LIGHT AND ELECTRONMICROSCOPICAL INVESTIGATIONS ON THE CYTOPROTECTIVE EFFECTS OF PROSTAGLANDIN E_2 AND DIMETHYL SULFOXIDE IN CARBON TETRACHLORIDE-INDUCED LIVER NECROSIS

(Received July 31, 1991)

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SUMMARY

Studies have shown that prostaglandin E_2 (PG E_2) can prevent liver necrosis due to several agents. Dimethyl sulfoxide (DMSO) is also known with its cytoprotective efficiency. To determine the degree of cytoprotective effectivenesses of these two agents, 20 female rats were divided into 5 groups.

Livers of rats treated only with carbon tetrachloride showed severe necrosis. In groups pretreated with prostaglandin E_2 and dimethyl sulfoxide, degree of necrosis was very low. Especially prostaglandin E_2 could perform this cytoprotection very well.

It is concluded that these two cytoprotective agents could prevent carbon tetrachloride-induced liver cell necrosis in the rats at different degrees.

Key Words: Carbon tetrachloride, prostaglandin E_2, dimethyl sulfoxide, cytoprotection.

INTRODUCTION

Prostaglandins are 20-carbon oxygenated fatty acids present in most mammalian cells and tissues (1). Beside several actions, they are known to protect many tissues against a variety of damaging agents (2-4). Thus, they perform cytoprotection.

On the other hand, dimethyl sulfoxide is known having beneficial therapeutic effects (3, 7). It is reported that it may act as analgesic and anti-inflammatory agent when applied to the skin; it also acts as a diuretic and may inhibit blood coagulation. Dimethyl sulfoxide is also known to be a cytoprotective agent (5-7).

In this study, we want to describe how prostaglandin E_2 and dimethyl sulfoxide could prevent the necrosing effect of carbon tetrachloride and we intend to compare the cytoprotective degree of these two agents.

MATERIALS AND METHODS

20 female Wistar albino rats of 180-200 gr average body weight were used in this study. All rats were accomodated in the same room and fed a regular diet and water ad libitum. Rats were divided into 5 groups: In group I (n=4), animals were sacrified after 24 hours following i.p. injection of saline solution (6 ml/kg). In group II (n=4), animals were injected a single dose of carbon tetrachloride i.p. (6 mg/kg). In group III (n=4), rats were pretreated with prostaglandin E_2 (20 ug/kg) i.p. given 1 hour before i.p. carbon tetrachloride (6 mg/kg) administration. In group IV (n=4), rats were injected a single dose of i.p. dimethyl sulfoxide (4 ml/kg). In group V(n=4), rats were pretreated with dimethyl sulfoxide i.p. (4 ml/kg) given 1 hour before i.p. carbon tetrachloride (6 mg/kg) administration.

In all experimental groups, rats were decapitated after 24 hours following the carbon tetrachloride injection.

For light microscopic examinations, liver tissue specimens were fixed in Bouin's and 10% formalin solutions. The sections from paraffin blocks (4-5 μ) were stained with Haematoxylin and Eosin and Periodic acid-Schiff dyes. Then, they were investigated at Olympus BH-12 light microscope. For ultrastructural examinations, tissue specimens were fixed in 2.5 % phosphate-buffered glutaraldehyde and then postfixed in 1 % OsO_4 solution for 1 hour. The sections taken from Vestopal W blocks (400-600 A°) were stained with uranyl acetate and Reynold's method. All sections were examined at Jeol 100 C electronmicroscope.

RESULTS

In group I (control group), at light microscopical level, hepatocytes with rounded nuclei, sinusoids and sinusoidal walls were in their normal structures (fig. 1). At the ultrastructural examination of that group, rounded hepatocyte nuclei, mitochondria with cristae, granular and agranular endoplasmic reticulum, Golgi complex, few lipid droplets and glycogen granules were noted (fig. 6).

In group II, a severe fatty degeneration in hepatocytes and cell infiltration in parenchyme was quite evident (fig. 2). In Periodic acid-Schiff stained sections, a decrease in glycogen content within hepatocytes was revealed. In electronmicrographs of that group, wide perinuclear spaces around hepatocyte nuclei were seen. Golgi complex cisternae together with those of the endoplasmic reticulum seemed to be quite enlarged. A loss both in ribosomal content and mitochondrial matrix were noticed together with a severe lipid accumulation

and decreased glycogen content within the hepatocyte cytoplasm (fig. 7 and 8).

In group III, minimal fatty degeneration and cell infiltration were observed within the liver parenchyme (fig. 3). Normal glycogen content of the hepatocytes were determined at Periodic acid-Schiff stained sections. At electronmicroscopical level, only a slight dilation in agranular endoplasmic reticulum was present. Other organelles seemed to be normal with an obvious glycogen content in rosette form and few lipid droplets (fig. 9).

In group IV, the hepatocytes were observed to reflect normal histology at both light and electronmicroscopical levels (fig. 4 and 10).

In group V, some fatty degenerations and a slight cell infiltration were observed at light microscopical examinations. Glycogen content was noted slightly decreased in Periodic acid-Schiff stained sections. At the ultrastructural level, while some hepatocytes seemed to be normal; dilated perinuclear spaces, ribosomal loss in granular endoplasmic reticulum, a decrease in glycogen and lipid droplets were present in cytoplasms of other hepatocytes (fig. 11). So, various degrees of cellular degeneration were evaluated within that group.

DISCUSSION

The aim of this study was to investigate how prostaglandin E_2 (PG E_2) and dimethyl sulfoxide could prevent the degenerative effect of carbon tetrachloride.

As it is well known, carbon tetrachloride and some other agents are used in order to produce severe degeneration in liver for best understanding of how cytoprotective agents work (8-11). For this purpose, we used a high dose of carbon tetrachloride (6 mg./Kg.) in this study.

In group treated only with carbon tetrachloride our findings such as variations in hepatocytes sizes, intense fatty degeneration and a decrease in glycogen content were parallel with those of some studies (3, 8, 11-13). At the ultrastructural examinations, extremely dilated endoplasmic reticulum membranes, loss in ribosomal content, swollen and degenerated mitochondria, many lipid droplets, decrease in glycogen showed similarities with the findings reported in some studies (11, 14, 15). We also noticed the close relationship of the lipid droplets with Golgi complex.

In many studies (14, 16-18), a biochemical approach was done in order to explain the mechanism of toxic effect of carbon tetrachloride. It was mentioned that, the mechanism was due to the enzymatic activation of carbon tetrachloride to a CCl. free radical within the membranes of endoplasmic reticulum. That phenomenon was followed by chloromethylation, saturation, peroxidation and destruction of unsaturated fatty acids of endoplasmic reticulum membranes. So, endoplasmic reticulum was said to be the target side for carbon tetrachloride (19, 11). We have already noticed in group II extremely dilated endoplasmic reticulum membranes as a morphologic reflection of those events. In some studies (19, 17, 18, 14)), it was concluded that carbon tetrachloride-induced necrosis resulted in an increase in surface tension. Then, a following loss in ribosomal content was mentioned. We also observed that loss at the ultrastructural examination of group II.

A high increase in lipid droplets within hepatocyte cytoplasms in group II was related in some studies (3, 8, 12, 16) to the increased triglyceride synthesis and decreased oxydation of fatty acids as a result of mitochondrial degeneration.

In group III, our evaluations implied that prostaglandin E_2 could protect liver tissue from carbon tetrachloride-induced necrosis. In many studies (1, 2, 4, 11, 20-24), this protective effect of prostaglandin E_2 was evaluated and it was concluded that a stabilization process of lysosomal and plasma membranes of hepatocytes exists. But, this cytoprotective mechanism was not clearly understood (2, 5, 1, 11).

In group IV, we administered only dimethyl sulfoxide (4 ml/kg) to the rats. In that group, there was no morphological change within the liver tissue. This means, in this dose range, dimethyl sulfoxide had no toxic effect on liver cells. Similar results were reported in related studies (5, 4, 20).

In group V, pretreated with dimethyl sulfoxide, especially ultrastructural eavluations implied a less degree of cytoprotection compared with group III pretreated with prostaglandin E_2. While some hepatocytes seemed to be intact; in others, lipid droplets, mitochondrial degeneration and absence of glycogen granules suggested that dimethyl sulfoxide could not perform its cytoprotective effect in the whole liver tissue. In studies where higher doses of dimethyl sulfoxide were used, a good cytoprotection was reported (24 - 26). It is declared that dimethyl sulfoxide performs it through decreasing the toxic effects of free radicals.

We can conclude that PGE2 has a strong cytoprotective effect. Dimethyl sulfoxide effect is thought to be dose-dependent. We think a higher dose may provide further cytoprotection and we aim to investigate it in further studies.

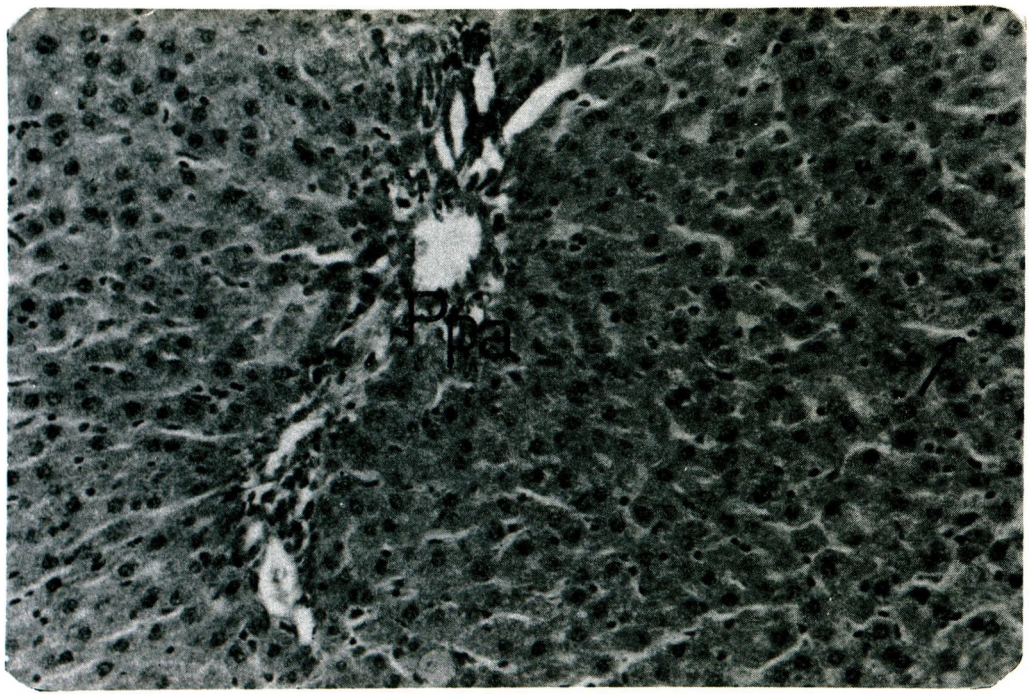

FIGURE 1: In control group micrograph, portal triad (Ppa), liver parenchymal cells, sinusoids and Kupffer cells (arrow) are seen. Haematoxylin-Eosin staining. X 200.

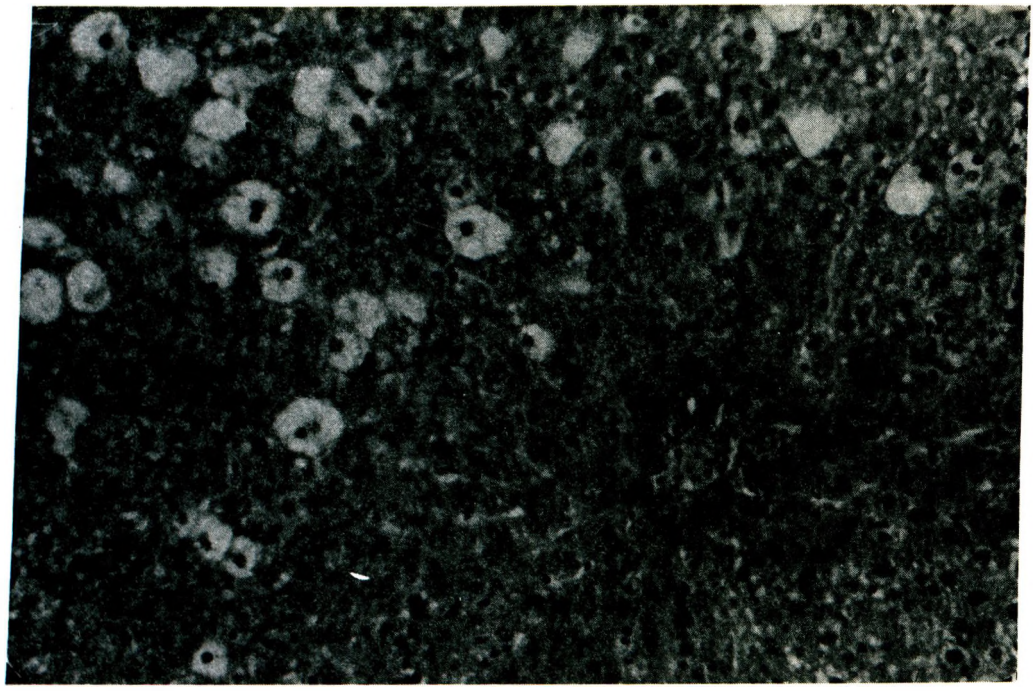

FIGURE 2: In carbon tetrachloride injected group, fatty degeneration (arrow), swollen hepatocytes and cell infitration in the liver parenchyme are observed. Haematoxylin-Eosin staining. X 200.

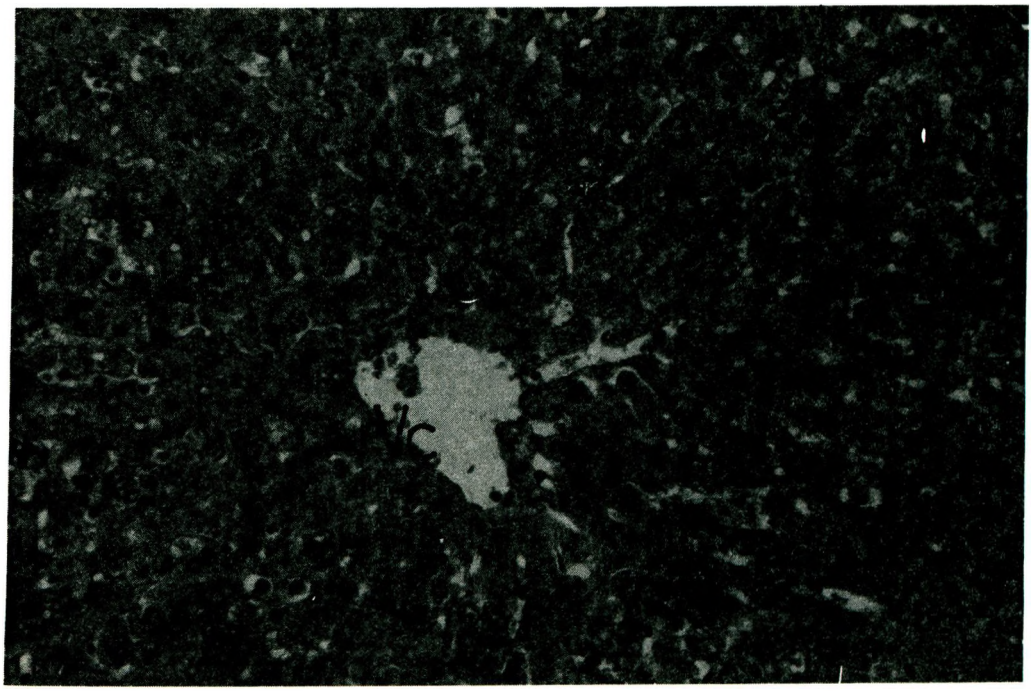

FIGURE 3: In liver of the rat pretreated with PG E_2, no necrosis is observed. Vc: Central vein. Haematoxylin-Eosin staining. X 200.

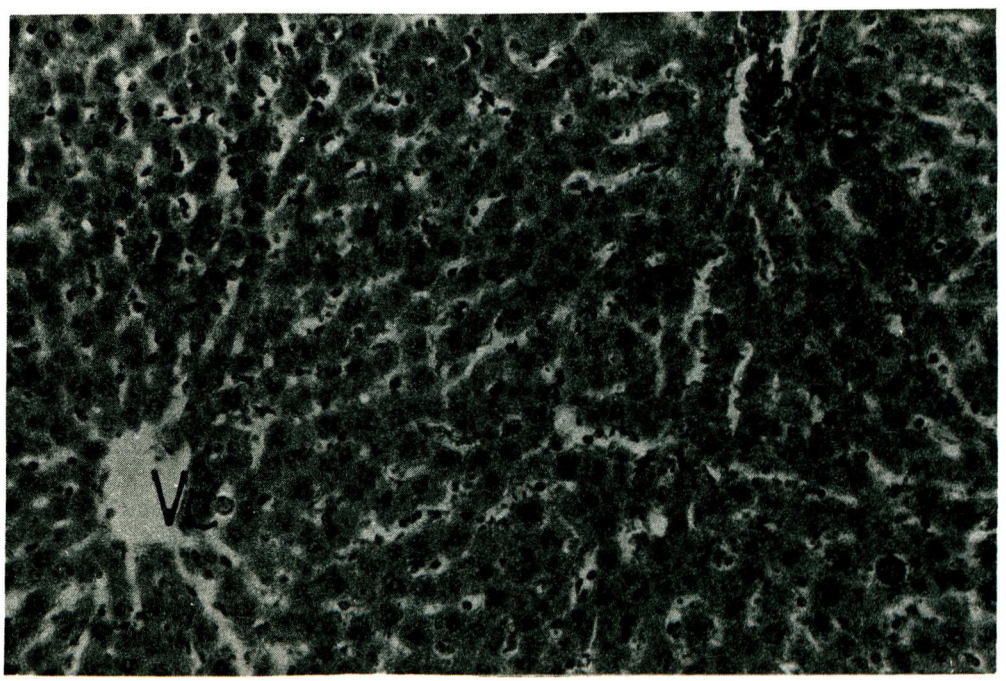

FIGURE 4: In liver of rat treated only with dimethyl sulfoxide, a normal liver histology is observed. Vc: Central vein; Ppa: Portal triad. Haematoxylin-Eosin staining. X 200.

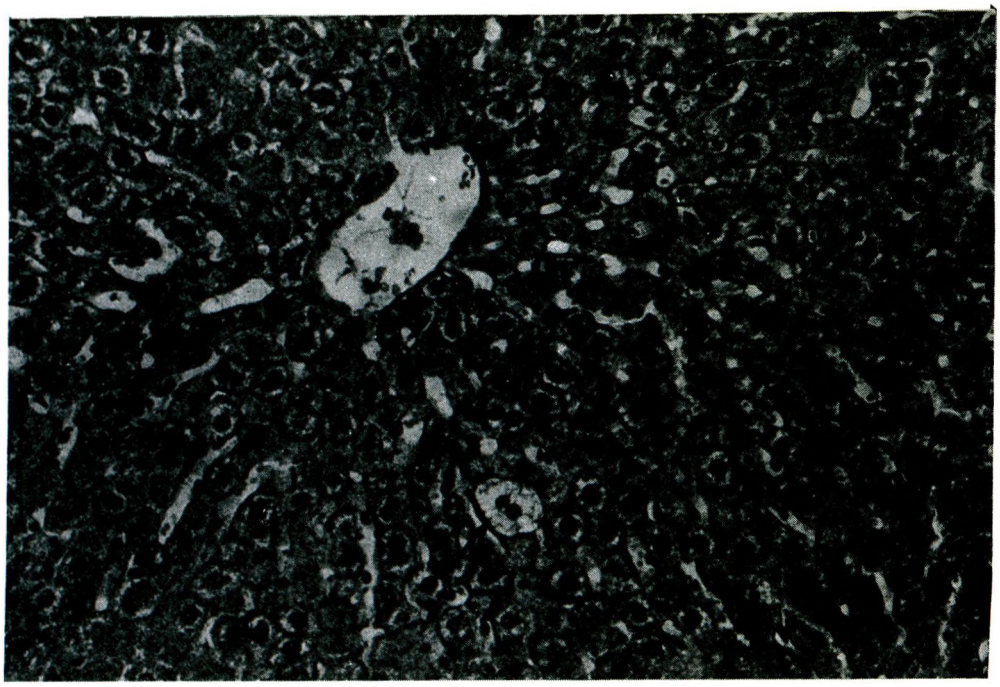

FIGURE 5: In group pretreated with dimethyl sulfoxide, fatty degenerations in some hepatocytes (arrow) are observed. Haemotoxylin-Eosin staining. X 200.

FIGURE 6: In control group, hepatocytes, sinusoids (Si) and bile canaliculi (Sk) are seen. Nu: Nucleus; Mi; Mitochondrion; GER: Granular endoplasmic reticulum; SER: Agranular endoplasmic reticulum; G: Golgi complex; Gl: Glycogen. X 10.000.

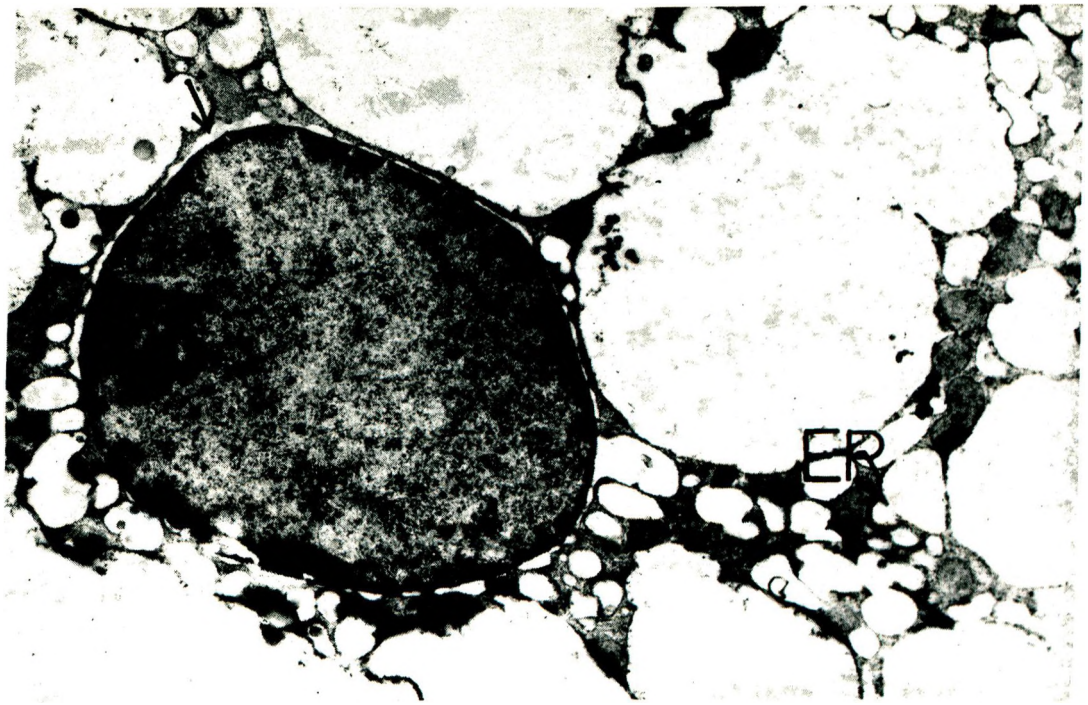

FIGURE 7: In group treated with carbon tetrachloride, extremely hypertrophied endoplasmic reticulum membranes (ER) and dilations at the perinuclear spaces (arrow) are present. X 10.000.

FIGURE 8: In group treated with carbon tetrachloride, hepatocyte nucleus (Nu), nucleolus (Nk), giant lipid droplets (Li) and their relationship with Golgi complex are observed. X 10.000.

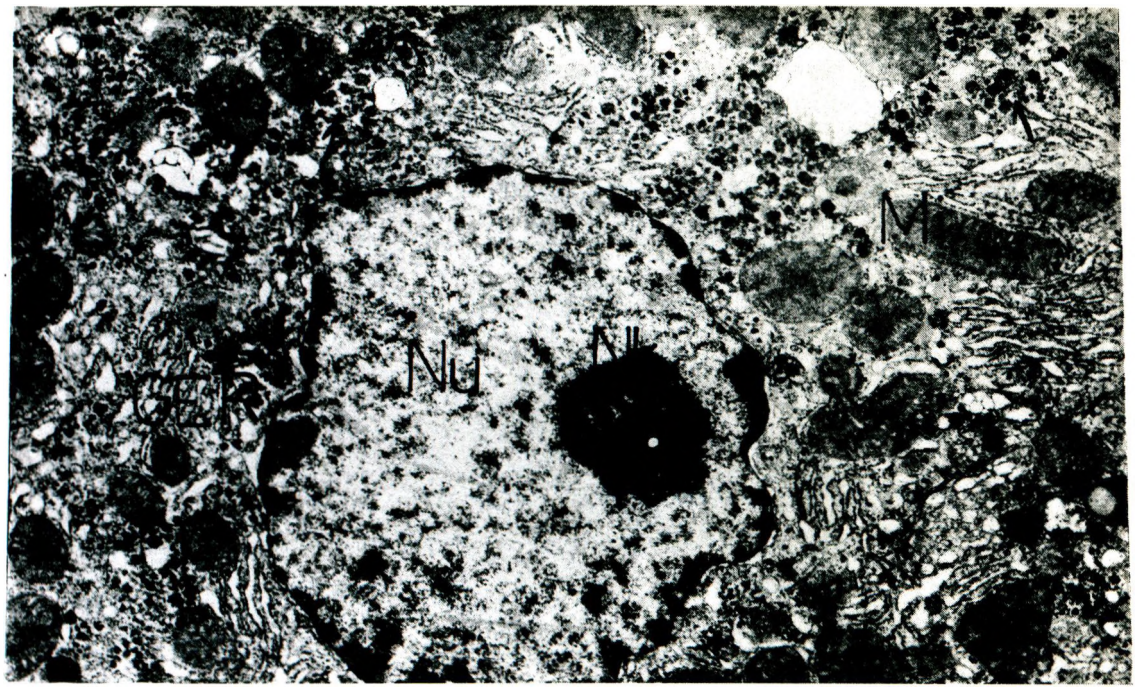

FIGURE 9: In group pretreated with PG E_2 before carbon tetrachloride administration, nucleus (Nu), nucleolus (Nk), mitochondria (Mi), well developed granular endoplasmic reticulum (GER) and glycogen in rosette form (arrow) are present. X 13.200.

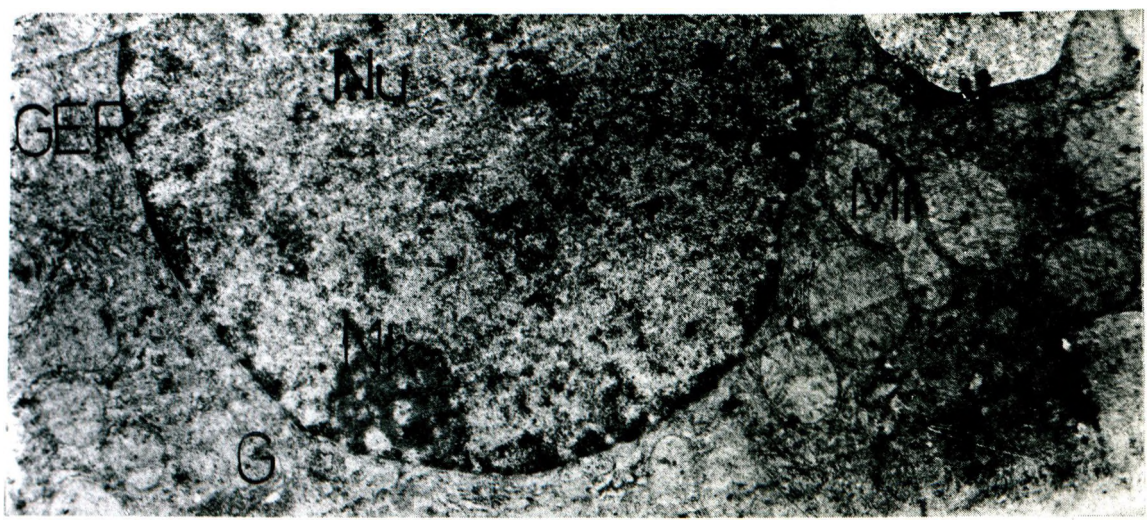

FIGURE 10: In group treated only with dimethyl sulfoxide, no necrosis in liver cells is observed. Nu: Nucleus; Nk: Nucleolus; Mi: Mitochondrion; G: Golgi complex; GER: Granular endoplasmic reticulum. X 13.200.

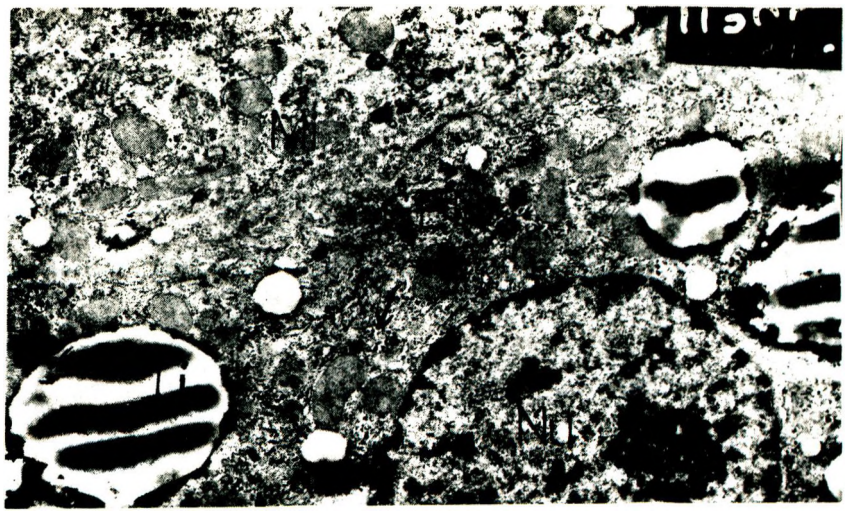

FIGURE 11: In liver of rat pretreated with dimethyl sulfoxide before carbon tetrachloride administration, some lipid droplets (Li), poorly developed granular endoplasmic reticulum (GER) and glycogen particles (arrow) are seen. Nu: Nucleus; Mi: Mitochondrion. X 10.000.

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