Evaluation of the anti-inflammatory, analgesic and anti-ulcerogenic potentials of *Achillea fragrantissima* (Forssk.)


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Keywords: *Achillea fragrantissima* Anti-inflammatory Analgesic Anti-ulcerogenic Egyptian folk medicine

**A R T I C L E   I N F O**

Article history:
Received 17 November 2014
Received in revised form 1 February 2015
Accepted 8 February 2015
Available online xxxx

Edited by AK Jäger

**A B S T R A C T**

*Achillea fragrantissima* is a perennial herb grown in Egypt and traditionally employed medicinally for its anti-inflammatory and analgesic properties among Sinai inhabitants. Non-polar and polar extracts were obtained by successive foliar extraction with dichloromethane:methanol (1:1) and 70% aqueous methanol, respectively. *Achillea* extracts were assayed in rodents for anti-inflammatory, anti-ulcerogenic and analgesic activities.

**Materials and methods:** Acute toxicity of non-polar and polar extracts of *A. fragrantissima* was evaluated in mice. Anti-inflammatory activity was assessed in carrageenan-induced rat-paw edema test while analgesic activity was explored centrally and peripherally using hot plate and writhing tests, respectively. In addition, anti-ulcerogenic activity was assayed in colon and gastric tissues.

**Results:** Foliar extracts of *A. fragrantissima* exhibited anti-inflammatory, central and peripheral analgesic activities. Moreover, both non-polar and polar fractions revealed protective effects against rat ulcerative colitis and gastric ulcers.

**Conclusion:** *A. fragrantissima* extracts possess anti-inflammatory, central and peripheral analgesic activities in addition to protective properties in colonic and gastric tissues.

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1. Introduction

The genus Achillea (Asteraceae) comprises more than 100 species and is chemically characterized by an accumulation of sesquiterpene lactones and flavonoids. *Achillea fragrantissima* is a white-wooly plant, with erect stems which grows up to one meter in height. The species grows in limestone valleys of the Egyptian north eastern desert and Sinai region. Previous phytochemical investigations of *A. fragrantissima* report the presence of volatile oils, tannins, monoterpenes ketones and sesquiterpene lactones (Batanouny et al., 1999; Al-Gaby, 2000; Al Samarrai et al., 2003).

The volatile oil prepared from the flowers exhibits broad spectrum antimicrobial activity. In addition to traditional folk medicine in which plant extracts are used to reduce fever, headache and weakness, an activity against the poliomyelitis-1 (POLIO) virus has been demonstrated. Additionally, cirsiliol isolated from *A. fragrantissima* caused relaxation of contracted rats’ proximal aorta, trachea, urinary bladder and uterus (Puerta de la et al., 1996; Batanouny et al., 1999; Soltan and Zaki, 2009). Furthermore, ethanolic extract of *A. fragrantissima* was tested for its anti-inflammatory effects on lipopolysaccharide (LPS)-activated primary cultures of brain microglial cells. It was found that phytochemicals present in the *A. fragrantissima* extract could be beneficial in preventing/treating neurodegenerative diseases (Elmann et al., 2011). The objective of the present study was to assess the potential anti-inflammatory, analgesic and anti-ulcerogenic activities of non-polar and polar extracts obtained from *A. fragrantissima* due to medicinal claims for its efficacy in Egyptian folk medicine.
2. Material and methods

2.1 Plant material

_A. fragrantissima_ plants were collected from Wadi Gebal district in Saint Catherine protectorate, southern Sinai. The plant was identified and authenticated by Dr. Mohammed El Bialy at the research station of wild plants in Saint Catherine protectorate. A voucher specimen (SK-115) was prepared and deposited in the herbarium of the National Research Centre – Egypt.

2.2 Plant extracts

Above ground tissue (1620 g) of _A. fragrantissima_ was air-dried, powdered and exhaustively extracted with dichloromethane:methanol (1:1) to obtain a non-polar extract. The extract was concentrated in vacuo into a dark oily green residue (56 g). The extracted powder was powdered and exhaustively extracted with 70% aqueous methanol to obtain a polar extract that was concentrated in vacuo into a dark yellowish brown residue (70 g). Each extract was freshly suspended in 1% v/v Tween 80.

2.3 Animals

Mature albino mice and Wistar rats (27–30 g and 150–180 g, respectively) of both sexes were obtained from the Animal House Colony at the National Research Centre (NRC), Egypt. Rodents were housed under standard conditions of natural 12 h light and dark cycle with free access to food and water; cages were equipped with raised bottoms of wide wire mesh in order to avoid cannibalism/coprophagy. Animals were allowed to adapt to the laboratory environment for one week before experimentation. All animal procedures were performed after approval from the Ethics Committee of the NRC and in accordance with the recommendations of the proper care and use of laboratory animals and followed the recommendations of the National Institutes of Health (NIH) guide for care and use of laboratory animals (Publication No. 85-23, revised 1985).

2.4 Acute toxicity experiment

A preliminary experiment was performed to determine the median lethal dose (LD50) of non-polar and polar extracts of _A. fragrantissima_. To determine the LD50 of both extracts, 60 Albino mice were randomly allocated into 6 groups, each of 10 animals. Mice were given the tested extracts orally in graded doses from 1000 to 4000 mg/kg b.wt. Control animals received only the vehicle (Tween 80) and kept under the same environmental conditions. Toxic symptoms and mouse viability after 48 h were recorded. Extract oral LD50 was calculated according to Gad and Weil (1982).

2.5 Anti-inflammatory activity

Extract anti-inflammatory activity was determined according to Winter et al. (1962). Rats were divided into 6 groups of six individuals and food withheld overnight before the experiment. Treatment groups included oral administration of the non-polar and polar plant extracts in doses of 200 and 400 mg/kg as well as a non-steroidal anti-inflammatory drug that inhibits prostaglandin, indomethacin (20 mg/kg) and a control group (C + ve) was treated orally with the vehicle alone (5 mL/kg). After 30 min, inflammation was induced by a single sub-planter injection of carrageenan (0.1 mL of 1% w/v in normal saline), into the left hind paw of each rat. The paw edema volumes were measured using a plethysmometer before as well as 1 h intervals from 1 to 4 h after carrageenan exposure. Percent edema and inhibition rates were calculated based on the following equation:

\[
\text{Edema}(\%) = \frac{V_t - V_o}{V_o} \\
\text{Inhibition} (\%) = \frac{E_c - E_t}{E_c} \times 100
\]

where: \(V_o\) is the volume before carrageenan injection (mL), \(V_t\) is the volume at t hour after carrageenan injection (mL), and \(E_c\) and \(E_t\) are the edema rates of control and treated groups, respectively.

2.6 Analgesic activity

Plant extract analgesic activity was carried out in mice based on two different models. The central analgesic activity of plant extracts was tested using a hot-plate apparatus as described by Turner (1965). As with the previous assay except for the use of mice, rodents were divided into 6 groups and oral treatments included non-polar and polar plant extracts in doses of 200 and 400 mg/kg. In this assay an inhibitor of cyclooxygenase (COX) activity, acetyl salicylic acid (150 mg/kg) was employed as well as a control group was treated orally with the vehicle alone (5 mL/kg). One hour post-treatment, mice were placed on a hot plate maintained at 53 ± 0.5 °C and the thermal-stimulus reaction time (i.e. fore- or hind-paw licking or jumping) was recorded. Reaction times were measured 0, 30, 60 and 90 min after treatment. A cut off time of 60 seconds was set to avoid paw damage.

The peripheral analgesic activity (also known as the writhing test) as described by Collier et al. (1968) has the same mice setup and treatment exposure as the hot plate method except that shock was induced by an intraperitoneal injection of acetic acid (0.7% aqueous solution) in a dose of 10 mL/kg b.wt and writhes per animal were recorded for 20 min after the injection. Percent protection was calculated based on the ratio:

\[
\text{Protection}(\%) = \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \times 100
\]

2.7 Rat ulcerative colitis assay

As with previous assays, rats were divided into 6 groups and oral treatments included non-polar and polar plant extracts in doses of 200 and 400 mg/kg. Normal and colitis control rats were given the vehicle only in a dose of 5 mL/kg. Assay samples were administered orally, once daily for 7 successive days with the aid of an orogastric cannula and the last dose was administered 2 h before colitis induction. Rodents were fasted overnight, with access to water _ad libitum_, before being anesthetized with ether inhalation for surgery. To induce ulcerative colitis a polyethylene catheter (2 mm diameter) was inserted 8 cm into the lumen of the colon _via_ the anus (Mascolo et al., 1995). An acetic acid solution (2 mL, 4%, v/v in saline) was slowly instilled into the rectum of each rat. Normal control rats received an equivolume saline solution devoid of acetic acid. Two days after colitis induction, animals were sacrificed with ether and laparotomy was performed. Colonic segments (8 cm in length and 3 cm proximal to the anus) were excised, opened along the mesenteric border, washed with saline, and scored macroscopically.

2.7.1 Colonic lesion assessment

Colon specimen wet weight/length (g/cm) ratios were calculated and visually inspected with a dissecting microscope. Mucosal lesions were quantified by a Buell and Berin (1994) 0–5 scoring system with 0 for no damage; 1 for localized hyperemia; 2 for ulcers or erosions with no significant inflammation; 3 for ulcers or erosions with...
inflammation at one site; 4 for two or more ulceration sites and/or inflammation; and 5 for two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending >1 cm along the length of the colon. Ulcer area was measured using a plane glass square. Each cell on the glass square was 1 mm² in area and the number of cells was counted and the ulcer area was determined for each colon. Ulcer index was measured by summing the lesion score and the ulcer area for each colon specimen (Minaiyan et al., 2006). Sections of colon specimens (0.5 cm long) were fixed in 10% buffered formalin and then embedded in paraffin for sectioning. Several sections were stained with hematoxylin and eosin (H and E) to evaluate histological features.

2.8. Induction of gastric ulceration by pylorus ligation

As with previous assays, rats were divided into 6 groups and oral treatments included non-polar and polar plant extracts in doses of 200 and 400 mg/kg. Normal and ulcerated control rats were given the vehicle only in a dose of 5 mL/kg. Rodents fasted overnight, with access to water ad libitum, before being anesthetized for surgery. Assay samples were administered orally 30 min prior to pyloric ligation. The abdomen of each rat was opened under light ether anesthesia and the pylorus was ligated (Anoop and Jegadeesam, 2003) and then sutured closed. Four hours after ligation, animals were sacrificed with excess ether and stomachs were dissected.

2.8.1. Gastric volume and titratable acidity

Gastric juices were collected and volume measured; liquid was then centrifuged (4000 rpm, 5 min) and a supernatant aliquot (1 mL) was titrated with sodium hydroxide (0.01 N) to pH 7.0 using the colorimetric indicator phenolphthalein. Titratable acidity was calculated:

Titratable acidity (mEq/L) = \( V_1 \times 1000 / V_2 \times 100 \)

where: \( V_1 = \) volume of 0.01 N NaOH (mL) and \( V_2 = \) volume of gastric juice.

2.8.2. Assessment of ulcer index (UI) and % gastric protection

The stomach was opened along the greater curvature and rinsed with water to measure the ulcerated area (mm²). The UI was calculated according to the method of Ganguly (1969) based on the formula:

UI = 10/X where, \( X = \) total mucosal area/total ulcerated area.

Percent gastric protection was estimated based on Navarrete and Trjo-Miranda (2002):

% gastric protection = \( \frac{\text{UIC} - \text{UIT}}{\text{UIC}} \times 100 \)

where, UIC and UIT = ulcer indices of control and treated groups, respectively.

2.9. Statistical analysis

Results were analyzed using a one-way ANOVA followed by a Dunnett’s multiple comparison test using SPSS statistics 17.0 (Chicago, USA), and expressed as means ± standard error.

3. Results

3.1. Acute toxicity evaluation

Oral administration of A. fragrantissima extracts in doses up to 4000 mg/kg did not produce symptoms of acute toxicity and mice mortality did not occur during the 48 h observation period. Moreover the mice did not exhibit diarrhea, hematuria, restlessness, uncoordinated muscle movements or respiratory distress. Accordingly, the oral LD₅₀ of A. fragrantissima extracts was determined to be higher than the highest tested dose (4000 mg/kg); ten and 20 fold dilutions of the highest dose were used for evaluating anti-inflammatory, antinociceptive and anti-ulcerative effects.

3.2. Anti-inflammatory activity

Pretreatment with either the non-polar or polar plant extract significantly suppressed edema rates in sub-plantar region of the hind paw induced by a carrageenan injection in a dose-related manner. The maximum anti-inflammatory effect of both extracts was induced at 2 h after a carrageenan injection with the non-polar extract doses of 200 and 400 mg/kg resulting in inhibition rates of edema of 26% and 29%, respectively (Fig. 1). The polar extract at 400 mg/kg showed the most potent anti-inflammatory effect with an inhibition rate of 45% compared with a 47% inhibition with the positive control, indomethacin treatment. The maximum edema rate of the injected foot pad of non-pretreated control rats (80 ± 6%) was obtained 4 h after carrageenan injection.

3.3. Analgesic activity

The tested extracts were shown to produce dose-dependent analgesic activity in mice in hot plate and writhing assays. Both non-polar and polar plant extracts as well as acetyl salicylic acid significantly prolonged the reaction time against the thermal stimulus in the hot-plate assay as compared to controls after 30, 60 and 90 min of administration (Fig. 2). Maximum protection against the thermal stimulus was observed at 90 min following the administration of 400 mg/kg of the non-polar extract (81%), which was not statistically different compared to the reference drug (89%).

In the acetic acid induced writhing assay, both non-polar and polar extracts at the tested doses of 200 and 400 mg/kg showed significant reduction in the number of writhes (Table 1). Maximum protection was observed with a polar extract dose of 400 mg/kg (55%), which was not statistically different than the acetyl salicylic acid reference drug (58%).

3.4. Ulcerative colitis

Rodent intestinal damage induced by acetic acid was associated with a significant increase in the wet weight/length ratio of the colon specimens as an indicator of inflammation (Table 2). The wet weight/length ratio increased 2.2-fold in rats with acetic acid colitis compared to normal rats (0.13 ± 0.01 versus 0.28 ± 0.01 g/cm, respectively). This ratio was reduced in individuals pre-medicated with 200 or 400 mg/kg of non-polar extract (0.17 ± 0.01 and 0.19 ± 0.01 g/cm, respectively) as
well as with 200 and 400 mg/kg of the polar extract (0.17 ± 0.01 and 0.16 ± 0.0 g/cm, respectively). In the untreated control group, abnormal changes were not observed suggesting that handling procedures did not impact the experimental outcome. Histopathological analysis was consistent with weight/length data in which acetic acid treatment alone increased coagulative necrosis of mucosa, hemorrhage in lamina propria and submucosa, submucosal edema and leucocytic cell infiltration whereas pre-treatment with either the non-polar or polar extracts significantly attenuated the extent and severity of the histological features of cell damage (Fig. 3).

While colitis rats exhibited lesion scores, ulcer areas and ulcer index values of 4.7 ± 0.2, 3.7 ± 0.3 cm² and 8.4 ± 0.3, respectively (Table 2), these inflammatory changes were significantly improved by oral dosing of the non-polar and polar plant extracts at both doses for 7 days prior to ulcer induction. Polar extract in a dose of 400 mg/kg produced the greatest reduction in the wet weight/length ratio, lesion score, ulcer area and ulcer index compared to the control colitis group. On the other hand, 200 mg/kg of non-polar extract had a lower anti-ulcerative efficacy.

3.5. Effect on pylorus ligation-induced gastric ulceration in rats

Pyloric ligation caused an accumulation of gastric secretions of 3.3 ± 0.1 mL/100 g with total acidity of the gastric secretions 59 ± 2.7 mEq/L in a control group. The polar extract at either dose had no significant effect on the volume of gastric secretions, total acidity or the ulcer index, however pre-treatment with the non-polar plant extract significantly reduced gastric secretions 2.5 ± 0.2 and 2.2 ± 0.1 mL/100 g at the doses of 200 and 400 mg/kg, respectively (Table 3).

Circular and linear lesions were frequently observed in the stomach of all the pyloric ligation control animals. Administration of both doses of non-polar extract significantly decreased the ulcer index to 5.4 ± 0.1 and 5.1 ± 0.2, respectively compared to 7.4 ± 0.2 in the positive control group. The percentages of gastric protection induced by both doses were 27 and 31%, respectively. Plant extract gastric protective effects were supported by histopathological examination of gastric tissues (Fig. 4).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of writhes/20 min</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.4 ± 3.89</td>
<td></td>
</tr>
<tr>
<td>Acetyl salicylic acid (150 mg/kg)</td>
<td>24.4 ± 1.33 *</td>
<td>58.22</td>
</tr>
<tr>
<td>Non-polar (200 mg/kg)</td>
<td>34.6 ± 2.68 *</td>
<td>40.75</td>
</tr>
<tr>
<td>Non-polar (400 mg/kg)</td>
<td>33.2 ± 1.43 *</td>
<td>43.15</td>
</tr>
<tr>
<td>Polar (200 mg/kg)</td>
<td>26.6 ± 1.03 *</td>
<td>54.45</td>
</tr>
<tr>
<td>Polar (400 mg/kg)</td>
<td>26.0 ± 1.03 *</td>
<td>55.48</td>
</tr>
</tbody>
</table>

* P < 0.05: Statistically significant from control (Dunnnett's test).

**P** < 0.05: Statistically significant from acetyl salicylic acid (Dunnnett's test).

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>W/L ratio (g/cm)</th>
<th>Lesion score (0–5)</th>
<th>Ulcer area (mm²)</th>
<th>Ulcer index (UI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.126 ± 0.009</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Colitis control</td>
<td>0.277 ± 0.012</td>
<td>4.66 ± 0.21</td>
<td>3.70 ± 0.32</td>
<td>8.36 ± 0.32</td>
</tr>
<tr>
<td>Non-polar (200 mg/kg)</td>
<td>0.170 ± 0.008 *</td>
<td>3.16 ± 0.16</td>
<td>2.45 ± 0.10</td>
<td>5.61 ± 0.20 *</td>
</tr>
<tr>
<td>Non-polar (400 mg/kg)</td>
<td>0.190 ± 0.009 *</td>
<td>3.50 ± 0.22 *</td>
<td>2.71 ± 0.19 *</td>
<td>6.21 ± 0.35 *</td>
</tr>
<tr>
<td>Pol (200 mg/kg)</td>
<td>0.166 ± 0.008 *</td>
<td>3.16 ± 0.16 *</td>
<td>2.46 ± 0.11 *</td>
<td>5.63 ± 0.09 *</td>
</tr>
<tr>
<td>Pol (400 mg/kg)</td>
<td>0.156 ± 0.008</td>
<td>3.00 ± 0.25</td>
<td>2.35 ± 0.15</td>
<td>5.35 ± 0.19 *</td>
</tr>
</tbody>
</table>

* P < 0.05: Statistically significant from ulcerogenic control (Dunnnett's test).

### 4. Discussion

The present investigation outlines the anti-inflammatory, analgesic and anti-ulcerogenic activities of an *A. fragrantissima* extract. The anti-ulcerogenic effect was further confirmed by histological preservation of the colon and gastric architecture. All rats treated with different doses of non-polar and polar plant extracts were alive during the 48 h of observation indicating an extract LD₉₀ higher than 4000 mg/kg. Since substances possessing LD₉₀ greater than 50 mg/kg are classified as non-toxic (Buck et al., 1976), the tested plant extract can be categorized as benign.

Carrageenan-induced paw inflammation is a standard assay for acute inflammation that is effectively employed to evaluate drug or plant extract anti-inflammatory activity. The release of histamine or serotonin occurs in the first phase (up to 1 h) and the second phase (over 1 h) is associated with the production of bradykinins, prostaglandins, and lysosomes (Crunkhorn and Meacock, 1971). The cyclooxygenase inhibitor, indomethacin as well as the non-polar and polar extracts showed significant edema reduction during the second phase of the edema process. This preliminary study suggests a possible role of the plant extract in prostaglandin synthesis inhibition. Indeed, previous plant extract studies have shown that flavonoids can play a pharmacological role in inflammation and allergies (Vinson et al., 1998). Moreover, future studies will specifically examine the role of extracted flavonoids in regulating inflammation.

The analgesic activity of the tested extracts was tested in mice using two different models: hot plate and writhing methods. The hot plate test in mice is an efficient analgesic model for the screening of centrally acting compounds against acute noxious thermal stimulation (Abbott and Melzack, 1982). In the present study, both non-polar and polar plant extracts possessed a central analgesic effect as they produced protection against thermal stimuli in mice after oral administration. Since lipid-insoluble or highly ionized drugs fail to enter the brain in significant amounts (Craig and Stitzelu, 2003), exclusion of polar over non-polar acting compounds against acute noxious thermal stimulation (Abbott and Melzack, 1982).

A potential source of analgesic activity is sesquiterpene lactones (Valerio et al., 2007) which are known to be abundant in plants. However, future studies will specifically examine the role of extracted sesquiterpene lactones in regulating inflammation.
abdominal muscles accompanying an extension of the forelimbs and elongation of the body. These symptoms are believed to be mediated by the prostaglandin pathways (Ronaldo et al., 2000). In the present study, non-polar and polar extracts produced peripheral analgesic activity in mice and thus indicates the presence of analgesic components that might influence the prostaglandin pathways. The polar extract is rich in phenolic compounds; mainly flavonoids and caffeoylquinic acid derivatives (Elgamal et al., 1991; Bimbiraitė et al., 2008). Flavonoids are known for their anti-inflammatory activity due to their influence on the metabolism of arachidonic acid and histamine release. Such metabolites can inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipooxygenase cyclooxygenase, and phospholipase A₂. Such arachidonic acid inhibition by inflamed cells could reduce endoperoxides, prostanoids, prostacyclin, and thromboxanes from the lipooxygenase pathway as well as hydroperoxy- and hydroxyeicosatetraenoic acids and leukotrienes from the cyclooxygenase pathway (Gabor, 1986).

The model of acetic acid induced colitis shares many of the histologic features of ulcerative colitis in human beings including mucosal edema and submucosal ulceration (Sharon and Stenson, 1985). The anti-ulcerogenic activity of the tested extracts was tested using an acetic acid-induced colitis model. The model shares many of the histologic features of ulcerative colitis in humans including mucosal edema, leukocyte infiltration of the mucosa and submucosal ulceration (Benedek et al., 2007). Induction of ulcerative colitis in rodents was achieved by intra-rectal application of acetic acid, resulting in severe inflammation and ulcers in the colonic tissue, compared with the normal control group. Pre-treatment of rats with ulcerative colitis by A. fragrantissima extracts resulted in significant improvement of colon ulcers by decreasing the lesion score and decreasing W/L ratio. The anti-ulcerogenic effect of the plant extract may be associated with observed anti-inflammatory properties. Interestingly, dicaffeoylquinic acid and a flavonoid fraction isolated from Achillea millefolium L inhibit in vitro proteases such as neutrophil elastases and metalloproteinases that can be involved in inflammatory bowel diseases like ulcerative colitis human (Benedek et al., 2007).

Peptic ulcer disease is one of the major gastrointestinal disorders which occur due to an imbalance between offensive factors (acid, pepsin and Helicobacter pylori) and defensive factors (mucin, prostaglandin and bicarbonate). Consequently reduction of gastric acid production as well as reinforcement of gastric mucosal protection has been the major therapeutic approach of peptic ulcer disease (Lakshmi et al., 2010). Furthermore, gastric acid is an important factor for the genesis of ulceration in pylorus ligation model. In this model, auto-digestion of mucosa by gastric acid and pepsin results in the development of ulcers (Shay et al., 1945). The obtained results are in accordance with previous studies obtained by Cavalcanti et al. (2006). They found that oral administration of A. millefolium was effective in protecting the gastric mucosa against acute gastric lesions induced by ethanol and indomethacin. Results of the present study are consistent with traditional uses of A. fragrantissima in the treatment of gastrointestinal disorders, an effect perhaps associated with the antioxidant protection and radical scavenging activity, which antagonizes oxidative stress-induced gastric damage (Potrich et al., 2010).

### 5. Conclusion

In conclusion, non-polar and polar extracts of A. fragrantissima were well tolerated following acute treatment and they neither produced death nor any signs of toxicity. The tested extracts possess anti-inflammatory and analgesic properties comparable to indomethacin and acetyl salicylic acid, respectively. Moreover, they attenuated the macroscopic colonic damage induced by acetic in rats. Phytochemical studies should provide greater insight into active principles and modes of biological activity.

#### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Gastric volume (mL/100 g)</th>
<th>Total acidity (mEq/L)</th>
<th>Ulcer index (UI)</th>
<th>% gastric protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.08 ± 0.07</td>
<td>7.3 ± 0.11</td>
<td>0.00 ± 0.00</td>
<td>100</td>
</tr>
<tr>
<td>Pyloric ligation control</td>
<td>3.33 ± 0.14</td>
<td>58.6 ± 2.74</td>
<td>7.43 ± 0.18</td>
<td>–</td>
</tr>
<tr>
<td>Non-polar (200 mg/kg)</td>
<td>2.45 ± 0.15*</td>
<td>38.3 ± 1.79</td>
<td>5.41 ± 0.14*</td>
<td>27.18</td>
</tr>
<tr>
<td>Non-polar (400 mg/kg)</td>
<td>2.16 ± 0.12*</td>
<td>31.4 ± 1.98*</td>
<td>5.13 ± 0.19*</td>
<td>30.95</td>
</tr>
<tr>
<td>Polar (200 mg/kg)</td>
<td>3.13 ± 0.04</td>
<td>58.3 ± 4.01</td>
<td>7.16 ± 0.14</td>
<td>3.63</td>
</tr>
<tr>
<td>Polar (400 mg/kg)</td>
<td>3.06 ± 0.10</td>
<td>49.1 ± 2.00</td>
<td>7.11 ± 0.17</td>
<td>4.30</td>
</tr>
</tbody>
</table>

* P < 0.05: Statistically significant from ulcergenotic control (Dunnett’s test).
Fig. 4. Histological gastric mucosal sections with gastric ligation only (A) showing submucosal edema. Pre-treatment with 400 mg/kg of non-polar extract (B) or 400 mg/kg of polar extract (C) attenuated the extent and severity of cell damage (H and E staining, original magnification × 200).

Acknowledgment

We gratefully acknowledge staff members of Histopathology Unit, Faculty of Veterinary Medicine – Cairo University for carrying out the histopathological investigation.

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