Hepatoprotective Effect of Echinops tenuisectus (Compositae) on CCl₄ Induced Hepatic Damage in Rats

Munaf H. Abdulrazzaq* , Enas J. Khadeem**, Suhad S. Al- Muhammadi**

*Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq
**Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract

Flavonoids are known to play a vital role in the management of various liver disorders. They are a large family of compounds synthesized by plants; they belong to a group of natural substances with variable phenolic structures. In this study we aim to scan the types of flavonoids in a newly studied, wild Iraqi plant named Echinops tenuisectus of Compositae family. The medicinal importance of flavonoids on one hand, and the absence of any phytochemical investigation on tenuisectus species of Echinops genus on the other hand, acquired this study its importance. Three flavonoids were identified in the seed’s extract of this plant (Silymarin, Rutin, Quercetin) by two chromatographic methods, first Thin layer chromatography (TLC) using TLC ready made GF254 plates, UV detector at 254 nm, and two different solvent systems in which the Rf value of the standards (Silymarine, Rutin, Quercetin) matched with the Rf value of the Silymarin, Rutin and Quercetin found in the plant seed’s extract. High pressure liquid chromatography (HPLC) was the other chromatographic method that used to identify the presence of these flavonoids in the plant seed. The plant seed’s aqueous extract was evaluated for its efficacy in rats by inducing hepatotoxicity with CCl₄. Single oral dose of 250mg/kg of Seeds Extract was given to rats for 7 days. Serum activities of transaminases (ALT and AST) were used as the biochemical marker of hepatotoxicity. Histopathological changes in rats liver section were also examined. The results of the study indicated that, the pretreatment of rats with Echinops extract before the hepatotoxins agent (CCl₄) offered a hepatoprotective action.

Key words: Echinops, Flavonoids

Introduction

The Echinops tenuisectus belong to the Family Compositae (Fig1) is a wild, Iraqi plant first studied in Iraq. The Echinops genus consist of 100 spp. (3) which are widely distributed in Sharaban, Diyala, Badrah (Upper Tigris Plain) (2). Preliminary investigation indicated that, this plant contain different types of flavonoids in accepted amount. Among these flavonoids: Silymarin (figure 2) which is a flavonolignan that has been introduced fairly recently as a hepatoprotective agent (3,4,5,6,7). Silymarin has been found to provide hepatoprotection through its antioxidants properties (scavengers and regulators of the intracellular content of glutathione) (8,9,10), as cell membrane stabilizers and permeability regulators that prevent hepatotoxic agents from entering hepatocytes (11,12). Also as inhibitors of the transformation of satellite hepatocytes in to myofibroblasts, the process responsible for the deposition of collagen fibers leading to cirrhosis (13, 14, 15).

1 Corresponding author : E-mail : enassara@yahoo.com
Received : 21/11/2007
Accepted : 24/5/2008
Hepatoprotective effect of Echinops flavonoids

The other flavonoids found in this plant are the Quercetin and Rutin (Figure 3,4), both of them possess a powerful antioxidant activity which help to prevent free radical oxidative damage to cells, also help in the treatment and prevention of alcohol and chemical - induced hepatotoxicity by increase glutathione in the liver\(^{(16)}\). Glutathione responsible for detoxifying a wide range of hormones, drugs, and chemicals. High level of glutathione in the liver increases its capacity for detoxification. Quercetin and Rutin increase the level of the important antioxidant enzyme superoxide dismutase in the cell cultures\(^{(17)}\). In addition they stimulate protein synthesis in the liver, which results in an increase in the production of new liver cells to replace the damaged one\(^{(18)}\). Shoskes 1999 demonstrate that Quercetin and Rutin also inhibit the synthesis of leukotrienes (mediators of inflammation, which can result in psoriasis)\(^{(19)}\). Recently, flavonoids can help in prevention of cancer through several pathway: inhibiting proliferation and inducing apoptosis\(^{(20,21)}\) or through inhibiting tumor invasion and angiogenesis\(^{(22,23)}\). This wide variety of beneficial health effects of these flavonoids acquired this study its importance in finding a new uninvestigated source of these important flavonoids, contained within Echinops tenuisectus of the Family Compositae and evaluate their possible protective effect against the experimentally- induced liver damage in rats by CCl\(_4\). Liver, the largest organ in vertebrate body, is the major site of intense metabolic activities. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Herbal drugs are playing an important role in health care programs world wide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy\(^{(24)}\). CCl\(_4\) is reported to produce free radicals which affect the cellular permeability of hepatocytes and it causes massive histopathological changes like necrosis, congested vessel and fatty changes (steatosis). So, the reverse of these phenomenon can be considered as the index of hepatoprotective\(^{(25)}\).
Materials and Methods

A. Plant materials:

The plant material was collected during July 2005 from Sharaban, Dyala city. The plant was identified by the Department of Pharmacognosy, College of Pharmacy, University of Baghdad; and authenticated by the Herbarium of Baghdad University. Fifty grams of the powdered plant material (seed part) were first defatted by reflux with 100 ml of petroleum ether (60-80°C) for one hour and filtered. The defatted dried plant material was then extracted by reflux using 100 ml of 70% ethanol for three hours. This step was repeated for four times, then the combined filtrates were evaporated under reduced pressure using Buchi rotatory evaporator attached to vacuum pump at 40°C, to a thick residue of ethanol extract (F1). This residue was then hydrolyzed with 2N HCl in aqueous methanol (1:1) under reflux for three hours; the resultant solution was then partitioned with 100 ml of ethyl acetate (F2). This fraction was evaporated under reduced pressure to dryness, as shown in the following diagram (Figure 5).

---

**Figure-5** [Schematic representation of flavonoids extraction from Echinops tenuisectus]

F2 (ethyl acetate fraction) → evaporation to dryness under reduce pressure → black-greenish oily residue, TLC and HPLC indicated that this fraction contain three compounds which are silymarin, rutin and quercetin and by preparative thin layer chromatography and HPLC we can separate each one and calculate the percentage of each one by weighting.

F2 (oily residue fraction) → dissolved in water → suspension (ready for hepatoprotective study)
B. Identification of Silymarin, Quercetin and Rutin in the plant seed extract.

The Identification of these flavonoids in the seed extract was performed by:

1. Identification of Flavonoids by TLC:

Using TLC ready made Gf254 plates, UV detector at 254 nm, Standard flavonoids and two different solvent systems that were (26):

Solvent (1): chloroform: acetone: formic acid (75:16.5:8.5) (Figure 6)
Solvent (2): n.butanol: glacial acetic acid: water (40: 10:50) (Figure 7)
(Table-1) showed the Rf values of the standards Silymarin, Quercetin and Rutin, and the Rf value of plant seed part extract.

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Standard Silymarin</th>
<th>Standard Quercetin</th>
<th>Standard Rutin</th>
<th>Seed extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.43</td>
<td>0.35</td>
<td>0.28</td>
<td>0.4,0.33,0.2</td>
</tr>
<tr>
<td>S2</td>
<td>0.2</td>
<td>0.81</td>
<td>0.56</td>
<td>0.21,0.8,0.5</td>
</tr>
</tbody>
</table>

Table 1: Rf values of standard silymarin, rutin and quercetin and seed extract.

Figure 6: TLC Gf254 plate of the seed extract and standards using S1 mobile phase.

Figure 7: TLC Gf254 plate of the seed extract and standards using S2 mobile Phase

Figure 8: HPLC of plant seed extract of Echinops tenuisectus.
Figure 9: HPLC of standard Silymarin.

Figure 10: HPLC of standard Quercetin.

Figure 11: HPLC of standard Rutin.
2. Identification of Flavonoids by HPLC:
Silymarin, Quercetin and Rutin were authenticated by HPLC. (Figures 8-11) The HPLC conditions are listed in the following table. (Table-2)

<table>
<thead>
<tr>
<th>Table 2: HPLC conditions.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC Conditions</td>
</tr>
<tr>
<td>Mobile phase</td>
</tr>
<tr>
<td>Column</td>
</tr>
<tr>
<td>Flow rate</td>
</tr>
<tr>
<td>Detector</td>
</tr>
</tbody>
</table>

C. Hepatoprotective studies:
1. Experimental animals:
Eighteen – Albino rats of both sexes weighting 150-200 gm (both sex) were used in this study. Animals were kept in the animal house of the College of Pharmacy/University of Baghdad, under standardized condition (12 hr light dark cycle at room temperature). The animals were fed standard chow and given water ad libitum.

2. Experimental design:
The animals were divided in to three groups (each group have 6 animals) and treated as follows:
Group (1): Six rats received normal saline for 7 day orally and secreted at along 7, saved as control
Group (2): Six rats received single oral dose of CCl4 (1mg/kg) diluted by corn oil in ratio of 1:1 v/v for the induction of liver damage and animals were sacrificed after 24 hr of CCl4 administration.
Group (3): Six rats received oral dose of the seed extract of Echinops tenuisectus Plant in amount equivalent to 250mg/kg by gavages tube for 7 days, befor CCl4 (1mg/Kg diluted by corn oil in a ratio of 1:1 v/v), then the rats were sacrificed after 24 hr, after CCl4 administration.

3. Biochemical estimation:
Serum was prepared from the collected blood and subjected to biochemical estimation of ALT and AST.

4. Histopathology:
Portion of liver tissue in each group was fixed in 10% formalin (Formalin diluted to 10% with normal saline) and proceeded for histopathology. After paraffin embedding and block making, serial section of 5µ thickness were made, stained with Haematoxylin and Eosin and examined under microscope.

5. Statistical analysis:
The significance of difference between the mean values was calculated using unpaired student's t-test. P-value less than 0.05 were considered significant for all data showed in our results.

6. Results:
A) Biochemical parameters:
Table-3 showed a significant elevation in the activities of both ALT and AST in CCl4-treated rats compared to control group. Pre-treatment rats with seed extract of Echinops tenuisectus (250mg/kg) showed a significant decline in the activities of ALT and AST compared with CCl4 treated rats (Table 3, Figure 12 and 13).

<table>
<thead>
<tr>
<th>Table 3: Effects of seed extract of Echinops tenuisectus on the activities of serum ALT and AST in rats treated with CCl4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>CCl4-treated</td>
</tr>
<tr>
<td>Seed extract + CCl4</td>
</tr>
</tbody>
</table>

- Each value represents Mean ± standard deviation.
- Values with non-identical superscripts (a,b) within each parameter are significantly different ( P< 0.05)
- N= Number of animals.

Figure 12: Bar chart comparing the effects of seed extract of Echinops tenuisectus pre-treated with CCl4 on serum ALT activity.
Hepatoprotective effect of Echinops flavonoids

Figure 13: Bar chart comparing the effects of seed extract of *Echinops tenuisectus* pretreated with CCl₄ on serum AST activity.

B) Histological examination:

Histological examination of rats liver treated with CCl₄ showed that, there was centrilobular hemorrhage, with heavy inflammation and necrosis. In addition to steatosis and individual necrosis were observed compared with control (Figure 14 and 15). Pre-treatment of rats with seed extract of *Echinops tenuisectus* before CCl₄, exhibit variable degrees of recovery with slight centrilobular congestion, marked reduction in inflammatory reaction. Furthermore, neither necrosis nor steatosis was observed in rats liver section (Figure 16).

Discussion:

Many compounds exhibit hepatoprotective activity, demonstrated either by decreasing the harmful effect of hepatotoxic compound or by maintaining the normal hepatic physiology. The present study showed that, the seed extract of *Echinops tenuisectus* have good hepatoprotective effect against CCl₄-induced hepatotoxicity in rat manifested by attenuating the increases in the serum activities of ALT...
Acknowledgment

The authors gratefully thank Prof. Dr. Ali-Al- Mussawi for supporting this project.

References:


