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Evaluation of Anti-Ulcer Activity of Polyherbal Extracts in Wistar Rats

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***Abstract*: A peptic ulcer is a sore on the lining of the stomach or duodenum. The two common types are gastric ulcers and duodenal ulcers. Medicinal plants have wider range of therapeutic advantages in the proper management of disease, as they have better pharmacological activity along with low incidence of side effects or the adverse effects. *Murraya koenigii* or curry leaf is used in various disorders like Anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic and hepatoprotective activities. *Trigonella foenum* is used in anti-hyper lipidemic, anti-diabetic, chronic heart diseases, reduce blemishes, for long and lustrous hair, anaemia. The present study investigates complete blood profile and anti-ulcer activity of chloroform extracts of *Murraya koenigii* and *Trigonella foenum* at different doses were compared with omeprazole (20mg/kg) treated animals.**

***Biochemical parameter:* Ulcer index, total acidity, volume of gastric contents, acidity and the macroscopic examinination of stomach of both treated and control animals were compared.**

***Keywords*: *Murraya koenigii*, *Trigonella foenum*, Peptic ulcer, ulcer index.**

1. Introduction

An ulcer is disease of skin both which is lining the human body externally and its internal viscera which can be an open sore in the lining of epithelial cells or deep lesion in the specific region which would lead to bleeding and turn out as bleeding sore or bleeding ulcer.

A. History [1]

The earlier description of ulcer dates back to an era of 3000 BC and even before that *Hippocrates*(460-360BC) known as "father of medicine", described an ulcer while defining cancer and termed it as *carcino*s and *carcinoma* referring them as non-ulcer forming and ulcer forming tumors. In pre-16th century Hippocrates himself described gastric. The great Arabic physician Avicenna studied the relationship between gastric pain and mealtimes. The first recorded case of

gastric ulcer was described by the Italian physician named Marcello Donati(1538-1602) in1586. In 1875, G. Bottcher and

M Letulle hypothesized that ulcer was caused by a bacteria, however, no one believed them at that time. As it was thought that bacteria couldn't survive the acidic conditions and the use of antibiotics was deemed as quackery. T. Schwann (1810 1882) discovered pepsin in 1834 and it was clear to the scientific fraternity as another known cause of peptic ulcer. During the age 1889 spiral-shaped bacteria had been identified in both mucosa and gastric contents of ulcer patients, this was followed by the observation of Helicobacter pylori by Howard steer in biopsies of patient with the ulcer in the year 1971. Revolution in the medical science came in the year 1982, when Helicobacter pyroli was first identified and cultured separately and was revealed as one of the known cause of peptic ulcer by John Robin Warren and Barry Marshal the two Australian physicians, for which they were also awarded Nobel prize in 2005, which proved Koch's 3rd postulate that the pathogenesis of ulcer and many diseases including cancer lied the presence of bacteria. The scientific investigations were followed by treatment methodologies applied by medical practitioners, the treatment goals and management of diseases have seen drastic changes including proper management and cure of ulcer.

During 1920's to 1960's vagotomy or surgical removal of parts of stomach were thought to be the treatment options to eradicate ulcer from the diseased individual.

During 1980's cimetidine was found to cure gastric ulcer and with more knowledge on the pathogenesis of ulcer i.e. due to acid, cimetidine and ranitidine were introduced to cure ulcer as by then it was clear that acid was the main cause in ulcer. After the revolutionary discovery of pathogen i.e. H.pyroli the treatment goals changed to the use of antibiotics and bismuth followed by use of acid blockers. After the discovery of H.pyroli during the age of 1995, almost 75% patients with ulcer were treated with anti-secretory agents or medications and only 5% received antibiotic therapy.

It took 2 decades to understand that H.pyroli was causative agent for an ulcer, more than half of the population were affected by H.pyroli, only 5-10% develop ulcer. [2]

B. Types of Ulcer [3]:

There are different types of ulcers most common are the peptic ulcer, gastric ulcer, which appeared to be due to damage to the lining of the stomach, and duodenal ulcer, which was associated with excessive acid secretion by the stomach.

2. Plant Profile

A. Curry Leaves [4]

- Botanical name : *Murraya koenigii*
- Kingdom : Plantae
- Order : Sapindales
- Family : Rutaceae
- Genus : *Murraya*
- Species : *koenigii*

Uses: Anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, and hepatoprotective.

Fig. 1. *Murraya koenigii*

B. Methi Leaves [5]

- Botanical name : *Trigonella foenum*
- Kingdom : Plantae
- Order : Fabales
- Family : Fabaceae
- Genus : *Trigonella*
- Species : *foenum*

Uses: Anti-hyperlipidaemic, anti-diabetic, chronic heart diseases, reduce blemishes, for long and lustrous hair, anaemia.

Fig. 2. *Trigonella foenum*

3. Materials and Methods

A. Collection of plant material

The mature leaves of *Murrayya koenigii* and *Trigonella foenum* are collected from local areas and are shade dried for 10 days. Dried leaves are powdered separately by using electric grinder and sieved in order to get the fine powder. The mature leaves of *Murrayya koenigii* and *Trigonella foenum* are collected from local areas and are shade dried for 10 days. Dried leaves are powdered separately by using electric grinder and sieved in order to get the fine powder.

B. Plant material extraction

The crude powders were weighed, mixed and placed in soxhlet apparatus separately by using 70% chloroform. 500 gms of each powder was extracted with 2.5 lit 70% chloroform by continous hot percolation using soxhlet apparatus for 6 hours and the extract was dried at 50ºc. The powdered extract was weighed and stored in tightly closed container until use.

C. Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on the aqueous extract of the leaves of Murraya Koenigii for qualitative identification.

D. Identification of Anti-Ulcer activity

Selection of animals: Adult, healthy, male rats of Wistar strain (Rattus norvegicus) weighing 150-200 g were used in the present study. The animals were housed in polypropylene cages under standard conditions (12 hrs light / dark cycle; 25±3°C temperature). Rats were provided with water and nutritionally adequate pellet diet ad libitum**.** All the animals were fasted prior to all assays were allocated to different experimental groups each of 6 animals. All the experiments were carried out according to the guidelines for care and use of experimental animals

Chemicals: Diclofenac sodium, Omeprazole tablets were purchased from the medical store. All other chemicals and reagents used were of analytical grade.

Acute Toxicity Studies Poly Herbal extract: Acute toxicity studies carried out according to 425 OECD guidelines, wistar albino rats (150-200 g) were divided into five groups. The rats were fasted for 6 h with only access to water ad libitum before experimental study. Group I, II, III and IV animals were administered various doses of poly herbal extract i.e. 250, 500, 1000, 2000 mg/kg. Group V received 0.5% CMC only. All the doses and vehicle were administered by oral route. The animals were observed for 14 days for mortality.

Screening methodology

NSAIDs Induced gastric ulcers in rats: [6] Gastric ulceration is produced in rats by certain drugs. the ability of the test drug to protect against the ulceration is observed.

Principle: Inhibition of endogenous prostaglandin production and consequent loss of gastric mucosal defence.

Induction of ulcer: Diclofenac sodium was suspended in 1% carboxy methyl cellulose and administered orally in a dose of 20mg/kg in overnight fasted rats.

Mechanism: They inhibit the activity of COX, the key enzyme in prostaglandin production. Various studies indicates that NSAIDS helps in the progression of ulceration by overcoming the expression of enzyme cyclo oxygenase (COX) which inhibit the conversion of amino acids to prostaglandins, that impairs the mucosal barrier and results in progression of peptic ulcers.

Procedure:[6] Healthy female Wister albino rats of weighting between 160-200gm were taken for the studies. The animal

were divided in to groups (each contain 6 animal).

- The animal in all the groups were kept for overnight fasting after that animal of all groups' received diclofenac sodium (NSAIDs, 20mg/kg). Group I animals receive CMC and diclofenac, the oral feeding of water and diclofenac sodium was continued for 4 days.
- Group II animals were administered with standard drug Omeprazole (20 mg/kg) respectively after 3h. of diclofenac sodium administration.
- Group III animals were administered with 250mg/kg of polyherbal extract.
- Group IV animals were administered with 500mg/kg of polyherbal extract.
- Group V animals were administered with 0.5 CMC.
- On 15th day the animals were sacrificed, stomach were removed and cut along the greater curvature to measure the ulcer index.

E. Evaluation of ulcer score and ulcer index: [7]

16 Stomachs of animals were cut opened along the curvature, washed with saline and ulcer index was recorded as follows,

Ulcer score

- 0 = Normal coloured stomach
- 0.5 = Red colouration
- 1.0 = Spot ulcer
- 1.5 = Haemorrhagic streaks
- 2.0 = Ulcers ≥ 3 but ≤ 5
- 3.0 = Ulcers >5

Mean ulcer scores for each experimental group were calculated and expressed as ulcer index.

No. lesions × ulcer score = Ulcer index Measurement

Total acidity from Gastric juice: Total acidity was determined by titrating 1mi of gastric juice with 0.1 NaOH using phenolphthalein indicator till a faint pink colour is obtained. Unit of total acidity is the mL of 0.1 N NaOH required for titration of 1 mL of gastric juice and is expressed as milliequivalents per mL of gastric juice. It was calculated by the formula.

Acidity = (volume of NaoH × Normality × 1) / 0.1

Volume of gastric juice: [8] The gastric juice was collected by ligating the pyloric and fundic end of the stomach. 3ml of distilled water was injected then the gastric juice was collected in the test tube and subjected to centrifuge for 500rpm for 5 min. The total acidity of gastric juice and volume was measured using a graduated cylinder. 6 The %decrease in gastric juice volume was calculated by vol. of gastric juice in control- treated group divided by vol.og gastric juice in control × 100.

4. Results

A. Soxhlet extraction of the plants

A=weight of the powder taken; B=Weight of the extract; C= % Yield(w/w)

$$\%yield(C)=(B/A)\times 100$$

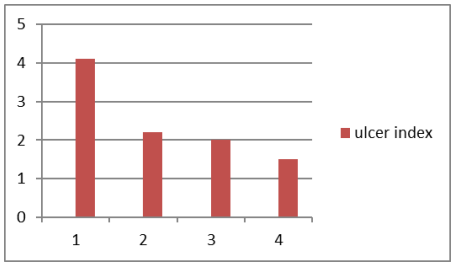
B. Acute toxicity studies

Acute toxicity studies on wistar albino rats show no mortality at a dose of 2000mg/kg for a period of 14 days. These studies help to predict that it does not have any toxicity and it is completely safe. So 250mg/kg b.w (i.e 1/8th) and 500mg/kg b.w(i.e 1/4th) were selected for the screening of anti-ulcer activity.

C. Effect of poly herbal extract on gastric volume in diclofenac induced model

In the present study the poly herbal extract (*Murraya koenigii and Trigonella foenum*) was evaluated for its Anti-ulcer activity against NSAID (Diclofenac) induced ulcers and the results were tabulated.

D. Comparison of the study



x-axis=groups, y-axis=ulcer index
Fig. 3. Comparision of the study

Table 1
Soxhlet extraction of the plants

Plant	Plant part	Method of extraction	Solvent used	A(gm)	B(gm)	C (gm)
Murraya koenigii	Leaf	Continous hot percolation by soxhlet extraction	70% chloroform	500	35	7
Trigonella foenum					29	5.8

Table 2
Effect of poly herbal extract on gastric volume

Group	Wt. of rats	Drugs given	Gastric volume	Free Acidity	Total Acidity	Ulcer index	% Protection
Group I	183±0.6	Diclofenac + CMC	1.9±0.01	13.5±0.02	25±0.02	4.1±0.64	0.0
Group II	189±0.3	Omeprazole + Diclofenac	1.3±0.2	10±0.01	18±0.01	2.2±0.6*	46.3
Group III	208±0.5	poly herbal extract (250mg/kg) + Diclofenac	1.1±0.1	8.6±0.03	15±0.03	2±0.5*	51.2
Group IV	182±0.3	Polyherbal extract(500mg/kg) + Diclofenac	0.95±0.02	7±0.1	12±0.04	1.5±0.05**	63.4

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)
P values: **< 0.001 - Highly Significant, * <0.05 - Significant, N S: Non Significant

E. Macroscopic examination of gastric mucosa in diclofenac induced ulcers

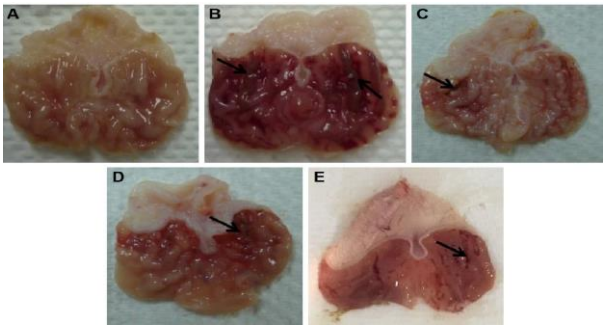


Fig. 4. Macroscopic examination of gastric mucosa

Note: (A) Normal control(CMC); B) Diclofenac induced ulcer control; (C) Standard (omeprazole 20mg/kg) treated group; (D, E) stomach treated with 250 and 500 mg/kg of polyherbal extract.

5. Discussion

The results of this study found that poly herb established a cytoprotective action against diclofenac induced cellular damage in the gastric mucosa of rats. Cytoprotection of anti-ulcer drugs has been recognised due to the generation of prostaglandins. It has also been observed that poly herb significantly and dose dependently reduced the extent of gastric ulceration in diclofenac treated rats without affecting the gastric secretion or pepsin activity. The defence potential of mucus perimeter of gastric mucosa depends upon a delicate balance between the processes affecting the synthesis and secretion of mucin constituents. The modern approach towards a potent antiulcer agent involves a delicate balance of controlling the synthesis, secretion and metabolism of proteins, glycoproteins and lipids, so as to strengthen the mucosal integrity.

Several scientific studies revealed that the phytoconstituents like flavonoids, tannins, terpenoids and saponin were responsible for gastro protective agents. Tannins possess as an antiulcer agent by its astringency property and vasoconstriction effects. Due to precipitation of micro proteins on the ulcer site, a protective layer was formed which hinders gut secretions and protects the mucosa from toxins and other irritants. Previous studies have recommended that these above active compounds had ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Therefore, poly herb possess antiulcer activity, may be due to presence of tanins, flavonoids and terpenoids.

Acute toxicity studies on wistar albino rats show no mortality at a dose of 2000mg/kg for a period of 14 days. Hence 250mg/kg bw and 500mg/kg bw were used in the present study.

The blood sample obtained from all the 5 groups through retro orbital route is analysed for various parameters on the intial day i.e. day 0 and the last day i.e. 15th day and they showed an increased levels of RBC, WBC, PLT and Hb.

A. Diclofenac induced ulcers

1) Effect of Gastric Volume

Administration of the extract and poly herb significantly decreased the gastric volume in comparison with rats treated with omeprazole. Comparing the gastric volume and gastric acidity, the gastric volume gets decreased, simultaneously the gastric acidity also decreased significantly.

2) Effect of Free Acidity and Total Acidity

The free acidity and total acidity were determined based on the titre values. The free acidity and total acidity of poly herb on albino rats decreased significantly in comparison with the standard group treated with omeprazole.

3) Ulcer index

The ulcer index was calculated by taking the mean ulcer score of each groups. Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-axis. The histograms of different groups were then interpolated by comparing the ulcer index of group I with group II, III and IV. It was noticed that the ulcer index of Dose group (Dose-IV 500mg/kg bw) was significantly less when compared to the standard group (Group-II) treated with omeprazole.

6. Conclusion

The study indicates that chloroform leaf extracts of *Murraya koenigii* and *Trigonella foenum* are effective in the treatment and prevention of diclofenac induced ulcers. The results also show that the polyherbal extract at the dose of about 500 mg/kg b.w was found to be moderately effective than 250 mg/kg b.w and standard diclofenac 20mg/kg b.w doses. Thus the presence of flavonoids, tannins, terpenoids and saponin in the herbal extracts might be responsible for the anti-ulcer activity.

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