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Histopathological Assessment and hematotoxicity of Dietary acrylamide on Wistar rats

Almoez Y. Hammad¹, Maha E. Osman² and Warda S. Abdelgadir³,*

¹ Faculty of pharmacy, Sudan International University, P.O.Box; 12769, Khartoum, Sudan
² Commission for Biotechnology and Genetic Engineering, National Centre for Research, Ministry of Science and Communication, P.O. Box 2404, Khatoum, Sudan.
³ Food Research Centre, Ministry of Science and Communication, P. O. Box 213, Khartoum, Sudan.

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Abstract
Acrylamide is a toxic, cancer-causing, industrial chemical used primarily in the preparation of polycrylamide, which is used principally in water and wastewater treatment and in pulp and paper processing. A significant source of dietary acrylamide is foods cooked at high temperature. Till recently, only known human health effect is neurotoxicity at relatively high doses occurring through occupational exposures. Numerous gaps in knowledge concerning the formation, dietary exposure, and potential for adverse health risks of acrylamide were identified, and the resulting recommendations called for additional research on these topics. Hence the objective of this study was to assess the effects of dietary acrylamide at different doses on tissues and blood of Wistar rats. The results showed no evidence of carcinogenic effect of acrylamide on any of the vital organs, but the main features were growth depression and pathological changes on the liver, kidneys and intestines sufficient to impose significant haematological changes.

Introduction
Acrylamide (C3H5NO) is an odorless, white crystalline solid at room temperature. It is an important industrial chemical, used primarily in the production of polymers and copolymers. Polymers of acrylamide have a wide range of applications e.g. waste water treatment, paper and pulp processing, mineral processing, cosmetics and in scientific laboratory for the separation of macro molecules (Nordian et al. 2003).

Occupational exposure of acrylamide has been reported in many studies (EIMS, 2002), and potentially toxic acrylamide is largely derived from heat-induced reactions between the amino group of the free amino acid asparagine and carbonyl groups of glucose and fructose in cereals, potatoes, and other plant-derived starchy foods (Friedman and Levin, 2008). When entre the body, acrylamide is easily absorbed and binds to hemoglobin and then it is distributed to different organs by body fluid (Shipp et al. 2006). Acrylamide is largely oxidized to glycidamide in mice, rats and humans by its oxidizing agent CYP450 2EI (Summer 1999). In humans, at relatively low doses of acrylamide, glycidamide is formed at higher extent than in rats, because of the higher levels of CYP450 2EI. Both compounds, acrylamide and glycidamide, are detoxified by glutathione conjugation and to some extent glycidamide is detoxified by hydrolysis. During detoxification acrylamide reacts rapidly with SH groups, and also with proteins SH groups and amino groups. The most important reaction of acrylamide with proteins is the adduction to hemoglobin (Hb), SH groups of proteins and the NH2 groups of the N-terminal valines of cytoskeletal proteins and protamines (Lapadula et al. 1989, Sega et al. 1989).

Epidemiological studies of human industrial and accidental exposures suggest that the nervous system is the principal site for toxicity in human (Central and peripheral nervous system) (Gold et al. 2004). The carcinogenic action has been confirmed by the experimental studies on laboratory animals. Further more, acrylamide was found to be a genotoxicant and a reproductive and developmental toxicant (IARC, 1994). The main objective of the present study was to assess the Histopathological and hematotoxic effects of dietary acrylamide on Wistar rats.

Materials and Methods
Experimental animal feeding and dosing
Forty male Wistar rats were obtained from the Aromatic Herbs and Medicinal
Plants Research Institute (A.H.M.P.R.I), Khartoum, reared within the premises of the Institute under 12 hours photoperiod with feed (Table 1) and drinking water provided ad libitum before the commencement of experimental feeding. Room temperature was maintained at 25±2 °C at adequate house ventilation. Then the animals were randomly allotted to five groups 1, 2, 3, 4 and 5 each of eight rats. Group 1 was designated as the control group. Extraneous Acrylamide powder (Promega Co.Ltd, Iran) was thoroughly mixed with the basal diet and fed to rats at 10 mg/kg (Group 2), 30 mg/kg (Group 3), 60 mg/kg (Group 4) and 90 mg/kg (Group 5) whereas Group 1 was fed the basal diet and served as control. Experimental feeding was continued for 6 weeks and a recovery period was adopted thereafter for 4 weeks.

Table 1. Percent inclusion rates (fresh basis) of ingredients of the basal diet fed to experimental rats

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat meal</td>
<td>42.5</td>
</tr>
<tr>
<td>Grain starch</td>
<td>39.2</td>
</tr>
<tr>
<td>Granulated Sugar</td>
<td>05.0</td>
</tr>
<tr>
<td>Cellulose Powder</td>
<td>03.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>05.0</td>
</tr>
<tr>
<td>Super Concentrate</td>
<td>05.0</td>
</tr>
<tr>
<td>Dimethionine</td>
<td>00.3</td>
</tr>
</tbody>
</table>

Hematological parameters

Blood samples were collected in dry test tubes containing EDTA (Ethylene diamine tetra acetic acid) and examined for hemoglobin concentration (Hb), red blood cells (RBC) counts, packed cell volume (PCV), mean corpuscular volume, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and total white blood cell (WBC).

Pathological examination

Necropsies were made on all rats to identify gross lesions and specimens of the liver, kidneys, intestines and spleen were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5μm and stained with haematoxylin and eosin (H and E) for histopathological examinations.

Statistical analysis

Mean values in body weight, blood and serum data were compared using student’s t-test after (Snedecor and Cochran, 1989).

RESULTS

Haematological findings

These data are presented in Table 2. The PCV was higher (p<0.05-0.001) and MCHC was lower (p<0.05-0.01) in the test groups than the controls. The Hb, and RBCs were higher (p<0.05-0.01) in rats fed on 10 mg/kg dietary acrylamide. Significantly higher (p<0.01-0.001) MCV values were shown in Groups 2, 3 and 4, but lower (p<0.05) record was observed in Group 5 fed on the highest dose, 90 mg/kg dietary acrylamide, than the controls. The WBC were significantly (p<0.01-0.001) higher in Groups 2, 3, and Sand the values of Hb and MCHC were significantly lower (p<0.05-0.01) in Groups 4 and 5 than the control rats.

After the end of the recovery period, the values of Hb in Groups 2, PCV in Groups 2 and 4, MCV in Group 4 and MCHC in Group 2 were lower (p<0.05) than the controls. In rats of Group 2, the values of MCV and MCHC were higher (p<0.05-0.001) than those of other groups and the controls.

Pathological findings

Generalized fatty change and hepatocytic degeneration were observed in the liver of the rats on diet containing 10 mg/kg acrylamide (Group 2) (Fig. 1). Congestion and fatty cytoplasmic vaculation of the centrilobular hepatocytes (Fig. 2) as well as mild congestion of the blood vessels of the heart were seen in the rats fed on acrylamide at 30 mg/kg (Group 3). In the rats fed a diet containing 60 mg/kg acrylamide (Group 4), there was congestion and scattered hepatic cell necrosis (Fig3), and generalized necrosis of the hepatic cells was seen in Group 5 (Fig. 4). In the rats fed a diet containing acrylamide at 90 mg/kg (Group 5) lymphocytic infiltration, degeneration and necrosis of the glomeruli and the proximal convoluted tubules were observed (Fig. 5). Shrinkage and degeneration of the glomerular tuft with dilatation and degeneration of the renal tubules were appeared in Groups 2 and 4 (Figs. 6) and Congestion and degeneration of the renal tubules were observed in the rat fed on diet containing 30 mg/kg acrylamide (Fig 7). There was catarrhal enteritis, desquamation of the epithelial cells into the lumen (Fig. 8) and packing of the intestinal lamina propria with lymphocytes. Desquamation of the intestinal epithelium and degeneration of the intestinal villi were seen in rats of Group 4 receiving diet containing 60 mg/kg acrylamide (Fig 9). The red pulp of the spleen revealed varying amounts of haemosiderin deposits and congestion (Fig. 10 and Fig. 11). Although, acrylamide was withdrawn four weeks after treatment, some histopathological lesions are still there though mild (Fig. 12, Fig 13 and Fig. 14). Organ lesions were correlated with changes in hematology. No lesions were seen in the control rats.

Haematological findings

These data are presented in Table 2. The PCV was higher (p<0.05-0.001) and MCHC was lower (p<0.05-0.01) in the test groups than the controls. The Hb, and RBCs were higher (p<0.05-0.01) in rats fed on 10 mg/kg dietary acrylamide. Significantly higher (p<0.01-0.001) MCV values were shown in Groups 2, 3 and 4, but lower (p<0.05) record was observed in Groups 4 and 5 than the control rats. After the end of the recovery period, the values of Hb in Groups 2, PCV in Groups 2 and 4, MCV in Group 4 and MCHC in Group 2 were lower (p<0.05) than the controls. In rats of Group 2, the values of MCV and MCHC were higher (p<0.05-0.001) than those of other groups and the controls.

DISCUSSION

It has been found in the present study, that dietary acrylamide in different doses produce structural and functional changes in body organs e.g. liver, kidneys, spleen and intestines. There was a significant decrease of Hb concentration in Groups 4 and 5. The decrease in hemoglobin content might be either due to decrease in hemoglobin synthesis or due to an increase in hemoglobin destruction. Low hemoglobin concentration reduces the oxygen carrying capacity of blood, which may result in clinical sign such as the
Figure 1: Histopathological responses. 
A: Generalized fatty change and hepatocytes degeneration in a rat receiving dietary acrylamide at 10 mg/kg; 
B: Congestion and fatty cytoplasmic vacuolation of the centrilobular hepatocytes of a rats in Group 3; 
C: Congestion and scattered hepatic cell necrosis of rats receiving diet with acrylamide at 60 mg/kg; 
D: Generalized hepatic necrosis of a rat receiving diet with acrylamide at 90 mg/kg; 
E: Lymphocytic infiltration, degeneration and necrosis of the glomeruli and the proximal convoluted tubules of a rat receiving diet with acrylamide at 90 mg/kg; 
F: Shrinkage of the glomeruli and degeneration of the glomerular tuft with degeneration of the renal tubules of a rat receiving diet with acrylamide at 10 mg/kg; 
G: Congestion and degeneration of the glomeruli and renal tubules in a rat fed on diet containing 30 mg/kg acrylamide; 
H: Catarrhal enteritis and desquamation of the intestinal epithelium of a rat receiving a diet containing 90 mg/kg acrylamide; 
I: Desquamation of the intestinal epithelium and degeneration of the intestinal villi of a rat receiving diet containing 60 mg/kg acrylamide; 
J: Haemosiderin precipitation and congestion of the blood vessels in a spleen of a rat fed a diet containing 10 mg/kg acrylamide; 
K: Haemosiderin precipitation and congestion of the spleen of a rat fed a diet containing 60 mg/kg acrylamide; 
L: Congestion and mild degeneration of the renal tubules in a rat fed on diet containing 30 mg/kg acrylamide after 4 weeks recovery period; 
M: Fatty change in a rat fed on dietary acrylamide at 60 mg/kg after 4 weeks recovery period; 
N: Haemosiderin precipitation of the spleen of a rat fed a diet containing 10 mg/kg acrylamide after 4 weeks recovery period. (H & E x100)
Table 2. Average (mean ± SE) hematological values in rats during treatment (6 weeks) and recovery (4 weeks) with dietary acrylamide

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb [g/dl]</th>
<th>RBCs [x10^6 mm^-3]</th>
<th>PCV (%)</th>
<th>MCV [m^3]</th>
<th>MCH [pg]</th>
<th>MCHC (%)</th>
<th>WBC [(x10^3 mm^-3)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>13.75</td>
<td>7.80</td>
<td>48.0</td>
<td>61.15</td>
<td>17.63</td>
<td>28.65</td>
<td>3.85</td>
</tr>
<tr>
<td>(control)</td>
<td>± 0.18</td>
<td>± 0.20</td>
<td>± 0.70</td>
<td>± 0.18</td>
<td>± 0.21</td>
<td>± 0.41</td>
<td>± 0.18</td>
</tr>
<tr>
<td>Group 2</td>
<td>14.93*</td>
<td>9.30*</td>
<td>59.48**</td>
<td>63.96**</td>
<td>16.05</td>
<td>25.10**</td>
<td>7.90**</td>
</tr>
<tr>
<td>(10mg)</td>
<td>± 0.25</td>
<td>± 0.13</td>
<td>± 0.98</td>
<td>± 0.86</td>
<td>± 0.11**</td>
<td>± 0.61</td>
<td>± 0.36</td>
</tr>
<tr>
<td>Group 3</td>
<td>13.17</td>
<td>7.2</td>
<td>52.65**</td>
<td>73.13***</td>
<td>18.29</td>
<td>25.01*</td>
<td>5.40**</td>
</tr>
<tr>
<td>(30mg)</td>
<td>± 0.66**</td>
<td>± 0.38**</td>
<td>± 0.76</td>
<td>± 0.25</td>
<td>± 0.16**</td>
<td>± 0.37</td>
<td>± 0.20</td>
</tr>
<tr>
<td>Group 4</td>
<td>11.05*</td>
<td>7.05</td>
<td>51.90*</td>
<td>73.62***</td>
<td>15.67*</td>
<td>21.29**</td>
<td>4.35</td>
</tr>
<tr>
<td>(60mg)</td>
<td>± 0.38</td>
<td>± 0.29**</td>
<td>± 0.55</td>
<td>± 0.30</td>
<td>± 0.34</td>
<td>± 0.27</td>
<td>± 0.37**</td>
</tr>
<tr>
<td>Group 5</td>
<td>12.98*</td>
<td>9.26*</td>
<td>56.10**</td>
<td>60.58</td>
<td>14.02**</td>
<td>23.14**</td>
<td>9.20***</td>
</tr>
<tr>
<td>(90mg)</td>
<td>± 0.48</td>
<td>± 0.24</td>
<td>± 1.44</td>
<td>± 0.61**</td>
<td>± 0.34</td>
<td>± 0.44</td>
<td>± 0.23</td>
</tr>
</tbody>
</table>

NS = not significant, * Denotes mean values significant at (P<0.05), **Significant= (P<0.01), ***Significant= (P<0.001).

general weakness of the body. Significant reduction of hemoglobin and other hematological parameters was observed by Lai et al (2011) when acrylamide was given in different doses through gavage to Swiss albino mice. Barber et al, (2001) reported that acrylamide and its reactive metabolite glycidamide are electrophilic in nature and form adducts with sulphydril group of hemoglobin resulting in degradation of haem part of hemoglobin, another cause of reduction in hemoglobin content.

CONCLUSION

1. The incorporation of acrylamide in the diet at 10, 30, 60 and 90 mg/kg is undoubtedly hazardous to Wistar rats and capable of producing enterohepatonephrotoxicity, anaemia and, leukocytosis.

2. Histopathological changes were evident even after the 4 weeks acrylamide withdrawal and sufficient to impose significant haematological changes.

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