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# Evaluation of the Effect of Moringa oleifera on Diclofenac Sodium Induced Ulcer in Male Wistar Rats

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**Abstract**: Aims: In the present study, an ethanolic leaves extract of Moringa Oleifera was examined for its antiulcer potential in experimental albino rats using two models: ethanol induced and pylorus ligation-induced gastric ulceration. The extract was orally administered at three different doses (100, 150, and 250mg/kg) for 15 consecutive days. The anti-ulcer effect in rats treated with different doses of Moringa oleifera leaves extract and omeprazole (30 mg/kg, p.o.). The study was carried out between November 2020 to February 2021 in the Pharmacology Laboratory of Department of Pharmacology. Bharat Technology, West Bengal, India, Methodology: The ethanol leaf extract of Moringa Oleifera was prepared. The doses of the extract administered to the rats were 100,150, 250 mg/kg body weight respective, Ulcer was induced with diclofenac sodium (100mg/kg; b.w.). omeprazole (30 mg/kg, p.o.) were determined and statistically compared with the anti-ulcer effects in the control rats treated with saline (NaCl, 0.9%). The MO at doses of 100, 150 and 250 mg/kg decreased the ulcer index significantly as compared to the control group ($p<0.01$). The MO significantly reduced the free acidity, total acidity, and ulcer index ($p<0.01$) and increased the pH of gastric content compared with the control group. This study suggeststhat MO possesses valuable antiulcer, anti-secretory, and cytoprotective activity. Thus, anethanolic leaves extract of Moringa oleifera can be used as source for an antiulcer drug.

**Keywords**: Moringa Oleifera leaves, Gastric ulcer, Diclofenac sodium induced ulcer.

## I. INTRODUCTION

Peptic ulcer disease is the most common GI tract disorder which are usually acid related and thus, extremely painful. They can take many forms and can appear both on the inside and the outside of your body.The pathophysiology of peptic ulcer disease involves an imbalance between offensive factors (pepsin, acid and H. pylori) and defensive factors (bicarbonate, prostaglandin, mucin, nitric oxide and growth factors)

Moringa Oleifera also known as the drumstick tree and horseradish tree is one of the most important topical plants which belongs to the family of Moringaceae. Various parts of this plant (roots, leaves, barks, seeds kernel) are used in all traditional medicine throughout the world. Phytochemical research reported that different part of Moringa Olifera has showed the presence of phenolic constituents, flavonoids, polyphenols, phytosterols, triterpenes. Traditionally it is used as analgesic, anti-inflammatory and antioxidant immunostimulant spasmolytic, anti-diarrhoea, anti-diabetic, anti-helminthic, antiamebic, antiallergic dyslipidermic and antibacterial application.

There are Several anti ulcer drugs which Reduction of gastric acid secretion like Cimetidine, ranitidine Misoprostol, Amoxicillin, clarithromycin, metronidazole enprostil etc. The Miracle Tree, as almost every part of it is useful for humans) 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 (Lugmanet al. 2012). Moringa oleifera plants are used as a food source in humans. This plant contains vitamin A, vitamin C, calcium, potassium, iron, and protein .

The current study seeks to establish the scientific validity of the anti-ulcer properties of Moringa oleifera leaves extracts in diclofenac sodium induced ulcer in albino rats.

**Figure 1: Moringa oleifera**

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## II. MATERIALS AND METHODS

### A. Plant material collection

The leaves of M. oleifera collected from Uluberia, Howrah, West Bengal, India within the month of November, 2021. After collection of plant, it was identified and authenticated by Dr. Sanjit Das, assistant professor, department of pharmacognosy, Bharat Technology, Uluberia, Howrah, West Bengal, India. The plant materials were separated from unwanted materials. Then it was cleaned, washed by distill water and shaded dried at room temperature and crushed powdered form.

### B. Preparation of extract

Collected the leaves and washed with distill water, remove moisture and shade dried ,crush, removed moisture by hot air oven in the temperature $400^{O}$ C. Produce coarce powder and added to petroleum ether for 24 hrs. mixture free from petroleum ether was extracted by Soxhlet apparatus using ethanol and incubated for 48 hours. Then it was filtered, removed solvent form extract by simple distillation and evaporated to dryness. The extracts were collected carefully and packed in eppendorf, stored in refrigerator until use. Now these extracts were diagnosed to preclinical screening.

### C. Experimental animal and mode of administration

Adult male and non-pregnant female albino rats, (average weighing 160 to 200 gm respectively) were used in this study. The animals were obtained from the department of pharmacology, Bharat Technology at Uluberia, Howrah, West Bengal, India. The rats were kept in solid covered polypropylene cages and kept under standard envionmental conditions. The rats were fed with standard diet and water ad libitum. The experiments were designed and conducted in accordance with the ethical norms approved by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The doses of 100, 150, 250 mg/kg, respectively. All treatments were given orally. Male albino rats were deprived of food for 24 hours before pyloric ligation, but had free access to water. Under light ether anesthesia, the abdomen was disection by using a small incision. The stomachs were removed, and the contents were drained into tubes and centrifuged at 1000 rpm for 10 minutes. Then it was observed to analysis for pH, free acidity, gastric volume, and total acidity. The stomach was examined for ulceration and the ulcer index (UI) was calculated.

### D. Dose selection

All the animal fed by oral administration with the help of feeding tube. The extracts are dissolved in water and administered orally and the non-steroidal anti-inflammatory drugs diclofenac sodium dissolved in water used as the ulcerogenic agent and the standard antiulcer drugs pantoprazole dissolved in water used as the gastro protective agent. The albino rats were divided into 6 groups each group, containing 6 rats. Before the experiment, all the animals were fasted for 18 hours, though water was given ad libitum till 2 hours before the experiment. The group of animals were treated as follows:

Group I rats were treated as the normal control

Group II (control) rats were administrated with diclofenac sodium (100mg/kg of body weight)

Group III (standard) rats were administrated with omeprazole (30 mg/kg of body weight) for 15 days

Group IV,V,VI (test) rats were treated with ethanolic extract of M. oleifera (100, 150 and 250 mg/kg of body weight) for 15 days

30 minutes later groups III-VI were administrated with diclofenac sodium 100 mg/kg of body weight. After the experimental period animals were sacrificed by cervical deception. The stomach where is dissected and examined for ulcers.

### E. Calculation of ulcer score

The stomach was dissected along the greater curvature and then washed slowly under running tap water. It was put on a glass slide and observed under 10X magnification for ulcers. Mean ulcer score in each group was calculated and was design as ulcer index and they are calculated by following formula:

Calculation of ulcer index, $UI = UN+US+UP \times 10^{-1}$

Where,

UI = ulcer index

UN = average of number of ulcers per animal

US = average of serverity score

UP = percentage of animal sufferd ulcer

Percentage to protection was calculated by following formula:

%Protection = (C-T/C) X 100

Where C= ulcer index in control group; T= ulcer index in treated group.

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**Table -1: Ulcer scoring [34, 35]**

| Ulcer | Scoring System Criteria |
|---|---|
| 0.0 | Normal |
| 0.5 | Punctuate or pinpoint haemorrhagic ulcer |
| 1.0 | Two or more small haemorrhagic ulcer less than 3mm |
| 2.0 | Ulcer greater than 3mm in diameter |
| 3.0 | Several ulcers |

### F. Statistical analysis of data

The data obtained were analysed using the Graphpad Prism, version 9.1.1. All the values were presented in the table and were expressed as mean ± standard error mean (SEM) of six animals. The significant difference between the mean ulcer index of treated group and that of the control group was tested with one-way analysis of variance (ANOVA) followed by Dunnett's post-test and p values <0.05 considered significant.

## III.RESULTS AND DISCUSSION

### A. Phytochemical screening

The leaves extract of Moringa oleifera contained dark green residue. The yield of ethanolic leaf extract of M. oleifera was found to be 22.4%. The result of preliminary phytochemical analysis of the ethanol leaves extract of Moringa oleifera showed the presence of tannins, glycoside, alkaloids, steroids, flavonoids, carbohydrate, terpenoids.

### B. Pharmacological study

Table -2 shows the dose dependent effect of the leaf extract of Moringa oleifera. The antiulcer effect of extract of Moringa oleifera leaves was found to have increased with increasing dose. The highest effect was observed at 250 mg/kg body weight. Administration of diclofenac sodium shows ulceraion. However, ethanol leaf extract of Moringa oleifera decreases the severity and incidence of gastric erosions in diclofenac sodium treated rats. The ulcer index of group I animal which served as normal (water) was 0.0059± 0.00018. The ulcer index for group II which served as disease control (diclofenac sodium) was 12.59±0.17. Omeprazole, the reference standard group III had ulcer index was 1.37±0.13. The ulcer index for group IV (100 mg/kg), group V (150mg/kg) and group VI (250 mg/kg) which served as test (leaves extract) was 3.84±0.015, 3.76±0.012, 2.73±0.012 respectively.

**Table -2: Ulcer parameters of diclofenac sodium-induced ulcerated rats treated with M. oleifera ethanol extract.**

| Group | Treatment | No. of animal | Dose | Ulcer Index | %Protection |
|---|---|---|---|---|---|
| I | Normal control with water | 6 | ---------- | 0.0059 ± 0.00018 | ---------- |
| II | Disease control with diclofenc sodium | 6 | 10 mg/kg | 12.59 ± 0.17 | ---------- |
| III | Standard with omeprazole | 6 | 30 mg/kg | 1.37 ± 0.13 | 89.1% |
| IV | Test with ethanol leaves extract | 6 | 100 mg/kg | 3.84 ± 0.015 | 69.5% |
| V | Test with ethanol leaves extract | 6 | 150 mg/kg | 3.76 ± 0.012 | 70.1% |
| VI | Test with ethanol leaves extract | 6 | 250mg/kg | 2.73 ± 0.012 | 78.3% |

Values are expressed as Mean± SEM; (n=5); Significance relative to control: ***p< 0.001

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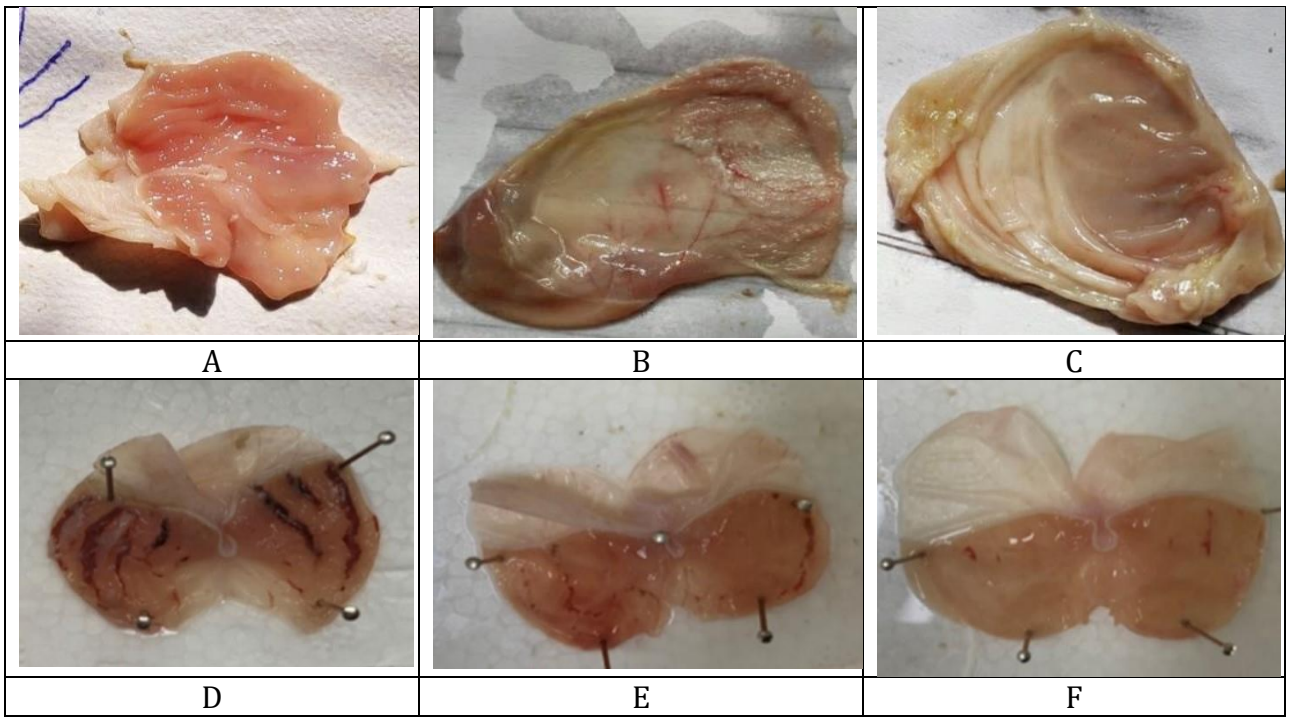


**Figure 2: Effect of gastric ulcer in rats: (A) Normal control with water; (B) Disease control with diclofenc sodium; (C) Standard with omeprazole; (D) Test with 100 mg/kg ethanol leaves extract; (E) Test with 150 mg/kg ethanol leaves extract; (F) Test with 250 mg/kg ethanol leaves extract**

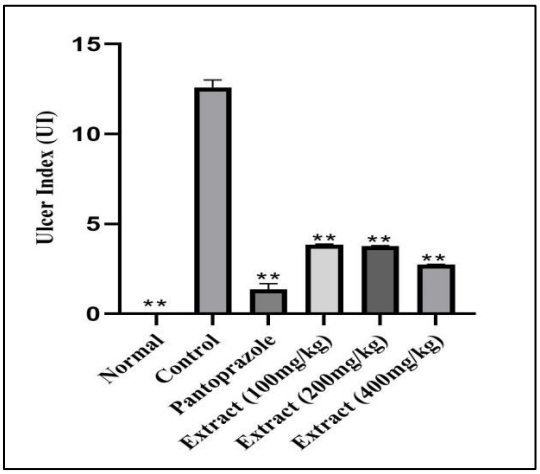


**Figure 3: Dose dependent studies of the extract of Moringa oleifera leaves using rats in diclofenac sodium-induced ulcer model**

## IV. DISCUSSION

Peptic ulcer is associated with multi pathogenic factor and could be due to imbalance between the aggressive factor and defensive factor.

The results of current investigation showed the ethanol leaves extract of Moringa oleifera significantly inhibited the development of diclofenac sodium induced gastric ulcer in a dose-dependent manner.

Diclofenac sodium cause ulceration and bleeding in the upper gastrointestinal tract which first documented by endoscopic study of Douthwaite and Lintott in[36]. In this study, the significant increase in ulcer index following oral administration of diclofenac sodium in the rats maybe inhibit the prostaglandin synthesis through the cyclocoxygenases pathway [37]. Decreased prostaglandin level cause increased gastric acid secretion which is important etiology of mucosal ulceration.

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Our result comply with previous reports, where omeprazole was found to enhance healing of ulcer due to it potent anti-secretory effect. It is a proton pump inhibitor which increases the level of prostaglandin synthesis in the gastric mucosa and it is considered to be chief mediators in gastric cytoprotection. Our results agree with reports of Kawano et al.[38], Okabe et al[39]. and Ruwart et al[40].

In preliminary phytochemical screening, the plant extract has been shown the presence of flavonoid, alkaloid, tannins, glycoside, carbohydrate may accounts antioxidant and anti-ulcer potential. Flavonoids have been reported to offer some anti-ulcer effect by increasing capillary resistance. Flavonoids improve micro circulation which renders the cell less injurious to precipitating factors [41,42].

## V. CONCLUSION

The present study indicated that the leaves of Moringa oleifera has dose-dependent ulcer reducing properties in ulcer induced by diclofenac sodium (non steroidal anti inflammatory drugs - NSAIDs).

## VI. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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