ǂǂ	0.14±0.002*ǂǂ	11.64±0.99*ǂǂ	19.88±4.04*ǂǂ

Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student's t test. ǂp <0.05 is significant when control, oral and combined routes were compared and ǂǂ p < 0.05 is significant when control, dermal and combined routes were compared using ANOVA. Abbreviations: GSH-reduced glutathione; GSSG-oxidized glutathione; GSH/GSSG- reduced/oxidizes glutathione ratio; MDA- malondialdehyde.

Table 2: Serum activities of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione S transferase

	Cat (µmol H_2O_2 consumed/(min·mg protein))	SOD (U/mg protein)	Gln-Per (µmol GSH consumed/(min·mg protein)	Gln Red (U/mg protein) (U/mg protein)	GST (U/mg protein)
Control	2.66±0.41	15.96±1.09	13.60±1.56	49.48±7.97	0.78±0.09
Combined route	1.61±0.40*	10.06±0.99*	8.62±2.02*	36.23±4.05*	0.58±0.05*
Oral route	1.92±0.31*ǂ	11.18±0.84*ǂ	10.67±1.11*ǂ	40.23±5.06*ǂ	0.60±0.05*ǂ
Dermal route	2.27±0.52*ǂǂ	13.90±1.03*ǂǂ	11.05±0.95*ǂǂ	45.54±7.22*ǂǂ	0.69±0.06*ǂǂ

Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student's t test. ǂp <0.05 is significant when control, oral and combined routes were compared and ǂǂ p < 0.05 when control, dermal and combined were compared using ANOVA. Abbreviations: Cat-catalase; SOD-superoxide dismutase; Gln-Per- glutathione peroxidase; Gln Red- glutathione reductase; GST- glutathione S transferase.

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