Full Length Research Paper

Hepatoprotective activity of *Trapa acornis* shell extracts against CCI₄-induced liver injury in rats

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Trapa acornis (TA) pulp is one of the most popular vegetables and fruits with unique taste in China. Our previous study showed that *T. acornis* shell (TAS) had strong antioxidant activity *in vitro*. Polyphenols and flavonoids have contributed to antioxidant activity; and hepatoprotective activity also has relationship with antioxidant activity. Nevertheless, there have been no reports on total phenolic content (TPC) and total flavonoid content (TFC) of extracts of TAS and hepatoprotective activity of TAS extracts against CCl₄-induced liver injury in rats. There was intragastric administration of ethyl acetate extract (TASEA) and *n*-butanol extract (TASBU) of *T. acornis* shell in rats for 8 days; and the last administration was done after 2 h, except for the normal control group. Other groups were all given 0.4% CCl₄ at the dose of 100 mL/kg diluted in olive oil by intraperitoneal injection. Results showed that the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in each treatment group were significantly decreased in liver tissue (*P*<0.001). In addition to TASEA (200 mg/kg b.w.), the level of superoxide dismutase (SOD) in liver tissue for treatment group was significantly increased (*P*<0.05, respectively). It is demonstrated that extracts of TAS had hepatoprotective effect on CCl₄-induced liver injury, which may be attributable to its antioxidant activity.

Key words: Trapa acornis, total phenolic, total flavonoid, antioxidant activity, hepatoprotective effect.

INTRODUCTION

CCl₄ is widely used to induce liver injury in animal models and is a potent lipid-soluble hepatotoxic agent. It produces peroxidative degeneration of many tissues (Szymonik-Lesiuk et al., 2003). The mechanism of CCl₄ hepatotoxicity rests with its biotransformation by the cytochrome P-450 system, giving rise to the trichloromethyl radical (CCl₃) which is further converted to the trichloromethyl peroxyl radical (CCl₃O₂⁻). CCl₄ metabolites react with polyunsaturated fatty acids and form covalent adducts with lipids and proteins. These events lead to lipid peroxidation and destruction of cell membranes with the consequent liver injury (Clawson, 1989; Recknagel et al., 1989). Reactive oxygen species (ROS) lead to various diseases such as angiocardiopathy, diabetes, cancer, neurodegenerative diseases, liver cirrhosis and the ageing process (Sabir et al., 2012). Natural constituents of plant can be derived from any parts of plants like bark, leaves, roots, fruits, seeds and fruit shells. These parts of a plant may contain active components (Gordon and David, 2001). Many studies have shown that polyphenolic and flavonoid compounds were found in tea, fruits, vegetables and herbs, several of which display antioxidant and ROS scavenging properties (Abdelly, 2009; Ksouri et al., 2010; Prasad et al., 2009; Komes et al., 2010; MIcek and Rop, 2011).

Trapa acornis Nakano (Chinese name is Nanhuling) belongs to the Trapaceae family and is one of the most popular vegetables and fruits with unique taste and medical functions in China. The genus *Trapa* was widely distributed in the tropical, subtropical and temperate regions

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of Asia, Africa and central Europe (Institute of Botany, Chinese Academy of Sciences, 2004). In China, this genus is mainly distributed in South of Yangtze River and many of its species have been used as traditional medicinal herbs to treat ulcer and cancer. Phytochemical research showed that flavonoids, polyphenols alkaloids and sterols were main compounds in this genus (Niu et al., 2003; Shang et al., 2007), and pharmacological investigation showed that extract of shells of Trapa species had anti-cancer activity, antimicrobial activity, antioxidant activity and hepatoprotection (Parekh and Chanda, 2007; Chiang and Ciou, 2010; Xie, 2011; Wang et al., 2011). Our previous studies showed that the extracts of TAS had good antioxidant activity (Kang et al., 2011).

However, there was no report concerning its polyphenols and flavonoids content, and hepatoprotective of TAS against CCl₄-induced liver injury in rats *in vivo*. In this work, the relationship between hepatoprotective properties and antioxidant compounds extracts of TAS was evaluated.

MATERIALS AND METHODS

Plant material and extract preparation

TAS was obtained in September 2009 in Jiaxing, Zhejiang Province, China, and identified by Professor Changqin Li (Institute of Chinese Materia Medica, Henan University). A voucher specimen was deposited in the Institute of Chinese Materia Medica, Henan University (No. 0910103). The air dried *T. acornis* shell (3.9 kg) was extracted three times with 80% ethanol for 2 d at room temperature. After evaporation of solvent *in vacuo* (Rotary Evaporators), the concentrated extract was suspended in water and extracted successively with petroleum ether, ethyl acetate extract and *n*-butanol respectively. Solution was concentrated under reduced pressure to yield petroleum ether extract (TASPE), ethyl acetate extract (TASEA) and *n*-butanol (TASBU).

Chemicals

CCl₄ was purchased from Tianjin Hongyan Chemical Reagent Factory. Bifendate was obtained from Zhejiang Pharmaceutical Co., Ltd, Wenling, China; ALT (alanine aminotransferase), AST (aspartate aminotransferase), Maleicdialdehyde (MDA) and Superoxide dismutase (SOD) were obtained from NanJing JianCheng Bioengineering Institute, Nanjing, China. Coomassie brilliant blue G-250 was obtained from Shanghai Chemical Reagent Company, Shanghai, China. Bovine serum albumin was obtained from Beijing Aoboxing Bio-tech Co., Ltd, Beijing, China.

Animals

Male Kunming mice, weighted 20 ± 2 g, were obtained from the Experimental Animal Center of Henan Province (Zhengzhou, China). Room was maintained in a temperature of $22 \pm 2^{\circ}$ C, humidity of 55–60% and controlled in 12-h light/dark cycle. Animals were housed in plastic cages with a standard commercial rat chow diet and water *ad libitum*. All animal procedures were approved by the ethical committee in accordance to the Institute's Ethical Committee Guidelines for Animal Experimentation and Care (HNPR-2009-05003).

Determination of total phenol (TPC) and flavonoid contents (TFC)

Total phenolic content (TPC) and total flavonoid content (TFC) of TASPE, TASEA and TASBU were determined by the method of Kang et al. (2010). Total phenolic content of *T. acornis* shell was expressed as milligram of catechol equivalents (CE) per gram of dry weight sample. Briefly, appropriate volumes were determined by Folin–Ciocalteu colorimetric method. Total flavonoid contents were measured according to aluminum nitrate colorimetric method. Results are expressed in rutin equivalents (mg RE/g of dry sample). The data are presented as the average of three measurements for different extracts of TAS.

Experimental design and treatment schedule

Mice were randomly divided into nine groups with 10 mice in each one. Group 1 (normal control) was treated with 0.5% CMC-Na (sodium carboxymethylcellulose); group 2 (liver injury model control), 0.5% CMC-Na; groups 3, 4 and 5, 400, 200 and 100 mg/kg of TASEA, respectively; groups 6, 7 and 8, 300, 150, 75 mg/kg of TASBU, respectively; group 9 (positive control), bifendate (70 mg/kg). The duration of treatment was 8 days by intragastric administration. After 2 h at the last administration, except for the group 1, the mice were all given 0.4% CCl₄ at the dose of 100 mL/kg diluted in olive oil by intraperitoneal injection. Group 1 was injected with an equivalent volume of olive oil. Blood was collected from the eyes after fasting for 16 h, and then the mice were sacrificed by cervical dislocation. The liver was removed promptly and weighed. A portion of liver was fixed by immersion in 0.9% NaCl. Blood samples were centrifuged (3000 rpm for 15 min at 4°C) for separating the serum. After that, the serum was stored at -20°C for analysis of ALT, AST.

Preparation of liver homogenates

Hepatic tissue was homogenized in 0.9% NaCl. The tissues were homogenized by homogenizer. The 10 and 1% supernatant was used for the determination of total protein and the activity of the following antioxidant enzymes: the content of MDA and the activity of SOD.

Biochemical analyses

The protein content in homogenates was assayed by the method of Lowry et al. (1951) using bovine plasma albumin as a standard. The levels of ALT, AST, SOD and MDA were measured following the commercial kit's instructions.

Statistical analysis

Statistical analyses were carried out by one-way ANOVA using SPSS 17.0. The overall significance of the results was examined using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. P<0.05 was considered statistically significant. All values were expressed as mean values ± SD.

RESULTS

Total phenolic and total flavonoid contents

TPC of TASPE, TASEA and TASBU varied from 0.07

Sample	Total phenolic content (mg/g) ^a	Total flavonoid content (mg/g) ^b	
TASPE	0.07±0.01 ^a	0.12 ± 0.03^{b}	
TASEA	17.16±0.10 ^a	2.66 ± 0.05^{b}	
TASBU	12.43±0.07 ^a	4.46±0.02 ^b	

Table 1. Total flavonoid and	polyphenol contents.
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^aResults are presented as mean \pm SD (*n*=3) and calculated as catechol equivalents. ^bResults are presented as mean \pm SD (*n*=3) and calculated as rutin equivalents.

Table 2. Effects of TASEA and TASBU on serur	m ALT and AST in liver injury mice.
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Group	Dose (mg/kg)	ALT (U/mL)	AST (U/mL)
Normal control		52.57 ± 9.98***	57.59±13.78***
Liver injury control		931.05 ± 128.25∆∆∆	573.38±138.95∆∆∆
TAGEA	400	400.52 ± 165.21***	259.61±98.60***
TAGEA	200	165.98 ± 70.79***	335.27±60.67**
TASEA	100	327.98 ± 26.93***	193.72±22.59***
TASBU	300	452.26 ± 51.47***	200.59±94.60***
TASBU	150	388.21 ± 94.71***	301.62±75.89***
TASBU	75	357.33 ± 130.45***	128.74±46.08***
Bifendate	70	381.83 ± 28.34***	169.18±78.56***

Data expressed as means \pm s.d (*n*=10). $\triangle P$ was compare with normal group. **P* was compare with Treated group compared with liver injury model control.

to17.16 mg/g (dry *T. acornis* shell) as shown in Table 1. The extracts appear to have the TFC varied from 0.12 to 4.46 mg/g and TPC varied from 0.07 to 17.16 mg/g (dry *T. acornis* shell). TASEA contains a higher polyphenol and TASBU, a higher flavonoids.

Effects of TASEA and TASBU on activity of ALT and AST in serum

Effect of TASEA and TASBU on serum ALT and AST in liver injury mice and the results are shown in Table 2. Compared with normal control group, the levels of ALT and AST in serum were highly increased within 12 h after CCl₄ treatment in the 8 days. The level of ALT and AST in serum of liver injury control group showed significant increase (P<0.001) and indicated that the model of CCl₄induced liver injury mice was established. Intragastric administration of TASEA and TASBU, ALT and AST levels were significantly decreased (P<0.001 and P<0.01, respectively) in liver injury mice. Bifendate (70 mg/kg) was less effective in attenuation of the enzyme activity of ALT and AST than that of TASBU (75 mg/kg). The level of ALT in the group treated with TASEA (200 mg/kg) was similar to normal control. Treatment with TASBU (75 mg/kg) is the most effective method in decreasing the level of AST. It had no dose-dependent inhibitory effects, as shown in Figure 1.

Effects of TASEA and TASBU on liver tissue SOD and MDA in liver injury mice

To test the effect of TASEA and TASBU on oxidation stress caused by intraperitoneal injection CCl_4 , hepatic SOD and MDA were measured, with results shown in Table 3, Figures 2 and 3. The level of SOD in liver injury control group was significantly lower than that of the normal control groups (*P*<0.001). Oral administration of TASEA and TASBU at dose of TASEA (400 and 100 mg/kg, respectively) and TASBU (300, 150 and 75 mg/kg, respectively) for 8 days can significantly reduce the level of SOD (*P*<0.05, *P*<0.01, *P*<0.001, respectively) compared to the liver injury control group. The level of MDA was reduced significantly in TASEA and TASBU (*P*<0.001) compared to liver injury control group.

DISCUSSION

ROS is generated from both endogenous and exogenous sources and may be involved in the aetiologies of several human diseases (Skandrani et al., 2010). In our previous studies, the extracts of TAS had good antioxidant activity (Kang et al., 2011). Phenolic compounds, biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. Dietary



Figure 1. Effects of TASEA and TASBU on serum ALT and AST in liver injury mice.



Figure 2. Effects of TASEA and TASBU on liver tissue MDA in acute liver injury mice.

polyphenol intakes from fruits and vegetables are known to reduce the risk of coronary heart disease and cancer (Reddy et al., 2010). Flavonoids have many biological activities such as the inhibition of plasma platelet aggregation and cyclooxygenase activity, the suppression of histamine release and SRS-A biosynthesis *in vitro*, potent nitric oxide radical scavenging activity and exhibiting antibacterial, antiviral, anti-inflammatory and antiallergenic effects (Tounsi et al., 2009). TAS was commonly discarded as fertilizer in China. Bravo had pointed out that polyphenolic compounds usually accumulate in the outer parts of plants such as shells and skins (Bravo 1998). Our research demonstrated that antioxidant activity was consistent with an increase of total phenolics. Some researchers have reported that fractions of water caltrop pericarps contained total phenolic content from 3.8 to 42.1 g GAE/ 100 g d.b, and total flavonoids from 9.2 to 47.7 g QE/100 g d.b. (Chiang and Ciou, 2010). Some studies have reported correlations between polyphenols and antioxidant activity measured by various methods (Awika et al., 2003; Rop et al., 2010) and have demonstrated that high degree correlation of total polyphenol content is in accordance to an increase in antioxidant activity. It shows that the assay for total phenolic content would be a useful technique for rapid evaluation of antioxidant activity in this plant (Velioglu et al., 1998). According



Figure 3. Effects of TASEA and TASBU on liver tissue SOD in acute liver injury mice.

to the results, the high total phenolic and total flavonoid of TASEA and TASBU suggested their potential uses as a potential source of antioxidants in food applications. Hepatoprotective studies showed that plants have active ingredients that are capable of free radical scavenging in living systems (Mitra et al., 1998). In this study, the antioxidant activity of TAS was assayed by estimation of MDA and SOD levels in vivo. Results showed that the level of MDA was decreased significantly significantly (P<0.001) and the level of SOD was increased significantly (P<0.001, P<0.01 and P<0.05, respectively). Reducing the level of MDA and increasing the level of SOD to decreased lipid peroxidation and/or decreased utilization enhance antioxidant capability and protect the body to further oxidative damage from free radicals (Gong et al., 2012).

CCl₄-induced liver injury is an experimental model widely used for hepatoprotective drug screening. Increase of free radical production has been implicated in of pathogenesis the acute hepatic disorders (Chandrashekhar et al., 2010). The metabolism of CCl₄ by cytochrome P450 leads to the formation of free radicals, inducing lipid peroxidation and impairing antioxidant status (Ye et al., 2009). Results showed that the levels the levels of ALT and AST were significantly decreased (P<0.001 and P<0.01, respectively) in administrating each dose group of TASEA and TASBU compared to liver injury control. Our results suggested that the high content of phenolic and flavonoids compounds may be responsible for hepatoprotective effect.

In conclusion, results indicated that TAS has a remarkable protective effect against CCl₄-induced liver injury in mice and its mechanism is related, at least in part, to its free radical scavenging and antioxidant activity on account of containing high polyphenols and flavonoids. The present study indicated that TASEA and TASBU possess potential hepatoprotective activity

against CCl₄-induced acute liver injury. Hence, they may be used in treating acute liver injury. Further work is necessary to isolate active ingredients and elucidate the actual mechanism involved in the hepatoprotective activity of this plant.

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REFERENCES

- Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L (2003). Screening methods to measure antioxidant activity of *Sorghum* (*Sorghum bicolor*) and *Sorghum* products. JAFC 51:6657-6662.
- Abdelly C (2009). Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. Food Chem. Toxicol. 47:2083-2091.
- Bravo L (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56:317-333.
- Clawson GA (1989). Mechanisms of carbon tetrachloride hepatotoxicity. Pathol. Immunopathol. Res. 8:104-112.
- Chiang PY, Ciou JY (2010). Effect of pulverization on the antioxidant activity of water caltrop (*Trapa taiwanensis* Nakai) pericarps. LWT-Food Sci. Technol. 43:361-365.
- Chandrashekhar VM, Muchandi AA, Sudi SV, Ganapty S (2010). Hepatoprotective activity of *Stereospermum suaveolens* against CCl₄-induced liver damage in albino rats. Pharm. Biol. 48:524-528.
- Gong F, Yin ZH, Xu QT, Kang WY (2012). Hepatoprotective effect of *Mitragyna rotundifolia* Kuntze on CCl₄-induced acute liver injury in mice. Afr. J. Pharm. Pharmcol. 6:330-335.
- Gordon MC, David JN (2001). Natural product drug discovery in the next millennium. J. Pharm. Biol. 39:8-17.
- Institute of Botany, Chinese Academy of Sciences (2004). Iconographia *Cormophytorum Sinicorum* (Tomus I). Science Press, Beijing. pp. 52-67.
- Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K,

Bakrouf A, Magné C, Komes D, Horź ić D, Belščak A, Ganić KK, Vulić I (2010). Green tea preparation and its influence on the content of bioactive compounds. Food Res. Int. 43:167-176.

- Kang WY, Li CF, Liu YX (2010). Antioxidant phenolic compounds and flavonoids of *Mitragyna rotundifolia* (Roxb.) Kuntze *in vitro*. Med. Chem. Res. 19:1222-1232.
- Kang WY, Li YY, Gu XZ, Xu QT, Huang X (2011). Antioxidant activities, a-glucosidase inhibitory effect *in vitro* and antihyperglycemic of *Trapa acornis* shell in alloxan-induced diabetic rats. J. Med. Plants Res. 5:6805-6812.
- Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S (1998). Effect of HD-03, a herbal formulation, on the antioxidant defence system in rats. Phytother. Res. 12:114-117.
- MIcek J, Rop O (2011). Fresh edible flowers of ornamental plants A new source of nutraceutical foods. Trends Food Sci. Technol. 22:561-569.
- Niu FL, Liu GL, Dong WY, Cheng K, Gui JT, Qu Y. Li CS (2003). Flavonoid compounds preliminary separation of the water chestnut. Food Sci. 6:91-93.
- Prasad KN, Yang E, Yi C, Zhao M, Jiang Y (2009). Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. Innov Food Sci. Emerg. 10:155-159.
- Parekh J, Chanda S (2007). *In vitro* antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. Afr. J. Biotechnol. 6:766-770.
- Recknagel RO, Glende EA Jr, Dolak JA, Waller RL (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacol. Ther. 43:139-154.
- Rop O, Mlcek J, Jurikova T, Valsikova M, Sochor J, Reznicek V, Kramarova D (2010). Phenolic content, antioxidant capacity, radical oxygen species scavenging and lipid peroxidation inhibiting activities of extracts of five black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) cultivars. J. Med. Plants Res. 4:2431–2437.
- Reddy VKC, Sreeramulu D, Raghunath M (2010). Antioxidant activity of fresh and dry fruits commonly consumed in India. Food Res. Int. 43:285-288.

- Szymonik-Lesiuk S, Czechowska G, Stryjecka Zimmer M, Slomka M, Madro A, Celinski K, Wielosz M (2003). Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. J. Hepatobiliary Pancreatic Surg. 10:309-315.
- Shang QK, Xuan YS, Zhu DX (2007). Separation and preparation of alkaloid derived from the hull of water chestnut by high performance preparative liquid chromatography. J. Northeast Normal Univ (Nat. Sci. Edit). 2: 82-86.
- Skandrani I, Limem I, Neffati A, Boubaker J, Ben SM, Bhouri W, Bouhlel I, Kilani S, Ghedira K, ChekirGL (2010). Assessment of phenolic content, free-radical-scavenging capacity genotoxic and antigenotoxic effect of aqueous extract prepared from *Moricandia arvensis* leaves. Food Chem. Toxicol. 48:710-715.
- Sabir SM, Ahmad SD, Hamid A, Khan MQ, Athayde ML, Santos DB, Boligon AA, Rocha JBT (2012). Antioxidant and hepatoprotective activity of ethanolic extract of leaves of *Solidago microglossa* containing polyphenolic compounds. Food Chem. 131:741-747.
- Tounsi MS, Ouerghemmi I, Wannesa WA, Ksouri R, Zemni H, Marzouka B, Kchouka ME (2009). Valorization of three varieties of grape. Ind. Crops Prod. 30:292-296.
- Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. JAFC. 46: 4113-4117.
- Wang SH, Kao MY, Wu SC, Lo DY, Wu JY, Chang JC, Chiou RYY (2011). Oral Administration of *Trapa taiwanensis* Nakai Fruit Skin Extracts Conferring Hepatoprotection from CCI4-Caused Injury. J. Agric. Food Chem. 59:3686-3692.
- Xie YR (2011). Trapa Extract Induced Apoptosis in Liver Cancer Cell Line SMMC-7721. J. Clin. Oncol. 1:34-37.
- Ye X, Feng Y, Tong Y, Ng KM, Tsao S, Lau GK, Sze C, Zhang Y, Tang J, Shen J, Kobayashi S (2009). Hepatoprotective effects of *Coptidis rhizoma* aqueous extract on carbon tetrachloride-induced acute liver hepatotoxicity in rats. J. Ethnopharmacol. 124:130-136.