Phytochemical and pharmacological studies on *Convolvulus fatmensis* Ktze.

Attia H. Atta*, Nawal H. Mohamed†, Soad M. Nasr‡, Samar M. Mouneir

Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Giza - 12211.

1. Aromatic and Medicinal Plants Department, Desert Research Center, Mataria, Cairo

2. Parasitology and Animal Diseases Department, National Research Center, Dokki, Giza, Egypt.

Abstract

Two aglycones flavonoid compounds were isolated from *Convolvulus fatmensis* Ktze. using column and preparative paper chromatography and identified by using 1 HNMR, 13 CNMR and UV shift reagent, these compounds were kaempferol and quercetin. Four coumarin compounds were isolated and identified as umbelliferone, scopoletin, ascleptin and scopoline for the first time. Two phenolic acids were also isolated in the same manner and identified by spectroscopic methods, they are ferulic acid and caffeic acid. The methanol extract of *Convolvulus fatmensis* was evaluated for its potential antiulcerogenic, diuretic and hepatoprotective effects as well as for its acute toxicity. The antulcerogenic effect was evaluated against acute (ethanol model) and prolonged (aspirin model)-gastric ulceration. The results revealed that, oral administration of the methanol extract (400 mg/kg bw) significantly reduced the alcohol-induced gastric ulcers (curative ratio; 32.6%). It also produced high curative ratio (75%), decreased the number of gastric ulcers, total acidity and total protein in aspirin-induced gastric ulcer (P < 0.05). Oral administration of 500 mg/kg bw methanol extracts did not affect the urine output, or sodium excretion while potassium and chloride excretion were significantly increased. The higher dose (1000 mg/kg bw) significantly increased the sodium, potassium and chloride excretion but did not affect urine volume. Moreover, it produced mild hepatoprotective effect against CCl₄-induced hepatotoxicity as indicated from serum biochemical and histopathological changes. No signs of acute toxicity were observed after oral administration of doses up to 2.75 g/kg bw.

Key words: *Convolvulus fatmensis*, Phytochemical, antiulcer, diuretic, hepatoprotective, medicinal plants

1. Introduction

Convolvulaceae is a glory morning family [1] includes 58 genera and 1650 species [2] distributed all over the world especially warm and temperate regions. It includes a number of very important medicinal plants that are used for treatment of various diseases such as jaundice, headache, constipation, rheumatic and skin diseases [3-5]. In folk medicine many other medicinal uses of plants from Convolvulaceae family for example as tonic (*Ipomoea digitata* and *Cressa cretica*), purgative (*Merremia alata*, *Argyreia capitata* and *Ipomoea pedicellaris*),
laxative (*Ipomoea indet*), for headache (*Ipomoea gracilis*) [6], toothache (*Convolvulus bidentatus*) [7] and to treat dermatitis caused by stink of Jelly fish (*Ipomoea pes caparis*) [8-9]. A large number of plants have been shown to produce promising antiulcerogenic effect, diuretic effects [10-11] and are used for various types of gastrointestinal pain [12]. Previous chemical or pharmacological studies have not been reported on *Convolvulus fatmensis* – a member of Convolvulaceae. The present work was carried out to investigate the phytochemical and the possible antulcerogenic, diuretic and hepatoprotective properties of *Convolvulus fatmensis* grown commonly in the Nile region, Mediterranean region and the Oases.

2. Material and Methods

2.1.1 Plant material

The aerial parts of *Convolvulus fatmensis* Ktze. were collected from North Sani (Wadi El-Arish), identified by comparison with Herbarium sample at Desert Research Center and by Prof. Dr. N. El-Hadidi and the staff members of the Herbarium of the department of Botany, Faculty of Science, Cairo University. A voucher sample was kept in the department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt. The air-dried plant was moderately pulverized, and stored for further use.

2.1.2 Adsorbents and solvent systems

Silica gel 60 F$_{254}$ was used for TLC (Pre-coated plates 20*20 cm) and silica gel 60 (0.063-0.200 mm) for column chromatography (Merck, Darmstadt, Germany). The solvent systems which used are:

(a) Benzene: ethyl acetate (86: 14 v/v), (b) ethyl acetate: methanol: water (30: 5: 4 v/v/v) and (c) chloroform: methanol (95: 5 v/v) obtained from Merck, Darmstadt, Germany.

2.1.3 Apparatus

Koffler’s hot stage apparatus for melting point. El- Ms (Chro N29 MY 5526) Ver. Ion Vie 22. UV - Visible spectrophotometer Schimadzu UV 240 was used for recording UV spectra and measuring the absorbance in UV range. NMR were carried out on a Buruker AMX-500 MHz,Varian Inova apparatus at the university of Texas at Austin and Varian mercury 300 MHz spectrometers.

2.1.4 Extraction

The defatted powder of the plant aerial part (1 kg) was extracted in a soxhlet apparatus with 90% methanol. The methanol extract was dried under reduced pressure and dissolved in water then successively extracted with ether, chloroform, ethyl acetate and n-butanol. Every extract was dried over anhydrous sodium sulphate and concentrated and left for phytochemical and pharmacological investigation.

2.2 Phytochemical studies

2.2.1 Isolation and purification of phenolic compounds

Different plant extracts were applied on precoated Silica gel G plates and run in the solvent system ethyl acetate: methanol: water (30: 5: 4 v/v/v).

TLC examination for all extracts was carried out and revealed that; n-butanol fraction contained a lot of resinous matter when chromatographed on TLC.

The ethyl acetate extract was subjected to column chromatography using silica gel G (0.063-0.200 mm) then precoated preparative TLC (F$_{254}$) plates using the system ethyl acetate: methanol: water (30: 5: 4 v/v/v). Bands corresponding to each flavonoid compound
were eluted with methanol, concentrated and submitted to sephadex LH 20 column (Amersham Pharmacia Biotech, Sweden) and eluted with methanol-water (70:30 v/v) where compounds 1 and 2 were isolated. Ether and chloroform extracts were approximately have the same spots.

Coumarins and phenolic acids were isolated from the combined ether and chloroform extracts using column chromatography packed with silica gel G and eluted by 100% benzene then the polarity gradually increased by 10% of ethyl acetate till reached 100% ethyl acetate then followed by preparative TLC plates (Silica gel 60 F254) using the solvent system Benzene: ethyl acetate (86: 14 v/v).

2.3 Pharmacological studies

2.3.1 Antiulcerogenic effect

2.3.1.1 Ethanol induced gastric ulceration

Fifteen male Sprague-Dawley rats (150-200 g bw) were kept under standard conditions before their use. Rats were randomly divided into 3 equal groups. Animals were starved for 48 h before use to ensure an empty stomach and were kept in cages with raised floors of wide wire mesh to prevent coprophagy [13]. To prevent excessive dehydration during the fasting period rats were supplied with sucrose (BDH) 8% (w/v) solution in NaCl (BDH) 0.2% (w/v) which was removed 1 hr before experiments [14].

In the first day, rats of group one were orally given two doses of 400 mg/kg bw (suspended in 2% Tween 80 in distilled water) of *Convolvulus fatmensis* extract with 6 h apart. A third dose was given in the 2nd day 1.5 h before oral administration of ethanol (Merck) 50% (v/v in distilled water) in a dose of 10 ml/kg bw. A control group was given equal volume of 2% Tween 80 in distilled water instead of the plant extracts but received ethanol in the same dose and route. In addition a third group was given ranitidine as a reference drug in a dose of 100 mg/kg bw [15] by the same route and at the same time intervals. One hour after ethanol administration, all rats were euthanized by an over dose of chloroform and the stomachs were rapidly removed, opened along their greater curvature and the long lesions were counted and measured and the petechial lesions were counted as described by Ogle et al [16]. The ulcer index (mm) and the curative ratio were calculated as described by Cho and Ogle [17] by the following formula:

\[
\text{Curative ratio} = \frac{(\text{Control ulcer index} - \text{Test ulcer index})}{\text{Control ulcer index}} \times 100
\]

2.3.1.2 Aspirin induced ulceration

Fifteen male Sprague-Dawley rats (150-200 g bw) kept under standard conditions were randomly divided into 3 equal groups; control, *Convolvulus fatmensis* and ranitidine-treated group (100 mg/kg bw). The modified method of Geol et al (18) was used for the production of experimental gastric ulceration. Aspirin (200 mg/kg bw) suspended in carboxymethylcellulose 1% was administered orally to all rats. Methanol extract of *Convolvulus fatmensis* (400 mg/kg bw) or ranitidine (suspended in 2% tween 80 in distilled water) was given 3 h prior to and after aspirin administration to each rat of the second or the third group respectively. Each rat of the first group (control) was given equal volumes of 2% Tween 80 instead of the drug at the same times and by the same route. Treatment continued for 3 days and the pylorus was legated on the fourth day. The abdomen was opened under ether anaesthesia and the pylorus was legated with silk suture then it was closed. Animals were left to recover and drinking water was withheld for 4 h. The rats were then killed with an overdose
of chloroform, the oesophagus was legated and the stomach was removed. The gastric mucosa was washed with 3 ml distilled water. The gastric juice and the washings were collected and centrifuged at 5000 rpm for 5 min. The volume of gastric juice was measured and expressed as ml /100 g bw. The stomach was opened and the glandular portion was examined, the number of ulcers was counted and the total length was measured. The curative ratio was calculated as mentioned before.

2.3.1.3 Determination of the total acid output
The method described by Oser (19) was used. One ml of the gastric juice in 10 ml of distilled water was titrated with 0.01 N NaOH using phenolphthalein as an indicator. Data were expressed as mEq/ml of the gastric juice.

2.3.1.4 Determination of protein content
Biuret reagents was applied to determine the total protein in the gastric juice [20] using kits from bioMérieux-France.

2.3.2 Diuretic Effects

2.3.2.1 Determination of urine output
A total of 20 Sprague-Dawley rats (males and non-pregnant females) of body weight 130 to 150 g were used. Rats were allocated randomly into 4 equal groups. The first and the second groups were used to test the diuretic effect of *Convolvulus fatmensis* methanol extract in an oral dose of 500 and 1000 mg/kg bw, respectively. Each rat of each group was placed into a separate metabolic cage; with wire mesh floor provided with a conical-shaped bottom underneath designed to collect urine in a receptacle without faecal contamination. The test animals were fasted overnight (12-14 h) but they had free access to fresh water. A third group was given equal volumes of 2% Tween 80 orally and kept as control. Rats of the 4th group were given an oral dose of furosemide of 7.5 mg/kg bw as a reference drug. The urine output through 24 hours was collected and measured in graduated cylinder.

2.3.2.2 Determination of urinary electrolytes
Sodium and potassium were estimated by flame photometer (Jenway England Model, PFP7). Chloride concentration was estimated spectrophotometrically according to Skeggs and Hichstrasser [21] using kit from QUMICA CLINICA APLICADA S.A. SPAIN after urine dilution when appropriate.

2.3.2 Hepatoprotective effect

2.3.3.1 Animals used:
Four groups of 5 Sprague-Dawley rats each were used. Rats of the first group (normal control) were given 2.5 ml/kg of 2% Tween 80 and on the 5th day 2.5 ml of corn oil/kg bw were given. Rats of the second group were given the same dose of corn oil and on the 5th day CCl₄ (50% in corn oil) was given in a single oral dose of 2.5 ml kg⁻¹ b wt. two hours after the last dose of the oil. The third group was pre-treated orally with 1000 mg/kg bw of *Convolvulus fatmensis* methanol extract. Doses were given in 2.5 ml of 2% Tween 80/kg bw daily for 5 days and CCl₄ was given two hours after the last dose. Moreover a 4th group was given 25 mg/kg bw silymarin (as a standard drug) daily for 5 days and 2 h after the last dose, CCl₄ was given as before.

Blood samples were collected after 24 h from the hepatotoxin administration by puncturing of retro-orbital plexus. Blood samples were placed in a plain centrifuge tube for serum separation and determination of the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [22], gamma glutamyl transferase (GGT) [23], glucose [24], bilirubin [25], triglyceride [26], cholesterol [27], total proteins [28] and albumin [29].
2.3.3.2 Pathological studies

Liver tissue specimens were collected from all groups immediately after sacrifice of rats and fixed in 10% normal saline. Paraffin sections of $5\mu$ thickness were prepared, stained by Hematoxylin and Eosin (H & E) and examined microscopically [30].

2.3.3.3 Acute toxicity study in mice

The acute toxicity of plant extract was tested using three doses (0.4, 1 and 2.75 g/ kg bw., orally) as described by Tanira et al. [31] but the observation period extended up to 72 h post administration. In addition, the general behavior of animals, signs of discomfort, or any nervous manifestations were observed.

2.3.3.4 Statistical analysis

Results are expressed as mean ± standard deviation (SD). Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan’s multiple range tests ($P \leq 0.05$).

3. Results

3.1 Phytochemical investigation

The isolated phenolic compounds from the different successive extracts were crystallized and identified by $R_f$, melting point and spectroscopic methods, they are:

- Kaempferol (1): yellow crystals, soluble in methanol, $R_f = 0.91$ and 0.94, m.p. 277°C.
  
  UV spectral data, $\lambda_{\text{max}}$ nm in (MeOH) = 268 and 367, (NaOMe) = 285, 322, 430, (NaOAc) = 275, 302 (sh), 385; (NaOAc + H$_3$BO$_3$) = 276, 320 (sh), 370; (AlCl$_3$) = 266, 305 (sh), 350, 422. (AlCl$_3$ + HCl) = 266, 305 (sh), 350, 422 [32].

- Quercetin (2): yellow needle crystals, soluble in methanol, $R_f = 0.85$ and 0.90, m.p. 315°C.
  
  UV spectral data, $\lambda_{\text{max}}$ nm in (MeOH) = 255, 270 (sh), 300 (sh), 370, (NaOMe) = 290, 330 (sh), 440, (NaOAc) = 260, 335 (sh), 381; (NaOAc + H$_3$BO$_3$) = 260, 295 (sh), 365; (AlCl$_3$) = 270, 312 (sh), 445; (AlCl$_3$ + HCl) = 265, 305 (sh), 425.

- Scopoletin (3): white crystals, $R_f = 0.33$, m.p. 203-204°C.
  
  UV spectral data, $\lambda_{\text{max}}$ nm in (MeOH) = 252, 260, 350, (NaOMe) = 277, 391.

- Umbelliferone (4): white crystals, $R_f = 0.43$, m.p. 224-226°C.

Table 1. Effect of methanol extracts of Convolvulus fatmensis (400 mg/kg bw) on ethanol induced gastric ulceration in rats (mean ± SD, n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index</th>
<th>Curative Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Ethanol)</td>
<td>41.10±4.00$^a$</td>
<td>-</td>
</tr>
<tr>
<td>Convolvulus fatmensis</td>
<td>27.70±7.80$^a$</td>
<td>32.60</td>
</tr>
<tr>
<td>Ranitidine 300 mg/kg bw</td>
<td>30.20±4.30$^b$</td>
<td>26.50</td>
</tr>
</tbody>
</table>

Means followed with different letters in the same column are significantly different at $P \leq 0.05$.
Table 2. Effect of methanol extract of *Convolvulus fatmensis* (400 mg/kg bw) on aspirin Induced-gastric ulceration in rats (mean ± SD, n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index</th>
<th>Curative ratio (%)</th>
<th>Number of ulcers</th>
<th>Volume/100g mEq/l</th>
<th>Total acidity mEq/l</th>
<th>Total protein µg/ml x 10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% Tween 80)</td>
<td>14.40±4.10b</td>
<td>0.00</td>
<td>2.40±0.70b</td>
<td>2.80±0.60a</td>
<td>35.60±6.80a</td>
<td>0.82±0.44b</td>
</tr>
<tr>
<td><em>C. fatmensis</em></td>
<td>3.60±1.50a</td>
<td>75.0</td>
<td>1.40±0.90a</td>
<td>3.10±0.30a</td>
<td>10.80±2.80a</td>
<td>0.31±0.04¹</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>8.80±5.00a</td>
<td>38.9</td>
<td>4.60±1.80a</td>
<td>2.60±0.20a</td>
<td>2.30±0.70a</td>
<td>0.81±0.20b</td>
</tr>
<tr>
<td>Furosemide</td>
<td>7.5</td>
<td>5.80±0.80a</td>
<td>144.20±14.10c</td>
<td>126.40±11.30ab</td>
<td>133.00±11.80b</td>
<td></td>
</tr>
</tbody>
</table>

Means followed with different letters in the same column are significantly different at P < 0.05

Table 3. Effect of methanol extracts of *Convolvulus fatmensis* on 24 h urine volume, and electrolytes concentrations in urine of rats (mean ± S.D, n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg bw</th>
<th>Volume (ml)</th>
<th>Sodium mEq/ml</th>
<th>Potassium mEq/ml</th>
<th>Chloride mEq/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% Tween 80)</td>
<td>-</td>
<td>3.30±0.40a</td>
<td>26.00±3.60a</td>
<td>74.30±16.80a</td>
<td>69.40±9.20a</td>
</tr>
<tr>
<td><em>C. fatmensis</em></td>
<td>500</td>
<td>2.80±0.60a</td>
<td>24.00±5.50a</td>
<td>117.30±15.90b</td>
<td>130.10±8.90b</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>2.98±0.20a</td>
<td>76.40±16.10b</td>
<td>270.80±29.60c</td>
<td>131.50±17.60b</td>
</tr>
<tr>
<td>Furosemide</td>
<td>7.5</td>
<td>5.80±0.80a</td>
<td>144.20±14.10c</td>
<td>126.40±11.30ab</td>
<td>133.00±11.80b</td>
</tr>
</tbody>
</table>

Means followed with different letters in the same row are significantly different at P ≤ 0.05

Table 4. Effect of methanol extract of *Convolvulus fatmensis* on some serum enzymes and biochemical parameters in rats after challenged with carbon tetrachloride induced hepatotoxicity (Means ± SD, n = 5).

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (2% Tween 80)</th>
<th>Carbon tetrachloride</th>
<th><em>Convolvulus fatmensis</em></th>
<th>Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/l)</td>
<td>211.50±19.70c</td>
<td>705.30±189.40c</td>
<td>517.60±70.60c</td>
<td>457.6±80.80b</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>76.00±11.90c</td>
<td>380.50±71.20d</td>
<td>236.20±2.90c</td>
<td>158.0±12.2c</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>7.70±0.50c</td>
<td>20.00±1.40c</td>
<td>4.80±0.80b</td>
<td>6.60±0.50b</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>129.40±20.40c</td>
<td>93.80±14.60b</td>
<td>110.40±4.40b</td>
<td>111.3±3.0b</td>
</tr>
<tr>
<td>T. bilirubin (mg/dl)</td>
<td>0.35±0.05c</td>
<td>0.41±0.02c</td>
<td>0.37±0.03b</td>
<td>0.42±0.03b</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>141.80±17.40b</td>
<td>75.80±18.00c</td>
<td>58.60±9.40b</td>
<td>66.40±2.80c</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>82.40±4.30c</td>
<td>74.80±8.60bc</td>
<td>63.50±3.80c</td>
<td>72.90±11.3bc</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.40±0.50b</td>
<td>6.80±0.50b</td>
<td>7.20±0.50b</td>
<td>7.90±0.60c</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.90±0.40b</td>
<td>4.30±0.20b</td>
<td>4.20±0.20b</td>
<td>4.60±0.20b</td>
</tr>
</tbody>
</table>

Means followed with different letters in the same row are significantly different at P ≤ 0.05
$^1$H-NMR (DMSO d$_6$) δ (ppm) 7.9 (1H, d, J = 9 Hz, H4), δ 7.2 (1H, d, J= 8.4 Hz, H5), δ 6.8 (1H, dd, J= 8.4, 1.5 Hz, H6), δ 7 (1H, d, J = 1.5 Hz, H8), and δ 6.2 (1 H, d, J=9.5 Hz H3).

Scopoline (5): white crystals, $R_f$ = 0.45, m.p. 126-128°C.

UV spectral data, δ max nm in (MeOH) = 280, 360, (NaOMe) = 260, 360.

$^1$H-NMR (DMSO d$_6$) δ (ppm) 7.9 (1H, d, J = 9 Hz, H4), δ 7.3 (1H, s, H5), δ 7.1 (1H, s, H8), δ 6.3 (1H, d, J = 9 Hz, H3), and δ 3.8 (3 H, s, OCH$_3$). Sugar moiety δ 5.1 (1H, d, J=9 Hz, H-1 glucose) and δ 3-3.8 (m. remaining sugar proton).

$^{13}$C-NMR: was done in DMSO-d$_6$, δ (160.4 for C-2), 112 (for C-3), 145.8 (for C4), 109.6 (for C-5), 113.2 (for C-6), 149.8 (for C-7), 102.9 (for C-8), 153.6 (for C-9), 112.2 (for C-10), 100.6 (for C-1’ glucose), 73.3 (for C-2’ glucose), 76.7 (for C-3’ glucose), 69.4 (for C-4’ glucose), 77 (for C-5’ glucose), 61 (for C-6’ glucose) and 55.9 (for OCH$_3$).

Asculetin (6) White needle crystals, soluble in chloroform and methanol, $R_f$ = 0.35, m.p. 267-270°C.

UV spectrum: UV δ max. nm in (MeOH)= 260, 310, 350 (NaOMe) = 260, 320, 390.

Fig 1.
(A) Liver of rat in control group showing the normal histological structure of the hepatocytes,
(B) Liver of rat administrated carbon tetrachloride,
(C) Liver of rat administrated carbon tetrachloride pretreated with silymarine and
(D) Liver of rat administrated carbon tetrachloride pretreated with methanol extract of
Convolvulus fatmensis (H and E X40).
$^1$H NMR: Spectrum in DMSO-$d_6$ δ ppm, 7.9 (1H, d, J= 9Hz, H-4), δ 7.1 (1H, s, H-5), δ 6.7 (1H, s, H-8) and δ 6.2 (1H, d, J= 9Hz, H-3).

$^{13}$C-NMR: $^{13}$C-NMR was done in DMSO-$d_6$, δ (161.2 for C-2), 110.7 (for C-3), 144.6 (for C4), 112.2 (for C-5), 148.4 (for C-6 appears shifted more down field by 24 ppm due to presence of OH attached to it), 150.3 (for C-7 appears also shifted more down field due to presence of OH attached to it) (33), 102.5 (for C-8), 142.8 (for C-9) and 110.7 (for C-10).

Ferulic acid (7): White needle crystals $R_f = 0.96$, m.p. 227°C.

UV spectrum: UV $\lambda$ max. nm in (MeOH)= 285, 312, (NaOMe) = 250 (sh), 290, 319.

$^1$H NMR: Spectrum in DMSO-$d_6$ δ ppm, 8.9 (S-OH), δ 7.5 (1H, d, J=1.7 Hz, H-7), δ 7.1 (1H, d, J=2.5, H-2), δ 7.05 (1H, dd, J=7.5 and 2.5 Hz, H-6), δ 6.95 (1H, d, J=7.5, H-5), δ 6.25 (1H, d, J=1.7 Hz, H-8) and δ 3.85 (3H, s, OCH$_3$).

Caffeic acid (8): Pale yellow powder $R_f = 0.78$, m.p. 221°C.

UV spectrum: UV $\lambda$ max. nm in (MeOH) = 240, 285 (sh), 325 (NaOMe) = 252, 301, 345.

$^1$H NMR: Spectrum in DMSO-$d_6$ δ ppm, 8.9 (S-OH), δ 12.1 (broad singlet- COOH), δ 9.5 (s- OH), δ 9.15 (s- OH), δ 7.4 (1H, d, J=17 Hz H-7), δ 7 (1H, d, J=2.5 Hz, H-2), δ 6.95 (1H, dd, J=7 and 2.5 Hz, H-6), δ 6.75 (1H, d, J=7 Hz, H-5) and δ 6.15 (1H, d, J = 17 HzH-8).

3.2 Pharmacological study

3.2.1 Antiulcerogenic Effects

*Convolvulus fatmensis* significantly (P<0.05) decreased the ulcer index with a curative ratio of 32.6% in alcohol-induced gastric ulcers (Table 1). Ranitidine decreased the gastric ulcer index from 41.1 to 30.2 with a curative ratio of 26.5%. The methanol extract of *Convolvulus fatmensis* significantly (P<0.05) reduced the gastric ulcer index in aspirin-induced gastric ulceration from 14.4 to 3.6. The volume of gastric juice is not significantly affected; however the number of gastric ulcers, the total gastric acidity and the total protein were significantly decreased (P <0.05). Ranitidine significantly (P <0.05) decreased the ulcer index and total acidity but did not affect volume of gastric juice and total protein content, although the number of ulcers was increased (Table 2).

3.2.2 The diuretic Effect

Oral administration of methanol extracts of *Convolvulus fatmensis* at a dose of 500 or 1000 mg/kg bw did not affect the urine output. Oral administration of methanol extracts of *Convolvulus fatmensis* in a dose of 500 mg/kg bw significantly (P < 0.05) increased the excretion of potassium and chloride only, while its administration in a dose of 1000 mg/kg bw significantly (P < 0.05) increased the excretion of sodium, potassium and chloride. Furosemide in an oral dose of 7.5 mg/kgbw significantly (P <0.05) increased the urine volume, the sodium, potassium and chloride excretion (Table3).

3.3 Hepatoprotective effect

3.3.1 Biochemical parameters

The CCl$_4$-treated animals exhibited a marked increase in serum enzymes activity as compared to control group (Table 4). A significant decrease was found in elevated AST, ALT and GGT values in both *Convolvulus fatmensis* and silymarine treated groups in comparison with CCl$_4$-treated group. There was a significant decrease in serum glucose level in CCl$_4$-treated group as compared to both *Convolvulus fatmensis* and silymarin-treated groups (Table 4). No significant changes in values of serum total proteins, albumin, globulin, triglyceride, cholesterol and bilirubin.
3.3.2 Pathological examination

Macroscopically, the livers in CCl₄-treated group were enlarged, very friable and pale in color. The livers of both silymarine and *Convolvulus fatmensis* -treated groups were slightly enlarged, moderate friable and mottled in appearance. Histopathological observation of liver from control group showed a normal hepatic architecture (Figure 1A). In CCl₄-treated group, very severe hepatotoxicity was evidenced by the appearance of centrilobular necrosis, ballooning degeneration in hepatocytes, congestion in the central vein and sinusoids, proliferation of Kupffer cells and mononuclear leucocytes inflammatory cells infiltration mainly surrounding the central vein (Figure 1B). In silymarine and *Convolvulus fatmensis* -treated groups, the centrolobular area of hepatic tissue showed moderate ballooning degenerative and necrobiotic changes in the hepatocytes with mononuclear leucocytes inflammatory cells infiltration inbetween, as well as proliferation of Kupffer cells and congestion in the central vein (Figures 1C and D).

3.3.3 Acute toxicity study in mice

Oral administration of *Convolvulus fatmensis* methanol extract in doses up 2.75 g/kg bw. did not cause any major signs of acute toxicity. No deaths were reported up to 72 hours after oral administration.

4. Discussion

The present data showed that *Convolvulus fatmensis* exhibited very useful pharmacological effects. The results showed that *Convolvulus fatmensis* significantly decreased the ulcer index in acute ulcer induced by alcohol as well as subacute ulcer induced by aspirin. Its effect in both cases was comparable or even higher than the reference drug Ranitidine (100mg/kg). This effect was indicated by the significant decrease in the ulcer index in both models and the number of gastric ulcers and the total gastric acidity in subacute gastric ulcers induced by aspirin. The effect of *Convolvulus fatmensis* extract may be due to mucosal protective effect since the gastric juice protein and the total gastric acidity were decreased but the volume was not. Phytochemical screening of *Convolvulus fatmensis* revealed the presence of unsaturated sterols, triterpenes, tannins, flavonoids and carbohydrates and/or glycosides [34]. Triterpenes are the active constituents that have been claimed to be effective as an anti-ulcer agent because it protects the mucosa against acid effects by selective inhibition of prostaglandins F₂α [35]. In this study two aglycones flavonoid compounds were isolated from *Convolvulus fatmensis* Ktz. namely kaempferol and quercetin. Therefore the gastric protective effect of methanol extracts is probably due to its content of flavonoids. The data showed that methanol extract of *Convolvulus fatmensis* had anti-ulcerogenic but not anti-secretory effect as indicated by the decreased number of ulcers, decreased total acidity and decreased total protein in gastric juice and non significant changes in the volume of gastric juice. Among the mechanism of aspirin-induced ulcerogenic, effect is the inhibition of the synthesis of endogenous prostaglandins [36] and consequently decrease the cytoprotective effect of prostaglandins. The anti-ulcerogenic effect of *Convolvulus fatmensis* points out to a possible cytoprotective effect as a mechanism of action but not due to anti-secretory effect. Similar conclusion has been reported for other plant extracts [37]. The anti-ulcerogenic active constituent (s) of these plants appeared to be extracted by methanol as it has been reported for other plant extracts [10]. The results revealed a remarkable diuretic effect. The increase in the electrolyte content of urine rather than the water volume, indicates a
saluretic effect. The lack of parallelism between urinary volume and electrolyte excretion has been observed for other plant extracts [38]. On the other hand the decreased volume of urine may be due to decreased glomerular filtration rate perhaps by decreased hydrostatic pressure as a result of relaxation of the afferent capillaries by one or more of the active constituents of the plant extract that have been proved to relax other smooth muscles [39].

The hepatoprotective effect of *Convolvulus fatmensis* was evaluated using the well described CCl₄-model [40]. Carbon tetrachloride induced acute pathological changes in the liver as indicated by the marked increase in serum aminotransferases (ALT, AST) and GGT activities and confirmed by histopathological observation compared to control (oil pre-treated) rats. The reported biochemical changes in serum are a result of CCl₄-induced mitochondrial damage [41] and damage of lysosomes [42]. Moreover it has been shown that reactive metabolites of CCl₄ e.g. trichloromethyl radical (CCl₃) or trichloroperoxyl radical (CCl₃O₃) are the probable inducers of lipid peroxidation, disturbance in calcium homeostasis and finally cell death [41]. Carbon tetrachloride-treated rats exhibited a significant decrease in concentrations of serum glucose, triglyceride and cholesterol compared to control (oil pre-treated) group. This is probably due to block of the secretion of hepatic triglyceride into plasma [43].

The protective effect of plant extract against CCl₄ may be attributed to the isolated flavonoids and phenolic compounds [44]. Flavonoids are known to be antioxidants, free radical scavengers and antilipid peroxidants leading to hepatoprotection [45]. Carotenoids are also known to be antioxidants and antihepatotoxic activity [44].

In conclusion the present study demonstrated that the *Convolvulus fatmensis* produced diuretic, antiulcerogenic and hepatoprotective effects with no adverse effects at high doses. Additional experiments are required to explore the exact mechanism of action of *Convolvulus fatmensis* methanol extract.

References


