

Screening the Anti-ulcer Activity of Polyherbal Extract of Selected Medicinal Herbs Against Albino Wistar Rats

V. Swathi¹, Pusapati Madan Ranjit*², M.Ramesh¹ and Y.A. Chowdary²

¹Division of pharmacology, Department of pharmacy and biotechnology, JNTUCEV, Vijayanagaram, A.P, India

²NRI College of pharmacy, Pothavarappadu (V), Agiripalli (M), Krishna Dist. A.P., India

Address for Correspondence

Assistant professor,
NRI College of
pharmacy
Pothavarppadu,
Agiripalli Mandal
Krishna Dist. A.P, India

E-mail: madanranjit@gmail.com

ABSTRACT

The main objective of this research was to elevate the better combination of known herbal drugs (*Curcuma longa*, *Coriander sativum* and *Occimum sativum*) for better anti-ulcer activity. Herbal extracts prepared from shade dried rhizomes, leaves using alcohol and water as solvents by soxhlet extraction, further all the extracts were subjected to preliminary phytochemical analysis. It was found out that, the extracts consists of alkaloids, glycosides, carbohydrates, steroids, proteins, amino acids, tannins, fixed oils and volatile oils. The anti-ulcer activity of polyherbral extracts was estimated in Albino rats weighing 150-200gms using pylorus ligation method. Biochemical parameters like ulcer index, total and free acidity, acid volume and pH were estimated in alcoholic and aqueous extracts. The alcoholic extract at a concentration of 400mg/kg produced more effect on ulcer induced models when compared with standard drug cimetidine (10mg/kg) with respect to other concentrations alcoholic extract significantly raised pH of gastric content, lowered the free and total acidity and ulcer index compared with control group. The results our conclusion was, the alcoholic extract exhibited more significant activity than aqueous activity.

Keywords: *Occimum sativum*; *Curcuma longa*; Amino acids; *Coriander sativum*.

INTRODUCTION

An ulcer is defined as “disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation”. Peptic ulcer is a serious gastro-intestinal

disorder¹. The formation of peptic ulcer depends on the presence of acid and peptic activity in gastric juice plus break down in mucosal defenses. Peptic ulcer and related acid peptic diseases affect up to 10% of the

population with sufficient severity to prompt victims to seek medical attention³. There are two major factors that can disrupt the mucosal resistant to injury: Non steroidal anti-inflammatory drugs (NSAIDS) and helicobacter pylori infections. A number of drugs are used widely for the treatment of ulcer.¹ The drugs show their affect mostly by inhibiting the *Cox* enzyme, protective mechanism, neutralizing mechanism and so on. In spite of its curative affects they show some side effects². Hence there came into practice the use of herbs for the treatment because of their less side effects and more beneficial effects. Hence, we opted to choose herbal drugs. The objective of the present study was to elevate the effectiveness of multi drug extract in preventing the formation of gastric ulcer experimentally by aqueous and alcoholic extracts of drug in pylorus ligation method in rats using Cemetidine as reference drug.

The drugs are *Coriander sativum L* (Family: Umbiliferaceae), *Curcuma longa* (Family: Zingiberacea) and *Ocimum santum* (Family: Lamiaceae). *Coriander sativum* has ulcer healing and anti bacterial activity. *Curcuma longa* has ulcer healing and anti-septic property⁴. *Ocimum santum* has anti-ulcer activity⁵. The choice of selecting these three drugs is that the individual's affects of these drugs are beneficial and their combination may also give beneficial results with fewer side effects. The anti-ulcer activity of this combination of drugs has no proof yet. Hence we opted to work on the combination of these crude drugs.

MATERIALS AND METHODS

Experimental animals

The albino-wistar rats of either sex obtained from Department of pharmacology, NRI College of Pharmacy, weighing 150-200gms were used for the study. Animals were divided into 6 groups each containing 5

animals. They were maintained in the departmental animal house at 22±3°C and relative humidity 44-55% respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Hindustan lever) and water ad libitum. The experimental procedure was approved by the *Institutional animal ethical committee* (CPCSEA approval number: 1032/AC/07/CPCSEA) and proceeded further.

The plants were collected from the paddy field of village Kantepudi, Sattenapalli, based the morphological characters(explained by standard monograph), microscopy and the chemical constituents, the plants were identified as *Ocimum sanctum*, *Curcuma longa* and *Coriandrum sativum*. Healthy adult male Wistar albino rats weighing between 150 and 200 gm were selected for the antiulcer studies.

Preparation of animals

The animals were acclimatized to standard laboratory conditions of temperature (22±3°C) and maintained on 12:12 h light: dark cycle.

Preparation of extracts

The air dried parts of the selected plants were powdered. The dried powdered plant of equal weight (20gms each) were mixed well with the use of a mixer and was refluxed by using ethanol and water (500ml).

Experimental procedure^{6,9}

The animals were placed in cages with grating floor to avoid copography and divided into six groups, each group with five animals. Group I with 0.9%(w/v) saline solution that serves as control group, Group II with Cemetidine (10mg/kg) I.P. serves as standard group, Groups III and IV received aqueous extracts of 200 and 400 mg/kg body weight and Groups V and VI with alcoholic extracts of 200 and 400 mg/kg body weight

which serves as test drugs. The animals were fasted for 48 hrs before the operative procedure was started.

Anaesthetized the fasted rats with anaesthetic ether and secured on the operation table. An incision of 1cm long was given in the abdomen just below the sternum. The stomach was exposed and a thread was passed around the pyloric sphincter and a knot was applied tightly. The abdomen was closed tightly with sutures and the rats were allowed to recover. After pylorus ligation the corresponding drugs to the respective groups were given and the animals were sacrificed after 17-19 hrs and the stomach was dissected out. The contents of the stomach were drained into a graduated centrifuge tube and their acidity was determined by titrating it with 0.1N NaOH. The stomach was opened along its greater curvature and pinned on a cork plate and inner surface was examined for ulceration with a microscope. The ulcer index was calculated by using the formula:

Ulcer Index = (Arithmetic mean of intensity in a group) x (number of ulcer positive animals) / Total number of animals

Procedure for phytochemical screening^{7,8}

The extract thus prepared by the continuous hot percolation was subjected for phytochemical screening by using the standard procedures for identification of various constituents present in it like alkaloids, glycosides, carbohydrates, steroids, proteins, amino acids, tannins, fixed oils, gums and mucilages and volatile oils. Table 1. Showed the preliminary phytochemical constituents of plant all extracts.

RESULTS

Estimation of acid secretory parameters like ulcer index, total and free acidity, pH and gastric volume were placed below in Table 2 and 3 showed the effects of

both alcoholic (Fig. 1) and aqueous (Fig. 2) poly herbal extract in curing peptic ulcer in comparison with standard drug cimetidine. The alcoholic extract showed significant increase in parameters like pH, free acidity, total acidity and gastric volume with reduction in ulcer index when compared with aqueous extracts. The alcoholic extract at dose of 400mg/kg body weight showed comparatively good results than alcoholic extract at dose of 200mg/kg body weight.

DISCUSSION

Estimation of pH of gastric contents

In control animals, without any drug the mean was 1.61 and 1.58. Alcoholic extract showed significant rise in pH 3.95 to 4.05 as compared to control. The rise in pH shown by the aqueous extract was 3.01. Cimetidine, a standard drug raised the pH to 4.13. This was more potent than the extracts used.

Estimation of free acidity of gastric contents in terms of ml of 0.1n HCL/ 100ml of gastric contents

Gastric free acidity was increased to 44.0 & 90.17mEq/litre in control animals. Alcoholic extract 20.0 mEq/litre showed significant decrease in free acidity as compared to control. The decrease in free acidity by aqueous extract was 84.61 mEq/litre respectively. When compared with Cimetidine, a known anti-ulcer drug, Alcoholic extract showed good results 19.66 mEq/litre, where as other extracts were less potent in decreasing gastric acidity.

Estimation of total acidity of gastric contents in terms of ml of 0.1n HCL / 100ml of gastric contents

Gastric total acidity was increased to 67.8 & 114.24 mEq/litre in control animals. Alcoholic extract 30.08 mEq/litre showed significant decrease in total acidity as compared to control. The decrease in total

acidity by aqueous extracts was 100.04 mEq/litre respectively.

Determination of ulcer Index

The ulcer index in control animals was 12.66 & 25.6. Alcoholic extract 4.5 significantly reduced the ulcer index as compared to control. The reduction in ulcer index by aqueous extracts is 21.6 respectively. Cimetidine, a standard anti-ulcer drug showed ulcer index 3.83 & 7.24 respectively.

Determination of gastric volume

The gastric volume has been decreased from 3.28 and 3.78 in both aqueous and alcoholic extract to 2.10 and 2.85 in aqueous and 1.83 and 1.88 in alcoholic extracts. The results are more comparable with the standard drug Cimetidine which results in gastric volume of 2.0 and 2.27 in aqueous and alcoholic extract.

CONCLUSION

In the present study, shade dried leaves and rhizomes of polyherbal extract of *Ocimum sanctum*, *Curcuma longa* and *Coriandrum sativum* having medicinally important bioactive constituents is reviewed, with special emphasis on the biological activities. The dried powder was subjected to phyto-chemical work. The preliminary phytochemical investigation showed presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, fixed oils, tannins, steroids and volatile oils. The two extracts viz. alcoholic and aqueous were employed for pharmacological screening.

From the above extracts alcoholic extract showed better anti-ulcer activity. The results of pharmacological activity indicated that, the alcoholic extract exhibited more significant activity than the aqueous extract. For assessing anti-ulcer activity Pylorus ligation induced ulceration model and acetic acid induced ulceration models were used and

various parameters like pH of gastric content, free acidity, total acidity, ulcer index were determined.

Finally histopathological observation was done on the basis of histopathological abdominal sections of photomicrographs of rat mucosa, stained with haematoxylin-eosin. Alcoholic extract significantly raised the pH of gastric contents. It lowered the free and total acidity and ulcer index as compared to control group. As per the above results, we concluded that, the polyherbal extract shows better activity against ulcer.

REFERENCES

1. Soll AH, 1990 Pathogenesis of peptic ulcer and implications from therapy, *N Engl J Med*,(322): 909-916
2. Chan FKL, Leung WK 2002, Peptic ulcer disease. *Lancet North Am Ed.* (360): 933-941.
3. H.P. Rang, M.M. Dale, J.M. Ritter, R.J. Flower; *Rand and Dales pharmacology* ; 6th edition; page no: 385-396.
4. S. Rafatullah *et al*; Evaluation of turmeric for gastric and duodenal antiulcer activity in rats; 1989.
5. Govind Pandey *et al*; pharmacological activities of *Occimum sanctum* (tulsi): A review; *Indian Journal of Pharmacology*; 2010; volume 5; issue 1.
6. P. Muthumani *et al*; study of phyto chemical, analgesic and anti-ulcer activity of extracts of aerial parts of *Cardiospermum halicacabum linn*; *IJPSR*; 2010; volume 1; Issue 10; page no:128-137.
7. C.K. kokate, A.P. Purohit, S.B. Gokhale pharmacognosy ; 43rd edition; June 2009; page no:6.16-
8. B. Uma *et al*; antimicrobial activity and phytochemical analysis of *Coriander sativum* against infectious diarrhea; *Ethanobotanical leaflets*; 2009; volume 13; page no: 590-594.
9. Rakesh k sindhu *et al*; Investigations into the anti-ulcer activity of rheum *ribes linn* leaves extracts; *IJPS* ISSN-0975-1491; volume 2, suppl 4 2010 page no: 91-92.

Table 1. Showed the preliminary phytochemical constituents of plant all extracts

| S. no | Phytochemical constituent | Alcoholic extract | Aqueous extract |
|-------|---------------------------|-------------------|-----------------|
| 1. | Alkaloids | ++ | ++ |
| 2. | Glycosides | ++ | ++ |
| 3. | Carbohydrates | ++ | ++ |
| 4. | Steroids | ++ | ++ |
| 5. | Proteins | ++ | ++ |
| 6. | Amino acids | ++ | ++ |
| 7. | Tannins | ++ | ++ |
| 8. | Fixed oils | ++ | -- |
| 9. | Gums & Mucilages | -- | -- |
| 10. | Volatile oils | ++ | -- |

Table 2. Showed the effects of alcoholic extract of poly herbal extract in curing peptic ulcer

| S. no | Parameters | Control | Standard | Test 1 | Test 2 |
|-------|----------------|------------|--------------|--------------|--------------|
| 1 | Ulcer index | 12.66±0.75 | 3.83±0.75** | 4.5±0.54** | 3.66±0.51** |
| 2 | Total acidity | 67.8±2.63 | 30.3±4.14** | 30.08±4.51** | 30.8±3.60** |
| 3 | Free acidity | 44.0±3.22 | 19.66±2.06** | 20.0±1.67** | 19.83±1.47** |
| 4 | pH | 1.61±0.17 | 4.13±0.25** | 3.95±0.18** | 4.05±0.25** |
| 5 | Gastric volume | 3.78±0.21 | 2.0±0.52** | 1.83±0.64** | 1.88±0.70** |

Control: Saline solution (0.9%w/v), standard: Cemetidine (10mg/kg), Test 1: 200mg/kg and Test 2: 400 mg/kg. Significance relative to control data: *P< 0.05, **P< 0.01

Table 3. Showed the effects of aqueous extract of poly herbal extract in curing peptic ulcer

| S. no | Parameters | Control | Standard | Test 1 | Test 2 |
|-------|----------------|-------------|-------------|-------------|-------------|
| 1 | Ulcer index | 25.67±0.84 | 7.24±3.38** | 21.06±0.61 | 10.10±0.01* |
| 2 | Total acidity | 114.24±0.22 | 34.65±0.13 | 100.18±0.34 | 30.8±3.60** |
| 3 | Free acidity | 90.17±5.28 | 20.24±6.78 | 84.61±0.31 | 36.71±0.11 |
| 4 | pH | 1.58±0.15 | 4.90±3.12 | 3.01±1.32 | 4.7±0.31 |
| 5 | Gastric volume | 3.28±0.10 | 2.27±0.20 | 2.10±0.27 | 2.85±0.52 |

Control: Saline solution (0.9%w/v), standard: Cemetidine (10mg/kg), Test 1: 200mg/kg and Test 2: 400 mg/kg. Significance relative to control data: *P< 0.05, **P< 0.01

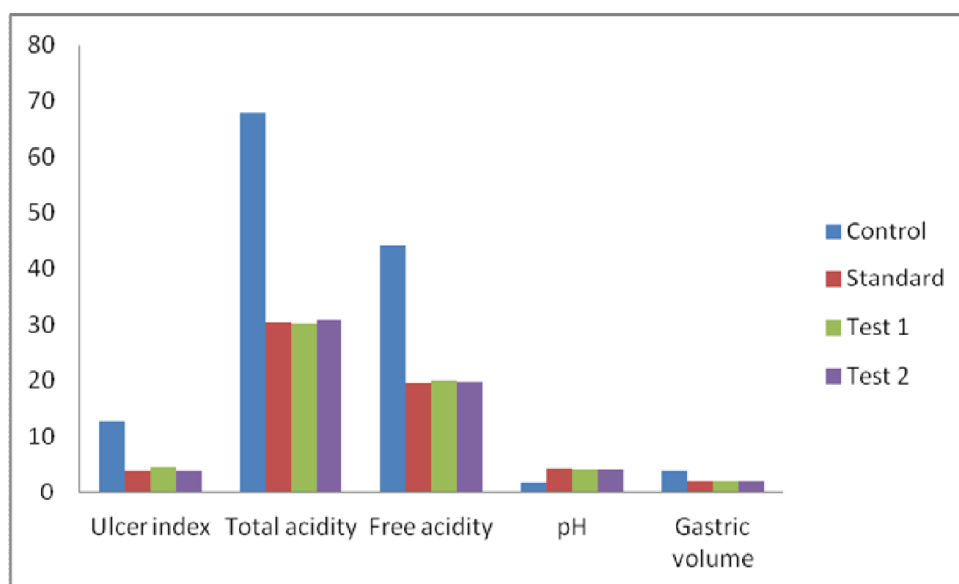


Figure 1. Showed the effects of alcoholic extract of poly herbal extract in curing peptic ulcer

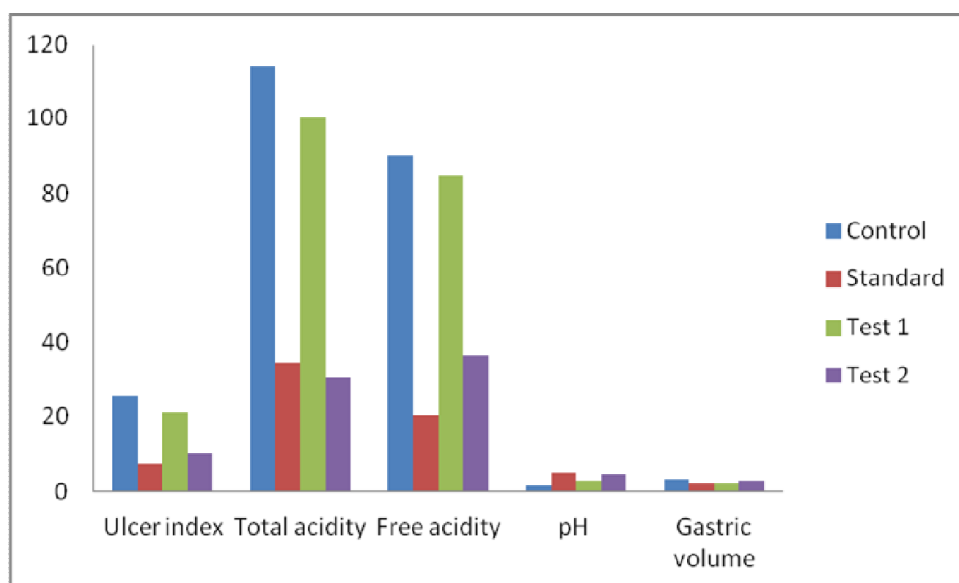


Figure 2. Showed the effects of aqueous extract of poly herbal extract in curing peptic ulcer