HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACTS OF *CHRYSANTHEMUM INDICUM* FLOWERS ON PARACETAMOL INDUCED LIVER INJURY IN ALBINO RATS

BHAVANI R¹, YAMUNA U², RAJESHKUMAR S*²

¹Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai - 632 506, Vellore, Tamil Nadu, India. ²School of Bio-Sciences and Technology, Vellore Institute Technology University, Vellore, Tamil Nadu, India.

Email: ssrajeshkumar@hotmail.com

Received: 08 February 2016, Revised and Accepted: 19 February 2016

ABSTRACT

Objective: The present investigation was evaluated that protective activity of aqueous extract of flowers of *Chrysanthemum indicum* studied against paracetamol-induced hepatotoxicity in animal model.

Methods: Bioactive functional groups, such as alcohol, carboxylic acid, and amines, were present in the aqueous extract of flowers of *C. indicum* identified by Fourier transform infrared spectroscopy. The animals were grouped into 5 and each group has 6 animals and induced the hepatic failure. Silymarin was used as reference standard. Aqueous extract of flowers of *C. indicum* treated in a different dose which was compared with control group of animals.

Results: Aqueous extract of flowers of *C. indicum* reduced the level of aspartate transaminase (AST), alanine transaminase (ALT), serum bilirubin, protein, triglycerides, and cholesterol compared than paracetamol treated Group II animals. Histopathological studies were confirmed that reduction of necrosis and inflammation in the liver cells.

Conclusion: Thus, these results revealed that the aqueous extract of flowers of *C. indicum* shown very significant (p<0.01) hepatoprotection against paracetamol-induced hepatic failure in animal model by reducing AST, ALT, serum total bilirubin, protein, triglycerides, and cholesterol levels.

Keywords: Hepatoprotective activity, *Chrysanthemum indicum*, Paracetamol.

INTRODUCTION

Plants are major sources of our earth playing a vital role in pharmacological applications such as antidiabetic [1], hepatoprotective [2], cardioprotective [3], nephroprotective [4], and anticancer activities [5]. *Chrysanthemum indicum* Linnaeus, a perennial herb of the family Compositae, is widely dispersed throughout China, north Russia, Southeast Asia and Europe. *C. indicum* Linn is traditionally used for many applications and now it is reported for various uses such as antimicrobial against bacteria (*Klebsiella pneumonia*, *Escherichia coli*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphyloccocus aureus*) and fungi (*Trichoderma viride*, *Candida albicans*, *Penicillium chrysogenum*, and *Aspergillus niger*) [6]. Larvicidal agents against dengue fever mosquito, *Aedes aegypti* L. [7,8], Antiarthritic [9], anti-inflammatory, and immunomodulatory activities [10].

The liver is one of the most vital organs in the human body that functions as a center of metabolism of nutrients, excretion, digestion, detoxification, etc. [11]. Liver damage was caused by the excess usage of drugs contains toxic chemicals, alcohol, and environmental pollutants. Liver damage involves cell necrosis, lipid peroxidation, and other oxidative damages [12]. In addition, the biochemical markers such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), protein, and bilirubin levels in serum were also increased [13].

Liver injury caused generation of free radicals by affecting the cellular membrane functions [14-17]. Many chemical drugs and radiation therapy were widely used for the treatment of liver damage. However, these were high expensive, poor compatibility, and cause many side effects [18]. To overcome these problems herbal derived drugs were formulated as hepatoprotective drug because of they are inexpensive, better cultural acceptance, better compatibility with the human body and minimal side effects. These herbal drugs have shown the ability to maintain the normal functional statues of the liver with or without fewer side effects [19].

The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used with better formulation by identifying their active chemical constituents. These curative properties of chemical constituents such as phenols, coumarins, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloid, and xanthenes were elevated and used to hepatoprotective drugs. In this study, biochemical markers (AST, ALT, bilirubin, lipid peroxidation, total proteins, etc.) in the injured liver was assessed that protective activity of aqueous extract of flowers of *C. indicum* studied against paracetamol-induced hepatotoxicity in animal model.

METHODS

Collection of plant

The plant *C. indicum* flowers were collected from Adhiparasakthi Agricultural College flower garden.

Preparation of plant extract

Fresh flowers were collected and were dried under shade. The dried flower was powdered by mixer grinder. About 5 g of *C. indicum* powder was taken into a beaker and added 100 ml of distilled water and boiled for 10 minutes after that they were filtered with Whatmann no 1 filter paper. The extracts were allowed to store and used for experimental animals.

Animals

Adult male Wistar albino rats maintained at the college weighing 170-200 g were used for the hepatoprotective studies. The rats were housed in polypropylene cages and kept under standard laboratory conditions.
for 5 minutes and centrifuged. 3.0 ml of the supernatant was taken

Parekh and Jung [23]. A value of 10 ml of ferric - uranyl acetate reagent

with different concentrations of bovine serum albumin solution. The

spectrophotometer after 10

The formation of blue color was read at 640

Estimation of serum total protein

The rats were randomly divided into five groups of 6 animals each.

Group I (Control): Control rats, received orally distilled water.

Group II (Induced): Induced rats, orally received paracetamol (2 g/kg

delay) dissolved in water for 7 days.

Group III (Paracetamol + Silymarin): Standard rats, orally received

paracetamol (2 g/kg body weight), followed by silymarin (100 mg/kg

delay) dissolved in water for 7 days.

Group IV (Paracetamol + ASN): Treated rats, orally received paracetamol

(2 g/kg body weight), and followed by aqueous extract of C. indicum

flowers (300 mg/kg body weight) dissolved in water for 7 days.

Group V (Paracetamol + ASN): Treated rats, orally received paracetamol

(2 g/kg body weight), and followed by aqueous extract of C. indicum

flowers (606 mg/kg body weight) dissolved in water for 7 days.

Collection of blood

Animals of all the groups were sacrificed by cervical decapitation on

the 8th day. Blood samples of each group were collected separately into

sterilized dry centrifuge tubes and allowed to coagulate for 30 minutes

at 37°C. The clear serum obtained after centrifugation was used for the

estimation of serum ALT, serum AST, serum bilirubin, serum protein,

cholesterol, and triglycerides.

Assay of serum AST (E.C.2.6.1.1) and ALT (E.C.2.6.1.2)

Assay of AST and ALT was performed according to the method of

Reitman and Frankel [20]. One ml of substrate was incubated for few

minutes at 37°C. Then, 0.2 ml of serum was added and incubated for

1 hr in the case of AST and 30 minutes for ALT. To the control, serum

was added after incubation. The reaction was arrested using 1.0 ml of

2.4-Dinitrophenylhydrazine solution and the tubes were kept at room

temperature for 20 minutes. About 10 ml of 0.4 N Sodium hydroxide

was added to all the tubes. A set of standards was also treated in a similar

manner. The development of color was measured colorimetrically at

520 nm. The enzyme activity was expressed as IU/L.

Estimation of serum bilirubin

The serum total bilirubin was estimated according to the method of

Malloy and Evelyn [21]. About 0.2 ml of serum was taken in a tube and
to this 1.0 ml of water was added. A 0.5 ml of HCl was added to the

blank tube. To the sample tube, 2.5 ml of methanol and 0.5 ml of diazo

reagent were added. The tubes were incubated at room temperature for

30 minutes. The development of blue color in the reaction mixture was

read at 540 nm in a Shimadzu spectrophotometer, along with bilirubin

calibrator solutions. The values were expressed as mg/dl.

Estimation of serum total protein

The serum total protein was estimated as per the method of Lowry

et al. [22]. Typically, 0.5 ml of serum was mixed with 4.5 ml of alkaline
copper reagent and then allowed to stand at room temperature for 10 minutes. Then, 0.5 ml of Folin’s phenol reagent was added.

The formation of blue color was read at 640 nm in a Shimadzu spectrophotometer after 10 minutes. A standard graph was obtained with different concentrations of bovine serum albumin solution. The values were expressed as g/dl.

Estimation of cholesterol

Serum total cholesterol was determined in serum by the method of

Parokh and Jung [23]. A value of 10 ml of ferric - uranyl acetate reagent

was added to 0.1 ml of serum sample, mixed well, allowed to stand for

5 minutes and centrifuged. 3.0 ml of the supernatant was taken

for analysis. Similarly, 0.1 ml of standard cholesterol was treated, and

3.0 ml aliquots were taken. Blank tube contained 3.0 ml of ferric - uranyl

acetate reagent. About 2.0 ml of sulfuric acid - ferrous sulfate reagent

was added to all the tubes and mixed well. After 20 minutes, the color

intensity was measured at 560 nm in a Shimadzu spectrophotometer.
The values were expressed as mg/dl.

Estimation of triglycerides

Serum triglycerides were estimated by the method of Foster and

Dunn [24]. To an aliquot of serum, 0.1 ml of methanol was added, followed by 4.0 ml of isopropanol. Then, 0.4 g of aluminia was added to all the tubes, shaken well for 15 minutes, centrifuged and then 2.0 ml of the supernatant fluid was transferred to labeled tubes. Exactly 0.6 ml of saponification reagent was added to the tubes and then placed in a water bath at 65°C for 15 minutes for saponification. Then, 1 ml of metaperiodate reagent and 0.5 ml of acetylace tone reagent were added to all the tubes, mixed well and incubated in a water bath at 65°C for 1 hr. A series of standards of 8- 40 µg triolein

was treated similarly along with a blank containing only the reagents.

All the tubes were cooled, and the color intensity was measured at

405 nm in a Shimadzu spectrophotometer. The values were expressed as mg/dl.

Histopathology studies

A portion of liver tissue in each group was fixed in 10% formalin
(formalin diluted to 10% with normal saline) and processed for

histopathology. After paraffin embedding, and block marking, serial

section of 5 µm thickness were made, stained with hematoxylin and
eosin and examined under microscope.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) using SPSS 16 software. The values are expressed as mean ± standard deviation and p<0.05 was considered significant.

RESULTS AND DISCUSSION

Phytochemical analysis using Fourier transform infrared (FTIR)

The presence of functional molecules in the aqueous extracts of

C. indicum flower was identified using FTIR spectroscopy spectrum

(Fig. 1). The broadband was observed at 3292.70/cm corresponding
to O-H stretching, H-bonded alcohols and phenols. A low absorption

peak was observed at 2919.01/cm indicates the presence of O-H

stretching carboxylic acids. Weak peaks were formed at 1733.58/cm
and 1602.36/cm due to the presence of C=O stretching carboxylic acid

and N-H bond primary amines, respectively. The presence of aromatic

amines was identified by showing peak at 1286.99/cm. A strong

narrow band was noted at 1013.11/cm assigned to C-O stretching

esters and ethers. However, it concluded that the aqueous extract

contained more phytochemicals such as carboxylic acids, primary

amines, aromatic amines, esters, and ethers could be actively involved in

the hepatoprotective activity.
**Hepatoprotective activity of C. indicum flowers**

The drug paracetamol has been used in this study to induce the cell necrosis in experimental model animals. This hepatotoxins increase the level of enzymes such as transaminases, lipid peroxidation, and ALP was the clear indication of cellular leakage and affects the functional integrity of the cell membrane [25].

Table 1 shows the effect of aqueous extract C. indicum flowers (300 mg/kg and 600 mg/kg) on serum biochemical markers like AST, and ALT in paracetamol induced liver toxicity. Fig. 2 and Table 1 show that serum AST, and ALT levels were significantly higher in animals receiving paracetamol (p<0.05) and decreased significantly in Groups IV and V which they received 300 mg/kg and 600 mg/kg of aqueous extract (p<0.01), respectively. Hepatic damage causes elevated level of liver enzymes such as serum AST, and ALT. Treatment with C. indicum flowers at 600 mg/kg revealed comparable activity with reference standard silymarin (25 mg/kg). Aqueous extract of C. indicum flowers decreased the liver markers AST (103.3±4.23 U/L), and ALT (45.93±1.94 U/L).

The level of total bilirubin, protein, triglycerides and cholesterol in paracetamol intoxicated animals were significantly increased when compared to control (Figs. 3-5 and Tables 2 and 3). The paracetamol treated showed increased levels of serum bilirubin, protein, triglycerides and cholesterol are due to the damage caused by paracetamol. These levels were restored significantly in flower extract treated animal groups Groups IV and V.

**Histopathology analysis**

Histopathological studies in control rat showed normal physiological cells (Fig. 6a). Fig. 6b showed focal necrosis and inflammation in paracetamol treated animals (Group II). Groups III, IV, and V showed minimum necrosis with swelling which were treated with standard silymarin, 300 mg/kg and 600 mg/kg of aqueous extract, respectively. The animals treated with aqueous extract of C. indicum flowers appeared to be normal as in Group I (Fig. 6a-e). These histopathological studies confirmed a hepatoprotective activity of C. indicum flower extract against paracetamol toxicated liver damage.

**CONCLUSION**

This present work evaluated that the hepatoprotective potential of aqueous extract of flowers of C. indicum against paracetamol induced hepatotoxicity. Aqueous extract of flowers of C. indicum was significantly reduced the

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>Level of significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>100.67±4.33</td>
<td>44.83±1.80</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group II (paracetamol)</td>
<td>200.50±10.17</td>
<td>80.67±4.82</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Group III (paracetamol+silymarin)</td>
<td>115.67±4.51</td>
<td>52.3±1.76</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Group IV (paracetamol+300 mg aqueous extract)</td>
<td>109.3±3.91</td>
<td>50.3±2.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group V (paracetamol+600 mg aqueous extract)</td>
<td>103.3±4.23</td>
<td>45.93±1.94</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

AST: Aspartate transaminase, ALT: Alanine transaminase

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bilirubin (mg/dL)</th>
<th>Total protein (g/dL)</th>
<th>Level of significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>0.19±0.06</td>
<td>7.49±0.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group II (paracetamol)</td>
<td>0.12±0.15</td>
<td>7.19±0.23</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Group III (paracetamol+silymarin)</td>
<td>0.15±0.06</td>
<td>7.33±0.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group IV (paracetamol+300 mg aqueous extract)</td>
<td>0.17±0.02</td>
<td>7.38±0.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Group V (paracetamol+600 mg aqueous extract)</td>
<td>0.18±0.03</td>
<td>7.44±0.38</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>Level of significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>121.67±5.62</td>
<td>108.50±5.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group II (paracetamol)</td>
<td>349.50±15.86</td>
<td>219.83±10.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Group III (paracetamol+silymarin)</td>
<td>115.00±4.23</td>
<td>112.33±5.40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group IV (paracetamol+300 mg aqueous extract)</td>
<td>130.00±5.24</td>
<td>125.00±6.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Group V (paracetamol+600 mg aqueous extract)</td>
<td>122.87±5.52</td>
<td>110.17±5.62</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

levels of AST, ALT, bilirubin, protein, triglycerides and cholesterol was confirmed by biochemical analysis. The necrosis and inflammation caused by paracetamol in liver was restored using aqueous extract of C. indicum flower analyzed by histopathological studies. Phytochemical constituents such as phenol, alcohol, amine, and ester are strongly strengthen the use as hepatoprotective plant. Further studies were required to characterize the active principle for proper drug formulations.

REFERENCES