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Evaluation of Antiulcerogenic Activity of Polyherbal Extracts of Medicinal Plants

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ABSTRACT

This study was carried out to investigate the ulcer healing activity of individual ethanolic extract of *Tinospora cordifolia* (Wild.) Miers (EETC), *Hemidesmus indicus* (L.) R. Br (EEHI) and Polyherbal combination (PHCE) of both in pylorus ligation induced gastric ulcer. Preliminary phytochemical screening and TLC were performed for the identification and separation of the active compounds. Animals were evenly divided into various treatment groups and were orally administered EEHI and EETC at different doses for 5days, pylorus ligation was performed on the next day (6^{th} day) in a 24 h fasted rats 60 minutes after drug administration i.e., group I served as control received 0.5% CMC (1 ml/kg) group II served as standard group given famotidine (20 mg/kg) and other groups in all type of treatments received test compounds, EEHI and EETC in the dose of 200 and 400 mg/kg as well as polyherbal combine extracts (PHCE), prepared by mixing these two plants extracts in a different ratio of 1:1, 1:2 and 2:1 at two different doses 200 mg/kg and 400 mg/kg. Various gastric mucosal and gastric secretion parameters including Ulcer index, preventive index, free acidity, total acidity, volume of juice (ml) and pH were estimated. Histopathological examination of rat stomach was further conducted to confirm the activity. Study revealed that the EEHI and EETC has shown significant anti-ulcer activity individually and in polyherbal form in pylorus ligated rats. Thus, extracts seems promising for future combination with modern drug therapy and study provides future research in the direction of screening and investigating the prominent active constituent responsible for antiulcer activity and possible mechanism.

INTRODUCTION

Peptic ulcer (PU), a profoundly predominant gastrointestinal disorder, includes gastric and duodenal ulcers. Gastric ulcers are situated in the stomach, characterized as pain; these ulcers are very common in older age people, symptoms may conclude nausea, vomiting, regurgitating and weight loss. On the other hand duodenal ulcers are found at the beginning of small intestine and characterized by severe pain with burning sensation in upper abdomen. In most cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain and cramps along with vomiting blood.[1] The pathophysiology of this gastro-intestinal disorder is seemed as an imbalance between mucosal defensive factors such as mucus, prostaglandins, nitric oxide, bicarbonates, peptides, growth factors and injurious factors like acid, pepsin, refluxed bile, LT, R.O.S and *Helicobacter pylori.* [2]

Peptic ulcer disease is one of the diseases in which quality of life is significantly impaired[3]. Almost 5-15% of adult populations of world are suffering from ulcer disease and its ramifications[4.] Peptic ulcer has now become major reason of morbidity and mortality[5]. In the United states, approximately 4 million people have suffered duodenal and gastric ulcer while 180000 patients are hospitalized each year due to peptic ulcer disease. There is 10% likelihood of developing peptic ulcer in about 10% males and 4% females[6,7]. Peptic ulcer incidence in India is 2.9% and for gastric ulcer is 2.7%.

Hemidesmus indicus (L.) R.Br (Periplocacea) is generally known as "Indian sarsaparilla" '*Hemidesmus indicus'* was previously placed under the family "Asclepiadaceae" but currently because of its pollinial features transisted to Periplocaceae[8]. Plant is also called "Anantmool" in Hindi means "eternal roots" as the roots are spread largely in a long way under the ground. Roots have sweet fragrance similar as camphor known as "karpoori"[9]. This is the most common medicinal plant regarded in the "Indian system of medicine"[10]and official drug in I.P[11] and B.P[12].

Tinospora cordifolia (Wild). Miers ex Hook. F & Thoms (Family: Menispermaceae) commonly known as "Amrita" or "Guduchi" is an essential herbs for "Indian system of Medicine" and has been used in Ayurvedic formulation for the treatment of various ailments throughout the decades[13]. Plants is considered to herbaceous vine indigenous to the tropical areas of Myanmar and Srilanka.

Literature survey reveals that despite with all the other essential medicinal properties ***Hemidesmus indicus (L.) R. Br.*** **(**root extract) and ***Tinospora cordifolia*** (Stem extract) also possess anti-ulcer activity. Based on pharmacological studies these two plants exhibited significant anti-ulcer activity. But no scientific evidence established is synergistic effect of these drugs. So the aim was to carry out the extraction and individual treatment as well as polyherbal combine treatment of these crude drugs to elevate the ulcer healing property.

Figure No. 1: *Hemidesmus indicus* (L.) R.Br a) Roots b) Leaves, *Tinospora cordifolia* (Wild) c) Leaves d) stems

MATERIALS AND METHODS

1. Collection and Authentication of Plants

Table No. 1: Indigenous medicinal plants whose botanical names, local names and parts have been used given below:

Sr. No.	Botanical names	Local names	Parts
1.	*Hemidesmus indicus*	Anantmool, Sariva	Roots
2.	*Tinospora cordifolia*	Guduchi, Giloy	Stems

Stems of *Tinospora cordifolia* (Wild.) Miers. were collected from botanical garden of Utthan near Shambhunath Institute of Pharmacy, Jhalwa Allahabad. Further Taxonomic identification was conducted by Dr. G. P. Sinha, Scientist-E/Head of Office, Botanical Survey of India, CRC, Allahabad (U.P). A plant specimen was deposited in the Herbarium of Botanical survey of India, CRC, Allahabad (U.P) for future reference, Accession No. (BSI) 98883.

The roots of *Hemidesmus indicus* (L.)R.Br. were purchased from authorized herbal store and authenticated by Scientist & Head Dr. A. K. S Rawat, Pharmacognosy & Ethano-pharmacology Division at CSIR-National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh (India). A voucher specimen (NBRI/CIF/536/2017) has been deposited in the Herbarium for future reference, Specification: NBRI-SOP-202.

2. Drugs and Chemicals

Hydrochloric acid, Sulphuric acid, Sodium hydroxide, Nitric acid, Chloroform, 99% Ethanol, Carboxymethyl Cellulose, Fehling solution A and B, Lead Acetate, Ferric chloride, Ethyl acetate, Formic acid were obtained from Merck specialties Pvt. Ltd. Mumbai. Methanol, Picric acid, Acetic acid, Ammonia(Solution), Chloroform, Potassium dichromate, Ammonia(solution), Hexane, Lead Sulphide, Copper sulphate, 10% buffered formalin were obtained from Fisher Scientific (Qualigens Fine Chemicals, Mumbai). Standard drug (Femotidine), Molish's reagent, Biuret's reagent, Benedict's reagent, Paraffin wax was obtained from Central drug House (CDH) Pvt. Ltd, New Delhi. α-napthol obtained from Fizmerk India Chemicals. Haematoxylin-eosin (Thermo scientific), Silica gel G, Formaldehyde were obtained from Renkem (Aventor Performance Materials India Ltd. Gujarat).

3. Procurement of experimental animals

Adult male Wistar albino rats 100-200 g of weight were purchased from committee for the purpose of control a supervision of experimental on animals (CPCSEA). The animals were brought, caged in a well maintained ventilated housed under standard laboratory conditions. They had given water and fed pellet diet. Animals were acclimatized to the laboratory condition for at least 5 days prior to the experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee IAEC, (REG No.-1451/PO/E/11/CPCSEA) and the experimental laboratory animal taken as per the protocol approval UIP/IAEC/April-2017/02.

4. Instruments

Weighing Balance, Electronic Weighing balance, Oven, Magnifying lens, Soxhlet Extractor, Centrifugal machine, Water bath, Heating mantle, Digital pH meter, U.V cabinet, Compound Microscope, Camera Lucida, Stage and Ocular micrometer. For Histopathology instruments used were "Automatic Tissue Processor" for dehydrating and hardening the tissues, "Rotary microtome" instrument used for sectioning tissue embedded in paraffin blocks, Light microscope (X4, X10 and X40) magnification.

5. Physicochemical parameters

Physicochemical parameters such as foreign matter, moisture content, alcohol and water soluble extractive value, total ash, water soluble and acid insoluble ash[14] were performed on *H. indicus* (L.) R. Br roots and *T. cordifolia* (Wild.) Miers. Stem powder and their standard limits were given in a reference[15,16].

6. Preparation of extracts

The whole roots of *Hemidesmus indicus* (L.) R. Br has been dried and powdered with the help of mechanical grinder from which 250 gm of coarse powder were extracted by "Soxhlet apparatus" with 500 ml of ethanol. The concentrated extract was placed 37°C for the residual ethanol to evaporate[17].

The stem of *Tinospora cordifolia* (Wild) Miers were taken with its bark and chopped off into big pieces of about 10-20 cm and rinsed with distilled water then it was powdered, air-dried and made it ready for Soxhlet extraction[18]. The extraction was done using 500 ml ethanol.

After extraction, extract was filtered and concentrated dried to obtain a crude ethanolic extract, which was further used for evaluation. The color, consistency and % yields of extracts were noted and extracts were further stored in tight bottles for experimental use.

7. Phytochemical analysis

Preliminary phytochemical analysis which was performed for ethanolic extracts of *Hemidesmus indicus* (L.) R. Br and *Tinospora cordifolia* (Wild.) Miers with its test solution prepared in 99% ethanol for the detection of phytoconstituents like carbohydrates, proteins, steroids, alkaloids, glycosides, saponin and flavanoids[19-21].

8. Thin layer chromatography

Preparative TLC was performed on ethanolic extracts i.e, *Hemisdesmus indicus* (L.) R. Br and *Tinospora cordifolia* (Wild) and in their combined extracts.

- **Procedures:** The chromatographic plates prepared in the laboratory are used activated.
- **Preparative of samples:** 200 mg of extracts was dissolved in 2 ml ethanol (99 %) respectively and were mixed by shaking for 3-30 minutes. Insoluble material was removed by centrifugation and solution was filtered with filter paper.
- **Preparation of adsorbent:** The slurry of the coating material and water was prepared using the spreading devices.
- **Saturation of the chromatographic chamber:** The chromatography is carried out in a standard chamber, to achieve saturation, poured into the chamber a sufficient quantity of the mobile phase to saturate the filter paper.
- **Applications of the test solutions:** Using a capillary, spots of the two extracts were placed into the starting line.
- **Preparation of mobile phase:** Different composition of mobile phase was tried for proper separation of constituent.
- **Development of chromatograms:** Spots were allowed to dry, plates was placed- inclined vertically as possible into the chamber, make sure that the points of application of extracts should remain above the surface of the mobile phase.

- **Observation and interpretation of the chromatograms:** The spots were seen in visible light, 254 nm and 365 nm. Centre of each spots highlighted with a needle. The distance from the area and center of each spot to the point of application was measured and recorded. Calculate the Rf value through the ratio of distance travelled by the solute to the distance travelled by leading edge of the solvent.

$$[\ Rf = a/b\ ]$$

Where a= the distance between the point of application and the centre of the spot of the material being examined; b=the distance between the point of application and the solvent front.

9. Acute oral toxicity studies of *Tinospora cordifolia* (Wild) Miers and *Hemidesmus indicus (L.) R. Br*

In Acute oral toxicity analysis as per OECD guidelines 423, the limit test dose of 5 g/kg orally have not shown any type of mortality or any sign of toxicity during the experimental period[22, 23].There is no sign of toxicity recorded even at the highest dose possible i.e.9 ml/kg for decoction and 8 gm/kg for whole plant material of *Tinospora cordifolia*[24]. On the basis of Acute oral toxicity testing, literature study revealed *Hemidesmus indicus* root and *Tinospora cordifolia* stem are relatively safe when administered orally in mice. Therefore, two different doses (200 and 400 mg/kg) were selected for the evaluation of Anti-ulcer activity.

10. Preparation of drug dose and test compounds

Doses of drugs were calculated for each animal based on their body weight and respective volumes were administered orally.

- **Standard drug:** Famotidine (standard) was suspended in 0.5% CMC (25 mg/ml) was given in the dose of 20 mg/kg.

- **Test compound**:

1) Ethanolic extract of *Hemidesmus indicus* (L.) R.Br (EEHI) was suspended in 0.5% CMC in water (100 mg/ml) in the doses 200 and 400 mg/kg. [25]

2) Ethanolic extract of *Tinospora cordifolia* (Wild) Miers (EETC) was suspended in 0.5% CMC in water (100 mg/ml).[26]

3) Polyherbal combine extracts (PHCE) were prepared by mixing these two plant extracts in a different ratio of 1:1, 1:2 and 2:1 at two different doses 200 mg/kg and 400 mg/kg. Before mixing, each extracts was suspended in 0.5% CMC in water (100 mg/ml stock sol. prepared for each) triturating till uniform suspension are formed.

11. Experimental induction of ulcer

- **Pylorus ligation**

A simple and effective method for production of gastric ulceration in the rat based on ligation of the pylorus by Shay model technique was used.

a) Individual treatment

Animals were taken and randomly divided into 4 groups each group containing 6 animals each for individual study.

Table No. 2: Test Drug 1- Ethanolic extracts of *Hemidesmus indicus* (EEHI)

Groups	Treatment	Dose
Group I	Vehicle	1 ml/kg
Group II	Famotidine	20 mg/kg
Group III	EEHI	200 mg/kg
Group IV	EEHI	400 mg/kg

Table No. 3: Test Drug 2- Ethanolic extracts of *Tinospora cordifolia* (EETC)

Groups	Treatment	Dose
Group I	Vehicle	1 ml/kg
Group II	Famotidine	20 mg/kg
Group III	EETC	200 mg/kg
Group IV	EETC	400 mg/kg

b) Polyherbal combined treatment

Animals were divided into eight groups, each group containing 6 animals each for polyherbal study.

Table No. 4: Test Drug 3- Polyherbal combine extract of *Tinospora cordifolia* and *Hemidesmus indicus* (PHCE)

Groups	Treatment	Dose	Combine Ratio
Group I	Vehicle control	1 ml/kg	_
Group II	Famotidine	20 mg/kg	_
Group III	PHCE= EETC + EEHI	200 mg/kg	1:1 = 100 mg + 100 mg
Group IV	PHCE= EETC+EEHI	200 mg/kg	1:2=66.67 mg +133.33 mg
GroupV	PHCE= EETC+EEHI	200 mg/kg	2:1=133.33 mg + 66.67 mg
GroupVI	PHCE= EETC+EEHI	400 mg/kg	1:1=200 mg+ 200 mg
GroupVII	PHCE= EETC+EEHI	400 mg/kg	1:2=133.33 mg + 266.67 mg
GroupVIII	PHCE= EETC+EEHI	400 mg/kg	2:1=266.67 mg + 133.33 mg

The first group served as control received 0.5 % CMC (1 ml/kg) the second group is a standard group received Famotidine (20 mg/kg), and other groups in all type of treatments received above test compounds at different doses once daily for 5 days Pylorus ligation were carried out on 24 h fasted rats on 6th day 60 minutes after drugs administration.

12. Surgical Procedure

The rats were fasted with free access of water and *ad libitum*. Under anesthesia Ketamine (100 mg/kg). The abdomen was opened by gradual incision of 1 cm long just below the sternum. Pyloric part of stomach was lifted out and tied with the help of thread for ligation and tightly applying the knot to avoid traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and abdominal wall closed tightly by uninterrupted sutures and the rats were allowed to recover. The animals are deprived of both food and water during the postoperative period. The animals are killed 18–20 hours later and ulcers are assessed[27]. Stomachs were dissected out and opened, contents were collected into clean tubes. Volume, pH, Free Acidity and Total acidity of gastric juice were determined[28].

13. Assessment of gastric ulcer

Macroscopic Evaluation: After opening along the greater curvature stomach was pinned in a wax tray to determine the ulcer index, inner surface was fairly examined for any lesions to be

seen both macroscopically and microscopically. The ulcer index was calculated macroscopically[29].

Ulcer index = 10/X

Where X = Total mucosal area/ Total ulcerated area

To measure the Total mucosal area &Ulcerated area dissected stomach was spread on cardboard with the mucus surface upwards avoiding corrugation; Tracing paper was placed over the stomach and the outline of stomach and the areas of erosions and ulceration were traced on it. This is then superimposed on graph paper having a millimetres scale[30].

Microscopic Evaluation (10X): Mucosal layer of glandular portion of stomach was scraped off through scalpel blade to prepare a T.S Slide then Draw a square of 1 mm in a drawing paper by means of stage micrometer in a microscope with the help of Camera lucida. Then trace and count no. of lesions and calculate its size by tracing all of forms lesions on a paper present in the area of $1mm^2$.

To calculate the Efficacy of extract and drug using the formula given by [31]

PREVENTIVE INDEX (%) $= \frac{\textbf{ulcer index (control)} - \textbf{ulcer index (test)}}{\textbf{ulcerindex (control)}}$ **X 100**

14. Histopathological studies

Tissue samples from the stomach of rats were fixed in 10% formalin saline for a minimum of 24 h and then dehydrated by washing in ascending grades of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned and stained with hematoxylin and Eosin (H and E). All sections were examined under light microscope (x4, x10 and x40) magnification.

15. Determination of biochemical parameters

1) Volume of gastric acid

Gastric juice was collected and centrifuged with the help of centrifugal machine at 3000 rpm for 10 minutes and finally, volume of juice was noted.

2) Determination of pH

pH of the gastric juice was measured by using digital pH meter.

3) Determination of free and total acidity

1 ml of gastric juice was collected into conical flask and titrated with 0.01 N NaOH using methyl orange as an indicator until yellow orange color was found. Further volume of the base or alkali was noted. This volume corresponds to free acidity. Then phenolphthalein was added and continues titrating until reddish color appears. Hence total vol. of alkali added showed total acidity[32].

Acidity was calculated using formula.

$$\text{Acidity} = \frac{\textit{Volume of NaOH X Actual Normality of NaOH X } 100}{0.1} \text{meq/1/100}$$

16. Statistical Analysis

Results expressed as the mean ± SD for six animals. Analysis was performed with the assistance of One- way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison test (Software "Graph pad prism") for determining the statistical significance between different groups. The results were judged significant, if $P<0.05$, $P<0.01$ and $P<0.001$[33].

RESULTS

1. Physicochemical parameters

Different Physicochemical parameters were performed on *Hemidesmus indicus* (L.) R. Br roots and *Tinospora cordifolia* (Wild.). Miers stem powder evaluated in Table No. 5.

Table No. 5.: Physicochemical parameters of *Hemidesmus indicus* (L.) R. Br roots and *Tinospora cordifolia* (Wild.) Miers stem powder

S.N.O	PARAMETERS	*H. indicus* (%)	*T. cordifolia* (%)
1.	Foreign matter	0.08%	0.7%
2.	Moisture content (Loss on drying)	4.5%	2%
3.	Alcohol Soluble Extractive value	26.08%	18.52%
4.	Water Soluble Extractive value	23.76%	15.28%
5.	Total ash	2.5%	8%
6.	Water soluble ash	3%	2.7%
7.	Acid insoluble ash	0.35%	1%

2. Percentage yield of ethanolic extracts of drugs

The color, consistency and % yields of ethanolic extracts of *Hemidesmus indicus* (L.) R.Br and *Tinospora cordifolia* (Wild.) Miers were observed after extraction, shown in Table No. 6.

Table No. 6: Percentage yield of extracts

Extracts	*H. Indicus* (L.) R Br.	*T. cordifolia* (Wild.) Miers
Color	Dark Brown	Green
Consistency	Solid	Semi solid
% yield	16.09 %	11.23 %

3. Phytochemical screening

Preliminary phytochemical screening was performed on ethanolic extract of root part of *H.indicus* (L.) R. Br (EEHI) and stem part of *T. cordifolia* (Wild.) Miers (EETC), results were shown in Table No. 7.

Table No. 7: Phytochemical screening of ethanolic extract of both drugs

Sr. No.	PHYTOCHEMICAL TEST	EEHI	EETC
A.	**Test for Carbohydrate**		
1.	Molish's test	+	+
2.	Fehling test	+	-
3.	Benedict's test	+	+
B.	**Test for Protein**		
1.	Biuret's test	+	+
2.	Millon's test	+	-
C.	**Test for Steroids**		
1.	Salkowaski test	+	-
D.	**Test for Glycosides**		
1.	Borntrager's test	-	+
2.	Keller Killani test	+	+
E.	**Test for Saponins**		
1.	Foam's test	-	+
F.	**Test for Alkaloids**		
1.	Hager's test	-	+
2.	Dragondorff test	+	-
G.	**Test for tannins and phenolic compounds**		
1.	Lead acetate test	+	-
2.	5 % $Fecl_3$ Solution	-	-
H.	**Test for Flavanoids**		
1.	Alkaline Reagent test	-	+
2.	Sulphuric acid test	+	-

+ = present, - = absent

Preliminary phytochemical screening was performed on Polyherbal Combine extracts (1:1) (PHCE), results were shown in Table No. 8.

Table No. 8.: Phytochemical screening of Polyherbal combine extract (PHCE)

Sr. No.	PHYTOCHEMICAL TEST	PHCE (1:1)
A.	**Test for Carbohydrate**	
1.	Molish's test	-
2.	Fehling test	-
3.	Benedict's test	+
B.	**Test for Protein**	
1.	Biuret's test	+
2.	Millon's test	+
C.	**Test for Steroids**	
1.	Salkowaski test	-
D.	**Test for Glycosides**	
1.	Borntrager's test	-
2.	Keller Killani test	+
E.	**Test for Saponins**	
1.	Foam's test	-
F.	**Test for Alkaloids**	
1.	Hager's test	-
2.	Dragondorff test	+
G.	**Test for tannins and phenolic compounds**	
1.	Lead acetate test	+
2.	5 % $Fecl_3$ Solution	-
H.	**Test for Flavanoids**	
1.	Alkaline Reagent test	-
2.	Sulphuric acid test	+

+ = present, - = absent

4. Thin layer chromatography

Different composition of mobile phases were tried for proper separation of constituents in ethanolic extracts of both the drugs and Polyherbal combine extracts (1:1), result was obtained as shown in Table No. 9 and 10.

Table No. 9: Thin layer Chromatography of ethanolic extracts of *Hemidesmus indicus* (L.) R. Br (EEHI) and *Tinsopora cordifolia* (Wild.) Miers (EETC)

Mobile phase	EEHI (No. of Spots)			EETC (No. of Spots)		
	Visible Light	U.V 254 (nm)	U.V 365 (nm)	Visible Light	U.V 254 (nm)	U.V 365 Nm
A.	-	-	3	-	-	2
A. n – Hexane : Chloroform : methanol (2:4.5:0.5 v/v/v)						
B.	-	-	1	1	1	1
B. Methanol: Ethyl acetate (1:9 v/v)						
C.	-	1	2	1	1	-
C. Chloroform : Methanol (9.5:0.5 v/v)						
D.	1	-	1	1	-	1
D. Hexane: Ethyl acetate (8:2 v/v)						
E.	-	1	-	1	2	2
E. Ethyl acetate : Formic acid : acetic acid : water (8.3:0.9:0.9:2.3 v/v/v/v)						
F.	-	-	-	1	2	1
F. Ethyl acetate: methanol: Acetic acid: water(6.5: 1.5:1:1 v/v/v)						

Table No. 10: Thin layer Chromatography of Polyherbal combine extracts (1:1) (PHCE)

	NO. OF. SPOTS		
Mobile phase	**Visible Light**	**U.V 254 (nm)**	**U.V 365 (nm)**
A.	-	-	3
A. n – hexane : Chloroform: methanol (2:4.5:0.5 v/v/v)			
B.	1	1	-
B. Methanol : Ethyl acetate (1:9 v/v)			
C.	1	-	1
C. Chloroform : Methanol (9.5:0.5 v/v)			
D.	-	-	1
D. Hexane : Ethyl acetate (8:2 v/v)			
E.	1	2	2
E. Ethyl acetate : Formic acid : acetic acid : water (8.3:0.9:0.9:2.3 v/v/v/v)			
F.	1	1	1
F. Ethyl acetate: methanol: Acetic acid: water(6.5: 1.5:1:1 v/v/v)			

On the basis of above result in ethanolic extract of both the plants and their Polyherbal combine extracts, maximum no. of spots were separated in mobile phase of composition n-hexane: chloroform: methanol (2: 4.5: 0.5 v/v/v) at 365 nm with their Rf values calculated in Table No. 11.

Table No. 11: TLC profile of three extracts with maximum no. of spots at 365 nm in a solvent n-hexane: chloroform: methanol (2: 4.5: 0.5 v/v/v)

Extracts	Solvent system	No. of spots (at 365 nm)	Rf value
EEHI	A.	3	0.78, 0.71, 0.38
EETC	A.	2	0.24, 0.42
PHCE (1:1)	A.	3	0.44, 0.79, 0.35

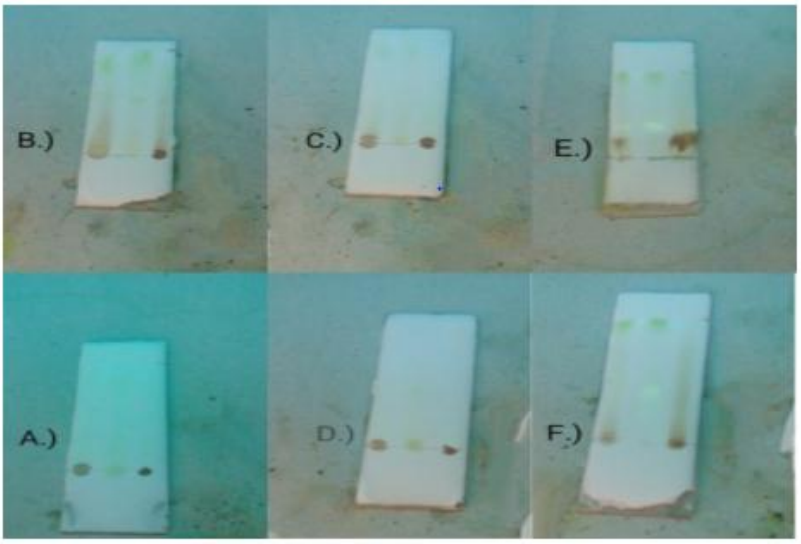


Figure No. 2: Preparative TLC of ethanolic extracts of three samples i.e *Hemidesmus indicus* (L.) R. Br (EEHI), *Tinsopora cordifolia* (Wild.) Miers (EETC) and thier Polyherbal Combine extracts (PHCE) at UV 254 nm in a above given 6 different solvents

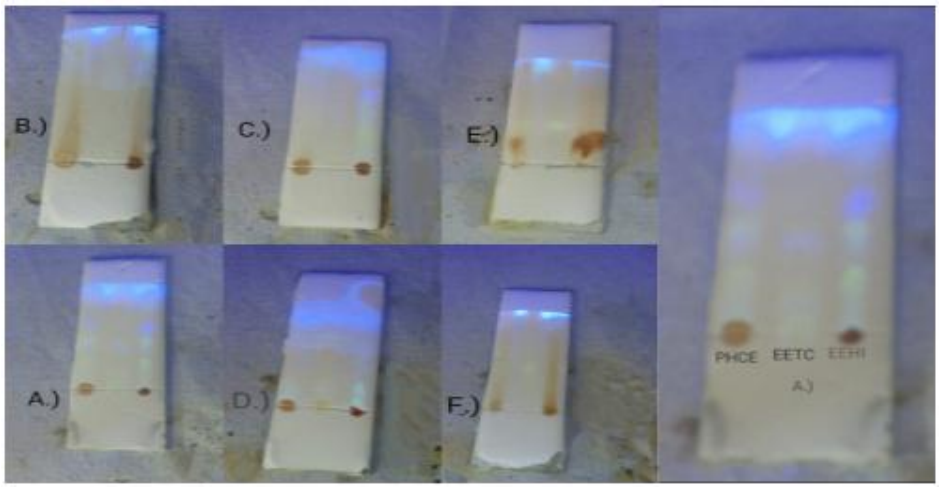


Figure No. 3: Preparative TLC of ethanolic extracts of three samples i.e *Hemidesmus indicus* (L.) R. Br (EEHI), *Tinsopora cordifolia* (Wild.) Miers (EETC) and thier Polyherbal Combine extracts (PHCE) at UV 365 nm in a 6 different solvents, solvent A maximum no. of spots were seen

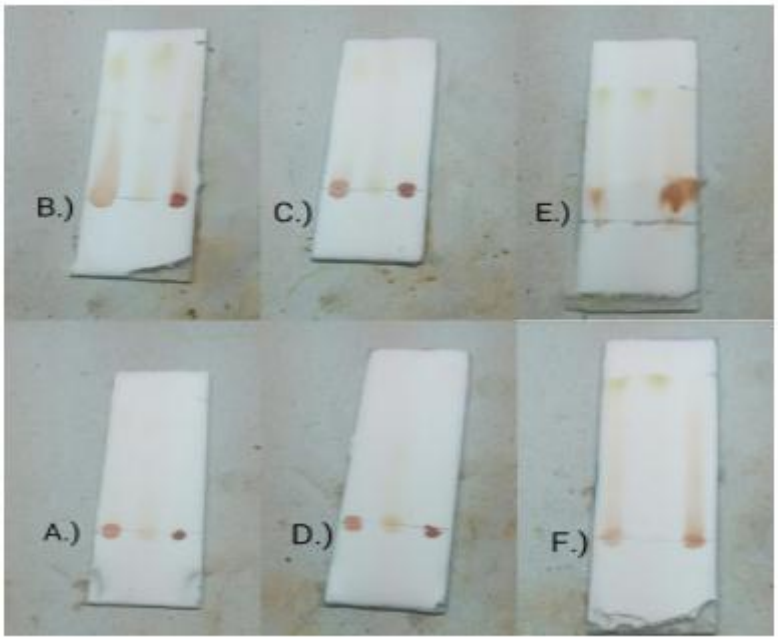
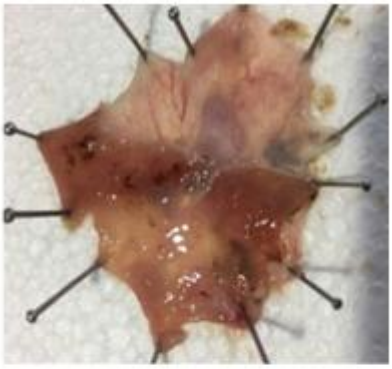
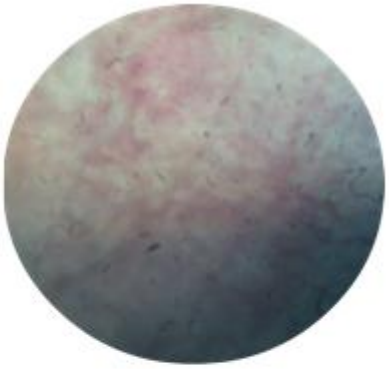


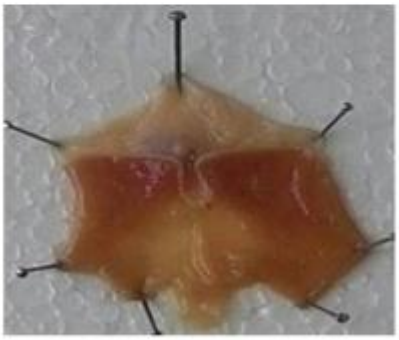
Figure No. 4: Preparative TLC of ethanolic extracts of three samples i.e *Hemidesmus indicus* (L.) R. Br (EEHI), *Tinsopora cordifolia* (Wild.) Miers (EETC) and thier Polyherbal Combine extracts (PHCE) at visible light in a 6 different solvents



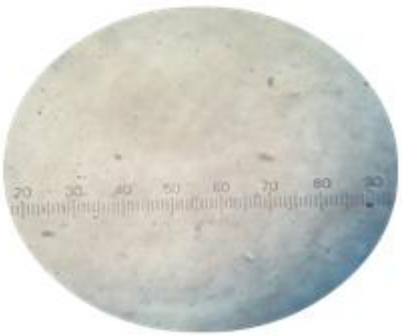
Vehicle control group (1ml/kg)



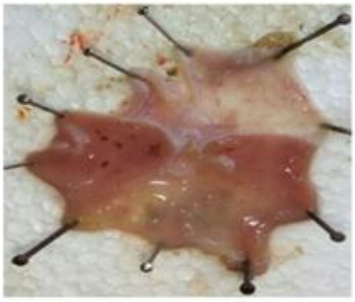
Redness seen all over the field(10X)



Standard drug Femotidine (20mg/kg)

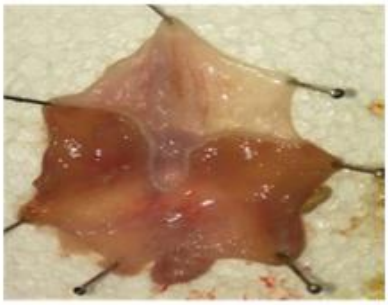


No ulcer appeared in a field (10X)

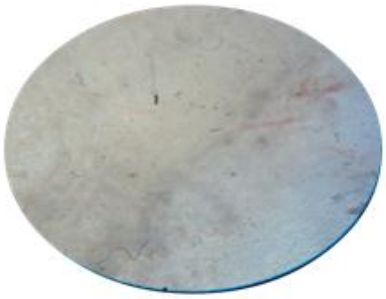


EEHI-200mg/kg

Moderate lesions (10X)

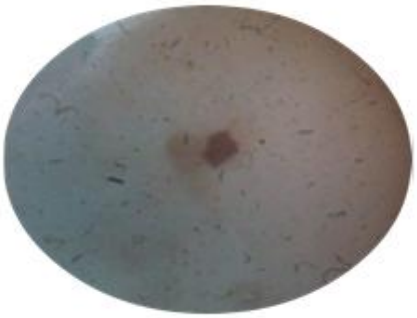


EEHI-400mg/kg

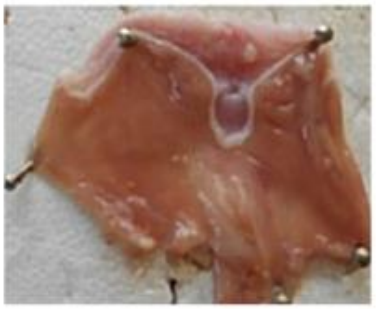


Few lesions appears on a right side

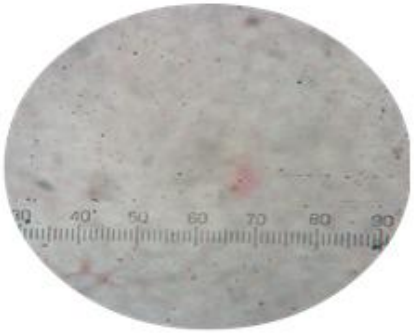
EETC-200mg/kg



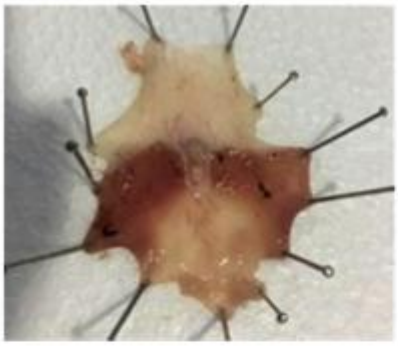
Red spots observed (10X)



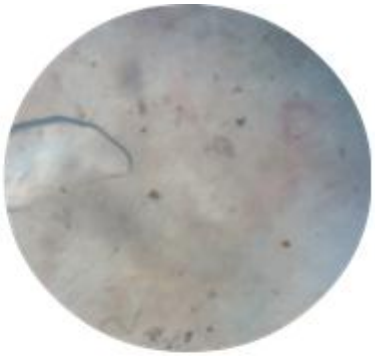
EETC-400mg/kg



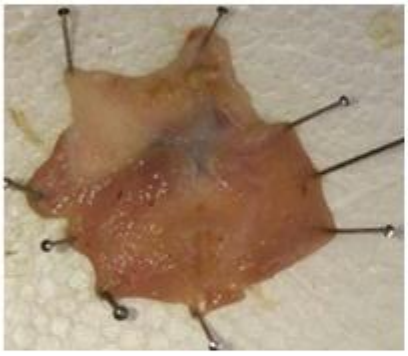
Less ulceration were found (10X)



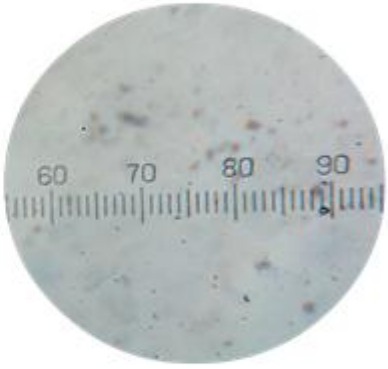
PHCE-200mg/kg (1:1)



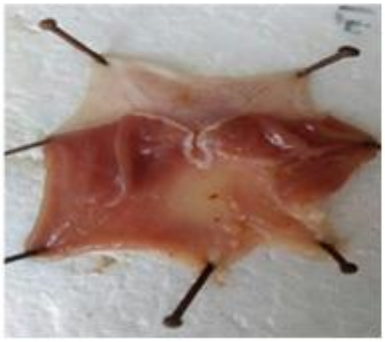
Lesions appeared on the field



PHCE- 200mg/kg (1:2)

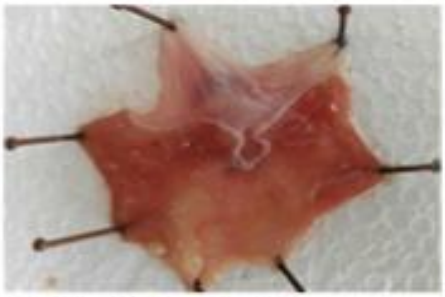


Small red lesions appeared



PHCE- 200mg/kg (2:1)

Red Coloration appeared (10X)



PHCE – 400mg/kg (1:1)

No Ulceration appeared in a Field

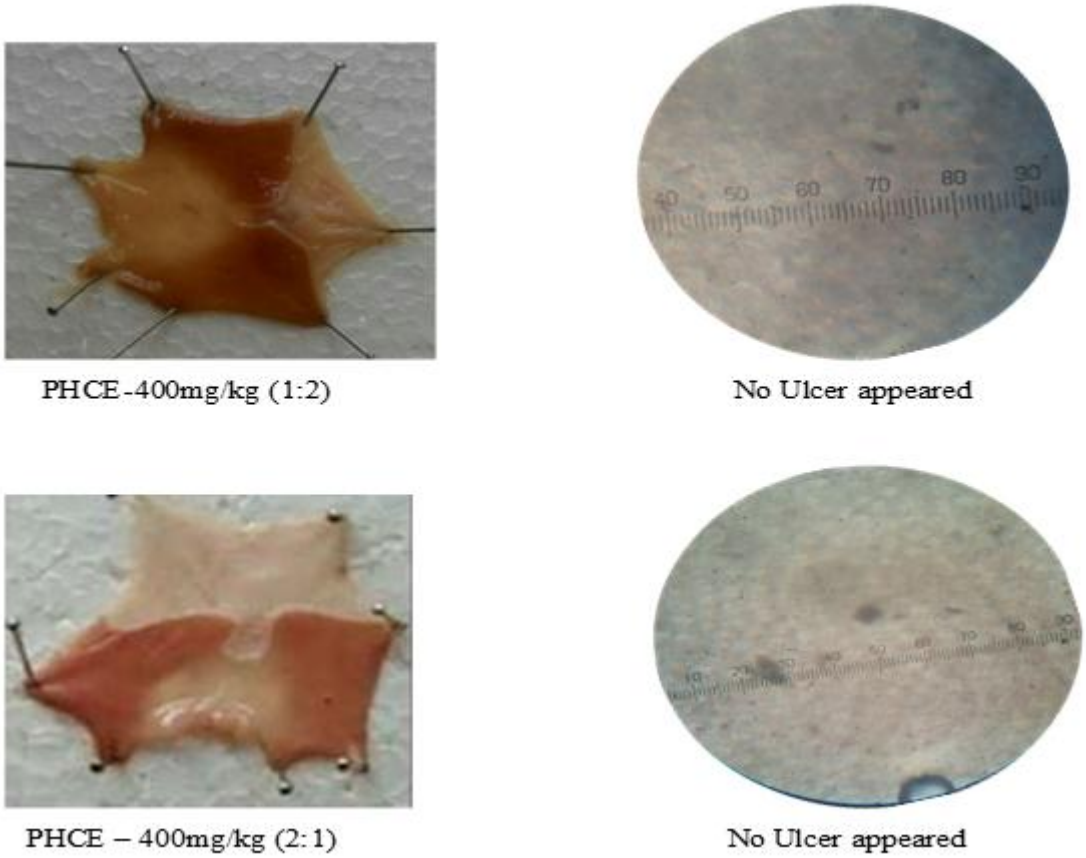


Figure No. 5: Observation of erosions in pylorus ligated stomach of rats.

5. EVALUATION OF ULCER AND BIOCHEMICAL PARAMETERS AGAINST PYLORUS LIGATION INDUCED MODEL

A. INDIVIDUAL TREATMENT

1. Test Drug 1- Ethanolic Extracts of *Hemidesmus indicus* (L.) R. Br (EEHI)

a) Effect of EEHI on Ulcer index and Percentage preventive index

The anti ulcer activity of EEHI in pylorus ligation induced ulcer is reported in Table No. 12 where EEHI were effective in significant reduction of ulcer index i.e., EEHI 200 mg/kg; (*P<0.05 vs control) as it reduced ulcer index to (0.4856±0.1694*), (EEHI400 mg/kg); (**P<0.01 vs control) reduced ulcer index to (0.261±0.0783**) and Standard drug (Famotidine); (***P<0.001 vs control) reduced ulcer index to (0.1658±0.0460***).

EEHI at high dose 400 mg/kg showed a statistical significant (**P<0.001) better protection when compared with control group. In comparison to pretreated famotidine group, EEHI did

not show any significant difference. Therefore, *Hemidesmus indicus* (L.) R. Br administered in doses 200 and 400 mg/kg orally observed a dose dependent decrease in ulcer index in pylorus ligated rats.

The percentage Preventive index for EEHI 200 mg/kg and 400 mg/kg was 44.32 % and 70.7 %, while standard drug was at maximum protection with 80.99%. Average number of ulcers and ulcer size (mm) were also significantly reduced in 200 mg/kg and 400 mg/kg EEHI i.e. (16.2±9.4180), (0.05728±0.0292) and (7.4±3.2093) (0.04688±0.00562) in comparison to the control (untreated group) group.

Table No. 12: Effect of Ethanolic Extract of *Hemidesmus indicus* (L.) R. Br on various ulcer parameters against Pylorus ligation induced ulcer

TREATMENT	MACROSCOPIC EVALUATION		MICROSCOPIC EVALUATION	
	Ulcer Index	Preventive Index %	Average no. of ulcers	Size (mm)
Group I Control (1 ml/kg)	0.8722±0.1005	00.00	25.4±5.941380	0.07712±0.003945
Group II Femotidine 20 mg/kg	0.1658±0.0460***	80.99%	5.8±2.387467	0.03546±0.006381
Group III EEHI 200 mg/kg (p.o)	0.4856±0.1694*	44.32%	16.2±9.418068	0.05728±0.029224
Group IV EEHI 400 mg/kg (p.o)	0.261±0.0783**	70.7%	7.4±3.209361	0.04688±0.005628

Data was represented as mean ± SD where n=6 rats per group. Values are significantly different from control group by one way- ANOVA followed by Turkeys' multiple comparison tests. *P<0.05; **P<0.01, ***P<0.001 were considered to be statistical significant.

b) Effect of EEHI on the biochemical parameters obtained from gastric juice of stomach.

Effect of EEHI on gastric volume, pH, free acid and total acid were studied in pylorus ligated rats in Table No. 13, where EEHI (200 and 400 mg/kg) has shown significant (*P<0.05 vs control) inhibition of volume of juice secreted in rats by, (2.64±0.7056*) and (2.0±0.5700*). Free acid were significantly (*P<0.05 vs control) reduced by the extract (200 mg/kg and 400

mg/kg) i.e.,(5.842±0.8881*) and (5.544±1.2234*) and Total acid were reduced (***P<0.001 vs control) by the extract (200 mg/kg and 400 mg/kg) i.e., (11.908±1.183***) and (10.818±0.921***). However EEHI (200 mg/kg and 400mg/kg induced significant (*P<0.05 vs control) increase in pH from (2.712±0.15139*) and (2.954±0.6390*) respectively.

Table No. 13: Effect of Ethanolic Extract of *Hemidesmus indicus* (L.) R. Br on gastric acid secretion in pylorus ligation method

Treatment	Vol. of gastric juice (ml)	pH	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)
Group I Vehicle 1 ml/kg	2.84±0.665582	1.974±0.553922	7.852±1.227	18.04±2.6034
Group II Femotidine 20 mg/kg	1.66±0.559*	3.626±0.563***	4.238±0.989***	6.766±0.5605***
Group III EEHI 200 mg/kg (p.o)	2.64±0.7056*	2.712±0.15139*	5.842±0.8881*	11.908±1.183***
Group IV EEHI 400 mg/kg (p.o)	2.0±0.5700*	2.954±0.6390*	5.544±1.2234*	10.818±0.921***

Data was represented as mean±SD where n=6 rats per group. Values are significantly different from control group by one way- ANOVA followed by Turkeys' multiple comparison tests. *P<0.05; **P<0.01, ***P<0.001 were considered to be statistical significant.

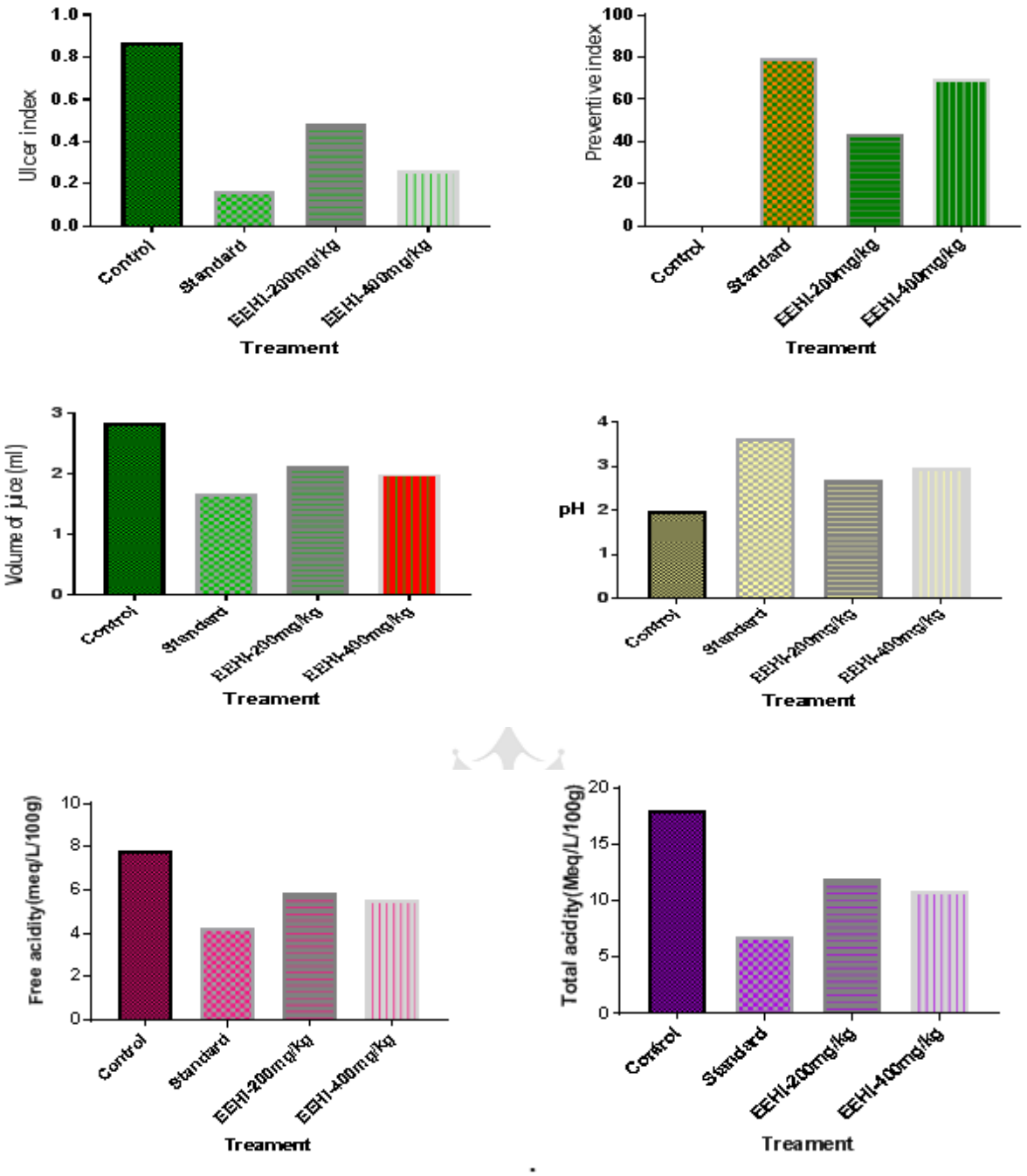


Figure No. 6: Effect of Ethanolic Extract of *Hemidesmus indicus* (L.) R.Br on various parameters in Pylorus ligation induced ulcers:-Ulcer index**,** Preventive index**,** Volume of juice (ml)**,** pH**,** Free acidity (mEq/L/100 g) and Total acidity (mEq/L/100 g).

2. Test Drug 2- Ethanolic Extracts of *Tinospora cordifolia* (Wild.) Miers. (EETC)

a) Effect of EETC on Ulcer index and Percentage preventive index

The anti ulcer activity of EETC in pylorus ligation induced ulcer is reported in Table No. 14 where EETC treatment produced a significant reduction of ulcer index (EETC 200mg/kg;(**P<0.01 vs control) reduced ulcer index up to (0.5112±0.0702**).(EEHI 400 mg/kg and standard drug (Famotidine); (***P<0.001 vs control) were reduced ulcer index much better up to (0.249±0.0719***) and (0.1658±0.0460***).

EETC at high dose 400 mg/kg showed a statistical significant (**P<0.001) better protection when compared with control group. In comparison to pretreated famotidine group, EETC did not show any significant difference. Therefore, *Tinospora cordifolia (Wild.) Miers* administered in doses 200 and 400 mg/kg orally showed a dose dependent decrease in ulcer index in pylorus ligated rats.

The percentage Preventive index for EETC 200 mg/kg was 41.38%, and for high dose 400 mg/kg was 71.45% while Standard drug (Famotidine) exhibited maximum 80.99 %. protection.

Average number of ulcers and ulcer size (mm) were also significantly reduced in 200 mg/kg and 400 mg/kg EETC i.e. (20.2±10.7098), (0.0657±0.0121), and (8.8±3.9623), (0.04692±0.0111) in comparison to the control group.

Table No. 14: Effect of Ethanolic Extract of *Tinospora cordifolia* (Wild.) Miers on ulcer parameters against Pylorus ligation induced ulcers

TREATMENT	MACROSCOPIC EVALUATION		MICROSCOPIC EVALUATION	
	Ulcer Index	Preventive Index %	Average no. of ulcers	Size (mm)
Group I Vehicle 1 ml/kg	0.8722±0.1005	00.00	25.4±5.941380	0.07712±0.003945
Group II Femotidine 20 mg/kg	0.1658±0.046***	80.99%	5.8±2.387467	0.03546±0.006381
Group III EETC 200 mg/kg (p.o)	0.5112±0.0702**	41.38%	20.2±10.709	0.0657±0.0121
Group IV EETC 400 mg/kg (p.o)	0.249±0.0719***	71.45%	8.8±3.9623	0.04692±0.0111

Data was represented as mean ± SD where n=6 rats per group. Values are significantly different from control group by one way- ANOVA followed by Turkeys' multiple comparison tests. *P<0.05; **P<0.01, ***P<0.001.were considered to be statistical significant.

b) Effect of EETC on the biochemical parameters obtained from gastric juice of stomach.

Effect of EETC on gastric volume, pH, free acid and total acid were studied in pylorus ligated rats in Table No. 15, where EETC (200 mg/kg) has shown significant (*P<0.05 vs control) reduction of volume of juice, free acid and total acid; (***P<0.01 vs control) in rats by, (2.74±0.4393*),(6.168±0.8429*) and (11.75±1.6612***) while in case of high dose (400 mg/kg) EETC has shown significant(**P<0.01 vs control) reduction of volume of juice, free acid and total acid; (***P<0.001vs control) in rats by, (1.92±0.7259**), (5.2±0.7478**) and (10.85±1.2669***). However EETC 200 mg/kg induced significant (*P<0.05 vs control) increase in pH from (2.352±0.5904*) and EETC 400 mg/kg significantly (**P<0.01 vs control) elevated pH from (2.864±0.3644**) respectively.

Table No. 15: Effect of Ethanolic Extract of *Tinospora cordifolia* (Wild.) Miers on gastric acid secretion in pylorus ligation method

Treatment	Volume of gastric juice (ml)	pH	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)
Group I Vehicle 1 ml/kg	2.84±0.665582	1.974±0.553922	7.852±1.227017	18.04±2.603459
Group II Femotidine 20 mg/kg	1.66±0.55946*	3.626±0.563***	4.238±0.989***	6.766±0.5605***
Group III EETC 200 mg/kg (p.o)	2.74±0.4393*	2.352±0.5904*	6.168±0.8429*	11.75±1.6612***
Group IV EETC 400 mg/kg (p.o)	1.92±0.7259**	2.864±0.3644**	5.2±0.7478**	10.85±1.2669***

Data was represented as mean ± SD where n=6 rats per group. Values are significantly different from control group by one way- ANOVA followed by Turkeys' multiple comparison tests. *P<0.05; **P<0.01, ***P<0.001 were considered to be statistical significant.

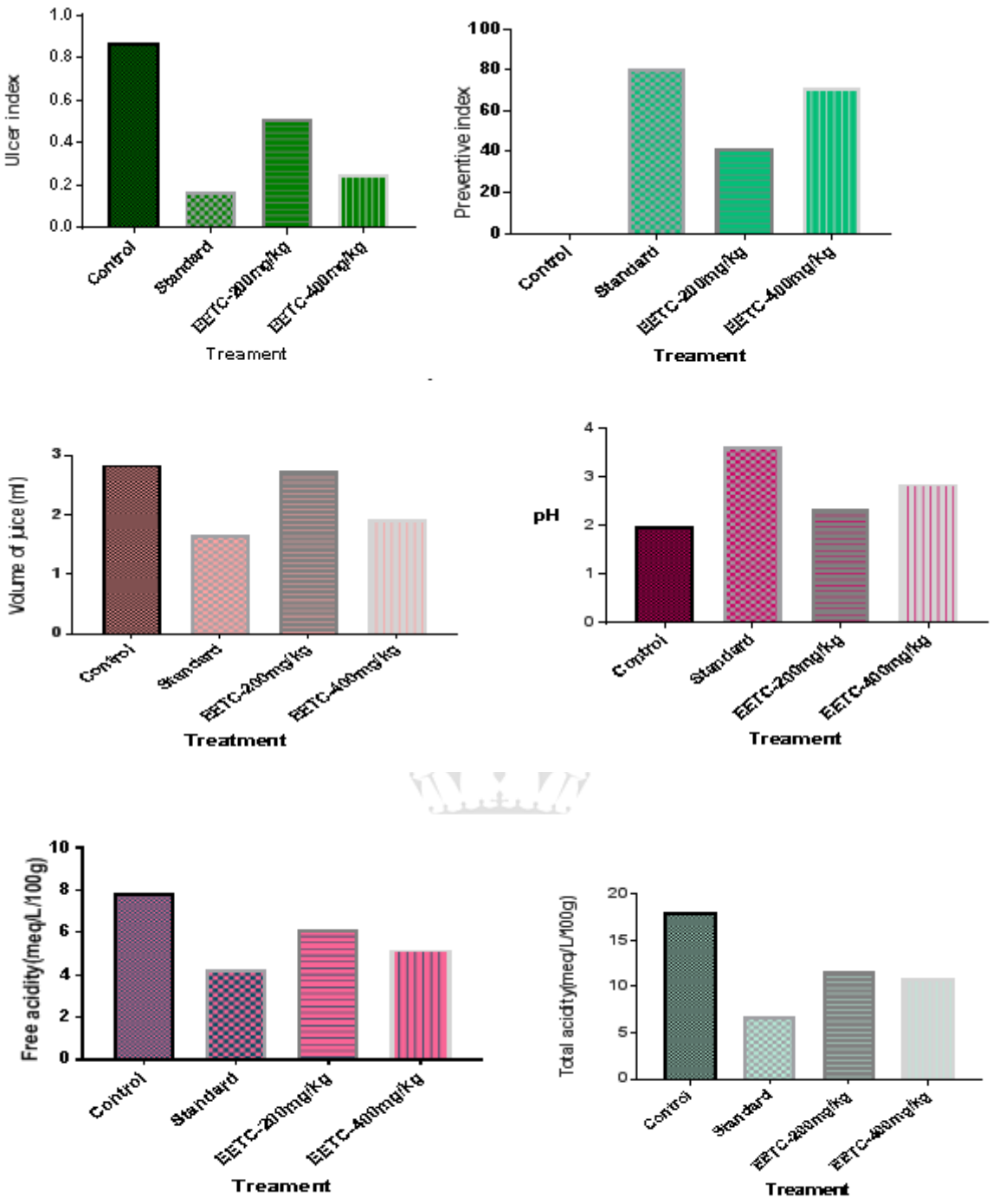


Figure No. 7: Effect of Ethanolic Extract of *Tinospora cordifolia* (Wild.) Miers on various parameters against Pylorus ligation induced ulcers: Ulcer index**,** Preventive index**,** Volume of juice (ml)**,** pH, Free acidity (mEq/L/100 g) and Total acidity (mEq/L/100 g).

B. POLYHERBAL COMBINED TREATMENT

1. Polyherbal Combine Extract of *Tinospora cordifolia* (Wild.) Miers (EETC) and *Hemidesmus indicus* (L.) R. Br (EEHI)

a) Effect of PHCE-200 mg/kg and 400 mg/kg (in a three combined ratio of (1:1, 1:2 and 2:1) on ulcer index and Percentage preventive index.

The antiulcer activity of (PHCE=EETC+EEHI) in Pylorus ligation induced ulcer is reported in Table No. 16, where PHCE were effective in a significant reduction of ulcer index, (PHCE=EETC+EEHI) 200 mg/kg in a three combined ratio of 1:1, 1:2 and 2:1 has shown significant reduction of ulcer index i.e., (**P<0.01 vs control) (0.5266±0.1488**), (0.3584±0.0791**) and (0.3914±0.0976**).

whereas (PHCE=EETC+EEHI) 400 mg/kg in three combined ratio of 1:1, 1:2 and 2:1 has shown significant reduction of ulcer index with better healing protection i.e., (***P<0.001vscontrol), (0.302±0.0854***), (0.274±0.0628***) and (0.277±0.0656***).

Therefore, PHCE of 400 mg/kg in combine ratio (1:1, 1:2 and 2:1) has shown dose dependent synergistic effect with better efficacy.

The percentage Preventive index for (PHCE=EETC+EEHI) 200 mg/kg in ratio 1:1, 1:2 and 2:1 was 39.62%, 58.90% and 55.12% However (PHCE=EETC+EEHI) 400 mg/kg in ratio 1:2, 2:1 has shown maximum inhibition i.e., 68.85%, 68.24% when compared to 1:1 i.e., 65.37%.

Average number of ulcers were significantly reduced in low combination of doses (PHCE=EETC+EEHI) 200 mg/kg in a combined ratio 1:1, 1:2 and 2:1 i.e. (24.0±5.403702), (20.2±8.671793) and (22.2±9.95992). However ulcer size (mm) were also decreased to (0.05356±0.017783), (0.05954±0.017313) and (0.05602±0.018312).

while in case of high combination of doses (PHCE=EETC+EEHI) 400 mg/kg in a combined ratio 1:1, 1:2 and 2:1, Average number of ulcers were (8.75±1.70782), (8.5±3.10912),(8.75±3.6855) and ulcer size(mm) were also significantly decreased to (0.05197±0.0115), (0.04855±0.0192), (0.04475±0.0110) in comparison to control group.

Table No. 16: Effect of dose of 200 mg/kg and 400 mg/kg Polyherbal Combine extracts on ulcer parameters against pylorus ligation induced ulcer

TREATMENTS (200 mg/kg and 400 mg/kg)	Microscopic evaluation		Macroscopic evaluation	
	Ulcer index	Preventive index %	Average no. ulcers	Size (mm)
Group I Vehicle 1 ml/kg	0.8722±0.1005	00.00	25.4±5.94138	0.07712±0.003945
Group II Femotidine 20 mg/kg	0.1658±0.0460***	80.99%	5.8±2.3874	0.03546±0.006381
Group III PHCE- 200 mg/kg (1:1)	0.5266±0.1488**	39.62%	24.0±5.403702	0.05356±0.017783
Group IV PHCE- 200 mg/kg (1:2)	0.3584±0.0791**	58.90%	20.2±8.671793	0.05954±0.017313
Group V PHCE- 200 mg/kg (2:1)	0.3914±0.0976**	55.12%	22.2±9.95992	0.05602±0.018312
Group VI PHCE- 400 mg/kg (1:1)	0.302±0.0854***	65.37%	8.75±1.707825	0.05197±0.0115
Group VII PHCE- 400 mg/kg (1:2)	0.274±0.0628***	68.85%	8.5±3.109126	0.04855±0.0192
Group VIII PHCE- 400 mg/kg (2:1)	0.277±0.0656***	68.24%	8.75±3.68555	0.04475±0.0110

Data was represented as mean±SD where n=6 rats per group. Values are significantly different from control group by one way- ANOVA followed by Turkeys' multiple comparison tests. *P<0.05; **P<0.01, ***P<0.001 were considered to be statistical significance.

b) Effect of PHCE 200 mg/kg and 400 mg/kg (in a three combined ratio of 1:1, 1:2 and 2:1) on the parameters obtained from gastric juice of stomach.

Effect of (PHCE=EETC+EEHI) on gastric volume, pH, free acid and total acid were studied in pylorus ligated rats in Table No. 17, where (PHCE=EETC+EEHI) of dose 200 mg/kg in a combined ratio 1:1 has shown significant reduction of volume of juice, free acid(*P<0.05 vs control) and total acid (***P<0.001 Vs control) i.e., 1:1 were reduced to (2.74±0.4258*), (6.574±0.65098*) and (12.206±1.46***). However (PHCE=EETC+EEHI) of dose 200 mg/kg of combined ratio 1:2 and 2:1 has shown significant reduction of volume of juice, free acid (**P<0.01 vs control) and total acid (***P<0.001 vs control) i.e., 1:2 were reduced to (2.34±0.67305**), (5.784±0.8909**) and (11.87±1.080***) and 2:1 were reduced to (2.47±0.463681**), (5.72±0.86385**) and (12.232±1.03***) respectively.

In case of (PHCE=EETC+EEHI) of dose 400 mg/kg in a combined ratio 1:1, 1:2 and 2:1 were produced significant reduction of volume of juice, free acid (*P<0.05 Vs Control) and total acid (***P<0.001 Vs Control) i.e., 1:1 were reduced to (2.04±0.6348*), (5.24±0.82036*) and (10.42±1.930***), 1:2 were reduced to (1.98±0.5932*), (5.4±1.35462*) and (9.34±1.4117***) and 2:1 were reduced to (2.08±0.4658*), (4.82±0.8871*) and (9.78±1.8267***) respectively.

pH were also significantly (*P<0.05 Vs Control) enhanced in case of both doses 200 mg/kg and 400 mg/kg of combined ratio 1:1, 1:2 and 2:1 i.e., (2.326±0.6171*), (2.488±0.5580*), (2.436±0.6674*) and (2.572±0.85318*), (2.466±0.50535*), (2.726±0.64967*).

Table No. 17: Effect of dose of 200 mg/kg and 400 mg/kg Polyherbal Combine extracts on the parameters of gastric juice

Treatment (200 mg/kg)	Volume of gastric juice (ml)	pH	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)
Group I Vehicle 1 ml/kg	2.84±0.665582	1.974±0.5539	7.852±1.227017	18.04±2.603459
Group II Femotidine 20 mg/kg	1.66±0.559464*	3.626±0.563***	4.238±0.989***	6.766±0.560***
GroupIII PHCE- 200 mg/kg (1:1)	2.74±0.4258*	2.326±0.6171*	6.574±0.65098*	12.206±1.46***
GroupIV PHCE- 200 mg/kg (1:2)	2.34±0.67305**	2.488±0.5580*	5.784±0.8909**	11.87±1.080***
Group V PHCE- 200 mg/kg (2:1)	2.47±0.46368**	2.436±0.6674*	5.72±0.86385**	12.232±1.03***
GroupVI PHCE- 400 mg/kg (1:1)	2.04±0.6348*	2.572±0.85318*	5.24±0.82036*	10.42±1.930***
Group VII PHCE- 400 mg/kg (1:2)	1.98±0.5932*	2.466±0.50535*	5.4±1.35462*	9.34±1.4117***
Group VIII PHCE- 400 mg/kg (2:1)	2.08±0.4658*	2.726±0.64967*	4.82±0.8871*	9.78±1.8267***

Data was represented as mean±SD where n=6 rats per group. Values are significantly different from control group by one way- ANOVA followed by Turkeys' multiple comparison tests. *P<0.05; **P<0.01, ***P<0.001 were considered to be statistical significant.

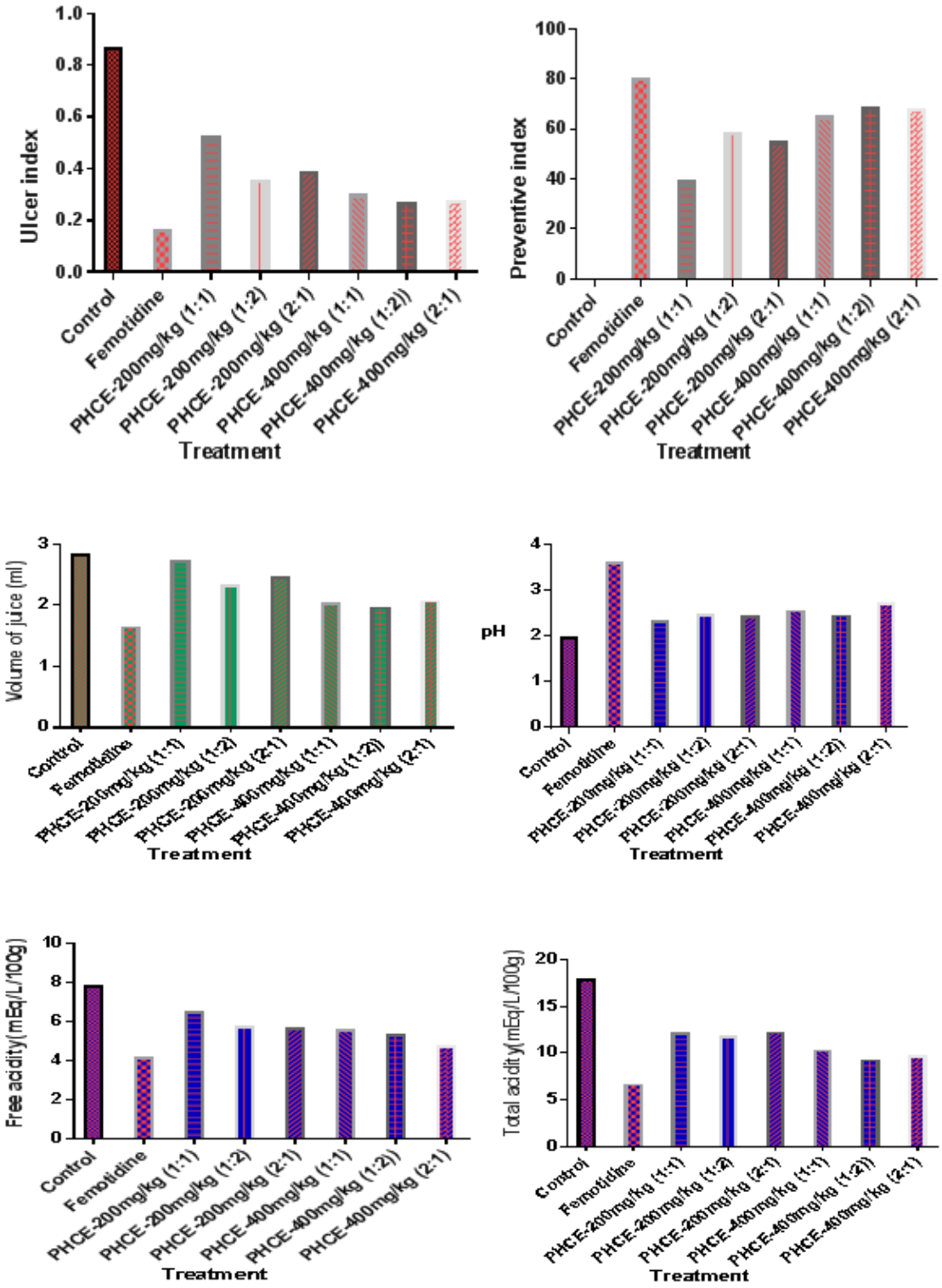


Figure No. 8: Effect of dose of 200 mg/kg and 400 mg/kg in a different combine ratios of Polyherbal Combine extracts of *Tinospora cordifolia* (Wild.) Miers. and ***Hemidesmus indicus* (L.) R. Br** on various parameters against pylorus ligation ulcers. Ulcer index, Preventive index**,** Volume of juice (ml)**,** pH**,** Free acidity (mEq/L/100 g) and Total acidity (mEq/L/100 g)

6. HISTOPATHOLOGY OF RAT STOMACH

1) When ethanolic extracts of *H. indicus* and *T. cordifolia* were administered

In Histopathology examination of stomach specimens of Group, I (Vehicle) were given only 0.5% CMC (1 ml/kg) orally prior to ulcer induction. The histopathology revealed chronic inflammation of mucosa with inflammatory cells (Lymphocytes) infiltration in Figure No. 9 and 10.

The albino rats from group II treated with Standard drug Famotidine showed no significance change in Histopathology almost normal appearance i.e., mucosa was seen fairly protected in Figure No. 11 and 12.

However, Rats from group III and IV treated with Test drug 1 Ethanolic Extracts of *Hemidesmus indicus* (L.) R. Br (EEHI), From the group III rats treated with dose of 200 mg/kg, histopathology showed superficial ulcers with desquamation of epithelium of the gastric mucosa with minimum variation from normal morphology in Figure No. 13 and 14. From the group 1V rats treated with the high dose of 400 mg/kg showed a better protection with less superficial ulceration in few areas of epithelium in Figure No. 15 and protected intact mucosal epithelium in other areas were seen in Figure No. 16.

Now in case of again rats from group III and IV treated with Test drug 2- Ethanolic Extracts of *Tinospora cordifolia* (Wild.) Miers (EETC), from the group III rats treated with the EETC dose of 200 mg/kg, histopathology revealed moderate ulcers with minimum variation from normal morphology of mucosa in Figure No. 17 and 18. From the group IV rats, EETC treated with the high dose 400 mg/kg showed no significant change in morphology i.e. normal appearance of mucosa in Figure No. 19 and 20.

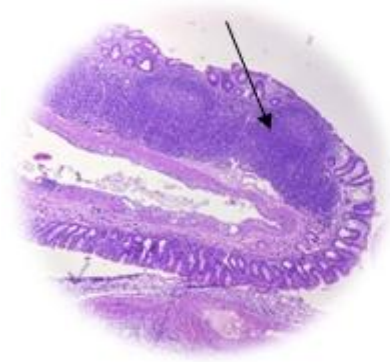


Figure No. 9 (X100)

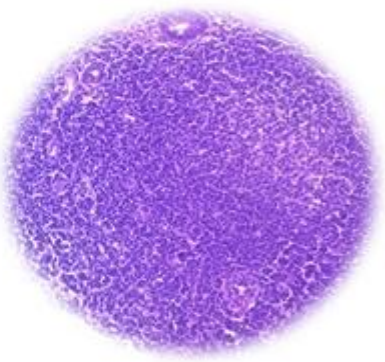
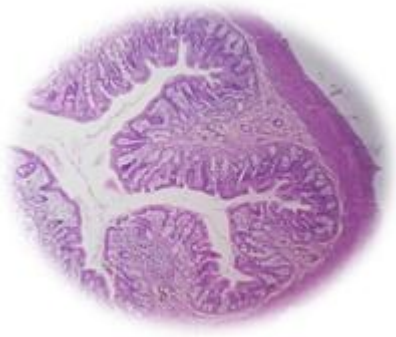


Figure No. 10(X400)

a) Control, Untreated group



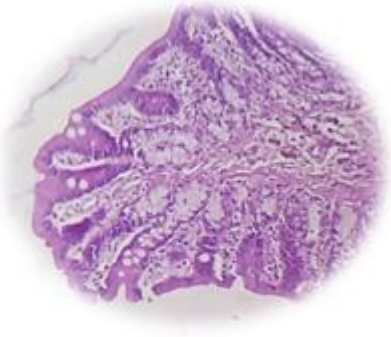
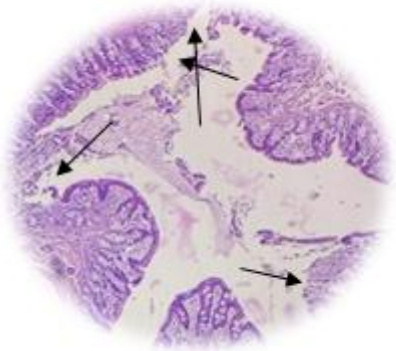


Figure No. 11(X100) **Figure No. 12 (X400)**

b) Famotidine -20 mg/kg



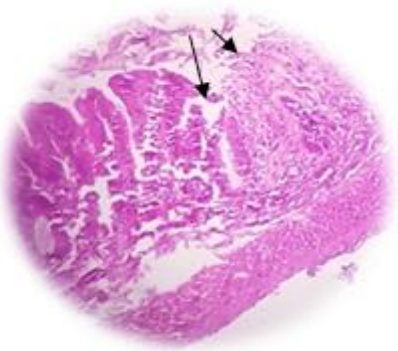
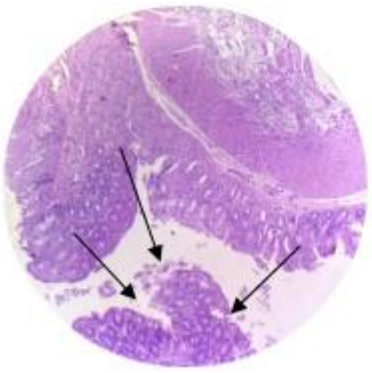


Figure No. 13(X100) **Figure No. 14 (X400)**

c) EEHI-200mg/kg



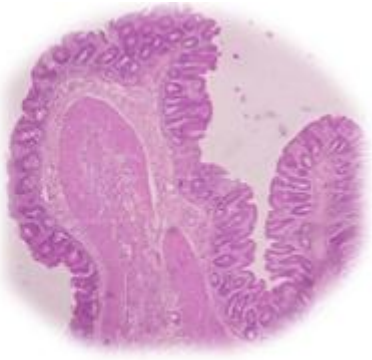
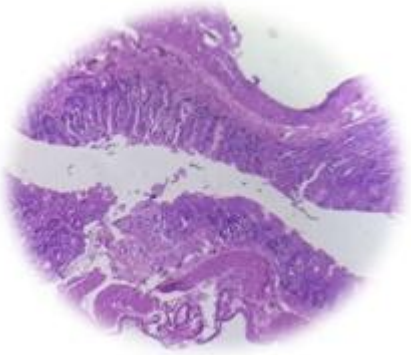


Figure No. 15(X100) **Figure No. 16 (X400)**

d) EEHI 400 mg/kg



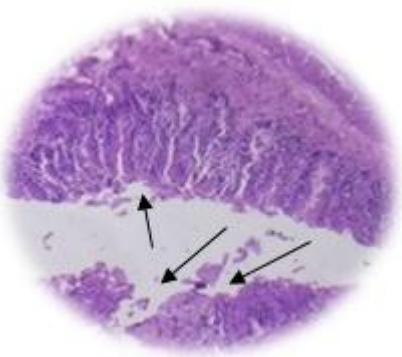
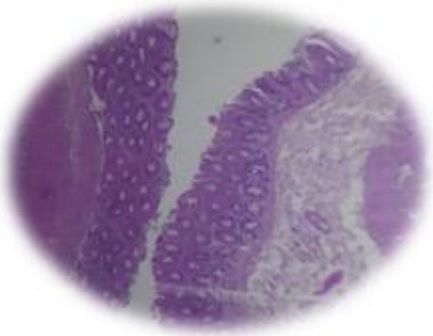


Figure No. 17(X100) **Figure No. 18 (X400)**

e) EETC 200 mg/kg



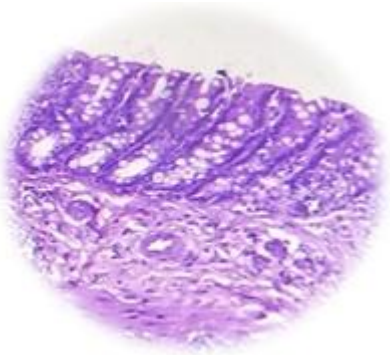
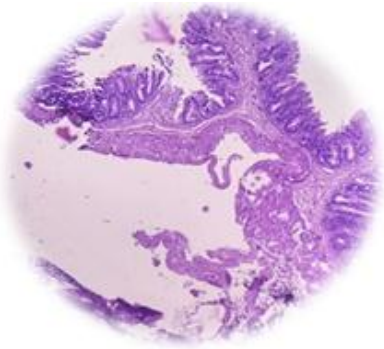


Figure No. 19 (X100) **Figure No. 20 (X400)**

f) EETC 400 mg/kg

2) When Polyherbal combine drug were administered

Histopathology of their polyherbal combine treatment of rats from the group III, IV and V has shown moderate to less ulceration. At a dose of 200 mg/kg and its low combination of doses in a ratio (1:1 in Figure No. 21 & 22, 1:2 in Figure No. 23 & 24 and 2:1 in Figure No. 25 & 26) has given significant dose dependent synergistic effect with less ulceration and minimum variation in morphology. But when same combine drug has given in high combination of doses 400 mg/kg at a ratio (1:1 in (Figure No. 27), 1:2 in Figure No. 28) and 2:1in Figure No. 29) to the rats from the group VI, VII and VIII histopathology revealed no significant change in morphology of gastric mucosa. Therefore, a High combination of doses has given high synergistic gastro-protective effect.



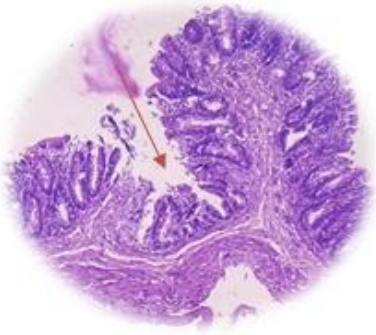
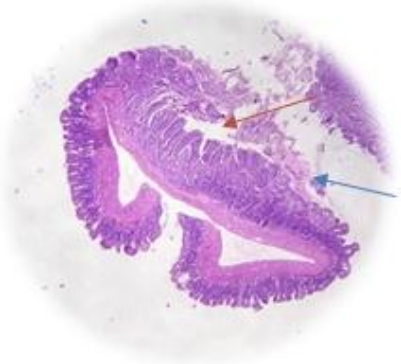


Figure No. 21(X100) **Figure No. 22(X400)**

a) PHCE-200 mg/kg (1:1)



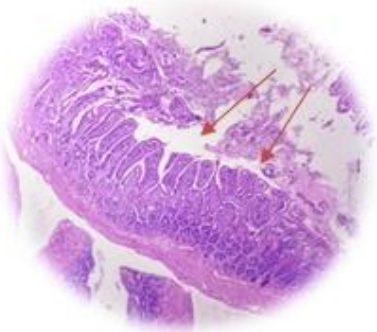
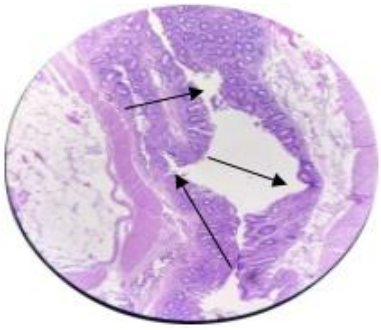


Figure No. 23(X100) **Figure No. 24(X400)**

b) PHCE-200mg/kg (1:2)



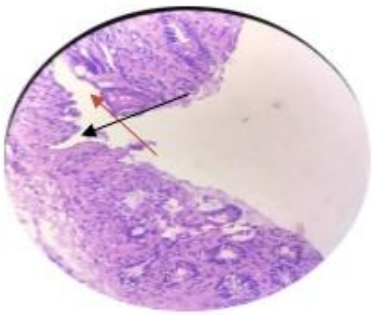


Figure No. 25(X100) **Figure No. 26(X400)**

c) PHCE-200 mg/kg (2:1)

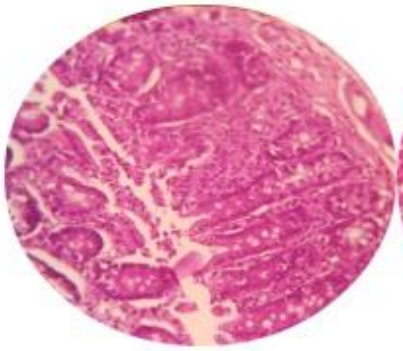
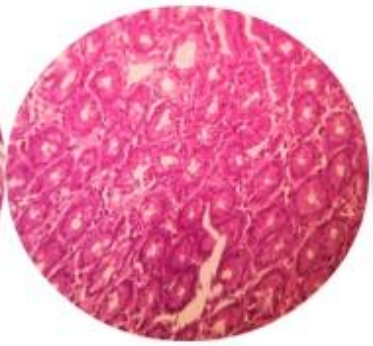
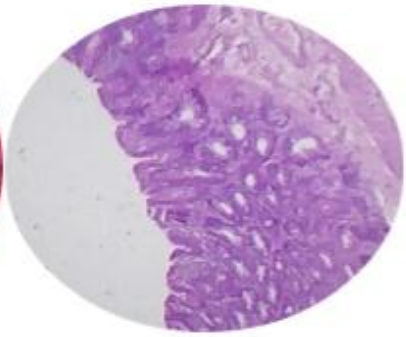




Figure No. 27 (1:1) **Figure No. 28 (1:2)** **Figure No. 29(2:1)**

d) PHCE-400 mg/kg (1:1, 1:2 and 2:1) observed in (X400)

DISCUSSION

Present study physicochemical parameters were evaluated including foreign matter which determines the presence of organic and inorganic matter, loss on drying investigating the amount of water in crude drug, extractive value has been evaluated for A.S extractive value and W.S extractive value were useful for estimation of constituents extracted with solvent required for extraction. Extractive values also provided an idea about the concept of the active compound present in the drug. determination of the ash value provides quality, purity and identity of the drug, sometimes total ash value is affected by inorganic variables like calcium oxalate, silica, carbonate content of the crude drug, Such variables are removed by determining acid insoluble ash value in which these variables are allowed to be treated with acid (as they are soluble in hydrochloric acid).

On preliminary phytochemical screening, the *Hemidesmus indicus* (L.) R.Br and *Tinospora cordifolia* (Wild.) Miers. and their polyherbal combined drug has revealed presence of Alkaloids, glycosides, Flavanoids, carbohydrate, proteins and some major compounds that might have contributed to its antiulcer activity. TLC were qualitative method which were performed for separation of these chemical constituents.

Pylorus ligation has been the ideal proposed model by Shay,(1945) which involves fasting of rats for 36 hours, followed by ligation of the pyloric end, Lesions produced by this model are generally located in Glandular portion in a pyloric antral of region below the limiting ridge.

Pylorus ligation model suppressed pain throughout the postoperative period followed by using anesthesia, as it served a greater purpose for investigating the efficacy of drugs on reducing

gastric ulceration. Pylorus ligation induced ulcers, resulted from autodigestion of the mucosa and breakdown of the gastric mucosal barrier. Ligation of the pyloric end of the stomach causes gastric ulcer due to stress induced increase in gastric acid secretion, volume of secretion could be the essential factor in the formation of ulcer because of constant exposure of the unprotected lumen of the gastric mucosa to the accumulated acid-pepsin[34,35]. Activation of the "vagus-vagal" reflux or vagal overactivity stimulates pressure receptors in the antral gastric mucosa could also be one of the reasons of hypersecretion of the acid due to pylorus obstruction[36].

Present study revealed that the EEHI and EETC possess gastro-protective activity as clearly evidenced by its significant reduction of following parameters such as ulcer index, no. of ulcers, ulcer size (mm), volume of juice (ml), F. acidity, T. acidity and Significant elevation of pH compared to control group during individual treatment in a same doses i.,e 200 mg./kg and 400 mg/kg. However standard drug Famotidine protected gastric mucosa much better by 80.99 % in comparison to EEHI (200 mg/kg-44.32% & 400 mg/kg-70.7%.) and EETC (200 mg/kg-41.38% & 400 mg/kg-71.45%) treated groups. Literature revealed tannins, flavonoids are present in the *Hemidesmus indicus* (L.) R. Br root and tannin-protein complex layer is reported to protect gastric mucosa and promote high resistance to any chemical and mechanical injury. These complexes also promote tissue repairment. Flavanoids on the other hand is the active constituents reported to exhibit gastroprotective effect act as a cytoprotective, anti-secretory and antioxidant agent[37,38]. Some research were undergone on the root of *H. indicus* (L.) R. Br on gastric ulcer reported healing protection of the drug might be because of high mucoprotective action leads to enhancement of mucosal defence factors such as prostaglandins synthesis, Drug provides alternative in ulcer healing to enhance the defensive factor so that normal balance is achieved[39,40]. Root extract is also reported to offer enhanced growth of mucosa due to Prostaglandins *E*2 which plays a vital role to maintain blood flow to mucosa and mucus production.[41] On the other hand, *Tinospora Cordifolia* (Wild.) Miers stem has been reported to possess antiulcer activity due to presence of isoprenyl flavonoid known as sofalcone. Sofalcone is reported to inhibit prostaglandins metabolizing enzymes 15-hydroxy-PGDehydrogenase, increasing PGE2 content of the mucosa in the rats[42,43]. Hence these are the possible mechanism based on previous studies supports the result that both the drug extracts EEHI and EETC on individual treatment exerted significant dose dependent anti-ulcer activity. In further study, combination of these two plant extract of stem and root part also exhibited synergistic effect in a dose dependent manner for which literature had no scientific evidence. So the result clearly stated that at the dose of 200 mg/kg an equal and unequal three combined

ratio 1:1-(100 mg of EETC + 100mg of EEHI, 1:2- (66.67 mg of EETC+133.33 mg of EEHI) and 2:1- (133.33 mg of EETC+66.67 mg EEHI) has shown remarkable anti ulcer activity even at low combination of doses when compared to untreated control group. Synergistic effect was found to be increased when high doses of combination has given i.e., 400 mg/kg in an equal and unequal combined ratio i.e., 1:1-(200 mg of EETC + 200 mg of EEHI), 1:2- (133.33 mg of EETC+266.67 mg of EEHI) and 2:1- (266.67 mg of EETC+133.3 EEHI).At this combination of dose 1:2 and 2:1 combine extract has shown maximum inhibition of ulcer (68.85% and 68.24 %.) in comparison with 1:1 ratio (65.37%), **(PHCE 1:2 > 2:1 > 1:1).**

Although, PHCE was not much potent than standard drug Famotidine. Hence dose dependent effect was clearly seen as the combined dose proportions were increasing. Therefore, poly herbal combine therapy of EEHI and EETC has demonstrated high synergistic ulcer healing property. This study was carried out to highlight the importance of combined herbal therapy that helps to facilitate the effectiveness as well as safety of drugs with less toxicity. Antiulcer activity of these plant extracts was further confirmed by the histopathological examination of rat stomach where different treated groups showed moderate to less ulceration then normal appearance of gastric mucosa. Although all the treated groups did not show any such extensive gastric damage. Except untreated control group where gross infiltration of lymphocytes was found to be appeared shows chronic inflammation.

CONCLUSION

Thus, the present study reveals antiulcer activity of individual and polyherbal ethanolic (99%) extract of root part of *Hemidesmus indicus*(L.) R.Br and stem part of *Tinospora cordifolia* (Wild.) Miers in pylorus ligation induced ulcer.

The above study has been justified through various aspects such as assessment of gastric ulcer parameters, biochemical and Histopathological studies.

Thus, further clinical studies of these two plant extracts can be utilized as an adjunct or combination to the formulated existing drugs in the pharmacotherapy of gastric ulcer.

The study also showed that the combination of *Hemidesmus indicus* (L.) R. Brand *Tinospora cordifolia (*Wild.) Miers as a polyherbal antiulcer remedy expressed better actions in reducing formation of peptic ulcer in the ratio of 1:2 and 2:1.at high dose 400 mg/kg. Thus, synergistic effect can further be enhanced by increasing combine dose proportion.

Plant extracts seems promising for in future combination with modern drug therapy and study provides future research in the direction of screening and investigating the prominent active constituent responsible for antiulcer activity and possible mechanism of actions.

ACKNOWLEDGMENT

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