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Research Article

Evaluation of Antiulcer and Antioxidant Activity of Polyherbal Formulation in Wistar Rats

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Abstract

The present study was carried out to investigate the antiulcer and antioxidant potential of poly herbal formulation (*Piper betel, Foeniculam vulgare, Acacia catechu, Eugenia caryophyllus*) in experimental rats. The aqueous extract of the formulation was subjected to acute oral toxicity studies according to OECD guidelines no. 423. The two dose levels were then selected i.e. 250mg and 500 mg/kg body weight and were further evaluated for antiulcer activity in aspirin induced gastric ulcer model and ethanol induced gastric ulcer model in wistar rats. The extent of ulcer formation was studied using ulcer index and was compared with the standard drug omeprazole. The aqueous extract of poly herbs showed maximum percentage inhibition ($p<0.01$) in aspirin induced gastric ulcer model i.e., 73% at the dose of 250 mg/kg body weight and the maximum percentage inhibition ($p<0.01$) in ethanol induced gastric ulcer model was 64% at the dose of 250 mg/kg body weight. Antioxidant activity was studied spectrophotometrically by 1, 1-diphenyl 2-picryl hydrazyl, reducing power and hydrogen peroxide free radical scavenging methods in vitro. The aqueous extract showed potent antioxidant activity as compared with ascorbic acid. The results of the present study suggest that the formulation shows synergistic effect in the treatment of ulcer. The antiulcer activity could be attributed to its antioxidant effect showed by different phytochemicals (alkaloids, flavonoids, tannins etc.) present in the extract.

1.0 Introduction

Gastrointestinal toxicity associated with nonsteroidal anti-inflammatory drugs (NSAIDs) is an important medical problem despite recent pharmaceutical advances. Besides being used as pain-killers, the NSAIDs are being increasingly used for prevention of malignancies, stroke, pre-eclampsia, Alzheimer's disease, and many other illnesses. The percentage of gastric ulcer cases induced by NSAIDs is emerging day by day, accounting for approximately 25% of gastric ulcers. In addition, various factors such as stress, hunger, *H. pylori* invasion etc. are also known to cause gastric ulcer. Consequently, prevention of gastrointestinal disorder continues to be of concern for both medical professionals and researchers. Various synthetic anti-ulcer drugs are presently available, and some of these like misoprostol are specifically used to prevent or treat the NSAID induced gastric ulcer [1]. However, each of these drugs confers simpler to severe side effects such as diarrhea, itching, skin rash, dizziness, and inactivation of some antifungal drugs (proton pump inhibitors), confusion in elderly patients, headache and anti androgenic effect (H_2 receptor blockers), constipation, vomiting, indigestion, back pain, and dizziness (Sucralfate), bleeding diathesis and abortion for pregnant women (Misoprostol). Thus, there is a growing interest on non-toxic, antiulcer formulations from medicinal plants, and many taxa of medicinal plants have been assessed worldwide for their anti ulcerogenic effects. In the developing nations, this turn of events has also been prompted, in part, by the high cost of the modern antiulcer medication[2].

The Indian traditional system of medicine has identified the *Piper betel* leaves with digestive and pancreatic lipase stimulant activities. The *Piper betel* leaves have strong pungent and aromatic flavor and widely consumed as a mouth freshener. In this study another 3 herbs are added to the piper betel. The 3 herbs are clove (*Syzygium aromaticum*), catechu (*Acacia catechu*) and fennel (*Foeniculum vulgare*). Here an attempt has been made to evaluate the synergistic activity of all the above on ulcer induced by aspirin and ethanol in wistar rats.

Betel leaves

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Black Catechu

Clove buds

Fennel seeds

Figure 1: Betel leaves, Black catechu, Cloves, Fennel

2.0 Materials and Methods

2.1 Plant Material

The betel leaves, clove buds, fennel seeds, black catechu were collected from the local Ayurvedic market of Hyderabad, Andhra Pradesh, India. It was authentified by Mr. P.V. Prasanna, Scientist-'E'-In–Charge, Botanical Survey of India (BSI), Hyderabad.

2.2 Experimental Animals

Thirty wistar rats weighing 150g - 180g were used for the study. The rats were on a diet of standard pellets and were allowed free access to water ad libitum. Animals were kept in standard plastic animal cages in groups of 5 animals. Prior to initialization of experimentation the animals were acclimatized to laboratory conditions for a week. The experiments were carried out according to guidelines of 'Committee for the Purpose of Control and Supervision of Experiments on Animals'(CPCSEA) New Delhi, India, numbered 1636/PO/a/12/CPCSEA and the procedures were approved by Institutional Animal Ethics Committee (IAEC), Sree Dattha Institute of Pharmacy.

2.3 Preparation of Extract

All the dried herbs clove, catechu, and fennel were finely powdered and the fresh betel leaves were triturated in mortar and pestle without adding water. Then all the powdered herbs were weighed about 20 g. The powdered material was subjected to maceration using different solvents for 48 hours. The extracts were filtered and evaporated to dryness and kept for further studies.

2.4 Phytochemical Screening

The different extracts obtained were subjected to phytochemical screening for the presence of alkaloids, carbohydrates, flavanoids, glycosides, proteins, tannins, terpenoids, coumarins, saponins according to standard procedures[3].

2.5 Acute Toxicity Studies

Acute oral toxicity was performed according to the OECD (Organization for Economic Co-operation and Development) Guideline No. 423. The animals in two groups of six each were taken and fasted overnight. The next day the aqueous extract (AEPH) (suspended in 5% tween 80 solution) was administered orally to one group at a dose level of 2000 mg/kg body weight and to another group, 5000 mg/kg body weight. The animals were continuously observed for the autonomic and behavioural changes for 24 hours for acute toxicity studies. The acute toxicity test reveals that the AEPH was safe up to 5000 mg/kg body weight. Therefore two doses were chosen (500 and 250 mg/kg body weight) for further studies

2.6 Evaluation of Anti-oxidant Activity

2.6.1 Quantitative evaluation of 1,1-diphenyl 2-picryl hydrazyl (DPPH) scavenging activity

The antioxidant activity of aqueous extract of poly herbs (AEPH) was determined on the basis of scavenging activity of stable 1,1-diphenyl 2-picryl hydrazyl free radical[4].

2.6.2 Quantitative evaluation of hydrogen peroxide free radical scavenging activity

This activity was determined according to previously described method[5].

2.6.3 Quantitative evaluation of reducing power

The reducing power of the AEPH was determined according to the method previously described[6].

2.7 Antiulcer Activity

Antiulcer activity was evaluated by aspirin induced ulcer and ethanol induced ulcer in wistar rats[7].

2.7.1 Aspirin induced gastric ulcer model

2.7.1.1 Experimental design

Group 1: Positive control group (Standard drug Omeprazole 30 mg/kg body weight)
Group 2: Negative control group (Aspirin 250 mg/kg body weight)
Group 3: Treated group (AEPH 250 mg/kg body weight)
Group 4: Treated group (AEPH 500 mg/kg body weight)

2.7.1.2 Assessment of antiulcer activity

The animals were fasted for 24 hours before the experiment. Animals were divided into 4 groups each containing 6 animals. Group 1 served as a positive control and received omeprazole at the dose of 30 mg/kg body weight, group 2 served as a negative control received aspirin at the dose of 250 mg/kg body weight. Group 3 received the aqueous extract of polyherbs at the dose of 250 mg/kg body weight and group 4 received the aqueous extract of polyherbs at the dose of 500 mg/kg body weight. After one hour of extract administration, aspirin at a dose of 250 mg/kg body weight was administered orally to all four groups. Then after 6 hours animals were euthanized with excess of anesthetic ether and the stomach was excised and cut along the greater curvature, washed and the inner surface was examined for ulceration. Ulcer index and % ulcer protection were calculated by using the methods described in table-1.
The ulcer index was then calculated by adding the total number of ulcers per stomach and total severity of ulcers per stomach.

2.7.2 Ethanol induced gastric ulcer model

2.7.2.1 Experimental design

Group 1: Positive control group (Standard drug Omeprazole 30 mg/kg body weight)
Group 2: Negative control group (Ethanol 1ml/animal)
Group 3: Treated group (AEPH 250 mg/kg body weight)
Group 4: Treated group (AEPH 500 mg/kg body weight)

2.7.2.2 Assessment of antiulcer activity

The animals were fasted for 24 hours before the commencement of the experiment. Then the animals were divided into 4 groups each containing 6 animals. Group 1 served as a positive control and received omeprazole at the dose of 30 mg/kg body weight, group 2 served as a negative control received ethanol at the dose of 1 ml/animal, the animals of group 3 received the aqueous extract of polyherbs at the dose of 250 mg/kg body weight and group 4 received the aqueous polyherbs extract at the dose of 500 mg/kg body weight. One hour after extract administration, 1ml of ethanol was administered orally to all the groups. After one hour the animals were euthanized with excess of anesthetic ether and stomach was cut and opens along the greater curvature, residual matter was cleared with saline and the inner surface was examined for ulceration[8].
Ulcer index and % ulcer protection were calculated by using the methods described in table 1.

Type	Score
Normal coloured stomach	0
Red colouration	0.5
Spot ulcer	1
Heamorragic streaks	1.5
Deep ulcers	2
Perforations	3

Table 1: Types of ulcers and its score

The percentage of ulcer protection was determined as follows:
Ulcer index was measured using the following formulae

$$U_I = U_N + U_S + U_P * 10^{-1}$$

Where,
U_I - ulcer index
U_N - average no. of ulcers per animal
U_S - average no. of severity score.
U_P – percentage of animals with ulcers
Percentage inhibition of ulcer index was calculated as below:

$$\% \text{ inhibition} = \frac{\text{Ulcer index}_{control} - \text{Ulcer index}_{test}}{\text{Ulcer index}_{control}} * 100$$

2.8 Statistical Analysis

Results are expressed as mean ± S.E.M. of triplets. The groups were compared by two-way ANOVA using Graph Pad Prism, Version 6.0 (Graph Pad Software, San Diego, CA, USA). P-values <0.05 were considered significant.

3.0 Results

3.1 Preliminary Phytochemical Screening

Different extracts of polyherbal formulation was analysed for the presence of various phytoconstituents. The result of this preliminary phytochemical testing of different extracts showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, terpenoids, coumarins and saponins as shown in table-2.

Table 2: Phytochemical analysis of different extracts of polyherbs.

S. No.	Test	Chloroform	Pet ether	Ethyl acetate	Ethanol	Aqueous
1.	Alkaloids	_	_	+	+	+
2.	Anthraquinone	_	_	_	_	_
3.	Carbohydrates	_	_	+	+	+
4.	Flavonoids	_	_	+	+	+
5.	Glycosides	+	+	+	+	+
6.	Proteins	_	+	+	-	+
7.	Tannins	_	_	+	+	+
8.	Terpenoids	+	+	+	+	+
9.	Coumarins	_	_	+	+	+
10.	Saponins	_	_	_	+	+

The results of our study on AEPH revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, terpenoids, coumarins, saponins in the aqueous extract of polyherbs. Based on screening the aqueous extract was further evaluated for its antioxidant activity.

3.2 Evaluation of Antioxidant Activity by DPPH Free Radical Scavenging Method

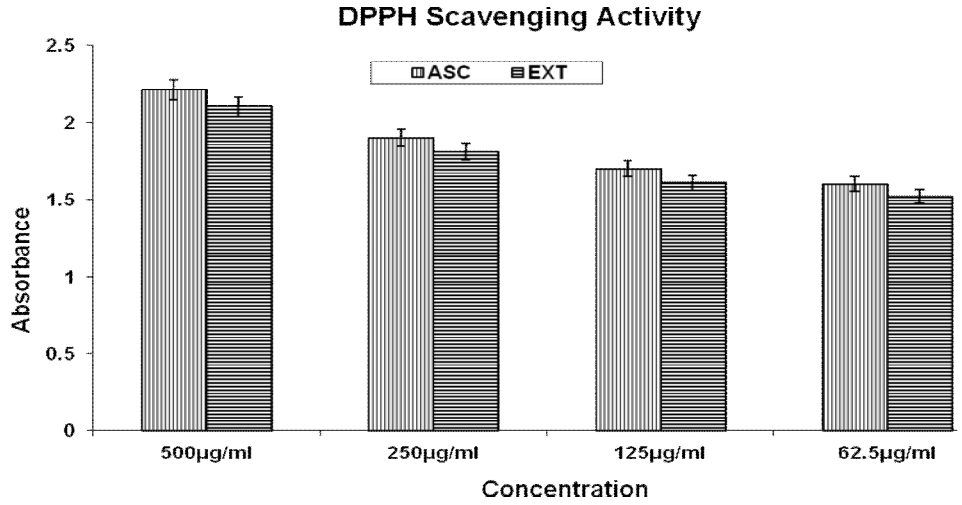


Figure 2: DPPH free radical scavenging activity of aqueous extract of polyherbs compared with that of ascorbic acid.

The aqueous extract of polyherbal formulation showed nearly similar results on DPPH free radical scavenging activity as that of standard ascorbic acid

3.3 Evaluation of Antioxidant Activity by Hydrogen Peroxide Scavenging Method

The graph below shows that the AEPH is a good scavenger of H_2O_2 when compared with standard ascorbic acid. The IC_{50} value of the extract was lesser than that of the standard.

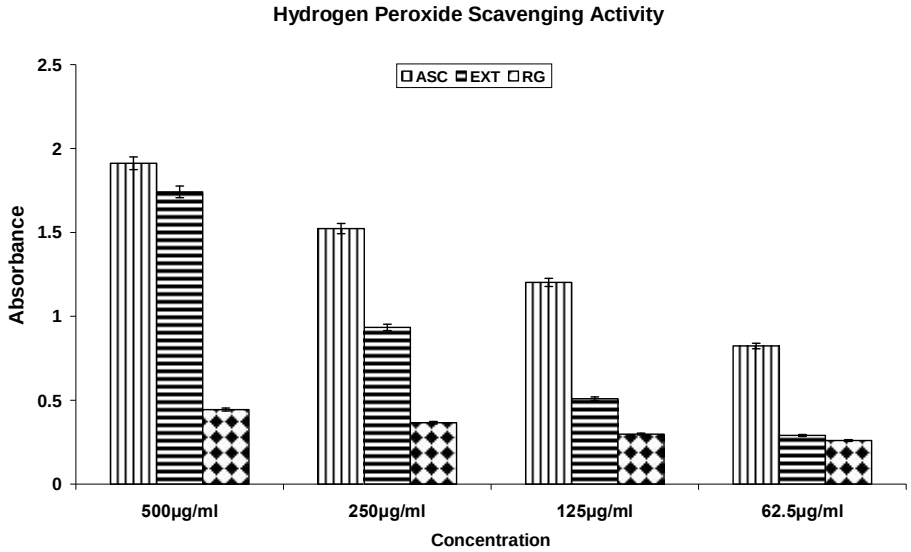


Figure 3: Hydrogen peroxide scavenging activity of the aqueous extract of polyherbs as compared to ascorbic acid and reduced glutathione as standard.

3.4 Evaluation of Antioxidant Activity by Reducing Power Assay Method

The graph shows the reducing power of the aqueous extract was comparable to that of ascorbic acid. The reducing activity was less than that of ascorbic acid and more than the reduced glutathione.

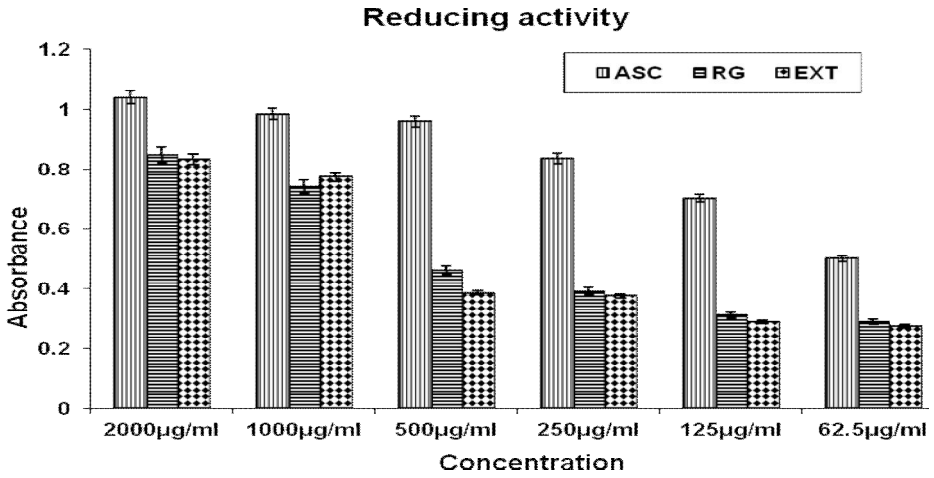


Figure 4: Reducing activity of aqueous extract of polyherbs in comparison to ascorbic acid and reduced glutathione

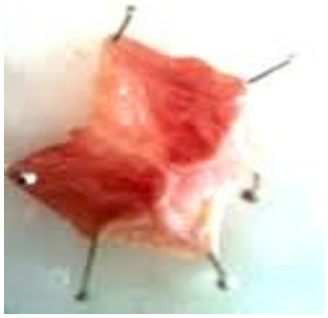
3.5 Effect of AEPH on Aspirin Induced Gastric Ulcers

Pretreatment of rats with AEPH showed protection from aspirin induced ulceration, as compared to control animals (Table 3). The aqueous extract of polyherbs at a dose of 250 mg/kg body weight was found to show significant decrease in ulcer index when compared to aqueous extract at a dose of 500 mg/kg body weight.
The % ulcer protection was studied in various groups and it was found that the % of ulcer protection in group 1 was 90, in Group 3 was 73 and Group 4 was 67 respectively as shown in table 3. The % ulcer protection was better in the group 3 when compared to group 4.

Table 3: Aspirin induced gastric ulcer index and % ulcer inhibition

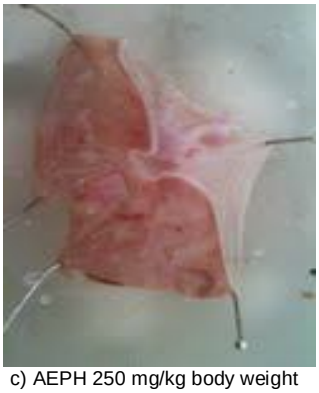
Treatment	Dose (mg/kg body weight, p.o.)	Dose (mg/kg body weight)	Aspirin	
			Ulcer index mean ±SEM	% ulcer inhibition
Group1	30 mg/kg (omeprazole)	30mg/kg	0.05±0.02	90
Group 2	-	-	0.48±0.02	0
Group 3	250 mg/kg(AEPH)	250mg/kg	0.13±0.009**	73
Group 4	500 mg/kg(AEPH)	500mg/kg	0.16±0.01**	67

Values are expressed as Mean ± S.E.M; no. of animals in each group (n) =6. One way ANOVA followed by turkey test done. p summary, ** p<0.01when compared with control.



a) Control aspirin 250 mg/kg body weight

b) Std omeprazole 30 mg/kg body weight



c) AEPH 250 mg/kg body weight

d) AEPH 500 mg/kg body weight

Figure 5: Aspirin induced gastric ulcers in sections of stomachs of various group

(a) Aspirin administered untreated rats, (b) Rats treated with standard omeprazole followed by aspirin administration, (c) Rats treated with AEPH with dose of 250 mg/kg body weight followed by aspirin administration, and (d) Rats treated with AEPH with a dose of 500 mg/kg body weight followed by aspirin administration.

3.6 Effect of AEPH on Ethanol Induced Gastric Ulcers

Pretreatment of rats with AEPH showed protection from ethanol induced ulceration, as compared to control group (Table 4). The aqueous extract of polyherbs at a dose of 250 mg/kg body weight was found to show significant decrease in ulcer index when compared to aqueous extract at a dose of 500 mg/kg body weight. The ulcer protection in group 1 was found to be 80%, 64% in group 3 and in group 4 was found to be 45%. The ulcer protection was better in the lower dose compared with the group 4 which was given 500 mg/kg body weight of AEPH. Omeprazole at a dose of 30 mg/kg body weight produced significant protection as compared to control group as shown in table-4.

Table 4: Ethanol induced gastric ulcers index and % ulcer inhibition:

Treatment	Dose (mg/kg body weight, p.o.)	Ethanol	
		Ulcer index mean +/-SEM	% ulcer inhibition
Group 1	30 mg/kg (omeprazole)	0.1458±0.003	80
Group 2	-	0.7458±0.04**	0
Group 3	250 mg/kg(AEPH)	0.2667±0.02	64
Group 4	500 mg/kg(AEPH)	0.4133±0.01**	45

Values are expressed as Mean ±S.E.M; no. of animals in each group (n) =6.
** p<0.01 when compared with control.

a) Control ethanol 1 ml/animal

b) Std. Omeprazole 30 mg/kg body weight

c) AEPH 250 mg/kg body weight

d) AEPH 500 mg/kg body weight

Fig 6: Ethanol induced gastric ulcers in sections of stomachs of various group.

(a) Ethanol administered untreated rats, (b) Rats treated with standard omeprazole followed by ethanol administration, (c) Rats treated with AEPH with dose of 250 mg/kg body weight followed by ethanol administration, and (d) Rats treated with AEPH with dose of 500 mg/kg body weight followed by ethanol administration.

4.0 Discussion

Today a large section of world's population relies on traditional remedies to treat plethora of diseases due to their low cost and less side effects. Peptic ulcer disease is a serious gastrointestinal disorder and is common in India. It has multifactorial causes in its pathophysiology including free radical generation and inflammation and hence requires a well-targeted therapeutic strategy. It is suggested that compounds containing antiulcer, antioxidant activity can prove effective in peptic ulcer diseases[9]. Some of the phytoconstituents now possess antiulcer activity for eg flavonoids, saponins, tannins, and terpenoids.

The study was under taken to investigate the synergistic effect of betel leaf, clove, fennel and catechu in the treatment of ulcer. From the phytochemical screening results of different solvents the aqueous extract of poly herbs showed the presence of flavonoids, alkaloids, tannins, terpenoids etc., and therefore aqueous extract was chosen for further studies.

Increases in the intercellular levels of reactive oxygen species (ROS) frequently referred as oxidative stress represent a potentially toxic insult which if not treated appropriately will lead to membrane dysfunction, DNA damage and inactivation of proteins. Chronic oxidative stress has numerous pathological consequences including cancer, arthritis, peptic ulcer and neurodegenerative disease. Under pathological conditions these deleterious species are removed by cellular antioxidant systems including antioxidants, vitamins and antioxidant enzyme etc. The antioxidant study was therefore undertaken to investigate the antioxidant potential of the poly herbal extract.

As it is well known that free radicals are one of the causative agents of gastric ulcers free radical scavenging activity was performed with the aqueous extract of poly herbs. It was observed that aqueous extract showed 80% reducing power as compared to standard ascorbic acid at a concentration of 500µg/ml, 95% DPPH scavenging activity as compared to standard ascorbic acid at a concentration of 500µg/ml and 91% hydrogen peroxide scavenging activity as compared to standard ascorbic acid at a concentration of 500µg/ml.

The extract was found to possess significant antiulcer activity as shown in table 3. % inhibition in aspirin induced gastric ulcers was found to be 73 and 67 respectively as in animals treated with aqueous extract of poly herbs at a dose of 250 mg/kg and 500 mg/kg body weight and the number of ulcer and their size was very less when compared to group 2 as shown in figure 5. Similarly as shown in table 4, the % inhibition in ethanol induced gastric ulcers was found to be 64 and 45 respectively in animals treated with aqueous extract of poly herbs at a dose of 250 mg/kg and 500 mg/kg body weight and even the number of ulcer and their size and red colouration was also very less when compared to group 2 as shown in figure 6.

The above results proved the fact that the aqueous extract of polyherbs is a potent antiulcer agent. Further this activity could be due to the presence of phytochemicals like flavonoids, alkaloids, tannins, terpenoids etc. which are well known for their antioxidant activity.

We propose here that the aqueous extract of polyherbs which includes betel leaf, clove, fennel and catechu can be used as antiulcer agent as well as therapeutic agent in the treatment and management of diseases caused by oxidative stress.

5.0 Acknowledgement

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