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In Vitro Evaluation of Antiulcer Activity of A Polyherbal Mixture

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ABSTRACT: The present study was performed to assess the in vitro acid neutralizing capacity and H^+/K^+ - ATPase inhibition activity of a chloroform extract of polyherbal mixture. In acid neutralizing capacity (ANC), the extract significantly reduced ANC to 7.3 at concentration of 1500mg as compared to 13.7 with standard Aluminium hydroxide+Magnesium hydroxide (500mg). While in H^+/K^+ - ATPase inhibition activity, the extract showed maximum percentage inhibition of 63.78% at the concentration 100µg as compared to 69.76% with standard Omeprazole. The IC 50 value of chloroform extract of polyherbal mixture is 53.43 µg in comparison with standard Omeprazole 56.45 µg. The study reveals that the chloroform extract of polyherbal mixture may contain compounds possessing antacid and enzyme inhibition activity and thus can be used as an alternative medicine for gastrointestinal diseases.

Keywords: Polyherbal mixture, In vitro, Acid Neutralizing capacity (ANC), H^+/K^+ - ATPase inhibition activity, IC 50 value.

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I. INTRODUCTION

There are several factors that may induce ulcer in human being such as stress, chronic use of anti-inflammatory drugs etc. Though in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defence mechanism.[1] The effective drug against peptic ulcer should basically act either by reducing the aggressive factors on gastroduodenal mucosa or by increasing mucosal resistance against them.[2] Thus, the search for a safe anti-ulcer drug that optimizes these properties is continuing and part of the search is the evaluation of medicinal plants for gastroprotective properties.

"*In vitro*" meaning "in glass", a Latin term refers to studies of biological properties that are done in a test tube (i.e., in a glass vessel) rather than in a human or animal. *In vitro* studies allow scientists to isolate specific cells, bacteria & viruses and study them without the distractions of having to look at a whole organism. Humans are much more complicated than test tube, however, as compared to *in vivo* studies, *in vitro* studies are substantially faster, less expensive, and can be done with fewer ethical & safety concerns.[3]

In context to this, the present study is thus performed to evaluate the *in vitro* antiulcer potency of the chloroform extract of polyherbal mixture C-AET by assessing the acid neutralizing capacity and H^+/K^+ - ATPase inhibition activity. Polyherbal mixture C-AET is a mixture containing leaves of *Argemone mexicana*, roots of *Echinops echinatus* and aerial parts of *Tricholepis glaberrima* (1:1:1).

Argemone mexicana (family - Papavaraceae) known as Ghamoya is an indigenous herb found in India. Ghamoya has occupied a pivotal position in Indian culture and folk medicine. It has been used in almost all the traditional system of medicine, such as in Ayurveda, Unani and Siddha. Traditionally, the plant is reported to be used as diuretic, purgative, anti-inflammatory, analgesic and believed to destroy worms, cures itching, various skin diseases and as antidote to various poisons. Some of the reported pharmacological activities are Wound healing, Anti-asthamatic, Anti-stress, Hepatoprotective, Anti-HIV, Anti- diabetic, Anti- inflammatory.[4]

Echinops echinatus, Indian Global thistle, belonging to the family Asteraceae is a shrub found in India, Pakistan and Sri Lanka. It contains various chemical constituents like Carbohydrates, Alkaloids, Flavonoids, Terpenoids, Steroids etc. Traditionally, the plant is reported to be used in the treatment of fever, inflammation, asthma, sexual disorders, in brain disorders etc. The root is abortifacient and aphrodisiac. Some of the reported activities are Antifungal, Analgesic, Anti-inflammatory, Diuretic, Antioxidant, Wound Healing etc.[5]

Tricholepis glaberrima DC (Asteraceae), commonly known as "Brahmadandi" is an important medicinal plant used in our traditional system of medicine to treat various diseases. It is used in Ayurveda for nervine tonic, aphrodisiac, skin disease and in cough. It is used because of the broad area of biological activities

like anti-inflammatory, urinary troubles, antiseptic activities. The plant is rich in many pharmaceutical active ingredients like flavonoids, triterpenoids, saponin glycosides and sterols.[6]

II. MATERIALS AND METHODS

2.1 Collection and Authentification of Plant Material

The leaves of *Argemone mexicana*, roots of *Echinops echinatus* and aerial parts of *Tricholepis glaberrima* was collected from Chittoor District and was authenticated by Dr. K. Madhava Chetty, Plant Taxonomist (IAAT:357), Asst. Professor, Department of Botany, Sri Venkateshwara University, Tirupati.

2.2 Extraction

300gm of dried plant materials(1:1:1) was extracted with chloroform by maceration. The extract/mixture thus obtained was subjected to evaporation on water bath until it becomes semisolid, then was stored in air tight container for further use.

2.3 *In vitro* Evaluation of Antiulcer Activity

2.3.1 Acid Neutralizing Capacity

The acid neutralizing capacity value for chloroform extract of polyherbal mixture C-AET (100mg, 500mg, 1000mg, 1500mg) was compared with the standard antacid Aluminium hydroxide + Magnesium hydroxide (500mg). To the 5ml quantity of this mixture, water was added to make up the total volume 70ml and then mixed for one minute. There after 30ml of 1.0N HCl was added into standard and test preparation and stirred for 15minutes, drops of phenophthalein solution was added and mixed. The excess HCl was immediately titrated with 0.5N Sodium hydroxide solution drop wise until a pink color is attained.[7,8]

The moles of acid neutralized is calculated by,

Moles of acid neutralized = (vol. of HCl ×Normality of HCl) - (vol. Of NaOH × Normality of NaOH)

$$\text{Acid neutralizing capacity(ANC) per gram of antacid} = \frac{\text{moles of HCl neutralized}}{\text{Grams of Antacid/Extract}}$$

2.3.2 H^{+}/K^{+} - ATPase Inhibition Activity

Preparation of H^{+}/K^{+} - ATPase Enzyme: To prepare H^{+}/K^{+} - ATPase enzyme sample, fresh sheep stomach was obtained from a local slaughterhouse of Hyderabad. The stomach was cut opened, the mucosa at gastric fundus was cut-off, and the inner layer was scraped out for parietal cells. Thus obtained cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 and centrifuged at 6000 g for 10 min. The supernatant (enzyme extract) was used to determine the H^{+}/ K^{+}- ATPase inhibition. Protein content of the cell extract was determined according to Bradford's method using the BSA as standard.

Assessment of H^{+}/K^{+} ATPase inhibition: The reaction mixture containing 0.1 ml of enzyme extract (300µg) and plant extract at different concentrations (20µg, 40µg, 60µg, 80µg, 100µg) was pre-incubated for 60 min at 37°C. The reaction was initiated by adding substrate 2 mM ATP (200µL), in addition to this 2mM MgCl2 (200µL) and 10mM KCl (200µL) was added. After 30 min of incubation at 37°C, the reaction was stopped by the addition of assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid followed by centrifugation at 2000 g for 10 min and inorganic phosphate released was measured spectrophotometrically at 660 nm by following Fiske-Subbarow method. Briefly, to the 1 ml of supernatant 4 ml of millipore water, 1 ml of 2.5% ammonium molybdate, 0.4 ml of ANSA was added and allowed to stand for 10 min at room temperature. Absorbance of released inorganic phosphate was measured at 660 nm. Enzyme activity was calculated as micromoles of Pi released per hour at various doses of chloroform extract of polyherbal mixture C-AET. Results were compared with the known antiulcer PPA inhibitor Omeprazole and expressed as Mean ± SEM.[9]

% enzyme inhibition was calculated using the formula:

Percentage of inhibition = $[\text{Activity(control)} - \text{Activity(test)}/\text{Activity(control)}] \times 100$

2.4 Statistical Analysis

The data is represented as Mean ± SEM. The data is analysed by one way ANOVA followed by Tukey test and the whole analysis was carried out using GraphPad PRISM 7 version 7.03 ©1992-2017. 'P' value were considered significant when *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 when the test and reference were compared with the control group.

III. RESULTS

3.1 Extraction

The % yield of chloroform extract of polyherbal mixture C-AET was found to be 6.27%.

3.2 *In vitro* Evaluation of Antiulcer Activity

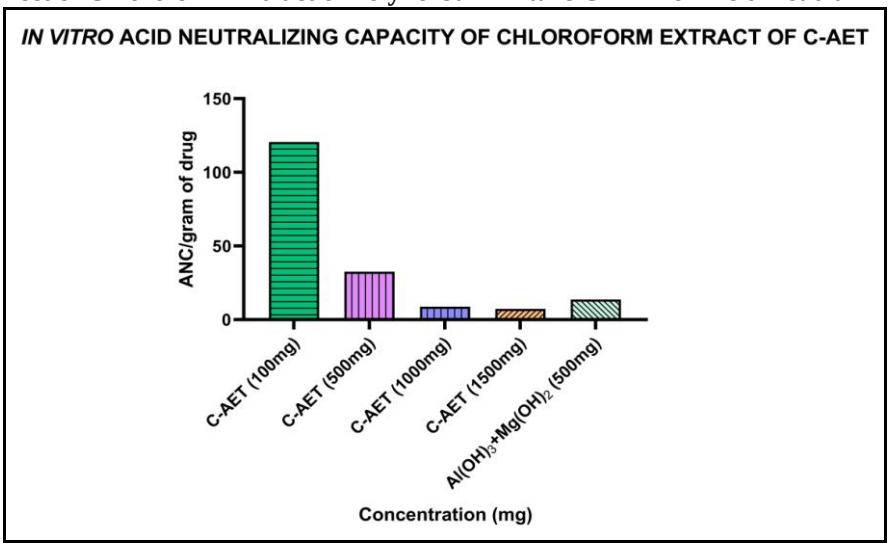
3.2.1. Acid Neutralizing Capacity

The neutralizing effect of the chloroform extract of polyherbal mixture C-AET was studied for four concentration (100mg, 500mg, 1000mg, 1500mg) and standard Aluminium Hydroxide + Magnesium Hydroxide [$Al(OH)_3$+$Mg(OH)_2$](500 mg). The results obtained envisage that the extract at concentration 100mg, 500mg, 1000mg and 1500mg showed significant reduction in acid neutralizing capacity (ANC) i.e., 120.5, 32.5, 8.75 and 7.3 respectively as compared to standard $Al(OH)_3$+$Mg(OH)_2$ (500 mg) which is 13.7. The extract at concentration 1500 mg was found to neutralize acid more significantly as compared to standard. The results are tabulated in Table & Graph 1.

Table 1: Effect of Chloroform Extract of Polyherbal Mixture C-AET on Acid Neutralizing Capacity

S.No.	Concentration (mg)	Volume of NaOH consumed (ml)	mEq of Acid Consumed	ANC per gram of Antacid
1	100mg C-AET	35.9	12.05	120.5
2	500mg C-AET	27.5	16.25	32.5
3	1000mg C-AET	42.5	8.75	8.75
4	1500mg C_3-AET	38	11	7.33
5	500mg $Al(OH)_3$+$Mg(OH)_2$	46.3	6.85	13.7

Graph 1: Effect of Chloroform Extract of Polyherbal Mixture C-AET on Acid Neutralizing Capacity



3.2.2 H^+/K^+ - ATPase Inhibition Activity

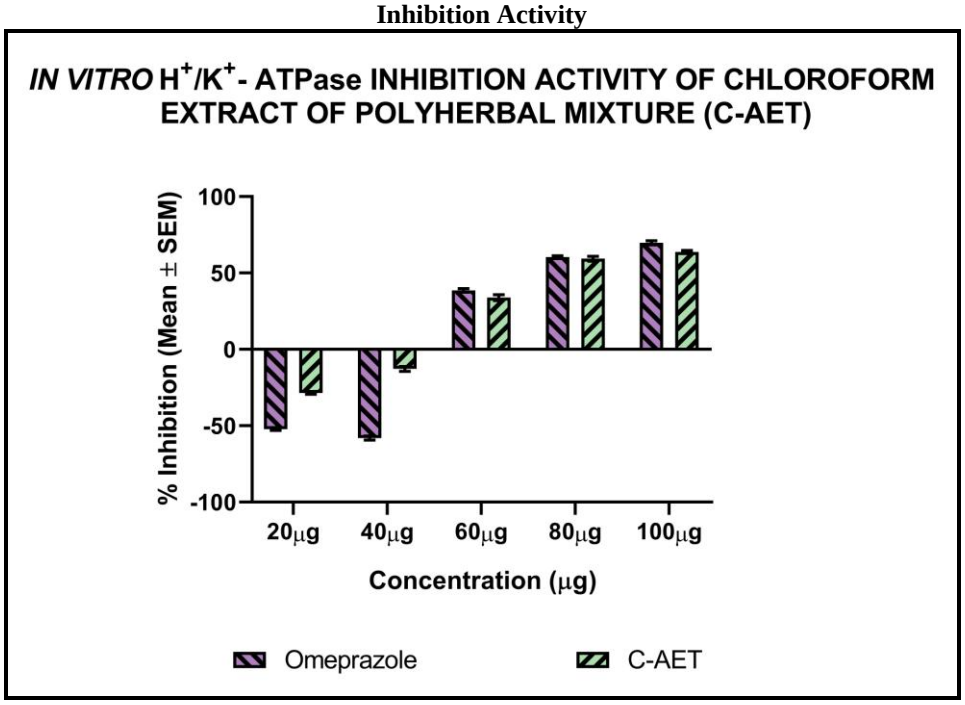
The H^+/ K^+ - ATPase inhibition activity of chloroform extract of polyherbal mixture C-AET at various concentration (20µg, 40µg, 60µg, 80µg, 100µg) was compared with Omeprazole as standard. The extract significantly showed activity in a dose dependent manner. Maximum percentage inhibition of 63.78±1.034% was observed for extract at a concentration of 100µg and standard Omeprazole showed 69.76±1.328%. The result have been tabulated in Table 2 and Graph 2(a). The half maximal inhibitory concentration (IC 50) i.e., the concentration of the extract which inhibits 50% of H^+/ K^+ - ATPase activity, IC 50 value of chloroform extract of polyherbal mixture C-AET is 53.43 µg in comparison with standard Omeprazole 56.45 µg. IC 50 graph shown in graph 2(b).

Table 2: Effect of Chloroform Extract of Polyherbal Mixture C-AET on *In Vitro* H^+/K^+ - ATPase Inhibition Activity

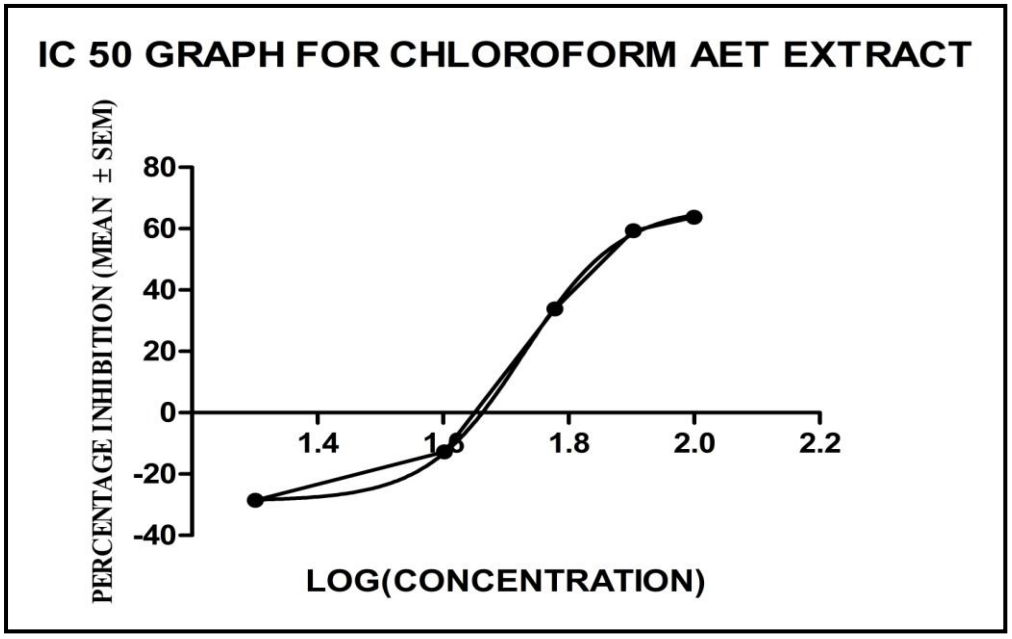
Concentration(µg)	Percentage Inhibition(%) (Mean±SEM)	
	Standard Omeprazole	Extract Chloroform Extract of C-AET
20µg	-52.25±0.83	-28.54±0.93
40µg	-58.10±1.33	-12.77±1.57
60µg	38.63±1.19	33.84±1.85***
80µg	60.47±0.70	59.36±1.58***
100µg	69.76±1.32	63.78±1.03***

Values are expressed as Mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared Standard Vs. Extract and analyzed by one way ANOVA followed by Tukey test.

Graph 2(a): Effect of Chloroform Extract of Polyherbal Mixture AET on *In Vitro* H^+/K^+ - ATPase Inhibition Activity



Graph 2(b): Graph representing IC 50 value for Chloroform extract of polyherbal mixture C-AET



IV. DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanism.[10] Acidity is a common gastrointestinal problem which is attributed to a functional disorder that can result due to a variety of reasons.[11] Excessive secretion of gastric acid or stomach acid (i.e., HCl), inflames the stomach lining and produces ulceration.[12] Antacids act by neutralizing gastric acid and thereby reduce the gastric pH.[11] The acid neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize and it is measured by a process known as back titration.[7] In ANC, the chloroform extract of polyherbal mixture C-AET at 1500mg concentration showed significant reduction in ANC of 7.3.

Hyperchlorhydria is a problem characterized by uncontrolled hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump. H^+/K^+ - ATPase is a key enzyme in inducing acidity, it is located on apical secretory membrane of parietal cells.[13] In H^+/K^+-ATPase inhibition activity, the extract showed maximum percentage inhibition of 63.78% at 100µg concentration. The IC 50 value of the extract C-AET and Omeprazole was found to be 53.43µg/ml and 56.45µg/ml respectively. The data reported here is indicative of that, the chloroform extract of polyherbal mixture C-AET may possess antacid, antisecretory, antiulcer property which may be due to presence of compounds in the mixture. However, further studies are required to establish its exact mode of action and the active principles involved in it antiulcer effect.

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