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EVALUATION OF GASTROPROTECTIVE POTENTIAL OF THE WHOLE PLANT *HEMIGRAPHIS COLORATA*

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ABSTRACT
Hemigraphis colorata (Red flame Ivy), popular in the name 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. Traditional medicine accounts for its use externally as a anti inflammatory and internally for anemia and as contraceptive. The aim of the study was to investigate gastroprotective and anti-ulcerogenic activities of alcoholic and aqueous extract of *Hemigraphis colorata* using rat model against absolute ethanol induced ulcer. Aqueous and ethanolic extract of whole plant of *Hemigraphis colorata* extract was prepared by continuous hot percolation method. Gastroprotective and anti-ulcerogenic activities of the extracts were examined by feeding the rats with extracts in the concentration of 200 and 400 mg/kg body weight (b.w.) following induction of gastric lesions by absolute ethanol. Stomach of the rats were cut opened, the gastric juice, pH of the gastric juice were determined. Ulcers were scored by analyzing the stomach walls under microscope and the ulcer index was determined. The results were compared to that fed by 20 mg/kg b.w. omeprazole, a known anti-ulcer drug. Omeprazole was used as the ulcer control drug. The aqueous extract of the whole plant showed significant protection against ulcer.

KEY WORDS: Gastroprotective, Anti-ulcerogenic *Hemigraphis colorata*, Omeprazole absolute ethanol, gastric lesions, ulcer index, aqueous extract, ethanol extract.

INTRODUCTION

During the past several decades, there has been a global trend for the revival of interest in the traditional system of medicine. Simultaneously the need for basic scientific investigation of medicinal plants using indigenous medical systems has become ever more interesting and relevant. A recent review of references and publications indicates that the anti- ulcerogenic effects of many taxa of medicinal plants have been assessed worldwide. The eating habits and stress of the modern civilized human is one of the major reasons for the numerous disorders developing in him like diabetes, hypertension ulcer *etc*. One such disorder is the GIT disorder especially, stomach or peptic ulcer. Gastritis and peptic ulcer disease involve damage to the lining of the stomach or duodenum (the first segment of the small intestine). Peptic ulcer is a gastro intestinal disorder occurring due to an imbalance between the aggressive factors like acid, pepsin, *Helicobacter pylori* and defensive factors like bicarbonate secretion, prostaglandins, gastric mucus, innate resistance of the mucosal cell factors **(**Dashputre *et al*., 2011). Normally peptic ulcer develops when aggressive factors overcome the defensive factors (Izzo *et al*., 2000) Synthetic drugs such as proton pump inhibitors, H2 receptors, cytoprotectants, demulcents, anti cholinergics, antacids and prostaglandin analogues are used for the treatment of ulceration but these drugs produce several side effects. For example, proton pump inhibitors (omeprazole, lansoprazole) may cause nausea, abdominal pain, constipation, diarrhoea and H_2 receptor antagonists (cimetidine) may cause gynaecomastia, loss of libido. Considering the several side effects (arrhythmia's, impotence, funaecomastia and haematopoeitic changes) of modern medicine (Muhammad Shoaib Akhtar *et al*., 1992) indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. Herbal medicines are considered as better alternatives for the treatment of peptic ulcer (Vanisree *et al*., 2002) Moreover they are considered as safe for the treatment of ulcers with lesser adverse effects, economical, effective and relatively less toxic, extensive research is carried out in search for potent antiulcer agents of plant origin (Sharma *et al*., 2011; Vinay *et al*., 2005)**.** There are many agents in alternative medicine, which have shown promising antiulcer activity without producing above mentioned adverse effects. The antiulcerogenic activity of many plant products is reported due to an increase in mucosal defensive factors rather than decrease in the offensive factors (Narayan *et al*., 2004). So an effort has been made in an idea of providing a plant based gastroprotective agent for the society. The study involves the evaluation of the gastroprotective potential of the aqueous and ethanolic extracts of the whole plant *Hemigraphis colorata.*

Hemigraphis colorata (figure 1) belonging to the family acanthaceae is a tropical perennial herb chiefly grown as an ornamental indoor and outdoor plant for its attractive and vivid foliage. The folk people ground the leaves in to a paste consistency and apply it on fresh wounds to

promote wound healing. They also use the leaves of *Hemigraphis colorata* to treat anaemia.

FIGURE 1. Habitat of *Hemigraphis colorata*

Traditionally the leaves of the plant has been used to mend gall stones, excessive menstruation and as a contraceptive. In Vanuatu, sap of the leaves is squeezed in water and drunk at dawn for four days, as contraceptive and to induce sterility. In Java, the leaves are used to treat bloody dysentery and hemorrhoids. The plant is also credited with diuretic competence (Devi Priya *et al*., 2013). *Hemigraphis colorata* has been reported to possess many pharmacological activities like antidiabetic (Gayathri *et al*., 2012), antioxidant, anti inflammatory & cytotoxic activity (Akhil *et al*., 2015). Traditional knowledge regarding the usage of the plant is wide but any scientific evidence to support it is very limited. Moreover review of literature finds no report on anti ulcer or gastro protective studies using *Hemigraphis colorata*. So, the present studies were carried out to prove the folk claim of its anti ulcer activity.

MATERIALS & METHODS

Collection of Plant Material

The whole plants of *Hemigraphis colorata* were collected from ABS Botanical Conservation, Research and Training Centre, Salem in Tamilnadu. The plants were collected during the month of july to september. Whole plant of *Hemigraphis colorata* were shade dried and powdered.

Preliminary Experiments

The ash values of the powdered drug, like total ash, acid insoluble ash, water soluble ash and sulphated ash, were determined according to the Indian Pharmacopoeia. The alcohol and aqueous soluble extractive values were also determined. The dried powdered whole plant of *Hemigraphis colorata* was extracted with ethanol and water (Kokate *et al*., 1997). Alcoholic and aqueous extracts were selected for the study. Both the alcohol and aqueous extracts were subjected to qualitative analytical tests for phytochemical characterization The drug was detected for various constituents like alkaloids, sterols, steroids, carbohydrates, fats, tannins, saponins and flavonoids.

Pharmacological screening: Gastroprotective evaluation- Ethanol inducer ulcer

The alcohol and water extracts were subjected to gastroprotective activity. Albino rats were used as the animal models. Experiments were performed complying with the rules of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), New Delhi, India and the study was permitted by IEAC of The Erode College of Pharmacy, Erode. The pharmacological study was conducted at the department of pharmacology, The Erode College of Pharmacy, Erode. Healthy Albino rats of either sex, weighing 120-150 g were selected and divided in to seven groups. Each group comprised of three animals. Before the study, the animals were kept in separate perforated steel cages to prevent coprophagy; The temperature was maintained at 25-30 º C. The animals were fasted for 24 hrs prior to the experiment, but allowed to have water. The seven groups of animals were used as follows:

Group I: Normal Control. Received 1% acacia (1.0 ml/kg)
Group II: Ulcer control. Received 99.9% ethanol (1.0 ml/kg)
Group III: Standard. Received Omeprazole as standard (20mg/kg)
Group IV: Received ethanol extract of *Hemigraphis colorata (*200 mg/kg) as the low dose.
Group V: Received ethanol extract of *Hemigraphis colorata* (400 mg/kg) as the high dose.
Group VI: Received aqueous extract of *Hemigraphis colorata* (200 mg/kg) as the low dose.
Group VII: Received aqueous extract of *Hemigraphis colorata* (400 mg/kg) as the high dose.

Induction of Ulcer

All the group of animals was orally treated with the above said. Ulcers were induced in animals of the group III to VII by administering 100% ethanol (5ml kg^{-1} b.w.) and animals were sacrificed after 1h of ethanol treatment (M. Jainu, 2006) after an hour the animals were sacrificed and

stomach was opened. The Stomach was carefully incised keeping the oesophagus closed and opened along the greater curvature. The luminal contents were removed. The gastric contents were collected and subjected to acidity studies explained later. The gastric mucosa was then flushed with saline and the stomach was pinned on to a board to assess the number of ulcers, the lesions in the glandular portion were examined under 10X. The LI of each animal was calculated by adding the following values obtained in the observation of mucosa considering the lesion degree produced scored as follows (Raju *et al*., 2009):

Scoring of ulcers & determination of ulcer index

1. 0 - Normal
2. 0.5 - Red ulcer
3. 1.0 - spot ulcer
4. 1.5 - Hemorrhage
5. 2.0 - Ulcer
6. 3.0 - Perforations

Ulcer index was determined by adding the value. The mean was determined and percentage inhibition was calculated using the formula:

Inhibition (%) = [(UAcontrol – UAtreated) / UAcontrol] ×100

Determination of total acidity in gastric fluid (S. Gopinathan *et al*., 2013)

The gastric contents were centrifuged at 3000 rpm for 10minutes. The volume of supernatant measured and expressed in ml /100 g body weight. The pH of the supernatant was measured using digital pH meter. An aliquot of 1ml of the gastric juice was treated with toppfers reagent and titrated with0.01N NaOH until all traces of red colour disappeared and turned to yellowish orange. The volume of sodium hydroxide was noted which signifies the free acid. Then 2-3 drops of phenolphthalein was added and titration was continued until a pale pink colour was developed. The volume of total alkali corresponds to total acidity.

$$\text{Acidity} = \frac{\text{volume of sodium hydroxide x Normalaity of sodium hydroxide x 100}}{0.1} mEQ/ml$$

Statistical analysis

All values are expressed as mean ±SEM, statistical differences among the experimental groups were assessed by one-way analysis of variance and the Dunnet test, with the aid of the Instat 2.06 test. The minimum significant level was $p< 0.001$.

RESULTS & DISCUSSION

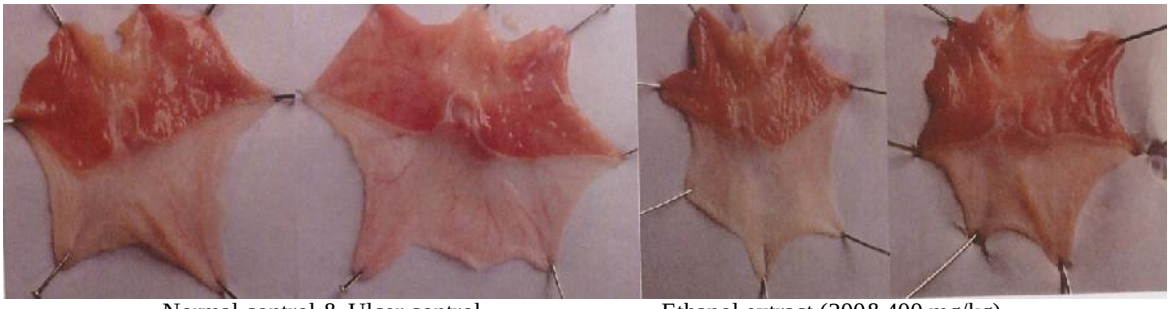
In recent years, considering the prominence of gastric injury and unpredictable side effects of the long-term use of synthetic drugs, interest in the use of herbal products has markedly elevated (Falcao *et al*., 2008). Whole plant of *Hemigraphis colorata* was subjected to phytochemical investigation. Phytoconstituents were extracted by applying continuous hot percolation method using solvents like ethanol and water. The water soluble extractive values and alcohol soluble extractive value was found to be 33.48% w/w and 15.16% w/w. The phytochemical analysis (Ravikumar *et al*., 2010) in both extracts confirms the presence of alkaloids, sterols, steroids, carbohydrates, tannins, flavonoids and fats.

The whole plant extract, both alcohol and aqueous extract of *H. colorata* were subjected to pharmacological studies, evaluation of gastroprotective efficiency against ethanol induced ulcer. Rats were used as animal model. The rodents that were administered with absolute ethanol, experienced severe macroscopic damage, compared to the normal control group, which was evidenced by development of hemorrhagic ulceration. The consequent hemorrhagic detriment was conspicuously attenuated by pretreatment with aqueous and ethanol extract of *Hemigraphis colorata* at the lower dose 200 mg/kg and higher dose of 400 mg/kg b.w. In addition, rats with prior administration of omeprazole (20 mg/kg) were able to suppress damage to the stomach, showing similar features to the normal control group. Macroscopic analysis of the gross appearances of the stomach as shown in figure 2, demonstrated that administration of aqueous extract of *Hemigraphis colorata*, especially at 400 mg/kg dose, had a protective effect against ethanol-induced gastric injury, which was comparable to the protective effect of omeprazole, as a standard antiulcer drug. The ulcers were scored and the comparative study is shown in figure 2 and ulcer index tabulated in table 1. The acidity parameters like gastric volume, total acidity, free acidity, free acid level quantified are tabulated in table 2.

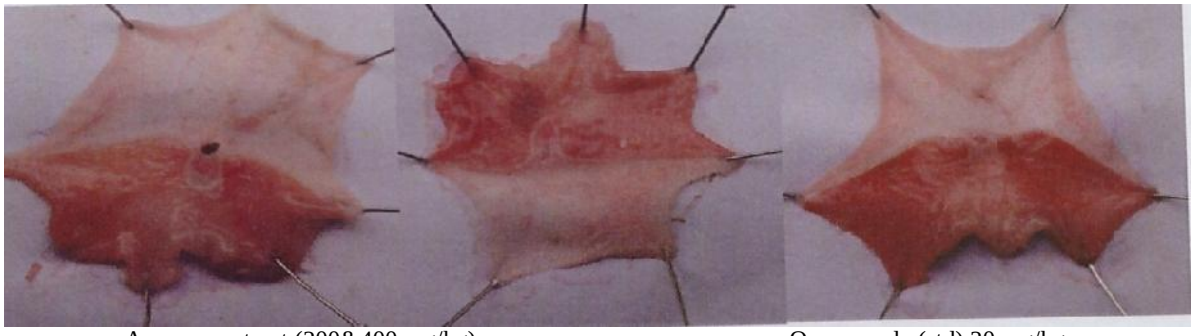
TABLE 1. Ulcer index and % inhibition in ethanol induced rats by *Hemigraphis colorata*

S.No	Group	Ulcer index	% inhibition
1.	Group I (Normal control)	00.00	-
2.	Group II (Ulcer control)	15.7 ± 3.55**	-
3.	Group III Omeprazole (std)	4.01 ± 1.61**	74.45
4.	Group IV Ethanol extract (200 mg/Kg)	2.95 ± 1.07**	81.21
5.	Group V Ethanol extract (400 mg/Kg)	4.53 ± 1.31**	71.14
6.	Group VI Aqueous extract (200 mg/Kg)	3.15 ± 1.25**	79.93
7.	Group VII Aqueous extract (400 mg/Kg)	2.10 ± 0.73**	86.62

n=6 ; **p<0.001; p<0.01



Normal control & Ulcer control Ethanol extract (200&400 mg/kg)



Aqueous extract (200&400 mg/kg) Omeprazole (std) 20 mg/kg

FIGURE. 2 Effect of anti ulcer activity of *Hemigraphis colorata* on ethanol induced ulcers in the rats

TABLE 3. Effect of *Hemighraphis colorata* on total acidity in ethanol induced ulcers in the rats gastroprotective study

S.No	Group	Gastric volume	pH of gastric juice	Total acidity	Free acidity
1.	Normal control	1.31 ± 1.09	1.83 ± 0.47	73.31 ± 4.25	41.31 ± 2.46
2.	Ulcer control	3.47 ± 0.19**	1.11 ± 0.40	94.29 ± 8.73**	76.41 ± 2.20***
3.	Ethanol extract (200mg/kg)	2.53 ± 0.65	2.05 ± 0.17	48.61 ± 4.05	36.51 ± 1.27***
4.	Ethanol extract (400mg/kg)	1.67 ± 0.12***	2.31 ± 0.09***	37.76 ± 3.17	22.16 ± 1.62***
5.	Aqueous extract 200mg/kg)	2.97 ± 0.51*	2.01 ± 0.31	44.74 ± 3.15	37.75 ± 1.53***
6.	Aqueous extract (400mg/kg)	1.83 ± 0.23***	2.14 ± 0.25	39.27 ± 3.23	24.15 ± 1.31***
7.	Standard	1.79 ± 0.10***	2.49 ± 0.17***	40.24 ± 2.01	20.41 ± 0.62***

n=6; ***p<0.001, **p<0.01, p<0.05.

Ethanol is a corrosive agent to the rat gastric mucosa. It promotes superficial cellular necrosis and release of histamine and leucotriene C_4. These tissue-derived mediators act on gastric microvasculature, starting events that result in mucosal and possibly submucosal tissue destruction (Oates *et al*., 1988) alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Soll, A.H. 1990; Surendra, 1999). The results of our study suggested that *Hemigraphis. colorata* prevented the necrotic action of these mediators on the gastric mucosa and protected the gastric mucosa from damage by acids. In this study, ranitidine was not used as standard substance, since the same is not effective in the model of lesions induced by ethanol (Oates *et al*., 1988). The gastro protective action mechanisms presented by *H. colorata* in the ulcer models could be related to the several chemical components of this plant, such as flavonoids and saponins, revealed in the phytochemical investigation part of the research. A dose of 400 mg/kg of aqueous extract showed larger effectiveness, since it was found to protect the animal models of gastric lesions.

CONCLUSION

Results from the study, suggest that aqueous extract of *Hemigraphis colrata* shows significant gastroprotective and anti-ulcerogenic activity. Further isolation and examination of the active constituent of the extract followed by depicting or analyzing its mechanism of action is warranted in the future.

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