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ANTIULCER ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF MITRAGYNA PARVIFOLI ROXB.

Madan L. Kaushik Professor and Head, Department of Pharmacology, CT Institute of Pharmaceutical Sciences Jalandhar 144020, Punjab.

Mamta kumari* Research Scholar, Department of Pharmacology, CT Institute of Pharmaceutical Sciences Jalandhar 144020, Punjab. *Corresponding Author

ABSTRACT

Mitragyna parvifoli Roxb has long been used in folk medicine in treatment of muscular pain, epilepsy, malaria, smallpox, fever, scabies, ulcer and stomach disorders. Therefore, the present study was designed to investigate the anti-ulcer effect of ethanolic extract of leaves of M. Parvifoli using different models of gastric ulceration in rats. Omeprazole (20 mg/kg/p.o.) has been selected as standard drug. The dose 200, 400mg/kg ethanolic extract of *M. Parvifolia* were selected for the present study. The ethanolic leaves extract of *M. parvifolia* showed significant anti-ulcer activity in a dose dependent manner (200 mg/kg and 400 mg/kg.b.w). The result indicate that 400 mg/kg dose of *M. parvifolia* produced a significant reduction in the ulcer index and significantly inhibit the gastric mucosal damage induced in pylorus-ligated rats. There is significant reduction in the basal gastric acid secretion, moreover the morphological score of stomach showed the anti-ulcer effect using ulcer index. The result of anti-ulcer activity of *M. parvifolia* was near to the standard drug Omeprazole. The above result shows that leaf extract of *M. parvifolia* probably contains active ingredients that could be helpful in the management of the ulcer.

INTRODUCTION

Peptic ulcer is major disorder of gastrointestinal track, define as a break off in the continuity of the mucosa of stomach or duodenum as a consequence of some medications like NSAIDs, gastric acids and pepsin finally causes lesions in internal mucosa [1]. It results probably due to an imbalance between the aggressive and the defensive factors such as acid-pepsin secrection, mucus secrection, blood flow and endogenous protective agents. In gastric ulcer, generally acid secretion is normal or low where in duodenal ulcer, acid secretion is high in half of the patients but normal in the rest [2-3]. It can be of either acute or chronic typically a recurrent condition affects up to 10% of the population with sufficient severity. An estimated 15,000 deaths occur each year as a consequence of peptic ulcer [4]. Many factors involve to induce the ulcer in gastrointestinal tract by increasing acid and pepsin secretion. Another factor which is responsible for the gastric ulcers is oxidative stress in gastric mucosa [5]. Recent studies have shown a correlation between free radicals and the extent of gastric ulceration in experimental animals. There are several types of medicines used to treat ulcer such as H_2 –blocker, proton pump inhibitors and mucosal protective agents. The ulcer due to H.pylori, these medications are prescribed in combination with antibiotics [6]. If medication is ineffective or complications arise, than surgery may be required. The side effects of antiulcer agents are dryness of mouth, achlorhydria, atrophic gastritis and encephalopathy. The cytoprotective agents can also be used for gastric ulceration .

However, all these treatments have limited efficacy and having undesirable side effects . In order to overcome the side effects associated with ulcer, alternative medicine is an method for the management of ulcer with the use a plant or its active constituents. These medicines having inherently high safety without any undesirable side effects [7]. The present study is based on the anti-ulcer activity of plant Mitragyna parvifoli Roxb. of family Rubiaceae. The leaves of M. parvifolia Roxb. reported several biologically active constituents indolic (tetrahydroalstonine, akkuamigine, hirsuteine) and oxindolic (mitraphylline, isomitraphylline, pteropodine, isopteropodine) alkaloids, corynanthedol, dihydrocorynanthedol and angustine. These active constituents are helpful for the treatment of peptic ulcer[8]. Mitragyna parvifoli Roxb. in pylorus ligated rats were tested rigorously by scientifically controlled experiment. Therefore the objective of this study was to investigate the effect of ethanolic extract on rats using pylorus ligation induction method. Hence this study was shown the antiulcer effect of ethanolic extracts (200mg/kg and 400 mg/kg.b.w). Both doses of extract showed the significant effect on rats having peptic ulcer. However, the ethanolic extract dose 400 mg/kg showed the greater antiulcer effect on pylorus ligation induced peptic ulcer in rats as compared to ethanolic extract dose 200mg/kg.

METHODS AND MATERIALS

A. Plant Material collection and authentication

The plant Material was collected from local area of Bhopal M.P. The leaves of M. parvifolia Roxb. were dried, powdered and subjected to extraction. The leaves were identified by Dr. Zia Ulttaran Professor and Head Department of Botany Saifia Science College Bhopal-462001, India.

B. Preparation of extracts

The leaves of M. parvifolia Roxb. was collected and dried in shade. The collected leaves were cleaned and then powdered. The leaf powder was extracted with hydro-alcohol (70-30) solvent by maceration for about 7 days. The solvent was concentrated under reduced pressure using rotary evaporation and dried below 40şC. The extract was brown in colour and percentage yield of the extract was calculated.

C. Preliminary Phytochemical screening

The extracts was obtained and followed the different qualitative chemical tests to establish the presence of a mixture of phytoconstituents i.e. alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins, amino acids, flavonoids and others by means of detection methods [9].

D. Acute toxicity studies

Acute toxicity study of extract was carried out according to OECD 425 guideline (Organization for Economic Co-operation and Development) which is based on a stepwise procedure with the use of a minimum number of animals per step [10].

E. Animal care and Handling

Male Wistar rats weighing 150-250g were used in the experiment. The experimental animals were maintained under standard laboratory conditions in an animal house. The research work was carried out under the guidelines of CPCSEA. The experimental work was performed in Pharmacological laboratory.

F. Antiulcer effect by Pylorus ligation

Animals are divided into four groups, each group consisting of six rats. Control group were received distilled water orally. The Omeprazole, in the dose of 20 mg/kg was be administered orally for Group-II with a reference drug for ulcer protective studies. Group-III and-IV received ethanolic extract of M. parvifolia Roxb in a dose of 200 and 400 mg/kg.p.o. After 45 min of ethanolic and Omeprazole treatment, pyloric ligation was be done by ligating the pyloric end of stomach of rats in respective groups using ether anaesthesia at a dose of 35mg/kg of body weight. The pylorus ligation was done without causing any damage to the blood

supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4hrs of surgery, rats were sacrificed and ulcer scoring was done. The gastric juice was collected for the gastric secretion studies [11-12].

The Scoring of ulcer will be made as follows
Normal stomach.....(0)
Red coloration.....(0.5)
Spot ulcer.....(1)
Hemorrhagic streak..(1.5)
Ulcers.....(2)
Perforation.....(3)
Mean ulcer score for each animal will be expressed as ulcer index.

STATISTICAL ANALYSIS

All represent mean ± S.E.M values. The data were analysed by means of variance (ANOVA).Whenever ANOVA was significant, further multiple comparisons were made by using Bonferroni's test. All the analysis was performed by using the statistical significance range from $p \leq 0.05$ to 0.001[9].

RESULTS

A. Phytochemical screening

Preliminary phyto-chemical screening indicate that the leaf extract of M. parvifolia Roxb. showed the presence of alkaloids, tannins, phenols and absence of fixed oils and terpenoids.

B. Pyloric ligation induced gastric ulcer

In pyloric ligation induced ulcer in rats, the oral administration of ethanolic leaves extract of M. parvifolia Roxb in two different dose showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. It was showing protection index of 40 % and 70 % at the dose of 200 and 400 mg/kg respectively in comparison to control whereas the standard group was reduction of ulcer 73.22%. (Table-1).

TABLE-1

Effect of *M. parvifolia Roxb*.extracts on Ulcer index and percentage inhibition

Treatment groups	Ulcer index and percentage inhibition in pylorus ligation ulcer model				
	Vol. of gastric Juice (ml)	Free acidity (MEQ/L)	Total acidity (MEQ/L)	Ulcer index	% inhibition
Control	8.40±0.22	88.2±2.60	120.4±2.90	9.8±0.46	-
Standard	2.84±0.23*	32.4±2.74*	38.24±2.32*	2.50±0.30*	73.22
Test-1	5.84±0.42	76.2±3.00	109.4±2.84	5.80±0.90	39.96
Test-2	2.94±0.41*	37.45±1.6*	46.90±1.34*	2.90±0.28*	70.12

Value are express as means ± SEM of 6 observations, Statistical comparision as follows significant at *$p<0.01$* compared to control.

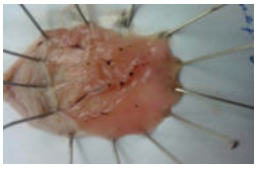

Figure 1: Stomach of control group showing ulcers.

Figure 2: Stomach of Standard group receving Omeprazole (20 mg/kg) and showing greater recovery in ulcer.

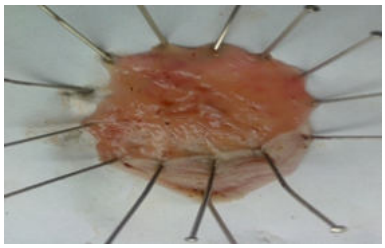

Figure 3: Stomach of Test-1 group receving 200mg/kg *M. parvifolia Roxb*.extract and showing mild recovery in ulcer.

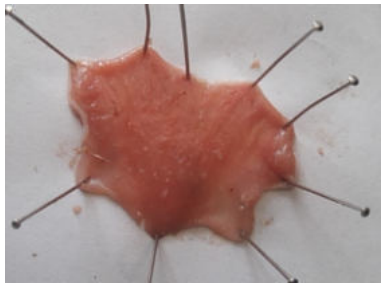

Figure 4: Stomach of Test-2 group receving 400mg/kg *M. parvifolia Roxb*.extract and showing marked recovery in ulcer.

DISCUSSION

Pylorus ligation induced ulcer was used to study the effect of leaves extracts of Mitragyna parvifoli Roxb on gastric acid secretion and mucus secretion. The pylorus ligation causes accumulation of gastric acid in the stomach and this results in increase gastric acid secretion which causes ulcers in the stomach [13]. The fasting of rats for 36 hours followed by ligation of pyloric end of rat stomach and the ulcer index is determined after 5 hours of the pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. Many authors have modified the original model [14-15]. In the present study the leaves ethanolic extract of M. parvifolia Roxb and Omeprazole significantly decreased the total acidity and free acidity. This suggests that M. parvifolia Roxb having an antisecretory effect. Its antiulcer activity is further supported by histopathological study shows that protection of mucosal layer from ulceration and inflammation models. The different constituents like flavanoids, tannins, steroids, saponins, alkaloids and glycosides have been reported to be responsible for antiulcer activity.

CONCLUSION

The results of the present study suggest that the ethanolic extracts 400 mg/kg of M. parvifolia Roxb. showed the significant effect in the recovery of ulcer. The 400mg/kg ethanolic extract showed the anti-secretory and cytoprotective effects. In the present study pylorus ligation method was used to evaluate the antiulcer activity. The plant showed the antiulcer activity due to its vital active constituents. The ethanolic extract of M. parvifolia Roxb. may be used for the treatment of ulcer.

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