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Protective effect of *Glycyrrhiza glabra* polysaccharides against carbon tetrachloride-induced liver injury in rats

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The objective of the present study was to investigate potential of *Glycyrrhiza glabra* polysaccharides to offer protection against acute liver injury in rats. Rats were administered a single oral dose carbon tetrachloride (CCl_4, 640 mg/kg b.w., 1:1 in groundnut oil) and sacrificed 7 days of post-treatment. Hepatic damage was assessed by employing biochemical parameters. Our results demonstrated that treatment of rats with *G. glabra* polysaccharides significantly prevented the increased activities of and aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP and LDH in serum. *G. glabra* polysaccharides treatment also restored CCl_4-induced altered caspase-3, TGF- 1 and TGF- 1 mRNA. Our findings provide evidences to demonstrate that *G. glabra* polysaccharides treatment significantly offsets CCl_4-induced liver injury in rats.

Key words: *Glycyrrhiza glabra* polysaccharides, rat, TGF- 1 mRNA, aspartate aminotransferase (AST), carbon tetrachloride.

INTRODUCTION

Licorice (*Glycyrrhiza glabra* L.; Family: Papilionaceae/ Fabaceae) is a traditional medicinal herb grows in the various parts of the world. It is a very sweet, moist, soothing herb. *G. glabra* is a hardy herb or under shrub, erect grows to about 2 m height. The roots are long, cylindrical, thick and multi-branched "(Hayashi et al, 1996).

Many biological activities such as antimuta-genic activity, anti-ulcer effects, protective action against hepatotoxicity, antitumor promoting activity, antimicrobial effects (Khattak and Simpson, 2010; Dhingra and Sharma, 2006; Yoon et al, 2009; Kaur and Arora, 2009), etc., were reported. Polysaccharides from *G. glabra* have been investigated in some studies.

Liver is the central organ for the detoxification and excretion of many xenobiotics including drugs. Drug excretion may be affected by liver dysfunction, leading to an alteration in the pharmacokinetics (Roche and Samuel, 2008). Investigation of the relationship between excretion and liver dysfunction is important for predicting the pharmacokinetics in patients with liver dysfunction to avoid drug adverse reactions.

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Carbon tetrachloride (CCl_4)-mediated liver injury is a well-established model for studying liver regeneration in rodents. It is mainly characterized by acute hepatocellular necrosis caused by alterations in permeability of cellular, mitochondrial and lysosomal membranes and it has been shown to induce hepatocyte apoptosis (Lee et al., 2008). CCl_4 injury activates many growth factors, such as tumor necrosis factor alpha (TNF-), transforming growth factor beta (TGF-) and hepatocyte growth factor (HGF) (Toshihiro et al., 1987).

The purpose of this study was to investigate the effect of *G. glabra* polysaccharides on the CCl_4-induced liver dysfunction in rats.

MATERIALS AND METHODS

Materials and equipments

Glycyrrhiza glabra was purchased from local herbs market and then ground to pass through 1 mm screen and stored overnight at 4°C in refrigerator.

Preparation of *G. glabra* polysaccharides

The procedure for *G. glabra* polysaccharide extracts was carried out consulting the scientific literature on this subject (Cai et al., 2008).

Table 1. Effect of *G. glabra* polysaccharides on ALT, AST, ALP and LDH level.

Group	ALT	AST	ALP	LDH
Normal control	35.17 ± 1.06	156.22 ± 11.25	185.31 ± 10.47	1704.53 ± 18.42
Untreated model control	240.51 ±11.43	409.66 ± 21.52	672.09 ± 27.17	2174.97 ± 87.45
Polysac.-treatment (200 mg/kg bw)	189.46 ±10.57	278.41 ± 18.59	462.36 ± 25.01	1875.32 ± 67.39
Polysac.-treatment (300 mg/kg bw)	106.37 ± 8.91	169.24 ± 10.52	231.54 ± 13.67	1732.84 ± 70.38

Around 15 g of those powder materials with distilled water were put in a vessel, heated and kept boiling for some time and stirred regularly. Then the extraction (liquid fraction) and the residue were collected separately, the residue was also re-extracted twice more. The extracted liquid fraction was collected and enriched, extracted with 90% ethanol for 12 h.

The precipitate was collected by centrifugation (3000 r/min, 20 min) and respectively, washed twice with acetone and tetrachloromethcme to remove lipid and then vacuum-dried at 40°C to obtain the desired polysaccharides. The crude polysaccharides was dissolved in distilled water again and then clarified by centrifugation (10,000 g, 10 min) and filtration and dialyzed against 0.01 M sodium acetate buffer (pH 5.0) at 4°C until the dialyzate was free of sugars. The dialyzed malt extract was then filtered to remove any precipitated material.

CCl_4-induced liver dysfunction in rat and medicine treatment

The present study was approved by the institutional animal care and use committee of our University. Male Wistar rats were intra-peritoneally injected with CCl_4 at a dose of 640 mg/kg (a volume of 2 ml/kg) or corn oil once every 2 days for 7 days. The degree of liver dysfunction was assessed by the serum levels of AST and ALT measured.

CCl_4-treated rats (n = 24) were randomly divided into 3 groups: untreated model control, 2 polysaccharides -treated groups. The untreated model group received a same volume of vehicle. Polysaccharides-treated groups received the same diet supplemented with lyophilized aqueous extract of *G. glabra* polysaccharides (200 or 300 mg/kg B.W.), for 4 weeks. Another 8 rats served as normal control, which received a same volume of vehicle.

Blood samples were collected for the assays of LDH and ALP etc. The livers were excised from the animals and assayed for biochemical indexes.

Biochemical assay

Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured with an autoanalyzer (Hitachi 7020 automatic analyzer, Hitachi Co., Tokyo, Japan) and expressed in IU/l.

Serum biochemical parameters (LDH and ALP) were assayed by the method of Reitman and Frankel (1957) using commercially available kits, product of Randox Laboratories Co. (Antrum, UK).

Total protein activity was measured by the method of Kano and Goto (2003). Levels of plasma albumin were assayed using clinical test kits (Roche Diagnostics; Germany) on a spectrophotometric analyzer (Cobas Mira Plus, Rotkreuz, Switzerland). Globin level was measured by the method of Yu et al. (1994).

Immunohistochemical assessments

The immunohistochemical procedure was followed as described previously (Oomman et al., 2005) . Sections were immunohisto-chemically stained for the presence of cleaved caspase-3 and TGF-1. The primary antibodies were subsequently recognized using a fluorescently tagged Alexa 488 or 594 secondary antibodies. For dual labeling, slices were exposed to both primary antibodies simultaneously followed by both secondary antibodies using the standard protocol. Negative controls (omission of primary antibody) consistently were included in each experiment in order to subtract background autofluorescence.

Reverse transcription and polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted by lysing cells with guanidium thiocyanate solution, pelleted by CsCl cushion centrifugation and purified by phenol/chloroform extraction. 2 µg of RNA was reverse-transcribed using oligo (dT) primer and the super Script TM preamplification system (GIBCOL BRL, Gaithersburg, MD, USA) in a final volume of 20 l at 42°C for 1 h. PCR reactions were conducted in a volume of 20 l containing 2 l of reverse-transcribed cDNA, 0.1 M forward and reverse primers each, 1 × PCR buffer (10 mM Tris HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% (v/v) Triton X-100), 0.5 mM dNTPs, 0.2 U/ l DyNAzyme TM II DNA polymerase (Finnzymes, Finland) and distilled H_2O. The primers used were designed based on references. The cycling conditions were 95°C for 2 min, followed by 35 cycles of 95°C for 15 s, 52°C for 20 s and 72°C for 1 min. PCR products were separated by electrophoresis with a 1.2% agarose gel. The PCR reactions were repeated twice.

RESULTS AND DISCUSSION

Effect of *G. glabra* polysaccharides on ALT, AST, ALP and LDH level

Alanine transaminase (ALT), also called serum glutamic pyruvate transaminase (SGPT) or alanine aminotrans-ferase (ALAT) is an enzyme present in hepatocytes (liver cells). When a cell is damaged, it leaks this enzyme into the blood, where it is measured (Bedogni et al., 2003; Yoksan and Akashi, 2009). ALT rises dramatically in acute liver damage, such as viral hepatitis or paracetamol (acetaminophen) overdose. Elevations are often measured in multiples of the upper limit of normal (ULN). Aspartate transaminase (AST) also called serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal muscle and is therefore not specific to the liver (Makarov et al., 1980; Marounek et al., 2010).

Table 2. Effect of *Glycyrrhiza glabra* polysaccharides on TP, GLB and ALB level.

Group	TP	GLB	ALB
Normal control	94.37 ± 6.21	62.27 ± 2.16	42.37 ± 1.68
Untreated model control	70.12 ±3.05	69.65 ± 2.98	38.67 ± 1.54
Polysac.-treatment (200 mg/kg bw)	70.07 ± 4.06	63.57 ± 4.09	37.84 ± 1.44
Polysac.-treatment (300 mg/kg bw)	68.94 ± 7.37	64.76 ± 6.83	36.94 ± 1.53

Table 3. Effect of *Glycyrrhiza glabra* polysaccharides on caspase-3, TGF- 1 and TGF- 1 mRNA.

Group	Caspase-3	TGF- 1	TGF- 1 mRNA
Normal control	3.67 ± 0.23	1.85±0.22	0.34±0.02
Untreated model control	1.37 ±0.11	10.64±0.64	1.08±0.09
Polysac.-treatment (200 mg/kg bw)	3.01±0.21	6.47±0.29	0.67±0.04
Polysac.-treatment (300 mg/kg bw)	4.34±0.26	3.01±0.13	0.31±0.04

A significant increase in ALT, AST, ALP and LDH levels was observed in untreated model control rats vs normal treatment lowered significantly plasma ALT, AST, ALP and control rats (Table 1). *G. glabra* polysaccharides LDH levels when compared with untreated model control group. This activity increased with the concentration of the *G. glabra* polysaccharides extract.

Effect of *G. glabra* polysaccharides on TP, GLB and ALB level

The liver produces most of the plasma proteins in the body making a measure of the amount of protein in the blood useful. Globulin is one of the 2 types of serum proteins, the other being albumin. Some globulins are produced in the liver, while others are made by the immune system. The term globulin encompasses a heterogeneous group of proteins with typical high molecular weight, and both solubility and electrophoretic migration rates lower than for albumin (Ishii et al., 2001; Tian et al., 2010). Albumin is a protein made specifically by the liver, and can be measured cheaply and easily. It is the main constituent of total protein; the remaining fraction is called globulin (including e.g. the immune-globulins) (Di Stefano et al, 2002; Kubiak et al, 2009).

Compared with the normal control, the plasma TP, and ALB levels were significantly ($p<0.05$) reduced in untreated model control rats, whereas (globulin) GLB level was significantly ($p<0.05$) increased. However, plasma TP, GLB and albumin ALB level remained unchanged between the polysaccharides-treated groups and untreated model control one (Table 2).

Effect of *G. glabra* polysaccharides on caspase-3, TGF- 1 and TGF- 1 mRNA

Among the identified caspases, caspase-3 (also called cpp32, Yama, or apopain) is a potent e.ector of apoptosis in a variety of cell types and promotes neuronal death during brain development and ischemic brain injury (Hao et al., 2008). Although the extent and the prolongation of caspase-3 gene over expression were distinct (Hayami et al., 2000), it was seen in both vulnerable neurons such as CA1 pyramidal cells (greater and prolonged) and less vulnerable neurons such as dentate granule cells (modest and transient).

Although the mechanisms underlying the progression of liver cirrhosis have yet to be fully elucidated, cytokines have been implicated as mediators of fibrosis in the liver. Among these cytokines, transforming growth factor- 1 (TGF- 1) is particularly well-studied and has been recognized as pro-fibrogenic in the case of liver injury (Ueberham et al., 2004; Chen et al., 2009). TGF- 1 is involved in the accumulation of ECMs for normal repair as a response to tissue injury, and is also responsible for fibrous changes due to aberrant overproduction of ECMs, including proteoglycans, collagens, fibronectin, and glycoproteins.

Compared with the normal control, the liver TGF- 1 and TGF - 1 mRNA were significantly ($p < 0.01$) enhanced in untreated model control rats, whereas liver caspase-3 was significantly reduced. *G. glabra* polysaccharides treatment lowered significantly liver TGF- 1 and TGF- 1 mRNA and increased caspase-3 when compared with untreated model control group. This activity increased with the concentration of the *G. glabra* polysaccharides extract (Table 3).

REFERENCES

Bedogni G, Miglioli L, Battistini N, Masutti F, Tiribelli C, Bellentani S (2003). Body mass index is a good predictor of an elevated alanine transaminase level in the general population: hints from the Dionysos study. Digest. Liver Dis., 35: 648-652.

Cai WR, Gu XH, Tang J (2008). Extraction, purification, and characterization of the polysaccharides from Opuntia milpa alta.

Carbohyd. Polym., 71: 403-410.
Chen X, Zhong HY, Zhu H, Zeng JH, Dai P (2009). Effect of Curcuma kwangsiensis polysaccharides on blood lipid profiles and oxidative stress in high-fat rats. Int. J. Biol. Macromol., 44: 138-142.
Dhingra D, Sharma A. (2006). Antidepressant-like activity of Glycyrrhiza glabra L. in mouse models of immobility tests. Progr. Neuro-Psychopharmacol. Biol. Psychiatr., 30: 449-454.
Di Stefano G, Busi C, Fiume L (2002). Floxuridine coupling with lactosaminated human albumin to increase drug efficacy on liver micrometastases. Digest. Liver Dis., 34: 439-446.
Hao ZM, Li XH, Qiao TD, Fan DM (2008). Successful expression and purification of human CIAPIN1 in baculovirus-insect cell system and application of this system to investigation of its potential methyltransferase activity. Int. J. Biol. Macromol., 42: 27-32.
Hayami S, Yaita M, Ogiri Y, Sun F, Nakata R, Kojo S (2000). Change in caspase-3-like protease in the liver and plasma during rat liver regeneration following partial hepatectomy. Biochem. Pharmacol., 60: 1883-1886.
Hayashi H, Hiraoka N, Ikeshiro Y, Yamamoto H (1996). Organ specific localization of flavonoids in *Glycyrrhiza glabra* L. Plant Sci., 116: 233-238.
Ishii M, Xu BQ, Ding LR, Fischer NE, Inaba T (2001). Interaction of plasma proteins with cytochromes P450 mediated metabolic reactions: inhibition by human serum albumin and -globulins of the debrisoquine 4-hydroxylation (CYP2D) in liver microsomes of human, hamster and rat. Toxicol. Lett., 119: 219-225.
Kano Y, Goto H (2003). Relationship between the occurrence of bitter fruit in cucumber (Cucumis sativus L.) and the contents of total nitrogen, amino acid nitrogen, protein and HMG-CoA reductase activity. Scientia Hortic., 98: 1-8.
Kaur R, Arora S (2009). Chemical constituents and biological activities of Chukrasia tabularis A. Juss. - A review. J. Med. Plants Res., 3(4): 196-216.
Khattak KF, Simpson TJ (2010). Effect of gamma irradiation on the antimicrobial and free radical scavenging activities of Glycyrrhiza glabra root. Radiat. Phys. Chem., 79: 507-512.
Kubiak A, Jakimowicz P, Polanowski A (2009). A Kazal-type serine proteinase inhibitor from chicken liver (clTI-1): Purification, primary structure, and inhibitory properties. Int. J. Biol. Macromol., 45: 194-199.
Lee HS, Kim HH, Ku SK (2008). Hepatoprotective effects of Artemisiae Capillaris Herba and Picrorrhiza Rhizoma combinations on carbon tetrachloride–induced subacute liver damage in rats. Nutr. Res., 28: 270-277.
Makarov VL, Kochkina VM, Torchinsky YM (1980). Polarized light absorption spectra of single crystals of aspartate transaminase from chicken heart cytosol. FEBS Lett., 114: 79-82.
Marounek M, Volek Z, Sk ivanová E, T ma J (2010). Effects of amidated pectin alone and combined with cholestyramine on cholesterol homeostasis in rats fed a cholesterol-containing diet. Carbohyd. Polym., 80: 989-992.
Oomman S, Strahlendorf H, Finckbone V, Strahlendorf J (2005). Non-lethal active caspase-3 expression in Bergmann glia of postnatal rat cerebellum. Dev. Brain Res., 160: 130-145.
Roche B, Samuel D (2008). Liver transplantation in viral hepatitis: Prevention of recurrence. Best Pract. Res. Clin. Gastroenterol., 22: 1153-1169.
Tian M, Chen F, Ren DW, Yu XX, Zhang XH, Zhong R, Wan CX (2010). Preparation of a series of chitooligomers and their effect on hepatocytes. Carbohyd. Polym., 79: 137-144.
Toshihiro K, Tatsuya H, Haruo K, Susumu I, Tsunemi S, Keiki O (1987). Alteration of chloral hydrate metabolism in rats with carbon tetrachloride-induced liver damage. Toxicol Lett., 37: 263-268.
Ueberham E, Arendt E, Starke M, Bittner R, Gebhardt R (2004). Reduction and expansion of the glutamine synthetase expressing zone in livers from tetracycline controlled TGF- 1 transgenic mice and multiple starved mice. J. Hepatol., 41: 75-81.
Yoksan R, Akashi M (2009). Low molecular weight chitosan-g-l-phenylalanine: Preparation, characterization, and complex formation with DNA. Carbohyd. Polym., 75: 95-103.
Yoon WJ, Ham YM, Kim KN, Park SY, Lee NH, Hyun CG, Lee WJ (2009). Anti-inflammatory activity of brown alga Dictyota dichotoma in murine macrophage RAW 264.7 cells. J. Med. Plants Res., 3(1): 1-8.
Yu JH, Bock JH, Slightom JL, Villeponteau B (1994). A 5 -globin matrix-attachment region and the polyoma enhancer together confer position-independent transcription. Gene, 139: 139-145.