

## **Electron Microscopic Study on the Effect of Liv.52 on Carbon Tetrachloride Treated Liver**

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### **INTRODUCTION**

The liver is a major and the largest organ in the body. It usually does not respond to any available medical treatment after reaching a certain stage of degeneration. Most drugs are metabolised in the liver after absorption from the intestines.

The introduction of Liv.52, an indigenous compound, has been claimed to be one of the remedies for the treatment of many liver disorders<sup>1,3,4,6,7</sup>. It has been further reported that it reduces the intrahepatic congestion and cholestasis. That liver regeneration was induced by this drug was claimed earlier by Sheth *et al.*, 1960<sup>11</sup>. This was later on confirmed by Prasad in 1974<sup>8,9</sup>, where explants of cirrhotic liver tissue have shown signs of regeneration, when grown in organo type culture, *in vitro*, in the medium containing Liv.52. According to him it also helps in glycogenesis in addition to the regeneration of liver cell and also decreases fibrotic changes. To find out the action of this drug on the liver cell at a subcellular level, this ultra-structure study has been carried out.

### **MATERIALS AND METHODS**

For this study 33 young male mice were selected. Out of these, three mice were kept as normal and the remaining 30 mice were divided equally into three groups. In group one all the ten mice were given subcutaneous injection of carbon tetrachloride (in the dose of 0.1 ml in arachis oil) twice a week upto the end of 60 days. Those animals kept in group two received Liv.52 drops orally on every alternate day upto 60 days. In addition, they also received subcutaneous injection of carbon tetrachloride in the same dosage and for the same period as in group one. The remaining animals of group three were given subcutaneous injection of carbon tetrachloride (in the same dose) upto 60 days. Thereafter they received Liv.52 drops orally on every alternate day for a further 30 days. During this period these animals did not receive the injection of carbon tetrachloride.

Five animals from each group were sacrificed at the end of 30 and 60 days by decapitation. The liver tissue was dissected out and cut into small pieces and fixed immediately in gluteraldehyde solution. After fixing in Osmium tetroxide the tissue was dehydrated in different grades of alcohol and embedded in Araldite solution. These sections were cut at the thickness of 600 Å by Portar Blum ultramicrotome. All the sections were stained with uranyl acetate and lead nitrate and then examined under Philips Electron microscope 300.

## OBSERVATIONS AND DISCUSSIONS

Fig. 1 – The Electron microscopy of normal mouse liver shows an oval nucleus (N) with nucleolus (NL). The cytoplasm contains a fair amount of glycogen granules of different size (GL). The rough endoplasmic reticulum (RER) is in parallel direction studded with fine grains of ribosomes. There are a fair number of mitochondrial (M) X 7700.

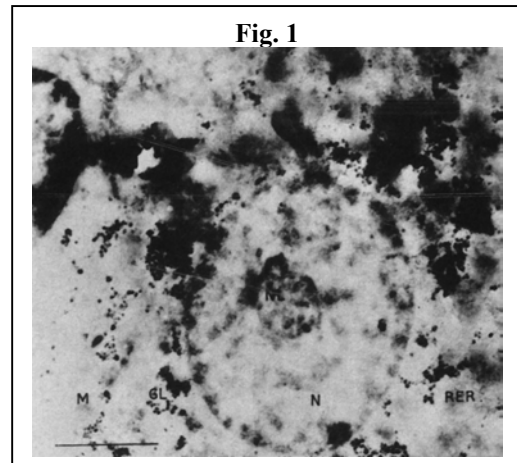


Fig. 2 – The Electron microscopic study of the liver treated with carbon tetrachloride for 60 days shows serrated appearance (indicated with an arrow) of the nucleus (N) with decrease in size. The nucleolus is not well defined. The cytoplasm shows destruction of rough endoplasmic reticulum (RER), which is devoid of ribosomes. Some scattered granular ribosomes could be seen, suggesting destruction of the nucleic acid i.e. RNA and DNA synthesis. No mitochondria could be located. The glycogen granules have become smaller in size and have clumped like droplets (GL). The cellular membrane is not well defined. Inter-cellular substance containing a fair amount of collagen fibre formation (CF) indicates the stage of fibrotic changes in the liver X 1300.

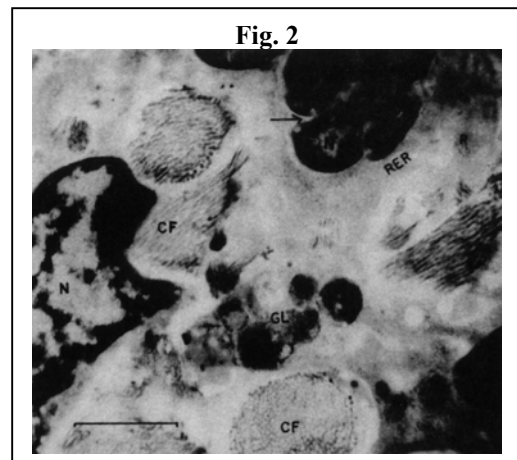


Fig. 3 – Similar picture showing extensive degenerative changes. The cytoplasm is devoid of its many contents including endoplasmic reticulum with increased number of vacuolation (V). The nucleus has reduced in size. Cell membranes are ill defined X 7700.

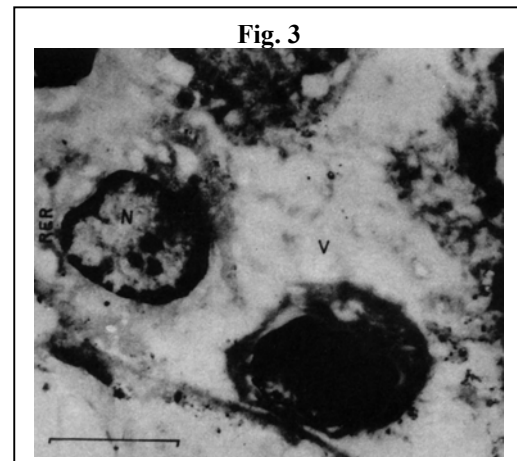


Fig. 4 – Those animals treated with carbon tetrachloride along with Liv.52 show reduction in nuclear (N) serration (indicated with an arrow) with a small nucleolus (NL). The cytoplasm shows reformation of rough endoplasmic reticulum (RER) studded with fine particles of ribosomes. Although the vacuolation still persists (V) fine granules of glycogen have reappeared (GL). The collagen fibre formation (CF) has reduced remarkably. A big secreting droplet (DL) probably biliary secretion can be seen. This suggests that Liv.52 helps to some extent in neutralizing the action of carbon tetrachloride, stimulating the liver function and reducing the fibrotic changes in the liver X 10400.

Fig. 5 – The Electron microscopic examination of those livers treated with Liv.52 after the administration of carbon tetrachloride, shows reappearance of nuclear shape (Oval) as seen in normal liver (N) with a nucleolus (NL). The serration of the nuclear membrane has disappeared. The cytoplasm contains increased amounts of both smooth and rough endoplasmic reticulum (GR). Mitochondria have started reappearing and are full of crystals (M). Few secreting droplets (G) can also be seen X 6300.

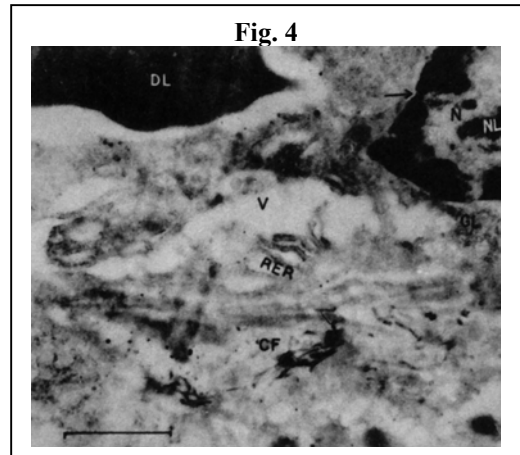
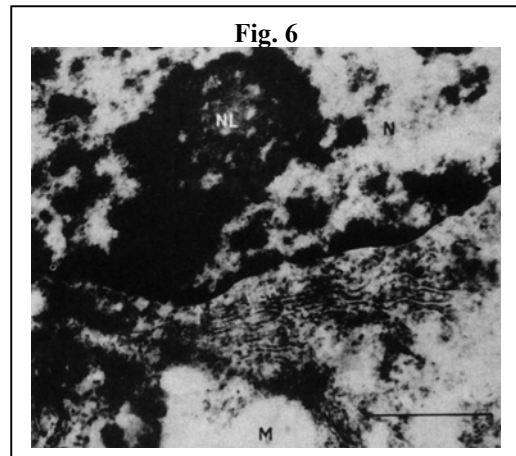
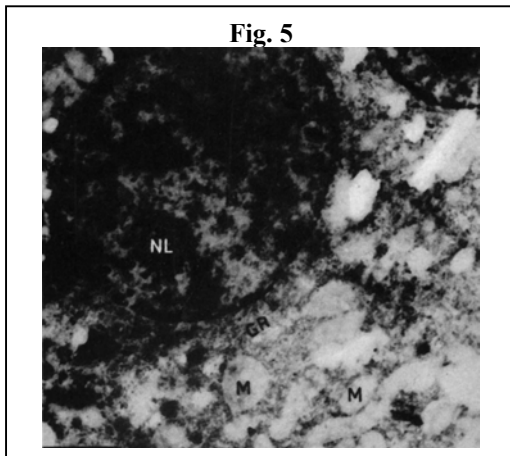


Fig. 6 – Shows well organized nuclear (N) membrane (indicated with an arrow) with a big nucleolus (NL) suggesting increased chromotive and nucleic acid synthesis. The rough endoplasmic reticulum (RER) has reappeared and is well organized, studded with dense ribosomes. X13000. There are increased number of mitochondria (M), which are hypertrophied and are having some organized crystals.



This suggests that the administration of Liv.52 probably stimulates the liver function not only in its biliary output<sup>5,10</sup> but also by activating the regeneration of the rough endoplasmic membrane and ribosomes. Thus, it increases the synthesis of nucleic acid and ribosomal enzymes. This confirms many earlier observations both *in vivo* and *in vitro*. Further, the liver damaged by carbon tetrachloride and not functioning may also start regenerating and functioning by increasing the nucleic acid synthesis. In addition, the glycogenesis has also increased remarkably by the administration of Liv.52, which corroborates our earlier observation made *in vivo*.

## SUMMARY

1. Liver damage was produced in mice by the administration of carbon tetrachloride. In addition, Liv.52 drops were given orally to these animals along with carbon tetrachloride and also after this treatment.

2. Degenerative and fibrotic changes produced by carbon tetrachloride could be prevented to some extent by the administration of this drug.
3. Further the damaged liver also showed signs of regeneration of nucleus, endoplasmic reticulum, mitochondria and also some glycogen granules, when treated with Liv.52.

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