Liver In Carbaryl Treated Rats- A Morphological And Morphometric Study

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Abstract: Carbaryl, a carbamate insecticide has been one of the most commonly and extensively used insecticide in the recent years. Since liver plays an important role in the first pass metabolism of carbaryl, which has seen to cause various disturbances in the liver enzymes, the present work was conducted to study the morphological and morphometric changes in the liver produced by the insecticide carbaryl. Inbred adult Wistar albino rats (150-200gm) were injected with 200mg/kg body weight of carbaryl intraperitoneally, five days a week for thirty days. Controls were maintained. Body weight of the animals recorded before the onset of the experiment and prior to their sacrifice revealed a significant increase in weight of the experimental rats (p<0.001), as compared to the controls. The animals were sacrificed within twenty four hours of the last injection, liver was processed and sections (7μ) cut and stained. Microscopically, the liver hepatocytic plates appeared disheveled and the hepatocytes appeared to be singly placed, probably due to dilatation of the bile canaliculi. Most of the liver cells appeared hypertrophied with an increased cytoplasmic basophilia and a large euchromatic nucleus, suggestive of carbaryl metabolism in these cells. Histomorphometry of these hepatocytes revealed a statistically significant increase in the mean long and short diameters in the experimental rats (28.02 ± 4.31 and 19.83 ± 3.94 microns, respectively), as compared to that of the controls (22.04 ± 3.64 and 12.29 ± 2.98 microns, respectively). Signs of hepatocellular degeneration such as councilman bodies, ballooning of the hepatocytes and aggregates of inflammatory cells were seen, predominantly around the portal triads and central veins. Binucleated hepatocytes were suggestive of regenerative attempts by the degenerative cells. The findings are highly conclusive of Toxic hepatitis.

Key Words: Carbaryl, Carbamates, Liver, Hepatocellular degeneration, Toxic hepatitis

Introduction:
Carbaryl, a synthetic 1-naphthyl-N-methyl carbamate insecticide is being used extensively due to its broad spectrum activity in commercial agriculture, poultry, pets, livestock, home and garden pest control. Carbaryl is the most frequently (58.6%) detected N-methyl carbamate in juice samples studied (Rawn et al, 2004). Humans are exposed either directly or indirectly to this pesticide causing various toxicities (M.Sittig, 1985; US Environmental Protection Agency, 1984). An occupational hazard like an increase in the sperm shape abnormality has been documented by Wyrobeck et al (1981) and Meeker et al (2004). Congenital malformations and teratogenesis in chicken embryos and beagle dogs (Marlac et al, 1965; Ghadir and Greenwood, 1966; Smalley et al, 1968; Robens, 1969), a follicular hypertrophy of the thyroid (Stemberg AM, 1970) and necrotic changes in testes and ovaries (Stemberg and Otonan, 1971) have been observed. Inflammatory infiltrations in the heart and the lungs has been reported by Toš-Luty et al (2001), while D.Bigot Lassere et al (2003) observed tumors in the heterozygous P53 knockout mice after oral administration of carbaryl. Sharma (1999) recorded an increase in the activities of transaminases and acid phosphatases suggesting hepatocellular damage. In contrast, Tripathi and Singh (2003) reported a significant inhibition of the liver enzymes including acid and alkaline phosphatases.

Keeping in mind the contradictory biochemical observations of the liver enzymes and the significant role of liver in the metabolism of carbaryl, the present experimental study was undertaken.

Material and Methods:
Inbred adult Wistar albino rats (150-200gm) were taken and divided into two groups. Group-I served as the experiment and Group-II as the control. The animals were group housed with ad libitum access to food and water. The body weights were recorded before the onset of the experiment and prior to their sacrifice. Group-I rats received 200mg/kg body weight of carbaryl intraperitoneally, five days a week for thirty days. The control animals received equal quantity of the vehicle by the same route. The animals were sacrificed within twenty four hours of the last injection. The liver was dissected and processed. Sections (7μ) were cut and stained with haematoxylin and eosin. Histomorphometry was done, data tabulated and statistically analysed by one way ANOVA method and Tukey’s test.

Observations:
The mean body weight of the carbaryl-treated rats was found to be significantly increased as compared to that of the control animals (P<0.001, Table1). Grossly, the liver was encapsulated with
connective tissue capsule and at places, areas of sub-capsular haemorrhage were seen (Fig.1).

### Table 1
Comparison of body weight (gm) in experimental and control rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (µ)</th>
<th>SD</th>
<th>p-value (one way ANOVA)</th>
<th>Significance (Tukey’s test at 5% level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>175.30</td>
<td>13.96</td>
<td>0.579 (&lt; 0.001)</td>
<td>Both the groups are not significantly different from each other.</td>
</tr>
<tr>
<td>Control</td>
<td>180.80</td>
<td>13.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior to sacrifice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>307.40</td>
<td>40.21</td>
<td>0.000 (&lt; 0.001)</td>
<td>Experimental group was significantly different from the control group.</td>
</tr>
<tr>
<td>Control</td>
<td>194.00</td>
<td>11.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Photograph of experimental rat liver showing areas of fatty tissue adherence (F) and numerous spots of subcapsular hemorrhage (shm).

Microscopically, these areas showed large blood filled spaces which replaced the normal liver parenchyma and the hemorrhagic blood was seen to extend into the neighboring sinusoids which became dilated. The liver hepatocytic plates appeared disheveled. The cells varied greatly in size and appeared to be singly placed (Fig.3). In most areas they appeared hypertrophied with an increased cytoplasmic basophilia and a large euchromatic nucleus having a prominent nucleolus (Fig.2). The mean long and short diameters of these hepatocytes was 28.02 ± 4.31 and 19.83 ± 3.94 microns respectively in the experimental group, whereas, it was 22.04 ± 3.64 and 12.29 ± 2.98 microns, respectively in the control group. The mean diameter was significantly increased in the experimental rats as compared to that of the control group (P<0.001, Table.2). There were foci of hepatocellular degeneration, where the cells appeared swollen and empty with lightly stained nucleus and indistinct cellular boundaries. Other degenerating hepatocytes appeared shrunken with dark and highly eosinophilic cytoplasm and a pyknotic nucleus, surrounded with a clear halo (Fig.3). These areas were infiltrated with macrophages and lymphocytes and were predominantly seen in the periportal regions (Fig.4).

Fig. 2: Photomicrograph of transverse section of the experimental rat liver showing hypertrophied hepatocytes(H) with increased cytoplasmic basophilia and a highly euchromatic nucleus (nu) with prominent nucleolus. The Kupffer cells (kup) appear enlarged and the sinusoids(sn) dilated. Haematoxylin and eosin stain (400x).

Fig. 3: Photomicrograph of transverse section of the experimental rat liver showing disheveled pattern of the hepatocytic cords and the degenerating hepatocytes with a highly eosinophilic cytoplasm and a pyknotic nucleus (nu) surrounded by a clear zone, known as the councilman body (cb). The hepatocytes appear to be singly placed. The sinusoid (sn) and the bile canaliculi (c) appear dilated. Haematoxylin and eosin stain (400x).

### Table 2
Comparison of the mean diameters (µ) of the hepatocytes in the experimental and control groups

<table>
<thead>
<tr>
<th>Diameters</th>
<th>Groups</th>
<th>Mean (µ)</th>
<th>SD</th>
<th>p-value (one way ANOVA)</th>
<th>Significance (Tukey’s test at 5% level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>28.02</td>
<td>4.31</td>
<td>0.000</td>
<td>(&lt; 0.001)</td>
<td>Both the diameters of the experimental group were significantly increased as compared to that of the control.</td>
</tr>
<tr>
<td>Control</td>
<td>22.04</td>
<td>3.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>19.83</td>
<td>3.94</td>
<td>0.000</td>
<td>(&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.29</td>
<td>2.98</td>
<td></td>
<td></td>
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</table>
Fig. 4: Photomicrograph of transverse section of the experimental rat liver showing dilated portal vein lined by endothelium (En) and surrounded by councilman bodies (cb) and inflammatory infiltrate (Inf). Haematoxylin and eosin stain (400x).

Discussion and Conclusion:

In the present study, carbaryl was seen to cause an increase in the body weight of the experimental rats, which was statistically significant (P<0.001) as compared to the control animals. Similar findings in rat were observed by Toš-Luty et al (2001) and Pant et al (1996). However, Branch et al (1986) reported a decreased weight in an elderly miner who was exposed to carbaryl dust (10 per cent) for a period of eight months. The difference in opinion could probably be due to a sustained exposure to higher amounts of carbaryl over a longer duration. Microscopically, the hepatocyte cords appeared disheveled and the hepatocytes were seen to be singly placed. This could be probably due to dilatation of the bile canaliculi between the hepatocytes and is suggestive of cholestasis. There was a significant increase in the mean long and short diameters of the hepatocytes, most of which appeared to hypertrophied. This was in accordance with the findings of Shetenberg et al (1968) who demonstrated an increase in cell size in the hypophysis and adrenal glands following carbaryl administration, which was due to an increase in the cellular activity.

The increase in the diameter of the hepatocytes was associated with a large euchromatic nucleus and a cytoplasmic basophilia, indicative of an increase in the cellular activity. Toš-Luty et al (2001) also observed an increase in the amounts of smooth endoplasmic reticulum in the hepatocytes and swelling of mitochondria in cardiomycocytes, postulating a possibility of the metabolism of carbaryl in these cells.

Foci of swollen cells with a lightly stained nucleus and indistinct cellular boundaries is probably suggestive of an ongoing 'ballooning degeneration'. The shrunken hepatocytes around the portal triads and the central vein with a highly eosinophilic cytoplasm and a dense pyknotic nucleus surrounded by a clear halo are the 'Councilman bodies', indicative of hepatocellular degeneration. Degenerative changes were observed in the stratum spinosum of epidermis and in the perikine cells of the cerebellum (Toš-Luty et al, 2001), in chick and duck embryos (Khara, 1966), in myocytes (Smalley et al, 1969), and in testes and ovaries (Sterberg and Otovan, 1971).

Hepatocytes were binucleated at places, which may be a regenerative attempt by the degenerating cells. Similar findings were documented by Smalley et al (1969). Areas of inflammatory infiltrations seen around the portal triads and in between the degenerating hepatocytes are in accordance with the findings of Toš-Luty et al (2001).

Various biochemical studies have revealed a disturbance in the carbohydrate and protein metabolism in the liver following carbaryl exposure, possibly caused by cellular hypoxia, cellular destruction, necrosis and a consequent impairment in the protein synthesis machinery leading to a hepatocellular damage (Kagan et al, 1970; Sharma, 1999; Tripathi et al, 2003; Sharma and Singh et al, 2004). In the present study, the disheveled pattern of the hepatocytic plates, evidence of increased cellular metabolism and regenerative attempts co-existing with ballooning degeneration, Councilman bodies and inflammatory infiltrations around the portal triads along with the dilatation of the bile canaliculi, are conclusive of Toxic Hepatitis.

References:


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