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# Anti-ulcer Activity of Polyherbal Formulation from Indian Medicinal Plants Cumin Cyminum, Trigonella Foenum Graecum, Piper Nigrum, Curcuma Longa

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## ABSTRACT

**AIM:**The objective of this study was to assess the Anti-Ulcer Activity of the Polyherbal Formulation made from the seeds of Cumin Cyminum, Trigonella Foenum Graecum, Piper Nigrum L. and rhizomes of Curcuma Longa in Wistar albino rats.

**METHODS:**Male Albino Rat of Wistar strain were used to evaluate the Polyherbal Formulation's anti-ulcer properties utilising an ethanol-induced gastric ulcer model.The effectiveness of PHF was assessed by determining, gastric pH and gastric mucus, GSH, malondialdehyde (MDA), and nitric oxide (NO) levels in stomach tissues.

**RESULTS:** The administration of the 400 mg/kg dosage of polyherbal formulation significantly exhibits the gastroprotective effects, when compared to the ethanol group.

**CONCLUSIONS:**PHF, containing seeds extracts of Cumin Cyminum, Trigonella Foenum Graecum, Piper Nigrum L. and rhizomes of Curcuma Longa was found to possess antiulcer properties in ethanol induced experimental animal models of gastric ulcers, and these findings suggest that the significant gastroprotective activity could be mediated by its antioxidant activity.

**KEYWORDS:**Ulcer, Polyherbal Formualation, Ethanol Induced

## 1.INTRODUCTION

Open sores on the stomach's muscularis mucosa or beyond, known as gastric ulcers, are a serious health risk. Gastric ulcers are a global health problem and poor healing is one the leading causes of recurrence. Although the pathophysiology of gastric ulcer is multifactorial, it is generally believed to result from an imbalance between protective and aggressive factors of the gastric mucosa. Protective mechanisms of the gastrointestinal tract include gastric mucosal integrity, mucus secretion, bicarbonate production, nitric oxide, gastroprotective prostaglandin synthesis, normal gastric motility and adequate tissue microcirculation, adverse factors include gastric acid and pepsin secretion, bile salts, reactive oxygen species (ROS), Helicobacter pylori infection, alcohol consumption, and long-term use of non-steroidal anti-inflammatory drugs (NSAIDs**) [1].** Phytomedicinal compounds have traditionally been used by herbalists and indigenous healers for the prevention and treatment of ulcers. Botanical compounds with

antiulcer activity include flavonoids, saponins, tannins, gums andmucilage**.[2]**Different medication therapies are available for treating the ulcer like-Antacids, Histamine (H2) Blockers, PPIs, Antibiotics.**[3]**

The natural drugs were found to be the safer alternatives to cure ulcers. In this study the antiulcer activity of a polyherbal formulation (PHF) containing the extracts of Cumin Cyminum, Trigonella Foenum Graecum, Piper Nigrum L. and Curcuma Longa was evaluated in ethanol-induced gastric ulcers in rats.

## 2. MATERIAL AND METHODS

### 2.1 MATERIAL

Fresh seeds of plant Cumin cyminum, Piper nigrum, Trigonella foenum graecum and the rhizomes of Curcuma longa were collected from the local market of Meerut. The seeds were packed and maintained in herbarium file and transferred to the botanical department of the CCS University for the authentication. The taxonomical identification of the plant was done by **Dr.Vijai Malik.** The solvents used for extraction process and chemicals used for phytochemical analysis were of analytical grade.

### 2.2. EXPERIMENTAL ANIMALS

30 Male Wistar albino rats weighing between 150 and 200 g were used collected from **Central AnimalFacility AIIMS Delhi**. The Institutional Animal Ethics Committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control, and Supervision on Experiments on Animals. Animals (albino rats) were kept in the departmental animal house at 23± 2°C and relative humidity 50 – 55%, and 12 hrs light and dark cycles for 1 week, animals were fed standard diet.

### 2.3.Induction of Acute Gastric injury by Ethanol

Gastric ulcer was induced by administration of 80% ethanol (5 ml/kg,), for 7 days, the animals were anesthetized with Diethyl ether.The stomachs were dissected and opened along greater curvature for evaluating the number and the length of gastric lesions.

### 2.4. Experimental Design

The animals (albino rats) were randomly divided into the 5 groups, 6 Animals in eachgroup:

- Group Ⅰ - Distilled water
- Group Ⅱ - Distilled water + Ethanol
- Group Ⅲ - Ethanol + PHF 100 mg/kg
- Group Ⅳ - Ethanol + PHF 200 mg/kg
- Group Ⅴ - Ethanol + PHF 400 mg/kg

The treatment groups were orally received PHF at doses of 100, 200, 400 mg/kg.

### 2.5. Determination of Malondialdehyde (MDA) in Gastric Mucosa

The level of lipid peroxidation was determined as malondialdehyde according to the commercial instructions of the colorimetrical assay kit by measurement of Thiobarbituric acid (TBA)-reactive the substance at 535 nm.

### 2.6. Determination of Nitric Oxide (NO) in Gastric Mucosa

The content of nitric oxide in gastric mucosa was measured according to the commercial instructions of the colorimetrical assay kit. The nitric oxide level was assessed quantitatively based on its metabolites and Griess reaction.

### 2.7. Histopathological Examination

For histopathology assessment, stomach tissues were fixed in 10% neutral formalin solution and were embedded in paraffin. Sections were deparaffinized and stained with hematoxylin and eosin (H&E).

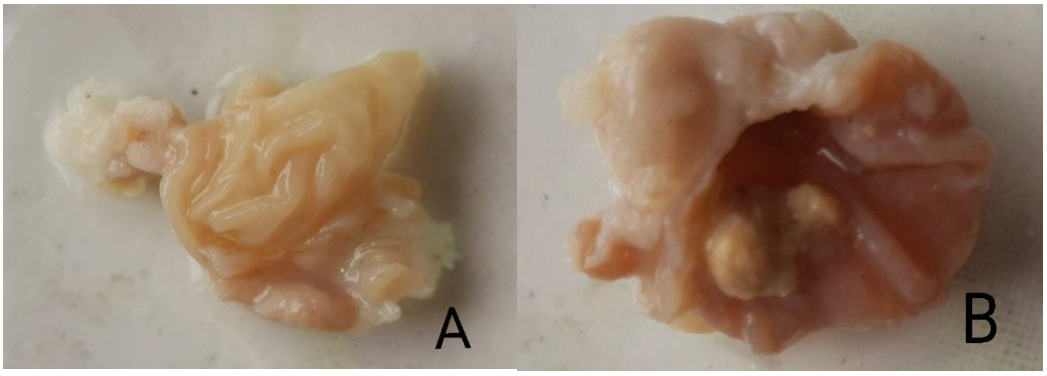
### 2.8. Statistical Analysis

All the data are expressed Mean ± SEM, one way analysis of variance (ANOVA) was used for the statistical analysis of data, followed by Turkey's post hoc. The level of significance was taken $P<0.05$.

## 3. Results

### 3.1. Effect of Polyherbal Formulation on Ethanol-Induced Gastric Lesions

The ethanol (80%) administration induced extensive long and hemorrhagic gastric ulcers in the rat. Oral pretreatment with PHF attenuated the number and the length of gastric lesions in a dose-depended manner. Among the tested doses, high doses of PHF (400 mg/kg) showed maximum inhibition on the number and length of gastric lesions. PHF (100, 200 mg/kg) had similar effects on the number and length of gastric ulcers. There was no significant difference between the gastroprotective activity of dose 100 mg/kg and dose 200 mg/kg PHF. In addition, high doses of PHF (400 mg/kg) alone had no side effects on stomach tissue.



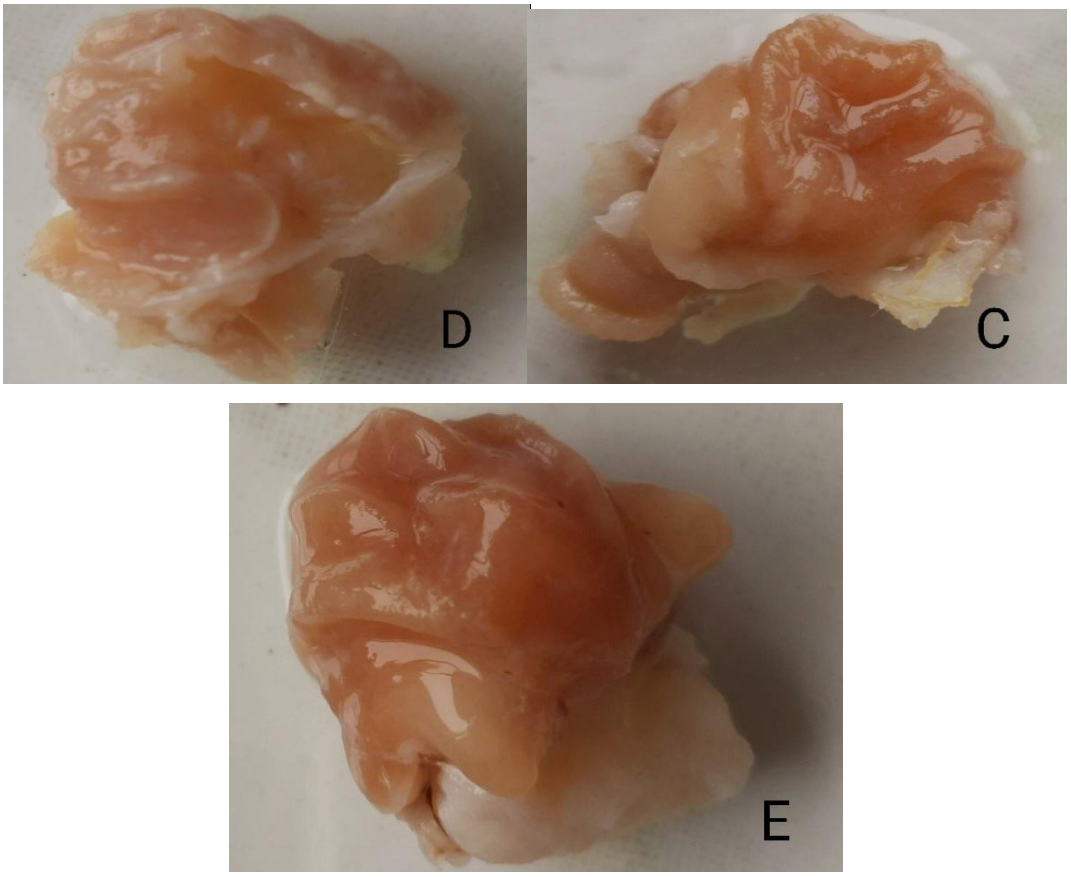



**Figure.1** The gross appearance of ulcers induced by ethanol (5ml/kg)and protective effects of Polyherbal formulation (100, 200, 400 mg/kg).

(A) Normal saline, the normal control group shows normal gastric mucosal tissues structure. (B) Normal saline + Ethanol, ethanol administered rats showed severe hemorrhagic gastric mucosal lesions.

(C) Ethanol + PHF 100mg/kg, shows the hemorrhagic lesions on the gastric mucosa.

(D) Ethanol + PHF 200mg/kg, shows mild hemorrhagic lesions on the gastric mucosa compared with normal control group.

(E) Ethanol + 400mg/kg, shows no hemorrhagic lesions, with slight inflammation of gastric mucosa compared with normal control group.

### 3.2 Effect ofPHF on Lipid Peroxidation

Lipid peroxidation level (MDA), ethanol increased gastric MDA level in disease control group compared with the normal control group, as shown in (Fig.2). Administration of PHF at 400 mg/kg dose significantly reduces MDA level in gastric tissue.

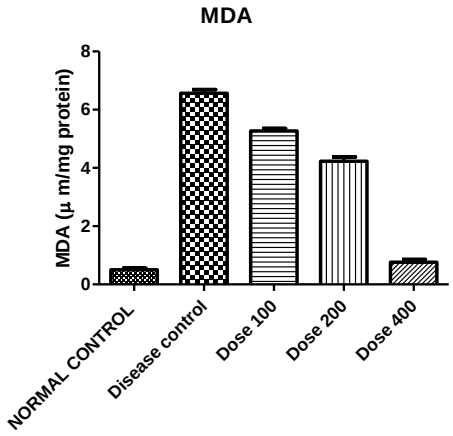



**Figure.2 MDA level in gastric tissue**

| GROUPS | MDA LEVEL |
|---|---|
| **Normal Control Group** | 0.5000 ± 0.05774 |
| **Disease Control Group** | 6.567 ± 0.1202 |
| **PHF Dose 100 mg/kg** | 5.267 ± 0.08819 |
| **PHF Dose 200 mg/kg** | 4.233 ± 0.1453 |
| **PHF Dose 400 mg/kg** | 0.7667 ± 0.08819 |

**Table. 1 MDA level in gastric tissue in different groups. Data expressed in Mean ± SEM.**

### 3.3 Effect of PHF on the levels of Nitric Oxide (NO) in gastric tissues.

The results (Fig.3) showed that administration of ethanol significantly breaks gastric oxide levels in rats, and administration of PHF at doses of 400 mg/kg or 200 mg/kg restored the nitric oxide levels.

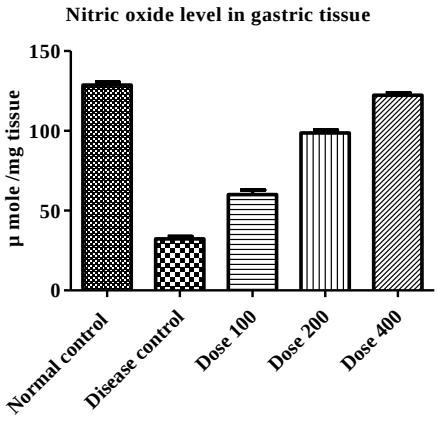



**Figure. 3 Nitric oxide level in gastric tissue.**

| GROUPS | NO2 LEVEL IN GASTRIC TISSUE |
|---|---|
| **Normal Control Group** | 128.7 ± 1.856 |
| **Disease Control Group** | 32.33 ± 1.453*** |
| **PHF Dose 100 mg/kg** | 60.00 ± 2.887*** |
| **PHF Dose 200 mg/kg** | 98.67 ± 1.856*** |
| **PHF Dose 400 mg/kg** | 122.3 ± 1.453 |

**Table. 2 Level of nitric oxide in different groups. Data expressed in Mean ± SEM.**

### 3.4 Effect of PHF on Gastric pH level

In comparison to the healthy control group, a substantial decrease in the pH of the gastric juice was seen in the disease control group. however, the treatment with doses 400 mg/kg or 200 mg/kg of the formulation dramatically raises the pH of gastric juice in rats. As shown in (Fig.4).

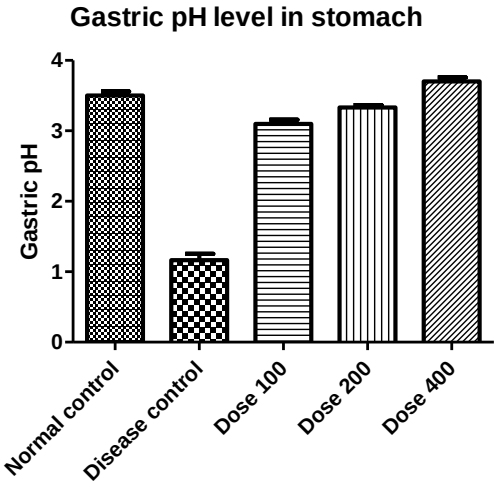



**Figure. 4 Gastric pH level**

| GROUPS | GASTRIC pH LEVEL |
|---|---|
| **Normal Control Group** | 3.500 ± 0.05773 |
| **Disease Control Group** | 1.167 ± 0.08819 |
| **PHF Dose 100 mg/kg** | 3.100 ± 0.05774 |
| **PHF Dose 200 mg/kg** | 3.333 ± 0.03333 |
| **PHF Dose 400 mg/kg** | 3.700 ± 0.05774 |

**Table.3 Gastric pH level in different groups. Data expressed in Mean ± SEM.**

### 3.5 Effect of PHF on Antioxidant Enzyme (GSH)

The disease control group had considerably lower levels of the defence antioxidant enzyme GSH. In contrast to the ethanol group, the decreased levels were restored by treatment with doses of 400 mg/kg or 200 mg/kg of formulation. Shown. in (Fig.5).

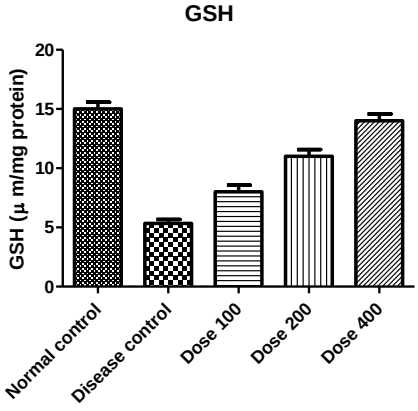



**Figure. 5 GSH level in gastric tissue**

| GROUPS | GSH |
|---|---|
| **Normal Control Group** | 15.00 ± 0.5774 |
| **Disease Control Group** | 5.333 ± 0.3333 |
| **PHF Dose 100 mg/kg** | 8.000 ± 0.5774 |
| **PHF Dose 200 mg/kg** | 11.00 ± 0.5774 |
| **PHF Dose 400 mg/kg** | 14.00 ± 0.5774 |

**Table.4 GSH level in gastric tissue in different groups. Data expressed in Mean ± SEM.**

### 3.6 Effect of PHF on Gastric Mucous

When compared to the normal control group, the disease control group had much less stomach mucus. In contrast to the ethanol-induced ulcer in rats, the treatment with 400 mg/kg and 200 mg/kg dosage enhanced this parameter. Shown in (Fig.6).

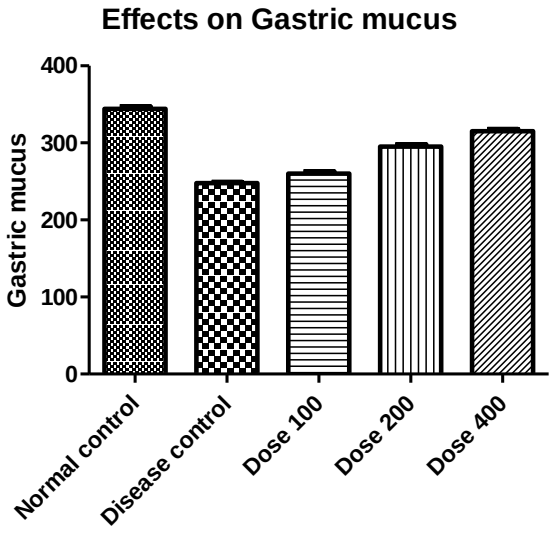



**Figure. 6 Gastric mucous level**

| GROUPS | GASTRIC MUCOUS |
|---|---|
| **Normal Control Group** | 344.0 ± 3.055 |
| **Disease Control Group** | 247.7 ± 1.453 |
| **PHF Dose 100 mg/kg** | 260.0 ± 2.887 |
| **PHF Dose 200 mg/kg** | 295.0 ± 2.887 |
| **PHF Dose 400 mg/kg** | 315.0 ± 2.887 |

**Table. 5 Gastric mucous level in different groups. Data expressed in Mean ± SEM.**

### 3.7. Effect of PHF treatment on histopathological examination of the Gastric Mucosa

The size and frequency of ulcers in the disease control group were larger than those in other groups, according to a histopathological analysis of gastric tissue sections, and epithelial and glandular cells had significant necrosis. The normal control gastric tissue slides displayed the stomach mucosal wall's normal structure and without any evidence of degradation. Ulcers were observed in the treatment groups;however, they were reduced at the polyherbal formulation dosages of 200 mg/kg and 400 mg/kg.

the reduction in cell infiltration as a result of the curative effect of dose 400 mg/kg was consider greater in comparison to 200 mg/kg and 100 mg/kg. Shown in (Fig.7).

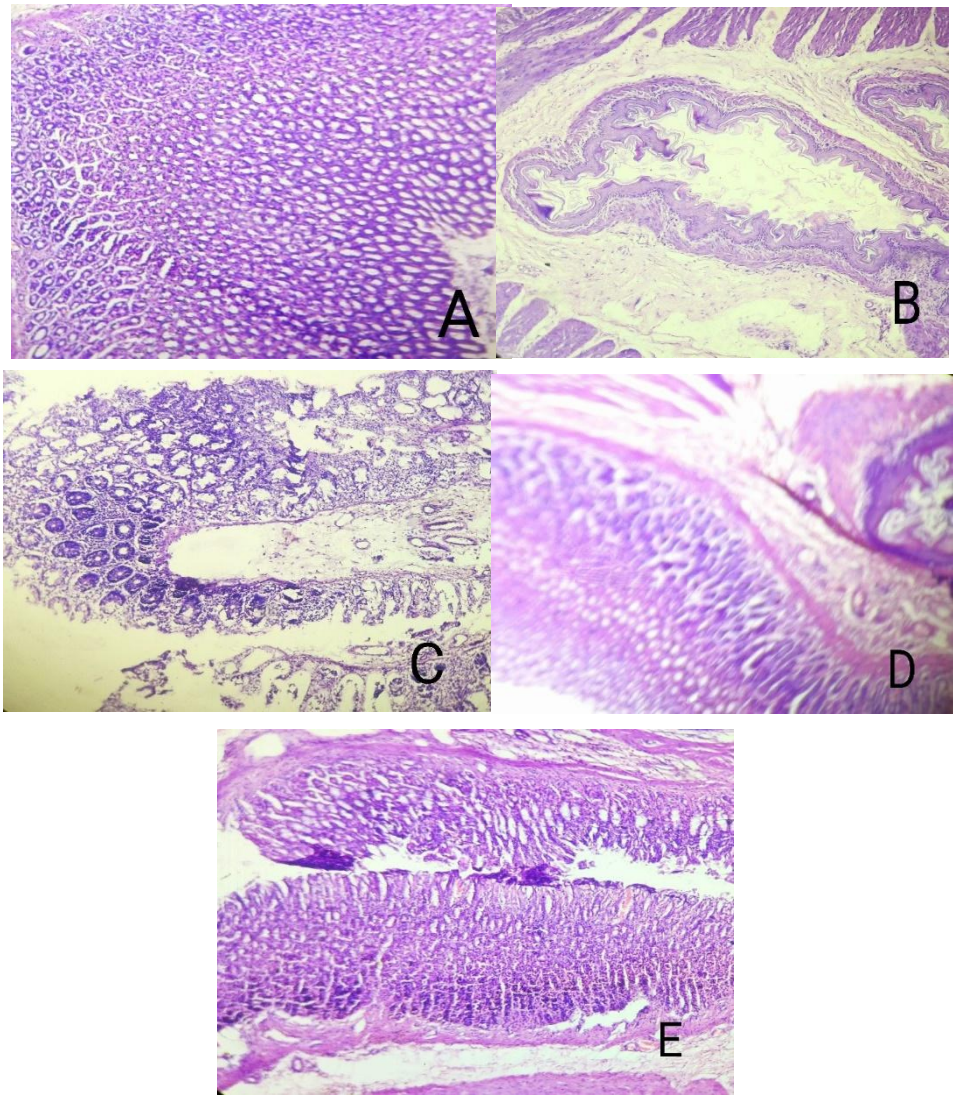



**Figure.7**Protective effects of Polyherbal Formulation (100, 200, 400 mg/kg) on histopathological changes of gastric tissue induced by ethanol. (A) Normal Control Group (B) Disease Control Group (C) Ethanol + PHF 100 mg/kg (D) Ethanol + PHF 200 mg/kg (E) Ethanol + PHF 400 mg/kg.

## 4. DISCUSSION

Ulcers are the one of the most common digestive diseases in present time. Ulcers are chronic, and even a single lesion of ulcer in any part of digestive tract may cause severe health issues. There are several numbers of natural sources that play an important role in preventing the gastric ulcer. The phenolic compounds exhibit many health promoting properties. Polyphenols shows a number of pharmacological properties in GIT area, they act as antioxidant, antisecretory and cytoprotective agent. Polyphenols are reported for their anti-ulcerogenic activity with good level of gastric protection. **[4]**This study shows the effects of Polyherbal Formulation on the Ethanol Induced Gastric Ulcers in Rats. In this study the polyherbal formulation consists of Cumin Cyminum, Trigonella Foenum Graecum, Piper Nigrum,

Curcuma Longa, these shows the antioxidant,anticarcinogenic, anti-inflammatory and many more pharmacological activities.

Ethanol-Induced gastric ulcer is a common animal model for investigating the new anti-ulcer drugs. The results of the present study exhibit that polyherbal formulation possesses the significant anti-ulcer activity in Ethanol-Induced gastric ulcer in wistar rats. Ethanol administration cause the gastric necrotic damage and subsequent inflammatory cell infiltration and reduces the secretion of bicarbonates, gastric mucus, and nitric oxide. It also reduces the gastric blood flow and induces the oxidative stress by increasing the production of MDA and reducing GSH production. **[5]**

The present study shows the polyherbal formulation at100, 200, 400 mg/kg dosage reduces the histopathological changes and the number and size of gastric ulcers induced by ethanol in rats. The study was done on 30 male wistar rats, divided into 5 groups, each group consists of 6 animals. In this study we found the anti-ulcer activity of our formulation by determining the parameters like: level of MDA and NO in gastric tissue, gastric pH level, level of GSH, effect on gastric mucous. And our findings suggest that, the polyherbal formulation possesses the anti-ulcer activity by reducing the **MDA** level (0.7667 ± 0.08819) and increasing the **NO** level (122.3 ± 1.453) in stomach at the dose of 400 mg/kg as shown in **Table No.1 and Table No.**2 respectively. On the administration of ethanol there is a significant reduction in the **pH** of gastric juice in disease control group (1.167 ± 0.08819) while the administration of PHF at the doses 400 mg/kg (3.700 ± 0.05774) and 200 mg/kg (3.333 ± 0.03333) enhances the pH of gastric juice shown in **Table No.3.** The antioxidant enzyme **GSH,** were significantly depleted on the administration of ethanol (5.333 ± 0.3333) while the treatment with PHF at the dose of 400 mg/kg replenished the depleted level of GSH (14.00 ± 0.5774), shown in **Table No.4.**

The polyherbal formulation also increases the level of **Gastric mucous** at the dose of 400mg/kg **(**315.0 ± 2.887) shown in **Table No.5,** when compared with disease control group.

Several reports suggest that nitric oxide plays a protective role in gastric ulcer, and treatment with NO donors can accelerate the healing of gastric ulcer **[6].**The fenugreek seeds contain flavonoids it is reported that fenugreek seeds exert their anti-ulcer activity through the flavonoid. Fenugreek seeds also prevented the rise in lipid peroxidation.**[7]** Curcumin has many pharmacological activities such as anti-inflammatory, antioxidant, anti-carcinogenic effects**[8]**Piper nigrum also exhibits anti-ulcer activity. This may be due to its antioxidant mechanism.**[9]**Flavonoids are said to provide protection against the development of ulcers by increasing capillary resistance. Flavonoids improve microcirculation, making cells less harmful to precipitating factors **[10].**

## 5. CONCLUSION

Our results indicates that polyherbal extract exerts anti-ulcer activity and may be effective in reducing the incidence of gastric ulcer and its complications. However, the further studies are required to determine the exact mechanism of anti-ulcer activity of this formulation.

## REFERENCES

1. Wendy Itzel Escobedo-Hinojosa, Erika Gomez-Chang, et al, Gastroprotective Mechanism and Ulcer Resolution Effect of CyrtocarpaProceraMethanolicExtracton Ethanol-Induced Gastric Injury, Evidence Based Complementary and Alternative Medicine, Volume 2018, Article ID 2862706, 12 pages

2. V.C Devaraj, B.Gopala Krishna, Antiulcer Activityof Polyherbal Formulation ( PHF ) from Indian Medicinal Plants, Chinese journal of natural medicines, Volume 11, Issue2, March 2013, Pages 145-148, https://doi.org/10.1016/S1875-5364(13)60041-2
3. Peptic ulcer disease treatment, https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/peptic-ulcer-disease-treatment
4. SabihaSumbul, MohdAftab Ahmad, AsifMohd, AkhtarMohd, Role of Phenolic Compounds in Peptic Ulcer: An Overview, J Pharm BioalliedSci, July 2011
5. Shohda A El-Maraghy, Sherine M Rizk, Nancy N Shahin. Gastroprotective Effect of Crocin in Ethanol-Induced Gastric Injury in Rats. Chem-Biol, Interact. 2015, 229,26-35
6. Neda Sistani Karampur, ArdeshirArzi, AnahitaRezaie, Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats. Medicina, March 2019
7. R. SujaPandian, C.V.Anuradha ,P.Viswanathan, Gastroprotective Effect of Fenugreek Seeds on Experimental Gastric Ulcer in Rats.Journal of Ethanopharmacology, April 2022
8. Dania Akbik, MalihehGhadiri, RaminRohanizadeh.Curcumin as Wound Healing Agent, Life Sciences, 2014.
9. 9.Ramnik Singh, JyotsanaMadan, Antiulcer Activity of Black Pepper against Absolute Ethanol Induced Gastric Mucosal Damage in Mice. Pharmacognosy magazine, July 2008
10. Paul A. Nwafor, F.K.Okwuasaba, L.G.Binda, Antidiarrhoel and Antiucerogenic effects of methanolic extract of AsparagusPubescens root in rats. Journal of Ethanopharmacology,2000