In vitro antioxidant activity and gastroprotective effect of ethanolic extract from Cucumis melo L. var. inodorus fruit on ethanol-induced gastric ulcer in rats

Amel Bouaziz*, Assia Bentahar, Salilha Djidel, Salilha Dahamma and Seddik Khennouf

Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, University Ferhat Abbas Setif 1, Setif, 19000, Algeria

ABSTRACT

The aim of this study was to estimate the content of polyphenols, flavonoids and tannins and to evaluate the antioxidant property and the antiulcer activity of the ethanolic extract of melon (Cucumis melo L. var. inodorus) pulp on ethanol-induced gastric ulcers in rats. Ferric reducing power and hydroxyl radical scavenging tests were applied to evaluate the in vitro antioxidant activity. The polyphenolic and flavonoids contents of melon extract were found to be 56.5 ± 2.49 mg GAE/g of dry extract and 0.43 ± 0.09 mg QE/g of dry extract, respectively. Whereas the tannins content was 48.3 ± 0.9 mg ETA/g of dry extract. Melon pulp extract exhibited a good reducing potential with an EC50 of 4.23 ± 0.08 mg/ml and high hydroxyl radical scavenging activity with IC50 of 1.83 ± 0.09 mg/ml. Oral administration of the melon pulp extract at doses of 200 and 600 mg/kg to rats reduced gastric mucosal lesions dose-dependently with percentage protection of 56.56 and 93.79% compared to omeprazole (95.92%) as reference drug. These results showed that the melon pulp extract had a good antioxidant activity and gastroprotective potential suggesting its use as an adjuvant in the treatment of gastric ulcer.

Keywords: Cucumis melo var. inodorus, Polyphenols, Flavonoids, Tannins, Ulcer, Rat.

INTRODUCTION

Gastric ulcer is an erosive lesion in the gastric mucosa characterized by a persistent inflammatory, oxidative and necrotic damage into stomach wall, which can perforate the submucosa and muscular layers and developed peritonitis or massive hemorrhage. The pathophysiology of this disease has a multifactorial process that is caused by the imbalance between aggressive factors, as acid and pepsin and mucosal defense factors, especially blood flow and prostaglandins. In addition, the incidence of peptic ulcer can be increased by many factors including stress, alcohol consumption, smoking, Helicobacter pylori, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Several medications were used in the treatment of ulcers such as antibiotics, proton pump inhibitors, prostaglandin analogs, and H2 receptor blockers. However, these agents are facing major problems due to their limited efficacy and severe side effects as gynecomastia, hypocoagulability, impotence, osteoporotic bone fracture, hypergastrinaemia and cardiovascular disease risks. Oxidative stress is caused by reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl, and nitric oxide (NO) radical. These ROS accumulations lead to damage to crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, carbohydrates in living system. Antioxidants apparently protect the living system from this oxidative stress, which is a hallmark feature of cancer, cardiovascular disease, diabetes, and ulcer. Most of the natural plant antioxidants such as vitamin C and E, carotenoids, polyphenols, flavonoids, and tannins have strong antioxidant capacity which play a vital role in the prevention and treatment of many diseases as gastric ulcer. Therefore, new therapeutic alternatives that present a good effectiveness as well as safer therapies are needed for the improvement of ulcer healing and prevention of disease recurrence. Hence, efforts are headed towards the finding of suitable treatment from natural product sources such as fruits, vegetables and plant extracts which possess the
antisecretory, cytoprotective and antioxidant property that play a key role in gastric mucosal protection.

Melon, which is also known as *Cucumis melo* L., belongs to the Cucurbitaceae family and it is one of the most widely cultivated and consumed fruits worldwide. Various studies have reported that *C. melo* L. is a delicious and juicy fruit offering numerous medicinal and nutritive functions. It contains polyphenols, organic acids, lignans and other polar compounds that are beneficial to human health. Melon pulp is rich in important vitamins, phytoene, β-carotene, and 5-methylytetrahydrofolic acid and it possesses high antioxidant and anti-inflammatory properties. However, there is little scientific data reported on the gastroprotective activity of *C. melo*. Hence, the current study was undertaken to evaluate the *in vitro* antioxidant activity and antiulcerogenic property of the ethanolic extract of *C. melo* var. *inodorus* pulp against ethanol induced ulcer model in rats.

**MATERIALS AND METHODS**

**Plant materials**

Fresh melon was purchased from the market in Setif region (Northeastern of Algeria). The whole fruit was washed, peeled and the pulp was separated from the flesh and then the rind was grated to reduce its size.

**Animals**

Male Wistar rats (150-200g) were purchased from Pasteur institute, Algiers. They were housed in an air-conditioned animal room (12 hours light/dark cycle, 23 ± 2°C) for one week for adaptation and have free access to commercial diet and water ad-libitum.

**Extraction of phenolic compounds**

The extraction of phenolic compounds was carried out according to method used by Markham. Briefly, 1kg of crushed pulp was mixed with 5 L of ethanol-water mixture (80:20, v/v) and kept at room temperature for 5 days to allow maximum extraction of bioactive molecules. The resulting solution was then filtered and the supernatant was evaporated using vacuum rotary evaporator at 40°C. The extract obtained was dried to obtain crude ethanolic extract and stored at 4°C until use.

**Determination of total polyphenols content**

The total polyphenols content was determined by the Folin-Ciocalteu method as described by Li et al. with slight modification. In brief, 0.1 ml of pulp extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 times). After 4 min, 0.4 ml of 7.5% sodium carbonate (Na2CO3) solution was added. The final mixture was shaken and then incubated for 90 min in dark at room temperature. The absorbance of all samples was measured at 760 nm and the results are expressed in milligrams of gallic acid equivalents per gram dried weight (mg GAE/g DW).

**Determination of total flavonoids content**

The total flavonoids content of each extract was determined by a colorimetric method as described by Bahourun et al. 12. 1 ml of melon pulp extract was mixed with 1 ml of aluminium chloride (AlCl3) solution (2%) and allowed to stand for 10 min. Absorbance of the mixture was then determined at 430 nm versus prepared methanol blank. Results were expressed as quercetin equivalent per gram dried weight (mg QE/g DW).

**Determination of tannins content**

The capacity to precipitate haemoglobin was determined by using bovine fresh blood according to the method described by Gharzouli et al. Briefly, a volume of melon pulp extract was mixed with an equal volume of hemolysed bovine blood (absorbance = 1.6). After 20 min, the mixture was centrifuged at 4000 rpm for 10 min, and the absorbance of the supernatant was measured at 756 nm. Results were expressed as mg equivalent tannic acid per gram dried weight (mg TAE/g DW).

**Evaluation of antioxidant activity**

**Ferric reducing power**

The reducing power of the melon pulp extract was determined according to the method of Chung et al. 14. A 0.1 ml aliquot of different concentrations of extract or BHT was mixed with an equal volume of 0.2 mol/l phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min to reduce ferricyanide into ferrocyanide. After that, 0.25 ml trichloroacetic acid was added into the mixture to stop the reaction, and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (0.25 ml) was added into distilled water (0.25 ml) and 0.1% ferric chloride (0.5 ml), and then the absorbance was measured at 700 nm. The EC50 value was defined as the effective concentration of the extract or standard which had the absorbance of 0.5.

**Hydroxyl radical scavenging assay**

Hydroxyl radical scavenging activity was measured by the ability of melon pulp extract to scavenge the hydroxyl radicals according to the method described by Smirnoff and Cumbes with slight modifications. The reaction mixture consists of 100 μl of varying concentration of samples or standard antioxidants, 1 ml of FeSO4 (1.5 mM), 0.7 ml of H2O2 (6 mM), 0.3 ml of sodium salicylate (20 mM). This mixture was incubated at 37°C for 1 h, after which the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated according to the following equation:

\[
\text{Scavenging activity} (\%) = \frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100
\]

Where A control was the absorbance of the control (without sample) and A sample was the absorbance in the presence of the sample.

**Evaluation of antiulcer activity by ethanol-induced ulcer model**

The gastroprotective activity of melon pulp ethanol extract was determined following the method used by Abdulla et al. with slight modifications. Rats were fasted for 24 h before the experiment but were allowed free access to drinking water till 1 h before the experiment. The groups were divided follows; Group I served as control and received distilled water (5 ml/kg); Group II was treated with omeprazole (20 mg/kg) as reference drug; Groups III and IV were treated with 200 and 600 mg/kg of melon pulp extract, respectively. One hour after this pre-treatment, all the groups of rats received a dose of absolute ethanol (2.5 ml/kg) orally. The animals were sacrificed after 30 min of ulcerogen administration, and their stomach were removed, opened along the greater curvature, washed with cold saline, and then flattened stomach samples were photographed. The total area of the lesions and the total area of the stomach was measured using Image J software. The percentage of ulceration was calculated according to the following formula:
% ulceration = (total ulcerated area / total mucosal area) × 100

Preventive Index (PI) was calculated for each treated group according to this formula:

\[ \text{PI} = \frac{\text{Uc} - \text{Ut}}{\text{SUc}} \times 100 \]

Statistical analysis

In vitro results were expressed as mean ± standard deviation (SD) and the in vivo results were presented as mean ± standard error of mean (S.E.M.). The differences between groups were determined by analysis of variance (one-way ANOVA) followed by Dunnet’s test. All results were analyzed using Graph Pad Prism version 5.00. Differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

Total polyphenols, flavonoids and tannins contents

Plant phenolic compounds are secondary metabolites with interesting properties for human health. The beneficial effects of these molecules are related to their antioxidant activity, particularly their ability to scavenge free radicals, to donate hydrogen atoms or electrons, or to chelate metal cations. Besides, phenolic compounds contribute largely to the color and sensory characteristics of fruits and vegetables. Therefore, it would be valuable to determine the amount of total phenolics present in the fruit extracts.

As seen in Table 1, the total polyphenol and flavonoids contents in melon pulp ethanolic extract were found to be 56.5 ± 2.49 mg GAE/g and 0.43±0.09 mg QE/mg extract. Whereas the tannins content was 48.3 ± 0.9 mg TAE/mg extract.

Table 1: Total polyphenols and flavonoids contents in melon pulp ethanolic extract

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic content (mg GAE/g)</th>
<th>Flavonoids content (mg QE/g)</th>
<th>Tannins content (mg TAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>56.5±2.49</td>
<td>0.43±0.09</td>
<td>48.3±0.9</td>
</tr>
</tbody>
</table>

GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent, TAE: Tannic Acid Equivalent. Results expressed as means ± SD (n = 3)

The literature reports many studies comparing polyphenolic, flavonoid and tannin contents of different varieties of melon as C. melo var. reticulates and C. melo var. cantalupensis, but polyphenolic, flavonoid and tannin contents of the variety C. melo var. inodorous were never assessed.

Antioxidant activity evaluation

Ferric reducing power

In reducing power assay, the yellow color of the test solution changes to various shades of green and blue, depending on the presence of reductants in the extract that might cause the reduction of the Fe³⁺/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl’s Prussian blue at 700 nm can monitor the Fe²⁺ concentration.

In this study, it was observed that the reducing capacity of melon ethanol extract and BHT increased dependently with increasing concentration. The melon ethanol extract showed a good reducing power with EC₅₀ of 4.23 ± 0.08 mg/ml; however, it remained significantly (p < 0.001) lower compared to BHT (EC₅₀ = 0.05 ± 0.006 mg/ml) (Table 2).

Table 1: Antioxidant activities of melon pulp ethanolic extract

<table>
<thead>
<tr>
<th>Extract/standard</th>
<th>EC₅₀ (mg/ml)</th>
<th>IC₅₀ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reducing power effect</td>
<td>Hydroxyl radical scavenging activity</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.23 ± 0.08***</td>
<td>1.83 ± 0.09***</td>
</tr>
<tr>
<td>BHT</td>
<td>0.05 ± 0.006</td>
<td>/</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.084 ± 0.004</td>
<td></td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD, n=3. *** p < 0.001, the comparison was realized against correspondent standards.

The reducing power of the extracts may provide a significant indication about the potential antioxidant capacity of the fruits and vegetables. The presence of antioxidants in the extract would result in the reduction of Fe³⁺ to Fe²⁺ by donating an electron. C. melo var. inodorous pulp extract showed good reducing power indicating the compounds in this fruit, performing as good electron donors and therefore should be able to terminate radical chain reaction by converting free radicals and ROS into more stable products. Also, the reductive abilities of this extract may be attributed to the presence of polyphenols. Several studies have shown the correlations between reducing power and polyphenolic contents in the plant extracts.

Hydroxyl radical scavenging activity

Hydroxyl free radicals have been implicated in the etiology of much pathology and eventually resulted in the cell injury/death. Therefore, the scavenging of hydroxyl radicals by extracts may provide a significant protection to biomolecules against free radicals.

As shown in Table 2, melon pulp ethanolic extract exhibited good in vitro scavenging activity against hydroxyl radicals generated in a Fenton reaction system with IC₅₀ of 1.83
±0.09 mg/ml, but this activity is relatively lower (p < 0.001) compared to that of vitamin C as standard (IC50 equal to 0.084 ±0.007 mg/ml).

Hydroxyl radical is the major active oxygen species in the biological systems and it can cause lipid peroxidation and enormous biological damage 26. In this study, melon pulp ethanolic extract displayed remarkable potential in scavenging hydroxyl radical which might be related to its amount of total phenolic and flavonoids content. According to many reports, there is a highly positive correlation between polyphenols, flavonoids, and antioxidant activities in many plant species, and this is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers 21.

**Effect of melon pulp extract on ethanol-induced gastric lesions**

The etiology of peptic ulcer is mainly due to an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To regain this balance, different therapeutic agents are used to inhibit the gastric acid secretion or to increase the mucosal defense mechanisms by increasing mucus production or interfering with prostaglandin synthesis 27. The present study investigated for the first time the antiulcer effect of ethanolic extract from *C. melo var. inodorus* pulp in experimental model of gastric ulcers induced by ethanol.

**Macroscopic evaluation of lesions**

As shown in Figure 1, the absolute ethanol administration induced extensive long and hemorrhagic gastric ulcers in the rats (Figure 1 A). Oral pretreatment with pulp melon ethanolic extract attenuated the number and the length of gastric lesions in a dose-dependent manner (Figure 1 B and C). Among the two tested doses, high dose of melon pulp extract (600 mg/kg) showed maximum inhibition on the number and length of gastric lesions. Omeprazole (20 mg/kg) (Figure 1D) and pulp melon extract (600 mg/kg) had similar effects on the number and length of gastric ulcers and restore the normal appearance of the stomach. In addition, high dose of melon pulp extract (600 mg/kg) alone had no side effects on stomach.

![Figure 1: Macroscopic appearance of gastric ulcers induced by ethanol and protective effects of melon extract (200 and 600 mg/kg) and omeprazole. (A): Ethanol treated control; (B): Melon extract treated rat (200 mg/kg); (C), Melon extract treated rat (600 mg/kg); (D): Omeprazole treated rat (20 mg/kg).](image)

**Evaluation of gastric protection**

Oral administration of melon pulp ethanolic extract (200 and 600 mg/kg), one hour before the induction of gastric lesions with ethanol, significantly reduced lesion area with percentage of ulceration of 13.88 ± 2.41 and 1.98 ± 0.63%, respectively, compared to the control group (31.96±2.41%) (Figure 2 A). Omeprazole (20 mg/kg) has been previously demonstrated to inhibit ethanol-induced gastric lesion formation and so it was used as positive control of lesion inhibition. Pretreatment of rats with omeprazole inhibited significantly (p < 0.001) the gastric lesions to 1.98 ±0.63% (Figure 2A). As shown also in Figure 2B, the protective indexes of melon ethanolic pulp extract of the two doses 200 mg/kg and 600 mg/kg groups were 56.56 ± 2.43 %, 93.79 ± 1.98 %, respectively, while the reference drug, omeprazole (20 mg/kg) showed protection ratio of 95.92 ±1.98 %. This protective index was similar to that of 600 mg/kg treated rat group (no significant difference).
Ethanol-stimulated gastric lesions model have been used commonly to investigate the pathogenesis of gastric ulceration and to evaluate the gastroprotective effect of various drugs and natural products. Ethanol is responsible for disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion, free radical production of superoxide anion and hydroperoxy free radicals, and ROS-mediated increased lipid peroxidation. Administration of an ethanolic extract of *C. melo* var. *inodorus* (200 and 600 mg/kg) exhibited a gastroprotective effect against ethanol induced gastric damage which is may be due to its polyphenolic, flavonoids and tannins contents which are free radical scavengers as shown in the present study and are also known to possess antiulcer activity and prevent ethanol-induced gastric ulcer. Therefore, the presence of flavonoids and tannins in *C. melo* var. *inodorus* pulp may be associated with the ulcer preventing action. It is suggested that these active compounds would be able to stimulate mucous bicarbonate and to inhibit prostaglandin secretion and counteract with the liberating effect of reactive oxygen in the gastrointestinal lumen whereas, tannins prevent ulcer development due to their protein precipitating and vasoconstricting effects. Their astringent action can help to precipitate microproteins on the ulcer site, thereby, forming an impervious layer over the lining, which hinders induced gastric ulcer.

**CONCLUSION**

The results of this study showed that pulp ethanolic extract from *C. melo* var. *inodorus* has a high content of total phenolic compounds and exhibited good reducing potential and hydroxyl radical scavenging activity. Also, melon pulp ethanolic extract exerted appreciable gastroprotective effect against ethanol-induced gastric lesions in rats. However, more studies are needed to determine the exact mechanisms of antiulcer activity of melon pulp.

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**REFERENCES**


