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Research Article

GASTRODUODENAL ULCER PROTECTIVE AND BIOCHEMICAL STUDY OF *HELIOTROPIUMINDICUM* ON EXPERIMENTAL RAT MODELS

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ABSTRACT

A peptic ulcer is an excoriated area of the mucosa caused by the digestive action of gastric juice in the stomach. The peptic ulcer is gastrointestinal tract multi factorial disease, which caused by tobacco smoking, alcohol intake, spicy foods, and NSIAD'S. T The several herbal formulations derived from Ayurveda and its additional systems of medicine, not yet to be scientifically validated that they have exhibited anti-ulcer activity. The present study was undertaken to investigate anti-ulcer activity of hydro alcoholic extract of *Heliotropiumindicum* on four different anti-ulcer models. The anti-secretary, acetic acid induced ulcer and cysteamine induced duodenal ulcer results have been expressed that the crude hydro alcoholic extract of ***Heliotropiumindicum*** dose levels 200 and 400mg/kg were dose dependent manner significantly decreased in the ulcer index and ulcer scoring (**P<0.05 and***P<0.001) when compared to control and positive control. The physicochemical studies results have suggested that the crude hydro alcoholic extract of ***Heliotropiumindicum*** dose levels 200 and 400 mg/kg, on offensive (Gastric PH & k^+ concentration) and defensive (Gastric volume, Free acidity, Total acidity, Na^+ concentration) factors were indicated (*P<0.01,**P<0.05 and***P<0.001), antiulcer activity when compared to control and positive control. The biochemical parameters results have been expressed that the crude hydro alcoholic extract of ***Heliotropiumindicum*** dose levels 200 and 400 mg/kg, offensive (Pepsin, Carbohydrate: Proteins, Total Carbohydrates, Total Hexoses, Hexosamine, Fucose, Non Sufhydryl group and Sialic acid) and defensive (Catalase, Superoxide Dismutase, Lipid Peroxidase, Mucus barrier) factors were indicated (*P<0.01,**P<0.05 and***P<0.001) antiulcer activity when compared to control and positive control. In another hypothesis, the pharmacological mechanism could be attributed either direct or indirect anti-ulcer activity.

Key words: *Heliotropiumindicum (HI)* Omeprazole, Ranitidine, Acetic Acid.

1. INTRODUCTION

Peptic ulcers are chronic, most often, solitary lesion that occur in any portion of the gastrointestinal tract exposed to the aggressive action of acid-peptic juices. It can be defined as a breach in the mucosa of the alimentary tract which extends through the muscular mucosainto the sub mucosa or deeper which leads to erosion[1-3]. .Peptic ulcer is one of the common diseases prevailing in human population and its incidence is increasing day by day due to

rapid development and civilization constraints[4]. The estimates of incidence of peptic ulcer vary ranging between 3-10%. In United States approximately 4 million peoples have ulcer (gastric & duodenal) and 3, 50,000 new cases are diagnosed by the every year. Around 10, 00,000 patients are hospitalized yearly and 3000 people die each year as a result of peptic ulcer. The male/female ratio for duodenal ulcer is 3:1 and gastric ulcer is about 2:1[5-8].The literatures have been showed that the no steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed medications. As NSAIDs relieve pain and reduce inflammation, most NSAID prescriptions are written for patients with arthritis. All though local irritation orally administrated NSAIDs allows back diffusion of gastric mucosa and induce the tissue damage has correlated with inhibition of prostaglandin especially PGI_2 and PGE_2 that serve as cytoprotective[9-11]. In ayurveda, peptic ulcer has mostly refers to*Amlapitta* or*Parinamasula*. *Amlapitta* and*Agni* are diseases of gastrointestinal tract. The herbal formulations were developed from Ayurveda, traditional system of Indian medicine and its additional systems of medicine, has been found to have antiulcer activity[12-14].The present study is attempted to develop the novel plant based drugs which have anti-ulcer property. The ***Heliotropiumindicum (HI)*** is a medium sized armed deciduous tree belonging to the family Boragenaceae[15-17]. The plant was collected from the eastern part of Kerala and authenticated from Survey of Medicinal Plants and Collection Unit, Ministry of Health and Family Welfare, Gov't of India, Nilgiris. Our present study is aimed to justify the traditional claim of the plant as anti-ulcer activity.

2.MATERIALS AND METHODS

2.1. PREPARATION OF EXTRACT

Plant description

Heliotropiumindicum(Linn)is small size weed, road side plant widely distributed moist areas and waste places of Tamil Nadu, Kerala ad Bihar and grows 15-60 cm in height with densely ascending branches. This plant contains alkaloids, flavonoids, phytosterols, phenolic compounds and tannins and being used for the treatment of cancer, gastrointestinal disorder and would healing purpose.

2.1.1 Collection of Plant material and Authentication

The plant material was collected from Kottakkal area, Kerala which was properly identified and authenticated by comparing with the voucher specimen available at thesurvey of Medicinal Plants, Kottakkal, Kerlala and also it was verified by Dr.Rajan, Botanist, Botanical survey of India, Emarald, Ootagamund. A Voucher specimen is deposited in the Dep't of Pharmacognosy for further reference.

2.1.2. Cold Maceration[18]

1000g of coarse powder of plants, mixed with 2000ml of solvent(hydro alcohol) in round bottom flask, which kept for 15days and shaken regularly 2 times per day and decanted. The extracts are dried under reduced pressure and stored in desiccators

2.2 *ANIMALS*

Inbred adult Wister rats of either sex (160-200 g) were obtained from the animal house of JSS College of Pharmacy. The animals were maintained in a well-ventilated room at a temperature of 25±2°C with 12:12 hour light/dark cycle in polypropylene cages. The Standard pellet feed (Hindustan lever, Bangalore) and tap water were provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory conditions 10 days prior to initiation of experiments. The project proposal was approved by JSSCollege of Pharmacy IAEC (Institutional Animal Ethical Committee) and the approval number being (IAEC No – JSSCP/IAEC/PHD/PH.COLOGY/02//2007-2008, DATED: 5/09/07).

2.3. ACUTE TOXICITY STUDIES[19]

The rats are fasted overnight, prior to dosing.The three dose levels of drugs are administered by the help of oral feeding needle over the period of 24 hours. After the drugs have been administered, food may be withheld for a further 3-4 hours in

rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study. The test substance is administered to single animal in a sequential manner following from the fixed dose levels of 5, 50,300 and 2000 mg/kg. The interval between dosing of each level is determined by the mortality, onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting study starting dose in main study is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg). Based on the mortality result on 14th day of observation, the doses for *in vivo* study are selected.

2.4. SELECTION AND PREPARATION OF DOSE FOR PHARMACOLOGICAL SCREENING

Based on the results of acute toxicity and gross behavioral study, the maximum tolerated dose was found to be 2000g/kg with no significant behavioral changes and toxicity caused in animals .Consequently, the dose was calculated 1/10, 1/5 for the experimental evaluation.

The hydroethanolic extracts of testing plants were suspended in 0.3%CMC suspension, two dose levels at 200mf and 400mg/kg body weight of the animals selected for screening the anti-ulcer activity.

2.5.MODIFIED PYLORUS LIGATED (SHAY) [21]

The adult albino Wister rats of either sex weighing about 180-250 g. The animals were divided into seven groups and each group consists of six animals. The individual animals were placed in cages with grating floor to avoid coprophagy and cannibalism. The rats were fasted for 48 hours, allowing free access of water and *adlibitum*. The groups were divided follows; Group I: served as control – 0.3% of Carboxy Methyl Cellulose (CMC) (1ml/kg, oral route), Group II: Served as Positive control –Ranitidine 27 mg/kg,

Group III: Served as Positive control-Omeprazole 2 mg/kg,Group IV: Served as Test extract- CP 200 mg/kg, GroupV: Served as Test extract- CP 400 mg/kg,Group VI: Served as Test extract- HI 200 mg/kg and Group VII: Served as Test extract – HI 400 mg/kg. The standard drugs and extracts were administrated (twice daily for two days) orally by using oral feeding needle. The last dose of standard drugs and extracts have administrated one hour prior to pyloric ligation. The animals were anaesthetized by using ketamine and xylazine ratio (100+5 mg/kg im route), (Jim gourdon, 2002). The abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by uninterrupted sutures. The animals were deprived of both food and water during the post-operative period. The animals were sacrificed using excess ether anesthesia, 4 h after the ligation. The stomach was excised carefully; keeping the esophagus closed, opened along the greater curvature and collected the gastric juice. The mucosa was flushed with saline and the stomach pinned on dissection table. The ulcer index was calculated according to the method (Anoop,et.al.,2003), the lesion were counted with the aid of hand lens (10x) and each given a severity rating as follows:

Ulcer score	Description
0	Normal
1	Scattered haemorragic spots
2	Deeper haemorragicspots and some ulcers
3	Haemorragic spots and ulcers
4	Perforation

The ulcer score was divided by a factor of 10 to get the ulcer index. The percentage of ulcer protection was calculated according to the standard formula.

$$\frac{\text{Ulcer index in control - Ulcer index in test}}{\text{Ulcer index in control}} \times 100$$

The collected gastric juice was purified by using centrifuge apparatus, which was subjected to various physicochemical and biochemical parameters. The free acidity, total acidity, volume and P^H were recorded in the general laboratory procedure. The bio-chemical parameters like total proteins, total hexoses, hexosamine, fucose, pepsin activity, total carbohydrate content and finally carbohydrate/protein were measured by using Hitachi spectrophotometer. The gastric acid sodium and potassium ions concentration were estimated by using flame photometer apparatus.

2.6.1.***Physicochemical Parameters***

2.6.1.1.**Gastric volume**

This was measured after centrifuging the gastric fluid, allowed to stand, decaned and poured into the measuring cylinder of graduation 0.01ml.

2.6.1.2.**Determination of pH**

The pH of the gastric juice was measured using the pH meter (Cyberscan, India)

2.6.1.3.**Determination of free acidity and total acidity**

l of gastric juice was pipette into a 100 ml conical flask. Added 2 or 3 drops of topfer' s reagent and titrated with 0.01N sodium hydroxide (NaOH-which was previously standardized with 0.01 N oxalic acid) until all traces of the red colour disappears and the colour of the solution was yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. This volume corresponds to total acidity.

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual Normality of NaOH} \times 100}{0.1} \text{ meq/l/100g}$$

2.6.2. **Biochemical Analysis**

2.6.2.1. **Estimation of total proteins**

The gastric juice was precipitated by using 90% alcohol (1:9 ratio). Then 0.1ml of alcoholic precipitate of gastric juice was dissolved in 1ml of 0.1N NaoH, and from this 0.05 ml was taken in another test tube, to this 4 ml of alkaline mixture was added and kept for 10 min then 0.4 ml phenol reagent was added and again 10 min was allowed for colour development. Reading was taken against blank prepared with distilled water at 610 nm in Hitachi spectrophotometer. The protein content calculated from standard curve prepared with bovine albumin and was expressed in term of µg/sml of gastric juice.

2.6.2.2. **Estimation of pepsin**

For each determination placed four tubes (1) and (2) containing 5 ml of substrate, (3) and (4) containing 10 ml of trichloroacetic acid. The gastric juice was mixed with an equal volume of HCl for maintain the PH 2.1 warmed at 37° C and added 1ml of mixture to the tubes (1) and (4) Incubated for 15 minutes. After the incubation tubes were allowed to stand for 4 min. (1) + (3) gives test and (2) + (4) gives blank. Filtered 25 min after the beginning of the filtration, 2 ml of filtrate was pipette into 10 ml of sodium hydroxide. Mixed by gentle rotation. After 30 min, the intensity of colour was measured at 680 nm in Hitachi spectrophotometer. The difference between test and blank gives a measure of peptic activity. As standard mixed 2ml of freshly

prepared phenol solution containing 50 µg/ml /ml with 10 ml of sodium hydroxide and 1ml of phenol reagent was added. After 5 to 10 min, the colour intensity was measured at 680 nm.

2.6.2.3. **Estimation of total carbohydrates**
The dissolved mucosubstances in gastric juice were estimated in the alcoholic precipitate
obtained by adding 1ml of gastric juice to 9 ml of 90 % of alcohol in the test tube and mixture was kept for 10 mins in the test tube stand and obtained supernatant solution was discarded. The precipitate was taken in a test tube and added 0.5 ml of 0.1 N sodium hydroxide and 1.8 ml of 6 N HCl. This mixture was kept in the boiling water bath for 2hrs. After the boiling, mixture was neutralized by added 5 N sodium hydroxide using phenolphthalein as a indicator and the volume was make up to required quantity with distilled water to form hydrolystate fraction. This hydrolysate fraction was used for the estimation of hexoamine, hexoses,fucose and sialic acid as described given below:

2.6.2.3. **Estimation of hexoseamine**
0.5 ml of the hydrolysate fraction was taken in a test tube and added 0.5 ml of acetyl-acetone reagent. The mixture was heated with the help of boiling water bath for 20 mins and cooling under running tap water. After the cooling mixed with 1.5 ml of 90 % alcohol and followed by addition of 0.5 ml of Ehrlich's reagent. The reaction was allowed for 30 mins. The colour intensity was measured by Hitachi spectrophotometer at 530 nm against the blank prepared by using hydrolysate fraction. The Hexosamine content was determined from the standard curve prepared by using D (+) glucosamine hydrochloride and concentration has been expressed in µg /ml of gastric juice.

2.6.2.4. **Estimation of total hexoses**
Taken 0.4 ml of hydrolysate fraction in a test tube and 3.4 ml of orcinol reagent was added. The mixture was heated with the help of boiling water bath for 15 mins. This was cooled under running tap water. After the cooling. The colour intensity was measured by Hitachi spectrophotometer at 540 nm against the blank prepared by using water. The total hexoses content was determined from the standard curve of D (+)-galactose-mannose and concentration has been expressed that the µg//ml of gastric juice.

2.6.2.5. **Estimation of Fucose**
In this method, three test tubes were taken. In one tube 0.4 ml of distilled water was taken to serve as control and in each of the other two tubes 0.4 ml of hydrolysate were taken. To all three tubes 1.8 ml of sulphuricacid:water (6:1) was added. The test tubes were kept under the ice cold water bath for prevent breakage of glass wares due to strong exothermic reaction. This mixture was heated with help of boiling water bath for exactly 3 mins. The test tubes were cooled with running tab water. To the blank and to one of the hydrolysate containing tube (unknown) 0.1 ml of cysteine reagent was added while cysteine reagent was not added to the last test tube containing the hydrolysate (unknown blank) . It was stand and allowed for 90 mins. After the 90 mins, Thecolour intensity was measured by Hitachi spectrophotometer at 396 and 430 nm against the blank prepared by using water. The optical density was calculated by using below formulae :

$$\text{True optical density} = \frac{(OD396 - OD430)\ \text{unknown} - (OD396 - OD430)\ \text{unknown blank}}{(OD396 - OD430)\ \text{water blank}}$$

The standard curve was prepared with D (+) fucose. The fucose content was expressed in terms of µg/ml of gastric juice.

2.6.2.6. **Estimation of sialic acid**

Taken 0.5 ml of the hydrolysate fraction in test tube and added few drorpsof 0.1 N H_2S0_4 and 0.2 ml of sodium periodiate was allowed and stand for 20 mins. 20 mins later 1 ml of sodium arsenate solution added to this mixture. The brown coloured mixture was disappeared after shaking. The disappeared mixture was treated with 3 ml of thiobarbituric acid and heated with the help of boiling water bath for15mins. After the heating, the test tube was cooling with the help of running tap water and added 4.5 ml of cyclohexanone allowed and stand for 15 secs. The mixture was centrifuged to get a clear pink layer of cyclohexanone. This supernatant was pipetted out and the intensity of colour was measured by Hitachi- spectrophtometer at 550 nm. The sialic acid content was determined from the standard curve of sialic acid and has been expressed in the terms of µg/ml of gastric juice.

2.6.2.7. **Estimation of sodium and potassium ion concentration**

The sodium stock solution was prepared by using 2.542 g of NaCl was dissolved 1L of distilled water in a beaker (1 mg/ ml). Stock solution was diluted to give four solutions containing 10, 5, 2.5, 1 ppm of sodium ions. Then Potassium stock solution was prepared by using 1.909 g of KCl was dissolved with the help of 1 L of distilled water in a beaker (1 mg/ ml). Stock solution was diluted to give four solutions containing 20, 10 5, and 2 ppm of potassium ions.The sodium and potassium concentrations were estimated by using flame photometer. c, The flame intensity of the gastric juice was noted. The concentrations of sodium and potassium ions were calculated by graphical method. The results are expressed in terms of mg/L.

2.7. **ASPIRIN PLUS SHAY RAT MODEL[21]**

The adult albino wistar rats of either sex weighing about 180-250 g. The animals were divided into seven groups and each group consists of six animals. The animals were placed in cages with grating floor to avoid coprophagy and cannibalism. The rats were fasted for 48 h, allowing free access of water and *adlibitum*. The groups were divided follows;Group I: served as control – 0.3% of Carboxy Methyl Cellulose (CMC) (1ml/kg, oral route),Group II: Served as Positive control –Ranitidine 27 mg/kg,Group III: Served as Positive control-Omeprazole 2 mg/kg,Group IV: Served as Test extract- CP 200 mg/kg,Group V : Served as Test extract- CP 400 mg/kg, group VI: Served as Test extract- HI 200 mg/kgandgroup VII: Served as Test extract – HI 400 mg/kg. The test extracts were administered twice daily for 3 days and standard drugs were administered once daily for 3 days by oral route. All the group of animals received asprin orally aqueous suspension at a dose of 200mg/kg, 1h before the last dose administration of solvent, extract and standard drugs. The animals were anaesthetized by using ketamine and xylazine ratio (100+5 mg/kg im route), (Jim gourdon, 2002). The abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by uninterrupted sutures. The animals were deprived of both food and water during the post-operative period. The animals were sacrificed using excess ether anesthesia, 4 h after the ligation. The stomach was excised carefully; keeping the oesophagus closed, opened along the greater curvature and collected the gastric juice.

The ulcer index was calculated according to the method of (Anoop,et.al.,2003) the lesion were counted with the aid of hand lens (10x) and each given a severity rating as follows: above specified method. The collected gastric juice was purified by using centrifuge apparatus and which was subjected to various physicochemical and biochemical parameters. The standard procedures were expressed above shay rat model. The free acidity, total acidity, volume and P^H were recorded in the general laboratory procedure. The bio-chemical parameters like total proteins, total hexoses, hexosamine, fucose, pepsin activity, total carbohydrate content and

finally carbohydrate/protein were measured by using Hitachi spectrophotometer. The gastric acid sodium and potassium ions concentration were estimated by using flame photometer apparatus.

2.8.**ACETIC ACID INDUCED CHRONIC ULCER MODEL**[22]

The adult albino Wister rats of either sex weighing about 180-250 g. The animals were divided into nine groups and each group consists of six animals. The animals were placed in cages with grating floor to avoid coprophagy and cannibalism. The rats were fasted for 24 h, allowing free access of water and *adlibitum.*The groups were divided as follows:

Group I: served as control – 0.3% of Carboxy Methyl Cellulose (CMC) (1ml/kg, oral route), Group II: Served as Positive control –Ranitidine 27 mg/kg, Group III: Served as Positive control-Omeprazole 2 mg/kg, Group IV: Served as Test extract- CP 200 mg/kg and Group V : Served as Test extract- CP 400 mg/kg. Laparotomy was performed by using combined ketamine and xylazine (100+5 mg/kg im route) anaesthesia on experimental rats. The fifty microlitre of 50% glacial acetic acid was administered by placing cylindrical plastic mould(6.5 mm in diameter) was firmly placed upon the wall of the stomach corpus at the region of the lesser curvature for 60 s, and the stomach wall wiped using cotton wool soaked in 0.9% NaCl solution. The abdominal incisions were stitched with the help of surgical thread and disinfectant (Povidine iodine) applied to the area each day to avoid infection. The animals then continued to receive their regular diet, with free access to water. Four days after the operation, control groups (group III) of six rats were sacrificed using ether and the stomachs were removed and cut open along the greater curvature in order to establish the degree of ulceration prior to the onset of treatment. The remaining rats were divided into seven groups and each group consists of six rats. The other groups were administered with respective solvent, drugs and extract once a day for the fifteen days. On the final day of the experiment, the rats were sacrificed by using excess anesthesia. The ulcer index was calculated according to the method of (Anoop,et.al.,2003) the lesion were counted with the aid of hand lens (10x) and each given a severity rating as follows: Above specified method.

2.8.1.**Gastric Mucosal Defensive Factors**

2.8.1.1.**Estimation of mucous barrier**

Glandular portion of stomach were soaked for 24 h in 10 ml of 0.1 % alcian blue 8GX dissolved in 0.16M sucrose buffered with 0.05M sodium acetate adjusted to pH 5.8 with HCl. The uncomplexed dye was removed by using two successive washes with the help of required quantity of 0.25 N sucrose. The washing has been done by different time intervals at 15 and 45 mins. The mucous was dilated by immersion with 10 ml aliquots of 0.5 M magnesium chloride for 2 h. The resulting blue solution was shaken with equal volume of diethyl ether and the optical density of aqueous phase was measured at 605 nm. The barrier mucous was expressed in terms of microgram of alcian blue dye/g of wet stomach glandular tissues

$$E\ 1\%\ 1cm\ X = \frac{\text{Absorbance X 105}}{\text{Wt of glandular tissues}}$$

E1% 1cm for alcian blue =189.

2.8.1.2**. Estimation of non-protein sulfhydryl (NP-SH) group**

The glandular part of the stomach was homogenized in ice-cold 0.02 M EDTA.The aliquots (5ml) were homogenate with the help of homogenizer and added 4 ml of distilled water and mixed with 1 ml of 50 % acid. The tubes were shaken intermittently for few mins. The mixed product was centrifuged at 3000 rpm/10mins and collected supernatant. Two ml of supernatant was mixed with 4 ml of tris Buffer pH 8.9, 0.1 ml of5,5dithio-bis-(2nitro benzoic acid) was added and the sampleswere shaken. The 5, 5 dithio-bis- (2 nitro benzoic acid) was added and set at 412 nm against non homogenated reagent. The absorbance was measured by using Hitachi spectrophotometer.

2.8.1.3. **Super oxide dismutase (SOD)**
Super oxide dismutase measurement was based on the ability of SOD inhibit spontaneous oxidation of adrenaline to form adrenochrome. The 2.78 ml of sodium carbonate buffer (0.05 mM) and 100 µl of stomach was homogenated either or sucrose (blank) a 30 ˚C for 45 mins and after the reaction was initiated by adding 100 µl solution (9.0mM). The absorbance was recorded at 480 nm for 8-12 min and temperature was maintained at 30˚C for throughout the experiment and the quantity was expressed by mg/ml protein.

2.8.1.4 **Estimation of lipid peroxide**
The method was used to estimate the total amount of lipid per oxidation product Thiobarbituric Acid Reacting Substance (TBARS) in the homogenate. For TBARS containing 0.1ml of tissue homogenate (Tris-buffer, PH7.5) was mixed with 2.0 ml of TBA-TCA-HCl reagent (thiobarbituric acid 0.37%: 0.25N HCl :15 % TCA mixed in 1:1:1 ratio). The resultant solution was placed in water bath for 5 min and centrifuged at 1000 rpm for 10 min. The absorbance of clear supernatant was measured against reference blank at 535 nm. The results were expressed as nM/min/mg tissue protein.

2.9. *CYSTEAMINE INDUCED DUODENAL ULCERS IN RATS*[23&24]
Wistar albino rats of either sex weighing 180-220 g were divided into nine group and each group consists of six animals. The groups were divided as follows: Group I: served as control – 0.3% of Carboxy Methyl Cellulose (CMC) (1ml/kg, oral route), Group II: Served as Positive control –Ranitidine 27 mg/kg, Group III: Served as Positive control-Omeprazole 2 mg/kg, Group IV: Served as Test extract- HI 200 mg/kg,Group V : Served as Test extract- HI 400mg/kg. The solvent, test extracts and standard drugs were administered 30 min before each dose of cysteamine hydrochloride. The duodenal ulcers were induced by the administration of cysteamine hydrochloride 400 mg/kg oral route for two times at intervals between 4 h before the surgery. The animals were fed with food and water throughout the experiment. The 1st dose of cysteamine administrated at 24 h later the duodenum was excised carefully and opened. The duodenal ulcers were scored for intensity, using a scale from 0 to 3. The ulcers scores after converting it into ulcer index were subjected to statically analysis by comparing with normal (solvent control).The duodenal ulcer scoring scale was referred as in pylorus ligated (Shay) rat model

3. **STATISTICAL ANALYSIS**
The tabulated parameters showed that the healing of ulcers was measured by (n=6) in each experiments in each group (P** <0.01 and P* <0.05) values were compared to solvent control and 4th day ulcerated group evaluated by using one way ANOVA followed by Turkey's multiple comparison test.

4. RESULTS

Table 1: Effect of Medicinal plant on Modified pylorus Ligated (Shay) Rat Model (MPL) expressed physicochemical and ulcer index

Group	Dose Mg/kg	Gastric Volume (ml)	pH	Ulcer index	Free acidity (Meq/l/100g)	Total acidity (Meq/l/100g)	Pepsin (µg/ml)	Total protein (µg/ml)
Solvent control		5.2±0.2	2.5±0.04	3.3±0.01	40.0±0.31	61.6±0.2	13.9±0.1	457.7±1.6
Ranitidine	27	3.7±0.1***	4.1±0.2***	1.4±0.02***	31.3±0.31***	44.5±0.2***	7.2±0.09***	320.3±1.4***
Omeprazole	2	3.0±0.1***	3.9±0.1***	1.14±0.02***	21.8±0.3***	31.94±0.2***	4.50±0.1***	255.0 ±0.9***
HI	200	4.5±0.02**	3.3±0.1***	3.15±0.04*	38.3±0.4*	64.01±1.0*	13.0±0.3**	450.1±1.9**
HI	400	2.6±0.2***b	4.1±0.2***b	1.23±0.01***b	22.8±0.2***b	33.8±0.23***b	6.12±0.1***a	243.1±0.3***a

Statistical analysis done by one way ANOVA followed by Turkey's multiple comparison tests (n=6); * P <0.05, ** P<0.01; *** P<0.001 VS Control; P<0.001[b] HI 200 VS HI 400

Table 2: Effect of Medicinal plant on Biochemical parameters of Modified Pylorus Ligated (Shay) Rat Model (MPL)

Group	Dose mg/kg	TCS	H (µg/ml)	F (µg/ml)	SA (µg/ml)	TH (µg/ml)	Na^+ C	K^+ C	C:P Ratio
Control		369.8±0.1	147.5±0.4	51.83±0.2	20.1 ±0.1	150.3±0.4	17.0±0.5	6.33±0.1	0.8± 0.003
Ranitidine	27	1022 ± 4.8***	407.5±2.1***	159.3±0.6***	37.3 ±0.3***	418±3.3***	7.3±0.1***	11.2 ±0.2***	3.2±0.02***
Omeprazole	2	**1120 ± 6.4*** **	467.5±2.1***	173.4±0.5***	42.9 ±0.3***	435.8±4.7***	5.7±0.1***	11.9 ±0.1***	4.4±0.03***
HI	200	708.4±2.5***	159.2±1.5*	56.33±1.7**	23.76 ±0.2**	269.2±3.5***	15.1±0.2**	8.2 ±0.1**	1.2±0.1**
HI	400	**898.7 ± 4.7***b**	420.8 ±2.9***a	135. ±0.6***a	42.4 ±1.5***a	310.8±3.0***a	6.2 ±0.2***a	13.75±0.7***a	3.7±0.02***a

Total Carbohydrates- **TCS**, Hexosamine- **H**, Fucose-**F**, Sialic Acid-**SA** and Total Hexoses-**TH.** Statistical analysis done by one way ANOVA followed by Turkey's multiple comparison test (n=6); * P <0.05, ** P<0.01; *** P<0.001 VS Control; P<0.001[b]: HI 200 VS HI 400

Table 3: Effect of Medicinal plants on Aspirin plus modified pylorus ligated (Shay) rat model (APL)

Group	Dose mg/kg	Gastric Volume (ml)	pH	Ulcer index	Free acidity (Meq/l/100g)	Total acidity (Meq/l/100g)	Pepsin (µg/ml)	Total protein (µg/ml)
Solvent control		6.2 ±0.12	2.1±0.1	2.9 ±0.06	63.08 ±0.4	90.2±0.3	11.23 ±0.2	545.9±3.9
Ranitidine	27	3.9 ±0.11***	4.0 0.2***	1.53 ±0.09***	40.52 ±0.4***	59.8 ±0.4***	8.3 ±0.2***	373.4 ±1.8***
Omeprazole	2	3.3±0.1***	4.32±0.1***	1.5 ±0.02***	36.57 ±0.6***	55.22 ±0.4***	5.3 ±0.2***	375.0 ±01.5***
HI	200	5.532±0.01**	2.7 ±0.02***	2.53±0.03*	59.16 ±0.1*	87.34±0.93*	10.32±0.2**	530.6±2.3**
HI	400	3.5 ±0.12***b	4.2±0.1***a	1.27±0.08***b	34.05±10.1***b[a]	39.69±0.4***b	5.5±0.03.***b	293.2.1 ±2.0***b

Statistical analysis done by one way ANOVA followed by Turkey's multiple comparison test P<0.001[a] HI 200 VS HI 400

Table 4: Effect of Medicinal plant on Biochemical parameters of Aspirin plus modified pylorus ligated (Shay) rat model (APL)

Group	Dose Mg/kg	TH (µg/ml)	H (µg/ml)	F (µg/ml)	SA (µg/ml)	TCS	Na^+conc	K^+conc	C:P-ratio
Solvent control		248.3 ±0.1	252.1 ±1.3	53.7 ±1.3	21.6 ±0.5	577 ±5.6	17.20 ±1.1	7.0 ±0.3	1.057 ±0.03
Ranitidine	27	465.0 ±3.7***	398.3 ±3.0***	190.5 ±4.5***	40.6 ±0.3***	1158 ±5.714***	7.93 ±0.1***	18.25 ±0.2***	2.898 ±0.07***
Omeprazole	2	452.0 ±6.9***	461.2 ±2.2***	192.5 ±2.4***	45.2 ±0.8***	1088 ±16.22***	6.42 ±0.2***	18.75 ±0.6***	3.100 ±0.01***
HI	200	272.3 ±1.5*	273.5 ±1.0*	66.5 ±1.0*	24.3 ±0.5*	693.2 ±2.6**	14.0 ±0.6**	9.1 ±0.11**	1.3 ±0.01**
HI	400	427.0 ±4.7***b	405.2 3.2***b	145.7 ± 2.2***b	40.03 ±0.6***b	1047.8 ±5.2***b	6.7 ±0.2***b	19.5 ±0.4***b	3.6 ±0.05***a

Total Carbohydrates- **TCS**, Hexosamine- **H**,Fucose-**F**,Sialic Acid-**SA** and Total Hexoses-**TH**

Table 5: Effect of Medicinal plant on Acetic acid inducedGastric mucosal damage model

Group	Ulcer index	Mucose barrier (mg/Alcian blue/gm)	Non protein sulfhydryl (NP-SH)glandular tissue/gm	Anti oxidant Estimation		
				Catalase (iu/mg)	Superoxide dismutase (iu/mg)	Lipid peroxidase (iu/mg)
Non-Ulcerated	-------	78.26 ±1.216	0.871 ±0.007	28.89 ±0.539	3.62 ± 0.282	0.29 ±0.02
Solvent control Ulcerated	2.91±0.06	31.88±0.942	0.271±0.010	15.17±0.313	1.91± 0.08	1.22±0.069
Ulcerated sacrificed on fourth day	3.2 ±0.07	22.58 ±0.282	0.1565 ±0.002	10.79 ±0.460	0.83 ±0.064	1.52 ±0.051
Ranitidine 27 mg/kg	1.13 ±0.39 *** ***	72.54 ±0.275 *** ***	0..819 ±0.01 *** ***	25.58 ±0.383 *** ***	3.01 ±0.207 *** **	0.40 ±0.010*** ***
Omeprazole 2 mg/kg	1.33 ±0.088 *** ***	75.31 ±0.248 *** ***	0.862 ±0.007*** ***	27.86 ±.477 *** ***	3.52 ±0.164*** ***	0.365 ±0.0195 *** ***
HI (200mg/kg)	2.23 ±0.04*** *	37.23 ±2.1*** **	0.321 ±0.018*** ***	17.08 ±0.2*** *	2.85 ±0.023*** *	0.972 ±0.053*** **
HI 400 mg/kg)	1.43 ±0.04*** ***	73.83 ±0.2*** ***	0.837 ±0.01*** ***	27.83 ±0.382*** ***	4.42 ±0.033*** ***	0.87 ±0.034*** ***

Table 6: Effect of Medicinal plants on Cysteamine induced duodenal ulcer model (CIDU)

Group Drug/Dose	Ulcer Incidence		Ulcer index
	Number	%	
Solvent control 0.3% w/v conc	6/6	100	0.7880 ± 0.003
Ranitidine(27 mg/kg)	2/6	33.3	0.4120 ± 0.0037***
Omeprazole (2 mg/kg)	0/0	0	0.3652 ± 0.0018***
Heliotropiumindicum (200 mg/kg)	3/6	50	0.752 ± 0.001**
Heliotropiumindicum (400 mg/kg)	2/6	33.33	0.343 ± 0.014***b

5.DISCUSSION

5.1.MODIFIED PYLORUS LIGATED (SHAY) RAT MODE

The data are shown in table no 1 & 2. The test extracts results showed that there was significant decrease in gastric volume [HI 4.5,2.6 ml], ulcer index [HI 3.2,1.2], free acidity [HI 38.3,22.8], total acidity [HI 63.7, 33.8] , Na^+ ion concentration [HI 15.1,6.2], pepsin [HI 13.0,5.9] ($P< 0.05$, $P <0.01$& $P<0.001$) when compared to control and positive controls. The pH (HI 3.3,4), carbohydrates (total hexose, hexosamine, fucose, sialic acid), K^+ ion concentration (HI 8.2, 12 and HI 8.2, 13.75) and carbohydrates/protein (CP ratio 1.2, 3.2 and HI ratio 1.2, 3.7 ($p< 0.01$ and $P<0.001$) were reflected to significantly increased when compared to control and positive control.

The causative of ulcer in the gastric mucosa due to pyloric ligation may be two fold. Stress induced increase in gastrin Hcl secretion ad stasis of the acid. The presence of acid in the stomach has shown to be a perquisite for the formation of stress ulcer. Also, development of ulcers in pylorus model may be due to increased metabolism of carbohydrates, synthesis of nucleic acids and exhaustion of carbohydrates. It has been postulated that histamine may be involved in the formation of pylorus ligated ulcers and play a mediating role in the gastric secretion, stimulated by gastrin, vagal excitation and cholinergic agents.

Ulcer index parameter was used for the evaluation of anti-ulceractivity since ulcer formation is directly related to factors such reduction in gastric volume, decrease in free and total acidity. It is significant ot note that when the P^H reached 3, the ulcer score appeared less, this is borne out by the decrease in free acid which might be

contributed to the anti-ulcer property of the plant extract.

The mucosal defense mechanism may be due to the epithelial cells of the gastric mucosa, which are imperable to hydrogen ions thereby forming a physical barrier. Carbohydrates/Proteins ration also supports the above observation. The anti-ulcer effect is also supported by the decrease in the aggressive factors like pepsin, protein and an increase in the resistance factors like Ph, hexose, hexosamine, fucose and sialic acid. Also, hexosamine concentration is an index of mucin content of the gastric juice. The pylorous ligated ulcer model caused by enhanced acid-pepsin secretion leading to auto digestion of the gastric mucosa and breakdown of mucosal barrier which supports our present findings that there was a increase in carbohydrate / protein ratio.(mucin activity) The ulcer healing property may be assumed that the plant extract has coating protective property on the gastric mucosa.

5.2. ASPIRIN PLUS PYLORUS LIGATED (SHAY)S RAT MODEL

The results have expressed that the table no 3 & 4. The extract treated groups parameters like gastric volume [HI 5.5,3.4], free acidity [HI 59.2, 34.0], total acidity [HI 87.2, 39.7.2] ulcer index [HI 2.5,1.3] and pepsin levels [HI 10.3,5.5] were significantly reduced (*P<0.05, &P**<0.01, P***0.001)when compared to control group and positive control. The table no 3,4 have showed that the extract treated groups offensive parameters like gastric pH, total carbohydrates and C:P ratio were significantly increased (P**<0.01 and P* <0.05) (P**<0.01, (P***<0.001) when compared to control group and positive controls. The table number 4 was indicated that the estimation of Na^+ and K^+ ions present in gastric acid. The extract treated groups gastric acid Na^+ levelswere decreased (P<0.01 and P <0.05, P<0.01 and P<0.001) when compared to control group and positive controls. The gastric acid K^+ ions level were gradually increased (P<0.01 and P <0.05, P<0.01 and P<0.001) when compared to control group and positive controls.

5.3.ACETIC ACID INDUCED CHRONIC ULCER MODEL

5.3.1. Mucous barrier

Animals in the solvent group showed a significantly decreased (P<0.01) in the mucous barrier when compared with those of the non-ulcerated control group and another group of ulcerated animal were sacrificed on the 4th day the mucous membrane size was reduced (22.58 ±0.2817/µg) alcian blue/g of glandular tissue P<0.01 when compared with vehicle control. Administration of *Heliotropiumindicum* (HI) was produced significant (P<0.01) recovery of the mucous membrane size in the dose dependent manner when compared to control group. The size of the mucous membrane increased up to 37.23± 2.1 µg and 73.83± 0.2 µg alcian blue /g of glandular tissue when the ulcerated animals treated with *Heliotropiumindicum* at 200 and 400 mg/kg b.w respectively. The recoveries of mucous membrane in the different groups of ulcerated animals were treated to standard drugs like omeprazole (2mg/kg) and ranitidine (27mg/kg). The size of the mucous membrane in the animals were treated to Omeprazole 2mg/kg (75.23±0.25 µg) and Ranitidine 27mg/kg (72.54±0.275 µg) alcian blue/g of glandular tissue respectively (Table no: 5).

5.4.Non-protein sulfhydryl group

Ulcerated animals were treated to *Heliotropiumindicum* dose levels of 200 and 400mg/kg body weight, showed a dose dependent and significant (P<0.01) increase in the non-protein sulfhydryl level when compared to control and positive controls. The non-protein sulfhydryl levels were indicated in the table no: 5

5.5.Catalase

The catalase results have showed in the table no:5 Ulcerated animals were treated to *Heliotropiumindicum*at dose levels of 200 and 400mg/kg body weight, showed a dose dependent and significant (P<0.01)

decreased in the catalase level when compared to control and positive controls.

5.6.**Super oxide dismutase**
The SOD results have been expressed that the*Heliotropiumindicum*at dose levels of 200 and 400mg/kg body weight, showed a dose dependent and significant (P<0.01) decreased in the SOD level when compared to control and positive controls.

5.7.**Lipid peroxidase**
The *Heliotropiumindicum* dose levels were found to be produced significant (P<0.001) decrease in the lipid peroxidation activity when compared to control and positive controls.. (Table no: 5)

5.8.**Cysteamine induced duodenal ulcer**
The results have shown in the table no: 5 The results revealed that the *Heliotropiumindicum* dose levels at 200 and 400 mg/kg were significant decreased (P**< 0.01 and P***< 0.001) in the ulcer index when compared to control and positive controls The extracts of *Heliotropiumindicum* exhibited a equipotent effect of Omeprazole at a dose level of 400mg/kg and 200 mg/kg also showed that the similar effect (equipotent) to ranitidine.

6. **SUMMARY AND CONCLUSION**
The hydroethanolic extracts of *Heliotropiumindicum* plant was found to be effective on experimental and drug induced ulcer models such as Modified pylorus ligated (Shay) rat model, Aspirin plus Modified pylorus ligated (Shay) rat model, Acetic induced chronic ulcer model and Cysteamine induced duodenal ulcer model. The effect of whole plant extracts of *Heliotropiumindicum*expressed on offensive and defensive factors of gastric content was studied on Modified pylorus ligated (Shay) rat model and drug induced ulcer model. The herbal extracts might control the ulcer formation by decreasing the secretion, drug induced damage and also by psychological stress. The herbal extracts were found to be more effective in controlling the ulcer in both the acute and chronic models. Further, cysteamine induced duodenal ulcer study indicated that the extracts possess anti-ulcer activity against duodenal ulcers in dose dependent manner. Hence it can be stated that herbal extracts may be beneficial in preventing the stress (including psychological or environmental stress), drug induced ulcers and also act as anti-secretary which may be attributed to the presence of phytoconstitiuents in plants. Reduction of gastric emptying time, Synthesis of mucosa, Inhibition of hyper-secretion of gastric acid are the possible mechanisms through which the herbal extracts are possessing anti-ulcer activity.
Even though the present study revealed the activity of herbal extracts and the mechanism of action, the future studies are needed to be carried out to isolate and screen the phytoconstiuents, which are responsible for the anti-ulcer and mucoprotective activity and to correlate them with the mechanism of action. Reduction of gastric emptying time, Synthesis of mucosa, Inhibition of hyper-secretion of gastric acid are the possible mechanisms through which the herbal extracts are possessing anti-ulcer activity and this work will be useful for further antiulcer research workers.

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