Effect of *Moringa oleifera* leaves extract on the oxidative stress and gastric mucosal ulcer induced by indomethacin in rats

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Indomethacin is commonly used as an anti-inflammatory and pain relieving medication; however, it has the side effect of gastric ulcer formation which is an actual common gastrointestinal illness that may result in dangerous complications and even death. Various diseases have been treated widely by the use of oriental herbal medicines, this study aims to evaluate the antiulcerative and antioxidative effect of two doses (100 and 50 of *Moringa oleifera* ethanolic leaf extract; MOELE) on indomethacin plus ethanol-induced oxidative gastric mucosal injury in rats. Sixty adult males Wistar rats weighing 170 to 200 g, were divided into equal six groups. First group of rats were administered saline as a vehicle, second group of rats were given indomethacin (15 mg/kg), third and fourth groups of rats were given MOELE 100 and 500 mg, fifth group of rats were given indomethacin+ MOELE 100 mg, and sixth group of rats were administered with indomethacin + MOELE (500 mg). To study the effect of MOELE on oxidative gastric mucosal injury in rats, two doses were administered 2 h before ulcer induction by indomethacin plus ethanol. The administration continued for two weeks. All rats were sacrificed 24 h after the last dose. Indomethacin group showed significant increases in lesion index (LI) and increase in malondialdehyde (MDA) level, while there was a decrease in superoxide dismutase (SOD), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) activities when compared with the control group (G1). MOELE with two doses mentioned before (groups 5 and 6) were effective to reduce stomach LI and oxidative stress markers (MDA) while increasing significantly the antioxidant biomarkers (SOD, GST, and GPx) compared with indomethacin group (G2). A highly significant decrease in MDA accompanied by a marked increase in SOD, GST, and GPx were recorded in group 6. The results concluded that MOELE has an effective antiulcer and antioxidant activities. It can scavenge the free radicals and protect gastric against ulceration. Also, MOELE could ameliorate the ulcerative side effect of indomethacin.

**Key words:** *Moringa oleifera*, antioxidant enzymes, indomethacin, lesion index, lipid peroxidation.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) as indomethacin are the most prescribed group of drugs in the world. They are used primarily for pain relief in chronic inflammatory joint disease. They are the main cause of peptic ulceration and its use has been associated with the development of gastrointestinal (GI) symptoms ranging from simple dyspepsia to life-threatening GI bleeds and perforations (Yap et al., 2015).
Ulcer development destroys the mucosal barrier exposing the underlying stomach tissue to the destructive action of acid and pepsin (Vander, 1998). Numerous factors have been implicated in the pathogenesis of peptic ulcer disease, which may be acquired during life, although some of these may have already been determined (Ghasi, 2000).

Gastric hyperacidity and ulcer are very common, causing tremendous human suffering nowadays. It is an imbalance between damaging factors, within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns, and trauma are known to cause severe gastric irritation, the mechanism, however, is still very poorly understood (Rao et al., 2000). The problems of ulcer due to indomethacin could be prevented by herbal treatment. One of these promising medicinal therapy is *Moringa oleifera*. The advantage of choosing a medicinal plant includes its easy availability, low cost and nearly no side effect compared with the synthetic medications.

*M. oleifera* Lam (syn. *M. pterygosperma*; commonly known as "The Miracle Tree," as almost every part of it is useful for humans). It has medicinal and nutritional value; it is also widely distributed throughout the world in Himalayan tracts, India, Pakistan, and Africa. It could be found even in the harshest and driest of soils (Luqman et al., 2012). Moringa plants are used as a food source with valuable properties in humans. Genus moringa contains vitamin C, vitamin A, potassium, iron, calcium and the protein quality of moringa leaves is claimed to be similar to eggs and milk (Fahey, 2005).

There are 36 anti-inflammatory compounds (phenolic derivatives and isothiocyanate) and 46 antioxidants (carotenoids, ascorbic acid, phenolic compounds, and flavonoids). These compounds naturally occur in the moringa plant (Anwar et al., 2007; Goyal et al., 2007; Adedapo et al., 2009). The leaves are reported to have anti-inflammatory, diuretic, antispasmodic, and hypotensive activity (Fayazuddin et al., 2013). The antioxidant property of moringa may be due to the presence of phenolic compounds (Bharali et al., 2003).

The existence of reactive oxygen species (ROS) leads to oxidative stress. It causes disturbances in the cellular metabolism (Breitenbach and Eckl, 2015). Oxygen free radicals mediate tissue injury and destroy the integrity of biological tissues. Also, it is associated with lipid peroxidation, which causes tissue damage by destroying cell membranes and releasing some of their intracellular components. ROS also can cause mucosal damage through the retrogression of the epithelial basement membrane components. Indeed, biological system’s ability to repair oxidative damage or to neutralize the reactive intermediates including peroxides and free radicals (Demir et al., 2003; Suzuki et al., 2012).

The gastric mucosa plays an important role in the physiological function of the stomach. This mucosa acts as a gastric barrier, which protects deeper located cells against the detrimental action of the gastric secretory components. The pathogenesis of gastric mucosal damage includes ROS that cause tissue damage, mainly due to increased lipid peroxidation (Kwiecien et al., 2014).

Antioxidant, anti-inflammatory, and immunomodulatory properties of biophenols are abundant in *M. oleifera* Lam, suggesting that they may have beneficial effects on inflammatory bowel diseases like gastric ulcers (Mahajan et al., 2007; Shaila et al., 2010).

Recently, Omodanisi et al. (2017) reported that *M. oleifera* has effective phytochemical ingredients that offer protection action against diabetic-induced renal damage, ROS and inflammation and could, therefore, show a role in decreasing diabetic problems, mainly in developing nations such as Africa where the majority cannot afford to purchase medicines.

The present study aims to use inexpensive, effective and readily accessible medication with a low side effect for the ulcer therapy. Therefore, the current study was carried out to assess the antioxidant activity of *M. oleifera* ethanolic leaf extract (MOELE) using in vivo acute models of ulcer that cause oxidative gastric damage in rats. Also, this study aims to scientifically confirm the use of *M. oleifera* leaves in the treatment of gastric ulcer.

**MATERIALS AND METHODS**

**Chemicals**

Indomethacin was purchased from Chiesi Pharmaceuticals SPP, Parma, Italy. Ethanol and kits used for measurement of malondialdehyde (MDA), glutathione-S-transferase (GST), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were purchased from Diamond and Sigma Company.

**Plant material extraction**

Air-dried powder (200 g) of *M. oleifera* leaves were soaked in 70% ethanol for 2 days and filtered. The filtrate was distilled using a rotary evaporator until dryness. The remaining solid residue was dissolved in distilled water, filtered and the filtrate was evaporated until dryness (dry mass 10 g). The dried mass was diluted with normal saline (100 mg/ml) and used in the experiments.

**Animals**

This study includes sixty adult males Wistar rats weighing 170 to 200 g. Wire bottomed cages were used for housing of the animals.
under controlled conditions of temperature (20 to 24°C), humidity and 12/12 h light/dark periods. Rats were fed with chow pellets and tap water was freely accessible. Animals were prevented from food overnight before the experiment. The animal experiments were approved by the Committee of Scientific Ethics at University of Dammam and consistent with its guidelines (IRB-2016-10-155).

Animals were randomly divided into six groups (10 rats each) as follows: Control group, received vehicle (0.5 ml vehicle) for two weeks; Group 2 (Indomethacin), rats were given oral administration of indomethacin at a concentration of 15 mg/kg-body weight/0.5 ml water in addition to 0.5 ml absolute ethanol for induction of gastric mucosal haemorrhagic injury (De La Lastra et al., 1999); Group 3 (100-MOELE), rats were given a single dose of MOELE, 100 mg/kg-body weight orally three times/week for 2 weeks; Group 4 (500-MOELE), rats received a single dose of M. oleifera extract, 500 mg/kg-body weight, orally three times/week for a period of 2 weeks; Group 5 (100-MOELE+Indomethacin), rats received a single dose of MOELE, 100 mg/kg-body weight; 2 h prior to induction of gastric mucosal haemorrhagic injury as in group 2; Group 6 (500-MOELE+Indomethacin), rats received a single dose of MOELE, 500 mg/kg-body weight, 2 h prior to induction of gastric mucosal haemorrhagic injury as in group 2.

Rats were sacrificed under ether anaesthesia at the end of the experimental period (the day after receiving the last dose). Abdomens were opened and stomachs were exposed. Then stomachs were opened along the greater curvature. The tissue of the stomach was washed using normal saline. Examination of tissue and mucosal injury can be carried out microscopically using a light microscope (Morini and Grandi, 2010). Scores/ratings as described by Okabe et al. (1970), was used to determine the ulcer index as a marker for the severity of gastric lesions a scoring system based on the length and number of hemorrhagic mucosal erosions, a lesion index (LI) of gross mucosal injury was performed as follows: stomach was dissected out, inflated with 12 ml of 2% formalin, placed in 2% formalin to fix both the inner and outer layers, and then opened along the mesenteric attachment or along the greater curvature. The incidence of animals with lesions was noted, and the damaged area (in square millimetres) was measured under a dissecting microscope with a square grid. The sum of the area of all lesions in gastric for each animal was calculated and served as the lesion index. Ulcer index = 10/X; where X = total mucosal area/total ulcerated area.

Gastric mucosa was removed using a skin-scraping spoon and then homogenized for biochemical assay.

### Biochemical analysis

Gastric mucosal preparations were used for measuring the lipid peroxidation product as MDA according to Draper and Hadley (1990). Activity of GST was assayed using the method of Habig et al. (1974), SOD was assayed by Giannopoulis and Ries (1977), and GPx was assayed by Rotruck et al. (1973). The enzyme activity was expressed as unit/mg of protein.

### Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer methods for post-hoc analysis. A value of P<0.05 was considered statistically significant. Statistical analyses were carried with the aid of a digital computer, using STAT and SPSS version 16.0 programs. Data were presented as mean ± standard deviation (SD).

### RESULTS

Generally, an analysis of the results compared the control group with indomethacin to examine its effect as model for the LI and oxidative stress, while groups 3 and 4 were used to monitor if the MOELE alone has a role or not. So compared with (-ve) control groups, no changes were observed due to the administration of MOELE alone. Meanwhile, the effect of treatment MOELE + indomethacin tested in comparison with indomethacin as a control +ve group. Observations revealed that there were no changes in the control group which suggests that handling and surgical procedures had no interference with experimental results.

Administration of indomethacin induced increased in LI (Table 1), elevation in MDA while decreasing the activities of antioxidant markers of GST, SOD, and GPx, indicating rises in the oxidative stress compared with control group (Table 2).

Oral administration of MOELE prior to administration of indomethacin plus ethanol highly significantly reduced the lesion index (P<0.001) compared with indomethacin group (Table 1). In addition, the lesion index was significantly reduced with the high doses of MOELE (500 mg/kg) (Table 1).

As shown in Table 1, gastric hemorrhagic lesions had improved in the groups 5 and 6 which received MOELE for 2 h before oral administration of indomethacin and ethanol. These lesions were accompanied by a highly significant rise in the lipid peroxidation level that expressed a high MDA level (P<0.001) and highly significant decreases in antioxidant enzymes (P<0.001) in group 2 (indomethacin group) when compared with the control (Table 2). While, oral administration of MOELE prior to administration of indomethacin plus ethanol significantly reduced the lesion index of groups 5 and 6 (P<0.001) (Table 1), as they might have significantly decreased the rise in MDA concentration and restored the activities of the antioxidant enzymes of GST, SOD and GPx in gastric mucosa when compared with indomethacin-treated rats (group 2) (Table 2). In addition, the administration of M. oleifera leaf extract three times/week for 2 weeks (group 6) has more pronounced effect. Moreover, the levels of antioxidant enzymes were not changed in rats of groups 4 and 3 when compared with control due to the administration of MOELE with different concentrations (Table 2).

### DISCUSSION

The peptic ulcer is one of the major gastrointestinal disorders; the treatment of peptic ulcer is directed against either reduction of the aggressive factors or enhancement of defensive mechanism. A number of drugs, including proton pump inhibitors and H₂ receptor antagonists, are available for the treatment of peptic ulcer, but the clinical evaluation of these drugs has shown the incidence of relapse, side effects and drug interactions (Anoop and Jagadeesan, 2003).
Phytomedicinal agents have traditionally been used by herbalists and indigenous healers for the prevention and treatment of ulcers. The natural drugs were found to be the safer alternatives to cure ulcers. In this study the antiulcer activity of *M. oleifera* ethanolic leaf extract was evaluated in indomethacin-induced gastric ulcers in rats.

The results of the present study showed that MOELE possesses significant anti-ulcer activity, it showed a significant reduction in ulcer index (Table 1) compared to control (P < 0.01). Indomethacin is known to produce erosions and ulcers in the stomach due to inhibition of cytoprotective prostaglandins (Vedavyasa, 1999).

Although, many products are used for the treatment of gastric ulcers, e.g. antacids and antihistamines; most of these drugs, however, produce several adverse reactions, like arrhythmias, impotence, gynecomastia and hematopoietic changes. Extracts of many herbal plants have been shown to produce promising results for the treatment of gastric ulcer (Verma et al., 2012).

MOELE was effective as a gastric cytoprotective agent; it may be due to its direct action on the mucus secretion or by increasing prostaglandins, thus protecting the stomach from indomethacin injury. It may be altering the antioxidant factors like total tissue sulfhydryl group (glutathione) suggesting that the healing of ulcers or prevention of the development of gastric ulcers in the model organisms, rats, is due to its antioxidant action.

The cytoprotective and antioxidant effects of MOELE may be contributed to the presence of some active phytochemical compounds such as alkaloids, sterols, glycosides, flavonoids, and terpenoids (Mahajan et al., 2008). Also, its leaves are rich in benzyl isothiocyanate which has anti-inflammatory activity (Lee et al., 2009).

In the present study, the antioxidant property, of 2 doses of *M. oleifera* leaf extracts exert its action via alteration in SOD, GPx, and MDA levels in rat gastric mucosa. During the ulcer condition, there is an increase in gastric mucosal SOD and lipid peroxidation (LPO) activities. This indicated that the generation of ROS during stress might be the causative factor for the inactivation of gastric peroxidase. *M. oleifera* leaf extracts exert their antioxidant defense mechanism probably by metabolizing lipid peroxides and scavenging endogenous H$_2$O$_2$ (Bhattacharya et al., 2000).

The superoxide anion (O$_2^-$), H$_2$O$_2$ and hydroxyl radical (OH) are the major ROS which induce cell degeneration by increasing lipid peroxidation of cell membrane lipids. The toxic end products of peroxidation induce damage of the structural and functional integrity of cell membranes, break DNA strands and denature cellular proteins. The natural cellular antioxidant enzyme includes SOD, which scavenges superoxide radicals by speeding up their dismutation.

Detoxification of the superoxide anion is not a terminating step in free radical scavenging, since the enzyme-catalysed dismutation results in the production of H$_2$O$_2$ which ultimately accumulates in the mitochondria and cytosol.

The results of the present study are similar to the finding of Mizui et al. (1986) which showed that the necrotizing substance like ethanol-induced gastric damage could be due to the formation of oxygen-derived free radicals resulting in lipid peroxidation and damage of cellular membrane with the release of intracellular component like lysosomal enzymes leading to further damage of leaves extract. *M. oleifera* was found to possess ulcer protective. Moreover, Moringa leaves contain isothiocyanate, which has anti-inflammatory as well as immune-modulatory activities (Shaila et al., 2010; Matsuda et al., 2007).

Biswa et al. (2012) reported that the presence of flavonoids in MOELE decreases the gut ulceration by improving microcirculation and increasing capillary resistance, so that the cells become more able to resist to the inflammatory factors.

LPO in the biological system has been demonstrated to be very important in mammalian physiology and pathophysiology. Increasing the rate of lipid peroxidation indicates the initiation of oxidative stress, which leads to various tissue injuries and cell death causing the

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**Table 1.** Effects of MOELE on macroscopic ulcer (Lesion index) in various groups in comparison with indomethacin-treated and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>LI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>-</td>
</tr>
<tr>
<td>Group 2 (Indomethacin)</td>
<td>42.8±4.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 3 (100mg-MOELE)</td>
<td>-</td>
</tr>
<tr>
<td>Group 4 (500mg-MOELE)</td>
<td>-</td>
</tr>
<tr>
<td>Group 5 (100mg-MOELE+Indomethacin)</td>
<td>19.3±2.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 6 (500mg-MOELE+Indomethacin)</td>
<td>7.98±1.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LI*: Lesion index (Bands 4 mm in length was multiplied by 3, where 2-4 mm was multiplied by 2, and bands <2 mm multiplied by 1). Values are given as mean ± standard deviation (SD) for ten animals in each group.

*Indicated significant differences at P<0.05 among control, indomethacin and groups 3 and 4. The different superscript letters (a, b, c) indicated a significant difference at P<0.05, among groups 5 and 6 compared with group 2 (indomethacin).
progression of many acute and chronic diseases. The products of LPO such as malondialdehyde (MDA) are more cytotoxic to cells and have an effect on the membrane structure and function (Basu, 2003).

The results of the present study had shown that MOELE can restore the antioxidant activities of GST, GPx, and SOD and decrease the LPO which is induced by oral administration of indomethacin. Also, there was a notable decrease in gastric lesions. It is interesting to note that *M. oleifera* leaf extracts when given to healthy animals enhanced the level of antioxidants. The results could be explained by Sreelatha and Padma (2009) who conclude that, the leaves extract of moringa prevents oxidative damage to major biomolecules by scavenging the free radicals, so it can protect the biological cells against oxidative damage. Also, the presence of both vitamin C and A, can increase the efficiency of this plant in preventing the oxidative damage to the cell membrane of the biological cells (Bharali et al., 2003).

The current results are also in agreement with Devaraj et al. (2007) who observed that *M. oleifera* leaf extracts when given to normal animals enhanced the level of antioxidant condition. Verma et al. (2009) reported that the scavenging and antioxidant activities of *Moringa* leaves extract are due to the hydrogen proton donation of that compound.

The overall results of the present study are in consensus with the earlier observation of Bello and Balaraba (2012) who demonstrated that in stressed rats, *M. oleifera* leaves extract significantly attenuated the stress-induced gastric ulcerogenesis. Moreover, the leaves have quercetin, a flavonoid compound that is suggested to have a gastric cytoprotective effect and considered as antiulcer agents (Casa et al., 2000; Choudhary et al., 2013). In conclusion, the findings suggest a useful therapeutic activity for MOELE as an antioxidant and anti-ulcerative medicinal plant for gastric ulcer treatment. Oral administration of MOELE, even with low doses, blocks and disrupted free radical metabolism. The extract of *M. oleifera* ameliorated the ulcer lesion and SOD, catalase (CAT), and MDA levels in rat gastric mucosa due to an antioxidant property of MOELE. The antioxidant defense mechanism of the extract was probably due to metabolizing lipid peroxides and scavenging H₂O₂. More studies required to distinguish the exact mechanism and isolation and characterization of active ingredients from crude extract.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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REFERENCES


