



# International Journal of Pharmacology

ISSN 1811-7775

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## Preliminary Phytochemical and Hepatoprotective Studies on Turnip *Brassica rapa* L.

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**Abstract:** In the present study, the pre-treatment of rats with Turnip (*Brassica rapa* L.), juice protected the animals against CCl<sub>4</sub>-induced hepatotoxicity. The treatment significantly reduced the serum GOT, GPT, alkaline phosphatase (ALP) and bilirubin level at a dose of 16 mL kg<sup>-1</sup> body weight. Besides, the juice also replenished the lowered nonprotein sulfhydryl (NP-SH) concentration in the liver tissue after CCl<sub>4</sub> treatment. The preliminary phytochemical screening showed the presence of flavonoids, anthocyanins and sulfur containing constituents. The present results reveal that turnip possesses a hepatoprotective action, through its antioxidative potentials.

**Key words:** Turnip, *Brassica rapa*, phytochemical screening, hepatoprotection

### INTRODUCTION

The liver is an organ of paramount importance. It plays a pivotal role in the metabolism of xenobiotics (foreign compounds entering the body) (Rajesh and Latha, 2004). Herbal medicines have received great attention as an alternative to conventional therapy and the demand for these remedies has currently increased (De Smet, 2002). Consumption of herbs and vegetables is known to benefit life via the prevention of style-related diseases including liver disorders.

Plants of the genus *Brassica* belong to the family *Brassicaceae* and include several of the most commonly consumed vegetables all over the world (Focciolo, 1988). The commonly consumed species include 'turnip' *Brassica rapa* locally known as Liffit or Shaljam, which is used as a vegetable. Turnip is medicinally used as an antiscorbutic, antiarthritic, resolvent and stimulant. It is also used as a stomachic and laxative (Chopra *et al.*, 1956). It is known in the Unani and Arab traditional medicine for its use in chronic gastritis, constipation, cholecystitis, cholelithiasis and in liver diseases (Pithford, 2002). Hartwell (1971) reported that it is a remedy for cancer. In this communication, we present the results of preliminary phytochemical screening and the antihepatotoxic and antioxidative effects of *B. rapa* on carbon tetrachloride (CCl<sub>4</sub>)-induced liver damage in rats.

### MATERIALS AND METHODS

**Juice preparation:** Fresh turnip was purchased from a local vegetable market in Riyadh, Kingdom of Saudi

Arabia and identified. The pink bulbs/roots were washed, soaked by blotting paper, then squeezed in an electrical blender to obtain the juice.

**Animals:** Wistar 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±2°C), humidity (55%) and light-dark conditions (12/12 h light/dark ratio). 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.

**Phytochemical screening:** A phytochemical analysis of the underground parts of turnip was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil, cyanogenic glycosides, coumarins, sterols, triterpenes and sulphur containing compounds (Farnsworth, 1966).

**Carbon tetrachloride-induced liver toxicity:** Rats were divided into five groups (I, II, III, IV and V) (N = 6 animals/group). Group I was kept as a control group. Groups II, III, IV and V received 0.25 mL of CCl<sub>4</sub> in liquid paraffin (1:1) 100 g<sup>-1</sup> body weight intraperitoneally (Sen *et al.*, 1993). Group III was administered silymarin

10 mg kg<sup>-1</sup>, body weight orally and groups IV and V were treated with turnip juice 8 and 16 mL kg<sup>-1</sup> body weight. Drug treatment was started 5 days prior to CCl<sub>4</sub> administration and continued until the end of the experiment. After 48 h, following CCl<sub>4</sub> administration, animals were sacrificed using ether anaesthesia. Blood was collected by heart 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 (GOT), glutamic-pyruvate transaminase (GPT), alkaline phosphatase (ALP) and total bilirubin activities were determined using Reflotron® Plus analyzer and Roche kits.

**Estimation of nonprotein sulfhydryl groups (NP-SH):** The activity of 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 was added. The tubes were shaken intermittently for 10-15 min and centrifuged at 3,000 g. Two milliliters of supernatant were mixed with 4 mL of 0.4 M Tris buffer, pH 8.9 and 0.1 mL of 0.4% (5, 5-dithio-bis-[2-nitrobenzoic acid]) (DTNB) was added and the sample was shaken. The absorbance, using a spectrophotometer, was read within 5 min of addition of DTNB at 412 nm against a reagent blank with no homogenate.

**Measurement of phenobarbital sleeping time:** Mice were divided into four groups of 10 animals each. Group I received the vehicle (0.3 mL of saline); group II received CCl<sub>4</sub> only. Groups III and IV received turnip juices 8 and 16 mL kg<sup>-1</sup> body weight. Thirty minutes later, the animals of groups II, III and IV were treated with sodium Phenobarbital (50 mg kg<sup>-1</sup>, intraperitoneally).

The time interval between the onset and the regaining of the righting reflex was measured as the sleeping time (Dandiya and Collumbine, 1959).

## RESULTS

**Phytochemical screening:** The preliminary phytochemical screening of the underground part of the turnip revealed the presence of flavonoid derivatives, sulphur-containing compounds and anthocyanins.

**Effects of turnip juice on SGOT, SGPT, ALP and bilirubin activities:** The results of hepatoprotective effects of turnip juice on CCl<sub>4</sub> intoxicated rats are shown in Table 1. In the CCl<sub>4</sub>-treated group, serum GOT, GPT, ALP and bilirubin were significantly increased as compared to normal rats group. The groups treated with 8 and 16 mL kg<sup>-1</sup> body weight of turnip juice showed significant decreases in the elevated levels of SGOT, SGPT, ALP and bilirubin in a dose-dependent manner. Treatment of rats with silymarin, a known hepatoprotective drug used as a reference standard also exhibited significant protective effect against CCl<sub>4</sub>-induced liver damage.

**Effect of turnip juice on NP-SH activities:** Nonprotein-sulfhydryl activities in CCl<sub>4</sub>-treated in rats were decreased drastically when compared with normal group. Treatment with turnip juice restored this decrease in content produced by CCl<sub>4</sub> towards normalization in higher dose (16 mL kg<sup>-1</sup>) (Table 2).

**Effect of turuip juice on Phenobarbital-induced sleeping time:** There was a significant lowering of Phenobarbital-induced sleeping time following the turnip juice administration (16 mL kg<sup>-1</sup>); where, the lower dose (8 mL kg<sup>-1</sup>) showed an insignificant reduction in sleeping time in CCl<sub>4</sub>-induced acute liver toxicity (Table 3).

Table 1: Effect of turnip juice on some enzymes and bilirubin in rats with CCl<sub>4</sub>-induced liver damage

Treatments (n = 6)	Dose (mL kg <sup>-1</sup> )	GOT (U L <sup>-1</sup> )	SGPT (U L <sup>-1</sup> )	ALP (U L <sup>-1</sup> )	Bilirubin (mg dL <sup>-1</sup> )
Control	Normal saline	65.75±8.04	33.7±7.78	482.66±21.89	0.61±0.07
CCl <sub>4</sub> only	1.25	426.00±35.42****a	339.00±14.22****a	875.50±39.33****a	2.40±0.19****a
Silymarin+CCl <sub>4</sub>	10	224.00±32.29****b	125.91±20.15****b	589.83±27.44****b	1.12±0.19****b
Turnip juice+CCl <sub>4</sub>	8	320.00±16.18**b	255.16±30.58**b	703.00±26.87**b	1.95±0.20
Turnip juice+CCl <sub>4</sub>	16	266.00±30.18**b	203.66±24.12**b	644.83±37.30**b	1.56±0.26**b

\*p<0.05; \*\*p<0.01, \*\*\*p<0.001 Student's t-test; <sup>a</sup>as compared with the control (normal saline) group <sup>b</sup>as compared with the CCl<sub>4</sub> only group. GOT = glutamic-oxaloacetic transaminase; GPT = glutamic-pyruvate transaminase; ALP = alkaline phosphatase

Table 2: Effect of turnip juice on the level of nonprotein sulfhydryl (NP-SH) groups in the liver of rat treated with CCl<sub>4</sub>

Treatments (n = 6)	Dose (mL kg <sup>-1</sup> )	NP-SH (mean±SE) mmol g <sup>-1</sup> of tissue
Control (normal saline)	-	6.60±4.81
Control CCl <sub>4</sub>	1.25	4.18±0.24****a
Turnip juice+CCl <sub>4</sub>	8.00	4.64±0.24 <sup>b</sup>
Turnip juice+CCl <sub>4</sub>	16.00	5.29±0.18**b

<sup>a</sup>As compared with the control (normal saline) group; <sup>b</sup>as compared with the control (CCl<sub>4</sub>). \*\*p<0.01; \*\*\*p<0.001 Student's t-test

Table 3: Effect of turnip juice on duration of Phenobarbital sleeping time in mice treated with CCl<sub>4</sub>

Treatment (n = 6)	Dose (mL kg <sup>-1</sup> )	Sleeping time (min)	Reduction in sleeping time (%)
Phenobarbital	50	40.80±6.46	-
CCl <sub>4</sub> +Phenobarbital	50	107.10±12.08****	-
CCl <sub>4</sub> +Turnip juice+Phenobarbital	8	74.00±10.11 <sup>b</sup>	30.9
CCl <sub>4</sub> +Turnip juice+Phenobarbital	16	42.10±4.58*** <sup>ab</sup>	60.6

\*\*\*p<0.001 student's t-test. \*As compared to the Phenobarbital group; <sup>b</sup>as compared to the CCl<sub>4</sub>+Phenobarbital group

## DISCUSSION

Various pharmacological and chemical substances which belong to the intrinsic or idiosyncratic group of hepatotoxins may induce a level of hepatic damage varying from asymptomatic hepatic functional disturbances to widespread liver necrosis. 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 (CCl<sub>4</sub>) to rats causes severe liver injury which 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 CCl<sub>4</sub> hepatotoxicity have long been investigated. It is generally believed that it is due to lipid peroxidation caused by carbon trichloromethyl radical, (CCl<sub>3</sub>). CCl<sub>4</sub> is biotransformed by cytochrome P450 to the trichloromethyl-free radical that induces membrane lipid peroxidation and disturbs Ca<sup>2+</sup> homeostatis to produce hepatocellular injury (Recknagel *et al.*, 1989). Earlier experimental studies have shown that CCl<sub>4</sub> administration caused an increase in serum levels of GOT, GPT and ALP in mice (Al-Shabanah *et al.*, 2000).

In the present study, turnip juice treatment significantly reduced the elevated serum levels of GOT, GPT, ALP and bilirubin. In some earlier studies, it has been reported that some Brassica species, including turnip have anti-carcinogenic activity (Kristal, 2002). Brassica vegetables are consumed for health improvement, which is related to their antioxidant activity (Plumb *et al.*, 1996; Verhoeven *et al.*, 1997). These beneficial effects have been attributed to the compounds which possess antioxidant activity. The major antioxidants of turnip and related vegetables are likely to be phenolic compounds such as flavonoids. These antioxidants scavenge radicals and inhibit the chain initiation or break the chain propagation (the second defense line) (Robards *et al.*, 1999; Shi *et al.*, 2001). Additionally, it has also been shown that CCl<sub>4</sub>-induced depletion of hepatic NP-SH is significantly prevented by fresh juice of turnip. Furthermore, the inhibitory effect of the juice on CCl<sub>4</sub>-induced prolongation of sleeping time suggest the ability of juice constituents to reverse the

damage exerted by CCl<sub>4</sub> on the cytochrome P450 involved in metabolism of phenobarbitone. In conclusion, the present study indicates that turnip juice treatment prevents CCl<sub>4</sub>-induced liver damage in rats, possibly through its antioxidant action and further supports the earlier findings on other *Brassica* species. These findings also substantiate the claims of herbal and Unani medicine practitioners, using turnip in liver ailments.

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