Evaluation of Antioxidant, Antiinflammatory and Antiulcer Potential of *Momordica charantia* Methanolic Seed Extract

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ABSTRACT

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. Several developing countries as well as developed countries are using natural herbal medicines. The widespread use of herbal remedies and healthcare preparations has been described in ancient texts such as the Vedas and the Bible. The aim of the present study was to evaluate the antioxidant, antiinflammatory and antiulcer potentials of *Momordica charantia* seeds. Phytochemical screening of the seed extract showed presence of such phytoconstituents which have remarkable potential for the prevention as well as cure of some diseases. Antioxidant property of the extract was studied by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The extract showed maximum percentage inhibition of 61.43% by 1,1-diphenyl-2-picrylhydrazyl method at a concentration of 100 μg mL⁻¹ as compared with the ascorbic acid. Further, it was evaluated for antiinflammatory activity by using carrageenan induced rat paw oedema. The extract showed significant reduction in the paw volume (62.79% reduction) at a dose of 500 mg kg⁻¹. In case of antiulcer activity maximum % inhibition of gastric ulcer was found to be 62.85% in ethanol induced ulcer model and 69.6% in non steroidal antiinflammatory drug induced ulcer model at a dose of 100 mg kg⁻¹ as compared with the standard ranitidine. The result of present study showed that seeds of *Momordica charantia* have been proven to be good natural source of antioxidant, antiinflammatory and antiulcer potentials.

Key words: *Momordica charantia*, antioxidant, antiinflammatory, antiulcer, bitter melon

INTRODUCTION

Plants have been a major source of phytoconstituents which have been used to treat various diseases since ancient times (Grover et al., 2002; Das et al., 2009). Many traditional systems of medicines like Ayurveda, Unani and Chinese medicine have flourished the traditional medicine system (Ismail et al., 2011). Literature survey on Cucurbitaceae family shows that the plants of this family have been used for various health benefits. It is an important plant family including a variety of plants having a number of medicinal uses.

Cucurbitaceae family is composed of pumpkins, gourds and melons. This family consists of 118 genera and about 825 species (Lima et al., 2010). Many species of this family such as pumpkin (*Cucurbita* sp.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.) and West Indian gherkin
(Cucumis anguria L.) are cultivated because of their medicinal and nutritional properties (Milind and Kaur, 2011). Among all these species, Momordica is an important genera. Momordica charantia is edible plant of this category and is widely cultivated.

Momordica charantia belongs to the family cucurbitaceae. It is also known as bitter melon, bitter gourd in English, karela in Hindi and Peria in Chinese. It is a plant widely cultivated in many tropical and subtropical regions of the world as food and medicinal plant (Hakim et al, 2011). Since antiquity, the fruits of this plant have been used as vegetable in India. It is a rich source of nutrients such as essential amino acids, vitamin A, carotenoids, folic acid and vitamin C and the whole plant contains many bioactive compounds (Krawinkel and Keding, 2006). It has been known to exhibit blood sugar lowering potential. Diabetic patient use it in various forms e.g., Juice of Momordica charantia as home remedy against diabetes mellitus.

Charantin, a steroidal saponin isolated have hypoglycemic potential. It is also used as antiviral, anthelmintic, anticancer, antimalarial, immunomodulatory, antipsoriatic and antimicrobial remedy (Dasgupta et al., 2011). Two proteins, α and β monomorcharin, have been isolated from the seeds of bitter gourd. These proteins have shown to act as immunosuppressive without having any cytotoxic effect. They also modulate the activity of both α and β lymphocytes (Singh et al., 2011). The fruit part of the plant has shown considerable activity in reducing oxidative stress caused by Reactive oxygen species (Rezaiezadeh et al., 2011).

Reactive Oxygen Species (ROS) play an important role in oxidative stress which is related to the pathogenesis of various serious disorders like neurodegenerative disorders, cancer, rheumatism, arteriosclerosis etc. Oxidative stress is generated when there is imbalance between the amount of free radicals and antioxidant level in the body (Shyur et al., 2005). ROS are known to damage cellular membranes by inducing lipid peroxidation. They also can damage DNA, proteins and lipids. The most popular ROS are superoxide radical (O2–), hydrogen peroxide (H2O2) and hydroxyl radical (OH–) (Kunwar and Priyadarshini, 2011). As antioxidants have been reported to prevent oxidative damage caused by free radicals, it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers. Antioxidant property of medicinal plants and their purified constituents have shown beneficial therapeutic potential in reducing oxidative stress (Lobo et al., 2010). The above ideas had prompted this investigation to search for potent and cost-effective antioxidants and antiulcer constituents from Momordica charantia.

MATERIALS AND METHODS

Plant material: The seeds were collected in the month of August, 2011 from the local market of Ropar, Punjab (India). It has been authenticated from the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar with a voucher specimen number 1212. A sample of seeds has been deposited there in the department.

Drugs and chemicals: Ascorbic acid and carrageenan were obtained from Central Drug House Pvt. Ltd., Mumbai, India. 1,1-diphenyl-2-picrylhydrazyl was obtained from Sigma Chemical Co., USA. Methanol was purchased from SD. Fine Chem. Ltd, Mumbai. Ranitidine was collected as free sample from Jackson Laboratories, Amritsar.

Experimental animals: The animal study was carried out during the period of Nov. 2011 to April 2012. Wistar albino rats of either sex (average weight 130-180 g) were purchased from Punjab
Agricultural University, Ludhiana. They were kept at standard laboratory diet, environmental temperature (24.0±1.0°C), relative humidity (55-65%) and 12 h light/12 h dark cycle. They were allowed free access to standard dry pellet diet. The experimental protocol was duly approved by Committee for the Purpose of Control and Supervision of Experiments on Animals.

**Extraction**: Seeds were shade dried and finely powdered. The powdered seeds were then macerated in methanol for a period of 72 h and repeated it for 3 times. The filtered solvent was then allowed to evaporate in the rotary evaporator at a temperature of 45°C. This crude extract was defatted by adding hexane to it and separated in separating funnel and concentrated. The extract was stored in refrigerator for further use in the evaluation of various activities.

**Phytochemical screening**: The extract was subjected to phytochemical screening for determining various phytochemicals present in the extract. The tests for the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, tannins and coumarin glycosides. Triterpenoids were tested by Salkowski test. Alkaloids were tested by Mayer’s reagent, Dragendorff’s reagent. Glycosides were tested by Borntrager’s reagent (Ayoula et al., 2008). Tannins were tested by ferric chloride test. Flavonoids were tested by Shinoda test (Wani et al., 2011).

**Antioxidant activity**

**Quantitative evaluation of 1, 1-diphenyl-2-picrylhydrazyl scavenging activity**: The free radical scavenging activity of methanol extract was determined by using 1,1-diphenyl-2-picrylhydrazyl method (Gill et al., 2010). A solution of 0.05 mM 1,1-diphenyl-2-picrylhydrazyl in methanol was prepared and 1.5 mL of this solution was added to 0.5 mL of extract in methanol in different concentrations (25-100 μg mL⁻¹). This mixture was shaken vigorously and kept in dark for 30 min. Then the absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-1700 phamraspec). Methanol solution of 1,1-diphenyl-2-picrylhydrazyl was taken as control. Triplicate of every absorbance was taken. Inhibition of 1,1-diphenyl-2-picrylhydrazyl was measured by the following equation:

\[ I(\%) = \frac{V_0 - V_s}{V_0} \times 100 \]

Where:
- \( A_c \) = Absorbance of Control
- \( A_s \) = Absorbance of Sample
- \( I \) = Inhibition of 1, 1-diphenyl-2-picrylhydrazyl

**Antiulcer activity**

**Experimental design of ethanol induced ulcer rat model**: Animals were divided into 5 groups each containing 6 Wistar rats was used to study the anti-ulcer activity of methanol extract:

**Group 1**: Administered vehicle (normal saline 0.9% w/v p.o.) 1 h before induction of ulcers

**Group 2**: Disease control group administered absolute ethanol (0.5 mL kg⁻¹) for the induction of ulcer

**Group 3**: Administered standard ranitidine (50 mg kg⁻¹ p.o.) 30 min before induction of ulcers
Group 4: Administered dose (300 mg kg⁻¹ p.o.) 30 min before induction of ulcers
Group 5: Administered dose (500 mg kg⁻¹ p.o.) 30 min before induction of ulcers

All animal were kept on fasting for 18 h before the performance of activity. After 15 min of ethanol administration, all the animals were sacrificed. Each stomach was opened along the greater curvature and washed with normal saline to clear the gastric content (Dashpurte and Naikwade, 2011). Each stomach was examined for the ulcer index according to the method of Kore et al. (2011).

Experimental design of NSAID (indomethacin) induced ulcer rat model: Animals were divided into 5 groups each containing 6 rats:

Group 1: Administered vehicle (normal saline 0.9% w/v, p.o.) 30 min before induction of ulcers
Group 2: Disease control group administered indomethacin (25 mg kg⁻¹, p.o.) for the induction of ulcers
Group 3: Administered standard (ranitidine 50 mg kg⁻¹, p.o.) 30 min before induction of ulcers
Group 4: Administered dose (300 mg kg⁻¹, p.o.) 30 min before induction of ulcers
Group 5: Administered dose (500 mg kg⁻¹, p.o.) 30 min before the induction of ulcer

Normal saline, ranitidine, extract were given orally and 30 min later indomethacin was administrated to all the groups. Six hours later, the animals were killed by decapitation. The stomachs were removed, opened along the great curvature and washed with saline to remove gastric contents and examined for the formation of ulcers (Gill and Bali, 2011). For each stomach, the ulcer index was calculated according to the method of Kore et al. (2011).

Antiinflammatory activity
Carrageenan-induced rat paw oedema: The rats were divided into five groups, each group consisting of six Wistar rats weighing 160-180 g each. Oedema was induced by subplantar injection of 0.1 mL of freshly prepared 1% carrageenan suspension into the right hind paw of each rat. The paw volume was measured at 0 and 3 h after injection of carrageenan by using a plethysmometer. The methanol extract of Momordica charantia Linn seeds at 300, 500 mg kg⁻¹ were administered orally to first three groups of rats. While the fourth and fifth group of animals received 5 mL kg⁻¹ propylene glycol as vehicle control and 10 mg kg⁻¹ diclofenac as drug control, respectively, for comparative pharmacological assessment. Drug pretreatment was given 1 h before the injection of carrageenan (Ganesan et al., 2008). The percentage inhibition of oedema was calculated by using the following formula:

\[ \text{Inhibition} \% = \left( \frac{V_o - V_s}{V_o} \right) \times 100 \]

Where:
- \( V_o \) = The average increase in the paw volume of control
- \( V_s \) = The average increase in the paw volume after test/standard drug
**Statistical analysis:** All the results were expressed as Mean±Standard Errors of Means (SEM). The data was analysed by one way Analysis of Variance followed by Tukey multiple range test. The (p<0.01) was considered to be statistically significant.

**RESULTS**

The phytochemical screening of methanol extract of *Momordica charantia* seeds revealed the presence of alkaloids, carbohydrates, triterpenoids and flavonoids (Table 1). Triterpenoids and flavonoids in plants may play a significant role in antioxidant, antiulcer, antiinflammatory activities (Gill et al., 2011).

The evaluation of antioxidant activity by 1,1-diphenyl-2-pierylhydrazyl (DPPH) method showed significant results. DPPH is one of the stable organic nitrogen free radicals, which is widely used for testing preliminary radical scavenging activity of a compound or a plant extract. It has a maximum absorbance at 517 nm. Absorbance decreases when antioxidants donate protons to DPPH, thereby reducing the latter (Guha et al., 2009). The % scavenging was found to be 61.43±0.01361% at a dose of 100 µg mL⁻¹ as compared to the standard ascorbic acid in case of DPPH free radical scavenging activity (Table 2).

The extract also showed significant reduction in the gastric ulcers in NSAID induced and ethanol induced ulcer models. The maximum % inhibition of gastric ulcer was found to be 62.85 and 69.8% at a dose of 500 mg kg⁻¹ in case of ethanol induced ulcer model and NSAID induced models, respectively (Table 3, 4).

| Table 1: Phytochemical screening of *Momordica charantia* methanol seed extract |
|---------------------------------|-------------------------------|
| Plant constituents             | Results                       |
| Alkaloids                      | +                             |
| Carbohydrates                  | +                             |
| Flavonoids                     | +                             |
| Triterpenoids                  | ++                            |
| Tannins                        | -                             |
| Coumarin glycosides            | +                             |

- Absent, + Present, ++: Higher presence of respective constituents

<table>
<thead>
<tr>
<th>Concentration (µg mL⁻¹)</th>
<th>Scavenging of DPPH (%)</th>
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<tbody>
<tr>
<td></td>
<td>Methanol extract</td>
</tr>
<tr>
<td>25</td>
<td>47.36±0.01457</td>
</tr>
<tr>
<td>50</td>
<td>57.67±0.02635</td>
</tr>
<tr>
<td>100</td>
<td>61.43±0.01361</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Mean±SEM

<table>
<thead>
<tr>
<th>Table 3: Antiulcer activity by ethanol induced ulcer model</th>
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<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Ulcer control</td>
</tr>
<tr>
<td>Ramiidine</td>
</tr>
<tr>
<td>MEMC (300 mg kg⁻¹)</td>
</tr>
<tr>
<td>MEMC (500 mg kg⁻¹)</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Mean±SEM. All values are significant at *p<0.05* compared to control and *p<0.05* compared to ramiidine (Tukey’s test). MEMC: Methanol extract of *Momordica charantia*
Table 4: Antiulcer activity by NSAID induced ulcer model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulcer index</th>
<th>Age inhibition of gastric ulcers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer control</td>
<td>2.5±0.088</td>
<td>-</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>0.43±0.633&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.68</td>
</tr>
<tr>
<td>MEMC (300 mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.03±0.688&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>58.80</td>
</tr>
<tr>
<td>MEMC (500 mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.76±0.658&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>69.60</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Means±SEM. All values are significant at *P<0.05 compared to control and **P<0.05 compared to ranitidine (Tukey’s test). MEMC: Methanol extract of Momordica charantia

Table 5: Antiinflammatory activity of MEMC by carrageenan induced rat paw edema

<table>
<thead>
<tr>
<th>Group</th>
<th>Paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
</tr>
<tr>
<td>Disease control</td>
<td>0.53±0.007</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>0.38±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MEMC (300 mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.44±0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>MEMC (500 mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.40±0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Means±SEM. All values are significant at *P<0.05 compared to control and **P<0.05 compared to diclofenac sodium (Tukey’s test). MEMC: Methanol extract of Momordica charantia

Antiinflammatory study was done by carrageenan induced rat paw edema. Carrageenan induced rat paw edema is a suitable experimental animal model for evaluating the anti-edematous effect of natural products. This model is believed to be triphasic. The first phase (1 h after carrageenan administration) involves the release of serotonin and histamine from mast cells, the second phase (2 h) is provided by kinins and the third phase (3 h) is mediated by prostaglandins, the cyclooxygenase products and lipoxygenase products (Ullah et al., 2012). The maximum % inhibition in paw volume was found to be 62.79% at a dose of 500 mg kg<sup>-1</sup> (Table 5).

DISCUSSION

In the present study, Momordica charantia seeds extract have been investigated for their antioxidant, antiulcer and antiinflammatory potentials and the extract showed optimum potential. Studies have been done for antioxidant activity on the whole fruit of Momordica charantia (Semiz and Sen, 2007; Rezaei zadeh et al., 2011). The leaf and stem part of Momordica charantia has been found to possess antioxidant potential (Kubola and Siriamornpun, 2008). The influence of ripening stages of fruit on antioxidant properties of Momordica charantia has also been studied (Aminah and Anna, 2011). Seeds of Momordica charantia have not been evaluated for antioxidant property by DPPH method. Results have shown that seed extract possess remarkable antioxidant activity. This activity may be due the presence of Triterpenoids in the present extract. The free radical scavenging activity of the extract may suppress the factors which may result into the reduction of gastric ulcers.

The seed extract was further investigated for antiulcer activity. The fruit part of Momordica charantia has been evaluated for antiulcer potential by indomethacin induced ulcer model in rats (Dengiz and Gursan, 2005). The methanol fruit extract of bitter melon has shown antiulcer potential (Alam et al., 2009). Antiulcer potency of fruit extract of Momordica cymbalaria has been reported (Dhasan and Jagadeesan, 2010). No study on the seeds of Momordica charantia has been reported. The results on antiulcer activity of seeds of this plant in the present research paper establish their antiulcer potential.
Seeds have also been investigated for their antiinflammatory potential. Antiinflammatory activity has been evaluated on the fruit without seeds (Lii et al., 2009), leaf part (Umukoro and Ashorobi, 2006) of this plant. Induction of antiinflammatory responses by dietary *Momordica charantia* has been reported (Manabe et al., 2003). The seed part has not been evaluated for antiinflammatory activity. The present research showed that seeds of *Momordica charantia* have considerable antiinflammatory activity.

**CONCLUSION**

Present study concluded that the methanol seed extract of *Momordica charantia* showed significant antioxidant activity by free radical scavenging. As it proved to be a potent antioxidant, antiulcer potency and antiinflammatory activity has also been shown by the extract. Thus the methanol seed extract of *Momordica charantia* can be used as a natural source of antioxidant, antiulcer and antiinflammatory agent.

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