HEPATO-RENAL TOXICITY OF KEROSENE FROM DERMAL AND ORAL EXPOSURE.

*Ayobola Abolape Iyanda

Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.

ABSTRACT

Objective: Due to lack of access to adequate medical care in Nigeria, kerosene or some other petroleum products are being used for the treatment of a number of clinical conditions. This therefore necessitates the need to study the impact of continuous exposure to trace amount of kerosene in female Wistar rats over a period of 3 weeks. Animals: Twelve rats were assigned to Groups 1 & 2 and administered with 0.3 ml/kg body weight of kerosene through either the oral and dermal route with the study being terminated after 1 week. Twelve other rats were assigned to Groups 3 (oral) and four (dermal) and given similar treatment as groups 1 and 2 with the study being terminated after 3 weeks. Six rats served as the control. Procedures: Blood was collected from each animal through retro-orbital bleeding and serum obtained after centrifugation. Serum activities or levels of hepatic and renal indices were determined. Results: By the end of three weeks, all indices were significantly altered (p<0.05) in both oral and dermal groups compared with control whereas, after 1 week of exposure in oral and dermal group total protein, globulin, ALT, AST were significantly changed (p<0.05); ALP, GGT, urea, creatinine and uric acid were not significantly changed (p>0.05) compared with control. Conclusion: These results suggest both hepatotoxic and nephrotoxic effects of kerosene even at dose level of 0.3 ml/kg body weight.

Keywords: female rats; liver, kidney, kerosene.

INTRODUCTION

Kerosene is a petroleum product that is commonly available in Nigeria. As a result of its non-harmful appearance (near-clear, colorless nature), it is widely used not only as fuel for
cooking purposes but it has been reported as being potent in the treatment of a number of ailments. There are reports to suggest its usage in the treatment of snake bites, arthritis, gastrointestinal disorders, burns, foot rot and leg ulcers.\textsuperscript{[1,2]} Other medical conditions to which this product has been widely used include skin and eye infections (conjunctivitis), eczema and scabies\textsuperscript{[3]} as well as cuts and scrapes, head lice, athlete's foot, animal health problems including cracked or infected hooves and worm infections.\textsuperscript{[4]}

Petroleum products are hydrocarbon compounds that are foreign to human system; being foreign they are metabolized to yield excretable non-toxic metabolites. The enzyme system cytochrome P450 is known to play a role in biotransformation of these compounds. Either the end or intermediate metabolites have been identified to be free radicals or highly reactive oxygen/nitrogen species that bind with cellular biomolecules to induce cellular damage in susceptible tissues like the liver or kidney. In many instances, xenobiotic exposure may not cause instantaneous tissue damage, especially if small doses are administered in an acute setting but become pronouncedly toxic at chronic setting even at the same level of small dose exposure. Kerosene toxicity in a mammalian species has been described with high dose at acute setting as well as low repeated dosing in chronic settings.\textsuperscript{[5]} In most of the cases of repeated small dose exposure of kerosene at chronic setting, the response at early stage of exposure has not been determined.

Although an earlier study has demonstrated that continuous exposure to small quantity of kerosene was both hepato and nephrotoxic by the end of the 30\textsuperscript{th} day of exposure, in most cases human exposure to the product dose not last that long especially if exposure is for therapeutic purpose, yet its harmful effect can not be discounted. The aim of this study therefore is to investigate using Wistar rats - the response to daily administration of small dose as early as the end of the first week of exposure and to compare that response with that of the third week, so as to establish how early in the course of exposure, hepatocellular and renal damage commences in a mammalian species.

**MATERIALS AND METHODS**

**Experimental Animals:** Mature male Wistar rats (230 g) treated in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985) were used for the study. These rats were kept in the Animal House attached to the Department of Veterinary Physiology, University of
Ibadan, Nigeria. Animals were housed in cages at ambient temperature of 23±3°C and a 12 h light, 12 h dark cycle and given unrestricted access to their respective feed and water.

**Experimental Animals:** Twelve of the rats that were employed for this study were randomly selected and divided into 2 groups comprising of 6 rats per group and each group was treated with kerosene either through dermal route or oral route (as contaminant of feed) and the study terminated by the end of the first week of daily exposure. Another group of twelve rats were given the same treatment but the study ended after the 21st exposure of daily administration. Six other rats served as the control. Trace quantity of kerosene, of 0.3 ml of kerosene/kg body weight was adopted as quantity sufficient to study the toxic effect of trace amount of kerosene after an earlier study\(^5\). Contamination of feed was carried out daily because of the volatility of the components of kerosene; dermal exposure was by discharging kerosene directly on the skin of each rat. The kerosene used for this study was obtained from Mobil filling station located in Osogbo, Nigeria. The blood obtained from each rat through retro-orbital bleeding and dispensed into anti-coagulant free bottle was centrifuged and for ten minutes at 3000 g and stored at - 20ºC. The serum obtained was used for clinical chemistry study.

**Clinical Chemistry**
Activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and \(\gamma\)-glutamyl transferase (ALT, AST, ALP & \(\gamma\)-GT) were determined in the serum of both the treated and control rats. Bergmeyer et al.\(^6\) method was employed to determine the serum activities of AST & ALT, whereas alkaline phosphatase (ALP) quantified using the method of Mc Comb and Bowers.\(^7\) while serum bilirubin and albumin were quantified using modified Jendrassik-Groff\(^8\) & standard bromocresol methods respectively. Biuret’s method was used to establish the level of total protein.\(^9\) Creatinine was estimated using Jaffé reaction while the level of urea was also measured by the diacetyl monoxime oxidase method. Hitachi® 902 automated machines (Roche Diagnostic, Germany) was used for these estimations.

**Statistical analysis**
Data obtained are expressed as mean ± SD (standard deviation). Degree of tissue damage was determined by establishing the level of significant difference between each of the treatment group and the control was determined using Student’s t-test. Analysis of variance
was employed for inter-group comparison. SPSS package version 15 was used for this purpose. \( P \leq 0.05 \) was considered significant.

**RESULTS**

Bilirubin, total protein, globulin and albumin were significantly different in orally kerosene administered rats as revealed in Table 1. Specifically, while bilirubin and globulin were significantly increased (\( p<0.05 \)) albumin and total protein were significantly decreased (\( p<0.05 \)) in 1 and 3 weeks kerosene exposure groups. On the other hand, in dermal exposed rats, while bilirubin and albumin were not significantly different (\( p>0.05 \)), both total protein and globulin were significantly increased after one week of kerosene exposure but at 3 weeks while bilirubin and globulin were significantly decreased, albumin and total protein were significantly decreased. ANOVA study revealed significant inter-group differences (\( p<0.05 \)) among the three exposure groups with respect to bilirubin, albumin, total protein and globulin. In Table 2, AST and ALT are significantly increased (\( p<0.05 \)) in rats in either oral or dermal routes compared with control after 1 and 3 weeks of kerosene administration but ALP and \( \gamma \)-GT were only significantly increased (\( p<0.05 \)) at 3 weeks but not 1 week. Significant inter-group differences were observed for all these hepatic enzymes at 3 weeks but only for ALT and AST at 1 week period of exposure. Results in Table 3 showed that at the end of the first week of kerosene administration the serum of none of the renal indices was significantly different (\( p>0.05 \)) compared with control whereas by 3 weeks of exposure all the renal indices were significantly increased (\( p<0.05 \)).

Table 1: Serum levels of bilirubin, total protein, albumin and globulin in kerosene treated rats.

<table>
<thead>
<tr>
<th></th>
<th>1 week</th>
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<th>3 weeks</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bilirubin (( \mu \text{mol/L} )) **</td>
<td>Total protein (( \text{g/dl} )) **</td>
<td>Albumin (( \text{g/dl} )) **</td>
<td>Globulin (( \text{g/dl} )) **</td>
<td>Bilirubin (( \mu \text{mol/L} )) **</td>
</tr>
<tr>
<td>Control</td>
<td>6.44±0.95</td>
<td>6.36±0.87</td>
<td>3.58±0.59</td>
<td>2.79±0.38</td>
<td>6.44±0.95</td>
</tr>
<tr>
<td>Oral route</td>
<td>16.06±1.12*</td>
<td>6.05±0.55*</td>
<td>2.86±0.42*</td>
<td>3.11±0.26*</td>
<td>35.16±5.78*</td>
</tr>
<tr>
<td>Dermal route</td>
<td>6.54±0.94</td>
<td>6.60±0.62*</td>
<td>3.61±0.36</td>
<td>2.99±0.29*</td>
<td>24.39±4.89*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation. *\( P < 0.05 \) is significant when compared with control using Student’s t test. **\( P < 0.05 \) when control, dermal and oral groups were compared using ANOVA, \( n=6 \).
### Table 2: Serum levels of hepatic enzymes in kerosene treated rats

<table>
<thead>
<tr>
<th></th>
<th>1 week</th>
<th>3 weeks</th>
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<tbody>
<tr>
<td></td>
<td>AST (IU/L) **</td>
<td>ALT (IU/L) **</td>
</tr>
<tr>
<td>X ± SD (control)</td>
<td>34.08±6.37</td>
<td>29.64±7.14</td>
</tr>
<tr>
<td>Oral Route</td>
<td>48.06±6.07*</td>
<td>50.29±5.42*</td>
</tr>
<tr>
<td>Dermal route</td>
<td>40.04±7.33*</td>
<td>34.99±3.66*</td>
</tr>
</tbody>
</table>

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

### Table 3: Serum levels of urea, creatinine and uric acid in kerosene treated rats

<table>
<thead>
<tr>
<th></th>
<th>1 week</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea (mmol/L)</td>
<td>Creatinine (µmol/L)</td>
</tr>
<tr>
<td></td>
<td>Urea (mmol/L) **</td>
<td>Creatinine (µmol/L) **</td>
</tr>
<tr>
<td>Control X ± SD</td>
<td>3.52±0.48</td>
<td>49.81±11.55</td>
</tr>
<tr>
<td>Oral route</td>
<td>3.70±0.40</td>
<td>56.03±20.83</td>
</tr>
<tr>
<td>Dermal route</td>
<td>3.50±0.52</td>
<td>51.04±7.64</td>
</tr>
</tbody>
</table>

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

**DISCUSSION**

Human exposure to kerosene is common in many parts of Nigeria. Kerosene is used in various homes for a variety of purposes. The biochemical parameters used to investigate both hepatic and renal response in rats exposed to small quantities of kerosene showed a significant increase (p≤0.05) when compared to the control. Treatment of rats with kerosene resulted in a significant hepatic damage as shown by a significant increase in the levels of serum marker enzymes: AST, ALT and ALP. Being non-functional plasma enzymes with their origin being intracellular, precisely cytoplasmic rather than extracellular, these significant increases suggest leakage through the plasma membrane. Lin et al.[10] have
suggested that an increase of these enzymes in the circulation is suggestive of hepatocellular membrane damage. These significant increases resulting from changes in the cell membrane may not be unassociated with reactive free radical species from the metabolism of aliphatic and aromatic hydrocarbons which are the major constituents of petroleum products.\textsuperscript{[11,12]}

Results of this study showed that 3 weeks after daily administration of kerosene, liver and kidney damage was already established in the rats in both dermal and oral groups. The kidney known to be a source of the enzymes that metabolize different components of kerosene, showed significant degree of damage at 3 weeks when compared with control using renal biochemical indices as markers of study. Urea, creatinine and uric acid, were significantly higher in treated rats than the control. On the other hand, rats in oral and dermal groups (1 week) did not manifest renal toxicity but hepatocellular membrane damage was evident as early as the first week. This difference in renal and hepatic manifestation may not be unassociated with differences in tissue distribution of cytochrome P450s, which are more abundant in liver than kidney. The fact that only AST and ALT were significantly increased but not ALP and γ-GT is an indication that at first week kerosene damages plasma membrane more than it produces post-hepatic obstruction. In addition, the level of plasma serum proteins was significantly lower in rats in both dermal and oral routes compared with controls while the level of bilirubin was slightly increased. This further portrays possible hepatic damage. The pattern of significant change in level of albumin was similar that of total protein, this is understandable since albumin constitutes about 50% of total protein concentration.

It is not surprising that both renal and hepatotoxic effect of kerosene was observed in these rats; Tsujino et al.\textsuperscript{[13]} by analyzing the systemic distribution of kerosene components in blood and tissues discovered that following dermal exposure to kerosene at least four types of trimethylbenzenes (TMBs) and aliphatic hydrocarbons (AHCs) with carbon numbers 9-16 (C\textsubscript{9}-C\textsubscript{16}) were detected in blood and all tissues subsequent to capillary gas chromatography/mass spectrometry (GC/MS) technique. The amounts of TMBs, a toxic metabolite detected were higher than those of AHCs although these increases were found more in adipose tissue in an exposure duration-dependent manner. The presence of these components in the adipose tissue will suggest that the toxic effects of kerosene will be felt for longer period since fat cells are not as rapidly turn-over as other cells. And this may be the
reason why the damaging effects of kerosene on the tissues studied are more pronounced at the end of the 3rd week compared with 1st week.

That kerosene applied through the dermal route was able to gain entry into these organs to effect damage can be deduced from the submissions of Dugard and Scott,[14] Flynn[15] as well as Jepson and McDougal.[16] These workers revealed that chemicals enter into (absorption) and pass through (penetration) the skin based on their chemical characteristics and that heavy molecular weight chemicals tend to move more slowly though the skin since both polarity and lipid solubility play significant role in it.[17] McDougal et al.[18], McDougal and Rogers[19] and McDougal and Robinson[20] have observed that charged chemicals usually do not passively cross membranes, including the skin efficiently; therefore chemical agents that have an affinity for lipids can often pass through the primary skin barrier, i.e. the stratum corneum and in most cases proceed into the blood stream for extensive distribution to different organs of the body.

The liver has a great potential to repair itself, a phenomenon that is well known with chronic acetaminophen toxicity, in which both biochemical and histologic features showed no liver damage and proteins responsible for liver repair and regeneration were significantly increased with sub-toxic level of acetaminophen exposure over a period of thirty days. This phenomenon was not evident in these rats probably because of the immunosuppressive effects of kerosene. When the immune response of the skin was studied subsequent to kerosene exposure (via the dermal route), Ullrich & Lyons[21] observed that application of JP-8, a kerosene based fuel induced immune suppression. Specifically, they identified that 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. Ullrich & Lyons[21] noted that suppression of T-cell proliferation occurred as early as 3 to 4 days after a single JP-8 treatment and continued for another 3 weeks, after which T-cell proliferation returned to normal. According to their submission, it seemed that only cellular immune reactions appear to be more susceptible to the immunosuppressive effects of JP-8, this is because antibody production in JP-8-treated mice was identical to that of control mice. This may be the reason why even though immunosupression is associated with kerosene exposure, the globulin fraction of the total protein was significantly higher in kerosene exposed rats
compared with control. This high level of globulin therefore can be deduced to have arisen from increase in either α or β component of the globulin fraction.

Moreover, they also reported that 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. By blocking the production of prostaglandin E(2) with a selective cyclooxygenase-2 inhibitor, JP-8-induced immune suppression was abrogated. Neutralizing the activity of interleukin-10 on the other hand with a highly specific monoclonal antibody also blocked JP-8-induced immune suppression. In addition, when JP-8-treated mice were injected with recombinant interleukin-12, a cytokine that drives cell-mediated immune reactions in vivo overcame the immunotoxic effects of JP-8 and restored immune function.[22,23] The findings of Ullrich & Lyons,[21] therefore suggest that immune suppressive cytokines, which probably was produced by JP-8-treated epidermal cells, are responsible for immune suppression in JP-8-treated mice and that by blocking and/or neutralizing their production in vivo the immunotoxic effects of JP-8 was overcome. Some other interleukins (interleukin-1α, interleukin-1β, interleukin-6) have also been implicated with decrease albumin synthesis. Significant decrease in albumin concentration was observed in the serum of kerosene exposed rats by three weeks of exposure.

CONCLUSION
Kerosene induced tissue damage was observed in rats treated with kerosene for three weeks. The results also suggest that hepatotoxicy proceeded nephrotoxicity; with plasma membrane damage being an earlier event than post-hepatic obstruction. Moreover, oral kerosene administration is more toxic than exposure through the dermal route.

REFERENCES


