Evaluation of *Momordica charantia* L. Fruit Extract for Analgesic and Anti-inflammatory Activities using *in vivo* Assay

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**ABSTRACT**

*Momordica charantia* L. fruits, leaves, seeds and roots are considered as valuable traditional medicine. It is considered antitodal, antipyretic, tonic, antispasmodic, mild hypotensive. It builds immunity, relieves fever and reduce cholesterol level. To give a scientific basis for medicinal usage of this medicinal plant, the fruit extract was evaluated for the analgesic and anti-inflammatory activities. In this study, the acute toxicity study, analgesic and anti-inflammatory activities of *Momordica charantia* L. fruit extract were evaluated in a series of *in vivo* assay. Acetic acid induced writhing test and tail immersion test in mice were used to study the analgesic effect, while the effect of the extract on acute inflammation was investigated by carrageenan-induced paw edema in rats. For phytochemical analysis *in vitro* assay were performed according to the slandered procedure. The oral administration of *M. charantia* extract upto 2 g kg⁻¹ in mice was found to be safe. The extract significantly (p<0.001) inhibited acetic acid induced writhing and tail-immersion test induced pain at dose 500 mg kg⁻¹, p.o. The extract also produced a moderate anti-inflammatory activity which was found to be significant at all the doses tested. The ethanolic extract showed 42.10% anti-inflammatory effect at dose 500 mg kg⁻¹, p.o. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, saponin, glycosides, steroids and sterol. The results obtained in this study lend credence to the ethnomedical use of the plant in the management of pain and inflammatory conditions. Thus, supporting the development of the biologically active substances as analgesics and anti-inflammatory agents. *M. charantia* may therefore, be a good candidate for functional foods as well as pharmaceutics.

**Key words:** *Momordica charantia*, anti-inflammatory, analgesic, ethanolic extracts

**INTRODUCTION**

Bitter melon (*Momordica charantia* L.) is an annual tendril herbage plant belonging to family Cucurbitaceae. Presence of ascorbic acid and iron in high concentration make this plant an important and valuable vegetable (Behera *et al*., 2008). Bitter melon is in use as a traditional medicine for diabetes in Central America, China and India and it also has antimicrobial properties (Grover *et al*., 2002; Yeh *et al*., 2003; Saeed and Tariq, 2005). *M. charantia* contains chemical constituents like charantin, linoleic acid, linolenic acid, momordicins, oleic acid, oxalic, trypsin inhibitors, v-insulin, ascorbigen, b-sitosterol-β-glucoside, lycopene, piperolic acid as well as the fruit pulp has soluble pectin but no free pectic acid (Dhalla *et al*., 1961). Researchers found
that, Bitter gourd fruit powder, in the form of an ointment showed a statically significant response in terms of wound contracting ability, wound closure time, tensile strength of the wound and regeneration of tissues at wound site when compared with a reference drug povidone iodine ointment in an excision, incision and dead space wound model in rats (Jayshri and Jolly, 1993). The phenolic compounds of bitter gourd have been reported to exhibit antioxidant activity (Horax et al., 2005; Budrat and Shotipruk, 2009). Grover and Yadav (2004) have reported anti-diabetes, anti-inflammatory, anti-bacterial and anti-cancer effects of *Momordica charantia*. Most of the synthetic drugs used at present for analgesic and anti-inflammatory effect that cause many side and toxic effects. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. Plant medicine are in use since a long time for analgesic and anti-inflammatory activity because of the reason that they are devoid of side effects (Ahmad et al., 1992). According to WHO there is about 82% population that depends upon herbal drugs and these are gaining popularity because of less side effects and low-priced (Kumara, 2001). The present study was therefore undertaken to investigate the secondary metabolite contents, acute toxicity, analgesic and anti-inflammatory potential of the ethanolic extract of *M. charantia* Linn. in experimental animal models.

**MATERIALS AND METHODS**

**Collection of plant materials:** The fruits of *Momordica charantia* (variety: charantia) were collected during the month of July, 2009 from the Rangamati District, Chittagong Hill tracts, Bangladesh and were taxonomically identified by standard procedure in the Department of Botany, University of Chittagong, Bangladesh.

**Preparation of the plant extract:** Fresh fruits were cleaned and dried under shade in clean dust free environment, grinded and stored in air-tight container. About 750 g powders were soaked in 4.5 L pure ethanol for about 7 days at room temperature with occasional stirring. After 7 days, ethanol extract was filtered using muslin cloth and Whatman’s filter paper No.1. The solvent was concentrated by evaporating ethanol using a rotary evaporator. The ethanol extract thus obtained transferred to a petri dish and kept in an oven at 30°C until the solvent is completely evaporated. Finally, a brownish colored residue was obtained (yield 3.23% w/w) which was kept in refrigerator at 4°C for experimental purposes.

**Phytochemical screening**

**Phytochemical analysis:** Freshly prepared *Momordica charantia* ethanol extract was subjected to preliminary qualitative phytochemical investigation using the procedure as reported (Khandelwal, 2007).

**Experimental animals:** Swiss Albino mice of both sexes weighting between 25 to 30 g and Wister Albino rats of the either sex weighting between 150-200 g obtained from animal house of BCSIR laboratories, Chittagong, Bangladesh were used for the present study. All the animals were kept in standard polypropylene cages under standard conditions in animal room with alternating light-dark cycle of 12 h. The animal were acclimatized to the laboratory conditions for at least five days prior to the experiments. During the entire period of the study the animals were supplied with standard pellet diet and water *ad libitum*. In this study, all the animal experiments was carried out according to the guidelines of Institutional Animal Ethics Committee (IAEC).
Acute toxicity study: Acute toxicity study was carried out according to the Organization of Economic Corporation Development (OECD) guidelines No. 425. Ethanol extract of *Momordica charantia* was administered orally in doses of 100, 200, 400, 800, 1000 and 2000 mg kg⁻¹ to the group of mice (n = 3) and the percentage mortality was recorded for a period of 24 h. During the first 1 h after the drug administration, the mice were observed for any gross behavioral change and the parameters observed were hyperactivity, grooming, convulsions, sedation and loss of righting reflex, respiration, salivation, urination and defecation (Vogel, 2002). Based on the above toxicity study, direct limit test was done. Initially at a particular dose, on the basis of the above study was administered to a single female rat and the rat was observed for 48 h with close surveillance up to initial 4 h (same as in case of first rat) and after 48 h (of the second administration), same dose was administered in two more female rats and the observation was done similarly as for the previous rat. The rats were observed for 14 days and no adverse observation was found morphologically. The weight of the animals was recorded on 7th and 14th day. Animals were divided into five groups of six animals each. The first group (Group I) served as a control group. And the second (Group II) was used as the reference standard. Three groups (Group III, IV and V) received ethanol extract at three different doses (100, 250 and 500 mg kg⁻¹ p.o.). The doses were selected on the basis of our preliminary screening. The research was conducted in accordance with the ethical rules on animal experimentation, approved by Institutional Animal Ethics Committee (IAEC).

Drugs and chemicals: Indomethacin (Opsonin Pharma limited, Bangladesh) and Tramadol Hydrochloride (SQUARE Pharmaceuticals Limited, Bangladesh) were used in the study. Distilled water was used as vehicle. All the chemicals and solvents were of analytical grade. Carrageenan was purchased from Sigma-Aldrich, Germany, acetic acid was obtained from Merck, Germany.

Analgesic and anti-inflammatory activity

Acetic acid induced writhing model in mice: Acetic acid induced writhing test model as described by Koster *et al.* (1959). This was performed to evaluate the analgesic activity of ethanol extract. In this model, the animals were pretreated with drugs 30 min before induction of writhing. The Group I animals received vehicle and Group II animals received the reference standard drug indomethacin (10 mg kg⁻¹ p.o.). Analgesic activity of ethanol extract at the doses of 100, 250 and 500 mg kg⁻¹ p.o. (Group-III, IV and V) was assessed by counting the number of writhing induced using 1% acetic acid. The number of writhing per animal was counted for 20 min. Percent reduction in writhing syndrome was calculated and compared with the standard drug. Percent reduction indicates the percentage protection against abdominal constriction which was taken as an index of analgesia. It was calculated by using the formula:

\[
\{(W_c - W_t) \times 100 \}/ W_c
\]

Where:

\(W_c\) = Number of writhing of the control group

\(W_t\) = Number of writhing of the treated group

Tail immersion method: In this method (Turner, 1971) the animals were pretreated with drugs for 60 min before tail immersion. The animals received vehicle (Group I) and the standard drug Tramadol Hydrochloride (50 mg kg⁻¹, i.p.) (Group II) which served as reference standard. Analgesic
activity of ethanol extract of *Momordica charantia* at doses 100, 250 and 500 mg kg\(^{-1}\) p.o. (Group III, IV and V), respectively was assessed by observing the reaction time in the treated groups. The distal 2-3 cm portion of mouse-tail was immersed in hot water maintained at 55±1°C. The time taken by the mouse to withdraw the tail from hot water was noted as reaction time. The cutoff time was considered about 10-12 sec.

**Carrageenan-induced hind paw edema in rats:** This test was conducted as per the method described by Winter *et al.* (1962). The paw thickness (0 h) was measured, in millimetres, using digital Plethysmometer (UGO Basile, Italy). The test substances and standard drug were administered one hour prior to the injection of a phlogistic agent via the oral route. The phlogistic agent carrageenan was prepared as 1% suspension, in sterile normal saline, a day before the study, to get a proper suspension. Carrageenan 0.1 mL\(^{-1}\) was injected subcutaneously into the right hind paw of each mouse. The paw volume was measured at 0 and 3 h after injection of carrageenan by using a Plethysmometer (Arunchalam *et al.*, 2002) after carrageenan injection. The edema thickness (mm) was calculated by the following formula Olumayokun *et al.* (1999):

\[
\text{Percentage inhibition} = \frac{(C_t - C_o)_{\text{treated}}}{(C_t - C_o)_{\text{control}}} \times 100
\]

where, \(C_t\) is the right hind paw thickness volume (in mm\(^3\)) at time t, \(C_o\) is the right hind paw thickness volume (in mm\(^3\)) before carrageenan injection. \(C_t - C_o\) is paw edema, \((C_t - C_o)_{\text{control}}\) is edema or paw size after carrageenan injection to control rats at time t. \((C_t - C_o)_{\text{treated}}\) is edema or paw size after carrageenan injection to treated (reference or sample drug) rats at time t.

**Statistical analysis:** The statistical analysis of data was done using the SPSS software (version 11.5). p<0.001 was considered as highly significant.

**RESULTS**

**Phytochemical analysis:** The crude bitter gourd extract was found to be positive for the presence of alkaloids, saponin, glycosides, steroids and sterol and phenolic compound as shown in Table 1.

**Acute toxicity study:** It was found that oral administration of *Momordica charantia* extract up to 2 g kg\(^{-1}\) did not produce any toxic effects in the normal behavior of the mice. Further no mortality was observed and from this observation it might be suggested that the plant extent in safe at this given dose.

<table>
<thead>
<tr>
<th>Table 1: Phytochemical screening of ethanol extract of <em>Momordica charantia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the phytochemical</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Alkaloids</td>
</tr>
<tr>
<td>Saponin</td>
</tr>
<tr>
<td>Glycoside</td>
</tr>
<tr>
<td>Steroids and sterol</td>
</tr>
<tr>
<td>Phenolic compound</td>
</tr>
</tbody>
</table>

+: Presence of the constituents
Table 2: Analgesic activity of *Momordica charantia* extract in acetic acid induced writhing method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Dose (mg kg⁻¹, p.o.)</th>
<th>No. of writhing (Count/20 min)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>--</td>
<td>69.8±1.65</td>
<td>----</td>
</tr>
<tr>
<td>II</td>
<td>Indomethacin</td>
<td>10</td>
<td>17.2±1.46*</td>
<td>75.36</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract</td>
<td>100</td>
<td>49.2±1.92*</td>
<td>29.51</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanol extract</td>
<td>250</td>
<td>43.1±1.7*</td>
<td>42.62</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract</td>
<td>500</td>
<td>34.8±2.37*</td>
<td>50.14</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n = 6 in each group). All values are significant at *p<0.001 compared to control group (Student t-test). Percentage inhibition of inflammation is given within parentheses.

Table 3: Analgesic activity of *Momordica charantia* extract on tail-immersion tests in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Drugs</th>
<th>Dose (mg kg⁻¹, i.p.)</th>
<th>Reaction time (s)</th>
<th>Latency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>control</td>
<td>--</td>
<td>2.90±0.77</td>
<td>--</td>
</tr>
<tr>
<td>II</td>
<td>Tramadol hydrochloride</td>
<td>50 (mg kg⁻¹, i.p)</td>
<td>4.95±0.27*</td>
<td>70.68</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract</td>
<td>100</td>
<td>3.11±0.23*</td>
<td>7.24</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanol extract</td>
<td>250</td>
<td>3.40±0.31*</td>
<td>17.24</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract</td>
<td>500</td>
<td>3.23±0.45*</td>
<td>35.51</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n = 6 in each group). All values are significant at **p<0.001 compared to control group (Student t-test).

Table 4: Anti-inflammatory activity of *Momordica charantia* extract in Carrageenan induced paw edema model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Dose (mg kg⁻¹, p.o.)</th>
<th>Increase in paw volume (mm³) at 3rd hour</th>
<th>Inhibition of paw volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>--</td>
<td>0.75±0.07</td>
<td>----</td>
</tr>
<tr>
<td>II</td>
<td>Indomethacin</td>
<td>10</td>
<td>0.32±0.06*</td>
<td>57.89</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol extract</td>
<td>100</td>
<td>0.65±0.01*</td>
<td>27.68</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanol extract</td>
<td>250</td>
<td>0.48±0.01*</td>
<td>36.84</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract</td>
<td>500</td>
<td>0.44±0.05*</td>
<td>42.10</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SEM (n = 6 in each group). All values are significant at *p<0.001 compared to control group (Student t-test).

**Effect on acetic acid induced writhing model in mice:** The extract produced significant (p<0.001) reduction in the number of writhing in mice in dose dependent manner. The ethanolic extracts (250 and 500 mg kg⁻¹, p.o.) significantly reduced the acetic acid-induced writhing by 42.62 and 50.14%, respectively but the standard drug indomethacin (10 mg kg⁻¹ p.o.) showed a reduction of 75.36% (Table 2).

**Effect on the tail immersion test:** From Table 3, It was observed that the ethanolic extracts (500 mg kg⁻¹) being the more active compared to 100 and 250 mg kg⁻¹.

**Effect on carrageenan-induced edema in rats:** The edema swellings induced by carrageenan in rats was significantly (p<0.001) inhibited by the fruit extract (100, 250 and 500 mg kg⁻¹, p.o.) as well as by Indomethacin (10 mg kg⁻¹, p.o). As shown in Table 4 the extract 500 mg kg⁻¹ reduced the edema swellings by 42.10% as compared to 57.89% reduction produced by indomethacin (10 mg kg⁻¹, p.o.) (Table 4).

**DISCUSSION**

The preliminary phytochemical screening of *Momordica charantia* extract showed the presence of alkaloids, saponin, steroids glycoside and phenolic compound which are much biological
importance. Dhall et al. (1981) also reported the presence of alkaloids, saponins-glycosides and phenolic constituents in *Momordica charantia*. The ethanol extract of *Momordica charantia* did not show any toxicity and behavioral changes in mice up to 2000 mg kg\(^{-1}\) and might be considered to be safe as a herbal drug (Patel et al., 2010) reported that the ethanol extract possessed high safety profile as no death was observed at doses > 5000 mg kg\(^{-1}\), p.o. in mice. The analgesic and anti-inflammatory effect of ethanol extract of *Momordica charantia* fruits in various models of pain and inflammation were found to be analogous. The stimulus may be thermal (tail immersion) mechanical (tail or paw pressure tests), or chemical (writhing and formalin tests) (Ainooson et al., 2009).

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs (Hasan et al., 2010). The crude extracts of *Momordica charantia* showed significant analgesic action compared to the reference drug Indomethacin. The ethanol extract at dose (500 mg kg\(^{-1}\), p.o.) reduced the pain by 50.14%. On the other hand, Patel et al. (2010) reported 59.99% at the same dose.

Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed et al., 2006) via cyclooxygenase (COX) and prostaglandin biosynthesis (Duarte et al., 1988). In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Derraedt et al., 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria et al., 2008). The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte et al., 1988).

The significant pain reduction of fruit extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways. The abdominal writhing induced by acetic acid was also reported to be less selective (Collier et al., 1968) and proposed to act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents (Toma et al., 2003).

Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins (Narayana et al., 2001; Ramesh et al., 1998). There are also reports on the role of tannins in anti-nociceptive activity (Ramprasath et al., 2006). Besides alkaloids are well known for their ability to inhibit pain perception (Uche and Aprioku, 2008). The extracts of the plants and indomethacin (10 mg kg\(^{-1}\)) also presented longer latency time than the control group in the tail immersion test in a dose related manner.

The tail immersion method have been found to be suitable for evaluation of centrally acting analgesics (Woolfe and MacDonald, 1994). The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model. In centrally acting analgesic methods, the extract containing 250 and 500 mg kg\(^{-1}\) doses was found to be effective.

Carrageenan-induced rat paw edema has been a popular inflammatory model to investigate anti-inflammatory effect of compounds (Vinegar et al., 1996). It has a biphasic effect. The first phase is due to release of histamine and serotonin (5-HT) (0-2 h), plateau phase is maintained by a kinin like substance (3 h) and second accelerating phase of swelling is attributed to PG release
(>4 h) (El-Shenawy et al., 2002). In our study, ethanol extract of *Momordica charantia* (100, 250, and 500 mg kg⁻¹, p.o.) significantly reduced edema induced by carrageenan. The ethanol extract at dose (500 mg kg⁻¹, p.o.) reduced the edema swellings by 42.10%. The ability of the extract to reduce the size of edema produced by carrageenan suggests that it contained chemical components that might be active against inflammatory conditions.

**CONCLUSION**

The results of the study suggest that *Momordica charantia* fruit extract might offer some beneficial effects in the management of analgesic and inflammatory conditions. Thus, further work is essential to fractionate, purify and identify the active principle(s) presenting this extract, as well as to understand the precise mechanism of action in analgesic and anti-inflammatory activities by the ethanol extract of *Momordica charantia*.

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**REFERENCES**


