



TOXICITY STUDIES ON CARBON TETRACHLORIDE AND N-NITROSOMETHYLUREA INDEPENDENTLY AND IN COMBINATION IN RATS*

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Abstract

This trial was designed to study the subchronic effects of carbon tetrachloride (CCl₄) and N-Nitrosomethylurea (NMU) independently as well as in combination. In the CCl₄ and CCl₄ + NMU groups, the lipid peroxidation (LPO) recorded the peak level on the 15th day while in NMU group it was reached only on the 75th day. Serum gamma glutamyl transpeptidase (GGT) levels were higher on the 15th day in CCl₄ group and on the 75th day in NMU and CCl₄ + NMU groups. Liver tissue GGT recorded the maximum level on the 15th day while it reached only by the 75th day in the NMU and CCl₄ + NMU groups. DNA strand breaks recorded an increase on the 45th day in CCl₄ group, 75th day in the NMU group and from 45th day onwards in the CCl₄ + NMU group. Initially there was cytoplasmic vacuolization in the hepatocytes followed by cholangio-fibrosis while kidney revealed degenerative changes. Congestion and haemorrhages were seen in the stomach during the initial period of the trial in the CCl₄ group. In the NMU group liver showed centrilobular necrosis, while kidney revealed tubular epithelial cytoplasmic vacuolations. Hyperplasia of squamous epithelium was seen in the stomach while testes showed degenerative changes. In the combined group, focal bile duct hyperplasia was prominent in the liver, while kidneys revealed glomerular swelling and exudation with degenerative changes in the tubules. Hyperplasia and

hyperkeratosis were seen in the stomach while testes showed degenerative changes.

Key words: N-Nitrosomethylurea, Carbon tetrachloride, toxicity, rats

Nitroso compounds represent a major class of important chemical carcinogens and mutagens. Their importance lies in the fact that these compounds can be formed *in vivo* from precursors and therefore lead to endogenous exposure to such compounds (Preussmann, 1980). N-Nitrosomethylurea (NMU) which belongs to nitrosamide group of N-nitroso compounds was shown to produce marked toxic changes in tissues with rapid growth of tissues such as bone marrow, lymphoid organs and gastrointestinal tract (Leaver *et al.*, 1969).

Carbon tetrachloride is a potent hepatotoxin. The inherent ability of carbon tetrachloride (CCl₄) to induce hepatic necrosis and liver cell regeneration together with concurrent *in vivo* formation of N-nitroso compounds were found to be conducive to the development of hepatocellular alterations and tumours by their combination. This study was undertaken to assess the toxicity of NMU in subchronic doses and to study the biochemical and pathological alterations.

Materials and Methods

The experimental animals were divided into five groups. The first group of 10

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rats was maintained as untreated control group. The second group of 20 rats was given CCl_4 at the dose rate of 0.2 ml/kg body weight mixed with olive oil in the ratio 1:1. The administration was daily for 10 days and observed for 10 weeks. The third group of twenty rats was orally administered NMU at a dose rate of 25 mg/kg body weight at weekly intervals for 10 weeks. The fourth group of 30 rats was administered CCl_4 orally at a dose rate of 0.2 ml/kg body weight in olive oil for a period of 10 days daily. This was followed by oral administration of NMU at a dose rate of 25mg/kg body weight at weekly intervals for 10 weeks. The fifth group of 20 rats was orally administered 0.1 ml of olive oil each, for a period of 10 days daily and kept for 10 weeks.

All the rats were kept under observation for 10 weeks. They were sacrificed regularly at fortnightly intervals. Estimations of lipid peroxidation (Ohkawa *et al.*, 1979) and protein (Lowry *et al.*, 1951) were carried out in freshly collected liver pieces. The activity of gamma-glutamyl transpeptidase in freshly collected blood serum (Jacob, 1971) and liver (Fiala *et al.*, 1976) were also estimated. Fluorometric analysis of DNA unwinding (Birnboim and Jevcak, 1981) was carried out in freshly collected blood Tissues were fixed

in 10% buffered formalin for histopathological studies. Tissues were processed and embedded in paraffin, sectioned at 5μ thickness and stained by hematoxylin and eosin.

Results and Discussion

The mean lipid peroxidation (LPO) values for the different treatment and control groups are furnished in Fig.1. In the CCl_4 group the peak mean LPO value was obtained by the 15th day which got reduced on the 75th day yet maintaining higher levels than controls. The increased levels of LPO were indicative of peroxidative deterioration of membrane lipids caused by CCl_4 leading to the genesis of hepatocyte injury and neurosis. This was observed by early workers (Comporti, 1985). The NMU group also showed a progressive increase in the mean LPO levels from the 15th day of observation. In the CCl_4 + NMU group, the highest LPO levels were obtained during the initial period when CCl_4 was administered. But following the administration of NMU during the later part of the trial although the increase in the LPO levels was significant they were on the decline when compared to the peak value at 15 days. These findings suggested that NMU was not as potent as CCl_4 in inducing

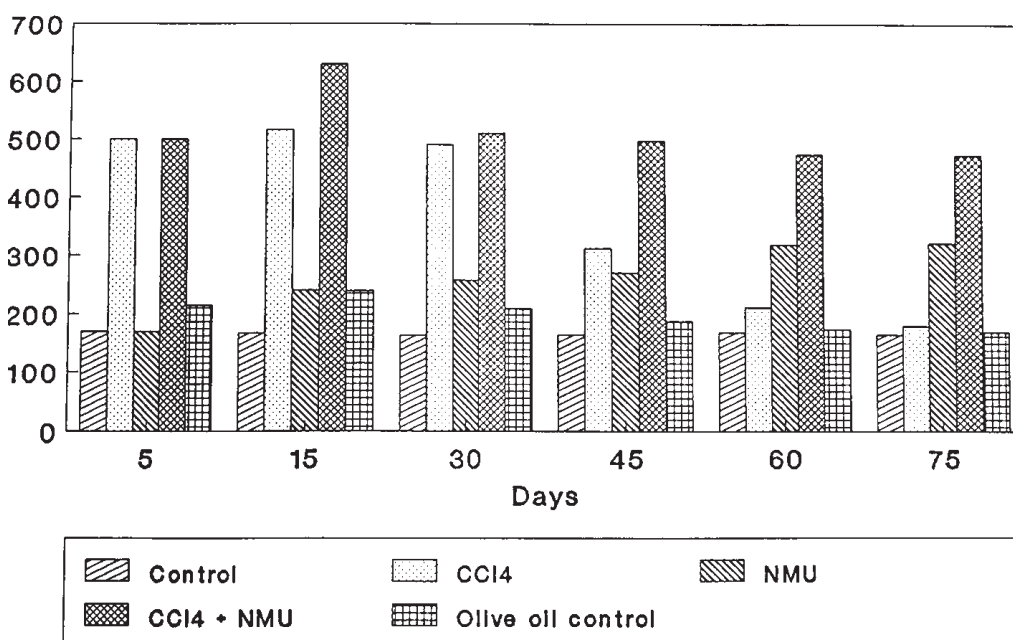


Fig.1. Sub Chronic Trial Mean liver tissue peroxidation values (n moles of MDA/100 mg of protein)

lipid peroxidation. In the olive oil vehicle control group, the peak LPO level was observed at 15 days and as a result of cessation of olive oil administration after the initial 10 days of trial, there was a progressive and steady decrease in the mean LPO values.

The mean serum GGT values of the animals belonging to the different treatment and control groups are furnished in Fig. 2. The mean serum GGT activity in the case of the CCl_4 group registered an increase up to 15th day of observation and subsequently there was a gradual fall in the level. In acute poisoning with CCl_4 , Rosalki (1975) observed a pronounced elevation of serum GGT values and values of up to 20 times the upper limit of normal were recorded. In the NMU group the pattern of increase was gradual till the end of the trial. In the case of the CCl_4 + NMU group the peak value was obtained only at the end of the trial. The serum GGT activity in the olive oil control group recorded only a very moderate elevation. Ivanov *et al.* (1976) attributed the increased serum GGT activity to the enzyme induction in the liver.

The mean values of liver tissue GGT activity for the different treatment groups are presented in Fig. 3. In the CCl_4 group, the peak level was obtained by 15th day and as a result of discontinuation of CCl_4 administration by the

10th day, there was a gradual decline till the end of the trial. The NMU group showed the peak value at the end of the trial as was also in the CCl_4 + NMU group. On the contrary, for the olive oil control group the peak value was obtained by 15th day. Ideo *et al.* (1972) reported a significant increase of serum and liver GGT in rats poisoned with carbon tetrachloride.

The DNA strand breaks assay is used for assessing the genotoxic potential of chemical compounds. The results obtained are summarised and furnished in Fig. 4. In the assay of DNA strand breaks with WBCs, the CCl_4 group showed only a marginal increase in the % "D value from the 45th to 75th day. In the case of the NMU group the increase was significant and with the CCl_4 + NMU group the values were still higher than when they were administered separately. NMU was known to induce toxic changes more markedly in tissues with rapid growth like the haematopoietic and lymphopoietic tissues (Leaver *et al.*, 1969).

In the CCl_4 group the pathological changes in the liver up to 15 days were severe vacuolar changes, with microvesicular fatty changes and nuclear pyknosis (Fig.5). In the kidneys, up to 15 days, renal congestion and tubular degeneration were observed. The

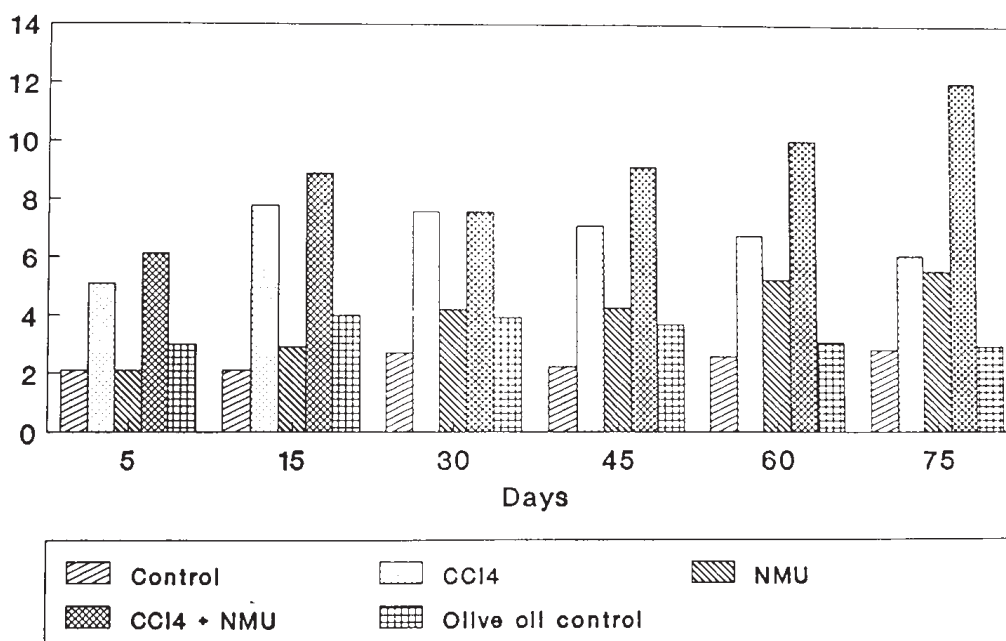


Fig.2. Sub Chronic Trial Mean serum gamma - glutamyl transpeptidase activity (I.U/L)

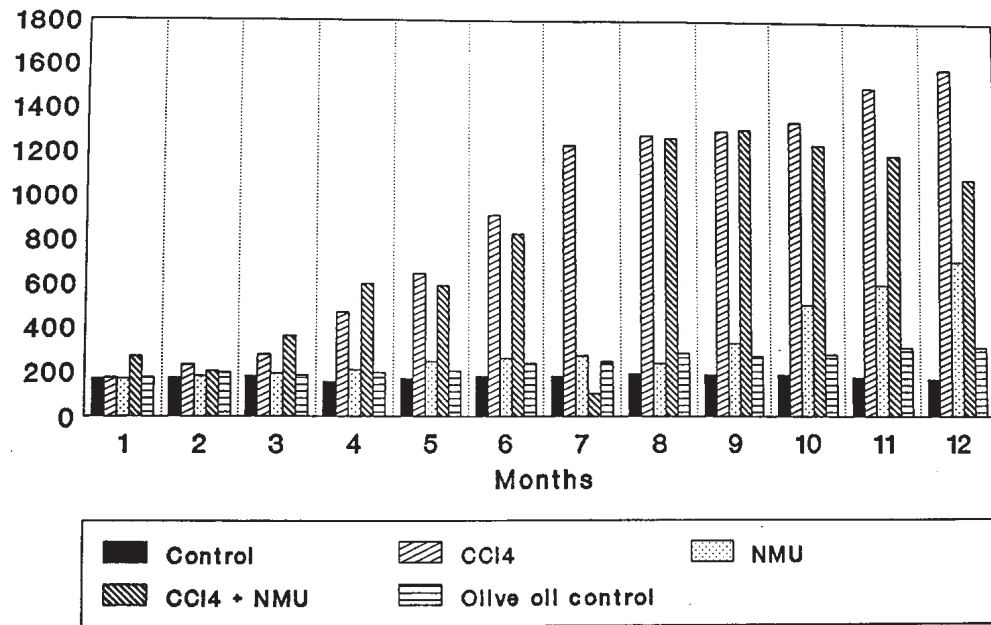


Fig. 3. Sub Chronic Trial Mean liver tissue lipid peroxidation Values (n moles of MDA/100 mg of protein)

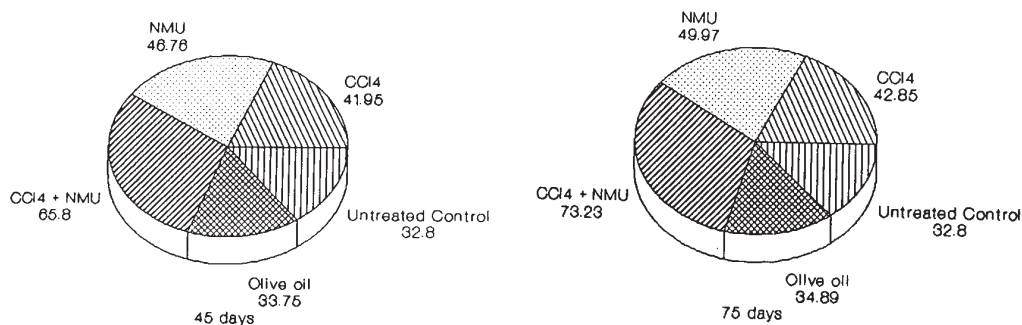


Fig.4. Sub Chronic Trial DNA double strand breaks detection by FADU in blood

stomach showed only mild hyperplastic changes.

In the NMU group, the hepatocytes developed prominent vacuolar changes and necrotic changes. (Fig.6). In the kidneys, epithelial cell swelling and later inflammatory changes could be observed. In the stomach, increased thickness of the squamous epithelial cell layer was seen.

In the case of CCl₄ + NMU group, the hepatocytes showed toxic changes in the form

of cytoplasmic granularity, nuclear pyknosis and fatty changes. These changes were persistent throughout the trial. The other pathological changes included renal tubular degeneration and squamous epithelial cell hyperplasia in the stomach.

In the olive oil vehicle control group the only changes observed in the hepatocytes were mild cytoplasmic vacuolation and occasional fatty changes.

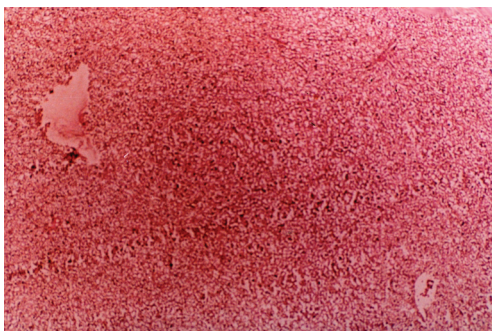


Fig. 5. Liver showing vacuolar and fatty changes and nuclear pyknosis.

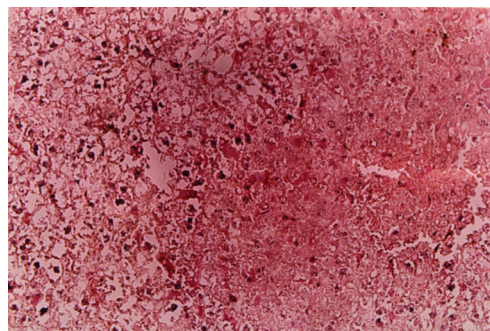


Fig. 6. Liver showing vacuolar and necrotic changes.

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