PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.3, pp 991-1000, July-Sept 2013

Evaluation Of Antiulcer Activity Of Alcoholic Extract Of Garuga pinnata Roxb. Leaves In Experimental Animal Models

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Abstract: Ulcers are the most common form of GI disorder that is often caused by either inflammation or infection that certainly affected 10% of the population at risk. Among all types of ulcers, peptic ulcer is the more catastrophic one. This point made an exemption objective to conduct an investigation for antiulcer activity of alcoholic extract of Garuga pinnata leaves in rats. Acute gastric lesions in rats was provoked by pylorus ligation, cold strain stress and indomethacin induction models. The experimental protocol assigned the animals (male albino wistar rats) into five groups of five animals each. The selected dose i.e. (100 and 200mg/kg p.o) of the Garuga pinnata leaf extract (GPLE) was administered to group IV and group V which was compared and scrutinized with the other groups. The anti-ulcer activity was evaluated by measuring the clinical parameters such as ulcer index, gastric content and total acidity of gastric fluid, pH value, gastric volume and antioxidant parameters such as lipid peroxidation level and glutathione parameters. The resultant data showed the noteworthy effect on ulcer induced by different ulcer induced models. In conclusion, the outcome demonstrated the antiulcer activity of GPLE which may be exhibited due to the presence of tannins and flavanoids.
Keywords: Peptic ulcer, Acute gastric lesion, Pylorus ligated, Garuga pinnata Leaves.

INTRODUCTION

Ulcer is defined as mucosal erosion equal to or greater than 0.5 cm in the lining of the gastrointestinal tract (GI) that are associated with the presence of gastric acid and pepsin, that is usually acidic and thus extremely detrimental. In general, the ulceration of the gastric and intestinal mucosa which is so called peptic lesion. The increasingly burden's of the people is prevalent in accordance with Helicobacter pylori infections which transmission is through Food, Contaminated ground water and through saliva [1],[2] eventually lead to an ulcer and large number of world population incurred to abdominal pain and gastritis[3]. Peptic ulcer diseases had a devastating effect on morbidity and mortality until the last decade of the 20th century, when epidemiological trends started to point to an impressive fall in its incidence. The lifetime risk for developing a peptic ulcer is approximately 10% [4]. The classification of peptic ulcer likely to be arises in different locations i.e. in Stomach, Duodenum, Esophagus, Meckel's Diverticulum (called Meckel's Diverticulum ulcer). The signs and symptoms observed are Abdominal pain, Nausea, Copious vomiting, Hematemesis, Melena (foul smelling feces due to oxidized iron from hemoglobin). The risks factors include are Heredity, Chronic pain, Alcohol abuse, Diabetes, Other lifestyle factors such as chronic stress, coffee drinking and smoking. Normally, Gastric mucosal defense include several local and Neurohormonal protective factors, which allow the mucosa to resist against frequent exposures of damaging factors; but when these protective mechanisms are underestimated due to overwhelmed by the noxious factors, a gastric lesion may develop. Peptic ulcer results from mucosal tissue necrosis triggers primarily by ischemia, with cessation of nutrient delivery and reactive oxygen species (ROS)

formation.Various medication and therapies have been implemented to reduce the causability of the serious conditions of ulcers. Some of the recent medications like Antacids, H_2 blocker and proton pump inhibitors are well responded as antiulcer drugs which are used to minimize the excessive acid secretion or by protecting the mucosal layer by exhibiting as cytoprotective activity [2]. However majority of the drugs have shown serious adverse affects which made an approach to research on natural sources for their beneficial, affordable, effective and devoid of adverse affects qualities. In recent years, there also has been rise in the interest of scientific community to explore the pharmacological actions or to confirm the veracity of claims made about several herbs which are believed to possess antiulcer activity. *Garuga pinnata* is among some of the plants which claimed to possess antiulcer activity. Recently, it has been proved for antioxidant activity, diabetes treatment etc. [5, 6]

It is also claimed to possess some medicinal properties like use in the treatment of obesity, splenomegaly, roundworm, gastropathy and foul ulcers [7, 8]. Currently, there is no scientific evidence available for *Garuga pinnata* as Antiulcer activity. This point made an attempt to determine *Garuga pinnata* as antiulcer activity. Therefore, the present study is undertaken to evaluate the antiulcer activity of alcoholic extract of *Garuga pinnata* roxb. Leaves in experimental animal models.

MATERIALS AND METHODS

Plant collection and Authentication

The leaves of *Garuga pinnata* Roxb. were collected in the month of November from the Kinnerasani Wildlife Sanctuary, District- Khamman, Andhra Pradesh, India. The plant was identified and authenticated by Dr. P. Jayaraman, Ph.D, Director, Plant Anatomy Research Centre (PARC). The Voucher specimen No. (PARC/2011/753) submitted to Department of Pharmacology SRM college of Pharmacy, Tamil nadu, India.

Extraction and Preparation of test sample

The leaves of *Garuga pinnata* Roxb. were shade dried and made to a fine powder. The powdered leaves of *Garuga pinnata* Roxb. were passed through sieve no. 40 and stored in airtight container for further use. The leaf powder was then defatted with petroleum ether. The defatted powder was extracted by soxhlet-extraction procedure with ethanol (70%) for 48 hours. Solvent elimination under reduced pressure afforded a Greenish-brown semisolid residue. [5]

Phytochemical Screening of extract

The alcoholic extract of *Garuga pinnata* Roxb. Leaves were subjected to preliminary phytochemical screening for their presence or absence of active phytochemical constituents.

Determination of MTD (Maximal Tolerated Dose)

OECD guidelines-420 stated about the Determination of MTD before establishing pharmacological activity of the New Chemical Entity (NCE), the purpose of the sighting study was to allow selection of the appropriate starting dose of NCE for the main study. The starting dose for the sighting study was selected from fixed dose levels of 5, 50, 300 and 2000mg/kg as a dose expected to produce evidence toxicity based, when possible on evidence from *in vitro* and *in vivo* data from the same chemical and from structurally related chemical in the absence of such info, the starting dose selected as 300mg/kg. The main study assigned two mice (one male and female) for each dose. Before administrating compound by oral route, mice should be fasted by withdrawing food for 3hrs and free to water *ad libitium*. Drug sample administered by oral route according to the body weight and observations recorded at ½ hr, 1 hr, 2hr and 4 hr after oral administration of the compound. Mortality was recorded for 14 days. When mortality was reported to remove the animal and performs necropsy so as to changes in the vital organ. At the termination of the study euthanization the living animal using approved CPCSEA method was done and performed the necropsy followed by absorptions of the compound from the site of administration. [9]

Experimental animals

The experiment was carried out on mice (male and female) weighing around 18-25 gm and male albino wistar rats, weighing 150-200g obtained from King Institute, Guindy. They were kept in quarantine for seven days and maintained in a 12h light/dark cycle at 23º± 2ºC temperature and 50±2% humidity and were fed with PETCARE-food pellets(Amruth Laboratories, Musherabad, Hyderabad) and water *ad libitium.* Each

experimental group has separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions prior to experimental protocol to minimize if any of nonspecific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of SRM College of Pharmacy was taken prior to experiment. All the experimental animals and the proposal was approved by committee for the purpose of control and supervision of experiments on Animals (CPCSEA).

Induction of ulcer in rats

Ulcer was induced by pylorus, inducing indomethacin orally or by cold water immersion method. Vehicle, standard and the extract were administered to respective group of animals which are overnight fasted.

Grouping of Animals

Male albino wistar rats 150-200g are divided into five groups, each group consisting of five animals. Animals rat were placed in cages with grating floor to avoid coprophagy in fasting period.

Group- I – Vehicle (10ml/kg)

Group- II – Control

Group- III – Standard (Omeprazole-20mg/kg, p.o)

Group-IV –low dose of GPLE (100mg/kg, p.o)

Group-V – High dose of GPLE (200mg/kg, p.o)

PHARMACOLOGICAL SCREENING

Pylorus ligated ulcer Technique

Animals were fasted for 24h. Animals were given Vehicle, Standard and plant extract by oral route, 1hr prior to pyloric ligation. Ligation was done without causing any damage to the supply of the stomach. Animals were allowed to recover in individual cages and were deprived of water during post-operative period. Animals were sacrificed 4 hr later and the stomach were excised and cut along the greater curvature, washed carefully and ulcer scoring was done. Gastric juice was collected and gastric secretions studies were performed. [10]

Indomethacin induced ulcers in rats

Animals were fasted for 24h having access to drinking water *ad libitium.* Animals were given Vehicle, Standard and plant extract by oral route, and 30 minutes later Indomethacin was induced in a dose of 25mg/kg (4mg/ml dissolved in 0.1 Tween 80 solutions) orally. After six hours, all the animals were sacrificed under light ether anesthesia and the stomach were excised and cut along the greater curvature, washed carefully and ulcer scoring was done. Gastric juice was collected and gastric secretions studies were performed. [11]

Cold-restraint stress-induced ulcers

Animals were fasted for 24h having access to drinking water *ad libitium.* One hour after test substance treatment, the experimental rats were immobilized by strapping all four limbs on a wooden plank and kept for 4 hours, at 4º ± 1ºC. After four hours, all the animals were sacrificed under light ether anesthesia and the stomach were excised and cut along the greater curvature, washed carefully and ulcer scoring was done. Gastric juice was collected and gastric secretions studies were performed. [10]

Ulcer scoring

Ulcer scoring measurement was considered same for all the induction models. A score for the ulcer was made as follows

- 0.5– Hemorrhage
- 1– Streaks
- 2–Spot ulcer
- 3– Severe ulcer
- 4–Erosions
- 5–Perforations

Evaluation

Mean ulcer for each animal was expressed as ulcer index

The percentage of ulcer protection will be determined as follows: [12]

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

ANTISECRETORY ACTIVITY OF GPLE

Volume of gastric content

The gastric content was collected through the esophagus. The gastric juice was centrifuged and the volume was noted. [13]

Biochemical evaluation of total acidity in gastric juice

The total volume of gastric content was measured. The gastric content were centrifuged at 1000rpm for 10 min. 1 ml of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N NaOH using Topfer's reagent as indicator, to the end point when the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further carried till the appearance of the pink color was regained. The volume of NaOH required was noted and was taken as corresponding to the total acidity. [13]

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality of NaOH x 100mEq/l}}{0.1}$$

Estimation of Pepsin activity

Pepsin activity was estimated using a method which a method which incorporates the digestion of hemoglobin solution by pepsin resulting in the formation of tyrosine. The formed tyrosine was separated and treated with alkaline reagent and phenol reagent so as to develop a blue color which was estimated using spectra-photometer at 610nm. [14]

ESTIMATION OF ANTIOXIDANT PARAMETERS

Estimation of Reduced Glutathione

Gastric tissue was scrapped and homogenized in ice-cold phosphate buffer (pH = 8.0) medium. The tissue homogenate was centrifuged at 3,000 rpm for 10 minutes and the supernatant was collected for the experiment. The tissue supernatant was then reacted with 10mM DTNB (5, 5'-Dithiobis- 2-nitrobenzoic acid) of pH 7.0. The resulting suspension was mixed thoroughly and kept at room temperature for 20 minutes. The absorbance was measured at 412nm in a UV- Visible spectrophotometer, 2mM of reduced glutathione (GSH) being used as standard. [15]

Estimation of Malondialdehyde (MDA)

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard method by Buege and Aust. 1.0 ml of the sample extract was added with 2.0 ml of the TCA-TBA HCL reagent (15% w/v TCA, 0.375 w/v TBA and 0.25 N HCL). The contents were boiled for 15 minutes, cooled and centrifuged at 10,000g to remove the precipitate. The absorbance was read at 535 nm and the malondialdehyde concentration of the sample was calculated using extinction coefficient of 1.56 x 105 M-1 Cm-1. [16]

Statistical analysis

Mean values ± SEM were calculated for each parameter. The data has to be subjected for statistical analysis by one way analysis of variance (ANOVA) followed by **Dunnet's "t" test** and *p< 0.05, 0.01* and *0.001* where considers as significant using Graph pad Prism 5.0.

RESULTS

Extract yield

500g of leaf powder was weighed and the alcoholic extract of the leaves yielded Greenish-brown semisolid residue and stored for experimental purpose

Preliminary Phytochemical screening

It was found that 70% Hydro Alcoholic Extract of Garuga Pinnata Leaf extract contained Terpenoids, Saponins, Phenolic Compounds, Tannins, Proteins, Amino Acids, Carbohydrates, Mucilage and Flavanoids.

Maximum Tolerated dose

Even at highest dose there were no physical signs of toxicity as evidenced by normal breathing and the absence of tremors, animals, convulsions, diarrhoea, salivation and paralysis in the treated animals. These observations revealed that the maximum tolerance dose of hydro alcoholic leaf extract of *Garuga pinnata* is 2000mg/kg.

Pylorus –ligated ulcer in rats

As shown in Table 1 depicting the measurement of Ulcer index, deemed the results showing the ulcer index of 3.2±0.20 whereas GPLE decreased the ulcer index significantly at the dose 100 and 200 mg/kg.

Table-1:

Groups	Dose(mg/kg)	Ulcer Index	% Protection
VEHICLE-I	-	0.2±0.20	93.7
CONTROL-II	-	3.2±0.20*	-
STANDARD(OMEPRAZOLE)-III	20mg/kg	$0.8\pm^{\#}$	$75^{\#}$
GPLE-IV	100mg/kg	$1.6\pm0.24^{\#}$	$50^{\#}$
GPLE-V	200mg/kg	$1.2\pm0.20^{\#}$	$62.5^{\#}$

All values are represented as Mean ± SEM (n=5), statistical significant when comparison was performed by using one way ANOVA couple with Dunnet's "t" test, *p < 0.05 pylorus ligation control Vs vehicle; $^{\#}p< 0.05$ treated groups Vs control.

Indomethacin-induced Ulcers

As shown in Table-2, Animals treated with GPLE exhibited significant reduction in the ulcer index. In measurement of ulcer index, the indomethacin induced control animals were showing the ulcer index of 2.8±0.20 whereas GPLE decreased the ulcer index significantly

Table-2:

Group	Dose(mg/kg)	Ulcer Index	%Protection
VEHICLE-I	-	0.40±0.24	85.7
CONTROL-II	-	2.80±0.20	0
STANDARD(OMEPRAZOLE)-III	20mg/kg	1.00±0.00*	65
GPLE-IV	100mg/kg	$1.60\pm0.24^{\#}$	42.8
GPLE-V	200mg/kg	$1.40\pm0.24^{\#}$	50

All values are represented as Mean ± SEM (n=5), statistical significant when comparison was performed by using one way ANOVA couple with Dunnet's "t" test, *p < 0.05 pylorus ligation control Vs vehicle; #p< 0.05 treated groups Vs control.

Cold-restraint stress induce ulcer

As shown in the Table-3, Animals treated with GPLE exhibited significant reduction in the ulcer index. In measurement of ulcer index, the cold restraint stress induce ulcer control animals were showing the ulcer index of 2.60±0.24 whereas GPLE decreased the ulcer index significantly.

Table-3:

Group	Dose(Mg/Kg)	Ulcer Index	%Protection
VEHICLE-I	-	0.2±0.20	92.3
CONTROL-II	-	2.60±0.24	0
STANDARD(OMEPRAZOLE)-III	20mg/kg	0.80±0.20***	69.2
GPLE-IV	100mg/kg	1.60±0.24*	38.4
GPLE-V	200mg/kg	1.20±0.20***	53.8

All values are represented as Mean ± SEM (n=5), statistical significant when comparison was performed by using one way ANOVA couple with Dunnet's "t" test, *p < 0.05; *** p<0.001 statistically significant relative to control group.

Antisecretory activity of GPLE

The results obtained when ulceration of the gastric mucosa was induced by pylorus ligation were shown in Table-4, in gastric secretion studies, it was found that pylorus ligation control rats having gastric pH of 2.10±0.14 was increased when treated with GPLE in dose dependent manner. Omeprazole the standard showed the highest gastric pH (4.89±0.16). The pylorus ligation control animals showed the intended increase in total acidity, gastric content, pepsin activity where as by GPLE decreased or lowered down the level of the total acidity, gastric content, pepsin activity at the dose of 100 and 200mg/kg.

Table-4:

Group	Gastric juice volume(ml)	Free acidity(mEq/l)	Total acidity(mEq/l)	Pepsin activity(mg/4hr)	pH
CONTROL-I	4.56±0.26	44.2±2.67	95.25±0.14	14.44±2.97	2.10±2.97
STANDARD-II	2.54±0.18***	14.6±1.16	31.25±3.68***	4.17±1.46	4.89±1.46
GPLE-III	3.34±0.24**	34.8±1.06	77±2.79**	7.02±1.54	3.22±0.14
GPLE-IV	3.04±0.20***	27.20±1.65	71.5±2.66***	5.78±1.46	4.46±0.18

All values are represented as Mean ± SEM (n=5), statistical significant when comparison was performed by using one way ANOVA couple with Dunnet's "t" test, *p < 0.05; **p<0.01 *** p<0.001 statistically significant relative to control group.

Figure-1

Gastric juice in pylorus ligated rats

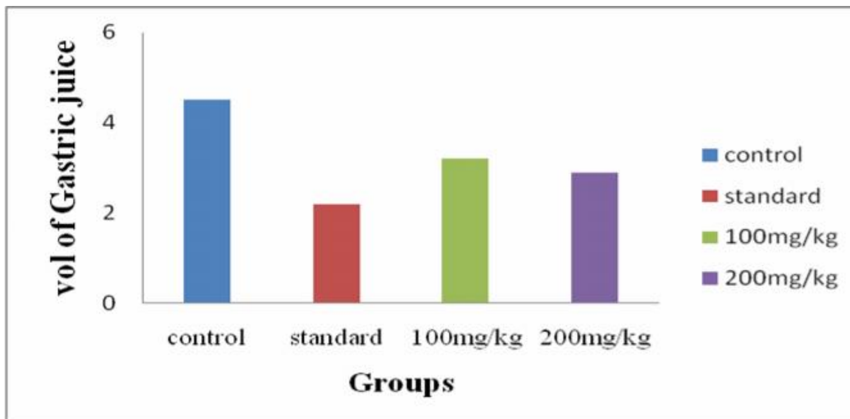


Figure-2

Free acidity (mEq/l) in pylorus ligated rats

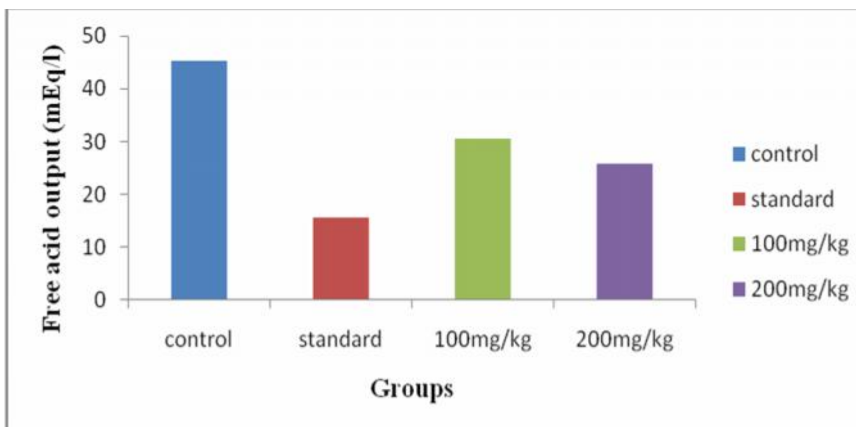


Figure-3

Total acidity in pylorus ligated rats

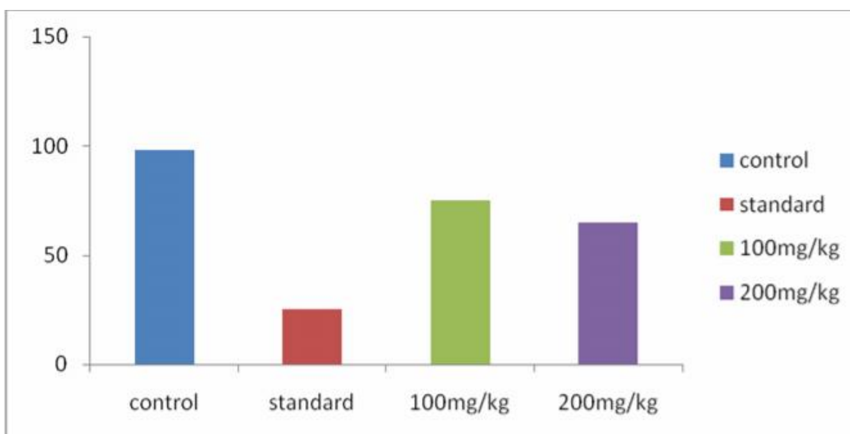
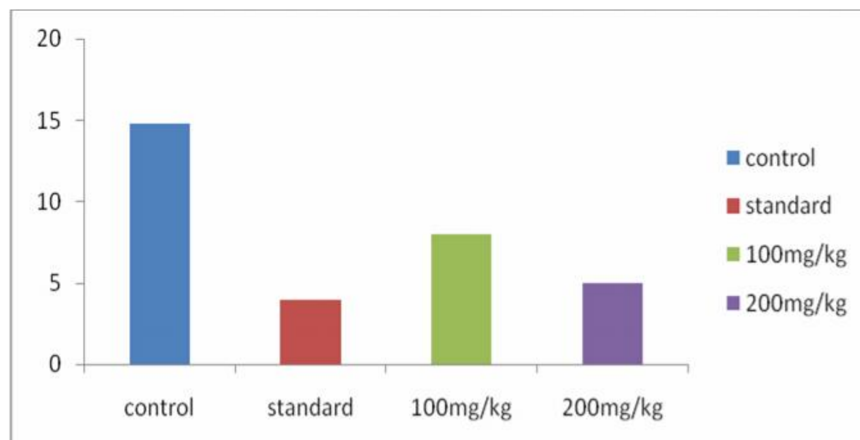


Figure-4

Comparison of pepsin contents



Estimated of Reduced glutathione and Lipid peroxidation

Table-5:

Group	GSH(mol/	MDA(nm/g)
VEHICLE-I	3.2±0.08	25.9±0.40
CONTROL-II	0.8±0.03	47.4±0.67
STANDARD(OMEPRAZOLE)- III	2.7±0.07**	29.6±0.58***
GPLE-1V	1.2±0.12*	38.4±0.90**
GPLE-V	2.2±0.12**	34.8±1.88**

All values are represented as Mean ± SEM (n=5), statistical significant when comparison was performed by using one way ANOVA couple with Dunnet's "t" test, **$p<0.01$ *** $p<0.001$ statistically significant relative to control group.

DISCUSSION

It is generally accepted that ulcer is the result of an imbalance between few adaptable aggressive factors and maintainance of the mucosal integrity through the endogenous defense mechanism. Pylorus ligation induced ulcer is one of the most widely used methods for studying the effects of drugs on gastric secretion [17]. In the present study, it involved fasting of the animals for 24h and pyloric ligation for 4hours. Omeprazole and GPLE (100 & 200mg/kg) significantly decreased the volume of gastric juice, ulcer index, free and total acidity exhibiting cytoprotective action. GPLE had also depicted significant effectiveness ($p<0.001$) in pylorus ligation induced gastric ulcer model. It was showing protection index of 50% and 62.5% at the dose of 100 and 200mg/kg respectively in comparison to control whereas standard drug Omeprazole has shown 75% protection. Pylorus ligation induced gastric ulcer occurs because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion and breakdown of the gastric mucosal barrier [10].Indomethacin is a cyclooxygenase inhibitor which reduces prostaglandin biosynthesis and disrupts mucosal barrier and produces erosions and ulcer in the stomach. It is also well known that prostaglandins synthesized in large quantities by gastrointestinal mucosa can prevent experimentally induced ulcer by ulcerogens. Thus, the gastric cytoprotective agents are effective in preventing in preventing ulcers and mucous content induced by indomethacin [11, 15]. Further, GPLE (100, 200mg/kg) produced an increased activity of antioxidant enzymes, suggesting that its effect on ulcer may partly be due to its antioxidant action. The pathophysiology of stress induced ulcer is due to the release of histamine, leading to an increase in acid secretion and a reduction in mucus production. Admittedly, Stress is also having a causability to disrupt the gastrointestinal tract.

The folds in the stomach are more susceptible to damage, when they come in contact with stress inducing factors. GPLE was tested in the level of 100 and 200mg/kg on Cold-Restraint Stress induced ulcers. In measurement of ulcer index, the cold-restraint stress induce ulcer control animals had raised the ulcer index, whereas GPLE decreased the ulcer index significantly. Free radicals have long been implicating as mediators of

tissue damage in gastrointestinal tract. Correspondingly, it has been shown that affected area is infiltrated by blood-derived cells, mainly neutrophills, macrophages and dendrite cells. In response to activation, these cells are responsible for the generation of reactive oxygen species which are released in large amounts into surrounding tissue. Lipid peroxidation is considered as critical mechanism of the injury that occurs during formation of ulcers. The large amount of malondialdehyde found in the control group is consistent with damage occurred by free radicals [16, 19]. The development of oxygen free radicals that occurs with development of ulcers in stomach leads to decreased GSH levels as a consequence of their consumption during oxidative stress and cellular lysis. This decrease contributes to increased cellular damage by favoring attack by free radicals. Omeprazole and GPLE blunted the depletion of GSH probably by competing in scavenging for free radicals, and as a result helped to preserve the integrity of cellular membrane [10].

Alcoholic extract of *Garuga pinnata* was effective in reducing the ulcer area and the ulcer score. As mentioned earlier, GPLE inhibits the development of induced gastric ulcers in rats. Overall, GPLE as shown a substantial and significant protection against gastric ulcer in all models. The protective effect might have been mediated by both anti-secretory and cytoprotective mechanism. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of GPLE and increase its usage in contemporary medicine.

RESULTS

The qualitative phytochemical study reveals the presence of Carbohydrates, Terpenoids, Flavanoids, Saponins, Phenolic compounds, Tannins, Protein and Amino acid. On the basis of the present results and available reports, it can be concluded that the alcoholic leaf extract of *Garuga pinnata* posses' anti-ulcer activity property in different induced model could be mainly due to modulation of defensive factors through an improvement of gastric cytoprotection and/or partly due to acid inhibition. The above effects of it may also due to the presence of Tannins and Flavanoids in the extract.

ACKNOWLEDGEMENT

The authors are thankful and heartful gratitude to Dr K.S Lakshmi, Dean, College of Pharmacy, SRM University, for her constant encouragement throughout the course of our investigation during the project work and the Management of SRM University for providing us the chemicals and the necessary facilities for our project work.

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