Effects of *Moringa oleifera* L. Herb and its extract on indomethacin-induced gastric oxidative stress in rats

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This study aims to evaluate the anti-inflammatory and anti-ulcerogenic potential of moringa leaf powder and extract by determining the anti-inflammatory profile of indomethacin-induced gastric oxidative stress in rats. Thirty six male Wistar rats of body weight 125±5 g were categorized into 6 groups, each comprising 6 rats. The first group was a control negative. The remaining groups were orally administered by indomethacin (30 mg / kg b.wt). One of these groups was a control positive and other ulcerated groups were treated with either 30 mg/kg b.wt. ranitidine or 10g/kg diet of moringa powder or 5 ml/kg b.wt of moringa extract or a mixture of moringa powder and extract for six weeks. Results revealed that administration of moringa powder, extract, and mixture showed significant increase in BWG, FER, stomach pH, hemoglobin, packed cell volume, RBCs count, Cox-2, gastric cytochrome P450 reductase, while, gastric juice volume, serum nitric oxide, Interleukin-1 and alpha tumor necrosis factor recorded a significant decline compared to control positive. The curative ratio percentage was insignificant between the groups of moringa powder or moringa extract or mixture of them compared to ranitidine group. Also, results revealed that moringa powder and extract is a rich source of natural antioxidants such as polyphenols and flavonoids (salicylic, querctin, ellagic acid, chlorogenic acid catechol and caffeic). This study demonstrated the anti-ulcerogenic effects of moringa and its extract on indomethacin-induced peptic ulcer suggesting that moringa powder and extract can be promising for treatment of gastric ulcers as a rich source of natural antioxidants.

Keywords: Anti-ulcerogenic - Curative ratio - *Moringa oleifera* L. - anti-inflammatory.

INTRODUCTION

*Moringa oleifera* L. is one of family Moringaceae members and it is commonly known as Drumstick tree, in Northwest India. Moringa possesses antimicrobial activity and is used for the traditional treatment of hypercholesterolemia and hyperglycemia, also as a nutritional supplement (Babu and Chaudhuri, 2005 and Asare, et al., 2012).

Moringa is a good source of natural antioxidants. Moringa leaves are a rich source of vitamin C, β-carotene, protein, potassium and calcium. It increases the shelf-life of fat containing foods as it contains a variety of antioxidant compounds such as, flavonoids, phenolics, ascorbic acid and carotenoids (Anwar et al., 2005 and Siddhuraju and Becker, 2003). Also, Sengev and Gernah, (2012) reported that moringa leaves contain high content of essential amino acids, minerals (Fe, Ca, P, Cu), riboflavin, folic acid, , pyridoxine, nicotinic acid and α-tocopherol. Indomethacin is a non-steroidal anti-inflammatory drug similar to ibuprofen and naproxen that accelerated healing in pain and inflammation.
Indomethacin works by reducing the production of prostaglandins that the body produces and which cause the fever and pain that are associated with inflammation (Takeuchi, et al., 1991) and (Dela Lastra, et al., 2002). The current study aims to evaluate the effect of *Moringa oleifera* leaves powder and extract on the reduction of stress-induced gastric ulcers by indomethacin in rats.

**MATERIALS AND METHODS**

**Materials**

**Chemicals**

*Indomethacin*
Drug tablets were obtained from SEDICO Pharmaceutical Co., Giza, Egypt.

*Ranitidine hydrochloride*
150 mg tablets, SEDICO Pharmaceutical Company, Giza, Egypt.

**Experimental plants**

*Moringa* (*Moringa oleifera*):
Flowers and leaves were obtained from the Agricultural Research Centre, Giza, Egypt.

**Experimental animals**

Animals used in this experiment were Wistar male albino rats of Sprague Dawly strain obtained from Helwan Farm of Laboratory Animals, Egypt. Animal experiment was carried out in compliance with the Guidelines for Animal Care and Ethics Committee of the NRC (Egypt) and the study protocol was approved.

Thirty six rats with average weight of 125±5 g were used. The animals were kept under observation for five days before experiment and fed on standard diet and water *ad libitum*. The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg), corn oil (50g/kg), mineral mixture (100g/kg), vitamin mixture (20g/kg) and DL-methionine (3g/kg).The standard diet was performed according to NRC, (1995).

**Methods**

**Chemical Analysis**

**HPLC analysis of polyphenols and flavonoids**
HPLC analysis of extracts was performed using an Agilent 1200 chromatograph equipped with a PDA model G1315B, a Bin pump model G1312A, an autosampler model G1313A and a RR Zorbax Eclipse Plus C18 column (1.8 μm, 150 mm×4.6 mm) were selected for detection according to (Merfort, et al., 1997).

**Indomethacin-induced gastric ulcer**
Gastric ulceration was induced in the animals according to the procedure described by (Sayanti et al., 2007). Briefly, rats were administered with a single oral dose of indomethacin (30 mg/kg body weight) to induce gastric damage.

**Experimental procedures**

The experiment was performed in Animal House in the Institute of Ophthalmology, Giza. All rats were fed for one week prior to the beginning of the experiment on basal diet, then divided into two main groups, the first group (n= 6 rats) was fed on the basal diet only as a negative control (C –ve) normal rats. The rats of second main group (n= 30 rats); indomethacin as 30 mg/kg was administered to all animals orally to induce gastric damage according to (Sayanti et al. 2007), after the administration they were divided into 5 sub-groups (each 6 rats) as follows:

**Sub-group (2):** Rats were orally administered by indomethacin and fed on basal diet without any treatment and considered as a positive control (C +ve).

**Sub-group (3):** Rats were orally administered by indomethacin and fed on basal diet and dose 30 mg/kg ranitidine drug of rat using a stomach tube.

**Sub-group (4):** Rats were orally administered by indomethacin and fed on basal diet and 10g/kg/diet moringa powder

**Sub-group (5):** Rats were orally administered by indomethacin and fed on basal diet and 5 ml/kg b.wt. moringa extract

**Sub-group (6):** Rats were orally administered by indomethacin and fed on basal diet and 10g/kg/diet and 5 ml/kg b.wt. Mixture of moringa powder and extract.

Daily food intake and weekly body weight gain were calculated. Food efficiency ratio (FER) was determined according to the method of (Chapman et al., 1959). At the end of the experimental period (6 weeks), rats were sacrificed after overnight fasting under ether anesthesia. Blood samples were taken from hepatic portal vein. Small portion of the blood sample was taken into heparinized tube and the remainder was left to clot by standing at room temperature for 15 minutes, and then centrifuged at 3000 rpm for 20 minutes. Serum was carefully separated and transferred...
into clean quite fit plastic tubes and kept frozen at -20°C until the time of analysis.

**Pylorus ligation-induced gastric ulcer**
Under inhalational ether anesthesia, the abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was identified, slightly lifted out and ligated, avoiding traction to the pylorus or damage to the blood supply. The stomach was then replaced carefully, and the abdominal wall closed by interrupted sutures. Amlodipine was given 4 hour prior, and ranitidine, normal saline were given 1 hour prior the ligation procedure to obtain best results (Bhave, et al., 2006). Animals were deprived of both food and water during the postoperative period (Parmar and Desai, 1993).

**Gastric mucosal injury was assessed**
Volume of gastric secretion (in ml), pH of gastric secretion and ulcer index (UI). Ulcer index was calculated according to Parmar and Desai, (1993) by the following formula:

\[ UI = 1 \times (\text{number of lesions of grade 1}) + 2 \times (\text{number of lesions of grade 2}) + 3 \times (\text{number of lesions of grade 3}). \]

With small nick, fundus of stomach was perforated on greater curvature of stomach. After ligation of cardiac and pyloric end, the greater curvature of stomach was opened. The accumulated gastric secretion fluid was collected, and volume and pH was measured. Gastric mucosa was observed under magnifying glass to calculate the ulcer index (Kilic, et al., 2006).

Curative ratio=(length of gastric ulcer in control positive group - length of gastric ulcer in treated group / length of gastric ulcer in control positive group ) x100.

**Biochemical analysis**

**Measurement of blood parameters**
Hemoglobin and packed cell volume (Drabkin, 1949 and Mc Inory, 1954). The rest part of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum.

**Measurement of serum parameters**
No, Interleukin-1 (IL-1), Tumor necrosis factor-alpha (TNF- α) were determined according to (Griess, et al.1982 Grassi, et al., 1991 and Beutler et al., 1985), respectively.

**Determination of gastric mucosal parameters:**
Gastric mucosal cyclooxygenase (Cox-2), prostaglandin E2 (PGE2), cytochrome P450 reductase (Cyto P450) were determined according to (Hemler and Lands, 1976 Hamberg and Samuelsson, 1973 and Mc-Lean and Day, 1974), respectively.

**Statistical analysis**
The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan’s multiple range test and p<0.05 was used to indicate significance between different groups (Snedecor and Cochran, 1967).

**RESULTS AND DISCUSSION**
The results of the HPLC analysis of polyphenols and flavonoids composition in moringa extract are summarized in Table 1.

### Table 1: Polyphenolic compounds and flavonoid (ppm) of moringa extract (mg/100g)

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>1211.19</td>
</tr>
<tr>
<td>Querctin</td>
<td>1855.11</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>867.76</td>
</tr>
<tr>
<td>Catechol</td>
<td>1104.18</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>250.41</td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>412.21</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>1582.01</td>
</tr>
<tr>
<td>Salicylic</td>
<td>2876.17</td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>412.84</td>
</tr>
<tr>
<td>Vanillic</td>
<td>203.37</td>
</tr>
<tr>
<td>Caffeine</td>
<td>386.21</td>
</tr>
<tr>
<td>Vanillic</td>
<td>241.10</td>
</tr>
<tr>
<td>Alpha-coumaric</td>
<td>82.18</td>
</tr>
<tr>
<td>Benzoic</td>
<td>29.49</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>34.63</td>
</tr>
</tbody>
</table>

The important identified polyphenols and flavonoids were: Salicylic (2876.17 mg/100g), querctin (1855.11 mg/100g), ellagic acid (1582.01 mg/100g), chlorogenic acid (1211.19 mg/100g), catechol (1104.18 mg/100g) and caffeic acid (867.76mg/100g) (Table 1). In other studies, Salicylic and querctin were reported as the major constituents in the moringa (Sengev, et al., 2012 and Ali and Hanaa 2016).
These differences indicate that the polyphenols and flavonoids of moringa varieties may vary according to different environmental and ecological conditions.

Table 2 showed that the control positive group recorded a significant reduction in feed intake (FI), gain in body weight (BWG) and feed efficiency ratio (FER) when comparing to their corresponding control negative group. These results may be attributed to loss of appetite, caused by gastric ulcer disturbance and pH in rats. These data are similar to that of Hunt, et al., (2006). Weight gain, feed intake and FER of all treated groups (10 g/kg, 5ml &mixture) of moringa powder and extract and drug group significantly increased as compared to the positive control. This is in agreement with the previous studies done some by Omotesho, et al., (2013) who reported that moringa leaves contain important polyphenolic compounds such as high level of salicylic, quercitin and ellagic acid. These results are in parallel with those reported by Mona, et al., (2013) and Masni, et al., (2017).

Results of the anti-ulcer studies Table 3 showed that pH, volume of gastric secretion and in gastric ulceration induced by indomethacin stress in rats. Also, a significant reduction was noticed in gastric secretion and in gastric ulceration induced by indomethacin in pylorus legated rats. The healing due to moringa which is attributed to its anti-inflammatory and antiulcer activity was reported previously (Farooq, et al., 2007).

The results in Table 4 indicated that hemoglobin concentration (HB), packed cell volume and RBCs were increased significantly in all groups that were treated when compared to the group of control (+ve). The groups treated with (10 g/kg, 5ml &mixture) moringa powder and extract and drug group showed non-significant difference compared to control (-ve) group. This might be attributed to the ability of moringa to prevent the oxidative damage of erythrocytes cell membrane as it contains antioxidants like ascorbic acids, organosulfur compounds, flavonoids and polyphenols (Rao et al., 2010 and Aja et al., 2014).

Results in Table 5 revealed a significant increase in the level of cyclooxygenase activity, prostaglandin (E2) concentration and cytochrome (P450) reductase activity in all treated rat groups compared with control positive group.
Table (3): Values of pH, volume of gastric secretion, ulcer index and Curative ratio % of control and indomethacin treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (-ve)</th>
<th>Control (+v)</th>
<th>Drug</th>
<th>Anti-ulcer with moringa powder and extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 g/kg</td>
<td>5ml/kg</td>
</tr>
<tr>
<td><strong>PH</strong></td>
<td>4.17±0.53 <strong>a</strong>*</td>
<td>2.03±0.41 <strong>c</strong></td>
<td>3.34±0.17 <strong>b</strong></td>
<td>3.57±0.17 <strong>b</strong></td>
</tr>
<tr>
<td><strong>Volume of gastric juice (1ml)</strong></td>
<td>2.14±0.08 <strong>b</strong></td>
<td>5.05±0.16 <strong>a</strong>**</td>
<td>1.93±0.16 <strong>c</strong></td>
<td>1.71±0.16 <strong>c</strong></td>
</tr>
<tr>
<td><strong>Ulcer index (mm)</strong></td>
<td>-</td>
<td>7.93±0.94**a****</td>
<td>2.39±0.14 <strong>c</strong></td>
<td>3.24±0.14 <strong>b</strong></td>
</tr>
<tr>
<td><strong>Curative ratio %</strong></td>
<td>-</td>
<td>-</td>
<td>55.63±4.16 <strong>a</strong></td>
<td>54.13±4.16 <strong>a</strong></td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD. Significance with control group * P<0.05 ** P<0.01 *** P<0.001
Values that share different letters at the same raw are significant.

Table (4): Hemoglobin concentration (HB), packed cell volume (PCV) and RBCs of control and indomethacin treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (-ve)</th>
<th>Control (+v)</th>
<th>Drug</th>
<th>Anti-ulcer with moringa powder and extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HB(g/dl)</td>
<td>PCV</td>
<td>RBCs (x10⁶/µl)</td>
<td>Anti-ulcer with moringa powder and extract</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 g/kg</td>
<td>5ml/kg</td>
</tr>
<tr>
<td><strong>HB(g/dl)</strong></td>
<td>14.34±1.99 <strong>a</strong></td>
<td>10.41±1.33 <strong>b</strong></td>
<td>13.41±1.51 <strong>a</strong></td>
<td>13.01±1.40 <strong>a</strong></td>
</tr>
<tr>
<td><strong>PCV</strong></td>
<td>40.11±4.77 <strong>a</strong></td>
<td>30.17±3.33 <strong>b</strong></td>
<td>36.32±3.59 <strong>a</strong></td>
<td>35.71±3.59 <strong>a</strong></td>
</tr>
<tr>
<td><strong>RBCs (x10⁶/µl)</strong></td>
<td>5.11±0.99 <strong>a</strong></td>
<td>2.29±0.82 <strong>b</strong></td>
<td>4.57±1.01 <strong>a</strong></td>
<td>4.51±1.01 <strong>a</strong></td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD. Mean values in each column having different superscript (a, b, c, d) are significant. Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Table (5): The Mean values ± SD of gastric tissues cyclooxygenase activity, prostaglandin (E2) concentration and cytochrome (P450) reductase activity, of control and indomethacin treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (-ve)</th>
<th>Control (+v)</th>
<th>Drug</th>
<th>Anti-ulcer with moringa powder and extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cox-2 (ng/mg)</td>
<td>PGE₂ (pg/mg)</td>
<td>Cyto P₄₅₀ (ng/mg)</td>
<td>Anti-ulcer with moringa powder and extract</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 g/kg</td>
<td>5ml/kg</td>
</tr>
<tr>
<td><strong>Cox-2 (ng/mg)</strong></td>
<td>4.56±0.91 <strong>a</strong></td>
<td>2.01±0.51 <strong>c</strong></td>
<td>3.92±0.34 <strong>b</strong></td>
<td>3.81±0.33 <strong>b</strong></td>
</tr>
<tr>
<td><strong>PGE₂ (pg/mg)</strong></td>
<td>375.34 ±55.14 <strong>a</strong></td>
<td>147.77±10.14 <strong>c</strong>**</td>
<td>373.12±30.12 <strong>a</strong></td>
<td>371.13±30.11 <strong>ab</strong></td>
</tr>
<tr>
<td><strong>Cyto P₄₅₀ (ng/mg)</strong></td>
<td>9.45±1.98 <strong>a</strong></td>
<td>19.34±3.14 <strong>b</strong>**</td>
<td>10.46±1.69 <strong>a</strong></td>
<td>10.36±1.68 <strong>a</strong></td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD. Values are significant with control group at * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant
Table (6): The Mean values ± SD of nitric oxide (NO), Interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) of control and indomethacin treated rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (+ve)</th>
<th>Control (-ve)</th>
<th>Drug</th>
<th>Anti-ulcer with moringa powder and extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO (pg/mg)</td>
<td>31.06±2.94 a</td>
<td>68.12±6.76 c***</td>
<td>43.67±4.19 b</td>
<td>47.67±5.29 b</td>
</tr>
<tr>
<td>IL-1 (pg/ml)</td>
<td>18.46±1.06 c</td>
<td>39.19±1.34 c***</td>
<td>24.64±2.52 b</td>
<td>26.64±2.69 b</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>6.20±0.81 d</td>
<td>17.75±1.94 c***</td>
<td>8.56±0.24 b</td>
<td>9.06±0.66 b</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD. Values are significant with control group at * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

The groups treated with (10 g/kg, 5ml & mixture) of moringa powder and extract and drug group showed non-significant difference compared to the control negative group. The effect of mixture on these biomarkers was found to be the highest among the other treated groups followed by 5ml, drug group and 10 g/kg of moringa powder and extract. Another study by Anjorin, et al., (2010) and Mangale et al. (2012) showed that the moringa has inflammatory and an antioxidant effects that help to support the body’s functions and maintain them in a normal range by neutralizing free radicals.

The results Table 6 revealed that the nitric oxide (NO), Interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) levels significantly decreased in all treated groups of rats as compared with the control positive group. In addition NO, IL-1 and TNF-α levels in all treated groups (except moringa powder and extract mixture treated group) have reached values similar to that of the normal group. The antioxidant effect is mainly due to the phenolic acids, flavonoids and anthocyanins present in moringa Farooq, et al., (2007) and Aja, et al., (2014).

CONCLUSION
In conclusion, the overall finding of this study demonstrates anti-ulcerogenic and antioxidant effects of moringa powder and extract on indomethacin induced peptic ulcer in rats suggesting that moringa powder and extract can be considered as a promising material for treatment of gastric mucosal injury and therefore further studies on this plant are encouraged.

CONFLICT OF INTEREST
Authors declared that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS
All authors equally contributed in the paper. All authors read and approved the final version.

REFERENCES
Anjorin TS, Ikokoh P, Okolo S. Mineral


Bhave AL, Bhatt JD, Hemavathi KG. Antiulcer effect of Amlodipine and its interaction with H$_2$ blocker and proton pump inhibitor in pylorus ligated rats. Indian J Pharmacol 2006; 38: 403–7.


Griess LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [$^{15}$N] nitrate in biological fluids. Anal Biochem 1982; 126 (1): 131-138.


Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and...


