

**PROTECTIVE EFFECT OF RAPHANUS SATIVUS USING BY
PHYTOCHEMICAL AND ANTIULCER ACTIVITY BY ASPIRIN INDUCED
GASTRIC ULCER**

ABSTRACT

Raphanus sativus, a widely growing, has been used in traditional medicine for treating many ailments. The objective of the present study was to evaluate the effects of Aspirinic extract of *R.Sativus* leaves on gastric ulcer. The antiulcer effects of MERS at 100 and 200 mg/kg doses were evaluated on Aspirin gastric ulcer models. The histological changes in gastric tissue of ulcer mice were also determined in aspirin induced models. *R.sativus* treatment significantly ($P < 0.01$) reduced the ulcer index and significantly ($P < 0.01$) increased the gastric pH of both Aspirin and aspirin-induced ulcer mice. The observations confirm that MERS whole plant has antiulcer activities.

Key words: *Raphanus sativus*, Ulcer, gastric ulcer, aspirin-induced ulcer

1. INTRODUCTION

1.1. General Introduction:

Life, illness, and plants all play a part in human birth. Early humans started learning about illnesses and breastfeeding. For the past 10 years, "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines (World Health Organization, 2000)" have been widely used, popular, and distributed despite their centuries-old existence (Rang. H.P et al 2003). Interest and support were not needed for research or education. Even when the patient's digestive system is functioning well, capsules are a great approach to absorb minerals-rich herbs (Field. M et al 2003).

India is home to several significant health systems, including Ayurveda, Siddha, and Unani, as well as a wealth of ancient knowledge. According to Anastasi, J.K. et al. (1997), some of the medicinal plants in India—roughly 7,500 of the 17,000 higher plants—are more numerous than those in other nations or regions. Since the term "standard" is not legally defined, its usage on product labels enhances the quality of the product (Saltz, L.B et al. 2000). *Phyllanthus amarus* and *Phyllanthus niruri* are the most often utilized. (IV) In the past, glycyrrhizin formulations were used to treat mixed liver disorders and stomach ulcers (Fathering, M. et al. 2009).

Radishes are grown and consumed throughout most of the world, being mostly raw as a crunchy salad vegetable with a pungent, slightly spicy flavor, varying in intensity depending on its growing environment. There are numerous varieties varying in size, flavor, color, and length of time they take to mature. Radishes owe their sharp flavor to the various chemical compounds produced by the plants, including glucosinolate, myrosinase, and isothiocyanate. Varieties of radish are now broadly distributed around the world, but almost no archeological records are available to help determine their early history and domestication. However, scientists have tentatively located the origin of *Raphanus sativus* in Southeast Asia, as this is the only region where truly wild forms have been discovered. India, central China, and Central Asia appear to have been secondary centers where differing forms were developed

1.2.Ulcer:

An ulcer is a rupture or discontinuity in a body membrane that prevents an organ from performing its regular activities. The stomach mucosa is continually exposed to potentially harmful substances such as acid, pepsin, bile acids, dietary components, bacterial products (*Helicobacter pylori*), and medications, which results in ulcers. These substances have been linked to the development of gastric ulcers, resulting in decreased stomach blood flow and motility, increased release of gastric acid and pepsin, and inhibition of prostaglandin synthesis and cell proliferative expansion (Singh, S. et al 1999).

With an emphasis on evidence-based practices, herbal treatments are being rigorously assessed for their possible advantages in treating common ailments. The efficacy of the herbal medicine in treating or preventing ulcers is being assessed.

Some risk factors, including blood type, diet, and spice consumption, were thought to be ulcerogens (helping cause ulcers) until the late 20th century, but they have been shown to be of relatively minor importance in the development of peptic ulcers, despite the fact that some studies have found correlations between smoking and ulcer formation (Watkinson, G. et al. 1988). These variables may include lifestyle choices (drugs, alcohol, stress, and cigarette smoking), infections (*H. pylori*), and natural causes (gastric cancer) (Kurata et al., 1997). Therefore, the main strategies for treating peptic ulcer disease have been to decrease the production of stomach acid and strengthen the protection of the gastric mucosa (Berardi, R.R. et al 2005).

The efficacy of the herbal medicine in treating or preventing ulcers is being assessed. In order to offer improved treatment alternatives, the focus is on addressing a variety of clinical disorders, most likely ulcers.

According to Cho, C.H. et al. (2002), the recurrence rate for ulcers treated with H₂ blockers and antacids is around 50% after six months and up to 95% after two years. Drugs used to treat peptic ulcers aim to either stimulate the mucosal defenses (mucus bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide), or counteract aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF," leukotrienes, endothelins, bile, or exogenous factors like NSAIDs) (Watkinson, G. et al., 1988). It is necessary to look for fresh antiulcer drugs from plant sources from the alternative medicine Ayurveda because of the aforementioned demerits that has been documented with the present antiulcer therapy (Kurata, M.P.H et al 1997). There are still effective ways to avoid a number of illnesses using traditional medicine (Sonnenberg et

al. 1981). Because of their perceived safety, fewer adverse effects, and natural nature, herbal products increase patient compliance and enhance overall treatment.

2. Aim and Objectives

- Gathering the plant material, drying it in the shade, and grinding it into a coarse powder
- The maceration technique is used to extract and the extract is separated using the distillation and evaporation processes.
- Assessment of phytochemistry using a range of chemical assays.
- To Identify the Antiulcer efficacy of plant extract.

3. MATERIALS AND METHODS

3.1. **Materials Required:** The following lists the materials needed for the planned study:

Glassware: Desiccator, test tubes, glass wine syringe, Petri dish, Soxhlet equipment, distillation set, watch glass, measuring Cylinder, MAspirin, petroleum ether, water, and ranitidine drug and the solvents.

3.2. **Animals:** Adult Albino mice of both sexes (18–25 g, and 6–8 weeks of age) were obtained from the animal house of Virchow Biotech Private Limited, Hyderabad. The mice were randomly kept in groups of 4 and 5 (n=5) in clean cages with a wire mesh top containing a hygienic bed of sawdust (regularly changed every 3 days) and retained in a well-ventilated room (25±1°C with 55±5% humidity) for excision and incision wound models, respectively.

They were kept in an animal home with a 12-hour light/12-hour dark cycle, at room temperature 23±1°C, and with a relative humidity of 55±5%. Throughout the trial, mice were given a commercial pellet meal and unlimited access to water. At least one hour before to the experiment's commencement, the animals were brought into the lab. Every animal procedure was carried out with the Institution of Animal Ethics Committee's (IAEC) approval and in compliance with guidelines for the responsible handling and care of lab animals (OECD-2001, Ghai. C.L.).

3.3. **Methodology:** Pharmacological investigation and phytochemical screening: The pharmacological investigation for evaluating The following models can be used to carry out diuretic action. 1. Acute toxicity 2. Antiulcer activity.

Based on factors such lab infrastructure and availability, animal facilities, time constraints, accuracy of results, results acquired quickly, and cost considerations, we have chosen just one model for the diuretic activity of plant extract.

3.4. **Phytochemical screening:**

Leaves of *Raphanus sativus* were collected from the College of Swami Vivekananda institute of pharmaceutical Sciences, Hyderabad. The plant material has been identified and verified by a taxonomist By JNTU University of Hyderabad

Raphanus sativus:

Family : Brassicaceae Species : *Raphanus sativus* L

Common Name : Garden radish

Status : Not Native

Classification Order : Brassicales

Genus : *Raphanus*

Species : *Raphanus sativus* L. - GARDEN RADISH

Being easy to grow and quick to harvest, radishes are often planted by novice gardeners. Another use of radish is as a cover or catch crop in winter, or as a forage crop. Some radishes are grown for their seeds; others, such as daikon, may be grown for oil production. Others are used for sprouting.

3.5. Extraction Process of *Raphanus sativus*

A Soxhlet device was used to extract 200 grams of powdered *Raphanus sativus* using 2-2.5 liters of methanol at 40 degrees Celsius with continuous hot percolation. The percentage yield figure was calculated after the extract underwent distillation and was kept on desiccators. Using several organic solvents, such as mAspirin, petroleum ether, and water, the same mark was maintained based on their polarity. The extracts' percentage yield was calculated.

3.6. Acute toxicity study

Using Swiss mice weighing 15-20 g were used in the study after acclimatization for a week under laboratory conditions at room temperature with standard rat chow and tap water *ad libitum* in polyacrylic cages. The animals were fasted overnight, to receive a single dose (2000 mg/kg BW) of *Raphanus sativus* extract paste next day and observation were carried out as per Organisation for Economic Co-operation and Development (OECD) guideline 423-2002. The animals were observed for first 24 hours (h) with special attention during the first 4 h and intermittent observation for next 14 days. On the 3rd day, the experiment was repeated in two more animals and observations were carried out as described earlier.

3.7. Induction of gastric ulcer

Mice were housed in metabolic cages with raised floors of wide mesh to prevent coprophagy, which could affect the induction of gastric ulcers. Animals were fasted for

24 h to empty the stomach of food and increase the level of gastric acid, thereby facilitating gastric injury under aspirin administration. One hour before the experiments, the water was also stopped. Gastric mucosal injury induced by a single oral dose of acetyl salicylic acid (500 mg/kg body weight)

3.8. Antiulcer Activity: Ulcer induced by absolute Aspirin

Four sets of six mice each were created from the animals. Absolute Aspirin (1 ml/animal) was given to Group I (toxicant control); ranitidine (100 mg/kg) was given to Group II; and MERS (100 and 200 mg/kg, respectively) was given to Groups III and IV^{58,59}. The mice were given 1 ml of 100% Aspirin orally after a 24-hour fast. Thirty minutes before to the Aspirin dosage, both the standard and test medications were taken orally. Following one hour of ulcerogen treatment, the animals were killed, their stomachs removed, and the contents aspirated. After centrifuging the contents for ten minutes at 1000 rpm, the pH was measured using a digital pH meter. For the analysis, the stomachs were preserved in 10% formalin after being cleaned with regular saline of ulcer index and histological studies (G. Ateufack et al 2015, L. D. Prazeres et al 2019).

3.9. Ulcer index is determined as follows:

Ulcer index = $10/x$, where “x” is total mucosal area/total ulcerated area.

3.10. Histological study

The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thickness were obtained using a microtome. The deparaffinized sections were stained with hematoxylin and eosin. Mucosal congestion, edema, desquamation and necrosis were observed.

Statistical Analysis

The toxicant group and the treatment groups were contrasted; the mean \pm SD of six animals per group was used to represent the results. Dunnett's test and one-way analysis of variance (ANOVA) were used for statistical analysis of the data. $P < 0.05$ was deemed significant when compared to the toxicant group.

4. RESULTS:

4.1. Phytochemical and Antiulcer:

4.2. The presence of alkaloids, phenols, proteins, carbohydrates, phytosterols, phenolic compounds, tannins, and flavonoids was revealed by the Methanolic Extract of Raphanus Sativus's phytochemical screening.

4.3. Impact of the Crude Extract on Ulcers Caused by Acidified Aspirin

With an ulcer index of 17.88 ± 0.23 , the inducer significantly increases the production of ulcers in groups who receive the vehicle (DW). However, groups of mice administered the extract at dosages of 100 and 200 mg/kg significantly reduced the ulcer index in comparison to the negative control. The ulcer index decreased to 12.96 ± 0.56 and 10.32 ± 0.07 for both dosages, respectively. But in mouse groups given 100 mg/kg of the test extract, the ulcer index decreased just little and not statistically.

The test extract's action was shown to be dose-dependent in this model. Using the percentage of ulcer inhibition, the cytoprotective effect was assessed and found to be 5.6%, 27.47%, and 42.24% for test doses of 100 and 200 mg/kg, respectively. In contrast, the percentage of ulcer inhibition in mouse groups administered the reference drug was 44.76%, which is equivalent to the test extract at the highest dose (42.24%). The results showed that although the action at 100 mg/kg was minimal, the crude extract had a significant cytoprotective impact at both dosages (100 and 200 mg/kg) (Table:1).

Table 1. The effect of MERS extract on ulcer number, ulcer score and ulcer index in acidified Aspirin induced ulcer model in mice.

Group	Mean ulcer number UN	Mean ulcer score US	Mean ulcer index UI	% UI
NC	29.81±1.23	49.00±1.15	17.87±0.22	-
Sucralfate	1.31±0.21	0.83±0.18	9.87±0.04	44.76%
MERS 100mg/kg	26.81± 1.75	42.11±3.79	12.96±0.56	27.47%
MERS 200mg/kg	1.51±0.21	1.67±0.68	10.32±0.07	42.24%

Values are expressed as mean \pm SEM (n=6) and analysed using one way ANOVA

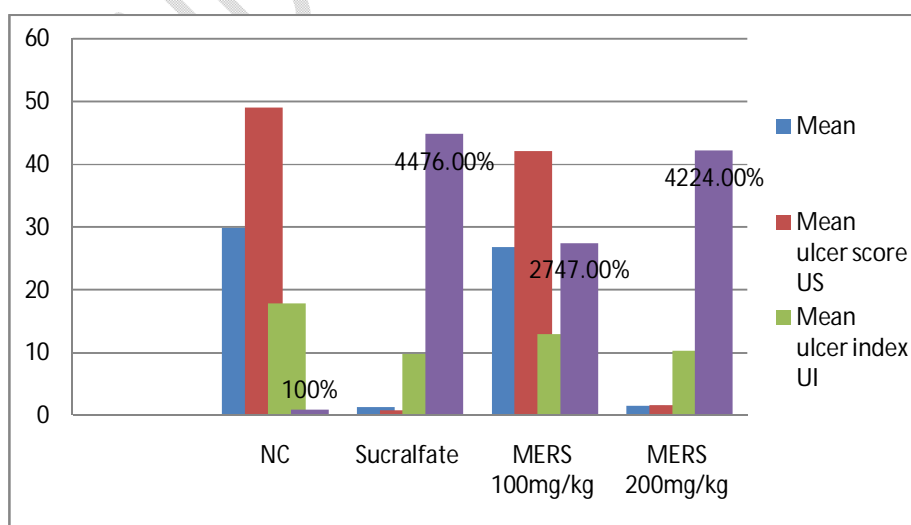


Figure 1. The effect of MERS extract on ulcer number, ulcer score and ulcer index in acidified Aspirin induced ulcer model in mice.

4.4. Histological study

Histopathology results figure 2 revealed mucosal congestion, edema, necrosis and desquamation in (aspirin and vehicle treated control group).

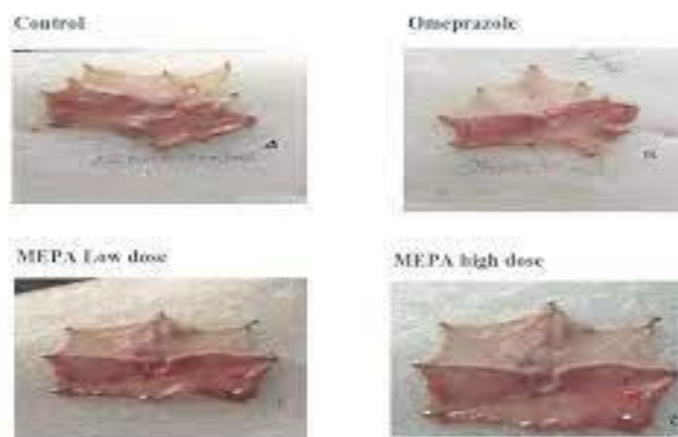


Figure 2: Effect of MEPA on Aspirin induced ulceration showing the ulcer index in different treated groups A) Control, B) Omeprazole, C) MERS low dose, D) MERS high dose

5. DISCUSSION:

During the rigorous 14-day follow-up period, the crude extract did not exhibit any discernible signs of delayed toxicity, according to the acute toxicity research. Furthermore, no mortality was seen at the limited test dose, which is five times the test extract's maximum dosage (200 mg/kg). Future study on the extract may be warranted if its fatal dosage (LD50) is more than three times the minimal effective dose. The plant leaf extract is therefore harmless, and its LD50 is probably far from the limit test dosage, as evidenced by the absence of mortality at 2 g/kg.

Raphanus sativus leaf extract may thus have cytoprotective effects via inhibiting leukotriene activity or the 5lipoxygenase pathway. The plant extract's capacity to cure ulcers was also investigated using the acetic acid-induced chronic ulcer technique. Similar to persistent ulcers in humans, the model results in a severe, hard-to-heal stomach wall ulcer. By blocking the gastrointestinal mucosa and creating an excessive buildup of acidic gastric juice, the inducer has been shown to produce stomach ulcers. At both test levels

(100 and 200 mg/kg), the plant extract dramatically reduced the ulcer index in this mouse after 14 days of therapy (Devaraj VC et al 2011).

Therefore, both antisecretory and cytoprotective qualities may be involved in the *Raphanus sativus* leaf extract's ability to relieve ulcers in people. Flavonoids, which have been demonstrated to have an antioxidant impact, may be responsible for the antiulcer action. Flavonoids are also linked to increased prostaglandin release, which in turn triggers important mucosal-protective mechanisms. Furthermore, saponins' ability to rebuild blood vessels through enhanced VEGF expression may be linked to their antiulcerogenic effect. Therefore, the antioxidant and angiogenic qualities of the plant extract may help heal ulcers (Y. Tsukimi et al 1996).

6. CONCLUSIONS

Excellent antiulcer activity was revealed by the investigation for both the solvent fractions and the *Raphanus Sativus* extract. The presence of a variety of bioactive compounds in the plant may have contributed to the found antiulcer potential. Although the exact mechanism of action of extracts is uncertain, the necessary ulcer-healing effect may in fact be caused by antisecretory and cytoprotective actions. **With an emphasis on evidence-based practices, herbal treatments are being rigorously assessed for their possible advantages in treating common ailments. The efficacy of the herbal medicine in treating or preventing ulcers is being assessed. In order to offer improved treatment alternatives, the focus is on addressing a variety of clinical disorders, most likely ulcers.**

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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