In vitro antioxidant activity and in vivo hepatoprotective activity of aqueous extract of Allium cepa bulb in ethanol induced liver damage in Wistar rats

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Abstract

The in vitro antioxidant and in vivo hepatoprotective effects of aqueous extract of Allium cepa (A. cepa) bulb were evaluated in male rats against ethanol induced liver damage in preventive and curative models. The antioxidant activity of A. cepa was assayed and activities were compared to standard antioxidant, ascorbic acid. The results revealed that the IC\textsubscript{50} values of A. cepa bulb extract for DPPH, hydroxyl, superoxide radical scavenging activities were 195.2 ± 0.2, 374.7 ± 0.4 and 182.5 ± 1.7 μg/mL, respectively. Liver injury was induced by 40% ethanol administration (3.76 g/kg bw, orally) for 25 days. In two different sets of experiments, the A. cepa extracts (100, 300 and 600 mg/kg bw) and silymarin (100 mg/kg bw) were administered orally in preventive and curative models. Ethanol administration caused severe hepatic damage in rats as evidenced by elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin levels. The A. cepa and silymarin administration prevented the toxic effect of ethanol on the above serum parameters in both preventive and curative models. The present study concludes that aqueous extract of A. cepa bulb has significant antioxidant and hepatoprotective activity against ethanol induced hepatotoxicity.

Keywords: Allium cepa; Antioxidant; Ethanol; Hepatoprotective; Rat

1. Introduction

Liver is the largest glandular organ of the body which plays a vital role in metabolizing carbohydrates, lipids, proteins and detoxifying xenobiotes and drugs. Thus the liver is prone to injury due to the chronic exposure to drugs, environmental toxicants and other xenobiotes [1]. Today, alcohol abuse is one of the major health problems worldwide. There is a close relationship between ethanol intake and alcoholic liver disease (ALD) as the 80% of ingested alcohol is metabolized in the liver [2]. In addition, ethanol is a main ingredient in most of the syrups, tinctures, and other medicines. In small doses it has a great medicinal value. But some people tend to have ethanol abuse [3]. In excess doses, it causes severe hepatic damage in humans and experimental animals. Chronic administration of ethanol is known to have a profound effect on the metabolism of lipids and lipoproteins. Moreover, ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase enzyme and acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase in the liver, giving rise to reactive oxygen species (ROS) via cytochrome P450 2E1 (CYP 2E1) [4,5]. This leads to oxidative stress in the hepatic cells which is the most striking initial manifestation of alcohol-induced liver injury [6,7]. When there is damage to the liver cell membrane, the cytosolic enzymes are leaked into the blood stream [8]. Therefore, the elevation of these cytosolic enzymes in the blood stream serves as a quantitative marker of hepatic damage.

In recent days, the use of herbal natural products has enhanced world-wide attentions. Many herbal supplements are claimed to assist in healthy lifestyle. Medicinally, herbal drugs have made a significant contribution to the treatment of hepatotoxicity [9]. Allium cepa, commonly known as garden onion, is the main representative genus of the Liliaceae family. A. cepa
is a bulbous plant widely cultivated with mass production in China, India and United States. It is rich in proteins, carbohydrates, sodium, potassium and phosphorus. Traditionally onion has been used to treat intestinal infections, eye infections, ear ache, urinary tract burning, headaches associated with drowsiness, ulcers on heels and cough resulted from inspiration of cold air. Many reports revealed that onion was found to have antibacterial, antiviral, antiparasitic, antifungal, anti-hypertensive, anti-hypoglycemic, antithrombotic, antihyperlipidemic, anti-inflammatory and antioxidant activities [10]. In recent decades, the extracts of various parts of the A. cepa have been extensively studied for their anti-diabetic [11], anti-tumor [12], hepatoprotective [13], and anti-nephrotoxicity [14] activities. However, protective roles of aqueous extract of A. cepa bulb, widely known as a culinary agent, in ethanol-induced hepatotoxicity have not been studied. Keeping these folkloric claims and reports in view, the present study attempted to evaluate the possible hepatoprotective effects of aqueous extract of A. cepa bulb in ethanol-induced hepatotoxicity in rats.

2. Materials and Methods

2.1. Chemicals and drugs

2.2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin estimated kits were purchased from Span Diagnostics, Surat, India. All other chemicals and reagents were of analytical grade.

2.2. Extract preparation

The fresh bulbs of A. cepa were washed and chopped into small pieces. 10 mL boiled distilled water was added to 5 g of coarsely powdered bulbs in a beaker. The mixture was kept in water bath with occasional stirring for 4 h. The dark brown aqueous extract was then filtered and reweighed with small volume of boiled distilled water, which was then added to the filtrate. The extract was then adjusted to 5 mL and used immediately.

2.3. Phytochemical analysis

Phytochemical screening of bulb extracts was carried out qualitatively for the presence of steroids, tannins, flavonoids, saponins, alkaloids, carbohydrates, glycosides, proteins, fats and oils [15].

2.4. In vitro antioxidant activities

2.4.1. DPPH scavenging assay

The DPPH radical scavenging activity was measured according to the method of Yang et al. [16]. The reaction mixture contained 2 mL of 95% ethanol, 0.1 mol/L DPPH and 2 mL of the A. cepa extract (50–300 μg/mL). The mixture was incubated at 25 °C for 15 min, and the absorbance was determined at 517 nm. Distilled water was used as the control. The scavenging activity of DPPH radicals in samples was calculated according to the following equation:

DPPH radical scavenging activity (%) = \( \frac{1 - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \),

where \( A_0 \) was absorbance of blank and \( A_1 \) was the absorbance of A. cepa extract.

2.4.2. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured according to the method of Winterbourn and Sutton [17]. The reaction mixture contained 1 mL of 0.15 mol/L phosphate buffer saline (pH 7.4), 1 mL of 40 g/mL safranin, 1 mL of 0.945 mmol/L EDTA–Fe (II), 1 mL of 3% (V/V) \( \text{H}_2\text{O}_2 \), and 0.5 mL of the A. cepa extract (50–300 μg/mL). After incubating at 37 °C for 30 min, the absorbance of samples was measured at 560 nm. The \( IC_{50} \) value of A. cepa is the effective concentration at which the hydroxyl radicals were scavenged by 50%.

The hydroxyl radical scavenging activity was expressed as:

Scavenging rate (%) = \( \frac{[A_0 - A_1]}{A_0} \times 100 \),

where \( A_0 \) was absorbance of the blank and \( A \) was the absorbance of samples.

2.4.3. Superoxide radical scavenging assay

Superoxide anion radical scavenging activity was determined according to the method of Stewart and Bewley [18]. The reaction mixture (3 mL) contained 13 mmol/L methionine, 10 mmol/L riboflavin, 75 mol/L nitroblue tetrazolium, 100 mmol/L EDTA, 50 mmol/L phosphate buffer (pH 7.8), and A. cepa extract (50–300 μg/mL). After illuminating the reaction mixture with a fluorescent lamp at 25 °C for 30 min, the absorbance of samples was measured at 560 nm. The scavenging rate was calculated using the following formula:

Scavenging rate (%) = \( \frac{[A_0 - A]}{A_0} \times 100 \),

where \( A_0 \) was absorbance of the blank and \( A \) was the absorbance of samples.

2.5. Animals study

Adult male albino Wistar rats (180 ± 20 g) were obtained from the Mahaveer Enterprizes, Hyderabad, India. They were kept under temperature of (23 ± 2)°C, humidity of 50% and light and dark cycles of 12:12 h. They were fed with commercial pellet diet (Rayon’s Biotechnology Pvt. Ltd., India) and water was provided ad libitum. The protocol was approved by Institutional Animal Ethics Committee and the lab was approved by CPCSEA, Government of India (Regd. No. 516/01/A/CPCSEA).
2.6. In vivo hepatoprotective study

2.6.1. Preventive study

The rats were divided into six groups, with 6 in each group. The feeding scheme was as follows:

Group 1: Normal control rats which received 2% gum acacia (0.1 g/200 g bw) for 25 days.
Group 2: Received 3.76 g/kg bw of ethanol (40%) for a period of 25 days.
Group 3: Received 3.76 g/kg bw of ethanol (40%) and 100 mg/kg bw of A. cepa extract simultaneously for 25 days.
Group 4: Received 3.76 g/kg bw of ethanol (40%) and 300 mg/kg bw of A. cepa extract simultaneously for 25 days.
Group 5: Received 3.76 g/kg bw of ethanol (40%) and 600 mg/kg bw of A. cepa extract simultaneously for 25 days.
Group 6: Received 3.76 g/kg bw of ethanol (40%) and 100 mg/kg bw of silymarin simultaneously for 25 days.

2.6.2. Curative study

Group 1: Normal control rats which received 2% gum acacia (0.1 g/200 g bw) for 50 days.
Group 2: Received 3.76 g/kg bw of ethanol (40%) for a period of 50 days.
Group 3: Received 3.76 g/kg bw of ethanol (40%) daily for a period of 25 days and then received 100 mg/kg bw of A. cepa extract for the next 25 days.
Group 4: Received 3.76 g/kg bw of ethanol (40%) daily for a period of 25 days and then received 300 mg/kg bw of A. cepa extract for the next 25 days.
Group 5: Received 3.76 g/kg bw of ethanol (40%) daily for a period of 25 days and then received 600 mg/kg bw of A. cepa extract for the next 25 days.
Group 6: Received 3.76 g/kg bw of ethanol (40%) for 25 days and then silymarin 100 mg/kg orally for the next 25 days.

Oral administration was applied in the study. Silymarin was used as reference hepatoprotective agent. In the preventive study, blood samples were collected on the 0 and 26th days and in the curative study, blood samples were collected on the 0, 26th and 51st days from retro-orbital plexus of rats. Blood samples were centrifuged at 3000 rpm for 30 min. The serum obtained was analyzed for aspartate aminotransferase (AST) [19], alanine aminotransferase (ALT) [19], alkaline phosphatase (ALP) [20] and total bilirubin [21] using semi-auto analyzer (Screen master-3000) and commercial diagnostic kits (Span Diagnostics, Surat, India).

2.7. Data and statistical analysis

All analyses were performed using statistical package for social sciences (SPSS) 13.0 for Windows (SPSS, USA). Data was expressed as mean with S.D. The significance was determined using Student’s paired t test. A P-value of less than 0.05 was considered statistically significant.

Table 1

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Aqueous extract of Allium cepa bulb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Proteins</td>
<td>−ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>−ve</td>
</tr>
<tr>
<td>Saponin</td>
<td>+ve</td>
</tr>
<tr>
<td>Fats and Oils</td>
<td>−ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>−ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>−ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
</tbody>
</table>

3. Results

3.1. The phytochemical screening of aqueous extract of A. cepa bulb

The phytochemical tests revealed that the bulbs of the plant contain carbohydrates, flavonoids, tannins and saponins (Table 1).

3.2. Effect of A. cepa extract against DPPH radicals

The free radical scavenging activity of A. cepa extract against DPPH radicals was shown in Fig. 1A. A. cepa extract and ascorbic acid standard showed antioxidant activity in a dose-dependent manner in the range of 50–600 µg/mL. The IC50 values for A. cepa and ascorbic acid were 195.2 and 159.7 µg/mL, respectively.

3.3. Effect of A. cepa extract against the hydroxyl radicals

The free radical scavenging activity of A. cepa extract against hydroxyl radicals was shown in Fig. 1B. A. cepa extract and ascorbic acid standard showed antioxidant activity in a dose-dependent manner in the range of 50–600 µg/mL. The IC50 values for A. cepa extract and ascorbic acid were 374.7 and 244.3 µg/mL, respectively.

3.4. Effect of A. cepa extract on the superoxide scavenging activity

The free radical scavenging activity of A. cepa extract against superoxide radical was shown in Fig. 1C. A. cepa extract and ascorbic acid standard showed antioxidant activity in a dose-dependent manner in the range of 50–600 µg/mL. The IC50 values for A. cepa and ascorbic acid were 182.5 and 97.2 µg/mL, respectively.

3.5. Determination of serum biochemical parameters

Results presented in Tables 2–5 indicated that levels of serum enzymes, namely AST, ALT, ALP and total bilirubin, were significantly (P<0.01) increased in ethanol treated rats compared with normal rats. However, levels of serum enzymes, like AST, ALT, ALP and total bilirubin, were significantly (P < 0.01)
increased in rats treated with A. cepa and silymarin compared to ethanol treated rats in both preventive and curative studies.

4. Discussion

Liver participates in a variety of enzymatic metabolic activities. Administration of ethanol causes elevation of serum ASP, ALT, ALP and total bilirubin levels in rats, indicating that ethanol may induce hepatocellular damages which in turn alters the structure and function of liver cells [22,23]. Our study on the ethanol induced hepatic damage are in accordance with previous reports [24]. Silymarin is a standardized extract of the milk thistle (Silybum marianum) chiefly contains flavonoid, including silybin, silybinin, silydianin and silychristin [25]. Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals. It functions through mechanisms of antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating [26]. Silymarin has been applied in alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxic and drug induced liver diseases and in diabetic patients in clinical settings. Silymarin may also be a

Table 2
Effect of aqueous extract of A. cepa bulb and silymarin on AST and ALT in Wistar rats (preventive study).

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST 0 day</th>
<th>26th day</th>
<th>ALT 0 day</th>
<th>26th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>28.5\textsuperscript{m} ± 2.35</td>
<td>29.5\textsuperscript{m} ± 3.57</td>
<td>29.5\textsuperscript{m} ± 4.87</td>
<td>30.6\textsuperscript{m} ± 4.62</td>
</tr>
<tr>
<td>ETH control (3.76 g/kg bw)</td>
<td>29.3\textsuperscript{m} ± 2.54</td>
<td>195.3\textsuperscript{m} ± 8.26</td>
<td>31.8\textsuperscript{m} ± 3.61</td>
<td>150.8\textsuperscript{m} ± 9.54</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (100 mg/kg bw)</td>
<td>28.8\textsuperscript{m} ± 3.57</td>
<td>140.8\textsuperscript{m} ± 5.84</td>
<td>30.6\textsuperscript{m} ± 4.62</td>
<td>110.5\textsuperscript{m} ± 7.86</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (300 mg/kg bw)</td>
<td>24.8\textsuperscript{m} ± 3.41</td>
<td>93.5\textsuperscript{m} ± 4.25</td>
<td>28.6\textsuperscript{m} ± 2.84</td>
<td>83.6\textsuperscript{m} ± 9.58</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (600 mg/kg bw)</td>
<td>27.5\textsuperscript{m} ± 5.24</td>
<td>80.4\textsuperscript{m} ± 3.68</td>
<td>32.7\textsuperscript{m} ± 3.42</td>
<td>60.8\textsuperscript{m} ± 5.81</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + silymarin (100 mg/kg bw)</td>
<td>22.7\textsuperscript{m} ± 4.32</td>
<td>65.2\textsuperscript{m} ± 5.51</td>
<td>30.6\textsuperscript{m} ± 4.12</td>
<td>53.8\textsuperscript{m} ± 8.25</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD of 6 rats.
ns = not significant; ETH = ethanol; A.C = A. cepa extract.
\* \( P<0.01 \), compared with normal control.
\# \( P<0.01 \), compared with ETH control.
Table 3
Effect of aqueous extract of A. cepa bulb and silymarin on ALP and total bilirubin in Wistar rats (preventive study).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP</th>
<th>Total bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>26th day</td>
</tr>
<tr>
<td>Normal rats</td>
<td>40.2±3.12</td>
<td>41.3±4.25</td>
</tr>
<tr>
<td>ETH control (3.76 g/kg bw)</td>
<td>38.2±4.62</td>
<td>229.6±5.34</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (100 mg/kg bw)</td>
<td>41.2±3.57</td>
<td>195.4±6.57</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (300 mg/kg bw)</td>
<td>42.6±4.91</td>
<td>154.6±7.54</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (600 mg/kg bw)</td>
<td>40.2±3.52</td>
<td>129.5±5.35</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + silymarin (100 mg/kg bw)</td>
<td>41.7±7.51</td>
<td>96.5±7.32</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD of 6 rats.
ns = not significant; ETH = ethanol; A.C = A. cepa extract.
* P<0.01, compared with normal control.
# P<0.01, compared with ETH control.

Table 4
Effect of aqueous extract of A. cepa bulb and silymarin on AST and ALT in Wistar rats (curative study).

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>26th day</td>
</tr>
<tr>
<td>Normal rats</td>
<td>29.6±2.5</td>
<td>35.5±4.7</td>
</tr>
<tr>
<td>ETH control (3.76 g/kg bw)</td>
<td>30.3±3.7</td>
<td>254.6±9.2</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (100 mg/kg bw)</td>
<td>27.8±2.8</td>
<td>195.8±7.3</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (300 mg/kg bw)</td>
<td>28.4±3.4</td>
<td>183.7±6.8</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + A.C (600 mg/kg bw)</td>
<td>30.8±4.5</td>
<td>170.5±6.2</td>
</tr>
<tr>
<td>ETH (3.76 g/kg bw) + silymarin (100 mg/kg bw)</td>
<td>30.6±5.6</td>
<td>157.3±8.4</td>
</tr>
</tbody>
</table>

Values are expressed as Mean± SD of 6 rats.
ns = not significant; ETH = ethanol; A.C = A. cepa extract.
* P<0.01, compared with normal control.
# P<0.01, compared with ETH control.

useful hepatoprotective drug for hepatobiliary diseases and hepatotoxicity induced by drugs. Moreover, it is used as a standard drug and exhibited potent hepatoprotective activity within the dose range of 25 to 200 mg/kg [27,28].

This study demonstrated that A. cepa aqueous bulb extract had reduced levels of AST, ALT and ALP which were elevated by ethanol administration. The results were in accordance with the findings of other investigators [13]. Moreover, it was reported that A. cepa leaf extract also can significantly restored the elevated AST, ALT and ALP enzyme levels to the normal levels [24]. Recently, Riyaz Shaik et al. [29] demonstrated that A. cepa leaves protected hepatocytes by preventing the release of these 3 enzymes. The study of Ogunlade et al. [30] demonstrated that administration of A. cepa by rabbits with alcohol abuse remarkably reduced serum levels of liver biomarker enzymes. Our results are consistent with earlier studies, which strongly suggest that A. cepa may protect the structural integrity of hepatocytes and prevent the release of cytosolic enzymes into bloodstream.

Phytochemical screening of aqueous extract of A. cepa bulb showed that it contains abundant carbohydrates, tannins, saponins, and flavonoids, a group of polyphenolic compounds [31]. Approximately 20 types of flavonoids were detected ub in onion species, with the two quercetin conjugates: quercetin-3, 40-O-diglucoside (QDG) and quercetin-40-O-monoglucoside.
(QMG) being the main flavonols representing up to 80–85% of the total flavonoid content [32]. The antioxidant activity in A. cepa bulb is mainly due to the presence of flavonoids [33]. The earlier studies have shown that the presence of organosulphur compounds like dipropyl disulphide, methyl-1-propanyl trisulphide and propyl-1-propenyl trisulphide in the A. cepa bulb [34]. In addition, phenolics, selenium, vitamin C and amino acids were also found to be responsible for antioxidant activity of two Egyptian onion varieties [35]. In the present study, A. cepa showed effective scavenging activities for DPPH, hydroxyl, and super oxide radical, suggesting that it could scavenge the free radicals generated during ethanol metabolism. This finding is consistent with previous studies that the antioxidant activity of A. cepa extract comes from rich sources of bioactive compounds, such as flavonoids [36–38], which quench ROS and regenerate membrane-bound antioxidants at both preventive and curative doses.

5. Conclusions

The present study demonstrated that the aqueous extract of A. cepa bulb protective against ethanol-induced hepatotoxicity which might be due to its antioxidant potential against DPPH, hydroxyl and superoxide radicals. The hepatoprotective role of A. cepa extract (600 mg/kg bw) was found to be comparable with Silymarin which might be due to the presence of flavonoid.

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References


