Pak. J. Agri., Agril. Engg., Vet. Sci., 2020, 36 (2): 141-148
ISSN: 1023-1072 (Print), ISSN: 2663-7863 (Online)
https://doi.org/10.47432/2020.36.2.8

GASTROPROTECTIVE AND ANTIOXIDANT EFFECTS OF POWDERED LEAVES OF MORINGA OLEIFERA AGAINST EXPERIMENTALLY INDUCED PEPTIC ULCER IN MALE MICE

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ABSTRACT

Moringa oleifera is used worldwide for its different pharmacological actions. In present study, the gastroprotective and antioxidant effects of *M. oleifera* were investigated in aspirin induced peptic ulcer in adult male mice. For that purpose, sixty healthy adult male mice were divided equally but randomlyinto 6 groups. Blood samples were drawn at 0 and 7 days of treatment. At the termination of experiment, the mice were slaughtered and stomach tissues were excised for histopathological examination. Statistical differences among treatment groups were determined by Duncan's Multiple Range test at 5% level of significance. The results suggested that aspirin induced gastric mucosal damage significantly (P<0.05) increased the level of total oxidant status and malondialdehyde, while significantly (P<0.05) decreased catalase and total antioxidant capacity. It was concluded that *M. oleifera* leaves' powder at the dose rate of 600 mg/kg is as efficacious as omeprazole by exhibiting gastroprotective and antioxidant effects against aspirin induced gastric damage in mice.

Keywords: aspirin, gastric, omeprazole, oxidation, stomach

INTRODUCTION

Stomach is a muscular, hollow and dilated part of the digestive system which is involved in the second phase of digestion, following mastication (chewing). Use of NSAIDs may lead to clinical manifestations and pathogenesis of gastroduodenal toxicity (Ijaz *et al*., 2016) especially aspirin including injury to the small and large intestines. Aspirin is a popular NSAID due to versatilityin its effectiveness like as antipyretic, anti-inflammatory and analgesic agent. In low doses, it is also used as an anti-thrombotic agent but it is unfortunate that aspirin and most other NSAIDs have capacity to cause injury of the gastric and duodenal mucosa with considerable morbidity and mortality (Feldman, 2015). Gastric ulcer is a most common gastrointestinal tract disorder including gastric hyperacidity and gastroduodenal ulcer which affects approximately 5-10% people during their life (Sabiha *et al*., 2011). The drugs currently in use for the treatment of ulcer produce adverse reactions which emphasizes us to make our more focus on the natural resources and to search for novel molecules in plants (Ukwe *et al.*, 2010).

Moringa oleifera is the member of family Moringaceae used as vegetable, spice and medicinal purposes. It acts as cardiac and circulatory stimulants, diuretic, anticancer, antihypertensive, antidiabetic, antioxidant, hepatoprotective and anti-hyperlipidemic. It is now used worldwide by researchers for its pharmacological activities that are ranging from anti-inflammatory to anticancerous agent (Anwar and Rashid, 2007). The leaves of *M. oleifera* are good source of protein, minerals, vitamin C, phenols, flavonoids and triterpenes. The flavonoid, quercetin and β-sitosterol are well known for their antiulcer and antioxidant activity supposed to reduce the development of gastric ulcer. Oxidative stress is one of the causes of tissue damage (ulcer) that results from the increased production of oxygen derived free radicals or reactive oxygen species (ROS) that take part in the pathogenesis of many diseases including ulcer (Ijaz *et al*., 2016). Proton pump

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inhibitors (PPIs) and H_2 receptor antagonists are available to treat the peptic ulcer but clinical estimation of these agents has shown incidence of slough relapse, side effects and drug interactions. Global estimates show that about 80% of the world population depends upon the traditional medicines derived from plant origin (Brinda *et al*., 2008). Side effects of allopathic drugs and development of resistance/tolerance to these drugs led to an increased use of plants as source of medicines in variety of diseases (Manjula *et al*., 2009; Ijaz *et al.*, 2016). Medicinal plants have always been proved as an alternative source of these drugs for the disease treatment (Aziz *et al*., 2014; Ijaz *et al*., 2016).

This study was planned to find out the gastroprotectivity of powdered leaves of *M. oleifera*in aspirin induced peptic mucosal ulcer with the aim to explore the antioxidant effect of *M. oleifera* in experimental animals and to ascertain any correlation between the antiulcer and the antioxidant activity of *M. oleifera* leaves' powder.

MATERIALS AND METHODS

In this research, the antiulcer effects of *M. oleifera* (Sohanjna) were investigated in adult albino mice having aspirin induced ulcer. The experimental protocol was as follows:

Plant material

Leaves of *M. oleifera* were obtained, authenticated and compared with its standard in the herbarium maintained by the botanical garden of University of Agriculture, Faisalabad. The samples were preserved in the pharmacology laboratory of Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad. Leaves were washed and dried under the shade and were finely powdered using a special electric grinder. This process was completed with precaution that the temperature did not raise up to 40ºC. The grinded powder was passed through a mesh (sieve no. 200) and then was stored in an airtight container for its further experimental use. Dried leaves powder was administered orally in 3 ml distilled water at the rate of 200, 400 and 600 mg/kg body weight for 0-7 days in the respective groups of mice (Kath and Gupta, 2006).

Study design

Sixty albino mice (n=10 in each group), weighing between 20-40 gram, were procured from the National Institute of Health (NIH) Islamabad and housed at experimental animal room in the Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan. The mice were housed in individual solid bottomed polypropylene cages at room temperature with 12/12 hours light/dark period. Prior to the experimentation, the mice were acclimatized for one week and were given standard feed and water ad *libitum.* After acclimatization, the animals were totally divided into six groups, each group consisting of ten animals. The experiment was conducted with the prior approval by the Directorate of Research and Advanced Studies and with the consent of the Society of Ethics of Animals, University of Agriculture, Faisalabad, Pakistan. Feeding schedule of normal routine feed, aspirin and powdered leaves of *M. oleifera*in adult male mice during the experimentation period (of 0 to 7) days has been presented as follows:

- Group I: Control untreated (on normal routine feed for 0-7 days).
- Group II: Treated with ulcerogenic drug, aspirin 150 mg/kg orally in 3 ml distilled water for 0-7 days.
- Group III: Treated with antiulcer drug; omeprazole 2.4 mg/kg orally alongwith aspirin (150 mg/kg) for 0-7 days.
- Group IV: Treated with *M. oleifera* leaves powder 200 mg/kg orally along with aspirin (150 mg/kg) for 0-7 days.
- Group V: with *M. oleifera* seed leaves 400 mg/kg orally along with aspirin (150 mg/kg) for 0-7 days.
- Group VI: Treated with *M. oleifera* leaves powder 600 mg/kg orally along with aspirin (150 mg/kg) for 0-7 days.

Mice were sacrificed at the end of experiment at day 7 post-treatment by using anesthetic ether. Blood samples were collected in serum gel tubes and serum was separated after centrifugation. Serum was collected in small clean Eppendorf tubes and then stored at refrigeration temperature. It was used for the evaluation of different biochemical parameters. The stomach of all animals was separated by using a sharp scissor. The stomach was cut along the greater curvature and its contents were collected into a small tube. Then, these gastric contents were centrifuged for 5 minutes at 3000 rpm. The supernatant was separated and its pH and volume was measured (Rajkapoor *et al*., 2002). This supernatant was used to estimate different biochemical

parameters *i.e.* acid output, ulcer index and ulcer inhibition measurements.

Examinations

Macroscopic evaluation

A. After emptying stomach samples, each sample was rinsed with normal saline. Each sample was marked with particular score after its macroscopic evaluation by using a magnifier and according to the severity of ulcer observed. Ulcer severity score was graded as: normal coloration as 0, red coloration 0.5, spot ulcer 1.0, hemorrhagic stress 1.5, deep ulcer 2.0 and perforations as 3.0 (Aminabee *et al.*, 2015; Gul *et al.*, 2015; Jafar *et al.*, 2018).

B. Antiulcer evaluation studies

Determination of pH and gastric volume

The pH of the gastric supernatant was determined with the help of a pH meter and gastric volume of gastric supernatant was measured precisely by using the micropipette (Rajkapoor *et al*., 2002).

Acid output

The measurement of acid output was allowed for quantitative analysis to estimate the concentration of an unknown acid or basesolution. Acid output was calculated by titrating the gastric supernatant fluid with 0.01N NaOH. Acidity was expressed as mEq/L per 100 gram of body weight (Maity *et al*., 2003).

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality X 100 mEq/l/100g}}{0.2}$$

Ulcer index calculations

The glandular portion of the stomach was taken out and was used for the estimation of different parameters (Majumdar *et al*., 2003). Stomach was opened at the greater curvature and contents were rinsed off with saline and the linings were examined with binocular magnifier. The ulcer index was determined by using the formula of Ganguly (1969).

Ulcer index (UI) = (UN + US + UP) X 10^{-1}

Where,

UN = Average number of ulcers per animal

UI= Ulcer Index

UP = Percentage of animals with ulcers US= Average number of severity score

Ulcer inhibition percentage

The curative ratio of treated groups from ulcer was calculated from equation given (Wasman *et al*., 2010).

$$\text{Percentage ulcer inhibition} = \frac{\text{Ulc} - \text{Ult}}{\text{Ulc}}$$

Where,

UIc = Ulcer index of control groups

UIt = Ulcer index of treated groups

C. Histopathological evaluation

Histopathology was performed on collected tissue samples according to the method as described by Bancroft and Gamble (2002).

D. Estimation of health biomarkers

The gastric tissues and serum samples were analyzed for the estimation of free radicals by performing the following analytical tests to determine antioxidant capacity and oxidant status.

Total oxidant status (TOS; µMol/L)

It was determined using the method as described by Erel (2005). Following formula was used to measure the TOS in the serum. The result obtained was expressed in µMol/L.

$$\text{Total Oxidant Status (TOC) µmol/L} = \frac{\text{Absorbance} - 022}{0.0815}$$

Total antioxidant capacity (TAC; mmol/L)

It was measured by using method established by Erel (2004). Following formula was used to measure TAC in the serum.

$$\text{Total antioxidant capacity (TAC)} = \frac{(Absorbance - 0.8366)}{-0.4991}$$

The result obtained was expressed in mmol/L.

Malondialdehyde (MDA; nmol/L)

Serum malondialdehyde (MDA) react with thiobarbituric acid (TBA) and concentration of thiobarbituric acid (TBA) was measured by the method described by Ohkawa *et al.* (1979). Following formula was used to measure the malondialdehyde (MAD) concentration in the serum.

$$\text{Malondialdyhyde (nmol/L)} = \frac{\text{Absorbance} - 0.1322}{0.94}$$

The results obtained were expressed in nmol/L.

Catalase (kU/L)

A serum level of enzyme catalase was determined by method established by Goth (1991). Following formula was used to measure the catalase enzyme activity in the serum.

$$\text{Serum catalase activity (kU/L)} = \frac{A\,(sample) - A\,(blank\,1)}{A\,(blank\,2) - A\,(blank\,3)} X\ 271$$

The result obtained was expressed in Kilo U/L.

Statistical analysis

The statistical analysis was carried out for estimating significance (P<0.05) of the difference between control and treated values. Mean ± SE was determined on the data which was normally distributed by one-way analysis of variance, followed by Duncan's multiple range test (DMR).

RESULTS

Antiulcer effect of *M. oleifera* dried leave powder at dose rate of 200, 400 and 600 mg kg^{-1} body weight has been assessed in in adult male albino mice. Experimental data and results have been systematized and reviewed as follows:

Ulcer parameters

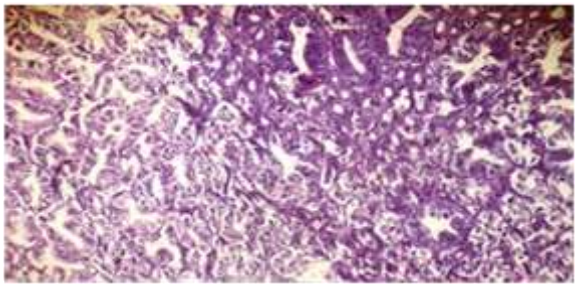
Mean ± SE values of all groups for ulcer parameters have been shown in Table 1. In all experimental groups, pH values have been significantly (P<0.05) increased, whereas, all other ulcer parameter values have been significantly (P<0.05) decreased than that of the respective values in drug treated group. However, ulcer index and gastric volume observed in experimental group 1 was non-significantly (P≥0.05) lesser than ulcer index and gastric volume of drug treated group. Percent ulcer inhibition was highest in the mice treated with the highest dose of *M. oleifera* dried leave powder (experimental group 3).

Oxidative stress biomarkers

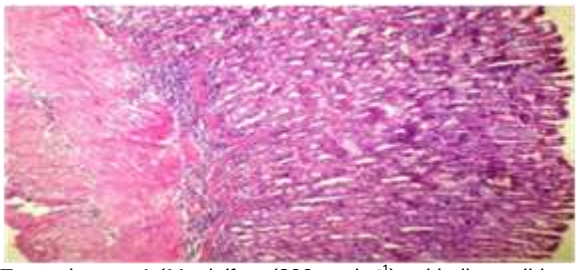
Mean ± SE values of all groups for oxidative stress biomarkers have been shown in Table 2. The values of total oxidant status and malondialdehyde in untreated toxic control group were significantly (P<0.05) higher than the values of all other groups except the TOS and MDA values of treated group 1. Total anti-oxidant capacity and catalase activity observed in toxic group was significantly (P<0.05) lesser that that of the respective values observed in all other groups except that of catalase activity observed in treated group 1.

Histopathology

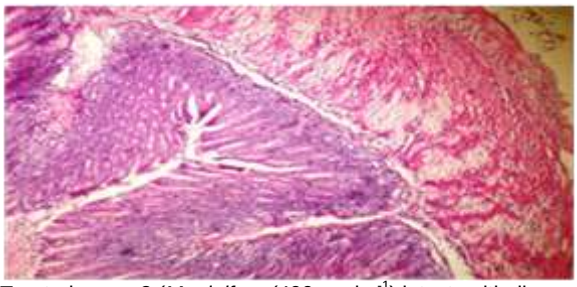
Histological view of the gastric epithelium of untreated toxic control group had infiltration of cells on muscularis mucosa. Villi also reduced in height and somewhere absent. In treated control group 1, superficial sloughing of epithelium was present at some spaces and in pyloric region sloughing was minimum. While in treated group 2 and 3 the tissues appeared normal on histological view. No pathology was observed. Epithelium was intact and did not show any sloughing (Plate 1).



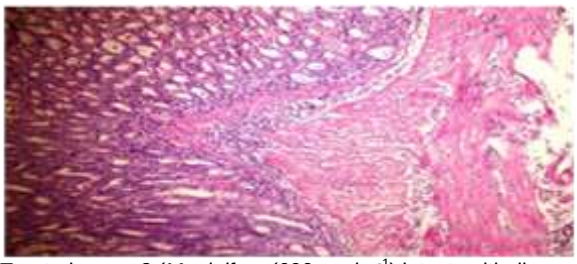
Untreated toxic group cellular infiltration in the glandular region



Treated group 1 (*M. oleifera* (200 mg kg^{-1}) epithelium mild sloughing



Treated group 2 (*M. oleifera* (400 mg kg^{-1}) intact epithelium



Treated group 3 (*M. oleifera* (600 mg kg^{-1}) intact epithelium

Plate 1. Photomicrograph of mice (treated and untreated)

DISCUSSION

Aspirin and certain other NSAIDs at toxic dose level has been reported responsible to inhibitcyclooxygenase enzymes which are necessary for prostaglandin synthesis. These prostaglandins are the master molecules for gastro-protection against all forms of abuses or harms to the gastric mucosa.So, aspirin worsens

the condition more by creating oxidative stress in tissues and mucosal erosion (Wallace, 2008; Fruchter *et al*., 2011; Ijaz *et al*., 2016). Aspirin also induces vasoconstriction of gastric vessels that generate hypoxic condition likeischemia which causes an increase in ROS (reactive oxygen species) generation in gastric mucosa. Oxidative stress such as lipid peroxidation leads to tissue damage that causes loss of membrane fluidity, membrane integrity, impaired ion transport and finally the loss of cellular functions. Thus, it initiates and aggravates many diseases including peptic ulcer and gastric carcinoma (Muhammad *et al.,* 2017). Decrease in gastric pH and percent ulcer inhibition while increase in ulcer score, gastric acid output and ulcer index parameters can help a lot for scrutinizing the drug induced gastric mucosal damage (Batu and Errol, 2008; Saranya *et al.,* 2011; Oluwabunmi and Tijani, 2015; Verma and Kumar, 2016). NSAIDs like aspirin induced erosion or injury to stomach lining leads to the occurrence of infection and production of free radicals such as hydrogen per oxide (H_2O_2) which in conjugation with O_2 release OH^- and H^+ ions (Dawud, 2008; Becker JC *et al.,* 2004; Chattopadhyay *et al.*, 2005). These ions will lead to decrease in pH and causes gastric mucosal erosion. To counter the effect of aspirin in present study, the graded doses (200, 400 and 600 mg kg^{-1}) of *M. oleifera* leaves' powder have been found to significantly (P<0.05) increase the gastric pH which is associated with the gastric mucosal protection and inhibition of HCl secretion. On the other hand, ulcer which is a visible lesion or breach in gastric mucosa, was scored on the basis of extent of severity *i.e.* from red coloration to hemorrhagic streaks. Ulcer score was measured by careful observation of open stomach of ulcerative models and was helpful in the estimation of ulcer index and percent ulcer inhibition. The results of this study revealed that ulcer scores and gastric content volume gradually decreased by increasing the doses (200-600 mg kg^{-1}) of M. Oleifera leaves' powder with respect to untreated toxic group. Both treated groups 2 and 3 have non-significant (P≥0.05) effect on ulcer score and ulcer index values as compared to treated control group. This action of M. oleifera leaves' powder is associated with its anti-secretory and mucoprotective activity. M. oleifera has been reported to be rich in carbohydrates and protein contents that in turn increased its mucus production. This mucus layer is a crucial factor in the gastric mucosal protection from the gastric lesions and it has been considered as an important defensive factor (Jainu *et al.,* 2006). Percent ulcer inhibition by the graded doses of plant leaves' powder have a potential effect to heal and treat ulceration as compared to omeprazole. This was assumed due to the cytoprotective activity of *M. oleifera* leave' extract and might be attributed to induction of mucus production that serves as a gastro-protective agent (Shetty *et al*., 2008).

Regarding the effect of *M. oleifera* leaves' powder on oxidative health biomarkers (TAC, TOS, MDA and catalase) (Shetty *et al*., 2008), the results suggested that gastro protective effect of plants were better than that of synthetic antiulcer agents *i.e.* omeprazole. It was attributed to the presence of flavonoids being antioxidants and natural phenols, tannins and fatty acids having antiulcer activity (Sehirli *et al*., 2008; Khanahmadi *et al*., 2010). The results revealed that total oxidative stress (TOS) and malondialdehyde (MDA) in treated groups were decreased significantly (P<0.05) as compared to that of untreated toxic group while total antioxidant capacity (TAC) and catalase levels were significantly (P<0.05) higher in treated groups. However, the results of treated groups were non-significant (P≥0.05) to synthetic drug (omeprazole) treated group. The reason for the decrease in oxidative stress is the presence of strong antioxidants such as; flavonoids, polyphenols and Vitamin C, etc. in the leaves of *M. oleifera* (Khalafalla *et al*., 2010).

Table 1. Ulcer parameters Mean ± SEM in control and experimental groups of albino mice at day 7 of the study

Groups	pH	Gastric Volume	Ulcer Scores	Gastric acid output (mEq/ L/100 gram of b.wt)	Ulcer Index	Percent Ulcer Inhibition
Blank/Untreated (N=10)	2.6 ± 0.1^{A}	3.2 ± 0.4^{BC}	0.1 ± 0.1^{C}	37.2 ± 6.4^{C}	3.7 ± 0.5^{C}	-
Ulcerogenic drug treated (N=10)	1.1 ± 0.1^{C}	4.4 ± 0.3^{A}	2.7 ± 0.2^{A}	118.1 ± 6.0^{A}	10.4 ± 0.6^{A}	-
Anti-ulcer drug Treated (N=10)	2.6 ± 0.2^{A}	2.2 ± 0.3^{C}	0.2 ± 0.1^{C}	31.6 ± 4.4^{C}	3.1 ± 0.3^{C}	69%
Experimental group 1 (N=10)	1.7 ± 0.3^{B}	4.2 ± 0.3^{AB}	1.4 ± 0.2^{B}	57.2 ± 8.0^{B}	9.1 ± 0.6^{A}	12%
Experimental group 2 (N=10)	2.3 ± 0.2^{A}	3.1 ± 0.2^{C}	0.4 ± 0.2^{C}	44.8 ± 4.2^{BC}	5.4 ± 0.7^{B}	48%
Experimental group 3 (N=10)	2.5 ± 0.2^{A}	2.7 ± 0.5^{C}	0.2 ± 0.2^{C}	29.0 ± 4.4^{C}	3.6 ± 0.5^{C}	65%

Means sharing similar letters in column are statistically non-significant (P≥0.05).

Table 2. Oxidative stress biomarkers (Mean ± SEM) values in control and experimental groups of albino mice at day 7 of study

Groups	TAC (mmol/L)	TOS (µMol/L)	MDA (nmol/L)	Catalase (kU/L)
Blank/untreated (n=10)	0.98 ± 0.01^{A}	2.65 ± 0.1^{B}	9.04 ± 0.3^{B}	9.13 ± 1.5^{A}
Ulcerogenic drug treated (n=10)	0.64 ± 0.1^{C}	4.51 ± 0.7^{A}	20.19 ± 1.6^{A}	5.80 ± 1.2^{C}
Anti-ulcer drugt treated (n=10)	0.97 ± 0.2^{A}	2.69 ± 0.1^{B}	10.39 ± 0.4^{B}	9.10 ± 0.2^{A}
Experimental group 1(n=10)	0.75 ± 0.1^{B}	3.90 ± 0.6^{A}	17.04 ± 0.8^{A}	6.65 ± 0.3^{C}
Experimental group 2 (n=10)	0.80 ± 0.3^{AB}	3.34 ± 0.1^{B}	13.21 ± 0.7^{B}	8.01 ± 0.6^{B}
Experimental group 3 (n=10)	0.95 ± 0.4^{A}	2.64 ± 0.3^{B}	11.35 ± 0.6^{B}	8.85 ± 0.6^{A}

Means sharing similar letters in column are statistically non-significant ($P \geq 0.05$)

The histological examination of gastric mucosa (Plate 1) revealed the extent of tissue damage by ulcerogens. Histopathological evaluation of the gastric mucosa of the untreated toxic group showed distorted gastric glands, inflammatory exudates, damaged mucosal epithelium and cellular debris. Congestion and infiltration were also seen in the glandular portion of the stomach epithelium. The histopathological assessment of *M. oleifera* leaves powder treated mice showed normal cellular construction in the gastric mucosa with no pathological changes that was almost identical to normal control and treated control groups. This is possibly attributed to the cytoprotective effect of *M. oleifera* leaves' powder (Akhtar *et al*., 2007).

The proposed mechanism of *M. oleifera* to produce gastroprotective effect might be due to its antioxidant properties. The leaves of *Moringa oleifera* contained many antioxidants such as flavonoids, vitamins C, vitamin E and polyphenols possessing radical trapping ability (Siddhuraju and Becker 2003). Moreover, polyphenolic compounds which covered majority of constituents detected in *M. oleifera* have well documented antioxidant potential through inhibiting ROS and lipid peroxidation in gastric tissues. Further, *M. oleifera* has been reported to restore depleted glutathione after toxic drug administration (Rehman *et al.,* 2017). Besides antioxidant activity of *M. oleifera* as gastroprotective plant, other mechanisms *e.g.* sympatholytic effect, inactivation of rennin-angiotensin system and modulation in nitric oxide synthesis cannot be over ruled. Further studies are obligatory to interpret these possible mechanisms.

CONCLUSION

Out of three gradual doses of *M. oleifera* (200, 400 and 600 mg kg^{-1}), last two doses possess significant ($P<0.05$) antiulcer activity as compared to toxic group and non-significant ($P \geq 0.05$) antiulcer activity than that of the synthetic antiulcer drug treated group. The possible reason is the presence of natural chemicals that not only have cytoprotective activity but also have anti-secretory, mucoprotective and antioxidant effects. Histopathological examination of stomach tissues of mice was normal with no pathological signs in gastric tissues of treated groups as compared to untreated toxic group that revealed the effectiveness of antiulcer activity of *M. oleifera*. It was concluded from present research that *M. oleifera* powdered leaves at dose rate of 400 and 600 mg/kg have sufficient antiulcer activity as compared to synthetic antiulcer drugs.

ACKNOWLEDGEMENTS

Authors are thankful to University of Agriculture, Faisalabad for providing chemicals and equipment at its well-established research labs.

STATEMENT OF CONFLICT OF INTEREST

Authors declare that they have no conflict of interest regarding publication of this article.

AUTHOR'S CONTRIBUTION

N. Latif: Researcher, data collector and writer
M. A. Naeem: Manuscript writing and data collection
A. Rashid: Research design and supervision
B. Aslam: Research design and supervision
M. M. Ashraf: Data analysis and manuscript evaluation and approval

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(Received: June 06, 2018; Accepted: December 22, 2020)