HEPATOPROTECTIVE EFFECT OF *MORUS ALBA* L. IN CARBON TETRACHLORIDE- INDUCED HEPATOTOXICITY IN MICE

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أجريت دراسات عديدة لمنع التسمم الكبدي في النماذج الحيوانية. ومن المعروف أن الأدوية العشبية تلعب دوراً هاماً في العلاج. وقد تم تقييم الخلاصة المائية الكحولية لأوراق نبات Morus alba من حيث تأثيرها الواقي للكبد في حالة التسمم الكبدي المستحث برابع كلوريد الكربون. وقد تم تحضير الخلاصة المائية الكحولية بطريقة النقع. وتم وزن الحيوانات وتقسيمها إلى خمس مجموعات في كل منها سبعة فئران. وقد تلقت المجموعة الأولى رابع كلوريد الكربون (المجموعة الإيجابية). وتلقت المجموعة الثانية زيت الزيتون فقط (مذيب رابع كلوريد الكربون كمجموعة سلبية). أما المجموعة الثالثة (A1)، والمجموعة الرابعة (A2)، والمجموعة الخامسة (A3) فقد تلقت الخلاصة الخام بجرعات 200 و 400 و 800 مغ/كغ، على التوالي وبعد ساعة تلقت رابع كلوريد الكربون بجرعة 0.2 مل/كغ لمدة خمسة أيام متتالية (المجموعات التجريبية) عن طريق الفم. وفي اليوم السادس تلقت الحيوانات صوديوم هكساباربيتال بجرعات 25 مغ/كغ عن طريق البريتون لتقدير زمن النوم. وبعد فترة النوم تمت التضحية بالحيوانات وسحب الدم من الوريد الودجي، وتم استخدام مصل الدم لتقدير مستويات إنزيمات أسبرتات أمينوترانسفيراز، وألانين أمينوترانسفيراز. وتم استئصال الأكباد وحفظها في فورمالين 10% بمحلول راصد لفحص أنسجتها المرضية. وأظهرت الخلاصة المائية الكحولية بجرعة 800 مغ/ كغ تأثيراً هاماً واقياً للكبد وذلك بخفض مستويات إنزيمات أسبرتات أمينوترانسفيراز وألانين أمينوترانسفيراز في مصل الدم، وتقليل زمن النوم، مما نتج عنه تدميراً أقل وضوحاً في بنية الكبد، ولم يكن هناك تليف أو التهاب بالمقارنة مع مجموعة رابع كلوريد الكربون ($p<0.05$).

Many studies have been performed to prevent liver toxicity in animal models. It is well known that herbal medicines play an important role in therapy. The crude hydroalcoholic extract of *Morus alba* L. leaves was evaluated for hepatoprotection against hepatotoxicity induced by carbon tetrachloride. The hydroalcholic extract was prepared by maceration technique. Animals were weighed and divided into five groups of seven mice. Group one received carbon tetrachloride (positive group). Group two received only olive oil (solvent of CCl_4 as negative group). Groups three (A1), four (A2), and five (A3) received crude extract in doses of 200, 400, and 800mg/kg, respectively and one hour later carbon tetrachloride in doses of 0.2ml/kg for five consecutive days (test groups). All administrations were made by p.o. in 0.2ml volume. Then on the day six animals received sodium hexabarbital in doses of 25mg/kg i.p. to determine the sleeping time. After sleep time determination animals were sacrificed and from the jugular vein, blood was taken. Serum was obtained for determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes levels. Livers were removed and kept in 10% formalin bufferd solution for histopathological

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examinations.The hydroalcoholic extract at dose of 800mg/kg exhibited a significant liver protective effect by lowering the serum levels of AST and ALT, decreasing the sleeping time and resulting in less pronouced destruction of the liver architecture, there was no fibrosis and inflammation, as compared with CCl_4 group ($p<0.05$).

Keywords: Carbon tetrachloride, liver toxicity, *Morus alba* L. (Moraceae)

Introduction

Liver is the largest organ in the vertebrate body and the site for intense metabolism. It plays an astonishing array of vital functions in the maintenance and performance of the body. The most important functions include carbohydrate, protein and fat metabolism, detoxification and secretion of bile. Unfortunately liver is often damaged by environmental toxins, poor eating habits, alcohol, medicines and Over The Counter (OTC) drugs.

Conventional medicine is pursuing the use of natural products such as medicinal plants to provide support to the liver on a daily basis to revitalize the liver and treat hepatic dysfunction (1). Liver diseases remain one of the serious health problems. To this point oriental therapy have put forward a number of herbal products and their formulations for liver disorders. In this modern age it is very important to provide scientific proof to justify the usage of various herbal drugs, as it is believed that they have less side effects and they are relatively cheap. However, examining the effectiveness of herbal medicines and looking for satisfactory remedies for serious liver diseases in order to develop effective and safe drugs is an area of interest (2).

Morus alba L. of Moraceae family (white mulberry) has long history of use in Chinese oriental medicine. It is claimed that almost all parts of this plant is useful in cardiovascular, liver and spleen disorders (3). Recent research has shown that this herb has free radical scavenging activities, hypolipidemic, antioxidant, antibacterial, antiviral, astringent, emollient and antiinflammatory properties (4-6). White mulberry leaf contains triterpenes (lupeol) Sterols (β-Sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids and organic acids (7).

Carbon tetrachloride, widely used as a dry cleaning solvent, in refrigerant and as fire extinguisher (8), can develop liver and kidney damage and could result in cancer (9-10).

In view of the reported hepatoprotective activity of *Morus alba* L. and traditional claims, the crude hydroalcoholic extract of *Morus alba* L. leaves was evaluated against carbon tetrachloride induced hepatotoxicity in mice with the aim of developing a natu-ral hepatoprotective drug.

Materials and Methods

Chemicals:

Carbon tetrachloride was purchased from Ekceer Pharmaceutical Company Iran. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) kits were supplied by Zist Ahimi Pharmaceutical Company Iran.

Experimental animals

Albino Swiss male mice weighing 35-40g were procured from Razi Research Center, Hasarak karaj Iran. Animals were given rodent chow and water *ad libitum* and allowed to acclimatized in an environment of controlled temperature, humidity and 12 h light / dark cycle. All procedures complied with the norms of the animal ethics committee of our university.

Plant extract:

Morus alba L. leaves were collected from Lorestan province (Dorood area and were authenticated by Taxonomist (Dr Noorollah Moallem) at Botany Department of Chamran University, Ahvaz, Iran. Leaves were dried in shadow and then were grinded to a uniform powder and weighed. The most important and essential part of extraction of plant material is the selection of a proper solvent which depends on the part and constituents of the plant. In this study a mixture of ethanol and water in the ratio of 7:3 was prepared. The dried leaf powder (350 g) was extracted by maceration for three days. The extracted material was filtered and the filtrate was concentrated under vacuum evaporator until dryness.

Treatment of animals:

Animals were weighed and divided in five groups of seven mice. Group one received carbon tetrachloride as positive group (+ve). Group two received olive oil (solvent of CCl_4) as negative group (-ve). Groups three (A1), four (A2) and five (A3) received plant hydroalcoholic extract, dissolved in normal saline, in doses of 200, 400, and 800mg/kg, respectively and one hour later carbon tetrachloride in doses of 0.2ml/kg. All administrations were made by p.o. in 0.2ml volume and repeated for five consecutive days. Then on day six animals received directly sodium hexabarbital in doses of 25mg/kg i.p. to determine the sleeping time (11-13). After determination of sleeping time animals were sacrificed and from the jugular vein blood was taken, serum was obtained for determination of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzymes levels. These biochemical parameters were assayed spectrophotometrically using a commercially available assay kits according to the manufacturer's protocol.

Livers were removed immediately weighed and kept in 10% formalin bufferd solution, dehydrated in gradual ethanol (50-100٪), cleared in xylene and embedded in paraffin. Sections (4-5 µm thick) were prepared and then stained with hematoxylin and eosin (H&E) dye for photomicroscopic observations.

Statistical analysis:

Data were analyzed by analysis of variance (ANOVA) followed by multiple comparisons using Tukey test to compare all groups against control. Results were considered statistically significant at $p<0.05$.

Results

Animals treated with toxic dose of carbon tetrachloride had markedly elevated levels of the serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), compared to normal mice, indicating acute hepatocellular damage ($p<0.05$). Serum enzyme values of the crude extract of *Morus alba* L. (white mulberry) at doses of 800mg/kg were significantly lower than those of toxic control values ($p<0.05$). Also serum enzyme values in other treated animals also showed a protective effect (Fig.1 and Fig. 2).

The effect of the hydroalcoholic extract of *Morus alba* L. on hexabarbital-induced sleep and liver weight are shown in Fig. 3 and 4 respectively.

The histopathological examination of the liver sections of negative control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (microscopic photograph A). Disarrangement of normal hepatic cells with centrilobular necrosis vacuolization of cytoplasm and fatty degeneration were observed in carbon tetrachloride intoxicated mice (microscopic photo-graph B).The liver sections of the mice treated with the crude extract of *Morus alba* L. at dose of 800mg/kg followed by carbon tetrachloride intoxication exhibited a significant protection as it was evident by the absence of necrosis, tissue damage and vacuoles (microscopic photograph C).

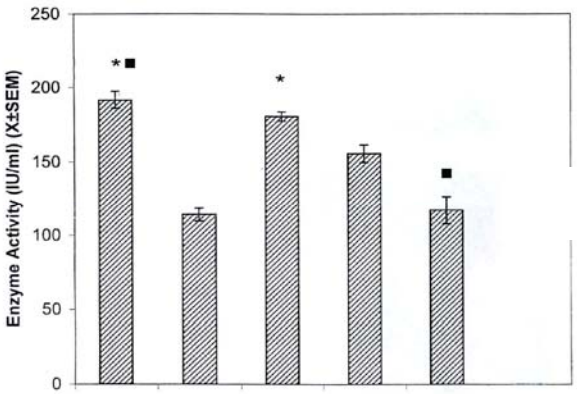


Fig. 1. ALT enzyme activity in different test groups.
+ve = positive control (CCl_4), -ve = negative control (olive oil) , A1 = 200mg/kg, A2 = 400mg/kg and A3 = 800mg/kg of herbal extract. * $p<0.05$ as compared with the negative group,
■ $p<0.05$ as compared with the CCl_4 control group.

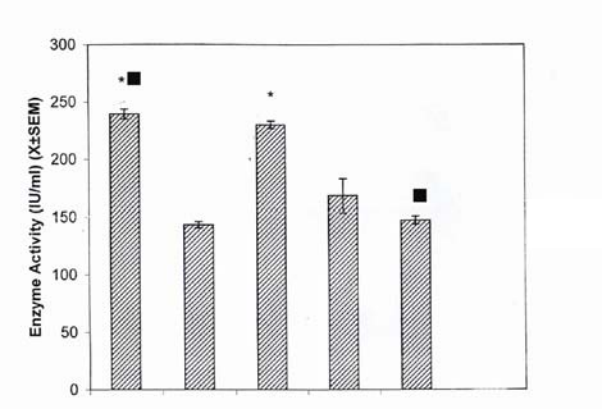


Fig.2. AST enzyme activity in different test groups.
+ve = positive control (CCl_4), -ve = negative control (olive oil), A1 = 200mg/kg, A2 = 400mg/kg and A3 = 800mg/kg of herbal extract. * $p<0.05$ as compared with the negative group,
■ $p<0.05$ as compared with the CCL_4 control group

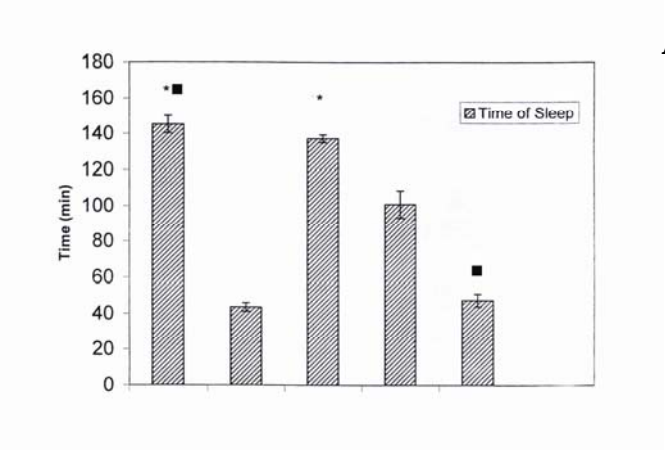


Fig. 3. Sleeping time in different test groups.
+ve = positive control (CCl_4), -ve = negative control (olive oil) , A1 = 200mg/kg, A2 = 400mg/kg and A3 = 800mg/kg of herbal extract. * $p<0.05$ as compared with the negative group,
■ $p<0.05$ as compared with the CCL_4 control group

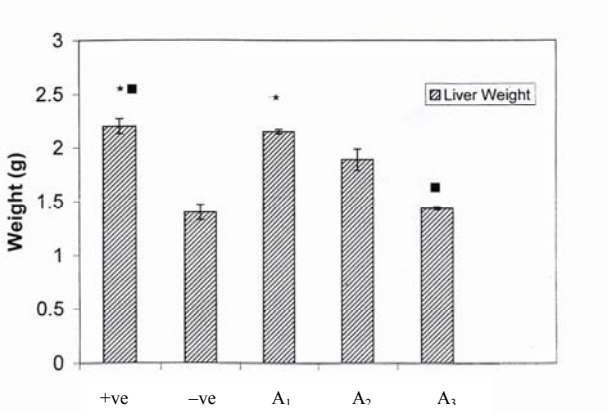
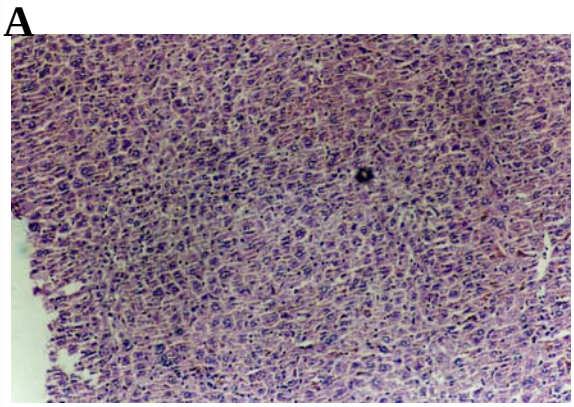
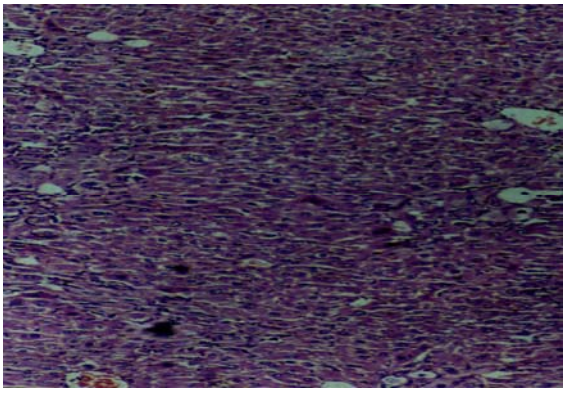
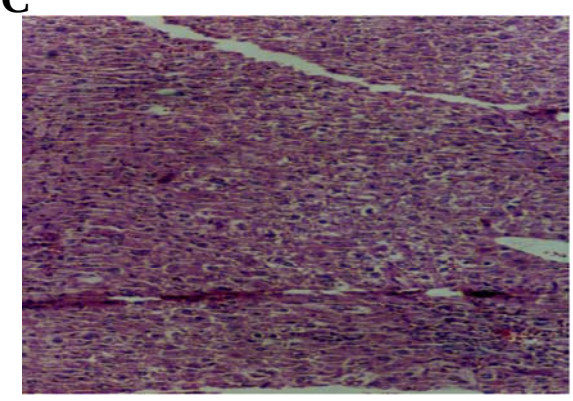


Fig. 4. Liver weight in different test groups.
+ve = positive control (CCl_4), -ve = negative control (olive oil) , A1 = 200mg/kg, A2 = 400mg/kg and A3 = 800mg/kg of herbal extract * $p<0.05$ as compared with the negative group,
■ $p<0.05$ as compared with the CCL_4 control group







Light microphotographs of H&E-stained sections of the formalin fixed livers. (**A**) Negative control (olive oil) group (**B**) Carbon tetrachloride control group, showing massive fatty changes, necrosis and broad infiltration of the lymphocytes and kupffer cells (**C**) Test group received 800mg/kg of the *Morus alba* L. extract. Magnification × 10.

Discussion

Carbon tetrachloride is bio-transformed by the cytochrome P450 system in the endoplasmic reticulum to produce trichloromethyl free radical. This free radical then combined with cellular lipids and proteins in the presence of oxygen to form a

trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxyl free radical leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death (14). For screening of hepatoprotective drug the commonly used model is carbon tetrachloride inducing liver damage (15). Increase in serum Aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT) level and liver weight have been attributed to the injured structural integrity of the liver as they are released into the circulation after cellular damages. Indeed we observed that pretreatment of mice with crude extract of white mulberry prevented the rise in the serum level transaminase, liver weight and also examination of histopathological sections of negative group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. Disarrangement of normal hepatic cells with centrilobular necrosis vacuolization of cytoplasm and fatty degeneration were observed in carbon tetrachloride intoxicated mice. The liver sections of the mice treated with the crude extract of *Morus alba* L. (white mulberry) at dose of 800mg/kg followed by carbon tetrachloride intoxication exhibited a significant protection as it was evident by the absence of necrosis, tissue damage and vacuoles. All these parameters conforming the protective potential of this extract against carbon tetrachloride induced hepatotoxicity and are in keeping with the previous work (15). Also these data are in keeping with the reported protective effect of *Morus alba* L. of Moraceae family (16). A possible mechanism of *Morus alba* L. extract as hepatoprotective may be due to it's antioxidant effect which impair the activation of carbon tetrachloride into the reactive form. Since flavonoides have hepatoprotective activities (17), it may be speculated that the constituents of *Morus alba* L., especially the flavonoids (rutin, moracetin, quercetin -3-triglucoside and isoquercitrin) were responsible for the observed protective effects.

In conclusion, these preliminary data suggest that under the present experimental conditions, *Morus alba* L. alcoholic extract exerts a protective potential in carbon tetrachloride induced liver injury which might substantiate it's use in medicine.

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