Anti-inflammatory and hepatoprotective activities of methanolic extract of *Anthemis scrobicularis* herbs

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ABSTRACT

The anti-inflammatory and hepatoprotective activities of the methanolic extract of *Anthemis scrobicularis* (ANS) herbs were evaluated in rats against carrageenan induced inflammation and carbon tetrachloride (CCl₄) induced hepatic injury. To evaluate the anti-inflammatory effects of ANS, twenty male rats were divided into four equal groups. Injection of 100 μl carrageenan in normal saline into the subplantar region of the hind paw of rats clearly induced paw edema. The volume of paw edema was attenuated following oral administration of ANS. For hepatoprotective effects, twenty five rats were equally divided into five groups. The hepatotoxicity, induced by a single dose of CCl₄, produced significant (p<0.001) increase of the levels of serum transaminase, phosphatase, bilirubin and a decrease in proteins were also noticed. The oxidative stress marker such as malondialdehyde (MDA) was increased and non-protein sulfhydryl (NP-SH) was decreased in the hepatotoxic tissues. Pre-medication of CCl₄-intoxicated rats with ANS at the doses 250 and 500 mg/kg reversed the abnormal liver diagnostic stricture. The results showed that ANS is toxicologically safe when orally administered and possess highly significant anti-inflammatory and hepatoprotective activities and the potentials usefulness of *Anthemis scrobicularis* in hepatic and inflammatory disease.

Key words: *Anthemis scrobicularis*, Anti-inflammatory, Hepatoprotective, Carbon tetrachloride, Histopathology.

INTRODUCTION

Anthemis is the second largest genus of the tribe Anthemideae, comprises of nearly 210 species.¹,² It is represented in Saudi Arabia by 19 species.³ *Anthemis scrobicularis* Yavin, Fam. Asteraceae is an annual herb, growing in sand dunes and sandy areas, Arabian peninsula Jordan, and Palestine.³ There is wide interest in the research of the plants of Anthemis, especially their active components, because many of the plants are reported to have antifungal⁴ antioxidant,⁵ antitumour, antiplasmodial, anthelmintic, schistosomicidal, cytotoxic, phytotoxic, analgesic activities⁶ and for treatment of cystitis and dental afflictions.⁷ Extracts, tinctures, salves, tisanes, infusion, decoction and other traditional formulations of same or related species are widely used for treatment of inflammation, dysmenorrhea, hepatotoxicity, hemorrhoid, abdominal pain and different types of skin inflammation in the European folk medicine.⁸⁻¹¹ The occurrence of sesquiterpene lactones, flavonoids, sterols, fatty acids, polyacetylenes and essential oils in various Anthemis species has been reported in previous works.¹²⁻¹⁶ These phytochemicals have been previously reported for anti-inflammatory, antioxidant and hepatoprotective activities.¹⁷⁻²⁰ Nonsteroidal anti-inflammatory drugs are among the most common drugs associated with drug-induced liver injury, with an estimated incidence of between 3 and 23 per 100,000 patient and the drugs like Nimesulide, sulindac, and diclofenac seem to be associated with the highest risk factor.²¹ As per our knowledge, there is no previous report on the activity of this plant. In the present study, we report the safety, anti-inflammatory and anti-hepatotoxicity effectiveness of the methanolic extracts of *Anthemis scrobicularis* for the first time.

MATERIALS AND METHODS

Materials

All the chemical and reagents procured were analytical grade. Carrageenan was purchased from BDH Chemicals Ltd., UK, while other chemicals were purchased from Sigma Aldrich.
Collection and Authentication of plant

The aerial parts of *Anthemis scrobicularis* Yavin, Fam. *Asteraceae* collected from the sandy areas near AlKharj governorate, Saudi Arabia in April 2013. The plant was kindly authenticated by Dr. Yousef Yaquob, College of Pharmacy, Department of Pharmacognosy, King Saud University, Al-Riyadh, Kingdom of Saudi Arabia. The plant material was air dried and reduced to fine powder.

Extraction

Powdered *Anthemis scrobicularis* (2 Kg) was extracted by percolation in methanol (3 × 4L) at room temperature for 3 days. The combined methanolic extract was concentrated in rotary evaporator at 45°C to 500 ml and then diluted with distilled water (500 ml). Lead acetate solution was then added drop wise until no more precipitate is formed then filtered. The filtrate was extracted with chloroform (4 × 200 ml) and concentrated in rotary evaporator at 45°C to afford yellowish brown residue (20 g).

Toxicological and Pharmacological evaluation:

Swiss albino mice (25–30 g b. wt) and Wistar albino rats (200-250g) of both sexes were used for anti-inflammatory and hepatoprotective studies. The animals were obtained from Lab Animal Care Unit, Pharmacy College, King Saud University, Riyadh, KSA. The animals were housed in the animal house of the Department of Pharmacology, College of Pharmacy, Salman Bin Abdulaziz University, Al-Kharj, KSA for acclimatization. The animals were kept in groups of five per cage under standard environmental conditions of temperature and light/dark (12/12 h) cycles, and provides commercial rat or mice feed and tap water given ad libitum. The animals were allowed to acclimate to the laboratory condition for one week before commencement of the experiment. The experiments and procedures used were approved by the Ethical Committee of the College of Pharmacy, King Saud University, Riyadh.

Determination of acute toxicity and median lethal dose (LD50) of the extracts

LD₅₀ of *A. scrobicularis* were determined according to the reported method.[24] Mice were divided into groups of 5 animals and the tested extracts were administered orally in doses of 0.1 to 5g/kg body weight. Signs of acute toxicity and number of death per dose within 24 h were recorded and the LD₅₀ was calculated.

Carrageenan-induced rat hind paw edema

Wistar rats were fasted for 16 h and were divided into 4 groups, each containing 5 individuals.[25] The control group was given 5 ml/kg of normal saline. The test groups of rats were treated orally by 250 and 500mg/kg ANS separately. The reference group was given 100mg/kg of an aqueous solution of phenylbutazone. One hour later, paw edema was produced by injecting 100 μl of 1% solution of carrageenan in saline into the left hind paw. Paw volume was measured before and after carrageenan injection up to 3 h, using a water displacement plethysmometer (plethysmometer (Ugo Basile 7150)).

Hepatoprotective study

The Hepatoprotective activity was evaluated in Wister albino rats using CCl₄ induced liver injury.[26] The rats were divided into 5 groups (5 animals each) and were treated in accordance, Group-one was served normal saline (control); Group-two was served 1.25ml/kg of CCl₄ (hepatotoxic), Group-three and four were served 250 and 500 mg/kg b.w. respectively (ANS extracts), Group-five was served silymarin (positive control). The animals were killed under light ether anesthesia 24 h after the last treatment. Blood was collected by cardiac puncture in plain tubes and their livers and kidneys were removed immediately after necropsy. Serum was separated by centrifugation at 3000 rpm at 4°C for 10 min. 10% (w/v) liver homogenate was prepared in 0.25M sucrose solution and centrifuged at 7000 rpm for 10 min at 4°C.

Assessment of liver function

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by Reitman and Frankel methods,[27] serum alkaline phosphatase (ALP) levels were estimated by King and Armstrong method,[28] gamma glutamyl transpeptidase (GGT) activity was determined by Szas method[29] and the bilirubin level in serum was determined by modified DMSO method of Walters and Gerarde.[30] The protein concentration was determined according to the Lowry et al., method[31] using bovine serum albumin (BSA) as a standard.

Assessment of oxidative stress

Malondialdehyde (MDA) was determined by Ohkawa et al., methods[32] and Non-Protein sulphydryls (NP-SH) was determined by according to the Sedlak and Lindsay method[33] in liver tissue.
Histopathological studies of liver and kidneys

Liver and kidneys were perfused with cold saline at 4°C and excised immediately. A small fragment of liver and kidney tissues was placed in 10% formalin (diluted to 10% with normal saline) for 1 hr. For histological studies, a portion of the liver or the kidney was fixed in ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight and 100% isopropyl alcohol for 1 hour and finally paraffin wax (four times 1h). Tissues were transferred into paraffin waxed filled moulds. The rotary microtome (Leitz 1512) was used for making the section (3 μm). The sections were placed on clean slides and placed onto warming table at 37-40°C. The slides were stained for 15min with Mayer’s haematoxylin solution; washed for 15 minutes in lukewarm running tap water then distilled water for 2 minutes, then finally with 80% ethyl alcohol. The slides were then counter stained for 2 minutes with eosin-phloxine solution. Histological observations were made under light microscope.

Statistical Analysis: Data recorded was analyzed as mean ± SEM (standard error of mean) in each group. Differences between groups were determined by unpaired Student’s t-test.

RESULT

Toxicity study

The results indicated that different doses of ANS (up to 5000 mg kg) did not produce any symptoms of acute toxicity.

Anti-inflammatory activity:

Oral administration of the crude extract of ANS (250-500 mg/kg) caused significant (P<0.001) inhibition of edema induced by the injection of carrageenan.

In (tabl-1), the carrageenan-induced rat paw edema at 2h was 2.17±0.02 mL. The mean reduction in rat paw edema carrageenan with phenylbutazone (PBZ) was 1.28±0.04 mL. The mean reduction in rat paw edema of 250 and 500mg/kg ofANS extracts was 1.70±0.05 and 1.34±0.03 mL respectively.

Assessment of liver function

The examination of ALT, AST and GGT were given in (Table 2). CCl₄ (1.25ml/kg) significantly (p<0.001) elevated the serum activities of ALT, AST and GGT when compared to the normal saline animals. Administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
<th>GGT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (2ml/kg, p.o.)</td>
<td>75.40 ± 13.26</td>
<td>32.86 ± 2.39</td>
<td>4.70 ± 0.28</td>
</tr>
<tr>
<td>CCl₄ (1.25ml/kg,i.p.)</td>
<td>211.33 ± 10.08***</td>
<td>194.16 ± 7.53***</td>
<td>11.25 ± 0.37***</td>
</tr>
<tr>
<td>A. scrobicularis +CCl₄ (250mg/kg, p.o.)</td>
<td>153.50 ± 5.51***</td>
<td>134.66 ± 2.45***</td>
<td>7.76 ± 0.19***</td>
</tr>
<tr>
<td>A. scrobicularis +CCl₄ (500mg/kg,p.o.)</td>
<td>134.00 ± 4.19***</td>
<td>123.66 ± 5.04***</td>
<td>6.65 ± 0.18***</td>
</tr>
<tr>
<td>Silymarin(10 mg/kg, i.p.)</td>
<td>124.16 ± 7.13***</td>
<td>112.93 ± 6.13***</td>
<td>5.83 ± 0.25***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n=5, ***P< 0.001. An anti-inflammatory extract of A. scrobicularis + CCl₄ (250 and 500mg/kg, bw) showed a statistically significant result.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Increased in rat paw edema (ml±SEM)</th>
<th>Net Reduction</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrageenan (1%)</td>
<td>1.05 ± 0.03</td>
<td>2.17 ± 0.02</td>
<td>1.12 ± 0.02</td>
</tr>
<tr>
<td>A. scrobicularis + Carrageenan</td>
<td>1.08 ± 0.03</td>
<td>1.70 ± 0.03</td>
<td>0.61 ± 0.02***</td>
</tr>
<tr>
<td>A. scrobicularis + Carrageenan</td>
<td>0.98 ± 0.04</td>
<td>1.34 ± 0.03</td>
<td>0.36 ± 0.03***</td>
</tr>
<tr>
<td>PBZ+ Carrageenan</td>
<td>1.08 ± 0.04</td>
<td>1.28 ± 0.04</td>
<td>0.19 ± 0.001***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n=5, ***P< 0.001. Ananti-inflammatory extract of A. scrobicularis + CCl₄ (250 and 500mg/kg, bw) showed a statistically significant result.
of ANS at doses of 250 and 500 mg/kg prior to CCl₄ significantly protected against the elevation of transaminases levels. The serum activities of AST, ALT and GGT in rats treated with ANS extract at a dose of 250 mg/kg + CCl₄ were 153.50±5.51, 134.66±2.45, and 7.75±0.18 IU/l, respectively and with 500 mg/kg plus CCl₄ were 134.00±4.19, 123.66±5.04 and 6.65±0.18 IU/l, respectively. These values were highly significant when compared with the intoxicated control rats (211.33±10.08, 194.16±7.53 & 11.25±0.37 IU/l respectively). Similarly, the elevated levels of serum ALP, bilirubin and total protein of tissue were significantly decreased in ANS extract (Table 3).

**Malondialdehyde and NP-SH assays**

The effect of ANS on the CCl₄-induced lipid peroxidation was examined through observation of the levels of MDA in liver tissues. Hepatic MDA and NP-SH level were significantly (p<0.001) changed in the CCl₄-intoxicated control group (5.10±0.3 nmol/g tissue and 1.10±0.09nmol/g of tissue) than the normal animals (1.077±0.05 nmol/g and 5.86±0.37nmol/g tissue). Treatment with ANS (250 & 500 mg/kg) and CCl₄ significantly (P<0.001) prevented the changed of MDA and NPSH. Silymarin treatment also prevented the CCl₄ Changed MDA (2.04±0.12 nmol/g tissue) and NP-SH(1.81±0.10nmol/g tissue) (Table-4).

**Liver and kidney histopathological studies**

Histopathological studies of liver of animal in the normal saline control group showed normal hepatic architecture (fig 1a), where the hepatocytes showed cytoplasmic vacuolization of hepatocytes and partial infiltration with inflammatory cells (fig 1b). Treatment with Silymarin and ANS (250 & 500 mg/kg) exhibited reversal of these changes (Figure 1c, d, e). However, protective effects were more pronounced at higher dose of ANS (500 mg/kg bw) and revealed no histological changes.Histopathological studies of kidney in saline control group showed normal histological structure of renal parenchyma (fig 2a), where the hepatocytes showed cytoplasmic vacuolization of epithelial lining renal tubules (fig 2b). Treatment with silymarin and ANS (250 & 500 mg/kg) exhibited reversal of these changes (Figure 2c, d, e).

**DISCUSSION**

The extract of ANS did not produce any symptoms of acute toxicity in mice, so the extracts are safe for animal’s use. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and the probable mechanism of action is bi-phasic; the release of histamine, serotonin, 5-HT and kinins in the first phase; while swelling is related to the release of

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**Table: 3. Effect of ANS on serum activity of GGT and bilirubin and tissue activity of total protein in CCl₄-intoxicated rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALP (IU/l)</th>
<th>Bilirubin (mg/dl)</th>
<th>Total protein (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (2ml/kg, p.o.)</td>
<td>294.33 ±6.17</td>
<td>0.54 ±0.02</td>
<td>125.51 ±3.54</td>
</tr>
<tr>
<td>CCl₄ (1.25ml/kg,i.p.)</td>
<td>514.16 ±11.75***</td>
<td>2.34 ±0.16***</td>
<td>56.55 ±3.47***</td>
</tr>
<tr>
<td><em>A. scrobicularis</em> +CCl₄ (250mg/kg, p.o.)</td>
<td>405.33 ±9.50***</td>
<td>1.50 ±0.04***</td>
<td>94.71 ±3.31***</td>
</tr>
<tr>
<td><em>A. scrobicularis</em> +CCl₄ (500mg/kg,p.o.)</td>
<td>340.66 ±4.19***</td>
<td>1.17 ±0.05***</td>
<td>111.26 ±3.94***</td>
</tr>
<tr>
<td>Silymarin(10 mg/kg, i.p.)</td>
<td>9.90.16 ±9.77***</td>
<td>0.90 ±0.06***</td>
<td>92.41 ±3.54***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n=5, ***P< 0.001. A Hepatoprotective extract of *A. scrobicularis* + CCl₄ (250 and 500mg/kg, bw) showed a statistically significant result.

**Table 4. Effect of ANS on MDA and NP-SH in liver tissue of rats with CCl₄ induced hepatotoxicity**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (nmol/g)</th>
<th>NP-SH (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (2ml/kg, p.o.)</td>
<td>1.077±0.05</td>
<td>5.86±0.37</td>
</tr>
<tr>
<td>CCl₄ (1.25ml/kg,i.p.)</td>
<td>5.10±0.3***</td>
<td>1.10±0.09***</td>
</tr>
<tr>
<td><em>A. scrobicularis</em> +CCl₄ (250mg/kg, p.o.)</td>
<td>3.28±0.14***</td>
<td>2.01±0.18***</td>
</tr>
<tr>
<td><em>A. scrobicularis</em> +CCl₄ (500mg/kg,p.o.)</td>
<td>1.99±0.11***</td>
<td>2.43±0.16***</td>
</tr>
<tr>
<td>Silymarin(10 mg/kg, i.p.)</td>
<td>2.04±0.12***</td>
<td>1.81±0.10***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n=5, ***P< 0.001. An Antioxidant extract of *A. scrobicularis* + CCl₄ (250 and 500mg/kg, bw) showed a statistically significant result.
Figure 1. Histopathological section of liver tissue of rats (H & E×400). (1a) Section of control rat showing the normal histology, (1b) Section of CCl₄ induced hepatotoxic rat showing cytoplasmic vacuolization of hepatocytes and partial infiltration with inflammatory cells. (1c) ANS + CCl₄ (250 mg/kg bw) showing slight granularity of the cytoplasm of hepatocytes and (1d) ANS + CCl₄ (500 mg/kg bw) showing no histopathological changes.

Figure 2. Histopathological section of renal tissues(H & E×400). (2a) Section of control rat showing the normal histology, (2b) Section of CCl₄ induced renal toxicity showing vacuolization of cytoplasm of epithelial lining renal tubules. (2c) ANS + CCl₄ (250 mg/kg bw) showing vacuolar degeneration of epithelial lining of some renal tubules and (2d) ANS + CCl₄ (500 mg/kg bw) showing no histopathological changes.
prostaglandin, bradykinins and lysozymes-like substances in 2-3 h in the second phase.\textsuperscript{[14]} The anti-inflammatory effect of the crude extract of the plant at the third hour after carrageenan injection strongly suggests its NSAID like activity. Similarly the standard drugs, diclofenac and indomethacin, produced a significant anti-edematous effect showed marked inhibition of carrageen induced edema in rats.\textsuperscript{[15]} Some of related species has already been reported for anti-inflammatory effects.\textsuperscript{[10,20,21]} Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the evaluation of hepatoprotective drugs or medicinal plant extracts.\textsuperscript{[26]} Generally, the extent of hepatic damage is assessed by histopathological evaluation and the level of cytoplasmic enzymes released into the circulation. Marked elevation of serum enzymes and total protein, MDA and NP-SH in liver tissue indicates damage to the hepatic tissue. The disturbance in the transport function of the hepatocytes, as a result of hepatic injury, causes the leakage of enzymes from cells due to altered permeability of the membrane that result in raised levels of enzymes.\textsuperscript{[37]} The normalization of the level of the corresponding enzymes is a definite indication of the hepatoprotective action of the compound under evaluation. ALT and AST are the most sensitive markers of hepatocellular injury and their elevation in serum is indicative of cellular leakage and loss of the functional integrity of cell membranes in liver.\textsuperscript{[38]} ALP is a membrane bound enzyme involved in active transport across the capillary wall. The increased level of ALP is also a reliable marker of liver damage.\textsuperscript{[39]} GGT is important in transport of amino acids required for the synthesis of GSH in cells. Bilirubin is an important degradation product of hemoglobin and is normally excreted into the bile. Increase in total serum bilirubin concentration after CCl\textsubscript{4} administration might be attributed to the failure of normal uptake, conjugation and excretion by the damaged hepatic parenchyma. Decline in enzymes levels after \textit{A. scrobicularis} administration indicated improvement in cellular integrity and status of hepatic cells. Non-protein sulfhydryls are known to be involved in several defense processes against oxidative damage; protect cells against free radicals peroxides and various poisonous substances.\textsuperscript{[40]} The increased TBARS after CCl\textsubscript{4} administration suggests enhanced LPO due to formation of excessive free radicals and failure of antioxidant defense mechanism leading to tissue damage. The phenolic compounds are known to exert protective effect against CCl\textsubscript{4} intoxication by reducing the MDA production, which is indicative of its antioxidant activity.\textsuperscript{[41]} The deficiency of GSH within the living organs can cause tissue injury and malfunction.\textsuperscript{[42]} In the current study, the liver NP-SH level in CCl\textsubscript{4}-treated groups was significantly diminished when compared with the control group. These findings are in accordance with earlier reports as sulfhydryl levels were significantly depleted in different organs of rats, when exposed to CCl\textsubscript{4}.\textsuperscript{[43]} Decline in oxidative levels after \textit{A. scrobicularis} administration also indicated the improvement in cellular integrity and status of hepatic cells. Some of related species \textit{Anthemis ruthenica} contains sesquiterpenes and flavonoids which are oxidized by radicals, resulting in more stable, less reactive radical and flavonoids can also inhibit the activity of many enzymes.\textsuperscript{[12–17]} Histopathological observations after CCl\textsubscript{4}-administration showed severe damage in liver and kidney. The prevention of liver and kidney cells texture also account safety and protective nature of ANS extracts in hepatotoxic conditions.

\section*{CONCLUSION}

The hepatoprotective and nephroprotective activities of ANS were probably due to free radical scavenging properties. The altered hepatic markers such as transaminases, ALP, bilirubin, total protein, MDA and NP-SH with CCl\textsubscript{4} exposure was reversed towards normalization with ANS extract. Bioactive components of ANS probably ameliorated the oxidative damage and had increased the restorative ability of liver and kidneys. So it is mandatory to explore the bioactive chemicals in ANS in order to develop the therapeutics that has a promising role in the treatment of hepatotoxic conditions.

\section*{CONFLICT OF INTERESTS}

There is no conflict of interests.

\section*{ACKNOWLEDGMENTS}

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\section*{REFERENCE}