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Research Paper

# Anti-Ulcer and Antioxidant Activity of Moringa *Oleifera* (Lam) Leaves against Aspirin and Ethanol Induced Gastric Ulcer in Rats

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## Abstract

Study was conducted to test the hypothesis that Moringa oleifera Lam. leave has hepatoprotective as well as antioxidant activity. For this purpose, a study was designed using alcoholic extracts of Moringa oleifera leave produce significant hepatoprotective and antioxidant activity. The activity was tested in wistar albino rats at dose levels of 125, 250 & 500 mg/kg, orally and compared with ranitidine (50 mg/kg) as standard. During the ulcer condition, there was an increase in gastric mucosal SOD and LPO levels. This indicated that the generation of relative oxygen species during stress might be the causative factor for the inactivation of gastric peroxidase. The alcoholic leaves extract of Moringa *oleifera* Lam. has shown ulcer protective effect as dose dependently against pylorus-ligation, ethanol, cold restraint stress, and aspirin-induced gastric ulcer in rats. The said extract of Moringa *oleifera* Lam. was found to decrease ulcer and acid pepsin secretion. A change was also seen in SOD, CAT, and LPO levels in rat gastric mucosa due to antioxidant property of alcoholic leaves extract of Moringa *oleifera* Lam. Antioxidant defense mechanism of the extract was probably due to metabolising lipid peroxides and scavenging $H_2O_2$.

***Keywords:*** *Ethanolic Extract, Moringa oleifera, Aspirin-Induced, Ulcer Protective Activity*

## 1. Introduction

Gastric hyperacidity and ulcer are very common, causing tremendous human suffering now days. 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). Oxygen derived free radicals implication, in the pathogenesis of a wide variety of clinical disorders and gastric damage, caused by physical, chemical and psychological factors that lead to gastric ulceration in human and experimental animals (Rao et al, 1999). Although many products are used for the treatment of gastric ulcers e.g. antacids and antihistaminics. Most of these drugs, however, produce several adverse reactions, like arrhythmias, impotence, gynecomastia and hematopoeitic changes (Ariypshi et al, 1986). Extracts of many herbal plants have been shown to produce promising results for the treatment of gastric ulcer (Pillai et al, 1978).

Plant extracts are some of the most attractive sources of new drugs, and have been shown to produce promising results for the treatment of gastric ulcer (Pillai et al, 1978). The precise biochemical changes during ulcer generation are not clear yet, although various hypotheses have been proposed from time to time. Increased gastric motility, vagal over activity (Cho et al, 1978), mast cell degranulation, low gastric mucosal blood flow and decreased prostaglandin level (Miller, 1987) during stress condition are thought to be the reasons of ulcer generation. Similarly role of oxygen-derived free radicals have also

been shown to play a role in experimental gastric damage induced by ischemia and reperfusion (Perry et al, 1986), hemorrhagic shock (Itoh et al, 1985) and ethanol administration (Mizui et al, 1986).

Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radical. Free radicals generated by the metabolism of toxicants initiate the toxicity cascade (Recknagel et al, 1983). The endogenous free radical scavengers; like reduced glutathione, vitamin C, vitamin E, catalase and super oxide dismutase, are present in the body. The uninhibited free radicals then initiate cell necrosis thus causes hepatotoxicity (Roy et al, 1994). Investigated new drugs have very little to offer for alleviation of these disorders. The whole Moringa *oleifera* plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, (Nadakami et al, 1973). The aqueous extracts of roots and barks of it were found to be effective in preventing implantation, (Shukla et al, 1988) whereas the aqueous extracts of its fruits have shown significant anti-inflammatory activity. Methanolic extracts of its leaves have shown anti-ulcer activity while ethanolic extracts of seeds exhibited anti-tumour activity (Guevara et al, 1999). Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β – carotene, amino and various phenolics acids (Farooq et al, 2007).

The Moringa plant, found in tropical and subtropical countries, provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals. It is very important for its medicinal value. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumour (Makonnen et al, 1997), antipyretic, antiepileptic, anti-inflammatory and antiulcer (Pal et al, 1995).

The objective of the present study was to investigate the antiulcer and antioxidant activities of the alcoholic extract of the leaves of Moringa *oleifera* using various animal models. Thus, the present research was undertaken to study the role played by mucosal offensive acid-pepsin and defensive mucin secretion, glycoproteins and free radical scavenging and antioxidant activity of the herbal drug (Moringa *oleifera* Lam) in animal models.

## 2. Materials and Methods

The present research work was designed to evaluate antioxidant and antiulcer activity of medicinal plant Moringa *oleifera* Lam. This study was performed at National Botanical Research Institute (N.B.R.I.), Lucknow, India. In order to see the antiulcer and antioxidant activity of Moringa *oleifera* Lam., the following main steps were involved:

### *2.1. Collection of the Plant Materials*

The plant leaves of Moringa *oleifera* Lam. (Family - Moringaceae) were collected from Botanical Garden of N.B.R.I. Lucknow, India in month of July 2007. The plant materials were authenticated by Dr. Sayeeda Khatoon, chemotaxonomist at Institute and the voucher specimens (NAB 182376) were deposited in the departmental herbarium of the Institute for future reference.

### *2.2. Preparation of Ethanolic Extract*

The freshly collected leaves (4 kg) of Moringa *oleifera* were first washed with distilled water dried in tray dryer under controlled conditions and powdered. The powdered plant materials (1000g) was macerated with petroleum ether to remove fatty substances and the marc was further exhaustively extracted with 50% ethanol for 3 days (3 X 5L). The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. The yield of dried extract obtained was 95.0 g (9.5 % w/w). The 50% ethanolic extracts obtained was further subjected to toxicological and pharmacological investigations.

### *2.3. Chromatographic Study (HPTLC)*

1 g of Moringa *oleifera* was dissolved in 25 ml of methanol and used for HPTLC analysis. 10 µl of the test solution was applied and the plate was then developed in Toluene, ethyl acetate and formic acid with a ratio of 7:2:1 respectively, to a distance of 8 cm. Developed plate was then observed under visible light after derivatisation with anisaldehyde-sulphuric acid reagent as shown in Figure 1.

### *2.4. Animals Used*

Studies were carried out using Swiss albino mice weighing 25-30 g. They were obtained from the Central Animal House Facility of Central Drug Research Institute, Lucknow. The mice were group housed in polyacrylic cages (38×23×10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 $^{0}$C) and relative humidity 44-56 %, with a dark and light cycle of 12 ± 1 hour. They were allowed free access to standard dry pellet diet and water *ad libitum*. All procedures described were reviewed and approved by the institutional committee for ethical use of animals (Zimmerman, 1983).

### *2.5. Acute Toxicity Studies (Ghosh, 1984)*

The adult Swiss albino mice of both sexes selected for acute toxicity study. The 50% ethanolic Leaves extract of Moringa *oleifera*, taken at various dose levels (i.e. 125, 250, 500, 1000, 2000 mg/kg body weight), was dissolved in 1 % carboxymethyl cellulose sodium and administered 10 ml/kg orally to the pairs of mice per dose level. The control animals received only 1 % carboxymethyl cellulose sodium in distilled water (10 ml/kg) orally. The animals were observed continuously for 2 hours, then occasionally for further 4 hours and finally overnight for mortality recorded. Behavior of the animals and any other toxic symptoms were also observed for 72 hours. Animals were kept under observation for up to 14 days. 50 % ethanolic Leaves extract of Moringa *oleifera* (2000 mg/kg) did not cause any mortality in mice. Therefore, the effective dose ($ED_{50}$) of 50% ethanolic extract of Moringa *oleifera* was decided as 1/10 of maximum dose (2000mg/Kg). Thus the doses of 50% ethanolic extract of Moringa *oleifera* such as 125, 250 and 500 mg/kg body weight, per oral (p. o.) were taken for the Antiulcer activity studies.

### *2.6. Antiulcer and Cytoprotective Studies*

**Group I-** Normal control

**Group II-** Ulcer induced control

**Group III-** 50% ethanolic extract Moringa *oleifera* (125 mg/kg body wt p.o.) in ulcer rats (not used for antioxidant activity)

**Group IV-** 50% ethanolic extract Moringa *oleifera* (250 mg/kg body wt p.o.) in ulcer rats

**Group V -** 50% ethanolic extract Moringa *oleifera* (500 mg/kg body wt p.o.) in ulcer rats

**Group VI-** Ranitidine (50 mg/kg body wt p.o.) in ulcer rats

50% ethanolic Leaves extract of Moringa *oleifera* and cytoprotective drug ranitidine were administered orally twice daily at 10:00 and 16:00 hrs respectively for five days before gastric ulcers were induced.

### *2.7. Drug Formulation*

The drug samples were prepared in 1 % carboxymethyl cellulose (CMC). Control group of animals received suspension of 1 % carboxymethyl cellulose in distilled water (10 ml/kg).

### *2.8. Pylorus Ligated (PL) - Induced Ulcers*

Gastric ulcers were produced in rats by following method as described by Sanyal et al (Sanyal et al, 1982). The rats were fasted for 24 hours before pylorus-ligation but water was allowed *ad libitum*. At the end of 24 h starvation, rats were anaesthetized with pentobarbitone sodium (35 mg/kg). Abdomen was opened by a midline incision and a ligature was placed at the pyloric end of the stomach taking care not to exclude any blood vessels. The abdomen was then closed in two layer and rats were left in a cage with a false bottom of wide mesh wire gauze to prevent coprophagy. Water was withheld from one hour before pylorus ligation and till the end of 4 h period when the rats were sacrificed by overdosing with ether. Abdomen of mice opened again immediately, cardiac end of stomach was ligated. To study the conditions, the stomach was taken out, cut open along the greater curvature and the mucosa was washed away under slow running tap water.

The ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows: 0 for no ulcer, + for pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion;, ++ for ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes;, +++ for ulcer size 1-2 mm with more than two-thirds of the mucosal thickness destroyed wih marked necrosis and congestion, muscular is remaining unaffected, ++++ for ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscular is still remaining unaffected. The pooled group ulcer score was then calculated according to the method of Sanyal et al (Sanyal et al, 1982). The results are given in Table 1.

### *2.9. Ethanol (EtOH)-Induced Ulcer (Hollander et al, 1985)*

The gastric ulcer was induced in rats by administering Ethanol (EtOH, 100%, 1ml/200 g, 1 hour). EtOH was administered on the day of the experiment. The animals were slaughtered by cervical dislocation and stomach was incised along with greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcer present in the glandular portion of the stomach millimeter square/rat (mm2/rat). The results are given Table 2.

### *2.10. Cold-Restraint Stress (CR Stress)-Induced Ulcers (Amar et al, 1981)*

Rats of either sex weighing 120-150 g were immobilized for 2 hours at 4° C following the method of immobilization as describe by Amar and Sanyal (1981). The animals were starved for 24 hours with free access to water and 60 minutes after receiving the corresponding treatment, they were fully stretched and strapped to a wooden plank with

adhesive tape after securing each limb to the plank individually. The animals were killed after 2 hours and ulcer were scored as described above. The results are given Table 3.

### *2.11. Aspirin (ASP)-Induced Ulcers (Goel et al, 1985)*

Aspirin was administered orally on the day of experiment at about 10 AM with the help of an orogastric tube in the form of an aqueous water suspension (200 mg/kg, per oral) and animals were sacrificed after 4 hours of administration. The stomach was incised along with the greater curvature and examined for ulcers as described earlier. The results are given Table 4**.**

### *2.12. Antioxidant Study*

#### *2.12.1. Estimation of Free Radical Generation*

The rats were given EtOH extract of herbal drug Moringa *olifera* lam. (125, 250 and 500 mg/kg), orally, daily for 3 days and on the day of experiment ($4^{th}$ day). One hour prior to subjecting the animal to Pylorus ligated. Ethanol, CRS, Aspirin, necrotizing concentration of respective irritants in the absence and presence of cytoprotective irritant or herbal drugs and the ulcer index is calculated as described earlier. The fundic part of the stomach is homogenized (5%) in ice-cold 0.9% saline with a Potter – Elvehjem glass homogenizer for 30 seconds. The homogenate was centrifuged at 800 rpm for 10 min followed by the centrifugation of the supernatant, at 12,000 rpm for 15 min in and the obtained mitochondrial fraction (Das et al, 1993).

#### *2.12.2. Reagents for Lipid Peroxidation and Antioxidants*

(a) Thiobarbituric acid (E. Merck), Trichloroacetic acid (BDH) and hydrochloric acid (0.27 M) (BDH)

(b) Dichromate – acetic acid reagent: 5 % potassium dichromate was prepared with acetic acid (1:3 v/v in distilled water)

(c) Phosphate buffer – 0.01 M, pH 7.0: Dissolved 173 g of disodium hydrogen phosphate in 61 ml and 122 mg of sodium dihydrogen phosphate in 39 ml of distilled water first and then made both solutions up to 200 ml with distilled water.

(d) Hydrogen peroxide – 0.2 M: 2.27 ml of hydrogen peroxide was made up to 100 ml with distilled water [BDH (E. Merck) India]. Other reagents used were of analytical grade.

#### *2.12.3. Measurement of Lipid Peroxidation (LPO)*

Lipid peroxidation product malondialdehyde (MDA) was estimated according to the method of Ohkawa et al (1979). 1.0 ml of sample was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20 % acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8 % thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at $85^{o}$ C for 1 hour. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 rpm for 10 minutes. Tetraethoxypropane was used as a standard.

#### *2.12.4. Preventive Enzymatic Antioxidants*

The fundic stomach was homogenized (5%) in 0.25 M sucrose and 50 mM phosphate buffer (pH 7.2) and mitochondrial fraction was prepared as described above (Das et al, 1993). The results are given Table 5.

#### *2.12.5. Measurement of Superoxide Dismutase (SOD)*

SOD was estimated by following the procedure of Kakkar et al (1984). Assay mixture contained sodium pyrophosphate buffer (0.052 M, pH 8.3), phenazine methasulfate (PMS, 6.2 M), Nitroblue tetrazolium (NBT, 30 M), potassium cyanide (KCN, 10 µm, pH 7.0) and 0.2 ml of sample fraction. Samples were preincubated for 5 minutes at 360 $^{o}$C prior to the addition of reduced nicotinamide adenine dinucleotide (NADH, 52 µm). Mixture was further incubated for 120 sec at $370^{o}$ C in a water bath and the reaction was stopped by adding 1 ml glacial acetic acid (17.4 M). The violet colour developed was extracted in 0.4 ml of n-butanol reagent blank. The activity was measured at 560 nm and the results have been expressed as unit n moles /mg protein /mg protein. The results are given in Table 6.

#### *2.12.6. Measurement of Catalase (CAT)*

Decomposition of $H_2O_2$ in the presence of catalase was followed at 240 nm (Aebi, 1983). A 50 µm sample was added to buffered substrate (50 mM phosphate buffer, pH 7.0 containing 10 mM $H_2O_2$ ) to make total volume 3 ml, a decrease in the absorbance was monitored at 370 $^{o}$C for 2.5 min at an interval of 15 sec. the activity was measured at 240 nm. Results are expressed as units (U) of CAT n moles / mg protein. The results are given Table 7.

Decomposition of $H_2O_2$ in the presence of catalase was followed at 240 nm (Aebi, 1983). A 50 µm sample was added to buffered substrate (50 mM phosphate buffer, pH 7.0 containing 10 mM $H_2O_2$ ) to make total volume 3 ml and decrease in the absorbance was monitored at 370 C for 2.5 min at an interval of 15 sec. the activity was measured at 240 nm. Results are expressed as units (U) of CAT as / mg protein. The results are given in Table 7.

### *2.13. Histopathological Examination*

For histopathological examination, the stomach was washed thoroughly with saline, dehydrated in gradual ethanol (50-100%), cleared in xylene and finally embedded in paraffin. Sections (size of 4-5 mm) were prepared and stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation (magnification 100x) as shown in Figure 2.

## 3. Results and Discussion

### *3.1. HPTLC Analysis of 50% Ethanolic Leaves Extract of Moringa oleifera Lam*

The 50 % ethanolic leaves extract of *Moringa oleifera*, on TLC plates were visualized under UV 254, 366nm and visible light. The plates were sprayed with anisaldehyde-sulfuric acid and heated at 110 °C for 10 minutes. These plates were scanned densitometrically using CAMAG TLC scanner at 584nm wavelength as shown in Figure 1.

### *3.2. HPTLC-Profile of 50 % Ethanolic Leaves Extract of Moringa oleifera Lam*

**Solvent system** : Toluene: Ethyl acetate: Formic acid (7:2:1)

**Visualization** : Observed the developed plate under UV light at 254 nm, 366 nm and visible light after sprayingwith anisaldehyde solution

**Scan wavelength** : 584nm

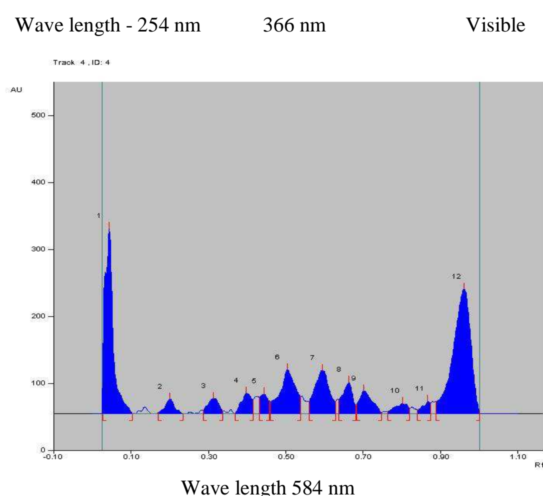


**Figure 1. HPTLC Finger Print Profile of 50 % Ethanolic Leaves Extract of *Moringa oleifera* Lam. in Toluene: Ethyl Acetate : Formic acid (7:2:1) Solvent system**

### *3.3. General Behavior and Acute Toxicity Studies*

50% ethanolic leaves extract of Moringa *oleifera* up to 2000 mg/kg did not cause any mortality in mice. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal out put, feeding behavior etc. during the period of observation.

### *3.4. Effect of 50% Ethanolic Leaves Extract of Moringa oleifera Lam. (Twice Daily for Five Days) on Pylorus Ligation-Induced Gastric Ulcers*

A dose-response antiulcer study has been done using 125, 250 and 500 mg/kg of 50% ethanolic leaves extract of Moringa *oleifera* Lam against pylorus ligation gastric ulcer models. Ulcers were scored and analysed. The results indicated that the ulcerogenic effect reduced from 19.6±1.5 $mm^2$/rat (pylorus ligation group) to 2.3±0.2 (P<0.001) (Moringa *oleifera* Lam extract group). Therefore, the results, in Table 1, showed dose-dependent antiulcerogenic activity in 50% ethanolic Leaves extract of Moringa *oleifera* Lam.

The present study of 50% ethanolic leaves extract of Moringa *oleifera* Pylorus-ligated-induced ulcer may be due autodigestion of gastric juice and decrease mucosal blood flow and break down of mucosal barrier (Goel et al, 1991). The Shay model (Shay et al, 1945) is simple, reproducible and highly predictable model for the screening and evaluation of antiulcer drugs. It utilises neither exogenous ulcerogens nor is induced by exogenous interfering factors. Ulcers are believed to develop due to excess of acid and pepsin for a given degree of mucosal defense.

### *3.5. Effect of 50% Ethanolic Leaves Extract of Moringa oleifera Lam. (Twice Daily for Five Days) on Ethanol-Induced Gastric Ulcers*

The 50% ethanolic extract of Moringa *oleifera* Lam were administered to various groups, orally, twice daily for five days. Experiments were carried out on 18-24 hours fasted rats on $6^{th}$ day. Ulcer were scored and analyzed as described earlier. The result indicated a dose-dependent antiulcerogenic activity from 17.9±1.7 $mm^2$/rat ethonol-induced gastric ulcer to 3.2±0.4 $mm^2$/rat (P<0.001) Moringa *oleifera* Lam extract group as shown in Table 2.

The effect of 50% ethanolic extract of *Moringa oleifera* lam on induce Gastric ulceration is mainly caused by the alteration of the antioxidant enzymes of the gastric mucosa with concomitant loss of cytoprotection due to decreased activity of the PG synthesis (Das et al, 1993). Development of a novel nontoxic potent antisecretory – antioxidant compound will prevent oxidative stress and it will be a suitable way to control this human suffering.

Mizui et al (1986) 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.

50% ethanolic leaves extract of Moringa *Oleifera* Lam was found to possess ulcer protective and decrease acid-pepsin secretion.

### 3.6. Effect of 50% Ethanolic Leaves Extract of Moringa oleifera Lam. (Twice Daily For Five Days) on Cold-Restraint Stress-Induced Gastric Ulcers

The 50 % ethanolic extract of Moringa *oleifera* Lam. was administrated to various groups, orally, twice daily for five days. On sixth day, experiments were carried out on 18-24 hours fasted rats. Ulcer were scored and analyzed as described earlier. The results indicated a dose-dependent antiulcerogenic activity from 23.6±1.5 $mm^2$/rat cold restraint stress-induced gastric ulcer to 3.0±0.4 $mm^2$/rat ($P<0.001$) Moringa *oleifera* Lam. extract group as shown in Table 3.

The present study of 50% ethanolic leaves extract of Moringa *oleifera* was effective in cold-restraint stress-induced gastric ulcer play an important role in the causation of gastroduodenal ulceration. Antistress drugs were found to be effective in stress-induced gastric mucosal damage. Oxidative stress has been proposed to be important etiopathological factor in genesis of peptic ulcer, while enhanced acid-pepsin appears to be directly related to Pylorus-ligated induced gastric ulcers (Senay et al, 1967 and Guth, 1971). Disturbance of gastric mucosal microcirculation, alteration in gastric secretion and abdominal gastric motility have been considered as pathogenesis mechanisms, responsible for the stress-induced gastric mucosal lesions (Mohammad et al, 1986).

### 3.7. Effect of 50% Ethanolic Leaves Extract of Moringa oleifera Lam. (Twice Daily for Five Days) on Aspirin-Induced Gastric Ulcers

The 50% ethanolic extract of *Moringa oleifera* Lam were administered to various groups, orally, twice daily for five days and experiment were carried out on 18-24 hours fasted rats on $6^{th}$ day. Ulcer were scored and analyzed as described earlier. The result indicated a dose- dependent antiulcerogenic activity from 23.3±1.8 $mm^2$/rat aspirin-induced gastric ulcer to 2.1±0.8 $mm^2$/rat ($P<0.001$) *Moringa oleifera* Lam extract group (Tables 4).

The present study of 50% ethanolic leaves extract of *Moringa oleifera* was effective in synthetic NSAIDs like Aspirin cause mucosal damage by interfering with prostaglandin synthesis, enhance acid secretion, increase back diffusion of $H^+$ ions, and breaking up of the mucosal barrier (Vane, 1971; St John et al; 1973 and Brzozowski et al, 1995). While ethanol perturbs superficial mucosal cells, notably mucosal mast cells to release vasoactive mediators notably $LTC_4/D_4$ and platelet activating factor (PAF) and histamine, which damage the gastrointestinal mucosa (Boughton et al, 1988).

### 3.8. Antioxidant Study-Effect of 50% Ethanolic Leaves Extract of Moringa oleifera Lam on Lipid Peroxidation (LPO), Catalase (CAT) and Superoxide Dismutase (SOD) Activities

A growing body of experimental and clinical evidence have shown that gastric mucosal damage by stress (Das et al, 1997), ethanol (Mizui et al, 1986), NSAIDs (Vaananenn et al, 1992 and Yoshikawa et al, 1993) and H. pylori is mediated through the involvement of reactive oxygen species. It has been shown that $OH^-$ radical plays a critical role in gastric mucosal tissue damage and causes ulcer. The present investigation of herbal drug was carried out to study their antioxidant and free radical scavenging activities.

The results, as shown in Table 5, 6, 7 & 8, of present study on free radical-mediated Lipid peroxidation and alteration in circulating enzymatic antioxidants, CAT and SOD, indicates the involvement of these enzymes in ulcer.

In our present study, the antioxidant property, of 50% ethanolic extract of Moringa *oleifera* Lam., was found to be changed in SOD, CAT, and LPO levels in rat gastric mucosa. During the ulcer condition there is an increase in gastric mucosal SOD and LPO activities. This indicated that the generation of reactive oxygen species during stress might be the causative factor for the inactivation of gastric peroxidase. Moringa *oleifera* Lam. exerts its antioxidant defense mechanism probably by metabolising lipid peroxides and scavenging endogenous $H_2O_2$ (Bhattacharya et al, 2000).

The superoxide anion ($O_2^-$), $H_2O_2$ and hydroxyl radical ($OH^-$) are the major reactive oxygen species (ROS) which induce cell degeneration by increasing LPO of cell membrane lipids. The toxic end products of peroxidation induce damage of the structural and functional integrity of cell membrane, break DNA strands and denature cellular proteins. The natural cellular antioxidant enzyme includes SOD, which scavenges superoxide radicals by speeding up their dismutation, CAT, a heame enzyme which removes $H_2O_2$. Detoxification of the superoxide anion is not a terminating step in free radical scavenging, since the enzyme catalysed dismutation results in the production of $H_2O_2$ which ultimately accumulates in the mitochondria and cytosol. Our result is thus similar with the finding that

**Table 1. Effect of 50% Ethanolic Leaves Extract of *Moringa oleifera* (Twice Daily For Five Days) on Pylorus Ligation-Induced Gastric Ulcers**

| Group | Treatment | Dose (mg/kg) | Ulcer index ($mm^2$/rat) | % Protection |
|---|---|---|---|---|
| I | Pylorus ligation | - | 19.6 ± 1.5 | - |
| II | Moringa *oleifera* extract | 125 | 12.8 ± 1.9[a] | 34.69 |
| III | Moringa *oleifera* extract | 250 | 5.1 ± 0.4[b] | 73.96 |
| IV | Moringa *oleifera* extract | 500 | 2.3 ± 0.2[b] | 88.26 |
| V | Ranitidine | 50 | 2.1 ± 0.3[b] | 89.28 |

*Values are mean ± SEM for 6 rats.*
*a=P < 0.01, compared to respective Pylorus ligation group.*
*b=P < 0.001 compared to respective Pylorus ligation group.*

**Table 2. Effect of 50% Ethanolic Leaves Extract of Moringa *oleifera* (Twice Daily For Five Days) on Ethanol-Induced Gastric Ulcers**

| Group | Treatment | Dose (mg/kg) | Ulcer index ($mm^2$/rat) | % Protection |
|---|---|---|---|---|
| I | Ethanol | - | 17.9 ± 1.7 | - |
| II | Moringa *oleifera* Extract | 125 | 13.5 ± 1.2[a] | 24.58 |
| III | Moringa *oleifera extract* | 250 | 6.3 ± 0.8[b] | 64.80 |
| IV | Moringa *oleifera extract* | 500 | 3.2 ± 0.4[b] | 82.12 |
| V | Ranitidine | 50 | 2.1 ± 0.3[b] | 88.28 |

*Values are mean ± SEM for 6 rats*
*a= P < 0.05, compared to respective Ethanolic group*
*b= P < 0.001 compared to respective Ethanolic group*

**Table 3. Effect of 50% Ethanolic Leaves Extract of *Moringa oleifera* (Twice Daily For Five Days) on Cold-Restraint Stress-Induced Gastric Ulcers**

| Group | Treatment | Dose (mg/kg) | Ulcer index ($mm^2$/rat) | % Protection |
|---|---|---|---|---|
| I | Cold-restraint stress | - | 23.6 ± 1.5 | - |
| II | Moringa oleifera extract | 125 | 13.3 ± 1.6[a] | 43.64 |
| III | *Moringa oleifera* extract | 250 | 10.3 ±. 0.8[b] | 56.35 |
| IV | *Moringa oleifera* extract | 500 | 3.0 ± 0.4[b] | 87.28 |
| V | Ranitidine | 50 | 2.6 ± 0.3[b] | 88.98 |

*Values are mean ± SEM for 6 rats*
*a= P < 0.01 compared to respective CRS group*
*b= P < 0.001 compared to respective CRS group*

**Table 4. Effect of 50% Ethanolic Leaves Extract of *Moringa oleifera* (Twice Daily For Five Days) on Aspirin -Induced Gastric Ulcers**

| Group | Treatment | Dose (mg/kg) | Ulcer Index ($mm^2$/rat) | % Protection |
|---|---|---|---|---|
| I | Aspirin | - | 21.3 ± 1.8 | - |
| II | Moringa *oleifera* extract | 125 | 15.2 ± 1.5[a] | 28.63 |
| III | Moringa *oleifera* extract | 250 | 6.3 ± 1.1[b] | 70.42 |
| IV | Moringa *oleifera* extract | 500 | 2.1 ± 0.8[b] | 90.14 |
| V | Ranitidine | 50 | 1.5 ± 0.6[b] | 92.95 |

*Values are mean ± SEM for 6 rats*
*a = P < 0.05, compared to respective Aspirin group*
*b = P < 0.001 compared to respective Aspirin group*

**Table 5. Effect of 50% Ethanolic Leaves Extract of Moringa *oleifera* on Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), and Catalase (CAT) in Pylorus Ligation-Induced Gastric Ulcers**

| Treatment | Dose (mg/kg) | UV Absorbance at 532 nm for LPO | UV Absorbance at 560 nm for SOD | UV Absorbance at 240 nm for CAT |
|---|---|---|---|---|
| Control | - | 0.52 ±0.03 | 98.38 ± 5.87 | 37.21 ± 1.13 |
| Pylorus ligation | - | 0.78 ± 0.05[y] | 207.12 ±11.64[x] | 19.13 ±1.33[x] |
| Moringa *oleifera* extract | 250 | 0.61 ± 0.04[a] | 156.13 ± 6.14[a] | 24.51 ± 1.18 |
| Moringa *oleifera* extract | 500 | 0.49 ± 0.02[b] | 127.21± 4.42[c] | 30.22 ± 2.12[b] |
| Ranitidine | 50 | 0.47 ± 0.03[c] | 113.24 ± 4.71[c] | 33.15 ± 1.42[c] |

*Values are mean ± SEM for 6 rats*
*x = P < 0.001 compared to respective control group*
*y = P < 0.01 compared to respective control group*
*a = P < 0.05, compared to respective pylorus ligation group*
*b = P < 0.01 compared to respective pylorus ligation group*
*= P < 0.001 compared to respective pylorus ligation group*

**Table 6. Effect of 50% Ethanolic Leaves Extract of *Moringa Oleifera* on Lipid Peroxidation (LPO), Superoxide Dismutase (SOd), and Catalase (CAT) in Ethanol-Induced Gastric Ulcers**

| Treatment | Dose (mg/kg) | UV Absorbance at 532 nm for LPO | UV Absorbance at 560 nm for SOD | UV Absorbance at 240 nm for CAT |
|---|---|---|---|---|
| Control | - | 0.42±0.04 | 107.13 ± 4.16 | 32.2 ± 2.17 |
| Ethanol | - | 0.62 ± 0.05[y] | 148.12 ±6.21[x] | 20.8 ± 2.32[x] |
| Moringa *oleifera* extract | 250 | 0.51± 0.03[a] | 117.24 ± 4.33[a] | 26.1 ± 1.16[a] |
| Moringa *oleifera* extract | 500 | 0.44± 0.02[b] | 104.26± 4.41[b] | 31.3 ± 2.17[b] |
| Ranitidine | 50 | 0.41 ± 0.04[c] | 101.32 ± 4.11[b] | 34.32 ± 2.21[c] |

*Values are mean ± SEM for 6 rats*
*x =P < 0.001 compared to respective control group*
*y =P < 0.01 compared to respective control group*
*a =P < 0.05 compared to respective Ethanol-induced group*
*b =P < 0.01 compared to respective Ethanol-induced group*
*c =P < 0.001 compared to respective Ethanol-induced group*

**Table 7. Effect of 50% Ethanolic Leaves Extract of Moringa *oleifera* on Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), and Catalase (CAT) in Cold Restraint Stress-Induced Gastric Ulcers**

| Treatment | Dose (mg/kg) | UV Absorbance at 532 nm for LPO | UV Absorbance at 560 nm for SOD | UV Absorbance at 240 nm for CAT |
|---|---|---|---|---|
| Control | - | 0.36 ± 0.03 | 98.11 ± 7.81 | 34.12 ± 2.41 |
| CR stress | - | 0.58 ± 0.05[x] | 187.62 ± 9.85 [x] | 18.21 ± 1.33[y] |
| Moringa oleifera extract | 250 | 0.46 ± 0.03[a] | 136.41 ± 4.32[b] | 24.11 ± 1.82[a] |
| Moringa oleifera extract | 500 | 0. 31 ± 0.02[c] | 115.32 ± 3.26[c] | 29.13 ± 1.61[b] |
| Ranitidine | 50 | 0.29 ± 0. 02[c] | 111.21 s± 3.05[c] | 31.21 ± 1.23[b] |

*Values are mean ± SEM for 6 rats*
*x =P < 0.001 compared to respective control group*
*y =P < 0.01 compared to respective control group*
*a =P < 0.05, compared to respective CR stress group*
*b =P < 0.01 compared to respective CR stress group*
*c=P < 0.001 compared to respective CR stress group*

**Table 8. Effect of 50% Ethanolic Leaves Extract of Moringa *oleifera* Extract on Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), and Catalase (CAT) in Aspirin-Induced Gastric Ulcers**

| Treatment | Dose (mg/kg) | UV Absorbance at 532 nm for LPO | UV Absorbance at 560 nm for SOD | UV Absorbance at 240 nm for CAT |
|---|---|---|---|---|
| Control | - | 0.42 ± 0.04 | 107.3 ± 14.3 | 32.2 ± 2.7 |
| Aspirin | - | 0.59 ± 0.06[y] | 228.6 ± 19.4[x] | 18.4 ± 1.2[y] |
| Moringa *oleifera* extract | 250 | 0.46 ± 0.03 | 168.2 ± 13.1[a] | 24.2 ± 1.9[a] |
| Moringa *oleifera* extract | 500 | 0. 40 ± 0.02[a] | 121.4 ± 11.5[c] | 29.4 ± 1.1[b] |
| Ranitidine | 50 | 0.36 ± 0.02[b] | 113.2 ± 10.7[c] | 33.2 ± 0.9[b] |

*Values are mean ± SEM for 6 rats*
*x =P < 0.001 compared to respective control group*
*y =P < 0.01 compared to respective control group*
*a =P < 0.05, compared to respective Aspirin-induced group*
*b =P < 0.01 compared to respective Aspirin-induced group*
*c =P < 0.001 compared to respective Aspirin-induced group*

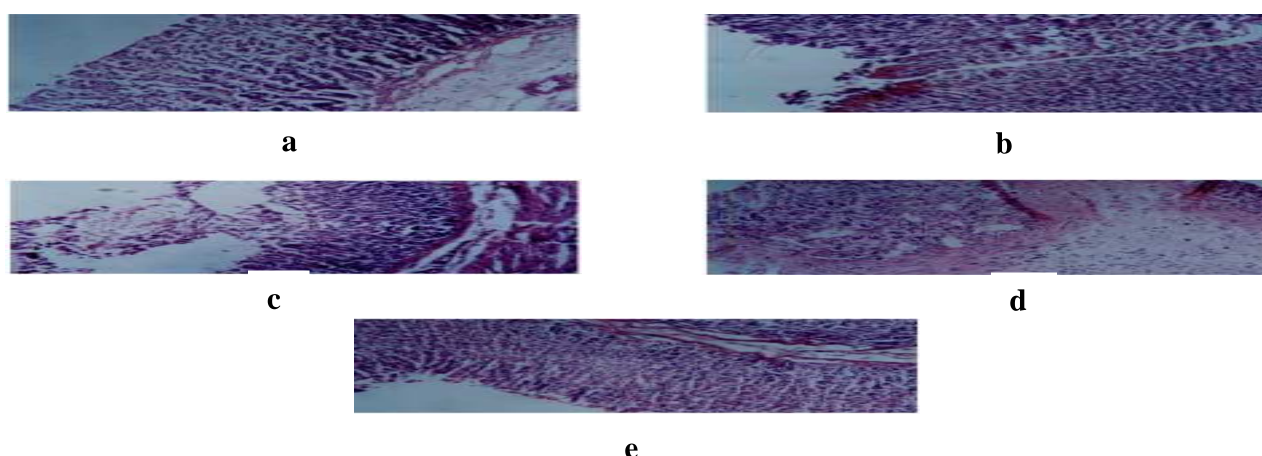


**Figure 2. Histopathological Sections of Stomach Mucosal**

*a. Normal – Normal mucosal and muscularis mucosal.*
*b. Induced – Shows hemorrhage and discontinuity in the lining epithelium hyperplastic mucosal glands.*
*c. 50% ethanolic leaves extract of Moringa oleifera 250 mg/kg- Shows discontinuity in the lining epithelium with exudates in the lumen sub-mucosal edema.*
*d. 50% ethanolic leaves extract of Moringa oleifera 500 mg/kg – No ulcer formation, small atrophic glands, thick muscularis, and edematous sub-mucosa with inflammatory infiltrate*
*e. Ranitidine– No ulcer formation, mild hyperplastic mucosa without any*

dimethylsulfoxide, an $OH^-$ scavenger, reduces cold restraint stress induced damage in the stomach (Cochran et al, 1983).

### *3.8. Histopathological Sections of Stomach Mucosa*

The section of stomach of normal, control animals show normal mucosal and muscularis mucosal. Ulcer induced animal shows hemorrhage and discontinuity in the lining epithelium hyperplastic mucosal glands. The treated animal with 50% Ethanolic Moringa *oleifera* leaves extract on dose of 250 mg/kg shows discontinuity in the lining epithelium with exudates in the lumen sub-mucosal edema. On the other hand the treated animal with 50% Ethanolic Leaves extract of Moringa *oleifera* on a dose of 500 mg/kg, shows no ulcer formation, small atrophic glands, thick muscularis, and edematous sub-mucosa with inflammatory infiltrate. Ranitidine, however, has great effect with no ulcer formation, mild hyperplastic mucosa without any edema formation as shown in Figure 2.

On the basis of the present study, we concluded that 50% ethanolic leaves extract of Moringa *oleifera* Lam. were found no toxic effect and 50% ethanolic leaves extract of *Moringa oleifera* Lam. was found to be passed ulcer protective effect against pylorus-ligation, ethanol, cold restraint stress, aspirin-induced gastric ulcer in rats. The 50% ethanolic leaves extract of Moringa *oleifera* Lam. was found to decreased ulcer and decreased acid pepsin secretion.

Along with above, in our present study, the antioxidant property of 50% ethanolic leaves extract of Moringa *oleifera* Lam. was also found and change in SOD, CAT, and LPO levels in rat gastric mucosa was observed. During the ulcer condition there were an increase in gastric mucosal SOD and LPO levels. This indicated that the generation of relative oxygen species during stress might be the causative factor for the inactivation of gastric peroxidase. 50% ethanolic leaves extract of Moringa *oleifera* Lam. showed antioxidant defense mechanism probably by metabolising lipid peroxides and scavenging endogenous $H_2O_2$.

The present investigation showed that the 50% ethanolic leaves extract of *Moringa oleifera* Lam. showed potent antioxidant activity as indicated the SOD, CAT were significantly increased and LPO levels reverted back in rat gastric mucosa.

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