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EFFECT OF ETHANOL EXTRACT OF SEED OF MUCUNA PRURIENS ON GASTRIC ULCERS

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ABSTRACT

The present study was carried out to evaluate the effect of ethanol extract of seed of Mucuna pruriens (0.5 g/kg, p.o. and 1 g/kg, p.o.) on gastric ulcers. The study was carried out on different gastric ulcer models such as pylorus ligation, indomethacin induced ulcers and stress induced ulcers. Ranitidine (50 mg/kg p.o.) and misoprostol (100 μg/kg, p.o.) were used as standard drugs. Both doses of ethanol extract of seed of Mucuna pruriens showed gastric ulcer healing effect and gastric antisecretory effect in pylorus-ligated rats and also showed gastric cytoprotective effect in indomethacin-induced ulcer and also produced a significant reduction in the development of stress induced gastric ulcers. The high dose of ethanol extract of seed of Mucuna pruriens (1 g/kg, p.o.) was more effective compared to the low dose (0.5 g/kg, p.o.). Ethanol extract of seed of Mucuna pruriens showed gastric antisecretory and cytoprotective effect and prevents the development of experimentally induced gastric ulcers in rats.

KEYWORDS: Mucuna pruriens, gastric ulcers, gastric antisecretory, gastric cytoprotection.

INTRODUCTION

Previous reports on the incidence of gastric ulcers in Indian population indicates that Chronic exposure to psychological stress is common cause a variety of patho-physiological changes in neuroendrocrine system, resulting in altered physiological activity of G. I. tract. Some drugs also cause gastric ulcer. Mucuna pruriens finds traditional use in number of diseases its various parts are used for various purposes. In tribal area of Chhatarpur Distt. Peoples used the bean of Mucuna for relieving from acidity. Roots are used for treatment ulcers, fever and delirium. They are useful in vitiated conditions of vata and pitta in Ayurveda. Furthermore, root of *Mucuna pruriens* is widely consumed in Shri Lanka, is reported to possess anti-ulcer

activity. However, no study has been conducted to scientifically prove that fruits of *Mucuna pruriens* possess any effect on gastric and duodenal ulcers. Hence, the present study will be undertaken to evaluate the effect of fruits of *Mucuna pruriens* on experimentally induced gastric ulcer. Last two decades have witnessed the introduction of a number of new drugs for the treatment of duodenal and gastric ulcers. Recent research during this period has also added considerably to our understanding of mucosal protection mechanisms and their role in the recovery of the upper gastrointestinal tract from acute damage. The nature and action of newer anti-ulcer drugs also mark a departure from the classical approach of seeking the anticholinergic- antisecretory types of drugs for the treatment of ulcers. These drugs have been shown either to directly promote the healing of ulcers or to possess H_2-antihistaminic activity, more selective antimuscarinic effects, inhibitory effect on gastric proton pump hydrogen-potassium ATPase, cytoprotective[3] or adaptive cytoprotective effects. Peptic ulcer is a condition associated with a number of factors involving autonomic nervous system, which occurs due to excessive acid secretion in the stomach. This condition is control with anti secretory drugs. The long-term uses of synthetic drug produce adverse effect on the human body. For safe and complete cure newer Ayurvedic drugs are used. Our object in the present study is to select a *Mucuna pruriens,* which may act by multiple mechanisms to reduce the ulcer formation and also reduce the relapse rate.

In our present study, we have selected *Mucuna pruriens to* evaluate its gastric antiulcer activity, which was not attempted by researchers earlier. *M. pruriens* have antioxidant activity.[24] Mucuna gives protection against superoxide generation, hydroxyl radical production and FeSO4-induced lipid peroxidation. These radicals are responsible for ulcer generation. Some bacteria such as *H.pylori* are responsible for ulceration The *M. pruriens* have anti microbial activity both against gram positive and gram-negative bacteria. So for above activity of Mucuna I have selected this plant for our investigation.

MATERIAL AND METHODS

Experimental animals

Male albino Wistar rats weighing between 200 and 250 gm were used. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were maintained under standard conditions in an animal house approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drugs and chemicals

Ethanol (Bengal Chemicals, Kolkatta, India), indomethacin (Sigma, St. Louis, MO, USA), ketamine hydrochloride (Troika Parentrals, Gujarat, India).

Gastric Ulcers

Pylorus ligation induced ulcers

The ligation of pyloric end of the stomach was performed on animals fasted for 36 h, under ether anaesthesia.[16,17] The two doses of CLO or ranitidine was administered intraduodenally, immediately after pylorus ligation. The animals were sacrificed six hours after pylorus ligation, through an overdose of ether anaesthesia. The stomachs were isolated and the content collected and centrifuged. The volume of the gastric juice was measured and this was used for the estimation of free acidity,[18] total acidity[18] pepsin content[19] and total proteins.[20] The ulcer index and gastric mucous content was determined.[21]

Healing of indomethacin induced gastric ulcers

The gastric ulcers were induced by administering indomethacin (5 mg/kg. p.o.) for five days.[22] The animals were then treated either with misoprostol (100 μg/kg, p.o),[23] low dose of CLO (0.5 g/kg p.o.) or high dose of CLO (1 g/kg p.o.), once daily for another five days, after the induction of ulcer, while the control group received only vehicle. The rats were sacrificed on the fifth day and the ulcer index was determined. The glandular portion of the stomach was taken and used for estimation of mucin content,[21] total proteins,[20] antioxidant factor's super oxide dismutase activity[24] and catalase activity.[25]

Cold restraint stress induced ulcers

The ulcer was induced by subjecting the animals to cold restraint stress. Ranitidine or CLO was administered 30 min prior to subjecting the animals to stress. The animals were placed in a restraint cage and the cage was placed at a temperature of 2°C for 3 h. The animals were sacrificed after three hours and the ulcer index was determined.[27,28]

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferrroni's comparison test. For comparing nonparametric ulcer scores, anova followed by non-parametric Dunn post test was used. The values are expressed as mean ± SEM and $P < 0.05$ was considered significant.

RESULT

Phytochemical Screening of the extract.

The ethanolic extract of *Mucuna pruriens* showed the following chemicals present in the extract.

Table No. 1 Phytochemical Screening of Ethanolic extract of seed of *mucuna.*

S.No.	Constituents	Test	Ethonalic extract
1.	Alkaloids	a) Mayers reagent b) Dragendroff's reagent c) Hagers reagent d) Wagners reagent	Present Present Present Present
2.	Carbohydrates	a) Molishs reagent b) Fehlings solution A and B c) Benedicts reagent d) Barfoeds reagent	Present Present Present Present
3.	Proteins	a) Biuret test b) Millons reagent	Present Present
4.	Steroids	a) Libermanns burchard test b) Keller Killani's test	Present Present
5.	Tannins	a) 10% Lead acetate solution b) Aqueous bromine solution	Present Present
6.	Flavonoids	a) Ferric chloride test b) Lead acetate test	Present Present
7.	Giycosides	Glacial acetic acid + ferric chloride + Con. Sulphuric acid	Present
8.	Saponins	Foam test	Present

Result on Experimental Models

Effect of *Mucuna pruriens* extract in pylorus ligation induced gastric ulcers

The ethanolic extract of *Mucuna pruriens Linn* and ranitidine showed a significant reduction in free acidity and total acidity when compared to control ($p<0.05$). All treatments produced significant effect on ulcer index. Also the treatments produced any significant effect on mucin content, pepsin activity and total proteins.

Table: 2 Effect of *Mucuna pruriens* extracts on free acidity, total acidity, and ulcer index.

Treatment	Free acidity mEq/litre	Total acidity mEq/litre	Ulcer Index
Control	6.74±0.3581	13.78±0.765	0.108±0.033**
Ranitidine	3.75±0.430*	8.25±1.838*	0.058±0.010**
Low dose	4.04±0.454*	10.23±0.742	0.098±0.025**
High dose	3.98±0.732*	8.83±1.765*	0.606 ± 0.186

All values are mean ± SEM, n = 6. * $p<0.05$, ** $p<0.01$ when compared to control group.

Table: 3: Effect of *Mucuna pruriens* extract on mucin content, pepsin content and total proteins.

Treatment	Mucin content	Pepsin content	Total proteins
Control	0.055 ± 0.007	0.355 ± 0.0206	11.86 ± 0.800
Ranitidine	0.127 ± 0.011**	0.183 ± 0.0091**	0.81 ± 1.298**
Lower dose	0.092 ± 0.009*	0.240 ± 0.0186**	9.00 ± 2.150**
Higher dose	0.108 ± 0.009**	0.207 ± 0.0105**	4.67 ± 0.957

All values are mean ± SEM, n = 6. *p<0.05, **p<0.01 when compared to control group.

Table: 4: Effect of *Mucuna pruriens Linn* extract on ulcer index in stress induced gastric ulcers.

Treatment	Ulcer Index
Control	0.177 ± 0.041
Ranitidine	0.020 ± 0.006**
Lower dose	0.075 ± 0.012*
Higher dose	0.021 ± 0.007**

All values are mean ± SEM, n = 6. *p<0.05, **p<0.01 when compared to control group.

Effect of *Mucuna pruriens e*xtract on healing of indomethacin induced gastric ulcers

The ethanolic extract of *Mucuna pruriens Linn* and misoprostol showed a significant reduction in ulcer index when compared to control (p<0.01). The higher dose showed more significant reduction. The lower dose of ethanolic extract of *Mucuna pruriens* and misoprostol showed a significant increase in the mucus content when compared to control (p<0.01). The higher dose of the extract showed a non-significant increase in mucus content when compared to control. None of the treatments produced any significant effect on total proteins and the ethanolic extract of *Mucuna pruriens* and misoprostol produced significant reduction in anti oxidant factors like SOD activity and catalase activity.

The animals became very weak after 5 days of administration of indomethacin (5mg/kg p.o.) and they showed symptoms of severe diarrhea and their stools were black in colour.

Table: 5: Effect of Mucuna pruriens seed extracts on mucin content, ulcer index and total proteins.

Treatment	Mucin content	Total proteins	Ulcer index
Control	0.585 ± 0.045	09.81 ± 0.960	0.606 ± 0.186
Misoprostol	1.597 ± 0.180**	32.22 ± 0.406	0.048 ± 0.010**
Lower dose	0.733 ± 0.795	11.93 ± 0.978	0.246 ± 0.046**
Higher dose	1.040 ± 0.045	17.03 ± 0.699	0.182 ± 0.049

All values are mean ± SEM, n = 6. *p<0.05, **p<0.01 when compared to control group.

Table: 6: Effect of Mucuna pruriens extract on anti oxidant factors in indomethacin induced ulcers.

Treatment	SOD Units/mg of proteins	Catalase Units/mg of proteins
Control	157.28 ± 9.72	816.54 ± 75.89
Misoprostol	37.92 ± 1.834**	253.61 ± 24.38**
Lower dose	98.41 ± 13.47**	652.45 ± 24.37*
Higher dose	92.03 ± 12.82*	459.45 ± 20.94**

All values are mean ± SEM, n = 6. * $p<0.05$, ** $p<0.01$ when compared to control group.

DISCUSSION

The present study dealt with the effect of *Mucuna pruriens Linn* seed on the gastric and duodenal ulcers. To evaluate the mechanism by which the seed extract increased the gastric ulcer healing, further studies on their effect on gastric secretion and gastric cytoprotection was evaluated using different gastric ulcer models.

Pylorus ligation induced ulcer was used to study the effect of extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 72 hours followed by ligation of pyloric end of the stomach. The ulcer index is determined 19 hours after pylorus ligation. The lesions produced by this method are located in the rumen region of the stomach.[44] In this model, the ulcers developed as lesion in the glandular portion of the stomach. The agents that decrease gastric acid secretion and increase mucus secretion are effective in protecting the ulcers induced by this method. The ethanolic seed extract of *Mucuna pruriens* and ranitidine significantly decreased the total acidity and free acidity. The ethanolic extract of *Mucuna pruriens* increased the mucus content but the increase in mucus content was not significant when compared with that of control.

Stress plays an important role in ulcerogenesis. The pathophysiology is complex in stress-induced ulcers. Stress induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production,.[58,59] In stress conditions, there is an increase in G.I. motility which causes folds in the G.I.T which comes in contact with acid secretion leading to induction of gastric ulcers (peripheral role) and the stress also brings central nervous system into play. The lesions produced by these methods are located in the glandular region of the stomach. The agents which decrease the G.I motility

and that have central actions are helpful in reducing the ulcers due to stress full conditions. The ethanolic extracts of *Mucuna pruriens* and ranitidine were effective in reducing the ulcers induced due to the stress. This suggests that some of the constituents present in the *Mucuna pruriens* seed extracts might have central actions, which are helpful in reducing the gastric ulcers or the reduction may be due to local effect on gastric motility or gastric secretion.

Indomethacin is known to produce erosions and ulcers in the G.I.T. of experimental animals such as rats and guinea pigs. A layer of mucus that apparently forms a barrier covers the gastric mucosa. The gastric mucus production is stimulated by prostaglandins. Prostaglandin deficiency has been regarded to be primarily responsible for ulceration. The administration of indomethacin results in the production of gastric mucosal damage mainly in the glandular portion of the stomach. Indomethacin is a known prominent inhibitor of prostaglandin synthesis that in turn damages the mucosal barrier; the damage in the mucosal barrier causes the permeation of sodium ions from the mucosa in to the lumen.[60] The agents having cytoprotective effect are effective in preventing the ulcers induced by indomethacin. The ethanolic extracts of *Mucuna pruriens* seed were effective in reducing ulcer index and significantly increased the mucus content. This model confirmed the cytoprotective effect of these extracts. The seed extracts of *Mucuna pruriens* were effective in altering the antioxidant factors like SOD activity, total tissue sulfhydryl group (Glutathione) and catalase activity suggesting that the healing of ulcers or prevention of development of gastric ulcers in different models is due to antioxidant action.

The ethanol extracts of the seeds were effective in all the tested models of gastric ulcers. *Mucuna pruriens* contains a number of flavonoids, triterpines, steroids, alkaloids and many other chemical constituents. The flavonoid, quercetin present in the seeds is a well-known anti-ulcer agent.[61] Further, the seeds contain rutin, a flavanoid that is reported to have gastric cytoprotective effect.[62] This explains more potent ulcer healing effect of ethanol extracts of the seeds. The ulcer healing effect obtained with ethanol extracts may be due to both anti-secretory and gastric cytoprotective constituents present in these extracts, as evident by a decrease in acidity in pylorus ligation and decrease in ulcer index in indomethacin gastric ulcers. Apart from flavonoids, the seed of the plant contain steroids such as beta sitosterol and beta-carotene and both of these are known to reduce the development of gastric ulcers.[63] This probably explains the activity of seed extract The results of the present study suggest

that consumption of the seeds of *Mucuna pruriens Linn* may be beneficial in healing of ulcers in patients suffering from peptic ulcer disease.

CONCLUSION

The ethanol extracts of the seeds also decreased the development of gastric ulcers in Shay rat model, indomethacin induced gastric ulcers, stress induced gastric ulcers. The antiulcer effect of ethanol extracts of *Mucuna pruriens Linn* may be due to both reductions in gastric acid secretion and gastric cytoprotection. The exact mechanism by which extract showed antiulcer effect can not be explained, it is speculated that this extract may cover the ulcer area and prevent the attack of gastric acid. The ethanolic seed extract of *Mucuna pruriens* and ranitidine significantly decreased the total acidity and free acidity. The ethanolic extracts of *Mucuna pruriens* and ranitidine were effective in reducing the ulcers induced due to the stress. This suggests that some of the constituents present in the *Mucuna pruriens* seed extracts might have central actions, which are helpful in reducing the gastric ulcers or the reduction may be due to local effect on gastric motility or gastric secretion. All these models suggested that some constituents are presented in the seed of Mucuna pruriens, which is responsible for ulcer healing. The extant of response vary in different models. In the stress induced gastric ulcer extract of *Mucuna* has greater action than other models. The ulcer healing effect obtained with ethanol extracts may be due to both anti-secretory and gastric cytoprotective constituents present in these extracts The present study deals with the study of effect of the ethanolic extract of seed of *Mucuna pruriens* on gastric and duodenal ulcers. Phytochemical analysis revealed that the ethanolic extract contains steroids and alkaloids, carbohydrates, proteins, alkaloids, tannins, flavonoids, glycosides, saponins In the acute toxicity study, no mortality was observed after treatment with the highest tested dose (5g /kg *p*.o) of the extract of fruits. Hence, $1/10^{th}$ of the tested dose (500 mg/kg *p*.o) for higher dose for experiments, and lower dose (100mg/kg p.o.) was selected for evaluation of anti-ulcer activity. The anti ulcer effect was evaluated using the following ulcer models pylorus ligation induced gastric ulcer, stress induced gastric ulcer and healing of indomethacin induced gastric ulcer. The extract of *Mucuna pruriens* was found to be effective in healing the ulcer induced by ligation of pylorus. In pylorus ligation induced ulcers the ethanolic extract of *Mucuna pruriens* fruit and ranitidine significantly decreased the total acidity and free acidity. The ethanolic extract of *Mucuna pruriens* increased the mucus content but the increase in mucus content was not significant when compared with that of control. This suggests that ethanolic extract of *Mucuna pruriens* having antisecretory effect. In stress-induced gastric

ulcers the ethanolic extract of *Mucuna pruriens (500mg/kg.p.o)* and ranitidine (50mg/kg.p.o) showed a significant reduction in ulcer index. The lower dose of the extract was more potent compared to the higher extract. This suggests that some of the constituents present in the *Mucuna pruriens* fruit extract might have central actions, which are helpful in reducing the gastric ulcers. In Indomethacin induced ulcers the ethanolic extract of *Mucuna pruriens* seed, the lower dose (100mg/kg.p.o.) effective in reducing ulcer index but in case of higher dose (500mg/kg.p.o.) it was found less effective both dosage significantly increased the mucus content. The results of the present study suggest that the seed of *Mucuna pruriens* Linn showed anti-ulcer activity. The antiulcer effect of *Mucuna pruriens* Linn may be due to both reductions in gastric acid secretion and gastric cytoprotection. The exact mechanism by which the extract showed antiulcer effect can not be explained.

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