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Effect of Thiola on Acetaminophen Induced Hepatic Necrosis in Mice

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Acetaminophen (paracetamol, N-acetyl -4- aminophenol) is a commonly used analgesic and antipyretic drug, substitute for aspirin (Beaver 1966). Acetaminophen was found to induce hepatotoxicity following both acute overdosage (Davidson and Eastham 1966) and chronic excessive use (Barker et al. 1977). The most common hepatic lesion resulted from acetaminophen treatment is centrilobular necrosis (Jollow et al. 1974). Although some variations in species susceptibility to acetaminophen occur (Davis et al. 1974), the characteristic hepatic necrosis is similar in man and experimental animals.

It has been established that acetaminophen - induced liver cell necrosis was not only mediated via a metabolite of the drug, but that the microsomal mixed - function oxidase system was importantly involved in its formation (Mitchell et al. 1973). In further support of this concept was the observation that the brunt of injury from acetaminophen in both laboratory animals and humans (Rose 1969) falls upon the centrizonal hepatocytes, in which the greatest lobular concentration of the microsmal mixed function oxidase system is located (Koudstaal and Hardnok 1971).

Many sulfhydryl compounds have been used extensively as protection against injurious effect of many toxic substances as well as ionizing radiations. Among these compounds found in the living body are cysteine, glutathione and ergothionine. Thiola (2-mercaptopropionylglycine), a new synthetic sulfhydryl compound originally developed as radioprotective agent, has a variety of pharmacological activities such as antiallergic and detoxicating action as well as enhancement of enzyme activities (De Pergola et al. 1982). Chiusoli et al. (1972) provided a survey on the toxic effects of thiola and they found that this compound did not induce any signs of suffering in experimental animals even at very high doses. The purpose of the present work was to study the possible protective role of thiola on acetaminophen - induced hepatotoxicity in mice.

MATERIALS AND METHODS

Male albino mice (25-30 g) were obtained from inbred strain in the Liver Research Institute, Menoufia University, Shebin El-Kom, Egypt. They were fed on a basal diet composed of 60% ground corn meal , 15% ground beans, 10% wheat bran, 10% corn oil, 3% casein , 1% mineral mixture and 1% vitamin mixture. Water was given adlibitum.

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The compounds tested were pure acetaminophen (Paracetamol, N-acetyl -4-aminophenol) and thiola (2-mercaptopropionylglycine). Acetaminophen was obtained from Kahira Pharm. Chemical company, Egypt, while thiola was supplied by Santen Pharm. Company Osaka , Japan.

Animals were divided into three groups .
Group 1 (15 mice) were given by oral gavage, once per day for 3 d with acetaminophen at a dose level of 450 gm / kg.

Group 2 (15 mice) were treated with acetaminophen as those of group 1 and after 3 hr of treatment, each mouse was injected intraperitoneally with thiola at a dose level of 25 mg / kg body weight. Thiola was dissolved in mammallian saline solution and neutralized with sodium bicarbonate before use.

Group 3 (10 mice) were considered as controls.
The treated animals and their controls were sacrificed by decapitation 24,48 and 72 hours after the beginning of experiment. For histological examinations, the liver was removed and fixed in Bouin's fluid. Sample of each of the liver lobes were embedded in paraffin wax, 5-um section were cut and stained with hematoxylin and eosin. Evaluation of the severity of the hepatotoxic effect was based on the number of deaths at 24 hr and assessment of the histological findings. One section from each lobe was examined and the percentage of necrotic area per total section area was ditermined using an ocular micrometer. The total percentage of necrotic area in the liver of each animal was derived from the mean of 10 sections of all the liver lobes. The statistical significance of the data was analyzed using Chi square test.

RESULTS AND DISCUSSION

Animals given acetaminophen showed general weakness and reduction in their spontaneous activity. The onset of observable intoxication was recorded between 3-4 hr following treatment. The mortality rates were reduced in animals given acetaminophen plus thiola compared with those given acetaminophen alone (Table 1). Treatment with thiola resulted in a significant reduction in acetaminophen - induced liver necrosis after 48 and 72 hr.

Table. 1. Effect of thiola on hepatic necrosis in male mice

Treatment	Death at 24 hr	Liver necrosis [a]		
		% of necrotic area after		
		24 hr	48 hr	72 hr
Control	1 / 10	0	0	0
Acetaminophen alone	5 / 15	15	38	70
Acetaminophen + thiola	2 / 15	12	16[b]	38[b]

a. Derived from the mean of 10 sections of all the liver lobes from each treatment.

b. Significant at $p<0.05$ in comparison with acetaminophen alone.

Histological examination of liver of control mice revealed that all the sections had a normal appearance (Fig.1). In the group of animals given acetaminophen alone there was sever hepatic necrosis. The degree of severity depends on the time of dosage . After 72 hr of treatment, the necrotic area often covered 70% of the section. The surviving hepatocytes adjacent to the necrotic areas had a clear vacuolated cytoplasm and occasionally an acinar arrangement (Fig. 2).

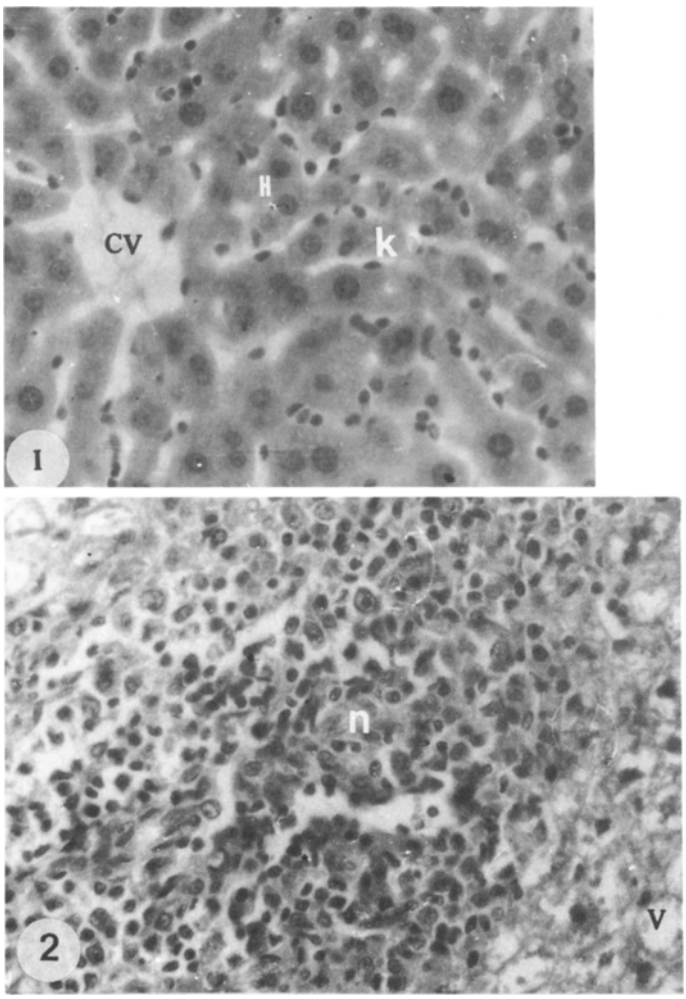
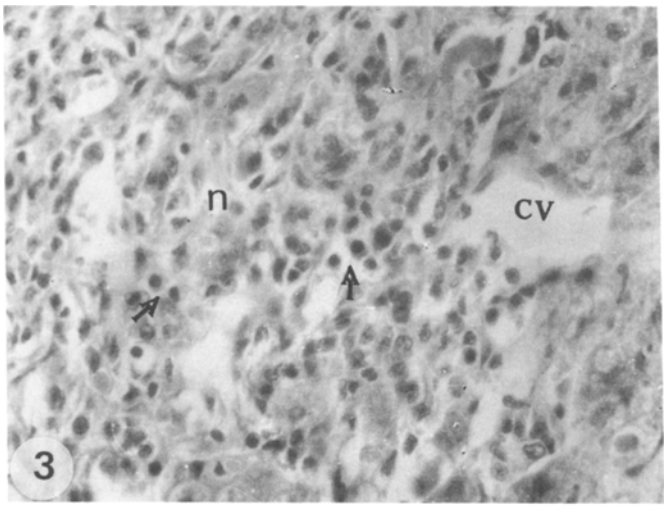


Figure. 1. Section of a control liver of mouse showing normal appearance of the hepatocytes (H), C.V : central vein, K:Kupffer cells, x300.

Figure. 2. Acetaminophen - treated liver showing large necrotic area (n), the hepatocytes around it showed cytoplasmic vacuolation (v), x300.



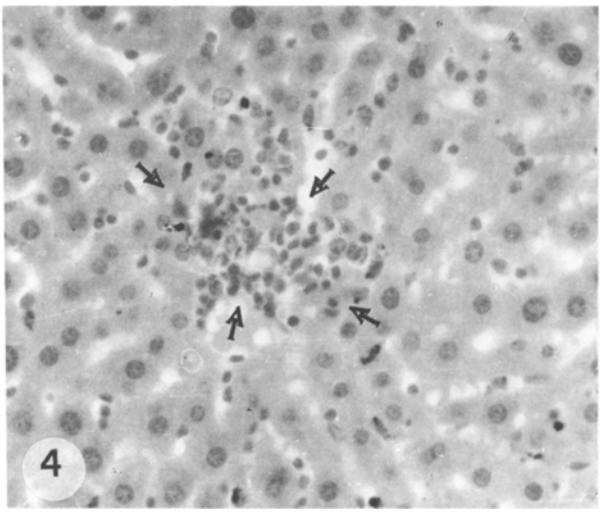


Figure. 3. Liver with necrotic area (n) adjacent to a central vein (CV), lymphocytic infiltrations are seen (arrows), x 300.

Figure. 4. Acetaminophen plus thiola - treated liver showing small necrotic area (arrows), the hepatocytes restored its normal appearance, x 300.

Inflammatory infiltrative cells were spread over several necrotic areas (Fig. 3). In animals treated with acetaminophen and injected with thiola, the percentage of sections with less severe necrosis was increased further and most of the sections were without necrosis, (Fig. 4). Moreover, an obvious degree of recovery and improvement in the various liver tissues was recorded in these specimens.

As observed in the present investigation, acetaminophen treament induced hepatic necrosis in experimental mice. Similar observations were reported in man and animals exposed to acetaminophen at high doses (Davidson and Eatham 1966, Barker et al. 1977, Portman et al. 1975, Clark et al. 1973).

Injecting mice with thiola was found to have a protective effect against acetaminophen - induced hepatic injury. Similarly, the sulfhydryl compounds L-methionine (McLean and Day 1975), L-cysteine (Strubett et al. 1974), and N-acetylcysteine (Piperno and Berssenbruegge 1976) showed a preventive role in hepatotoxicity of acetaminophen, but the therapy was not without unpleasant side effects (Dougles et al.. 1967) . Thiola differs from the other sulfhydryl compounds by its better stability in pharmaceutical preparation accounting for its greater handling capacity and lower toxicity. It has a detoxifying action against liver damage induced by CCl_4 (Horiuchi et al., 1979), ethionine (Chiba et al. 1979) and alcohol (De - Pergola et al. 1982).

The mechanisms of protection by sulfhydryl compounds are not clear. In an investigation to study the role played by glutathione in preventation of hepatotoxicity of acetaminophen, Mitchell et al. (1973) have indicated that the cellular injury is associated with the conversion of acetaminophen to a reactive metabolite by the cytochrome p-450 dependent mixed function oxidase system. At theraputic doses, this metabolite is detoxified by conjugation with glutathione. At higher doses or after depletion of liver glutathione levels (Potter et al. 1974), excess metabolite combines covalently with cellular macromolecules, resulting in hepatic functional impairment and cell necrosis. In the present work. it is speculated that thiola may be conjugated with the metabolite(s) of acetaminophen thereby reducing its covalente binding with proteins of hepatocyte membranes (Jollow et al., 1973), thus preventing hepatocyte destruction.

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