ABSTRACT
Liver is a dual organ having both secretory and excretory functions, is a vital organ of the body engaged in various metabolic, secretory and biotransformation activities. Any damage to liver can impair any all of these functions. In the present investigations histopathological changes with light and SEM on liver of mice intoxicated with nickel salts (NiSO₄), have been observed. The oral administration of different doses of nickel salts was made for 21 days. Histopathologically, the sinuses broadened, the number of binucleated cells increased as compared to control, and the cytoplasm was more diffused. With moderate and high doses haemolysis, a mass of damaged hepatic cells and nuclei was observed. Under SEM, the microvilli of hepatic cells were seen to be damaged and the sinusoids had less number of Kupffer cells. The debris of damaged cells increased with increase in dose. The effects were dose dependent after treatment with NiSO₄.

KEYWORDS: Kupffer cells, mice, nickel, SEM.

INTRODUCTION
Liver is an important organ as it performs multifarious activities like organic metabolism, cholesterol metabolism, digestive functions via bile production and secretion, clotting functions, endocrine functions, excretory and degradative functions, it biotransforms many endogenous and foreign organic molecules.¹

Nickel is present in many foods (e.g., cocoa, hydrogenated fats, ground nuts, soybeans and tea), and some dietary nickel is derived from the use of stainless steel cooking utensils and
Small amounts of nickel are present in fossil fuels, and their combustion is a major source of ambient nickel levels. Some workers have reported hepatic toxicity in animals caused by nickel salts. Histopathological lesions in kidney and liver in white fish have been proved to be the most sensitive and reliable indicators of Ni exposure. In liver, areas of focal necrosis and altered bile ducts were observed. Significant increases in lipid peroxide were also observed.

Comparative study of toxicity of nickel salts on the liver has not been made by any earlier worker. Hence the present studies have been undertaken to see the histopathological changes after NiSO₄ with light and scanning electron microscopy.

**MATERIAL AND METHODS**

Adult male mice, weighing 30-35 gm of Balb/c obtained from the Central Animal House, Babylon University, were orally administered different doses of nickel sulfate daily for 21 days through gavage i.e. 6.3 mg/kg b.wt., 25.8 mg/kg b.w. and 45.1 mg/kg of NiSO₄ ·. Light was maintained from 7 AM to 7 PM during summer and 7 AM to 6 PM in winter. The animals were divided into four groups each group having 5 mice (one control group on normal diet and water).

The weight of liver from treated groups was taken along with control after 21 days when the mice were dissected and percentage change was calculated by the following formula:

\[
\frac{\text{Weight of tissue of treated group} - \text{Weight of tissue of control group}}{\text{Weight of tissue of control group}} \times 100
\]

The liver from all these groups was cut into small pieces and put in 0.9% physiological saline. Fixation of liver tissue was done in Bouin. The tissues were dehydrated in different grades of alcohol, cleared in benzene and embedded in paraffin wax (60-62°C). Sections of 8µ thick were cut on microtome and stained in haematoxylin/eosin (H/E). The liver from all these groups was cut into small pieces, washed in phosphate buffer and fixed in 4% glutaraldehyde in phosphate buffer. The tissues were dehydrated in ascending acetone grade and finally taken to amylacetate and critical point dried. The dried samples were fixed on stubs for gold coatings with JFC 1100, ion sputter and then examined under JSM 6100 Scanning Electron Microscope (SEM).
Observations
The intake of feed and water by treated mice reduced as compared to control. Moreover, the decrease was dose dependent. The liver weight decrease after low doses salts was non-significant whereas after moderate and high doses the decrease in liver weight was significant and highly significant respectively as shown in Table 1. The control liver stained with H/E has been observed to be made up of number of lobules. Each lobule contains a central vein surrounded by endothelial layer. The hepatic cells of parenchyma are arranged in hepatic plates or hepatic laminae (Figure 1).

Table 1: In vivo effect of nickel sulfate (NiSO₄) administration on liver weight of male mice

<table>
<thead>
<tr>
<th></th>
<th>Control ( wt.gm )</th>
<th>Low Dose (6.3 mg/kg)</th>
<th>Mod. Dose (25.8 mg/kg)</th>
<th>High Dose (45.1 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (wt.gm)</td>
<td>1.5±0.05</td>
<td>1.51±0.13*</td>
<td>1.39±0.11**</td>
<td>1.36±0.11***</td>
</tr>
</tbody>
</table>

Value represents mean ± S.D., n=5 in each group.
The p-value was calculated between the test group and control group.
* Non-significant  ** Significant  *** Highly significant

Figure 1: Transverse section of liver of mice Showing nuclei (N), neucleoli (Nu) in nuclei, cytoplasm granules (CG) in hepatocytes and kupffer cells (KC) for the control animals stained with Haematoxylin / Eosin (magnification 400 X).

With low dose, a few spaces have been observed in the liver. The sinuses broadened. The number of binucleated cells increased as compared to control. The nuclear chromatin took darker colour (Fig.2).
With moderate and high dose, the above effects were more prominent. The nuclei took darker stain than control. The spaces increased. The two nuclei of the hepatic cells even showed fusion in some cases. The intercellular membranes were lost. The stellate Kupffer cells showed an increase in number as compared to control. The vacuoles in the hepatic cells showed an increase (Figure 3).

The SEM of liver the parenchymatous hepatic cells along with sinusoids showing the wide fenestrations (Figure 4) were observed.
With low dose, there were some changes in the hepatic cells resulting in the damage of microvilli. In the sinusoids, blood cells due to haemolysis can be observed (Figure 5).

**Figure 5: Scanning electron micrograph of liver of mice treated with low doses of NiSO$_4$**

With moderate and high dose, the damage to hepatic cells was more. The sinusoids had less number of Kupffer cells, the debris due to necrosis of cells could be observed. The microvilli were not clearly visible. A few blood cells were observed (Figure 6).

**Figure 6: Scanning electron micrograph of liver of mice treated with high doses of NiSO$_4$**

**DISCUSSION**

With low doses of NiSO$_4$, the sinuses broadened, some pycnosis of nuclei and necrosis of cytoplasmic contents of hepatic cells was observed by light and scanning electron microscopy.

With moderate and high doses, Kupffer cells showed increase in number and haemolysis along with a mass of damaged hepatic cells and nuclei were also observed. These observations are in conformity with those of Donskoy$^{[6]}$ after NiSO$_4$ treatment, who reported microvesicular fatty metamorphosis, mild hydropic degeneration and foci of inflammation.
Electron microscopy revealed microvesicular degeneration of hepatocytes and related increase in serum aspartate aminotransferase activity.\[^7\]

Areas of focal necrosis and altered bile ducts in liver after nickel exposure. The differences in the rough endoplasmic reticulum, decreased liver cholesterol and triacylglycerol accumulation were observed.\[^{10-11}\]

Histological examination of rat liver revealed microvesicular fatty metamorphosis, mild hydropic degeneration, and foci of inflammation. Electron microscopy revealed microvesicular steatosis of hepatocytes and dose-related increases in serum aspartate aminotransferase activity.\[^7\]

The histopathological changes in liver caused by nickel salts will interfere in the functions of liver as elaborated under the head ‘introduction’ and will also be responsible for decrease in liver weight as observed during present observation. These observations are in agreement with the observations made by\[^{12,13}\] who reported a decrease in liver to body weight after oral intake of nickel with diet.

From the study of effect of nickel compounds on the liver of mice it can be concluded that the toxicity of NiSO\(_4\) was dose dependen.

REFERENCES


