Full Length Research Paper

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

Liang Renjie*, Shi Shidi and Sun Changsen

School of Life Science, Taizhou University, Zhejiang, 317000, P. R. China.

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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 (CCl₄, 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 aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP and LDH in serum. *G. glabra* polysaccharides treatment also restored CCl₄-induced altered caspase-3, TGF-β1 and TGF-β1 mRNA. Our findings provide evidences to demonstrate that *G. glabra* polysaccharides treatment significantly offsets CCl₄-induced liver injury in rats.

Key words: *Glycyrrhiza glabra* polysaccharides, rat, TGF-β1 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 affects (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.

Carbon tetrachloride (CCl₄)-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). CCl₄ injury activates many growth factors, such as tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) 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 CCl₄-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).
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 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\textsubscript{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 intraperitoneally injected with CCL\textsubscript{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\textsubscript{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 \textit{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 (Oommen et al., 2005). Sections were immunohistochemically stained for the presence of cleaved caspase-3 and TGF-\(\beta\)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 \(\mu\)g of RNA was reverse-transcribed using oligo (dT) primer and the super Script TM preamplification system (GIBCOBRL, Gaithersburg, MD, USA) in a final volume of 20 \(\mu\)l at 42°C for 1 h. PCR reactions were conducted in a volume of 20 \(\mu\)l containing 2 \(\mu\)l of reverse-transcribed cDNA, 0.1 \(\mu\)M forward and reverse primers each, 1 \(\times\) 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/\(\mu\)l DyNAzyme TM II DNA polymerase (Finzymes, Finland) and distilled H\(_2\)O. The primers 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 \textit{G. glabra} polysaccharides on ALT, AST, ALP and LDH level

Alanine transaminase (ALT), also called serum glutamic pyruvate transaminase (SGPT) or alanine aminotransferase (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 1. Effect of \textit{G. glabra} polysaccharides on ALT, AST, ALP and LDH level.

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

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 tetrachloromethane to remove lipid and then vacuum-dried at 40°C (20 min) and respectively, washed twice with acetone and 90% ethanol for 12 h.
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
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 liver TGF-β1
and TGF-β1 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-β1 and TGF-β1 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).

Effect of G. glabra polysaccharides on caspase-3,
TGF-β1 and TGF-β1 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-β1
(TGF-β1) 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-β1 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.

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