HEPATO PROTECTIVE EFFECT OF ARTICHOKE LEAVES AQUEOUS EXTRACT IN CCL4 INTOXICATED RATS

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ABSTRACT

The phytochemical screening of Artichoke leaves aqueous extract (ALE) was undertaken and its protective effect against carbon tetrachloride (CCL4)-induced hepatotoxicity in rats was studied. Fifty mature male rats were randomized into 5 equal groups as follows: (1) negative (normal) control, (2) positive (intoxicated non-treated) control, (3) standard (Silymarin-pretreated, 50 mg/kg), (4) pretreated with ALE in dose 200 mg/kg and (5) pretreated with ALE in dose 400 mg/kg, respectively. CCL4 was subcutaneously injected to all groups except the negative control during the last week of experiment to induce hepatotoxicity. Silymarin and ALE were orally given to rats in daily doses for 6 weeks. At the end of experiment, rats were weighed, sacrificed and blood samples were collected for serum biochemical analyses. Livers of the sacrificed rats were taken for assaying activities of antioxidant enzymes and histopathology. The phytochemical screening revealed that ALE contains flavonoids, saponins, alkaloids, tannins, glycosides, and devoid of resins and triterpenes. Oral pretreatments with ALE increased body weight gain; normalized serum levels of AST, ALT and ALP enzymes, total cholesterol, triglycerides, low density lipoprotein, and total bilirubin, and increased levels of total proteins. There were also increases in activities of tissue superoxide dismutase, glutathione peroxidase and catalase antioxidant enzymes with mitigation of renal tubular necrosis induced by CCL4 in the liver. These results denote that ALE has hepatoprotective, hypolipidemic and antioxidant effects in hepatotoxic rats. Therefore, intake of Artichoke leaves as herbal tea may be beneficial for patients who suffer from liver diseases.

Keywords: Artichoke, Hepatoprotective, Hypolipidemic, Antioxidant, Liver histopathology.
INTRODUCTION
Liver diseases resulting from liver damage due to oxidative stress is a global health problem. Despite of its frequent occurrence and high morbidity and mortality, the medical management of liver diseases is still inadequate. Drugs such as paracetamol and chemicals as CCl4 increase formation of reactive free radicals that cause oxidative stress and lipid peroxidation, and so damage the membranes of hepatocytes leading to swelling, degeneration, necrosis and fibrosis of hepatocytes. [1]

Hyperlipidemia is a major risk factor for coronary heart diseases (CHD) and is now considered the most common cause of death in both Western and Eastern countries. [2] Because of the synthetic chemical drugs prescribed for treating liver diseases and hyperlipidemia have many adverse side effects; there is a great need to search for alternative safe natural agents from medicinal plants and herbs.

Medicinal plants are a promising natural source of hepatoprotective, hypolipidemic and antioxidant constituents and they are beneficial in the treatment of liver diseases, hyperlipidemia and gallbladder disorders and in protection against poisoning by chemical and environmental toxicants. [3,4]

Artichoke (Cynara scolymus, Family Asteraceae) is an important component of the Mediterranean diet. Artichoke is rich in bioactive polyphenol compounds mainly cynarin, luteolin and chlorogenic acid as well as dietary fibers, vitamins and minerals. [5] Traditionally, Artichoke leaves were used for the treatment and prevention of many diseases. Artichoke has been used to treat dyspepsia mainly because of its choleretic effect that is associated with increased bile formation. [6] Artichoke extracts have been found to exhibit hepatoprotective activity; [7,8] lipid lowering property; [9,10,11,12] antioxidant effect [13,14] and reduce postprandial blood glucose [15] in man and experimental animals. Artichoke extracts also produced protective effects against hepatocellular carcinoma both in vitro [16] and in vivo. [17] Artichoke plant is traditionally used for treating liver diseases, gallbladder disorders and dyspepsia [6,7,8,10] as well as some conditions such as hyperlipidemia, overweight and obesity. [11,12,18]

The present study was designed to determine the phytochemical constituents of the aqueous extracts of Artichoke leaves qualitatively, evaluate its protective effect against CCL4-induced acute hepatotoxicity in rats and clarify the potential mechanisms of action.
MATERIAL and METHODS

Plant
Fully mature Artichoke (*Cynara scolymus*, CV Balady, Family *Asteraceae*) plant was purchased from a local green grocery market. The leaves were removed, pulverized, dried by freezing and kept till preparation of aqueous extracts.

Carbon Tetrachloride, Silymarin and Biochemical Kits
Carbon tetrachloride was purchased from El-Gomhoryia Company, Egypt in the form of 10% liquid solution. It was diluted with liquid paraffin at a ratio of 1:1 (v:v). Silymarin was obtained from Shanghai Realmus Industry Co., China, in form of brown soft gelatin capsules each containing 100 mg of 80% silymarin. Biochemical kits for determination of serum and liver tissue biochemical parameters were purchased from Sigma-Aldrich Company, USA.

Rats
Fifty adult male Sprague-Dawley rats weighing 160-165 g body weight and 8-9 weeks old were used in this study. Animals were obtained from the Laboratory Animal Colony, Helwan, Egypt. Rats were housed in a well ventilated animal room at Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Egypt under standard conditions of 24 °C temperature, 50% relative humidity and 12 hr light/12 hr dark cycles. Basal diet and water were provided to rats *ad libitum*.

Preparation of Basal Diet
The dietary supply of protein, fat, carbohydrates, vitamins and minerals was in accordance with the recommended dietary allowances for rats. Basal diet was consisted of 20% protein (casein), 10% sucrose, 5% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fibers (cellulose). The remainder was corn starch up to 100%.

Preparation of Aqueous Extracts
The leaves of Artichoke were removed, dried in shade, and pulverized. The dried plant materials 200 g of leaves were soaked in 1 liter of hot water at 50 °C for 5 hours, then filtered through double layers of muslin and centrifuged at 8000 rpm for 15 minutes to remove any plant debris. The aqueous extract was freezing dried and stored at -20°C till used. This procedure was described by Shalaby and Hamowieh.
Detection of Active Constituents
The qualitative phytochemical determination of active constituents of Artichoke extract was performed using standard screening protocols according to Harborne. [21]

Experiment Design
The experiment was performed on fifty adult Sprague Dawley male rats randomly distributed into 5 groups, of 10 rats each. Group (1) was kept negative (normal) control; and rats of the other 6 groups were subcutaneously injected by 2 ml/kg/rat of CCL4 diluted with liquid paraffin (1:1, v:v) during the last week of experiment to induce acute hepatotoxicity. [22] Group (2) was kept positive control (model) group and group (3) was pretreated with Silymarin (reference hepatoprotective drug) orally in a daily dose 50 mg/kg b.wt for 6 weeks. Groups (4) and (5) were orally pretreated with the aqueous extract of Artichoke leaves (ALE) in daily doses of 200 and 400 mg/kg b.wt, respectively, for 6 weeks. At the beginning and end of experiment period, the initial and final body weights of rats were recorded and body gains were calculated. Rats were then euthanized by prolonged exposure to ether anesthetic and blood samples were withdrawn by cardiac puncture. Blood was left to clot at room temperature and centrifuged at 8000 rpm for 15 minutes for separating the serum which kept frozen till biochemical analyses. Rats were then sacrificed and halve of the livers was used for preparing tissue homogenates to assay the activity of antioxidant enzymes. The other halve was preserved in 10% formalin solution till processed for histopathological examination.

Biochemical Analyses
Serum aspartate aminotransferase and alanine aminotransferase [23] and alkaline phosphatase [24] liver enzymes; total cholesterol; [25] triglycerides [26] and high density lipoprotein cholesterol [27] were chemically determined using specific kits. Low density lipoprotein cholesterol was calculated using the formula of Friedewald et al., [28] Atherogenic index (LDL-c/ HDL-c) was calculated according to Nwagha et al. [29] Serum total protein (TP) was chemically determined [30] and albumin (Alb) and globulin (Glb) were determined according to the method of Fernandez et al. [31] Serum total bilirubin (TBil) was also estimated. [32]

Assay of Antioxidant Enzymes
One gram of liver tissue was washed with ice-cooled 0.9% NaCl solution and then homogenized in 100 ml of ice-cooled of 1.5% potassium chloride solution and 50 mMol potassium phosphate buffer solutions (pH 7.4) to yield 1% tissue homogenate (w/v).
Homogenization was performed using Sonicator, 4710 Ultrasonic Homogenizer. Liver homogenates were centrifuged at 8000 rpm for 15 minutes at 4°C. The supernatants were used for estimation of the activity of antioxidant enzymes glutathione peroxidase, \[33\] superoxide dismutase\[34\] and catalase.\[35\]

**Histological Procedure**

Halve of livers of the sacrificed rats was taken and preserved in 10 % neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol (60%, 70%, 80% and 90%). The specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H&E stain) then examined under microscope.\[36\]

**Statistical Analysis**

Data were presented as means ± SE. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Duncan’s multiple range tests \[37\]. The analysis was done with SPSS computer program (version 15, Chicago, USA). Differences between the experimental groups were considered significant at $P<0.05$ level.

**RESULTS**

The phytochemical screening of Artichoke leaves aqueous extract (ALE) revealed that it contains large amounts of flavonoids and saponins; moderate amounts of alkaloids and tannins and small amount of glycosides. The extract is devoid of resins and triterpenes as recorded in table 1.

<table>
<thead>
<tr>
<th>Active constituents</th>
<th>Phytochemical tests</th>
<th>Observation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>1-Mayer’s reagent</td>
<td>White precipitate</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2-Drangendorff’s reagent</td>
<td>Orange precipitate</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>3-Wagner’s reagent</td>
<td>Brown precipitate</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1-Shinoda test</td>
<td>Red color</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>2-Sodium hydroxide test</td>
<td>Yellow color</td>
<td>+++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>1-Benedict test</td>
<td>Bluish precipitate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2-Fehling test</td>
<td>Red precipitate</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>1-Shaking test</td>
<td>Persistent froth</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>2-Silver nitrate test</td>
<td>Mirror formation</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>1-Ferric chloride test</td>
<td>Bluish precipitate</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2-Lead acetate test</td>
<td>Whitish precipitate</td>
<td>++</td>
</tr>
</tbody>
</table>
Rats inflicted with acute hepatotoxicity induced by CCL4 gained less body weight when compared with normal control rats. Rats orally pretreated with Silymarin (reference drug) and ALE for 6 weeks had significant ($P < 0.05$) increases in the body weight gain when compared with the positive control group. The effect of Silymarin on body weight gain was better than ALE as recorded in table 2.

Acute intoxication of rats by CCL4 induced significant increases ($P < 0.05$) in serum levels of liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) when compared to the negative control group. Compared to the positive control, both Silymarin and ALE when orally given to CCL4-intoxicated rats significantly ($P < 0.05$) lowered the high serum levels of AST, ALT and ALP as depicted in table 3.

### Table 2. Effect of aqueous extract of Artichoke leaves (ALE) on body weight (B.wt) and body weight gain (BWG) in CCL4-intoxicated rats. (n= 10 rats).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial B.wt (gm)</th>
<th>Final B.wt (gm)</th>
<th>BWG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Group (1) Negative Control</td>
<td>Week 0</td>
<td>Week 6</td>
</tr>
<tr>
<td></td>
<td>180.0 ± 4.0$^a$</td>
<td>230.50 ± 2.55$^a$</td>
<td>28.05 ± 1.65$^a$</td>
</tr>
<tr>
<td></td>
<td>Group (2) Positive Control</td>
<td>185.0 ± 6.5$^a$</td>
<td>205.00 ± 3.52$^{ef}$</td>
</tr>
<tr>
<td></td>
<td>Group (3) Silymarin (50 mg/kg)</td>
<td>188.0 ± 5.4$^a$</td>
<td>228.00 ± 3.45$^{b_{es}}$</td>
</tr>
<tr>
<td></td>
<td>Group (4) ALE (200 mg/kg)</td>
<td>190.0 ± 8.1$^a$</td>
<td>225.50 ± 4.37$^b$</td>
</tr>
<tr>
<td></td>
<td>Group (5) ALE (400 mg/kg)</td>
<td>189.0 ± 5.3$^a$</td>
<td>224.00 ± 4.30$^b$</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test.

- $^\dagger$ Significant when compared with the negative group.
- $^\S$ Significant when compared with the positive group.
Table 3. Effect of aqueous extract of Artichoke leaves (ALE) on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in CCL4- intoxicated rats. (n= 10 rats).

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>42.0 ± 2.66\textsuperscript{d}</td>
<td>38.0 ± 1.12\textsuperscript{d}</td>
<td>95.5 ± 2.15\textsuperscript{d}</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (2)</td>
<td>81.0 ± 4.34\textsuperscript{a}</td>
<td>59.0 ± 2.14\textsuperscript{a}</td>
<td>112.0 ± 5.17\textsuperscript{a}</td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (3)</td>
<td>45.0 ± 3.67\textsuperscript{c}</td>
<td>44.0 ± 2.43\textsuperscript{c}</td>
<td>96.0 ± 4.56\textsuperscript{c}</td>
</tr>
<tr>
<td>Silymarin (50 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (4)</td>
<td>77.0 ± 3.32\textsuperscript{b}</td>
<td>50.0 ± 3.51\textsuperscript{b}</td>
<td>103.0 ± 5.11\textsuperscript{b}</td>
</tr>
<tr>
<td>ALE (200 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (5)</td>
<td>69.0 ± 5.14\textsuperscript{b}</td>
<td>47.0 ± 2.12\textsuperscript{b}</td>
<td>100.0 ± 4.77\textsuperscript{b}</td>
</tr>
<tr>
<td>ALE (400 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at \( P < 0.05 \) using one way ANOVA test.

\( \dagger \) Significant when compared with the negative control group.

\( \S \) Significant when compared with the positive control group.

It is clear from table 4 that rats acutely intoxicated by CCL4 had significant high serum levels of total cholesterol (TC) and triglycerides (TG) when compared with the negative control group. Oral pretreatments with Silymarin and ALE in CCL4-intoxicated rats for 6 weeks significantly (\( P < 0.05 \)) normalized the high levels of serum TC and TG when compared with the positive control group.

Acute intoxication of rats by CCL4 caused significant decreases in high density lipoprotein (HDL) cholesterol, and increases in low (LDL), very low density lipoprotein (VLDL) and atherogenic index (AI) when compared with the negative control group. Oral administration of Silymarin and ALE for 6 weeks to CCL4-intoxicated rats significantly (\( P <0.05 \)) increased serum HDL and decreased LDL, VLDL and AI when compared with the positive control group (Table 5).

Table 4. Effect of aqueous extract of Artichoke leaves (ALE) on serum levels of total cholesterol (TC) and triglycerides (TG) in CCL4-intoxicated rats. (n= 10 rats).

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>95.50 ± 3.6\textsuperscript{d}</td>
<td>80.00 ± 2.8\textsuperscript{d}</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test.

† Significant when compared with the negative group.

§ Significant when compared with the positive control group.

### Table 5. Effect of aqueous extract of Artichoke leaves (ALE) on serum levels of high (HDL), low (LDL) and very low density lipoprotein (VLDL) cholesterol and atherogenic index (AI) in CCL4-intoxicated rats. (n= 10 rats)

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>AI LDL/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) Negative Control</td>
<td>64.00 ± 3.5$^d$</td>
<td>15.50 ± 0.2$^d$</td>
<td>16.00 ± 0.2$^d$</td>
<td>0.242</td>
</tr>
<tr>
<td>Group (2) Positive Control</td>
<td>58.90 ± 5.1$^a^f$</td>
<td>46.80 ± 0.5$^a^f$</td>
<td>27.10 ± 0.4$^a^f$</td>
<td>0.794</td>
</tr>
<tr>
<td>Group (3) Silymarin (50 mg/kg)</td>
<td>73.10 ± 4.6$^c^f$§</td>
<td>28.50 ± 0.2$^c^f$§</td>
<td>19.00 ± 0.3$^b^g$</td>
<td>0.389</td>
</tr>
<tr>
<td>Group (4) ALE (200 mg/kg)</td>
<td>71.20 ± 4.7$^b$</td>
<td>25.90 ± 0.3$^b$</td>
<td>18.90 ± 0.3$^b$</td>
<td>0.363</td>
</tr>
<tr>
<td>Group (5) ALE (400 mg/kg)</td>
<td>71.80 ± 3.6$^b$</td>
<td>20.60 ± 0.2$^b$</td>
<td>18.10 ± 0.1$^b$</td>
<td>0.286</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test.

† Significant when compared with the negative group.

§ Significant when compared with the positive control group.

Subcutaneous injection of CCL4 in rats significantly ($P < 0.05$) decreased serum levels of total proteins (TP), albumin (Alb), globulin (Glb) and increased total bilirubin (TBil) when compared with the negative control group. Silymarin and ALE significantly ($P < 0.05$) normalized serum levels of TP, Alb, Glb and TBil when compared with the positive control group as recorded in table 6.
Table 6. Effect of aqueous extract of Artichoke leaves (ALE) on serum levels of total proteins (TP), albumin (Alb), globulin (Glb) and total bilirubin (TBil) in CCL4-intoxicated rats. (n=10 rats).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TP (g/dL)</th>
<th>Alb (g/dL)</th>
<th>Glb (g/dL)</th>
<th>TBil (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) Negative control</td>
<td>7.30 ± 1.07a</td>
<td>2.93 ± 0.22a</td>
<td>3.80 ± 0.30a</td>
<td>0.19 ± 0.04d</td>
</tr>
<tr>
<td>Group (2) Positive Control</td>
<td>3.16 ± 0.05df</td>
<td>1.40 ± 0.15ef</td>
<td>1.90 ± 0.60d†</td>
<td>0.58 ± 0.05a†</td>
</tr>
<tr>
<td>Group (3) Silymarin (50 mg/kg)</td>
<td>7.20 ± 0.09g</td>
<td>1.95 ± 0.11b§</td>
<td>3.70 ± 0.44b§</td>
<td>0.21 ± 0.02a§</td>
</tr>
<tr>
<td>Group (4) ALE (200 mg/kg)</td>
<td>5.20 ± 1.03b</td>
<td>1.80 ± 0.14b</td>
<td>2.55 ± 0.33c</td>
<td>0.37 ± 0.2b</td>
</tr>
<tr>
<td>Group (5) ALE (400 mg/kg)</td>
<td>5.70 ± 1.04b</td>
<td>1.80 ± 0.13b</td>
<td>2.60 ± 0.52b</td>
<td>0.33 ± 0.2b</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at P < 0.05 using one way ANOVA test.

† Significant when compared with the negative group.
§ Significant when compared with the positive control group.

Rats inflicted with acute hepatotoxicity by injection of CCL4 had significant (P < 0.05) decreases in the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes in liver tissue when compared with the negative control group. Oral administration of Silymarin and ALE to CCL4-intoxicated rats for 6 weeks increased the activity of SOD, GPx and CAT enzymes in hepatic tissue as compared to the positive control group (Table 7).

Table 7. Effect of aqueous extract of Artichoke leaves (ALE) on activities of hepatic superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes in CCL4-intoxicated rats. (n= 10 rats).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SOD (U/mg protein)</th>
<th>GPx (nmol/min/mg protein)</th>
<th>CAT (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) Negative Control</td>
<td>62.40 ± 2.30a</td>
<td>0.92 ± 0.04a</td>
<td>0.187 ± 0.02a</td>
</tr>
<tr>
<td>Group (2) Positive Control</td>
<td>47.60 ± 2.22af</td>
<td>0.42 ± 0.01ef</td>
<td>0.144 ± 0.01ef</td>
</tr>
<tr>
<td>Group (3) Silymarin (50 mg/kg)</td>
<td>60.55 ± 4.16bg</td>
<td>0.53 ± 0.02bg</td>
<td>0.175 ± 0.01bg</td>
</tr>
<tr>
<td>Group (4) ALE (200 mg/kg)</td>
<td>57.66 ± 2.55c</td>
<td>0.56 ± 0.03b</td>
<td>0.168 ± 0.03b</td>
</tr>
<tr>
<td>Group (5) ALE (400 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALE (200 mg/kg)</td>
<td>ALE (400 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (5)</td>
<td>59.33 ± 2.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.62 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.173 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at \( P < 0.05 \) using one way ANOVA test.

† Significant when compared with the negative group.
§ Significant when compared with the positive group.

Unit of GPx = nmol of GSH utilized/min/mg protein.
Unit of CAT = nmol of \( \text{H}_2\text{O}_2 \) utilized/min/mg protein.

Histopathological examination of liver sections of normal control rats showed normal histological structure of hepatic lobule with normal hepatocytes and hepatic sinusoids (Fig. 1). Injection of CCL4 in rats induced severe fatty degeneration with leukocyte inflammatory cells infiltration (Fig. 2), associated with coagulative necrosis of hepatocytes and dilated ventral vein (Fig. 3). Liver sections of rats given orally Silymarin (reference drug) revealed almost normal histological architecture of hepatic lobule (Fig. 4). The small dose (200mg/kg) of Artichoke leaves extract (ALE) showed relative regression of hepatic pathological lesions and only mild fatty degeneration and necrosis were seen (Fig. 5). Examination of liver of rats pretreated with the large dose 400 mg/kg of Artichoke ALE revealed almost normal histological architecture of hepatic lobule (Fig. 6).

Fig. 1. Liver section of a normal control rat showing normal architecture with normal central vein, portal tract, hepatocytes and sinusoids. (H&E X 200).
Fig. 2. Liver section of a rat injected with CCL4 (positive control) showing severe fatty
degeneration and necrosis (arrows) with leukocyte cells infiltration (arrow). (H&E X 200).

Fig. 3. Liver section of a rat injected with CCL4 showing coagulative necrosis of hepatocytes (arrow) and dilated central vein (arrow). (H&E X 200).
Fig. 4. Liver section of a rat pretreated with 50 mg/kg of silymarin showing almost normal histological structure of hepatic lobule. (H&E X 200).
Fig. 5. Liver section of a rat pretreated with 200 mg/kg of Artichoke leaves extract (ALE) showing mild fatty degeneration and necrosis of hepatocytes (arrow). (H&E X 200).
Fig. 6. Liver section of a rat pretreated with 400 mg/kg of ALE showing nearly normal histological structure of hepatic lobule. (H&E X 200).

DISCUSSION

The current study was designed to determine qualitatively the phytochemical constituents of aqueous extract of Artichoke leaves, to assess its effects against CCL4-induced hepatotoxicity in rats and clarify the potential mechanisms of action.

There is a great need to search for much safe natural agents to be used for treating liver diseases and hyperlipidemia because of the chemical drugs prescribed for therapy are usually associated with deleterious side effects. Medicinal plants, vegetables and fruits with hepatoprotective and antihyperlipidemic activities have gained much attention, especially those with low toxicity. The biological value of the plant materials depends on their bioactive chemical constituents such as saponins, anthocyanins, flavonoids, polyphenols, triterpenes and other phytochemicals. \[3,4\]

Artichoke (Cynara scolymus L., Family Asteraceae) is commonly eaten as a vegetable and its leaves are frequently used in folk medicine in the treatment of liver diseases, \[7, 8, 9\] hyperlipidemia, dyslipidemia and obesity, \[18, 38\] gall bladder disorders, \[6\] acute gastritis and gastric ulcer \[39\] and atherosclerosis. \[40\]

In this study, the phytochemical screening of Artichoke leaves extract (ALE) showed that it contains flavonoids, alkaloids, glycosides, saponins, and tannins, and is devoid of resins and triterpenes. These results were in accordance with the previous reports. \[39, 41, 42\] Moreover, the authors isolated and characterized the bioactive phenolic constituents from fresh and canned Artichoke by HPLC. \[41, 42\] It was reported that Artichoke plant can be regarded as a functional food and also as a promising source of potent antioxidant polyphenolic compounds. \[42\]
Carbon tetrachloride (CCl4) is a selective hepatotoxic chemical agent that commonly used for induction of hepatotoxicity in rats. [22] CCl4 produced reactive free radicals (trichloromethyl radical, CCl3) which initiate cell damage via either covalent binding to cell membrane proteins or by induction of lipid peroxidation which causes hepatic cell damage and leads to liver cirrhosis. [43]

Results of the present study showed that acute hepatotoxicity induced by CCL4 in rats characterized by decreased body weight gain; elevated serum levels of liver enzymes (AST, ALT, and ALP); total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL) and total bilirubin (TBil.); decreased serum levels of total proteins (TP) and decreased activities of hepatic tissue antioxidant enzymes. These serum biochemical alterations were confirmed by histopathological lesions manifested by presence of fatty degeneration and coagulative necrosis upon examination of liver sections. These results were similar to the previous reports. [22, 43, 44] The previous authors concluded that subcutaneous injection of CCL4 in rats causes body weight loss, elevates serum liver enzymes (AST, ALT and ALP) and produces hyperlipidemia. Acute intoxication by CCL4 in rats also damaged liver tissues causing fatty degeneration and necrosis. [7, 8, 44, 45]

Artichoke (Cynara scolymus) is one of the most ancient plants grown in the world and its extracts obtained from different parts of the plant (leaves, pulps and roots) have been used as medicaments from time immemorial. The present results revealed that aqueous extracts of Artichoke leaves (ALE) improved body weight gain in CCL4-intoxicated rats. This finding was similar to that previously reported by Mehmatcik et al. [7] who concluded that pretreatment with Artichoke extracts increased the body weight of CCL4-intoxicated rats. The authors attributed this effect to the antioxidant protection effect of Artichoke extract. The improvement of body weight gain by Artichoke was also explained by its choleretic effect that is associated with increased bile formation with subsequent improving digestion of food. [6]

In this study, oral administration of Artichoke leaves (ALE) to CCl4- intoxicated rats exhibited hepatoprotective effect. This effect was evident from the significant decreases in the elevated serum levels of liver enzymes (AST, ALT and ALP) in CCL4-intoxicated rats. This finding agreed with that previously reported by Mehmatcik et al. [7] in rats and by Sannia [8] in hypercholesterolemic patients. The hepatoprotective effect of ALE could be attributed to its antioxidant activity that is evident in this study and in previous studies in rats.
and in humans. The previous authors concluded that Artichoke could be regarded as a bioactive functional food and also as a promising source of antioxidant phenolic compounds.

The results showed that ALE lowered the elevated serum levels of total cholesterol, triglycerides, and low density lipoprotein cholesterol in CCL4-intoxicated rats. These results agreed with the previous findings in both rats and humans. The hypocholesterolemic activity of Artichoke extract was attributed to inhibition of HMG-CoA reductase (3-hydroxy-3-methyl–glutaryl CoA reductase), an enzyme that responsible for synthesis of cholesterol in the liver. The present results also showed that ALE normalized the lowered serum total proteins and the elevated total bilirubin in CCL4 intoxicated rats. These results were in agreement with those of the previous findings. It is well known that liver diseases are usually accompanied by hypoproteinemia and high serum total bilirubin levels.

In the current study ALE increased the activity of tissue antioxidant enzymes (SOD, GPx and CAT) in liver of CCL4-intoxicated rats. These findings were similar to the results of the previous studies. The previous authors concluded that the protective activity of Artichoke could be attributed to its constituents of many bioactive polyphenolic antioxidant compounds, mainly cynarin, luteolin, and chlorogenic acids which are abundant in leaves.

The alterations of serum biochemical parameters induced by ALE were confirmed by regression of histopathological degenerative changes seen in the liver of CCL4-intoxicated rats. The amelioration of histopathological lesions (fatty degeneration and necrosis) by oral administration of ALE was in accordance with those previous reports. In the present study, the serum and tissue biochemical changes induced by pretreatment of CCL4-intoxicated rats with ALE were to parallel to the observed histopathological findings, denoting hepatoprotective activity of Artichoke extracts.

CONCLUSION

The aqueous extract of Artichoke leaves produces hepatoprotective, hypolipidemic and antioxidant effects and mitigates the degenerative changes induced by CCL4 in liver of rats. These results affirm the traditional use of Artichoke plant for treating liver diseases. Intake of Artichoke leaves as herbal tea may be beneficial for patients suffering from liver diseases and hyperlipidemia.
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