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STUDY OF THE PROTECTIVE EFFECT OF *RAPHANUS SATIVUS* (RADISH) SEED IN LIVER TOXICITY INDUCED BY CARBON TETRACHLORIDE IN MICE

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Abstract

It is well known that some herbal medicine play an important role in therapy. The hydro-alcoholic extract of Radish seed was obtained by maceration technique. Animals were weighed and divided in eight groups (each group consists of seven mice). The control groups received normal saline and olive oil for four consecutive days. The positive control group received normal saline in days first and 4th and CCL_4 (0.2ml/kg) in days 2nd and 3rd. The test groups received oral crude extracts in doses of (100, 200, 400, 600, 800 mg/kg) on day first and day fourth. Then on day second and 3rd they received oral CCL_4 one hour before crude extract administration. On day fifth all groups were weighed and then administered intrapretoneal (ip) with hexobarbital sodium (0.25mg/kg) to determine the sleeping time. Blood samples was withdrawn and serum was prepared for AST and ALT activities. Liver was removed and kept in 10% formalin solution for histopathological studies. Results of histopathological findings showed that groups received (600 and 800 mg/kg) were significant (reduction in liver damage) as compared with positive control group. Also, results of serum enzyme activities were analyzed by one-way ANOVA method indicated that these two groups (600 and 800 mg/kg) were also significant as compared with CCL_4 group ($p<0.01$). In conclusion according to results obtained from crude extract of Radish seed in dose of (600 and 800 mg/kg) may be good enough to protect liver damage induced by CCL_4.

Keywords

Liver, Radish seed, Carbon tetrachloride, AST, ALT enzymes, Mice.

Introduction

Herbal medicine has been used for centuries by people all over the world to treat disease and promote health. Both the west and east have spent considerable time, research and energy developing the theories and applications within the field of herbal medicine. Herbal drugs are generally easy to administer when properly prescribed and used, have the advantage of being relatively cheap when compared to western pharmaceutical medicines (1). Radish has been grown as food crop, for long time but it also have various medicinal actions. The roots stimulate the appetite and digestion, having a tonic and laxative effect and also stimulating the flow of bile (2). The leaves and seeds are used in the treatment of

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asthma and other chest complaints (3,4,5). Carbon tetrachloride is used as a solvent in synthetic chemistry research. It is one of the most potent hepatotoxins and is widely used in scientific research to evaluate hepatoprotective agents (6). The liver and kidney are especially sensitive to carbon tetrachloride. In mild cases the liver becomes swollen and tender, and fat builds up inside the organ. In severe cases liver cells may be damaged or destroyed leading to a decrease in liver function. Exposure to high concentrations of carbon tetrachloride can affect the central nervous system, and may result in coma and even death (7,8,9). Chronic exposure to carbon tetrachloride can cause liver and kidney damage and could result in cancer. The International agency for research on cancer has determined that carbon tetrachloride is possibly carcinogenic to humans .The EPA has determined that carbon tetrachloride is a probable human carcinogen (10,11,12,13). Aim of this study was to find out the protective effect of Radish seed *(Raphanus sativus*) in hepatotoxicity induced by carbon tetrachloride.

Materials and Methods

Chemicals

Carbon tetrachloride, formaldehyde and sodium hexobarbital were purchased from Merck Co. (Germany). Alanine transaminase (ALT) and Aspartate transaminase (AST) kits were donated by Pars Azmun Co.Iran .

Animals

Swiss albino mice (35-40g) were supplied by Razi Research Center Hesarak Karaj Institute, Iran and housed in polypropylene cages at room temperature (22$\pm$2 C) with proper light, diet control and ventilation.

Plant materials

The plant materials (seed) were collected from local market in Ahvaz, Iran and were authenticated by the Botanist at Faculty of Agriculture of Shahid Chamran University, Ahvaz, Iran. The plant materials were extracted in 80% ethanol by maceration method for three days. The crude extract was filtered and the filtrated portion was concentrated under vacuum evaporator.

Animals were weighed and divided in eight groups (each group consists of seven mice). The control groups received normal saline and olive oil for four consecutive days. The positive control group received normal saline in days first and 4th and CCL_4 (0.2ml/kg) in days 2nd and 3rd. The test groups received oral crude extract in doses of 100, 200, 400, 600, 800 mg/kg for four days, (but on day 2nd and 3rd they also received oral CCL_4 one hour before administration of crude extract). On day fifth all groups were weighed and then they were given hexobarbital sodium (0.25mg/kg I.p.) to determine the sleeping time. Blood samples were withdrawn and serum was prepared for AST and ALT activities. Liver was removed and kept in 10% formalin solution for histopathological studies (13).

Staining method

For observation of histopathological changes and cell damage Hematoxiline and Eosin staining method were used (14).

Assay of ALT and AST

For determination of hepatic enzyme activities (ALT, AST) Frankel and Reitman method was applied. The absorbance of results were determined at 505 nm (15).

Statistical analysis

The data obtained were statistically analyzed using one-way ANOVA, and ($P<0.01$) was considered as the level of significance.

Results

After the completion of biochemical experiments and the histopathological study of the liver tissue the following results were obtained. Results of histopathological findings showed that in groups which received normal saline and olive oil (negative control groups) liver cells had normal nucleous and cytoplasm and there was no abnormality in the cells (Figure.1). But in the group that received carbon tetrachloride (positive control group) liver cells were inflammed, and cell necrosis, cell degeneration and cell injury were observed as shown in Figure.2.

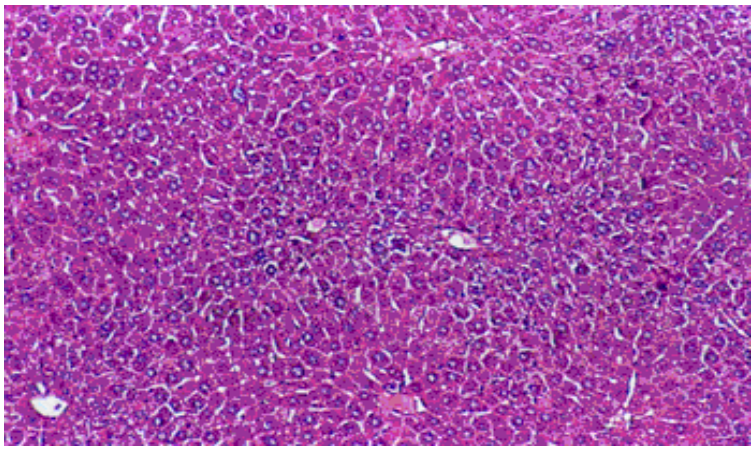

Fig.1: Microscopic photograph of liver treated with normal saline (negative control).

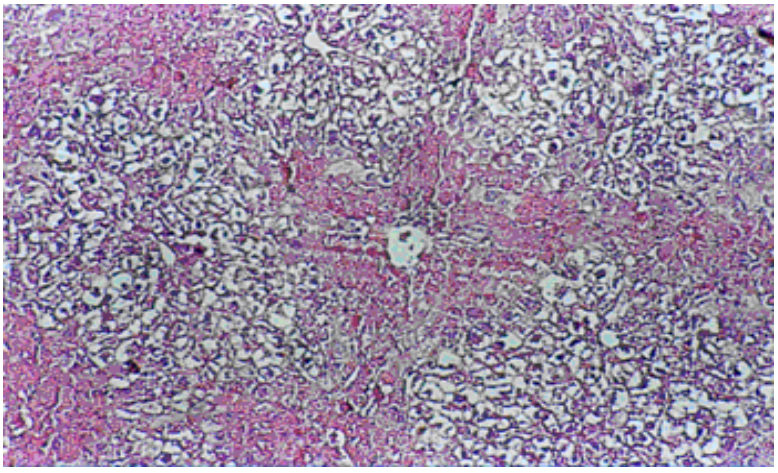

Fig.2: Microscopic photograph of liver treated with carbon tetrachloride (positive control group), massive centribular necrosis, accumulation of inflammation cells and congestion have been observed (H&E, X 100)**.**

In groups that received (600 and 800 mg/kg) crude extract showed significant liver protection as liver cells were in proper shape with normal cytoplasm, synosoides necleous as shown in Figure.3 and Figure.4.

Also, results of serum enzyme activities showed that these two groups (600 and 800 mg/kg) indicated significant reduction in AST and ALT activities as compared with CCL_4 group ($p<0.01$). (Table 1, Figure.5 and Figure.6).

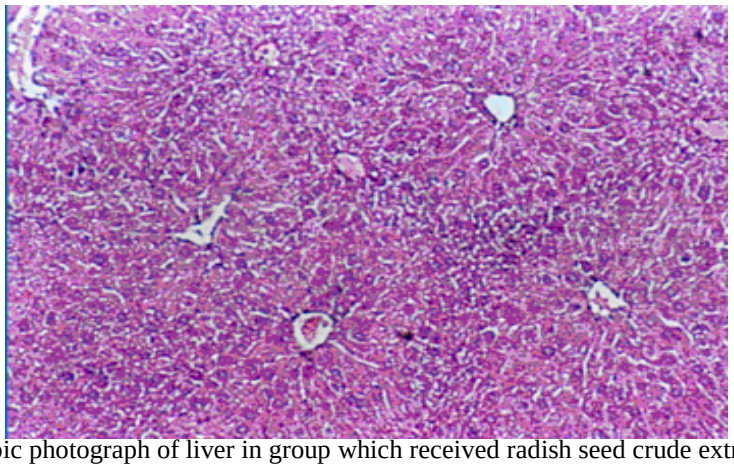

Fig.3: Microscopic photograph of liver in group which received radish seed crude extract (600mg/kg). Hepatic cell regeneration significantly increased and liver injury reduced, necrosis in the liver cells was reduced and liver structure shows normal shape (H&E, X100).

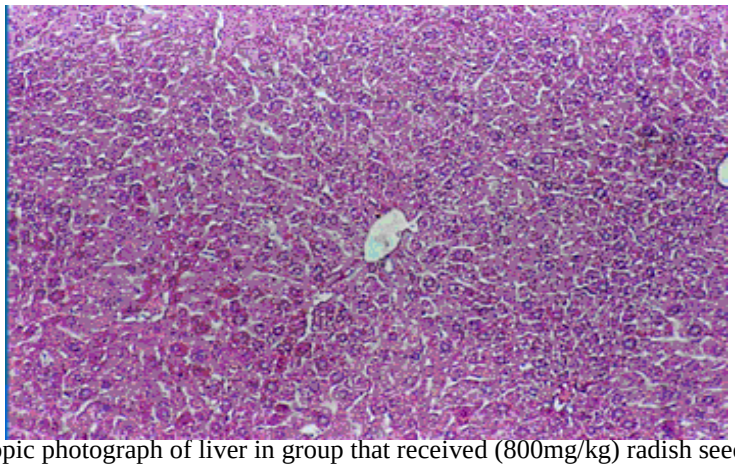

Fig.4: Microscopic photograph of liver in group that received (800mg/kg) radish seed crude extract. Hepatic cell regeneration significantly increased and liver injury reduced, necrosis in the liver cells was reduced and liver structure shows normal shape (H&E, X 100).

Results obtained for the parameter of sleeping time indicated that the sleeping time in the treated test group also decreased as compared to the sleeping time of positive control group (Table 1). In this study the mean of liver weight in the positive control group as compared with the negative control group increased significantly P<0.01(Fig.7).

Table 1: Mean of AST, ALT and sleeping time of different groups.

Groups	AST activity (IU/ml)	ALTactivity(IU/ml)	Sleeping time (min)
Negative control (Normal saline)	254	89	29
Negative control (Olive oil)	259	92	28
Positive control (CCL4)	459	253	38
Test1 (100mg/kg)	408	233	37
Test2 (200mg/kg)	394	204	36
Test3 (400mg/kg)	385	192	35
Test4 (600mg/kg)	255	107	30
Test5 (800mg/kg)	265	104	29

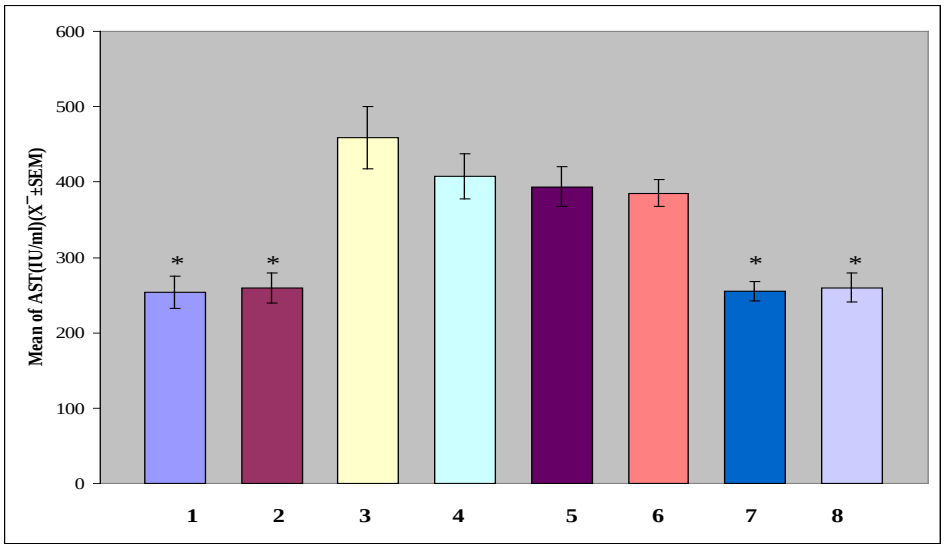

Fig.5: The comparison of aspartate aminotransferase (AST) activity in negative control groups (1&2), positive control group (3), different test groups: 100mg/kg (4), 200mg/kg (5), 400mg/kg (6), 600mg/kg (7) and 800mg/kg (8). (*P<0.01 relative to control responses, one-way ANOVA).

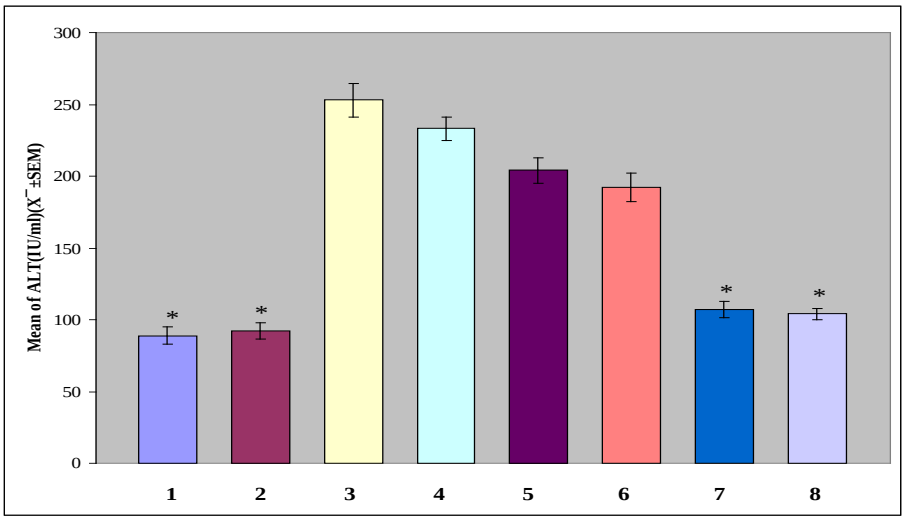

Fig.6: The comparison of alanine aminotransferase (ALT) activity in negative control groups (1 & 2), positive control group (3), different test groups: 100mg/kg (4), 200mg/kg(5), 400mg/kg(6), 600mg/kg(7), 800mg/kg(8). (*P<0.01 relative to control responses, one-way ANOVA).

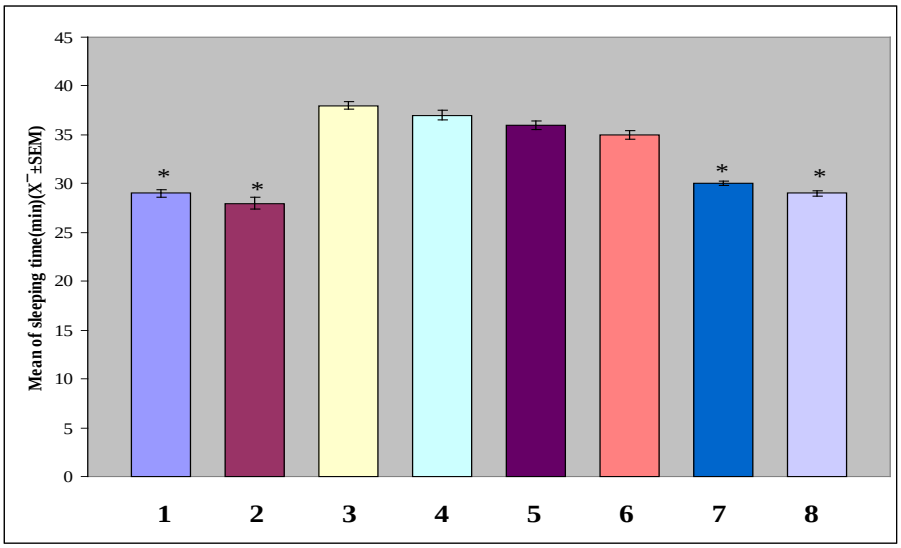

Fig.7: The comparison of sleeping time (min) in negative control groups (1 & 2), positive control group (3), different test group: 100 mg/kg (4), 200 mg/kg (5), 400 mg/kg (6), 600 mg/kg (7), 800 mg/kg (8). (*P<0.01 relative to control responses, one-way ANOVA).

Discussion

Carbon tetrachloride is one of the most potent hepatotoxic organic solvent and is widely used in scientific research to evaluate hepatoprotective agents. Human organs especially liver is very sensitive to carbon tetrachloride. In mild cases, the liver becomes swollen and tender, and fat builds up inside the organ. In severe cases, liver cells may be damaged or destroyed leading to a decrease in liver function. Ample experimental and epidemiological studies support the involvement of carbon tetrachloride in the pathogenesis and progression of several chronic diseases. It has been established that carbon tetrachloride in hepatic parenchymal cells is metabolically activated by cytochrome P450 dependent monoxygenases to form a trichloromethyl free radical which alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides leading to liver damage (16). It is reported that administration of 10 mg/kg to 1000 mg/kg carbon tetrachloride by gavage to rats resulted in increase of serum levels of hepatic enzymes and increase in centribular lesions in the liver (17). Many herbs and plant products have been shown to have liver protection. Due to its anti oxidant property as source of vitamin C and sulfur containing compound, radish seed can compete with the free radicals in the cell components and protect the liver from injury (18). In order to evaluate the liver protective activity of radish seed crude extract against carbon tetrachloride intoxication, different doses of crude extract of radish seed were tested, the obtained results showed that doses of (600 mg/kg and 800 mg/kg) significantly decreased AST and ALT activities ($P<0.01$) which were analyzed by one-way ANOVA method indicates protection of liver from injury and also the liver size was recovered as compared with the carbon tetrachloride treated group. The histopathological findings indicated that there was no abnormal fats, necrosis in the liver cells, and the liver structure was recovered to normal shape. Results obtained for the parameter of sleeping time which was analyzed by one-way ANOVA method indicated that the sleeping time in the treated test group also decreased as compared to the sleeping time of positive control group ($p<0.01$). It is well known that liver enzymes (Cytochrome P_{450}) have a potential role in the metabolism of pharmaceutical compounds but if there is any damage to liver the activities of these enzymes changes. A drug such as sodium hexobarbital which is an inducer of P_{450} enzymes could prolong the sleeping time in animals in which their liver has been damaged. In this study we observed the group which received the crude extract of radish seed in doses of (600 and 800 mg/kg) also showed decreased in sleeping time as compared with the positive control group which indicates that this crude extract could protect liver from carbon tetrachloride toxicity .This observation can relate to our previous study as point of protective effect (19, 20).

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