Polyphenols from *Taxus chinensis* var. *mairei* prevent the development of CCl4-induced liver fibrosis in rats

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**Introduction**

Liver fibrosis, a common consequence of chronic liver injury, is characterized by an excessive deposition of extracellular matrix (ECM), resulting in liver dysfunction and irreversible cirrhosis. The prospect that hepatic fibrosis is reversible has generated great interest for researchers to develop anti-fibrotic therapies (Povero et al., 2010). Because activated hepatic stellate cells (HSCs) play a critical role in the development of liver fibrosis, inhibition of HSCs activation and proliferation along with induction of HSCs apoptosis would be reasonable methods in the prevention or treatment of hepatic fibrosis (Son et al., 2009; Wang et al., 2011).

The activation of HSCs, marked by the expression of α-smooth muscle actin (α-SMA), involves the loss of lipid droplets, the remodeling of ECM and the production of pro-fibrotic cytokines, which stimulate quiescent HSCs in an autocrine and paracrine manner (Brenner, 2009). The balance between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) plays an important role in ECM degradation (Di Sario et al., 2004). In addition, the fibrotic growth factors in liver fibrosis, such as...
Several studies have demonstrated the hepatoprotective effects of (Fig. 2), amounting to respectively. The purity of the total TPs was assessed using HPLC 13.96%, 10.82%, 6.70%, 14.47%, 22.81%, 16.64%, 6.13%, 1.52%, nol-14-polyprenol-24, which accounting for 0.22%, 1.06%, 5.65%, 2.1. Drugs 2. Materials and methods 2.2. Treatment of animals

All procedures concerning animal treatment and experimentation were in accordance with the guiding principles in the care and use of animals and approved by the Animal Care and Use Committee of the Zhejiang Academy of Medical Sciences. Male and female Sprague-Dawley (SD) rats (200 ± 20 g) were provided from the Hangzhou Experimental Animal Center (Hangzhou, China). The animals were maintained under the standard conditions of the facility with a 12 h light/dark cycle at a temperature of 21 ± 3 °C and free access to food and water. After a week of acclimatization to the standard conditions, the animals were randomly divided into six (n=10–12 rats per group) experimental groups: (1) normal control with vehicles only (olive oil), (2) rat model given CCl4 only; (3) CCl4+low TPs (48 mg/kg); (4) CCl4+medium TPs (120 mg/kg); (5) CCl4+high TPs (300 mg/kg); and (6) CCl4+PP (120 mg/kg). The rat model of liver fibrosis was induced by subcutaneous injection of 40% (v/v) of CCl4 diluted in olive oil (3 mL/kg body weight) twice per week for 8 weeks as previously described (Lan et al., 2008). The dose of TPs was selected based on previous reports (Wang, 2007). All animals in the drug-treated groups were intragastrically (i.g.) administered their respective drug daily simultaneously with the CCl4 injection for 8 weeks. Animals were anesthetized with diethylether 24 h after the final treatment. Blood was collected from the celiac artery to determine the serum biochemical parameters. Liver tissue was rapidly dissected: the left lobe was fixed in 4% phosphate-buffered formaldehyde for histological analysis while the rest was snap frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.3. Serum biochemical analyses

Serum was obtained by centrifugation of blood at 3000 rpm for 15 min at 4 °C and used for the measurement of the activities of AST, ALT and ALP as well as the content of ALB by automated analyzer (Mindray, China).

2.4. Histopathologic examination

The paraformaldehyde-fixed liver tissue was embedded in paraffin and sectioned serially at a thickness of 5 μm. Pathological analysis was evaluated by hematoxylin eosin (HE) staining. Histological changes were evaluated in ten non-consecutive, randomly chosen histological fields at 200 × magnification. The criteria used for scoring fibrosis severity were listed as follows (Li et al., 2003): score 0, no fibrosis present; score 1, fibrosis present; score 2, mild fibrosis; score 3, moderate fibrosis; and score 4, severe fibrosis. Collagen accumulation was detected by Sirius red staining and quantified with Image-Pro Plus 6.0 software by analyzing ten non-consecutive and randomly histological fields at 100 × magnification per slide and calculating the ratio of connective tissue to the total liver area.

2.5. Immunohistochemical staining

Immunohistochemical studies were performed on paraffin-embedded liver tissue sections of 5 μm thickness, which were deparaffinized, rehydrated and heated in 0.01 M citric acid buffer (pH=6.0) for 20 min to restore antigen. The sections were then treated with 3% hydrogen peroxide in methanol for 10 min at room temperature to block endogenous peroxidase activity, and then washed three times with phosphate-buffered saline (0.01 M, pH=7.5) for 5 min. For immunohistochemical staining, the tissue sections were incubated overnight with anti α-SMA antibody (1:50, Abcam, Great Britain) at 4 °C followed by secondary
antibody for 30 min at room temperature. Negative control slides were treated with PBS only. After washing three times with PBS, the immunostaining was visualized with an EnVision System, Peroxidase/DAB kit according to the manufacturer’s instructions (Gene Tech Co., Ltd., China). After color activation was terminated by washing with water, the sections were counterstained with hematoxylin and finally mounted with a neutral resin. The stained slides were analyzed by Image-Pro Plus 6.0 software with ten non-consecutive, randomly chosen histological fields at 200× magnification. The volume fraction of each structure was calculated as the percentage of the number of points overlaying each structure in relation to the total number of counted points (Trebicka et al., 2010).

2.6. The hepatic lipid peroxidation (LPO) assay

MDA, the end product of lipid peroxidation, was analyzed using a spectrophotometric measurement of the level of thiobarbituric acidreactive substances (TBARS) at 535 nm by commercial assay kits according to the manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, China). Absorption was measured at 550 nm. Hydroxyproline concentration was determined using a standard curve for hydroxyproline and results were expressed as μg/g of wet liver tissue.

2.7. The hepatic antioxidant system assay

The level of GSH and the activities of SOD and GSH-Px were measured using commercial assay kits according to the manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, China), and were expressed as mgGSH/g protein, U/mg protein and U/g protein, respectively.

2.8. Hydroxyproline content of liver tissue

Hepatic hydroxyproline in liver hydrolysates was measured photometrically by commercial assay kits according to manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, China). Absorption was measured at 550 nm. Hydroxyproline concentration was determined using a standard curve for hydroxyproline and results were expressed as μg/g of wet liver tissue.

2.9. Isolation of total liver RNA and quantitative real-time polymerase chain reaction (qRT-PCR) for mRNA analysis

The levels of mRNA expression of α-SMA, Col α1(I), Col α1(III), MMP-2, TIMP-1, TIMP-2, PDGF-β, TGF-β1, CTGF, HGF and TNF-α were determined by qRT-PCR assays. Total RNA was extracted from liver tissues using Trizol according to the manufacturer’s protocol (Invitrogen Corp., USA). RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. The cDNA was amplified from 300 ng of total RNA using a HI-FI-MMLV Reverse Transcriptase (CoWin Biotech Co., Ltd, China). qRT-PCR was performed on the ABI StepOne/StepOnePlus (Applied Biosystems, USA) in a final volume of 15 μL containing
3.1. TPs attenuated CCl4-induced liver injury

A significant increase in the analyzed biochemical parameters (AST, ALP and ALT) and decrease in the ALB levels were observed in the CCl4-induced rats as compared to the normal group \((p < 0.01)\) (Table 2). Rats treated with both TPs and CCl4 showed a remarkable reduction of serum ALT, AST and ALP levels and a restoration of ALB levels compared to the model group \((p < 0.05\) or \(< 0.01)\).

The architecture of fibrotic livers with or without TPs treatment was determined by HE staining (Fig. 3). Compared to the normal group (Fig. 3A), chronic CCl4 exposure damaged normal liver structure and hepatocytes. Macrovesicular and microvesicular steatosis occurred, and collagen fibers presented with pseudo-lobe formations in different sizes (Fig. 3B). Several individual rats were in the early stages of cirrhosis and had severe collagen fibers with thick compartments and more pseudo-lobe formations. Compared to the model group, groups treated with TPs significantly attenuated the symptoms caused by CCl4, including less fatty degeneration and markedly reduced collagen deposition and pseudo-lobe formation \((p < 0.05\) or \(< 0.01)\) (Fig. 3D–F and Table 3). The severity of hepatic fibrosis was scored as previously described (Li et al., 2003). The medium dose of TPs (120 mg/kg) showed the most pronounced anti-fibrotic effect (Table 3).

3.2. TPs suppressed CCl4-induced oxidative stress

The lipid peroxidation was examined by determining the MDA content in frozen liver tissue homogenate. The MDA content in the CCl4 group was 4.8-fold higher than that in the normal group \((p < 0.01)\) (Table 4). Compared to the CCl4 group, the MDA levels of TPs-treated groups (48, 120 and 300 mg/kg) were significantly decreased by 58.3%, 68.8% and 66.7%, respectively \((p < 0.01)\) (Table 4).

To evaluate the antioxidant capacity of the liver tissue, the activities of superoxide dismutase (Cu/Zn SOD) and glutathione peroxidase (GSH-Px) as well as the amount of glutathione (GSH) were determined. TPs treatment significantly increased the levels of these parameters compared to the model group, indicating a protective role against oxidative stress.

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession no.</th>
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### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALB (g/L)</th>
<th>ALP (IU/L)</th>
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<tr>
<td>Normal</td>
<td>47.0 ± 8.0</td>
<td>121.5 ± 18.6</td>
<td>39.7 ± 1.1</td>
<td>128 ± 8.8</td>
</tr>
<tr>
<td>Model (CCl4)</td>
<td>686.5 ± 253.9 **</td>
<td>1191.5 ± 284.9 **</td>
<td>33.3 ± 2.8 **</td>
<td>864.5 ± 288.9 **</td>
</tr>
<tr>
<td>CCl4 + TP120</td>
<td>188.6 ± 64.3 **</td>
<td>338.1 ± 120.2 **</td>
<td>36.2 ± 2.1 **</td>
<td>627.3 ± 271.5 **</td>
</tr>
<tr>
<td>CCl4 + TP48</td>
<td>157.7 ± 38.5 ***</td>
<td>344.7 ± 104.6 ***</td>
<td>39.2 ± 2.4 ***</td>
<td>292.6 ± 144.7 ***</td>
</tr>
<tr>
<td>CCl4 + TP120</td>
<td>151.8 ± 112.6 ***</td>
<td>302.8 ± 168.7 ***</td>
<td>39.3 ± 0.7 ***</td>
<td>268.8 ± 99.9 ***</td>
</tr>
<tr>
<td>CCl4 + TP300</td>
<td>212.2 ± 151.1 ***</td>
<td>352.6 ± 185.6 ***</td>
<td>38.0 ± 1.1 ***</td>
<td>355.1 ± 192.9 ***</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Each group has a minimum of \(n=6\).  
* \(p < 0.05\) vs. the normal group.  
** \(p < 0.01\) vs. the normal group.  
*** \(p < 0.001\) vs. the normal group.  
* \(p < 0.05\) vs. the model group.  
** \(p < 0.01\) vs. the model group.  
*** \(p < 0.001\) vs. the model group.
in the liver tissue homogenate were measured. Compared to the normal group, CCl4 exposure resulted in a decrease of Cu/Zn SOD (p < 0.01) and GSH-Px (p < 0.05) activities as well as the GSH levels (p < 0.01) in the liver homogenate. However, TPs treatment significantly restored the activity of SOD and GSH-Px and the level of GSH in the liver homogenate (p < 0.05 or < 0.01). TPs also exhibited a significantly higher efficacy than PP at the same dose of drug.

Table 3
Histopathological grading for hepatic injury in liver after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dosage (mg/kg)</th>
<th>n</th>
<th>Severity score of hepatic fibrosis</th>
<th>Average</th>
<th>p</th>
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<tr>
<td>Normal</td>
<td>Olive</td>
<td>9</td>
<td>0</td>
<td>0.0</td>
<td>–</td>
</tr>
<tr>
<td>Model</td>
<td>Olive</td>
<td>8</td>
<td>0</td>
<td>2.0</td>
<td>–</td>
</tr>
<tr>
<td>TPs</td>
<td>48</td>
<td>12</td>
<td>0</td>
<td>2.1</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>11</td>
<td>1</td>
<td>1.8</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>9</td>
<td>0</td>
<td>2.1</td>
<td>0.014</td>
</tr>
<tr>
<td>PP</td>
<td>120</td>
<td>9</td>
<td>0</td>
<td>2.4</td>
<td>0.101</td>
</tr>
</tbody>
</table>

Note: Significance was determined by the Mann–Whitney test.

* p < 0.05 vs. the normal group.
** p < 0.01 vs. the normal group.
* p < 0.05 vs. the model group.
** p < 0.01 vs. the model group.

Table 4
Effects of TPs on hepatic SOD and GSH-Px activity, GSH and MDA content in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cu/Zn SOD (U/mg prot)</th>
<th>GSH-Px (U/g prot)</th>
<th>GSH (mgGSH/g prot)</th>
<th>MDA (nmol/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>109.6 ± 15.8</td>
<td>363.0 ± 71.4</td>
<td>0.92 ± 0.14</td>
<td>1.0 ± 0.39</td>
</tr>
<tr>
<td>Model (CCl4)</td>
<td>54.0 ± 7.9**</td>
<td>256.9 ± 38.2**</td>
<td>0.46 ± 0.06**</td>
<td>4.8 ± 1.08**</td>
</tr>
<tr>
<td>CCl4 + PP120</td>
<td>71.0 ± 14.2**</td>
<td>259.0 ± 41.4**</td>
<td>0.57 ± 0.07**</td>
<td>2.1 ± 0.82**</td>
</tr>
<tr>
<td>CCl4 + TPs48</td>
<td>79.7 ± 11.5**</td>
<td>325.2 ± 57.3**</td>
<td>0.60 ± 0.08**</td>
<td>2.0 ± 1.12**</td>
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<tr>
<td>CCl4 + TP120</td>
<td>102.2 ± 11.0**</td>
<td>385.4 ± 73.5**</td>
<td>0.80 ± 0.18**</td>
<td>1.5 ± 0.45**</td>
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<tr>
<td>CCl4 + TP300</td>
<td>70.6 ± 12.2**</td>
<td>297.7 ± 81.4</td>
<td>0.63 ± 0.05**</td>
<td>1.6 ± 0.44**</td>
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</tbody>
</table>

Note: Significance was determined by the Student–Newman–Keuls test.

* p < 0.05 vs. the normal group.
** p < 0.01 vs. the normal group.
* p < 0.05 vs. the model group.
** p < 0.01 vs. the model group.

Fig. 3. Effects of TPs and PP treatment on histopathological changes induced by CCl4 in the rat liver. Liver sections were stained using the hematoxylin–eosin (HE) method: (A) normal (vehicle only), (B) model (CCl4 only), (C) CCl4 + PP 120 mg/kg group, (D) CCl4 + TPs 48 mg/kg group, (E) CCl4 + TPs 120 mg/kg group, and (F) CCl4 + TPs 300 mg/kg. 200 × magnifications, scale bar = 200 μm.
3.3. TPs reduced CCl4-induced collagen accumulation in the liver

Compared to normal rats, CCl4 administration caused a significant increase in accumulation of collagen and ECM volume fraction as assessed by the hepatic hydroxyproline content and morphology of the Sirius red staining. CCl4 treatment dramatically increased the amount of hydroxyproline by 2.4-fold, whereas the coadministration of TPs or PP with CCl4 significantly decreased hydroxyproline levels ($p < 0.01$) (Fig. 4A). Sirius red staining showed that treatment with various doses of TPs could reduce the deposition of collagen in CCl4-induced fibrotic livers (Fig. 4B and C). The percentage of positive Sirius red staining area by image analysis was approximately 78–86% less in groups treated with TPs compared to the model group by image analysis. The data from hydroxyproline levels in the liver homogenate were consistent with the results from the Sirius red staining and image analyses.

3.4. TPs attenuated HSC activation

Immunohistochemical staining of $\alpha$-SMA was used to evaluate the degree of HSC activation. The image analysis of the immunohistochemical staining of $\alpha$-SMA showed that compared with normal group (Fig. 5A and F), chronic CCl4 treatment significantly increased the accumulation of activated HSC ($p < 0.01$) (Fig. 5B and F). Compared to the CCl4 group, different doses of TPs treatment significantly decreased HSC activation in the liver ($p < 0.01$) (Fig. 5C–E and F), with the medium dose (120 mg/kg) having the most pronounced effect on inhibiting HSC activation. These data parallel the results of the collagen accumulation experiment.

3.5. TPs reduced the expression levels of pro-fibrotic factor and elevated the hepatoprotective factor in chronic CCl4-induced liver injury

To elucidate the possible molecular pathways by which TPs suppressed hepatic fibrosis, we examined mRNA expression of

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**Fig. 4.** Effects of TPs and PP treatment on hepatic collagen accumulations induced by CCl4 treatment in the rat liver. Collagen accumulation was assessed by (A) hepatic hydroxyproline content and (B) & (C) Sirius-red stained of cryo-liver sections. Data in (A) and (B) are mean ± SD (each group has a minimum of $n=6$). For the hepatic Sirius-red staining (C), (a) normal (vehicle only), (b) model (CCl4 only), (c) CCl4 + PP 120 mg/kg, (d) CCl4 + TPs 48 mg/kg, (e) CCl4 + TPs 120 mg/kg, (f) CCl4 + TPs 300 mg/kg. CCl4 treatment increased collagen depositions, which were significantly reduced with different dosages of TPs and PP ($^#p < 0.05$, $^{##}p < 0.01$, vs. the normal group; $^&p < 0.05$, $^{&&}p < 0.01$, vs. the model group; the same below). 100 x magnifications, scale bar = 500 μm.
several fibrogenic-related genes. The mRNA expression of α-SMA (Fig. 6A) after different treatments was concordant with the results of immunohistochemical staining. As shown in Fig. 5, the hepatic mRNA levels of several factors involved in fibrillar extracellular matrix (ECM) synthesis, such as ColⅠa, ColⅢ, MMP-2, TIMP-1 and TIMP-2 (p < 0.01) (Fig. 6B–F), were increased in the CCl4-treated livers compared to normal liver. In the treatment with TPs + CCl4, the fibrotic-related cytokines TGF-β1, CTGF, PDGF-β and TNF-α were also significantly increased (p < 0.01) (Fig. 6G–I and K), whereas HGF levels decreased (p < 0.05) (Fig. 6J).

4. Discussion

The present study showed that the TPs (polyprenols that are isolated from the needles of Taxus chinensis var. mairei) had protective effect on liver injury and attenuated the progression of liver fibrosis induced by chronic CCl4 treatment. Sirius red staining showed a significant anti-fibrotic effect of TPs, confirmed by serum ALT, AST, ALB and ALP levels, but steatosis was still observed as shown by HE staining, which was concordant with the obvious difference of antioxidant activities between TPs 120 and TPs 300 is inconsistent with the insignificant difference of MDA production. Compared to the model, the treatment with TPs + CCl4 significantly increased the activity and/or content of Cu/Zn SOD, GSH-Px and GSH, which scavenging free radical scavenger, and simultaneously reduced production of lipid peroxides, which mildly alleviated the oxidative damage caused by CCl4. This might be due to the structure of polyprenols which comprise of a sequence of unsaturated isoprenic units. However, the obvious difference of antioxidant activities between TPs 120 and TPs 300 is inconsistent with the insignificant difference of MDA production, indicating a complex antioxidant mechanism in organisms (Galano et al., 2009; Qian et al., 2011). Therefore, we can only speculate that the anti-fibrotic action of TPs might be related with the free radical scavenging ability of TPs while the preliminary conclusion needs further experimental verifications. MMP-2 plays an important role in remodeling the basement membranes, as it degrades several ECM components. It is virtually undetectable in healthy liver tissue but is highly expressed in activated HSCs in liver fibrosis (Hemmann et al., 2007). Moreover, TIMP-1 and TIMP-2 are mainly produced by HSCs and are upregulated in experimentally induced liver fibrosis and various human liver diseases (Ikebuchi et al., 2011; Qian et al., 2011). We showed that chronic CCl4 treatment significantly induced HSC activation, as indicated by immunohistochemical semi-quantification and the mRNA expression level of α-SMA, as well as by hydroxyproline.
content and Sirius red staining. In addition, the mRNA expression levels of \(\alpha\)-SMA, Col \(\alpha\)1(I), Col \(\alpha\)1(III), MMP-2, TIMP-1, TIMP-2, TGF-β1, CTGF, PDGF-β, HGF, and TNF-α. In the CCl4-treated group, the expression levels of \(\alpha\)-SMA, Col \(\alpha\)1(I), Col \(\alpha\)1(III), MMP-2, TIMP-1, TIMP-2, TGF-β1, CTGF, PDGF-β and TNF-α were significantly increased \((p < 0.01)\) while HGF was significantly decreased \((p < 0.05)\). TPs treatment significantly restored the expression levels of these genes to the levels found in normal livers compared to the CCl4 group \((p < 0.05 \text{ or } < 0.01)\) \((\text{each group has a minimum of } n = 6)\). *\(p < 0.05\), **\(p < 0.01\) vs. the normal group; *\(p < 0.05\), **\(p < 0.01\), \#\#\(p < 0.01\), vs. the model group.

Fig. 6. mRNA expression levels of (A) \(\alpha\)-SMA, (B) Col \(\alpha\)1(I), (C) Col \(\alpha\)1(III), (D) MMP-2, (E) TIMP-1, (F) TIMP-2, (G) TGF-β1, (H) CTGF, (I) PDGF-β, (J) HGF, and (K) TNF-α. In hepatic cells subjected to chronic CCl4 treatment.

In chronic liver injury, the injured or damaged cells (especially HSCs) release various pro-fibrotic cytokines, such as TGF-β1, CTGF, PDGF and TNF-α (Tipoe et al., 2010; Trebicka et al., 2010; Domitrovic et al., 2011). We showed that TPs significantly
alleviated the fibrosis development caused by CCl4. Therefore, we proposed that the anti-fibrotic effect of TPs was partially due to the suppression of various pro-fibrogenic factors. TGF-β1 is a potent inducer of Col α1(I) and changes in positive proportion (Hellerbrand et al., 1999). However, Col α1(I) is also significantly increased in PDGF-β transgenic rats without any notable changes of TGF-β1 (Czochra et al., 2006). Although there is no definitive evidence indicating that PDGF-B can stimulate collagen expression in HSCs, it has been demonstrated that anti-PDGF strategies can efficiently inhibit Col α1(I) deposition (Borkham-Kamphorst et al., 2004). These factors are also closely related to ECM accumulation in addition to HSC activation, both of which contribute to the development of fibrosis. Our results indicated that TPs significantly decreased the expression of TGF-β1, CTGF, PDGF and TNF-α. The above results implied that the anti-fibrotic effects of TPs were a result of the suppression of various pro-fibrogenic factors, thus preventing the development of liver fibrosis in rats induced by chronic CCl4 treatment.

Hepatic growth factor, a potent mitogen for hepatocytes, plays a role in the regeneration and protection of the liver not only by decreasing apoptosis of hepatic parenchymal cells, but also by inducing apoptosis of activated HSCs (Kim et al., 2005; Li et al., 2007). Our results showed that HGF mRNA expression was significantly decreased by 8-week CCl4 treatment, concordant with the previously published results (Mu et al., 2009). Compared to the model group, the HGF expression in hepatic tissues was increased in the TPs-treated groups, indicating that TPs could protect liver parenchymal cells against injury caused by CCl4 and promote the self-repair capacity of the liver cells.

It should be mentioned that the anti-fibrotic effect of TPs might be due to their metabolites in vivo, as there were no positive results of TPs on HSC-T6 cell line (data not shown). It has been verified that the uptake of dietary polyprenols are reduced and phosphorylated into dolichols and dolichol phosphate (dolichol-P), which serves as the rate-limiting factor in glycosylation reactions (Chojnacki and Dallner, 1983). The reductase SRD5A3, which reduces the alpha-isoprene unit of polyenols to form dolichols, has also been detected (Cantagrel et al., 2010). Dolichol levels in isolated parenchymal and non-parenchymal rat liver treated with thioacetamide/1,2-dichloroethane were lower than the levels found in drug-treated liver (Bassi et al., 2004). The amount of dolichol and dolichol-P content was dramatically decreased in instances of cirrhosis and primary carcinoma (Egens and Elmenberger, 1985), indicating an important role of dolichols in liver disease. Moreover, dolichols might function as an endogenous lipophlic scavenger along with PUFA and Vitamin E (Chojnacki and Dallner, 1988). Therefore, we believe that the hepatoprotective effect of TPs is related to dolichols.

In conclusion, our studies suggest that TPs significantly attenuate the development of liver fibrosis induced by chronic CCl4 treatment. The hepatoprotective effect of TPs may be due to its resistance to oxidative stress and the mechanism of TPs might be related with the inhibition of HSC activation, the suppression of pro-fibrotic cytokines and the protection of hepatocytes. However, the precise mechanism of TPs in liver fibrosis remains to be elucidated and further studies will be required on the dose independent effect of TPs and provide scientific evidence for developing polyenols as anti-fibrotic or hepatoprotective drugs.

Acknowledgments

This work was financially supported by the key project of Provincial and Institutional Cooperation of the Zhejiang Province (no. 2008SY03) and the key project of the Science and Technology of Zhejiang Province (no. 2009C12044). The authors thank Prof. Shi-Ying Cai (Yale Liver Center, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT) for his advices on these experiments. We are also grateful to associate Prof. Huazheng Peng (Institute of Biology, Zhejiang Forestry Academy, Hangzhou, PR China) for his help with the qRT-PCR techniques and Prof. Xiaoli Zhao (Institute of Genetics, Zhejiang University, Hangzhou, PR China) for his help with the experiment of HSC-T6.

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