WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 23, 1208-1218. Research Article ISSN 2277–7105

EVALAUTION OF SYNERGISTICALLY GASTROPROTECTIVE EFFECTS OF ETHANOLIC LEAVES EXTRACTS OF ACHYRANTHES ASPERA & EUPHORBIA HIRTA

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Article Received on
18 October 2024,

Revised on 08 Nov. 2024,
Accepted on 28 Nov. 2024

DOI: 10.20959/wjpr202423-34670

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ABSTRACT

Objects: In the present study the Synergistically Gastroprotective activity of ethanolic leaves extract of *Achyranthes aspera & Euphorbia hirta* was investigated in the chemical induced ulcer. The efficacy of the both plants based upon leaves extract was compared with standard reference anti-ulcer drug Ranitidine. **Methods:** The extracts collected from the both plants of *Achyranthes aspera & Euphorbia hirta*. Firstly, Both plants leaves of *A. Aspera* & *E.hirta* was collecting and reduced to small size after drying in shade for one month or till dry and crushed to form coarse powder. The powdered drug (250 gm) was subjected to continuous hot extraction with the help of soxhlet apparatus using ethanol solvent. The plant material was dry in hot air oven at 50^0C for an hour. After the effective extraction, the solvents were distilled off, the both extracts were then concentrated on water bath to become dried. The obtained extract was weighing and stored in an air tight container. **Result:** The modal of absolute 99% ethanol induced ulcer, oral administration of ethanolic leaves extracts and standard drug (20mg /kg/bw) dose showed that reduction in ulcer index, collection of gastric juice, free acidity, total acidity, and also shows the pH of gastric juice and all parameters compared with the control group. It was showing protection index of 52.58%(A. aspera) 59.13% (E.hirta) and 64.10% (A. aspera + E hirta) at the doses of 300, 300, & (100 +300 mg/kg-bw). Omeprazole as reference standard drug and showing protection index of 68.95% at the dose of 20mg/kg-bw. The results are shown in tables and figure for illustration (Tables 1-2 and fig. 1-3).

KEYWORDS: *Achyranthes aspera*, *Euphorbia hirta*, Ranitidine, Ethyl alcohol.

INTRODUCTION

The peptic ulcer refers to a spectrum of disorders that includes gastric ulcers, duodenal ulcers or near the site of surgical gastrointestinal anastomosis.[1]

Causes of peptic ulcer[2,3]

When the stomach's natural system are disturbed due to any obstruction, such as the damaging effects of digestive juices (Including Acid and Pepsin, an enzyme that helps breakdown protein) stop working or the acid production is too over whelming for these protective defences to work properly, you can get an ulcer. And then, they are generated through an imbalance between mucosal aggressive & Protective factors.[4]

The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

Reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches to cure peptic ulcer disease. As a result, more and more synthetic drugs are introduced and offering newer options for treatment of peptic ulcer. The types of drugs vary from proton-pump inhibitor to H_2 antagonist or a cytoprotective agent. At the same time, each of these drugs confers simple or several side effects like arrhythmias, impotence, and gynaecomastia, hyperplasia and haemopoetic changes. Because of several side effects of synthetic medicines, there is new thought of better natural alternative for the treatment of peptic ulcer.

Alternative approach in recent days is the research of medicaments from Ayurvedic and traditional medicinal systems. The phyto-constituents available in the medicinal plants have proved to be clinically effective and relatively less toxic than the existing synthetic drugs and reducing the offensive factors and serving as tool in the prevention of peptic ulcers. Several herbal plants are reported to have antiulcer activity and several pre clinical (Animal) studies are reported on the efficacy of herbal medicines such as *Garcinia cambogia,*[5] *Cissus quaddrangularis* Linn,[6] *Tephrosia populnea,*[7] *Bambusa arundinacea,*[8] *Ocimum sanctum,*[9] *Emblica officinalis,*[10] *Pterospermum acerifolium,*[11] *Bauhinia variegate,*[12] *Terminalia chebula,*[13] *Spheranthus indicus,*[14] polyherbal extract containing *Curcuma*

longa, *Coriander sativum* and *Ocimum sanctum*[15] and Plant juices such as *Aloe vera*, banana stem juice and banana flower juice[16] and *Carica papaya* (papaya) fruit juice.[17]

The present study evaluates the gastro protective efficacy of *Achyranthes aspera* & *Euphorbia hirta* extract in alcohol induced ulcerated rats. Ranitidine is used as standard reference drug.

MATERIAL AND METHODS

Plant Selection and Collection of plant material

The Plant leave *A. Aspera* & *E.hirta* were selected of the exhaustive literature survey and collected from nearby area Sonepat, (Haryana), India in the month of March 2021.

Authentication

The plant leaves of *A. Aspera* & *E.hirta* leaves was authenticated by a senior Botanist Head of Department of Botany, Singhania University, Pacheri Bari, Jhunjhunu (Raj.), India.

Preparation of extracts

Both plants leaves of *A. Aspera* & *E.hirta* was collecting and reduced to small size after drying in shade for one month or till dry and crushed to form coarse powder. The powdered drug (250 gm) was subjected to continuous hot extraction with the help of soxhlet apparatus using solvent ethanol. The plant material was dry in hot air oven at 50^0C for an hour. After the effective extraction, the solvents were distilled off, the both extracts were then concentrated on water bath to become dried. The obtained extract was weighing and stored in an air tight container**.**

❖ Gastroprotective activity experimental animal

Albino Wistar rats of either sex weighing between 150-200 g were used. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water ad libitum. All procedures involving animals were carrying out under the institute ethics committee approval (667/PO/R/S/02/CPCSEA).

Ethanol induced ulcer

Swiss albino rats were divided into 6 groups (n=6). The different groups of animals are assigned as follows in tabular form.

S. No	Groups	Treatments
1.	Group 1	Received vehicle only
2.	Group 2 (Negative Control)	Served as control group and ulcer was induced with 1 ml /200gm-bw of 99% absolute alcohol (ethanol).
3.	Group 3 (Positive Control)	Drug control animals- alcohol induced ulcerated animals treated with Omeprazole (20mg/kg-bw).
4.	Group 4	Severed as treatment group and leaves extract of *Achyranthes aspera* (300 mg/kg/bw).
5.	Group 5	Severed as treatment group and leaves extract of *Euphorbia hirta* (300 mg/kg/bw).
6.	Group 6 (Combination)	Severed as treatment group and leaves extract of *Achyranthes aspera & Euphorbia hirta* (100 +300 mg/kg-bw) respectively.

The gastric ulcers were induced in rats of either sex weighing between 150-200 g by administrating absolute ethanol (99%); (1 ml / 200 g). All the animals were fasted for 36 h before administration of ethanol. The animals will divided into six groups, each consisting of six rats. Group I served as normal (no treatment). Group II served as negative control received with 1 ml /200gm-bw of 99% absolute alcohol (ethanol). Group III served as positive controls received omeprazole (20 mg/kg, p.o), animals of Group IV, V, VI received Ethanolic leaves extracts at the dose of 300, 300, (100+300) mg/kg respectively. The gastric ulcers were induced in rats by administrating absolute ethanol orally, and after 45 min of extracts and omeprazole treatment. They were keeping in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h later with anaesthetic ether and stomach was incised along the greater curvature and ulceration was score. A score for the ulcer were study.[18]

Macroscopic evaluation of stomach

The abdomen will open, cardiac end of the stomach were dissected out & the content were drained into the glass tube. The volume of the gastric juice was measured and its pH was determined. The isolated abdomen was examined by a 10X magnifier lens to assess the formation of ulcer. The numbers of ulcer were counted.[19]

Scoring of ulcer[20]

- 0 = Normal coloured stomach,
- 0.5 = Red colouration,
- 1 = Spot ulcer, 1.5 = Haemorrhagic streaks,
- 2 = Ulcers ≤ 3 but ≤ 5,

- 3 = Ulcers > 5

Calculation of ulcer index[21]

- $U_1 = UN + US + UP \times 10^{-1}$
- Where,
- U_1 = Ulcer index,
- U_N = Average of number of ulcer per animal,
- U_S = Average of animal severity score,
- U_P = Percentage of animal with ulcer

Determination of acid

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times \text{Meq /Lit/100gm}$$

Determination of percentage protection[22]

$$\% \text{ Protection} = \frac{\text{Control mean ulcer index-test mean ulcer index} \times 100}{\text{Control mean ulcer index}}$$

Biochemical estimation

Gastric acid collected from ethanol induced ulcer in rats. The gastric juice thus collected centrifuged and the volume of gastric juice as well as the P^H of gastric juice was noted. The gastric juice subjected to biochemical estimations as follows:-

Determination of free acidity and total acidity[23]

1. Gastric juice (1 ml) was taken in to a 100 ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01N NaOH solution until all traces of red colour disappears and the colour of solution turns yellowish orange (end point).
2. The volume of alkali added was noted. This volume corresponds to free acidity,
3. 2-3 drops of phenolphthalein solution was added and titration was continued until a definite red tings reappears.
4. The volume of alkali added was noted which corresponds to total acidity.

Free acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times \text{Meq /Lit/100gm}$$

Statistical analysis

The data of results obtained were subjected to statistical analysis and expressed as mean ± SD. the data were statically analyzed by one way analysis of various (ANOVA) and compare the means of the studied groups with standard. The data were statically analyzed by Graph pad prism Software version (7.1).

RESULTS AND DISCUSSION

Ethanol induced ulcer activity

The modal of absolute 99% ethanol induced ulcer, oral administration of ethanolic leaves extracts and standard drug (20mg /kg/bw) dose showed that reduction in ulcer index, collection of gastric juice, free acidity, total acidity, and also shows the pH of gastric juice and all parameters compared with the control group. It was showing protection index of 52.58%(A. aspera) 59.13% (E.hirta) and 64.10% (*A. aspera + E hirta*) at the doses of 300, 300, & (100 +300 mg/kg-bw) 20ml/kg/bw. Omeprazole as reference standard drug and showing protection index of 68.95% at the dose of 50mg/kg-bw. The results are shown in tables and figure for illustration (Tables 1-2 and fig. 3-11).

Table 1: Anti-ulcer activity of *A. aspera* & *E. hirta both plants* ethanolic leaves extract in ethanol induced ulcer model.

S. No	Treatment	Ulcer Index	Ulcer Protection
1.	Group 1	-	-
2.	Group 2 (Negative Control)	13.8833 ±0.3108	-
3.	Group 3 (Positive Control)	4.3100±0.1066	68.95 %
4.	Group 4 (*A. Aspera)*	6.5833±0.1306	52.58 %
5.	Group 5 (*E. hirta)*	5.6733±0.1276	59.13 %
6.	Group 6 (Combination)	4.9833±0.1376	64.10 %
F, df value		331.053, (2/15)	
P value		P<0.0001	

Value of mean SEM, n=6, p* p<0.01 when compared with control

Treated with *A. aspera* & *E. hirta both plants* ethanolic leaves extract treated with standard drug omeprazole

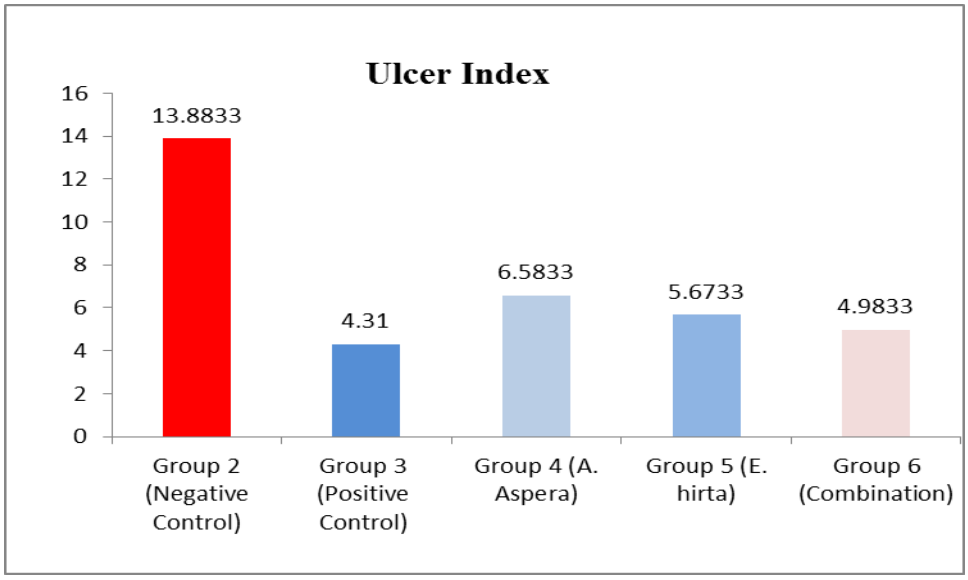

Fig. 1: Representing ulcer index.

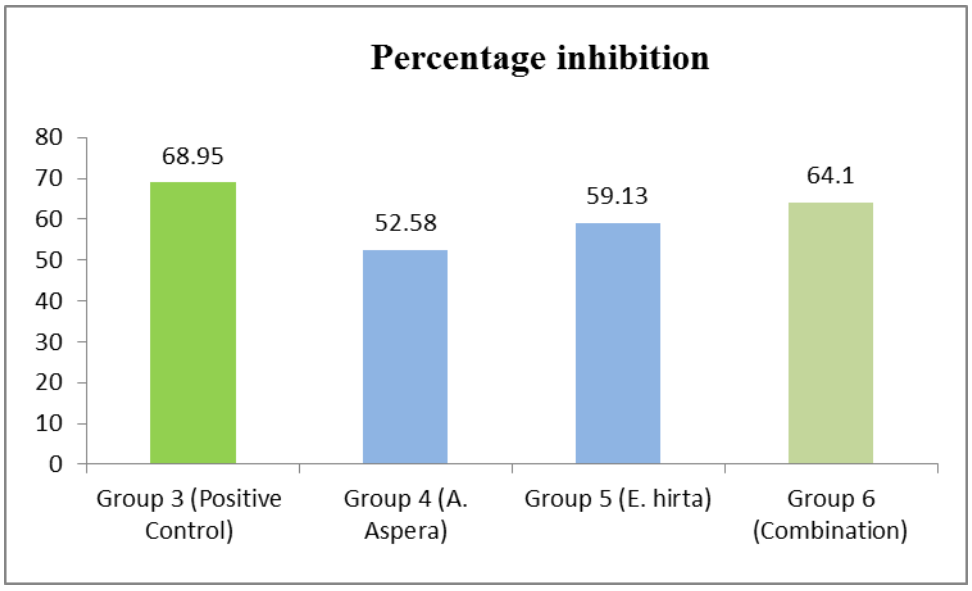

Fig. 2:- Representing percentage inhibition in various groups.

Table 2: Estimation of volume of gastric juice P^H, free acidity, total acidity of gastric juice.

S. No	Treatment	Volume of gastric Juice (ml)	p^H of gastric Juice	Free acidity	Total acidity
1.	Group 2 (Negative Control)	5.41210± 0.0966	3.3166± 0.2386	19.7333± 0.4395	47.1167± 1.8737
2.	Group 3 (Positive	3.3166± 0.0737	5.4666± 0.2403	10.8833± 0.4423	31.8667± 1.6177

	Control)				
3.	Group 4 (*A. Aspera)*	4.0500± 0.0921	5.333± 0.1429	13.2867± 0.2971	41.7700± 1.01422
5.	Group 5 (*E. hirta)*	3.9966± 0.0737	3.5166± 0.2386	10.6833± 0.4422	31.8667± 1.6177
6.	Group 6 (Combination)	3.8500± 0.0921	5.5666± 0.2403	13.2767± 0.2978	41.6700± 1.01422
F, df value		140.3, (2,15)	24.15, (2,15)	137.2, (2,15)	25.10, (2,15)
P Value		P<0.0001	P<0.0001	P<0.0001	P<0.0001

Value of mean SEM, n=6, p* p<0.01 when compared with control

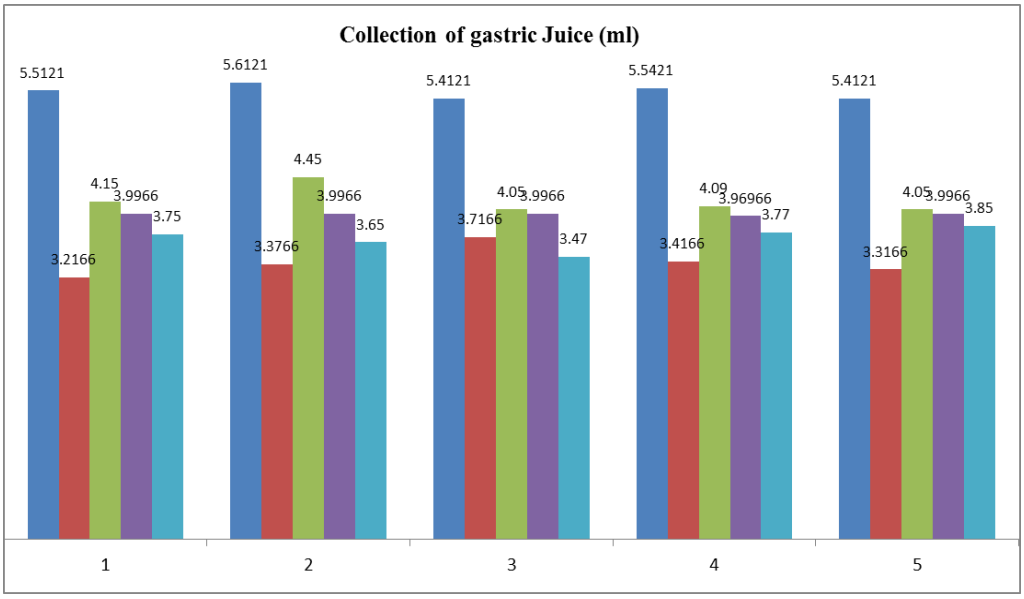

Fig. 3: Representing the collection of gastric juice (ml) in various groups.

CONCLUSION

Leaves extract has been traditionally used for a number of disorders. The literature survey on the plant described that the plant possessed various traditional medicinal properties. The purpose of this research work was to study anti-ulcer activity of both plants leaves extract and established the pharmacological characteristic of the *A. aspera* & *E. hirta both plants* ethanolic leaves extract. The obtained plant juice was subjected to pharmacological study by different experimental animal model to be used. *A. aspera* & *E. hirta both plants* ethanolic leaves extract and exhibited better anti-ulcer activity using chemical (99% Alcohol) induced ulcer, comparable to standard Omeprazole.

Hence it was concluded that the *both plant leaves extract* revealed more significant effect for anti-ulcer rather than individual extract of both plants when compared to the standard. Therefore it seems worthy to develop extract of both plants optimized affects in ulcer.

ACKNOWLEDGEMENT

Author are in debt to research guide Dr. Anand Singh, School of Pharmaceutical Science, SINGHANIA UNIVERSITY, Pacheri Bari, Jhunjhunu (Raj.), India to be providing laboratory facilities for PhD research work.

Author contribution statement

Mr. Jitendra Kumar conceptualized and gathered the data with regard to this work. Dr. Anand Singh analyzed these data and necessary inputs were given towards the designing of the manuscript. Both authors discussed the methodology and results and contributed to the final manuscript.

Conflict of interest

We declare that we have no conflict of interest.

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