Protective effects of different extracts of *Eucommia ulmoides* Oliv. against thioacetamide-induced hepatotoxicity in mice

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A comparison of analysis in evaluating the hepatoprotective action of fractional ethanolic (F₀), ethyl acetate (F₁), n-butanol (F₂) and aqueous (F₃) extracts of *E. ulmoides* Oliv. (EUO) against thioacetamide (TAA) induced hepatic damage was studied in mice. The extract (453 mg/kg-F₀, 104 mg/kg-F₁, 95 mg/kg-F₂ and 237 mg/kg-F₃ body weight, po, once daily for 15 days) restored serum marker enzymes levels to normal in TAA treated mice. The biochemical biomarkers viz., total protein, albumin and total bilirubin were also restored forward normal level expression pattern of liver protein profile of mice by using sodium dodecyl sulfate polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis showed 144 spots in TAA administered group which were significantly reduced in EUO extracts treated group. Among the four extracts ethyl acetate (F₁) and n-butanol (F₂) extracts showed more significant liver protection. TAA induced injury can be correlated with its high phenolic content in these extracts which may have hepatoprotective effects in regulating liver proteins by scavenging free radicals.

**Keywords:** Antioxidant activity, *Eucommia ulmoides*, Protective effect, Thioacetamide-induced hepatotoxicity

*Eucommia ulmoides* Oliv. (EUO, Du-Zhong in Chinese) is one of the tonic herbs in traditional Chinese medicine (TCM). According to ancient records, EUO is recommended to improve the function of the liver and kidneys, strengthen the muscles and lungs, prevent miscarriages, lower blood pressure and increase longevity¹. EUO tea reduced the frequency of chromosome aberration after mitomycin C treatment in CHO (Chinese hamster ovary) cells². The water extract from EUO leaves having a strong antioxidant function in vitro³, can decrease oxidative damage in biomolecules⁴, avoid low-density lipoprotein oxidative modification⁵, and inhibit H₂O₂-induced DNA damage in lymphocytes⁶. Zhu et al.⁶ reported the protective effects of water extract of Du-Zhong on CCl₄-induced chronic hepatotoxicity in rats⁶.

TAA is a potent hepatotoxicant. Acute TAA exposure results in hepatitis, whereas chronic TAA exposure causes cirrhosis, which resembles the important features of the human disease in experimentally-induced liver injury models⁷. TAA causes severe liver cell necrosis when treated in a single intraperitoneal dose of 6.6 mmol/kg⁸. Shortly after administration TAA undergoes an extensive metabolism to acetamide and to the hepatotoxic metabolite TAA-S-oxide (TAASO) by the mixed function oxidase system⁹. By means of cytochrome P-450 monooxygenase, TAASO is further metabolised by oxidative stress⁹. The free radicals generated by this oxidative pathway cause lipid peroxidation and increase cytosolic calcium levels and reduce glutathione levels. In addition, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities increase in the serum³.

Liver cirrhosis and drug induced liver injury may be an important cause of death in China and other developing countries. Although treatment of liver diseases with chemical medicine has rapid and strong efficacy, it has got the risk of adverse effects and high cost. Therefore, treatment of liver diseases with functional foods such as plant-derived extractions which are not only low cost, but also have less side effects. Keeping above in view, an attempt has been made to explore extracts of EUO, an old herb in TCM. It may be useful to develop alternative functional foods for prevention of different kinds of liver diseases.

In this study, we investigate the effects of ethanol (60 % w/v), ethyl acetate, n-butanol and water...
extracts from EUO on acute TAA-induced hepatotoxicity. The activities of ALT, AST, alkaline phosphatase (ALP) and albumin (ALB) and the serum amounts of total protein (TP) and total bilirubin (TB) were measured. SDS-PAGE and 2-DE approaches were applied to analyse the protein profiles of livers of mice administrated with different extracts of EUO. In addition, the free radical-scavenging potential of different extracts of EUO was determined by using 2, 2-diphenyl-1-picrylhydrazil (DPPH), 2, 2'-azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS). The superoxide anion radical scavenging activity and antioxidant activity on lard oil of different extracts of EUO were used to determine their antioxidant effects.

Materials and Methods

Plant material—EUO leaves used were purchased from Traditional Medicine Market in Guangzhou, Guangdong province, China, and identified by Professor Zhang DanYan of Guangzhou University of Traditional Chinese medicine.

Animals—KM mice (18-20 g body weight) supplied by the Guangzhou University of Traditional Chinese Medicine were used. All animal studies were reviewed and approved by the animal and ethics review committee of Guangzhou University of Traditional Chinese Medicine.

Preparation of plant extract—Powder (100 g) of dried plant material and 1000 mL ethanol (60%, w/v) were added into the vessel (soaked for 24 h), then put into ultrasonic extraction apparatus (Beijing Branson Ultrasound Co., LTD., China). The extract conditions were follows: the ultrasonic power was 100 W, the extraction time was 30 min at room temperature. After being extracted once, the residue was extracted twice under the same conditions. After the extraction was finished, the extract was filtrated, concentrated using a rotary evaporator at 45 °C, and dried in a vacuum oven at 50 °C overnight, the remain was suspended by 10 times (w/v) volume of distilled water degreased using petroleum ether. Then the suspension was extracted with ethyl acetate and n-butanol by an equal volume, 3 times. The acetic ether, n-butanol and water extracts were concentrated using a rotary vacuum evaporator at 45 °C and then vacuum-dried (50 °C). It got four kinds of extracts of EUO, ethanol extract (F0), ethyl acetate extract (F1), n-butanol extract (F2) and water extract (F3) were 62.70 mg/g, 79.43 mg/g, 88.29 mg/g and 56.79 mg/g (w/w), respectively.

TAA-induced hepatotoxicity—The effect of the EUO extracts was evaluated in a 15-day study using mice with TAA-induced hepatotoxicity. The mice were randomly divided into following 7 groups of 10 each. Group I (normal control) mice received normal saline. Group II (TAA control) mice were given normal saline for 15 days followed by TAA treatment. Group III was the positive control group and given 200 mg/kg bifendate (Guangzhou Baiyunshan Xingqun Pharmaceutical Co., LTD, China) for 15 days before the TAA treatment. Group IV to VII were prophylactically treated for 15 days with different extracts of EUO (F0: 453 mg/kg/day; F1: 104 mg/kg/day; F2: 95 mg/kg/day; and F3: 237 mg/kg/day, po, based on the average dosage used for human and acute toxicity studies). One hour after the last administration, group II-VII received TAA, 50 mg/kg 2p. The normal control group was (2p) treated with equal amount of normal saline. After 16 h of TAA and normal saline treatment, the blood samples were collected from the orbital sinus under ether anaesthesia, and the liver samples of the mice were obtained after sacrificing the animal.

Determination of liver function markers—The blood samples were allowed to coagulate for 10 min, and the serum was separated by centrifugation at 3500 rpm at 4 °C. The liver was immediately removed, washed with cold normal saline and stored at - 80 °C. The activities of ALT, AST, ALP and ALB and the amounts of TP and TB were determined using commercially available kits (SINNOWA Medical Science & Technology Co., LTD, China) to assess.
the protective effects of EUO extracts against TAA-induced hepatotoxicity in mice.

Protein separation and detection by SDS-PAGE and 2-DE—The mouse liver was cut into small pieces and washed thoroughly in cold normal saline to reduce contaminants and blood. Subsequently, 100 mg liver samples were homogenised for 30 min at 4 °C by using 1 mL of lysis buffer containing 7 M urea; 2 M sulfourea; and 4 \% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)
. The intact cells and the connective tissue were discarded by centrifugation at 8,000 g for 40 min at 4 °C. The supernatant was collected and then filtered thrice with a 0.45 \(\mu\)m filter to remove any large proteins. The supernatant was stored at -80 °C. The protein concentration of supernatant was measured using the Bradford assay\(^{10}\) with bovine serum albumin as a standard. To purify, 32 \(\mu\)g of protein was separated by SDS-PAGE gel electrophoresis using precast 15\% Tris-HCl resolving gels and applying a 10 mA current. For analysis using 2-DE, the protein samples were directly applied to the immobilized pH gradient (IPG) strips (7 cm, pH 4-7, Bio-Rad). According to the biochemical marker data, the protein samples were tested. The protein sample was mixed with a rehydration buffer, containing 7 M urea; 2 M sulfourea; 4\% (w/v) CHAPS; 65 mM DTT and carrier ampholytes. The IPG strips were rehydrated with 150 mL mixture at 20 °C and covered with mineral oil (12 h at 50 V). After a passive rehydration, an isoelectric focusing (IEF) was performed, which was run in a Bio-Rad cylindrical tube gel apparatus. The electrophoretic conditions during the IEF were as follows: a linear increase from 50 to 250 V for 30 min; fast increase from 250 to 500 V for 30 min; fast increase from 500 to 4000 V for 3 h; and 4000 V for 20,000 Vhr. As soon as electrophoresis was completed, the strips were removed from the mineral oil. The strips were equilibrated for 15 min with buffer A (50 mM Tris-HCl buffer, pH 8.8; 6 M urea; 30\% (v/v) glycerol; 2\% (w/v) SDS and 2\% (w/v) DTT) and buffer B (50 mM Tris-HCl buffer, pH 8.8; 6 M urea; 30\% (v/v) glycerol; 2\% (w/v) SDS and 2.5\% (w/v) iodoacetamide). The strips were directly loaded onto a 10\% SDS-PAGE gel using 0.5\% (w/v) agarose. The IPG strips were loaded onto the polyacrylamide gels, and a constant 10 mA current was applied. The gels were scanned using a transmissive flatbed scanner (UMAX) and then analysed using PDQuest software (Bio-Rad, version 8.0).

**DPPH scavenging** assay—DPPH was used to determine the free radical scavenging (antioxidant) activity on the EUO extracts concentration in the range of 1-1000 \(\mu\)g/mL. Vitamine C (Vc) was used as a positive control. DPPH solution (0.1 mM) and different EUO extract concentrations were prepared with ethanol, and then 900 \(\mu\)L of DPPH solution was added to 100 \(\mu\)L of the test solution. The reaction mixture was incubated at 37 °C for 30 min. The absorbance was measured at 515 nm, and the percentage of inhibition (\%) was calculated using the following equation: Inhibition (\%) = (1-A\(_1\)/A\(_0\)) \times 100 \%, where A\(_1\) is the absorbance of the mixture solution and A\(_0\) is the absorbance of the DPPH solution\(^{11}\).

**ABTS scavenging** assay—The ABTS assay is based on the ability of the antioxidants to scavenge the long-life radical cation ABS\(^{12}\). The extracts were tested in a concentration range of 1-1000 \(\mu\)g/mL. Vc was used as a positive control. This scavenging process produces a decreased absorbance level at 734 nm. The ABTS solution was prepared, and 440 \(\mu\)L of the 140 mmol/L potassium persulfate solution was mixed with 25 mL of the 7 mmol/L ABTS solution. The reaction solution was protected from the light for 24 h. Ethanol was then added until the absorbance of the ABTS solution was 0.7 ± 0.002 at 734 nm. The ABTS solution was prepared, and 440 \(\mu\)L of the 140 mmol/L potassium persulfate solution was mixed with 25 mL of the 7 mmol/L ABTS solution. The reaction solution was protected from the light for 24 h. Ethanol was then added until the absorbance of the ABTS solution was 0.7 ± 0.002 at 734 nm. Subsequently, 100 \(\mu\)L of the test sample solution was mixed with 900 \(\mu\)L of ABTS solution for 30 min and protected from the light. The absorbance at 734 nm was measured, and the percentage of inhibition (\%) was calculated using the following equation: Inhibition (\%) = (1-A\(_1\)/A\(_0\)) \times 100 \%, in which A\(_1\) is the absorbance of the mixture solution and A\(_0\) is the absorbance of the ABTS solution.

**Superoxide anion radical (O\(^{2-}\)) scavenging activity**—The superoxide radical scavenging activities of the EUO extracts were determined using the pyrogallic acid method\(^{13}\). Briefly, 100 \(\mu\)L of the sample solution was mixed with 450 \(\mu\)L of a 0.1 mol/L Tris-HCl buffer and 320 \(\mu\)L redistilled water, and the solution was incubated at 25 °C for 20 min. Pyrogallic acid (10 mmol/L, 30 \(\mu\)L) was added to the mixture, and the absorbance at 320 nm was measured at 0.5-min intervals. Chlorhydric acid (10 mmol/L) was applied as a blank control.

**Antioxidant activity on lard oil**—The activity of the EUO extracts in the lard oil antioxidant experiment was evaluated using the oven storage test\(^{14}\). In total, 15 g of lard oil was mixed with 6 mg of extract and Vitamin C. The mixture was baked in the oven.
(60 ± 2 °C), and the value of peroxide value (POV) was measured at 48 h intervals using the iodometric method (GB5009, 37-2003).

Statistical analysis—The data were expressed as the mean±SD. The results were analysed by a one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using R2.13.2 software; P values ≤ 0.05 were considered significant. The IC₅₀ values were determined using a linear regression analysis of the dose-response curve.

Results

Effects of the EUO extracts on liver function markers—According to the Chinese Pharmacopoeia, the human dosage of EUO was 6-9 g, and the mice dosage was 25- to 50-fold of the human dosage. The dosage of the four different extract was calculated using the following equation: dosage (g/kg) = (9/70) × 40 × extracting %. The dosages of different extracts of EUO were selected for in vivo experiments. Based on acute toxicity studies, the dosage of extracts did not show any sign and symptoms of toxicity or mortality up to 1000 mg/kg body weight, which could be considered relatively safe.

The effects of EUO extracts on serum enzymes, TB and TP levels in TAA-induced hepatotoxicity model mice were shown in Table 1. The activities of ALT, AST and ALB and the serum amounts of TP and TB of the TAA-treated group at an ip dose of 50 mg/kg were significantly elevated (P<0.01) after 16 h. The increased levels of these biochemical biomarkers clearly showed hepatic cell damage.

Compared with the TAA treated group, the ALT activities in the serum of four of the groups were significantly lower (P<0.01) when treated with 200 mg/kg bifendate, 104 mg/kg ethyl acetate extract, 95 mg/kg n-butanolic extract and 237 mg/kg water extract. The AST activities in the serum of ethyl acetate and n-butanolic extract of EUO treated group were markedly decreased (P < 0.01). The amounts of TP in the bifendate and ethanol treated group were significantly lower (P < 0.01). The amounts of TP in the ethyl acetate and water extract of EUO treated group were reduced compared with the TAA control group (P < 0.05).

Protein analysis—According to the biochemical marker data, ethyl acetate extract and n-butanolic extract significantly reduced the ALT and AST activities, which were effective on the TAA-induced hepatotoxicity in mice. The protein samples from normal control group, TAA control group, ethyl acetate and n-butanolic extract of EUO treated group were used to perform the protein analysis.

The silver staining analysis of the SDS-PAGE gels were shown in Fig. 1. Compared to normal group, the protein of TAA treated group markedly increased at 15 kDa and 70 kDa and had a small decrease at 26 kDa. The protein of ethyl acetate extract of EUO treated group significantly decreased from 28-250 kDa and 15 kDa. The protein pattern of n-butanolic extract of EUO treated group was similar with normal group.

To evaluate whether the extracts of EUO exhibited protective effects against TAA-induced hepatotoxicity, we carried out proteomic analysis on liver tissues of mice maintained on extracts of EUO with TAA. Fig. 2 show 2-DE gels of liver proteome map of mice on the effect of ethyl acetate and n-butanolic extracts of EUO with the administration of TAA. Different spot numbers were assigned by PDQuest 8.0 and shown in Table 2. The PDQuest analysis reported that more than 144 spots were differentially expressed. The EUO extract treatments significantly reduced the TAA-induced change as shown in the thirteen protein marks in Fig. 2C and D.

Table 1—The effects of EUO extract and bifendate on TAA-induced liver damage in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>ALB (U/L)</th>
<th>TB (μmol/L)</th>
<th>TP (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>24.16±6.92</td>
<td>117.17±30.08</td>
<td>246.87±74.04</td>
<td>37.48±0.69</td>
<td>4.03±2.67</td>
<td>58.88±1.90</td>
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<tr>
<td>II</td>
<td>14227.11±377.83†</td>
<td>7890.88±567.68†</td>
<td>288.63±11.70</td>
<td>81.01±1.94†</td>
<td>101.46±6.07†</td>
<td>198.30±1.66†</td>
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<tr>
<td>III</td>
<td>9170.38±3278.33**</td>
<td>7917.44±3442.20</td>
<td>359.03±51.66</td>
<td>80.66±4.52**</td>
<td>104.90±7.31</td>
<td>191.42±5.18**</td>
</tr>
<tr>
<td>IV</td>
<td>15277.76±3998.05</td>
<td>11349.54±3424.39</td>
<td>319.97±57.12</td>
<td>80.90±4.20**</td>
<td>104.17±3.83</td>
<td>191.11±2.52**</td>
</tr>
<tr>
<td>V</td>
<td>123.40±137.03**</td>
<td>123.31±49.26**</td>
<td>315.83±110.85</td>
<td>83.12±1.59**</td>
<td>114.93±7.09</td>
<td>194.76±1.57*</td>
</tr>
<tr>
<td>VI</td>
<td>90.57±82.83**</td>
<td>135.35±35.14**</td>
<td>284.52±87.15</td>
<td>83.76±2.49**</td>
<td>111.08±6.19</td>
<td>195.51±2.62</td>
</tr>
<tr>
<td>VII</td>
<td>11066.41±2078.25**</td>
<td>6526.76±1122.08</td>
<td>306.66±76.79</td>
<td>82.46±2.00**</td>
<td>101.94±4.58</td>
<td>194.29±1.98**</td>
</tr>
</tbody>
</table>

†Significantly different from the normal control group (P < 0.01).
** Significantly different from the TAA-induced control group (P < 0.01).
* Significantly different from the TAA-induced control group (P < 0.05).
Antioxidant activity of the EUO extracts—
The DPPH free radical scavenging effect of EUO extract was tested (Fig. 3A.) The EUO extracts exhibited a weaker DPPH radical scavenging activity. The IC\textsubscript{50} value of F\textsubscript{0}, F\textsubscript{1}, F\textsubscript{2}, and F\textsubscript{3} were 198.90, 84.60, 105.12 and 477.95 μg/mL, respectively. Used as reference antioxidant, the IC\textsubscript{50} value of V\textsubscript{c} is 1.85 μg/mL. Effect of extracts on ABTS free radical scavenging activities of ethanol, ethyl acetate, n-butanolic and water extracts of EUO was assayed at various concentrations (Fig. 3B). ABTS is used as a free radical to evaluate antioxidant. Mild ABTS free radical scavenging activity was evident in extracts of EUO, the IC\textsubscript{50} values of F\textsubscript{0}, F\textsubscript{1}, F\textsubscript{2}, F\textsubscript{3}, and V\textsubscript{c} were 65.41, 52.69, 43.27, 148.80, and 2.79 μg/mL, respectively. The pyrogallic acid method was used to evaluate the superoxide radical scavenging effect of the EUO extracts, and the result was presented in Fig. 3C. The IC\textsubscript{50} values of F\textsubscript{0}, F\textsubscript{1}, F\textsubscript{2}, F\textsubscript{3}, and V\textsubscript{c} were 657.42, 483.94, 580.66, 1415.33, and 25.04 μg/mL, respectively. The protective effect of the EUO extracts on the peroxide of lard oil was shown in Fig. 3D. The extracts of EUO all have antioxidant activity on lard; the capacity is as follow: F\textsubscript{2} > V\textsubscript{c} > F\textsubscript{1} > F\textsubscript{0} > F\textsubscript{3}.

Ethanol, ethyl acetate, n-butanolic, and water extracts of EUO have antioxidant activities as demonstrated using a variety of radicals, such as DPPH, ABTS, and \textsuperscript{O}_{2}^{-}, and antioxidant activities on lard oil. The ethyl acetate and n-butanolic extracts have greater activities compared with the other extracts.

Discussion
TAA is a commonly used chemical to induce experimental liver fibrosis which is similar to human liver cirrhosis, TAA-induced hepatotoxicity was chosen as an experimental model\textsuperscript{14}. In the present study, treatment with TAA (50 mg/kg) considerably attenuated the TAA-induced acute liver injury as indicated by elevated serum levels of ALT, AST and TB\textsuperscript{16} and severely affected the metabolism of the ALB and TP in the serum. The TAA-induced hepatotoxicity model was performed successfully. However, there were no changes in the serum level of ALP, which is the most sensitive and diagnostic marker of hepatic injury\textsuperscript{10}.

Early treatment with the ethyl acetate extract (F\textsubscript{1}, 95 mg/kg) and the n-butanolic extract (F\textsubscript{2}, 104 mg/kg) were effective to counteract the TAA-induced hepatic injury by reducing the elevated serum levels of ALT and AST to a normal functioning status. The water extract (F\textsubscript{3}, 237 mg/kg) and bifendate (200 mg/kg) also decreased the ALT and TP serum levels compared to the TAA-induced group. The ethanol extract (F\textsubscript{0}, 453 mg/kg), in dosage which is sum of F\textsubscript{1}, F\textsubscript{2} and F\textsubscript{3}, only prevented an increase in the TP. A possible explanation for this may be that the compounds in EUO (i.e., phenolic compounds, flavonoids, and iridoid compounds) possess strong antihepatotoxic activity. The ethyl acetate, n-butanolic and water extracts, which were separated from the ethanol extract, can effectively prevent TAA-induced hepatotoxicity compared to the ethanol extract, whereas the ALT and AST serum levels were within the normal range\textsuperscript{17}. A probable reason for this result was that the compounds in the same class caused an agonist reaction when they are mixed.

In the assessment of liver damage by TAA, the enhanced activities of these serum marker enzymes observed in TAA treated mice in our study correspond to the extensive liver damage induced by TAA. Results indicate that EUO leaves extract administration could prevent TAA-induced increase
in activities of different marker enzymes of heptocellular injury, viz. AST, ALT, TB, and TP, suggesting that EUO possibly has a protective influence against TAA-induced heptocellular injury and degenerative changes.

Rajeswary et al.\textsuperscript{18} reported that CCl\textsubscript{4}-induced hepatotoxicity in rats was useful in studying the effects of medicinal plant extract with therapeutic potential to be used in humans. In the present study thought the hepatoprotective effect of the extracts of EUO in TAA-induced acute liver damage was evaluated by similar method, proteomic studies have been used in the present studies to research the effects of extracts of EUO for TAA-induced liver damage. The summary of the EUO extracts treatment liver protein was shown as Fig. 1, which reported that the n-butanolic extract (F\textsubscript{2}) can reduce the TAA-induced protein changes, and the ethyl acetate extract (F\textsubscript{1}) improved the increase of TAA-induced proteins and lowered the expression of the large proteins (28-250 kDa). Using two-dimensional electrophoresis, the section of the medical treatment liver protein was presented as Fig. 2 with pH 4-7. Ethyl acetate extract (F\textsubscript{1}) and n-butanolic extract (F\textsubscript{2}) were effective in inducing changes originally caused by the TAA treatment; some protein spots decreased or disappeared, and some protein spots increased or appeared.

It is known that free radicals play a major role in the development of TAA-induced liver cirrhosis\textsuperscript{19}. In the present study, four EUO extracts were obtained using a hyperacoustic extraction and separation method. The phenolic compounds in ethanol (F\textsubscript{0}), ethyl acetate (F\textsubscript{1}), n-butanolic (F\textsubscript{2}) and water (F\textsubscript{3}) extracts were measured.

The present study demonstrates that the extracts of EHO had DPPH and ABTS free radical scavenging activities, as well as had the superoxide radical

<table>
<thead>
<tr>
<th>Group</th>
<th>Spots</th>
<th>Matched spots</th>
<th>Match rate (%)</th>
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<tr>
<td>I</td>
<td>147</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>II</td>
<td>225</td>
<td>81</td>
<td>36</td>
</tr>
<tr>
<td>V</td>
<td>150</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>VI</td>
<td>156</td>
<td>63</td>
<td>40</td>
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</table>

Table 2—Protein spot analysis of 2-DE about the test groups

Fig. 2—2-DE gels of liver proteome map of mice with the effect of 15 days feeding of extracts of EUO with the administration of TAA. Liver proteins were extracted and IPG strips (7 cm pH 4-7) were used for IEF, followed by SDS-PAGE (10% polyacrylamide). Thirteen significantly differentially expressed proteins are marked on the four gel images. A: normal control group; B: TAA-induced group; C: EUO ethyl acetate extract treatment group; D: EUO n-butanolic extract treatment group.
scavenging effect and antioxidant activity on lard oil. These results showed the ability to reduce free radicals which may stop the free radical initiation or retard free radical chain reaction in the propagation of the oxidation mechanism. The free radical scavenging activity of the four extracts was assessed using a variety of radicals: DPPH, ABTS, superoxide radical and antioxidant activity on lard oil. The activity sequence was similar to the phenolic content. N-butanolic extract (F2) > ethyl acetate extract (F1) > ethanol extract (F0) > water extract (F3). In a previous study, phenolic compounds were shown to play an important antioxidant role in living systems and are widely distributed in plants.

Conclusion

In conclusion, the results of present studies collectively demonstrate that EUO has significant hepatoprotective activity against TAA-induced acute liver toxicity in mice. The hepatoprotective action of EUO is certainly associated with their antioxidant properties acting as a scavenger of free radicals. The improvement in liver injury and liver functions by EUO extracts may be due to the presence of flavonoids and phenolic compounds which are reported to offer significant protection against liver toxicity. These preliminary findings on hepatoprotective and antioxidant actions reported herein would lend support to the use of EUO as a hepatoprotective agent. Further studies are in progress in our lab to characterize the active principles and for better understanding the mechanism of action.

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References


