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Phytochemical profile and pharmacological activities of *hemigraphis colorata*: a review Comparative study between ethanolic and chloroform extracts of mucuna *pruriens* seeds extract in male wistar rats for the screening of anti-ulcer activity

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Abstract

Background: Well documented neuroprotective profile as well as antioxidant potential of *mucuna pruriens seeds* with lesser side effects, we are intended to compare study between ethanolic (MPEE) and chloroform extracts (MPCE) of mucuna *pruriens* seeds extract in albino Wistar rats for the screening of anti-ulcer activity.

Materials and methods: The pylorus ligation induced ulcer was used to explore the activity of the ethanolic and chloroform extract. Male albino Wistar rats weighing 100-150g were selected and grouped them among normal control, standard (Omeprazole; 20mg/kg) and treatment groups (MPEE; 50 mg/kg and MPCE; 50 mg/kg) respectively.

Results: In our study, the chloroform and ethanolic extract of the seeds of Mucuna pruriens showed antiulcer efficacy as evidenced by a considerable decrease in gastric volume, free and total acidities, ulcer index, and pH. Consequently it might be predicted that both the extracts prevent suppress gastric damage induced by aggressive factors. Preventive anti-oxidants like catalase (CAT) enzymes and reduced glutathione (GSH) constitute the first line of defense in order to combat reactive oxygen species.

Conclusion: The ethanolic and chloroform extracts were significantly inhibited the growth of ulcers. Based on the findings, it was determined that the ethanolic extract had superior antiulcer activity over the chloroform extract. In comparison to ulcer control rats, the administration of herbal extract (50 mg/kg p.o.) raised CAT, SOD, and GSH levels, indicating its efficiency in reducing free radical-induced damage. The preliminary phytochemical investigation showed the presence of glycosides, flavonoids, carbohydrate, and tannins in the ethanolic extract, whereas glycosides, sterols, triterpines and tannins in the chloroform extract. The MPEE exhibited a better ulcer protective activity than the MPCE extract.

***Key Words*:** Mucuna *pruriens*, anti-ulcer, Omeprazole, Pylorus ligation, MPEE (50mg/kg) and MPCE (50mg/kg).

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Introduction

According to Giri *et al*., (2009) Peptic ulcer is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factor. Stomach ulcer is called gastric ulcer while duodenum ulcer is called duodenal ulcer and together peptic ulcer. The pathophysiology of peptic ulcer involves an imbalance between offensive and defensive factors.

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Exogenous aggressive factors such as smoke, anti-inflammatory drugs alcohol, stress, fatty foods and *Helicobacter pylori infection* triggered tissue necrosis through mucosal ischemia free radical generation and end of nutrient delivery, hydrochloric acid collectively with pepsin, pancreatic enzymes and bile decreased the defense mechanisms of gastrointestinal mucosa such as the intracellular junctions, bicarbonate secretion, mucus secretion, local blood flow and cellular growth by [1]. The prevalence of gastrointestinal ulcer differs around the world: duodenal ulcer is dominant in the western populations and gastric ulcers are more common in Asia. As the prevalence of this disease increases over the time, one would expect peptic ulcer to continue to have a significant global impact in the basic health and economic system and in patient quality of life, according to Heloina *et al.,* (2008). An estimated 15,000 deaths occur each as a consequence of peptic ulcer disease. Today there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection by [2]

Materials and methods

Plant collection and Extraction of plants

In this study, the seeds of plant mucuna *pruriens* was collected in and around Central Institute of Medicinal and Aromatic Plants (C-MAP -CSIR) Lucknow, Uttar Pradesh, India. These plants were authenticated at Goel Institute of Pharmacy and sciences Lucknow vide authentication number Tech./Herb/2021-22/02. The voucher specimens of plants are placed in department of pharmacognosy at our institution. The seeds of mucuna *pruriens* extracted by different solvents using soxhlet apparatus. Different extracts were obtained by using different solvents like chloroform and ethanol. The extracts were evaporated to dryness at low temperature (<40°C) under reduced pressure in a rotary evaporator. The percentage yield of all extracts were calculated and extracts were stored in air-tight desiccators for further analysis.

Development and optimization of extract

The extract of seeds of mucuna *pruriens* were prepared by using different ratios. The extracts of seed of plants mucuna *pruriens* was optimized by using different types of Antioxidant assay.

Test Animals

Male albino wistar rats weighing between 100-150g were used for the antiulcer activity. Animals were received from the animal house of Goel Institute of Pharmacy and Sciences, Lucknow, India. . Experimental animals were handled according to regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (2014/PO/Re/S/18/MP CSEA). The animals were maintained under standard conditions of humidity, temperature (25±5°C) and light (12hours light/dark). The animals were acclimatized to animal house conditions and fed with standard pellet diet and water *ad libitum*.

Grouping of Animals:

The pylorus ligation induced ulcer were used for exploring the activity of the extract. The animals were grouped A, B, C and D in each model. Group A was control hence no induction and no treatment was given to this group, group B was standard drug treated group they were administered standard drug such as Omeprazole (20mg/kg) Group C & D were adminisrered MPEE (50mg/kg) and MPCE (50mg/kg) respectively.

Results and Discussion

Phytochemical Tests

The different extracts obtained from seeds of mucuna *pruriens* by successive solvent extraction method were subjected to various qualitative tests to detect presence or absence of common phytoconstituents by (Sherif *et al.,* 2020) and OECD guidelines (2001).

Table1: Phytochemical Test

Tests	Ethanolic extract	Chloroform extract
I. Test for sterols	Negative	Positive
II. Test for Glycosides	Positive	Positive
III. Test for Saponins	Negative	Negative
IV. Test for Carbohydrates	Positive	Negative
V. Tests for Alkaloids	Negative	Negative
VI. Test for Flavonoids	Positive	Negative
VII. Test for Tannins	Positive	Positive
VIII. Test for Proteins	Negative	Negative
IX. Test for Triterpines	Negative	Positive

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Pylorus Ligation Induced Ulcer

The Ethanolic and Chloroform seeds extracts of mucuna *pruriens* showed a significant reduction in free acidity, total acidity gastric pH, when compared with control ($p<0.01$) both of the treatments produced significant effect on ulcer index ($p<0.01$). The Ethanolic extract showed 75.05% protection and Chloroform extract showed 64.90 % ulcer protection. The ethanol extracts also showed significant increase in antioxident levels of CAT, SOD and GSH ($P<0.001$), ($P<0.01$) respectively. The chloroform extract showed ($P<0.001$), ($P<0.01$), and ($P<0.05$) significance in CAT, SOD, and GSH levels. Both MPEE and MPCE showed ($p<0.01$) significance in Protein, mucin, carbohydrate, fucose, Hexoses and Hexosamine estimation.

Mucuna pruriens on Pylorus Ligation Induced

Table 2: Evaluation of Antiulcer Activity of Ethanolic and Chloroform Extracts of Seeds of Ulcer Model

S.No.	Groups	pH	Volume	Ulcer index	Free acidity	Total acidity	percentage protection
1.	Control	2.39±0.13	5.11±0.02	4.76±0.12	93.6±0.37	118.5±0.6	-
2.	Omeprazole(20mg/kg)	4.74±0.04	2.36±0.05	0.41±0.07***	30.2±0.20**	55.2±0.55**	95.31
3.	MPEE (50mg/kg)	4.15±0.03	2.94±0.07	1.21±0.05***	33.7±0.40**	61.4±0.33**	76.06
4.	MPCE (50mg/kg)	3.32±0.03	4.04±0.03	1.62±0.22***	41.2±0.34**	64.9±0.12**	65.87

Table 3: Evaluation of Antiulcer Activity of Ethanolic and Chloroform extracts of *Seeds of mucuna pruriens* seeds.

S.No.	Groups	Catalase	SOD	GSH
1.	Control	60.68±4.22	80.34±2.21	2.54±0.32
2.	Omeprazole (20mg/kg)	88.24±3.2	108.2±2.86	3.93±0.24
3.	MPEE (50mg/kg)	70.3±1.61	91.76±2.14	3.56±0.29
4.	MPCE (50mg/kg)	66.78±2.12	86.9±2.81	3.07±0.08

*Values are expressed as Mean ±SEM, n=6 animal in each group ***P<0.001, **P<0.01 considered as significant compared to control.

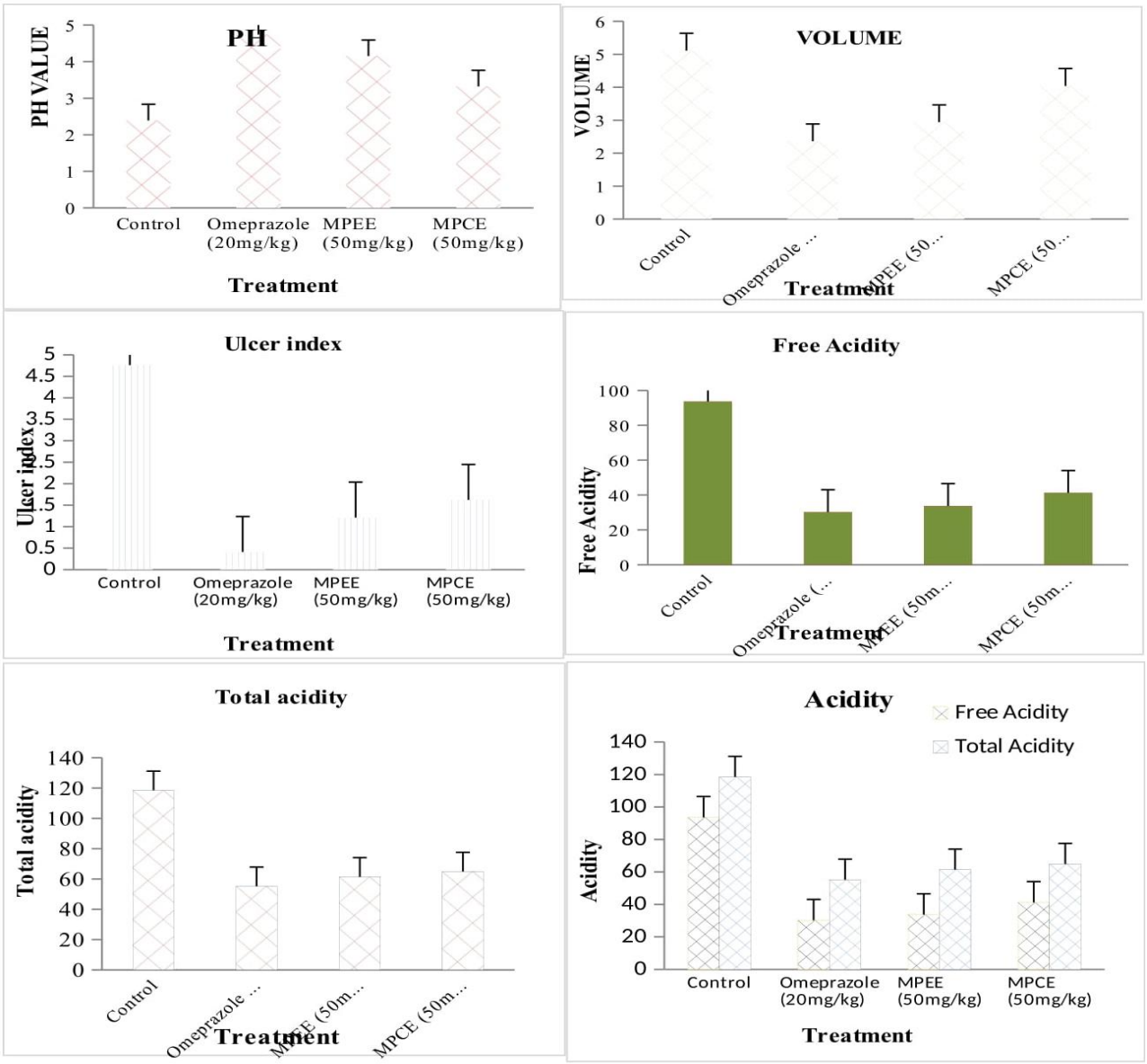

Fig. 1: Evaluation of pH, Volume, Ulcer Index, Total Acidity, Free Acidity and Free acidity and Total acidity on *Seed of mucuna pruriens* extracts by Pylorus Ligation Induced Ulcer Model.

*Values are expressed as Mean ±SEM, n=6 animal in each group ***P<0.001,**P<0.01 considered as significan compared to control

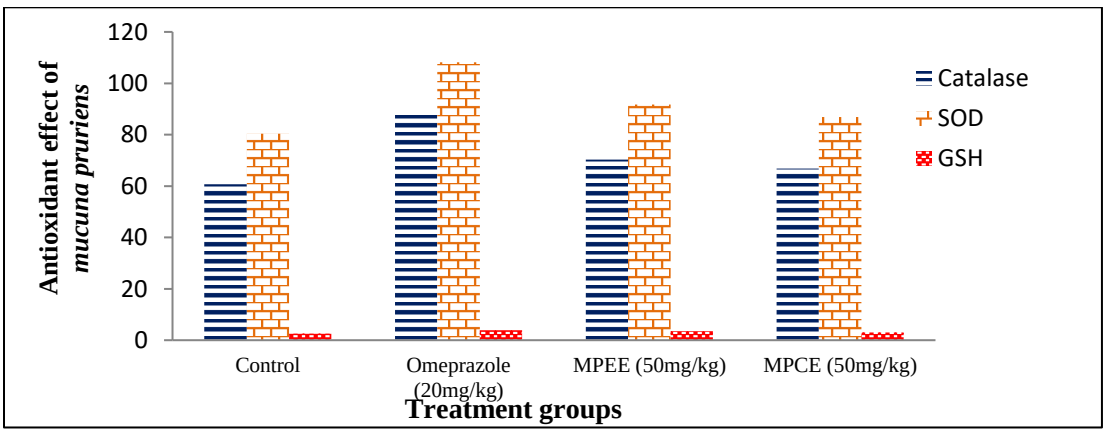

Fig.2: Antioxidant Assay of *Seeds of mucuna pruriens* extracts

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Discussion

In the current study, chloroform and ethanolic extract of *mucuna pruriens* seeds were screened out for their gastroprotective action using pylorus ligation-induced ulcer model in experimental rat. Reports have been documented that ulcer is predominantly produced due to the imbalance between aggressive (gastric juice, pepsin) and protective (mucosal blood flow, bicarbonate secretion, the secretion of mucosa integrality of cellular membrane, cell regeneration, prostaglandin and other hormones) factors. The general anti-ulcer drugs inhibit the acid secretion, protect the mucosa, and inhibit the *Helicobacter pylori*. According to Brodie et al. (1996), the induction of ulceration is caused by the digesting impact of stored gastric juice and interference with gastric blood circulation in pylorus ligation. In our study, the chloroform and ethanolic extract of the seeds of Mucuna pruriens showed antiulcer efficacy as evidenced by a considerable decrease in gastric volume, free and total acidities, ulcer index, and pH. Consequently it might be predicted that both the extracts prevent suppress gastric damage induced by aggressive factors. Preventive anti-oxidants like catalase (CAT) enzymes and reduced glutathione (GSH) constitute the first line of defense in order to combat reactive oxygen species. Further, Goel et al. (1992) revealed that CAT decreases peroxyl radicals produced by H2O2. According to Aebi H. et al. (1974), reduced glutathione (GSH) is a significant inhibitor of free radical-mediated lipid peroxidation and a prominent low molecular weight scavenger of free radicals in the cytoplasm (1974). The levels of CAT, SOD and GSH was considerably reduced in Ulcer control group as compared to normal group. In comparison to ulcer control rats, the administration of herbal extract (50 mg/kg p.o.) raised CAT, SOD, and GSH levels, indicating its efficiency in reducing free radical-induced damage. According to Halliwell et al. (1995), reduced secretion of gastric acid plays a central role in the development of ulcers in pylorus-ligated rats (1995). It has also been stated that the higher nucleic acid production, increased glucose and carbohydrate metabolism, and other compensatory processes all contribute to the development of the ulcer. The higher carbohydrate content of the test extract might be responsible for influencing mucous secretion, which in turn affect the state of the mucus secretion.

Conclusion

The ethanolic and chloroform extracts were significantly inhibited the growth of ulcers. Based on the findings, it was determined that the ethanolic extract had superior antiulcer activity over the chloroform extract. In comparison to ulcer control rats, the administration of herbal extract (50 mg/kg p.o.) raised CAT, SOD, and GSH levels, indicating its efficiency in reducing free radical-induced damage. The preliminary phytochemical investigation showed the presence of glycosides, flavonoids, carbohydrate, and tannins in the ethanolic extract, whereas glycosides, sterols, triterpines and tannins in the chloroform extract. The MPEE exhibited a better ulcer protective activity than the MPCE extract.

Statistical Analysis:

*Values are expressed as Mean ±SEM, n=6 animal in each group ***P<0.001,**P<0.01 considered as significant compared to control.

Conflict of Interest

Author declared that there is no conflict of Interest.

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