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Effects of Oral and Dermal Sub-Chronic Exposure of Kerosene on Biochemical Parameters in Male Wistar Rats

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Author's contribution

The corresponding author is solely responsible for this work.

Research Article

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ABSTRACT

Aim: Many of the studies that have been carried out to investigate the toxicity of kerosene have been large-dose, acute-setting experiments. Although the hepatic and renal damage as a result of kerosene exposure has been demonstrated in an earlier study in female Wistar rats, gender is known to play a role in an animal's response to a xenobiotic. Therefore, the aim of this study is to determine the effect of repeated exposure of trace amount of kerosene to male Wistar rats so as to establish if differences in gender of an animal will modulate the toxic response of kerosene in sub-chronic setting.
Methods: Twelve male rats were divided equally into 2 groups and administered with 0.4 ml/kg kerosene either through the oral or dermal route; six other rats served as control group. Kerosene administration lasted for 21 days after which blood was obtained through retro-orbital bleeding.
Results: Results of the study reveal that while the hepatic enzymes alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (γ-GT) as well as other biochemical parameters- bilirubin, urea, creatinine and uric acid were significantly increased, total protein and albumin were significantly reduced ($p<0.05$). Moreover, in most cases these changes were more significant for oral route than dermal route.
Conclusion: These results suggest nephrotoxic and hepatotoxic nature of kerosene in male rats and confirm that the oral route of administration is more dangerous than

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dermal, a finding that was similar to what was observed for female rats in an earlier study.

Keywords: Male rats; liver; kidney; kerosene; dermal and oral routes.

1. INTRODUCTION

Kerosene, a petroleum product distilled by fractional distillation is a mixture of aromatic and aliphatic hydrocarbons that may contain sulfur. Its percentage composition being 25% normal paraffins, 12% branched paraffins, 30% monocycloparaffins, 12% dicycloparaffins, 1% tricycloparaffins, 16% mononuclear aromatics and 5% dinuclear aromatics (NIOSH, 1977). It is used for diverse purposes; as jet-fuel, solvent for insect spray and a cheap source of fuel for non-electrified dwellings worldwide.

Kerosene, like most xenobiotics, is metabolized in different tissues by cytochrome P450s. Although most cases of unintentional exposure are linked to inhalation and dermal route of exposure, oral exposure of trace amount repeatedly does occur in Nigeria, where kerosene is sold in residential area by kerosene retailers. Sometimes, these retailers measure this product using bowls meant for domestic purposes, without given any consideration for its toxic effects. Data are available that suggest that large-dose kerosene exposure will cause hepato-nephrotoxicity in an acute setting, and trace quantity of kerosene has been demonstrated to alter the morphology of both hepatocytes and renal cells in female Wistar rats [1]. But the fact that gender is known to influence a species' response to metabolic processing of a xenobiotic necessitates a study of this nature [2]. Therefore the aim of this study therefore is to investigate the impact of trace amount of kerosene in male Wistar rats in a sub-chronic setting.

2. MATERIALS AND METHODS

2.1 Treatment

The study was executed in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985). Male albino rats (230 g), purchased from the Department of Veterinary Physiology, University of Ibadan (Nigeria) were used for the study. The rats were kept 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 supplied with water *ad libitum*.

Twenty-four rats were used for this study, eighteen of which were equally allocated into the three treatment groups while six others served as the control. Dermal, oral, or combine routes of exposure were appointed for each of the treatment groups, through which trace quantity of kerosene was administered to these rats. Oral exposure was ensured through contamination of the feed while dermal exposure was carried out by introducing kerosene directly on the skin of each rat [3]. Rats in the dermal group were kept in individual cages to prevent mate grooming and the kerosene was applied to the neck region to prevent self-grooming. The kerosene used for the study was purchased at Mobil filling station, Osogbo (Nigeria). The duration of the study was for 21 days, 0.4 ml of kerosene/kg body weight was adopted [4] for the present study as quantity adopted to 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. Kerosene was mixed thoroughly daily with the feed because of the volatile nature of this product.

2.2 Clinical Chemistry

At 10:00 h of the 22nd day, collection of blood commenced from the rats. Blood was obtained through retro-orbital bleeding. The serum activities and levels of the following indices of hepato-renal function were assessed; namely alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ-glutamyl transferase (ALT, AST, ALP & γ-GT). Others are bilirubin, total protein, albumin and uric acid. While serum activities of AST & ALT were estimated using the method of Bergmeyer et al. [5] that of alkaline phosphatase (ALP) was by the method of Mc Comb and Bowers. [6] On the other hand, serum levels of bilirubin and albumin were quantified using modified Jendrassik-Groff [7] & standard bromocresol methods respectively. Total protein was measured using Biuret's method. [8] Creatinine urea and uric acid were estimated by the Jaffé reaction, diacetyl monoxime oxidase and phosphotungstate methods respectively. Hitachi® 902 automated machines (Roche Diagnostic, Germany) was used for these estimations.

2.3 Statistical Analysis

Data obtained from this study are expressed as mean ± SD (standard deviation). While Student's t- test was used to establish the degree of significant difference between each of the treatment group and the control, analysis of variance was employed to establish inter-group comparison. SPSS package version 15 was used for this purpose. P≤ 0 .05 was considered significant.

3. RESULTS

All the results of the study are presented in Table 1. Administration of male rats with kerosene resulted in significant increases ($p<0.05$) in the serum levels of bilirubin and globulin and significant decreases ($p<0.05$) in the levels of total protein and albumin. Moreover, inter-group comparison between control, combine routes and oral route on one hand and inter-group comparison between control, combine routes and dermal route showed there were significant differences ($p<0.05$) among groups for bilirubin, globulin, total protein and albumin. Results showed significant differences between control and each of the exposure group, with ALT, AST, ALP and γ-GT being significantly increased ($p<0.05$). Using ANOVA significant inter-group differences ($p<0.05$) were observed for all the hepatic enzymes. Renal indices, urea, creatinine and uric acid were significantly different ($p<0.05$) with inter-group comparison as well as being significantly increased when each of the exposure group was compared with control.

Table 1. Biochemical parameters in Kerosene treated male rats

Parameter	Control	Oral route	Dermal route	Combine route
Bilirubin (µmol\L)	7.12±1.84	26.87±3.16*‡	15.21±4.04*‡	30.65±3.30*
Total protein (g/dL)	7.22±1.01	6.19±0.97*‡	6.54±0.58*‡	5.70±0.41*
Albumin (g/dL)	4.16±0.64	2.52±0.38*‡	3.40±0.56*‡	2.01±0.37*
Globulin (g/dL)	3.07±0.48	3.67±0.66*‡	3.14±0.38*‡	3.79±0.31*
Aspartate amino transferase (IU/L)	27.72±5.30	63.76±7.05*‡	49.03±9.47*‡	120.55±11.30*
Alanine amino transferase (IU/L)	38.58±8.17	77.54±5.78*‡	54.07±7.06*‡	117.48±15.09*
Gamma-glutamyl transferase (IU/L)	46.90±7.11	76.14±10.05*‡	58.72±3.38*‡	88.77±14.12*
Alkaline phosphatase (IU/L)	54.00±16 .01	74.06±8.09*‡	59.43±15.11*‡	89.69±7.68*
Urea (mmol\L)	4.09±0.65	8.51±1.05*‡	6.82±0.78*‡	11.26±1.73*
Creatinine (µmol\L)	52.99±15.09	94.64±10.53*‡	61.70±12.00*‡	116.92±20.64*
Uric acid (mmol\L)	240.66±83.92	314.42±43.07*‡	289.38±13.94*‡	497.72±48.02*

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

4. DISCUSSION

Some of the important functions of the liver include detoxification of bilirubin, epimerization of galactose to glucose as uridine-5-phosphate derivatives and synthesis of protein (albumin) and prothrombin. Many of these functions are derailed when hepatotoxic chemicals damage liver cells; damage that occurs from lipid peroxidation and other oxidative process. The degree of hepatic damage caused as a result of chemical exposure is usually assessed through the activities of released cytoplasmic enzymes such as AST and ALT in circulation. These two enzymes were significantly increased as a result of kerosene exposure, although results show that rats in combine routes of exposure featured a more significant degree of hepatic damage than rats in oral and dermal routes. Increase in the activity of plasma AST though not entirely specific to liver injury but do also occur in cardiac infarction, hemolytic anemia and muscle injury. The significant increase in serum activity of ALT, a better bio-indicator of liver injury than AST, [9] an enzyme that catalyses the conversion of alanine and glutamine to pyruvate and glutamate further suggests hepatic damage as a result of kerosene exposure. That the rats in oral group have higher activities of these enzymes suggest that exposure through oral route will provoke a more harmful response than dermal route.

Gamma-glutamyl transferase (GGT) another liver specific enzyme was also significantly increased as well as ALP as a result of kerosene exposure in all categories of kerosene administered rats. Moreover, bilirubin was also significantly increased. Serum bilirubin increased in these rats can be attributed to regurgitation of bile originating from obstruction within the liver resulting from damage or inflammation caused by kerosene. [10] Moreover, the regurgitation of bile can also be linked with increase in ALP activity as it has been

suggested by Sallie et al. [11] Serum ALP activity and bilirubin level conversely are related to the function of the hepatic cells. Whereas increase in the activities of AST/ALT in usually linked to plasma membrane damage, elevated activity of serum ALP is due to the increased synthesis in presence of increasing biliary pressure [12].

Lipid peroxidation seems to be the basis of destructive process in liver injury due to kerosene exposure [13]. The significant increase in serum ALT and AST activities occurring in association with decreased levels of serum total protein and albumin suggests not only plasma membrane disruption but derailment synthetic hepatic ability. The liver i.e. parenchymal cells are the major site of synthesis of source of most of the serum proteins, and as Pawlikowska-Pawlega et al. [14] have put it, the parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the α- and β-globulins. Therefore, the observed decrease in albumin level which occur post-kerosene administration could be a result of a decrease in the number of cells responsible for albumin synthesis in the liver which occurred as a result of necrosis. Apart from this, interference with the albumin-synthesizing process in the liver as result inflammation may also be implicated for decrease in albumin concentration.

There is also the possibility of the involvement of the inflammatory process; as it has been reported for other chemical-induced toxicity that has been accompanied by the inflammatory process. Chemical products of inflammation are known to shunt amino acids to enhance the synthesis of proteins that are important to the inflammatory process, thereby depressing albumin synthesis as it is not essential to inflammation [15,16]. These results are in agreement with what was observed for the female Wistar rats that were administered with trace amount of kerosene [17], an indication that sex does not affect hepatic and renal presentation in rats post-kerosene administration.

Another hepatic index, bilirubin that is not directly associated with inflammation is increased. Accumulation of bilirubin is indicative of possible alterations in binding, conjugation and excretory capacity of hepatocytes, such that the significant increase bilirubin level is usually an indication of biliary obstruction, hemolysis, and in some cases renal failure [14]. Our observation of increased level of serum bilirubin in these kerosene exposed rats suggests kerosene-induced hepatic damage, especially as other hepatic indices were also significantly altered compared with control.

Moreover, our results also revealed considerable renal impairment in animals exposed to kerosene, as revealed by the significant increase in serum levels of urea and creatinine, although nephrotoxic effects of the dermal route was quite lower than the oral route. This may not be unassociated with the differences in bioavailability of kerosene from different route of exposure. Data are available to substantiate that orally administered drugs in most cases have higher bioavailability than drugs or other agents applied through the dermal route [18].

That kerosene may be more nephrotoxic than hepatotoxic had earlier been suggested. Bruner et al. [19] related this to the binding of kerosene metabolites to α2μ-globulin, a low molecular weight serum protein synthesized in the liver of male rats. Accumulation of this complex has been linked to pathological responses within the kidney in the F344 rats, but unlike the result of the study of Bruner et al. [19] both hepato and nephrotoxicity were identified in the Wistar rats used for this study. This phenomenon has been observed in relation to the toxicity of other xenobiotics. Take for instance the data obtained from the

study of Tarloff et al. [20], in which the effect of acetaminophen on different strains of rats was studied.

5. CONCLUSION

The results of this study suggest that Wistar rats are susceptible to toxic effects of kerosene even at low level in a sub-chronic setting. Data obtained from this study of higher activities of hepatic enzymes in oral group than dermal group suggests that exposure through the oral route provoked greater toxic response than dermal route in male Wistar rats.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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