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Protective Effect of Fresh Radish Juice (*Raphanus sativus* L.) Against Carbon Tetrachloride-Induced Hepatotoxicity

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Abstract: The fresh juice obtained from the locally grown radish root was tested for possible hepatoprotective effect against carbon tetrachloride-induced hepatocellular damage in albino rats. The juice at two doses of 2 and 4 mL/kg/rat for five consecutive days, exhibited a significant dose-dependent protective effect. The magnitude of protection was measured by using biochemical parameters including determination of Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), bilirubin (Bil) and non-protein sulfhydryl content in the liver tissues (NP-SH), in addition to histopathological assessment. The protective effect was demonstrated in lowering the elevated serum levels of SGPT, SGOT, Bil, ALP and increasing NP-SH level. Silymarin, a known hepatoprotective agent was used as a positive control. Biochemical data were further supported by the histopathological results. The phytochemical examination of the fresh juice revealed the presence of sulfurated, phenolic and terpenoid compounds in radish.

Key words: Radish, fresh juice, *Raphanus sativus*, liver protection, carbon tetrachloride, Brassicaceae

INTRODUCTION

Raphanus sativus L. (radish) is a member of the family (Brassicaceae) is generally eaten raw for its pungent flavor. Radish is most valued by the inhabitants of many Western and Eastern countries as a food and a medicine (Mayer, 1981). In Greeko-Arab or Unani medicine and in Indian folklore, is administered as a household remedy for the prevention and the treatment of gall stone, jaundice, flatulence, indigestion and in various gastric ailments. In folklore of Asian countries, it is widely known to have beneficial effects such as improving digestion and alleviating constipation. An earlier study by Jung *et al.* (2000) has shown that radish extract stimulates gastrointestinal motility in rodents. In Mexico, radish is a popular folk remedy for the treatment of urolithiasis and a wide variety of urinary disorders (Burk, 1983). Other varieties of *Raphanus sativus* were also described as having many health beneficial effects when administered in various illnesses (Vargas *et al.*, 1999; El Sayed *et al.*, 1995). Eating a few slices of raw radish with salt and pepper thrice daily, decreases complaining of piles, constipation, indigestion, colic, dyspepsia, enlargement

of liver, spleen, jaundice and prolapse of the rectum (Prahoveanu and Esanu, 1990). The juice of radish has a tonic and laxative action on the intestine and indirectly stimulates the flow of bile (Aman, 1969). Regarding the usefulness of fresh juice of radish in protecting or curing liver ailments, no published studies have been found. It is worth mentioning that a related species, *Brassica* showed hepatoprotective activity (Rafatullah *et al.*, 2006). In addition, Radish and other members of the family Brassicaceae were found to contain indole-3-carbinol. According to several published reports, this substance exhibited strong hepatoprotective properties against various carcinogenic agents (Aggarwal and Ichikawa, 2005). The present investigation was undertaken to verify the claims of the Unani and Arab herbal medicine practitioners regarding the hepatoprotective effect of radish juice.

MATERIALS AND METHODS

Preparation of juice: The fresh radish was purchased from the local vegetable market in Riyadh and authenticated in the Department of Pharmacognosy,

College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The material was washed and squeezed in an electric blender (National, Japan) to obtain fresh juice.

Phytochemical screening: The phytochemical analysis of the roots of Radish was conducted for the presence and/or absence of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil, cyanogenic glycosides, coumarins, sterols, triterpenes and sulfur containing compounds.

Animals: Winstar albino rats of either sex and approximately the same age (8-10 weeks), weighing 180-200 g, obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, were used. Swiss albino mice were used for studies of sleeping time. The animals were kept at a constant temperature ($22\pm 2^\circ\text{C}$), humidity (55%) 12 h and light-dark conditions. The animals were provided with Purina chow and free access to drinking water *ad libitum*. The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Carbon tetrachloride-induced liver toxicity: Rats were divided into five groups (1, 2, 3, 4 and 5) ($N = 6$ animals/group). Group I was kept as a control one (no treatment). Group 2, 3, 4 and 5 received 0.25 mL of CCl_4 in liquid paraffin (1:1) 1 mL kg^{-1} body weight intraperitoneally (IP) (Sen *et al.*, 1993). Group 2 was administered only CCl_4 . Group 3 was administered silymarin 10 mg kg^{-1} body weight orally and group 4 and 5 were treated with radish juice, 2 and 4 mL kg^{-1} body weight, respectively. Juice treatment was started five days prior to CCl_4 administration and continued until the end of the experiment. The animals were sacrificed using ether anaesthesia, after 24 h following CCl_4 administration. Blood was collected by cardiac puncture, allowed to clot and serum separated. Liver was dissected out and used for biochemical studies.

Parameters measured: The serum levels of glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin were determined using Reflotron® Plus analyzer and Roche kits.

Estimation of Non-Protein Sulfhydryl Groups (NP-SH): The level of non-protein sulfhydryl groups (NP-SH) was measured according to the method of Sedlak and Lindsay (1968). The liver tissue was homogenized in

ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). Aliquots of 5 mL of the homogenates were mixed in 15 mL test tubes with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid. The tubes were shaken intermittently for 10-15 min and centrifuged at 3000 rpm. Two mL of supernatant were mixed with 4 mL of 0.4 M Tris buffer, pH 8.9 and 0.1 mL of 0.4% DTNB [5,5-dithiobis-(2-nitrobenzoic acid)] (DTNB) was added and the sample was shaken. The absorbance was read within 5 min of addition of DTNB at 412 nm against a reagent blank with no homogenate.

Measurement of Pentobarbital sleeping time: Mice were divided into four groups of ten animals each. Group I received the vehicle (0.3 mL of saline); group II received CCl_4 only. Group III and VI received radish juice, 2 and 4 mL kg^{-1} body weight, respectively. Thirty minutes later, the animals of groups II, III and IV were treated with sodium Pentobarbital (50 mg kg^{-1} , intraperitoneally). The time interval between the onset and the regaining of the righting reflex was measured as the sleeping time (Dandiya and Collumbine, 1959).

Histopathological studies: The liver tissue was fixed in 10% ethanol buffered formalin and processed through graded ethanol, xylene and impregnated with paraffin wax. Sections were made by microtome. After staining with haematoxylin and eosin, the different histopathological indices were determined (Culling, 1974).

Statistical analysis: The data were statistically analyzed using ANOVA.

RESULTS

The administration of CCl_4 to the animals resulted in a marked increase in serum transaminases (SGPT, SGOT), serum alkaline phosphatase (ALP) and total bilirubin activities. Toxic effects of CCl_4 were not shown in animals treated with silymarin and various doses of radish among the treated groups. A significant dose-dependent hepatoprotective effect was observed in the animals pre-treated with 2 and 4 mL radish juice by way of restoring the levels of liver enzymes and bilirubin (Table 1). An antioxidant activity was also evident, as the radish juice exhibited the ability to replenish the decreased NP-SH level in rats' livers after CCl_4 intoxication (Table 2). On the other hand, Pentobarbital-induced narcosis was found to be exacerbated by CCl_4 in mice, which was significantly decreased in the groups received radish juice (Table 3).

Histopathological assessment of liver tissues showed various histopathological changes in CCl_4 -treated rats'

Table 1: Effect of fresh radish juice pretreatment on serum GOT, GPT, ALP and Bilirubin activities on rats treated with CCl₄

Enzymes	Control	CCl ₄	Silymarin	Radish juice	Radish juice
SGOT (unit L ⁻¹)	97.05±12.71	422.83±39.13 ^{***}	187.83±16.35 ^{***}	320.66±31.92	249.83±25.51*
SGPT (unit L ⁻¹)	34.13±6.30	383.33±25.16 ^{***}	143.83±18.49 ^{***}	176.66±27.50 ^{***}	176.50±56.91 ^{**}
ALP (unit L ⁻¹)	417.50±23.72	898.66±36.84 ^{***}	573.50±34.90 ^{***}	768.00±124.16	645.50±93.2*
Bilirubin (mg dL ⁻¹)	0.60±0.06	2.22±0.15 ^{***}	1.12±0.09 ^{***}	1.37±0.13 ^{**}	1.25±0.066 ^{***}

*p<0.05, **p<0.01, ***p<0.001, ANOVA

Table 2: Effect of fresh radish juice pretreatment on the level of nonprotein sulfhydryl (NP-SH) in the liver of rat treated with CCl₄

Treatment (n = 6)	Dose (mL rat ⁻¹)	NP-SH (Mean±SE) mmol g ⁻¹ of tissue
Control (normal saline)	-	7.75±1.09
Control CCl ₄ only	0.25	2.92±0.34 ^{**}
Radish juice + CCl ₄	2.00	4.36±0.33 ^{**}
Radish juice + CCl ₄	4.00	5.55±1.03 ^{**}

*p<0.05, **p<0.01, ANOVA. *As compared with control group. ^aAs compared with CCl₄ only treated group

Table 3: Effect of fresh radish juice on duration of Pentobarbitone sleeping time in mice treated with CCl₄

Treatment (n = 6)	Dose (mL kg ⁻¹)	Sleeping time (min)	Reduction in sleeping time (%)
Only pentobarbital	50 (mg kg ⁻¹)	38.2±8.06	-
Pentobarbital + CCl ₄	50 mg kg ⁻¹	103.7±11.32 ^{***}	-
Radish juice + CCl ₄	2 (mL rat ⁻¹)	72.5±10.99 ^b	30.08
+pentobarbital			
Radish juice + CCl ₄	4 (mL rat ⁻¹)	55.6±7.02 ^{**}	46.38
+pentobarbital			

p<0.01, *p<0.001, ANOVA. *As compared to the pentobarbital ^bAs compared to CCl₄ + pentobarbital group



Fig. 1: Normal (control) rat liver. Normal liver parenchymal cells. H. and E. x 200

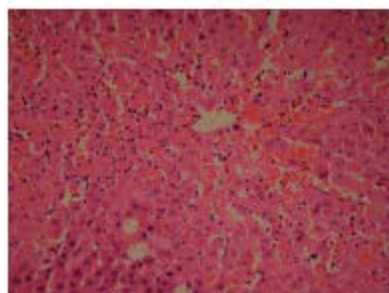


Fig. 2: CCl₄ only treated rat liver. Extensive zonal necrosis involving the perivenular zone (zone 3) around the central vein. Neutrophilic infiltration of the residual stroma with focal steatosis (fatty change) in the preserved parenchyma. H. and E. x 200

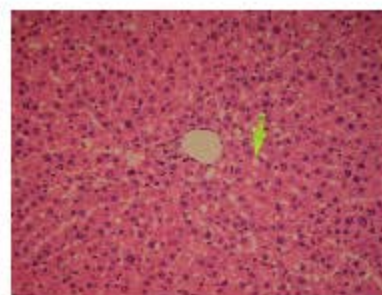


Fig. 3: Radish juice 2 mL/rat+CCl₄. Mild inflammatory infiltrate around the central vein with lipofuscin pigments. Mild steatosis present. Simple hepatocyte necrosis identified. H. and E. x 200

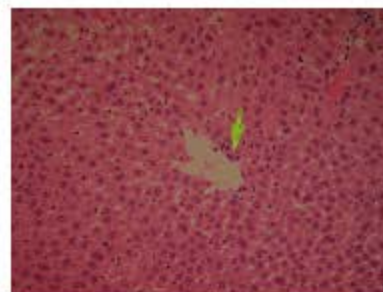


Fig. 4: Radish juice 4 mL/rat+CCl₄. Minimal inflammatory infiltrate only around the central vein. H. and E. x 200

livers, extensive zonal necrosis involving the perivenular zone (zone 3) around the central vein, focal steatosis (fatty change). The lower dose (2 mL kg⁻¹ plus CCl₄ administration caused mild steatosis and mild inflammatory infiltrate around the central vein. The higher dose (4 mL kg⁻¹) treatment resulted in the minimal inflammatory infiltrate and no steatosis of the liver (Fig 1-4). The preliminary phytochemical screening of radish revealed the presence of phenolic, terpenoidal and sulfated compounds.

DISCUSSION

Carbon tetrachloride has been used as a tool to induce hepatotoxicity in experimental animals. This toxic chemical caused peroxidative degradation in adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in transaminases and alkaline phosphatase

was a clear indication of cellular leakage and loss of functional integrity of cell membrane (Plaa and Hewitt, 1982). The increase in the level of serum bilirubin reflected the severity of jaundice (Lin *et al.*, 1997). Carbon tetrachloride, which is an intrinsic hepatotoxin, was used to induce hepatic damage in this study since it has previously been shown to exert its toxic effects on the liver (Kus *et al.*, 2004). Administration of CCl₄ causes severe injury in rats' livers. This damage is recognized by an increase in serum levels of the hepatic enzymes SGOT and SGPT, which are indices of liver cell damage (Teocharis *et al.*, 2001). The biochemical mechanisms involved in the development of CCl₄ hepatotoxicity have long been investigated. It is generally believed that it is due to lipid peroxidation caused by carbon trichloromethyl radical (CCl₃·). CCl₄ is biotransformed by cytochrome P-450 to the trichloromethyl-free radical that induces membrane lipid peroxidation and disturbs Ca²⁺ homeostasis to produce hepatocellular injury (Recknagel *et al.*, 1989). The administration of radish juice showed significant and dose-dependent hepatoprotective activity, which was comparable with the standard drug silymarin. It is known that SGOT can be found in the liver, cardiac muscle, kidney, brain, pancreas, lungs, skeletal muscle, leukocytes and erythrocytes (in decreasing order of concentration) (Rafatullah *et al.*, 1991). Whereas SGPT is present in highest concentration in the liver. In tissues, SGPT occurs in two locations, the cytosol and mitochondria (Rej, 1978). SGPT appears to be a more sensitive and specific parameter of acute hepatocellular damage than SGOT (Lin *et al.*, 1997). Therefore, the possible hepatoprotective mechanism of the radish juice on the CCl₄-induced liver injuries may be through the following actions; inhibition of the cytochrome P-450 activity, prevention of the process of lipid peroxidation, stabilization of the hepatocellular membrane and enhancing the protein synthesis (Poterton, 1983).

Our observation in the present study also indicated that the treatment with CCl₄ caused a significant reduction in non-protein sulfhydryl moiety (NP-SH) concentration in the rat liver. The radish juice however offered a significant replenishing of the NP-SH level. Thus, a possible role of the sulfhydryl seems to be implicated in hepatoprotection mechanism by its antioxidant potential (Burk, 1983). Additionally, pentobarbital-induced sleeping time is significantly prolonged in liver damage and this parameter may be employed as a measure of functional status of the hepatic drug metabolizing system. The present study showed the ability of radish juice to cause significant reduction in sleeping time. The later effect is presumably attributed to enhancement of detoxification mechanism by liver (Fujimoto *et al.*, 1960).

On the basis of the obtained results in this study, it can be concluded that radish juice has preventive effect against CCl₄-induced hepatocellular damage in rats. The present liver-protective effect of radish juice is presumably due to its contents of sulphurated, phenolic and terpenoid compounds. The effect of these compounds could be through preventing the accumulation of excessive free radicals and protecting the liver against CCl₄ intoxication. The protection of liver by radish juice against CCl₄-induced toxicity might be related to glutathione-mediated detoxification. The findings of the present study support the claims of Unani and Arab traditional medicine practitioners on the usefulness of radish in liver ailments caused by various etiologies.

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