

NIFEDIPINE IN THE TREATMENT OF LIVER TOXICITY INDUCED BY ACETAMINOPHEN OVERDOSE IN MICE

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Abstract - Acetaminophen is an analgesic and antipyretic drug which is widely used by public and poisoning with this drug is common. One of the most important adverse effects of acetaminophen poisoning is centrilobular necrosis in hepatic cells which depends on activity of microsomal cytochrome P-450 (CYP) enzymes. The aim of this investigation was to find out the protective effect of nifedipine against liver toxicity caused by acetaminophen overdose (700 mg/kg as calcium channel blocker). In this study doses of 5,50,100,250,500 mg/kg of nifedipine were administered to mice orally one hour before acetaminophen administration. The negative control group received normal saline. The positive control group was administered with acetaminophen at a dose of 700 mg/kg one hour after nifedipine administration. After 24 hours, enzyme activity (ALT, AST), histopathological examination and liver weight were compared with the control groups. The results revealed that nifedipine at dose of 500 mg/kg was the most effective and protected liver damage from acetaminophen toxicity.

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Key Words: Nifedipine, acetaminophen, liver toxicity

INTRODUCTION

Liver toxicity induced by chemicals have been well recognized as a toxicological problem. After acute exposure usually lipid accumulation in the hepatocytes, cellular necrosis or hepatobiliary dysfunction occurs, whereas cirrhosis or neoplastic changes are considered to be results of chronic exposure (1).

Acetaminophen is an analgesic and antipyretic drug well tolerated and widely used in therapy (2). However hepatic toxicity and occasionally necrosis is a well recognized outcome of acetaminophen overdose (3).

Acetaminophen is metabolised by liver before excretion and about 85%-95% of acetaminophen is conjugated to acetaminophen glucuronide and acetaminophen sulfate which are nontoxic metabolites. The remaining portion is metabolised through the cytochrom p-450 mixed function oxidase to a highly reactive intermediate N-acetyl-p- benzoquinoneimine (4,5,6). The extent of hepatic damage is assessed by the level of released cytoplasmic transaminases (GOT and GPT) in circulation (7).

The aim of this study was to find out the protective effect of nifedipine as calcium channel blocker to prevent the accumulation of calcium in liver cells. The major tests that have been proved useful for evaluation of hepatic injury in laboratory animals are serum enzyme activities and histopathological examination of liver (8,9). It is clear that nifedipine as a calcium channel blocker can prevent the entrance of Ca^{++} into liver cells. Therefore it is presumed that nifedipine may be able to abolish the destructive processes which are caused by toxic dose of acetaminophen.

MATERIALS AND METHODS

Albino Swiss white male mice were supplied by Razi Research Center in Hasarak, Karaj, Iran. Animals were maintained in proper light and diet control. Acetaminophen and nifedipine were purchased from Darupakhsh Iran. Alanine Transaminase (ALT) and Aspartate Transaminase kits were obtained from Zist Shimi Co. Iran. Centrifuge Beckman (USA). Microtome model 2045 (Germany). Salura tissue passage model RH 12. EP-2 (Japan). Spectrophotometer Bausch (Germany). Light microscope Nikon (Japan) and Vacuum evaporator Heidolph (Germany).

After preparation of acetaminophen and nifedipine in the appropriate doses animals were divided in nine groups and each group consisted of ten mice in the weight range 20 ± 2 grams. Animals were fasted overnight and then according to dose schedule experiments were carried out. The test groups received nifedipine in doses of 5 mg/kg (E), 50 mg/kg (F), 100 mg/kg (G), 250 mg/kg (H) and 500 mg/kg (I) orally. The negative control groups received normal saline (A) and the positive control group received acetaminophen in dose of 700 mg/kg (C). A group received N-acetylcysteine, and acetaminophen called (D) and group received acetaminophen vehicle (tragacant suspension), called (B). After 24 hours blood was collected from the jugular vein and serum was prepared and then the activities of Alanine Transaminase (ALT) and Aspartate Transaminase (AST) enzymes were measured according to Franked and Reitman method(4).

After blood collection liver was removed and fixed in 10% formaline solution for histopathology tests.

RESULTS

It is well known that acetaminophen at dose of 1 g/kg induced 100% lethality in mice (10); hence hepatic toxicity and necrosis can be produced by acetaminophen overdose. In case of acetaminophen poisoning the nontoxic routes of metabolism become saturated and proportionately more of the drug is metabolised through the toxic P-450 route, exhausting the supply of glutathione. Free toxic metabolite in liver cells can then bind to cytosolic proteins of hepatocytes leading to centrilobular hepatic cell necrosis (11,12).

In order to evaluate the protective property of nifedipine against acetaminophen intoxication, doses of 5 mg/kg, 50 mg/kg, 100 mg/kg, 250 mg/kg and 500 mg/kg were studied. As shown in Fig. 1 and 2 the ALT and AST activities in the test group receiving nifedipine as compared with the positive control group are significantly different. According to data obtained from AST and ALT a significant protection was observed. Liver weight in different test, normal and toxic groups are shown in Fig. 3.

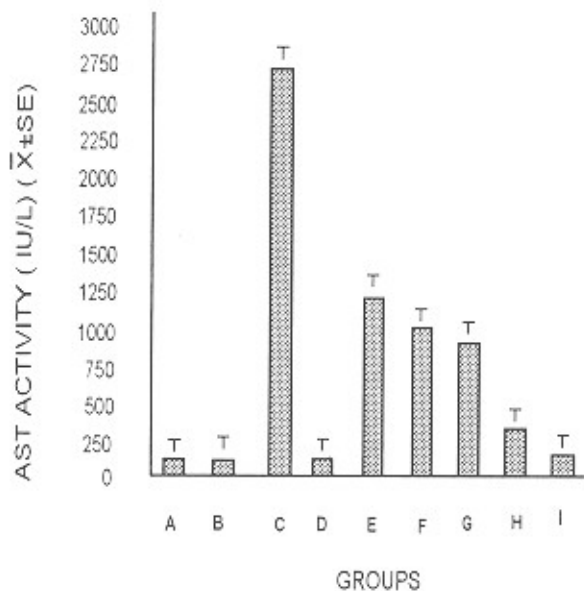


Fig. 1. AST activities in different test, normal and toxic groups

The histopathological studies showed that the nifedipine can protect liver damage but it is more effective at dose of 500 mg/kg because at this dose hepatic cells are mostly normal and there is no necrosis in liver as compared with the positive control group. Fig. 4 (normal liver) is from the group which received normal saline, Fig. 5 (acetaminophen) positive control group and Fig. 6 nifedipine at dose of 500 mg/kg (test group).

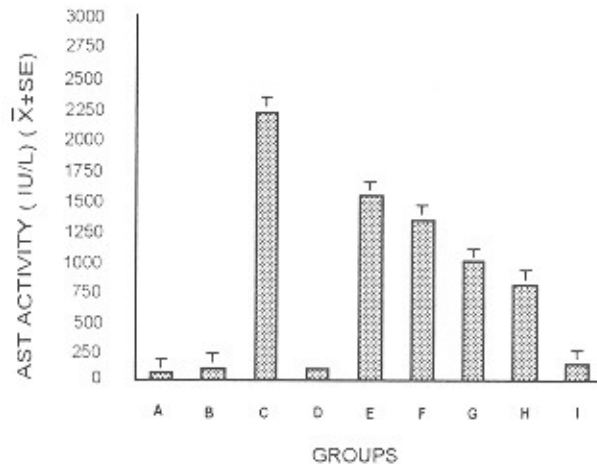


Fig. 2. ALT activities in different test, normal and toxic groups

- A: Normal saline (negative control)
- B: Acetaminophen solvent (tragacant suspension)
- C: Acetaminophen 700 mg/kg
- D: Acetaminophen and NAC (negative control)
- E: nifedipine 5 mg/kg
- F: nifedipine 50 mg/kg
- G: nifedipine 100 mg/kg
- H: nifedipine 250 mg/kg
- I: nifedipine 500 mg/kg

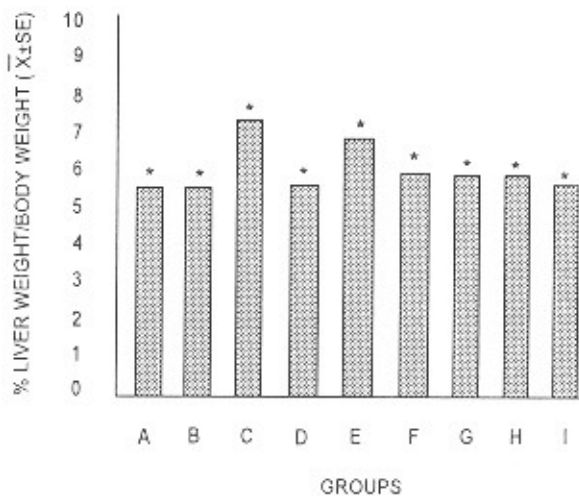


Fig. 3. Liver weight in different test, normal and toxic groups

- A: Normal saline (negative control)
- B: Acetaminophen solvent (tragacant suspension)
- C: Acetaminophen 700 mg/kg
- D: Acetaminophen and NAC (negative control)
- E: nifedipine 5 mg/kg
- F: nifedipine 50 mg/kg
- G: nifedipine 100 mg/kg
- H: nifedipine 250 mg/kg
- I: nifedipine 500 mg/kg

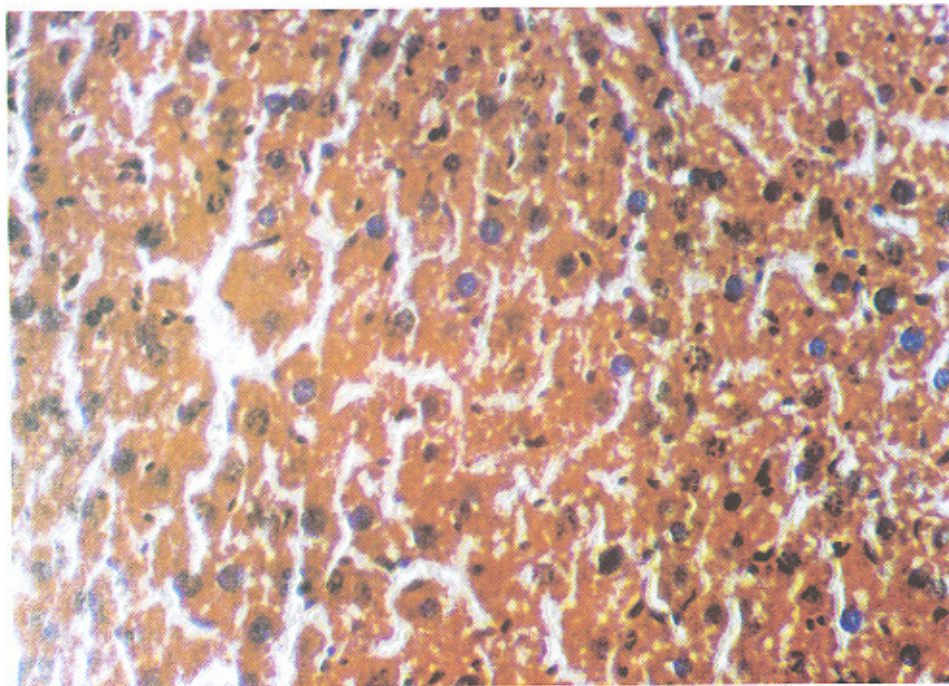


Fig. 4. Normal liver tissue in group receiving normal saline (negative control)

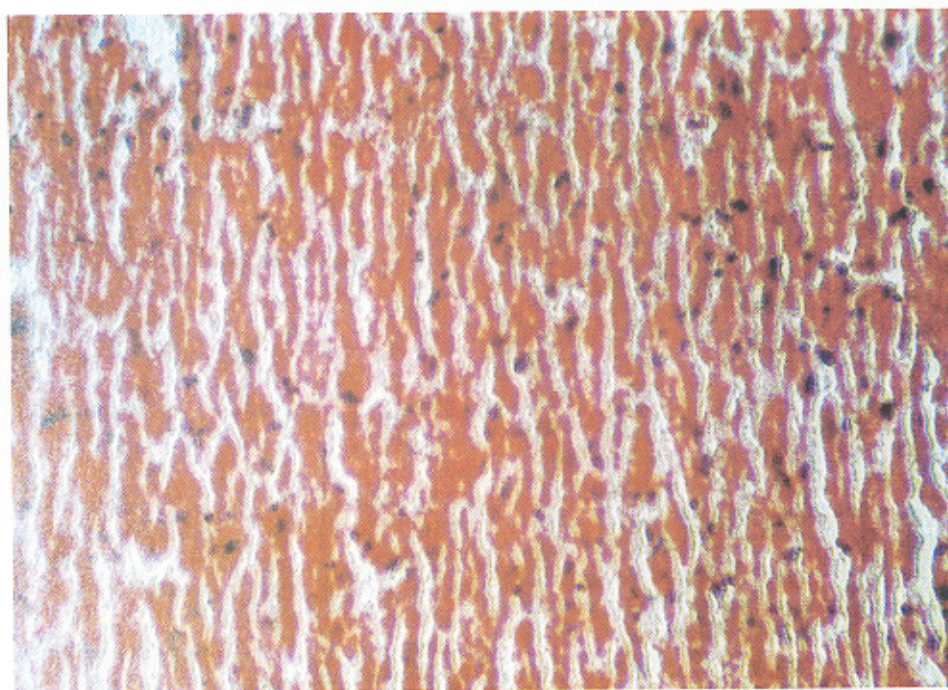


Fig. 5. Liver in group receiving 700 mg/kg acetaminophen (positive control)

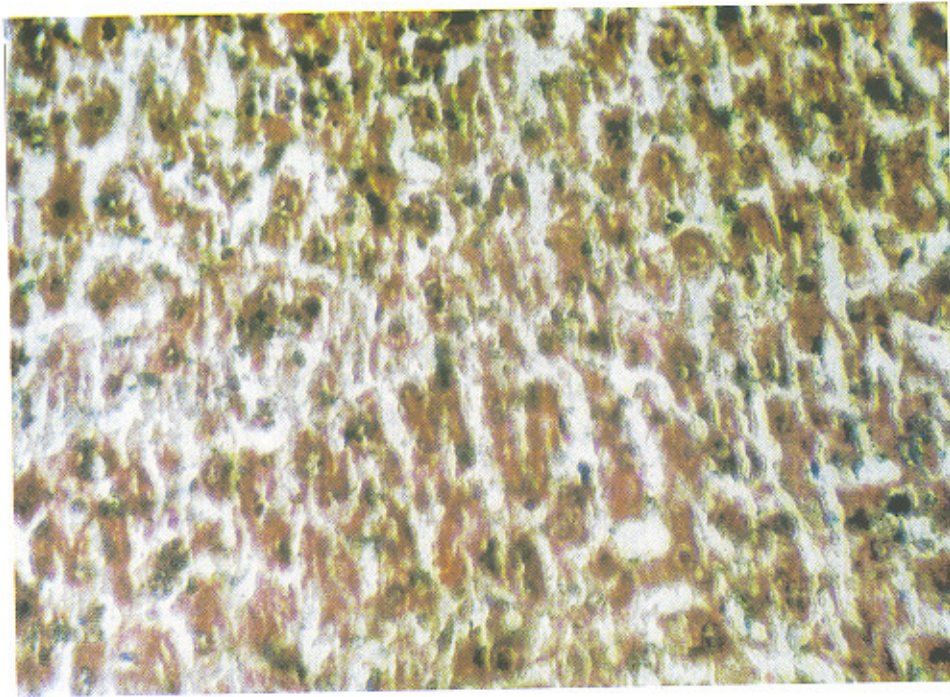


Fig. 6. Liver in group receiving 500 mg/kg nifedipine (test group)

DISCUSSION

With regard to results obtained in this study nifedipine can protect liver damage caused by acetaminophen. nifedipine at dose of 500 mg/kg was more effective because hepatic cell regeneration increased significantly and protection at the above dose as compared with positive control group was noted. In other doses protection was also observed but was not as effective as observed at 500 mg/kg of nifedipine. In the group which received acetaminophen at dose of 700 mg/kg the weight of liver because of hypovolemic shock increased also, AST and ALT were increased which is considered a primary death after acetaminophen poisoning (13). In histopathology study the toxicity effects of acetaminophen were observed, like necrosis in hepatic cells, and most of the damage was seen in the centrilobular hepatic cells because in this region microsomal enzymes are more therefore the cytochrome p-450 enhances the metabolism of acetaminophen (14).

It is important that in histopathological examination in this study there were not any abnormal fat necrosis in liver cells. The liver structure and shape recovered to normal size after it was been intoxicated with acetaminophen. In conclusion, it seems that nifedipine is suitable for protection of liver damage caused by acetaminophen in mice.

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