The protective effect of garlic oil on hepatotoxicity induced by acetaminophen in mice and comparison with N-acetylcysteine

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ABSTRACT

Objective: The aim and purpose of this study was to find out the protective effect of garlic oil in liver toxicity induced by acetaminophen overdose and the comparison of its effect with N-acetylcysteine in albino male mice (18-22g).

Methods: This study was undertaken during the period from January 1999 through to August 2000, at the School of Pharmacy, Ahwaz University of Medical Sciences, Ahwaz, Iran. All animals were fasted over night and were divided into 8 groups. Each group consisted of 10 mice. Garlic oil was administered intraperitoneally in doses of 100mg/kg, 200mg/kg and 500mg/kg. Immediately after this, a toxic dose of acetaminophen (500mg/kg orally) was administered followed by another administration one hour later (500mg/kg orally). Twenty-four hours after the last administration, blood was withdrawn from the juagular vein of the mice and serum enzyme activities were measured and compared with the control groups. The liver samples were studied for the histopathological examination.

Results: The results in group which received 200mg/kg of garlic oil showed good protection activity as compared with the positive control group. The histopathological observations also showed that the area of liver damage was reduced significantly as compared with the positive control group. The severity of injury was variable among the animals and there was less evidence of necrosis in this group. Some protection was observed in other doses of garlic oil but these were not much significant. The results obtained one hour after acetaminophen intoxication (post treatment) showed a less protective effect as compared with the group which received garlic oil simultaneously after acetaminophen intoxication.

Conclusion: Garlic oil, as similar to N-acetylcysteine, can eliminate electrophilic intermediates and free radicals through conjugation and reduction reactions. Therefore it protects the liver from toxic doses of acetaminophen. In the present study we also observed the protection by the garlic oil. The clearance of the toxic metabolites of the acetaminophen from the liver occurs much faster in immediate treatment with garlic oil (200mg/kg) .

Keywords: Garlic oil, acetaminophen, liver toxicity, mice.

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Acetaminophen is widely used as an analgesic and antipyretic drug. This medicine is safe when taken in the proper therapeutic doses.[1] However, it can cause acute hepatotoxicity and nephrotoxicity in human and experimental animals when taken in overdoses or in combination with other drugs or alcohol. At normal dose levels the major elimination pathways of acetaminophen are conjugation with glucuronide and sulfate. Approximately 10% of acetaminophen is oxidized by a cytochrome P450 dependent pathway to form an electrophilic metabolite. Several P450 enzymes are known to play an important role in acetaminophen bioactivation.[2] The cytochrome P450 2E1 and 1A2 are major enzymes responsible for acetaminophen bioactivation in human liver microsomes. Garlic (or *Alliume sativum)* belonging to the *Liliaceae* family are widely used in traditional medicines which contains sulfur,

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fats, vitamins, enzymes and many minerals.[3] It has been reported that garlic and related organosulfur compounds have antioxidant, antitumorgenic, antibiotic and detoxifying properties.[4] The detoxifying effect of garlic may be related to its ability to inhibit phase I enzymes, induce phase II enzymes or bind to exogenous toxins through sulfhydryl groups. Garlic contains more than 30 sulfur components such as alliin, allicine and ajuen, which are present in an oily extract of garlic.[5] A flavor component derived from fresh garlic has been shown to protect against chemical-induced toxicity in animals.[6] However, detailed information regarding the effect of garlic oil in acetaminophen-induced hepatotoxicity and related mechanisms is lacking. Hence, the aim of this study was to determine the protective effect of garlic oil on acetaminophen-induced liver toxicity in mice as compared with the use of N-acetylcysteine.

Methods. Albino Swiss male mice weighing 18-22 grams were obtained from Razi Research Center, Hasarak Karaj, Iran. They were given access to rodentchow, water and libitum and allowed to acclimatise in an environment of controlled temperature, humidity and 12 hours light/12 hours dark cycle. Acetaminophen was purchased from Daru parkhsh Co., Iran. N-acetylcysteine was obtained from Merck, Germany. Garlic oil was extracted from fresh garlic in our laboratory by direct steam distillation. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were purchased from Zist Shimi, Tehran, Iran and other materials were prepared as previously described.[7] Fresh garlic oil was prepared by a direct steam distillation method[8] and then doses of 100 mg/kg, 200 mg/kg and 500 mg/kg were administered intraperitoneal (ip) to mice. As acetaminophen does not dissolve in distilled water, an acetaminophen suspension was prepared by gum tragacant in physiological saline (this suspension exhibited no toxic effects) and administered in a 500 mg/kg dose.[9] The experimental animals were then divided into 8 groups (10 mice in each group). Mice were fasted overnight and according to the time and dose schedule, experiments were carried out. Group A mice received garlic oil at varying concentrations (100 mg/kg, 200 mg/kg and 500 mg/kg) immediately following acetaminophen administration. Group B mice received 500 mg/kg N-acetylcysteine and 200 mg/kg garlic oil immediately following acetaminophen administration. Group C mice received 500 mg/kg N-acetylcysteine and 200 mg/kg garlic oil one hour after initial acetaminophen administration. In all groups, the resultant effects were compared with the positive (acetaminophen) and negative (saline, gum/saline suspension, corn oil/saline) control groups. After 24 hours, all animals were sacrificed and blood was drawn from the jugular vein to prepare serum for measuring serum enzyme activities. The liver was removed and fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin for histopathological studies.[10] The hepatotoxicity was determined by measuring ALT and AST levels. Data was analyzed for significance ($P<0.005$) using analysis of variance.

Results. The positive control group that received a toxic dose of acetaminophen showed a significant increase in AST and ALT levels as compared with the negative control groups. The histopathological studies with hematoxylin and eosin staining indicated that most of the liver in the toxic group had extensive necrosis and there was congestion of blood cells at zone 3 (Figure 1). In this region there are increased numbers of microsomal enzymes therefore the cytochrome P450 enhances the metabolism of acetaminophen. In the group that received garlic oil at doses of 100 mg/kg, 200 mg/kg and 500 mg/kg immediately after acetaminophen administration, significant protection was observed at all doses compared with the negative control groups (as evidenced by the reduced levels of ALT and AST) (Figure 2a-2b). Furthermore, in addition to the reduced levels of serum enzymes, histopathological examination showed that the area of liver damage was greatly reduced at a dose of 200 mg/kg (Figure 3). These results indicate that garlic oil extract at a dose of 200 mg/kg is preferable for protection against acute hepatotoxicity. In the groups that received 200 mg/kg of garlic oil and 500 mg/kg of N-acetylcysteine immediately after acetaminophen administration and one hour later, the results showed that garlic oil and N-acetylcysteine appeared to have equivalent liver protective effects when given immediately following a toxic dose of acetaminophen (Figure 4a-4b). However, one hour after administration of acetaminophen, subsequent

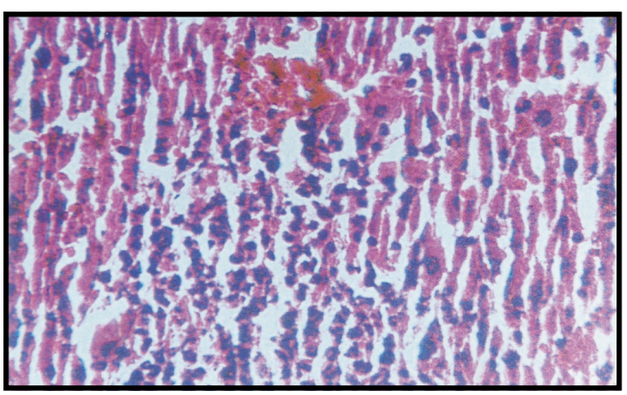

Figure 1 - Example of a liver section (stained with hematoxylin and eosin) taken from mice receiving a toxic 500mg/kg dose of acetaminophen (positive control group).

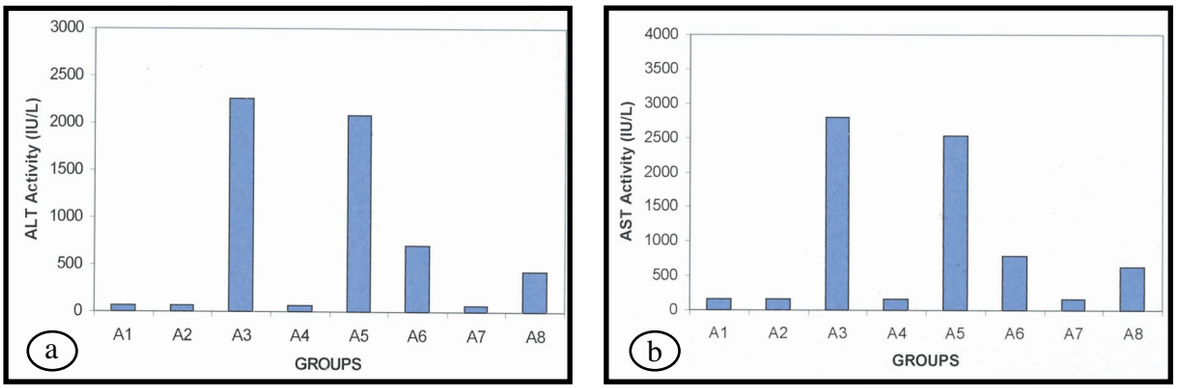

Figure 2 - The comparison of different aminotransferase activity in groups receiving acetaminophen 500 mg/kg and garlic oil in doses of 100mg/kg. 200mg/kg and 500mg/kg. **(a)** Alanine aminotransferase. **(b)** Aspartate aminotransferases. A1-normal saline, A2-suspension of gum tragacant and saline, A3-acetaminophen 500mg/kg and saline, A4-corn oil and saline, A5-acetaminophen 500mg/kg and corn oil, A6-acetaminophen 500mg/kg and garlic oil 100mg/kg, A7-acetaminop[hen 500mg/kg and garlic oil 200mg/kg, A8-acetaminophen 500mg/kg and garlic oil 500mg/kg.

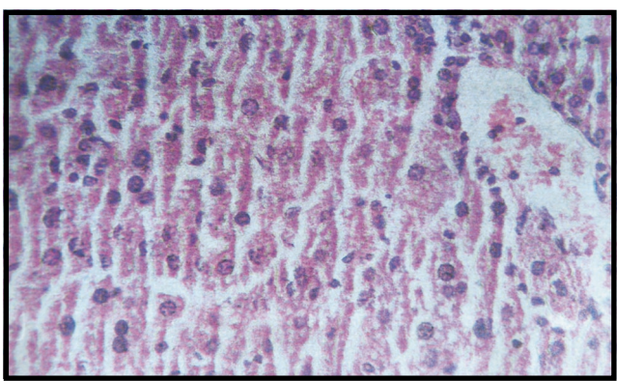

Figure 3 - Example of a liver section (stained with hematoxylin and eosin) taken from mice receiving 200mg/kg of garlic oil immediately following administration of a toxic 500mg/kg dose of acetaminophen.

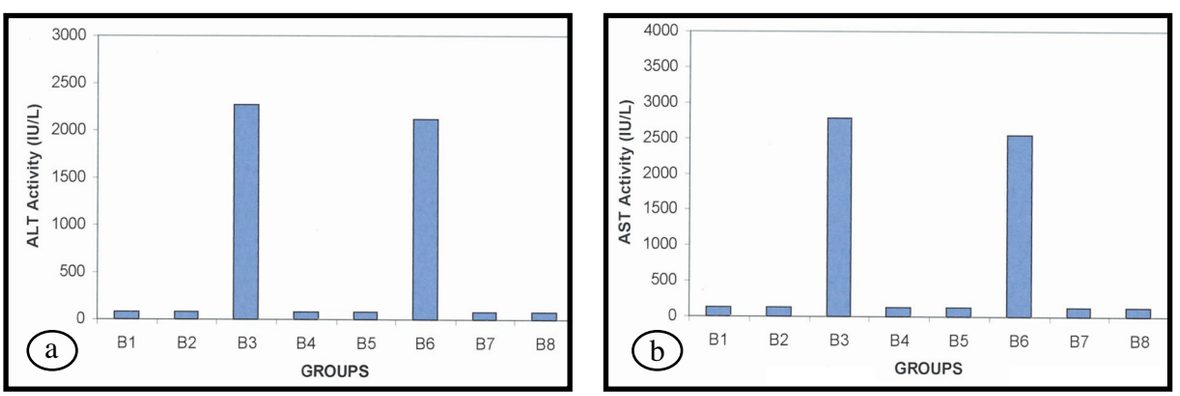

Figure 4 - The comparison of different aminotransferase activities in toxic groups N-acetylcylsteine and garlic oil groups immediately after acetaminophen treatment **(a)** Alanine aminotransferase **(b)** Aspartate aminotransferases. B1-normal saline, B2-suspension of gum tragacant and saline, B3-acetaminophen 500mg/kg and saline, B4-corn oil and saline, B5-Garlic oil 200mg/kg and saline, B6 - acetaminophen 500mg/kg and corn oil, B7- acetaminophen 500mg/kg and N-acetylcysteine 500mg/kg, B8-acetaminophen 500mg/kg and garlic oil 200mg/kg.

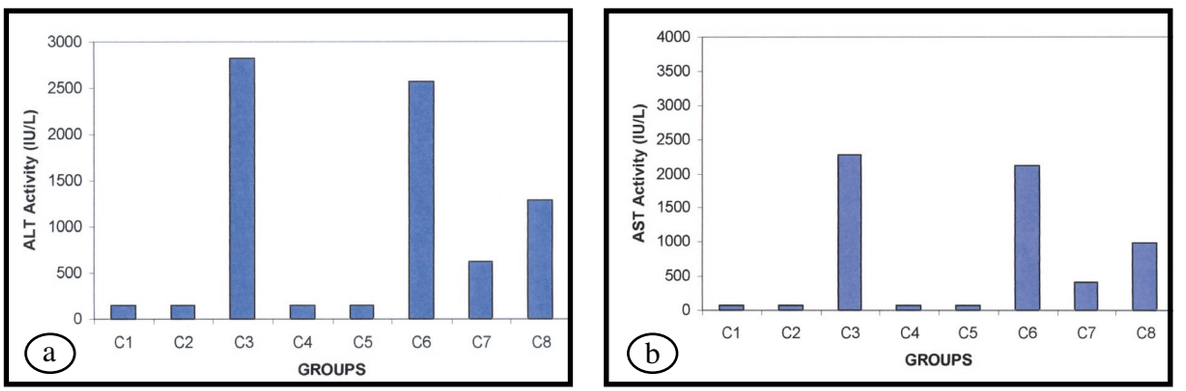

Figure 5 - The comparison of different aminotransferase activity in toxic groups N-acetyclysteine and garlic oil one hour after acetominophen treatment **(a)** Alanine aminotransferase **(b)** Aspartate aminotransferases. C1-normal saline, C2-suspension of gum tragacant and saline, C3-acetaminophen 500mg/kg and saline, C4-corn oil and saline, C5-garlic oil 200mg/kg and saline, C6-acetaminophen 500mg/kg and corn oil, C7-acetaminophen 500mg/kg and N-acetylcysteine 500mg/kg, C8-acetaminophen 500mg/kg and garlic oil 200mg/kg.

doses of garlic oil (200 mg/kg) and N-acetylcysteine (500 mg/kg) exhibited differing protective effects. As shown in Figure 5a-5b the levels of ALT and AST following N-acetylcysteine are half those attained following garlic oil administration. Therefore, it appears that N-acetylcysteine may provide increased protection against long-term liver toxicity, compared to garlic oil. However, the protective effect of garlic oil is still significant compared to the control groups.

Discussion. The extent of hepatotoxicity and liver damage is assessed by the level of released cytoplasmic transaminases in the circulation such as ALT and AST.[11] Acetaminophen is converted to its reactive metabolite N-acetyl p-benzoquinoneimine (NAPQI) and halogenated free radical by hepatic cytochrome P450. The massive production of reactive metabolite may lead to depletion of protective physiological moieties (such as glutathione and α-tocopherol) ensuring widespread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes.[12] Since the microsomal drug metabolizing enzymes (MDME) inhibitory activity is reported to be common in medicinal plants,[13] garlic oil was used in this study. In summary, the results of this study demonstrated that treatment of mice with garlic oil immediately after acetaminophen had a good protective effect (comparable to that of N-acetylcysteine) against acetaminophen-induced hepatotoxicity. Although garlic oil exhibited reduced protection one hour later, compared to N-acetylcysteine, the degree of protection suggests that garlic oil may still provide some protection against long-term liver damage. The previous studies in this area had shown that garlic plays a key role in the detoxification of the reactive toxic metabolite of acetaminophen. This could be due to the inhibition of the bioactivation of acetaminophen by garlic resulting in the decreased formation of a toxic metabolite (N-acetyl P-benzoquinoneimine). This protection may be due to the thiol groups in garlic oil conjugating with a toxic metabolite of acetaminophen.

In conclusion, from the results obtained in this investigation and previous studies it is suggested that garlic oil can reduce liver damage resulting from acetaminophen toxicity.

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