ANALGESIC ACTIVITY OF BOILED AND NON-BOILED VIGNA MUNGO SEEDS

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ABSTRACT

Background: The objective of this study was to evaluate the analgesic potential of seeds of Vigna mungo, a lentil crop whose seeds are eaten in the boiled form in Bangladesh. Methods: Analgesic activity was determined through decreases by the methanol extract of boiled and non-boiled seeds in intraperitoneally administered acetic acid induced writhings in Swiss albino mice. Results: At doses of 50, 100, 200 and 400 mg per kg body weight, the methanol extract of boiled seeds dose-dependently and significantly reduced the number of acetic acid induced writhings by, respectively, 21.4, 32.1, 35.7, and 46.4%. Interestingly, this effect was found to be reversed by methanol extract of non-boiled seeds, which at the aforementioned four doses, reduced the number of writhings, respectively, by 39.3, 35.7, 32.1, and 21.4%. By comparison, a standard analgesic drug, aspirin, when administered at doses of 200 and 400 mg per kg reduced significantly the number of writhings by 42.9 and 53.6%, respectively. The results suggest that the seeds may contain components, which can actually increase the feeling of pain; however, such components are inactivated by boiling. Conclusion: Crude extract of boiled Vigna mungo seeds are effective in alleviating pain.

KEY WORDS: Analgesic, Vigna mungo, lentil, Fabaceae.
BACKGROUND

*Vigna mungo* (L.) Hepper is a lentil crop cultivated in Bangladesh for its seeds, which are boiled and taken with rice in the cooked form. The plant is known in English as ‘black gram’ and in Bangladesh as ‘mashkalai dal’. It is a Fabaceae family plant.

Hydroalcoholic extract of seeds has been reported to result in a significant (p < 0.05) reduction of serum total cholesterol, triglycerides, very low-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels in diet induced hyperlipidemic rats.\(^1\) Seed husk extract reportedly protected DNA and erythrocytes from free radical induced oxidative damage.\(^2\) The anti-osteoarthritic activity of *Vigna mungo* hydroalcoholic extract has been shown in papain induced osteoarthritis model.\(^3\)

Ongoing research studies in our laboratory have mainly focused on screening of Bangladeshi plants and plant parts for their antihyperglycemic and analgesic activities.\(^4\)-\(^20\) Since *Vigna mungo* has been found to give anti-osteoarthritic activity (a disease causing severe pain), it was the objective of the present study to evaluate the analgesic potential of seeds of *Vigna mungo*. Since the seeds are consumed in the boiled form, it was of further interest to evaluate boiled seeds of the plant for their analgesic potential.

Methods

*Plant material collection*

Seeds were collected during May 2015 from a local market in Dhaka city.

*Preparation of methanolic extract of Vigna mungo seeds (MEVM)*

For preparation of boiled seed extract, seeds were first steamed for 15 min, which is the average time in Bangladeshi households for boiling or cooking the seeds to make them soft and edible. Following steaming, the steamed seeds were thoroughly dried in the shade and 150g of dried and powdered steamed seeds were extracted with methanol (w:v ratio of 1:5, final weight of the extract 5.069g). For preparation of non-boiled seed extract, 150g of dried and powdered seeds were extracted with methanol (w:v ratio of 1:5, final weight of the extract 6.686g).

*Chemicals and Drugs*

Aspirin was obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.
Animals
Swiss albino mice, which weighed between 14-18g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Analgesic activity evaluation through abdominal writhing test
Analgesic activity of extract was examined as previously described.[21] Mice were divided into eleven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MEVM (non-boiled) at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Groups 8-11 were administered MEVM (boiled) at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MEVM, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid following a slight variation of an earlier described method[22], following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

\[
\text{Percent inhibition} = (1 - \frac{W_e}{W_c}) \times 100
\]

where \(W_e\) and \(W_c\) represents the number of writhings in aspirin or MEVM administered mice (Groups 2-11), and control mice (Group 1), respectively.

Statistical analysis
Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.[11]

RESULTS
At doses of 50, 100, 200 and 400 mg per kg body weight, the methanol extract of boiled seeds dose-dependently and significantly reduced the number of acetic acid induced
writhings by, respectively, 21.4, 32.1, 35.7, and 46.4%. Interestingly, this effect was found to be reversed by methanol extract of non-boiled seeds, which at the afore-mentioned four doses, dose-dependently reduced the number of writhings, respectively, by 39.3, 35.7, 32.1, and 21.4%, thus demonstrating that the non-boiled extract may contain component(s), which instead of dose-dependently alleviating pain, dose-dependently reduces this effect. However, this latter reducing effect is destroyed by steaming or boiling, which probably inactivates these component(s). By comparison, a standard analgesic drug, aspirin, when administered at doses of 200 and 400 mg per kg reduced significantly the number of writhings by 42.9 and 53.6%, respectively. The results are shown in Table 1 and strongly suggest that seeds of this lentil crop should be consumed after boiling only to have pain alleviating effects. It is also interesting to note that in Bangladesh, the seeds are consumed only in the boiled form and never in the raw state.

Table 1: Analgesic effect of crude methanol extract of boiled and non-boiled Vigna mungo seeds (MEVM) in acetic acid-induced pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of abdominal constrictions</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml</td>
<td>5.6 ± 0.24</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>3.2 ± 0.58</td>
<td>42.9*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.6 ± 0.40</td>
<td>53.6*</td>
</tr>
<tr>
<td>(MEVM) boiled 50 mg</td>
<td>4.4 ± 0.51</td>
<td></td>
<td>21.4*</td>
</tr>
<tr>
<td>(MEVM) boiled 100 mg</td>
<td>3.8 ± 0.66</td>
<td></td>
<td>32.1*</td>
</tr>
<tr>
<td>(MEVM boiled) 200 mg</td>
<td>3.6 ± 0.24</td>
<td></td>
<td>35.7*</td>
</tr>
<tr>
<td>(MEVM boiled) 400 mg</td>
<td>3.0 ± 0.32</td>
<td></td>
<td>46.4*</td>
</tr>
<tr>
<td>(MEVM non-boiled 50 mg)</td>
<td>3.4 ± 0.51</td>
<td></td>
<td>39.3*</td>
</tr>
<tr>
<td>(MEVM non-boiled 100 mg)</td>
<td>3.6 ± 0.68</td>
<td></td>
<td>35.7*</td>
</tr>
<tr>
<td>(MEVM non-boiled 200 mg)</td>
<td>3.8 ± 0.58</td>
<td></td>
<td>32.1*</td>
</tr>
<tr>
<td>(MEVM non-boiled 400 mg)</td>
<td>4.4 ± 0.24</td>
<td></td>
<td>21.4*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05; significant compared to control.

DISCUSSION

The identification of components present in non-boiled Vigna mungo seeds responsible for the progressive non-alleviation of any analgesic effect with increasing doses awaits further study. It is also not clear as to why boiled seeds gave dose-dependent significant analgesic effects. It is possible that boiling destroyed components, which enabled other phytochemicals present in seeds to exhibit the observed analgesic activity. Whatever be the cause, this is an interesting phenomenon, which deserves further studies.
That boiled seeds can produce an analgesic effect can be a boon to people suffering from chronic or acute pain and who lacks affordability or access to modern pain alleviating drugs. *Vigna mungo* is one of the cheapest lentils available in Bangladesh and so can be afforded by most people. This makes this lentil crop seeds a potentially excellent resource for pain relief.

**CONCLUSION**
The results suggest that methanolic extract of boiled *Vigna mungo* seeds is effective in relieving pain.

**CONFLICTS OF INTEREST**
The author(s) declare that they have no competing interests.

**REFERENCES**


