Research Article

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EVALUATION OF ANTIULCER ACTIVITY OF *MUCUNA PRURIENS* BY *IN VIVO* METHOD

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ABSTRACT

The traditional system of medicine has developed faith and pronounced as effective and safe method of treatment. *Mucuna pruriens* use to treat impotence, diabetes mellitus and has anti-ulcer activity. On the other side NSAID's (Non-Steroidal Anti-inflammatory Drugs) is popular group of medicament use to treat inflammation and pain. Aspirin is the most common NASID in the practice which is well known to cause gastrointestinal ulcers. To evaluate the antiulcer activity of aqueous extract of *Mucuna pruriens* in aspirin induced ulcers. Preparation of extracts separately with Petroleum ether, alcohol and water. A total of thirty adult albino wistar rats were divided into four groups to evaluate the anti-ulcer effect. Group 1 Aspirin control (150 mg/kg) Group 2 Aspirin + Standard drug (Pantoprazole 40 mg/kg/per oral) Group 3 Aspirin + *Mucuna pruriens* aqueous extract (200mg/kg body weight) Group 4: Aspirin + *Mucuna pruriens* aqueous extract (400mg/kg body weight). The aqueous extract of *Mucuna pruriens* showed a dose- dependent protection against Aspirin induced gastric ulceration. The extract protected the rat stomach from ulceration by 58.3% at a dose of 200 mg/kg body wt. ($P \leq 0.001$) and this increased to 86.8 ($P \leq 0.001$) at 400 mg/kg body wt. as compared to solvent treated rats, respectively. This was the same percentage protection as 40 mg/kg body wt. of the proton pump inhibitor like pantoprazole. *Mucuna pruriens* showed significant antiulcer activity in experimentally induced ulcer in rat model by decreasing the gastric secretions and by enhancing glycoprotein levels.

Keywords: Antiulcer activity, Gastro-intestinal mucosa, Gastric-secretions

INTRODUCTION

Mucuna pruriens is very famous herbal plant of Ayurvedic system of medicine [1-6] generally use to treat Parkinson'S disease[7], impotence, diabetes mellitus and has anti-ulcer activity as mentioned in the various Ayurvedic texts. On the other side NSAID's is a popular group of medicament use to treat inflammation and pain. NSAID's are well known to cause gastrointestinal mucosal damage and aspirin is the most common NASID in practice, well known to cause gastrointestinal ulcers. All parts of *Mucuna pruriens* are generally used to treat impotence [8], diabetes mellitus [9] and cancer [10] whereas the seeds have multi-diversified functions like several free radical mediated diseases management, rheumatoid arthritis, diabetes, atherosclerosis, nervous disorders, analgesic, antipyretic activity and in the management of parkinsonism [11]. Acute toxicity and Gastro protective role of *Mucuna pruriens* in ethanol induced gastric mucosal injuries in rats is previously studied [12]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [13]. The chemical constituents may be used for the various purposes such as activity against bacteria.[14] Due to its multiple pharmacological uses and presence of medicinally active phyto-constituents allow us to write this research article and represent medicinal values of this plant to scientific communities.

Statistics from the Western population have shown that about 2% to 4% of non-selective NSAID users develop serious gastric mucosal erosions and about 20% of long-term NSAID users develop peptic ulcers. In addition, about 1% to 8% of elderly NSAID users are hospitalized for complications of peptic ulcers within 1 year of initiating therapy.

The mechanism of how NSAID's induce gastric lesions remains unclear and cannot be explicitly explained. The most probable mechanism suggests disruption of gastric mucosal integrity via the production of free radicals. The body has endogenous antioxidants, which under normal conditions are adequate to protect the organs. The NSAID's also inhibit the production of protective prostaglandins, which is another possible mechanism in the pathogenesis. In the present study we have used Aspirin as ulcer inducing agent, as the non-selective NSAID's are well known to cause gastrointestinal mucosal damage and albino wistar rats were used to study the anti-ulcer effect of *Mucuna Pruriens*. The primary objective of the present study is to evaluate the antiulcer activity of aqueous extract of *Mucuna pruriens* in aspirin induced ulcers and secondary objectives are to evaluate the extractive values of *Mucuna pruriens* separately with Petroleum ether, alcohol and water, for 24 hours. by maceration and physico-chemical analysis.

MATERIALS AND METHODS

Collection, authentication and preparation of seeds extract

Mucuna pruriens seeds were collected from Himachal Pradesh, India in December. The identity of the plant material was verified by Dr. H.B Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/ Consult/06/757/74) is deposited in the herbarium of National Institute of Science Communication and Information Resources, New Delhi, India. The seeds (30 gm) of

Mucuna pruriens were powdered and extracted separately with Petroleum ether, alcohol and water, for 24 hours. by maceration. The extracts were filtered, pooled and the solvent was removed under reduced pressure (yield was obtained 6.40, 6.35 and 24 % w/w respectively).

Physico-chemical analysis

Physico-chemical analysis i.e. percentage of ash values, loss on drying and extractive values were performed according to the official methods prescribed [15] and the WHO guidelines on quality control methods for medicinal plant materials [16].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Harborne [17]

Animals

Thirty adult albino wistar rats of either sex weighing between 180-230 g, were procured from the animal house of Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi housed in clean and disinfected cages at 25 ± 2 °C temperature and with a natural photoperiod of 12-hour light/dark cycle and 57 ± 7 % relative humidity under standard hygienic conditions. Commercial basal diet rat feed and water was given ad libitum to the animals. The study got its approval by the Institutional Animal Ethics Committee vide certificate number (IAEC/DIPSAR/2014-I/02) of DIPSAR, New Delhi.

Acute toxicity

Acute toxicity studies for the extract of *Mucuna pruriens* were performed according to the acute toxic classical method as per guidelines 423 prescribed by the Organization for Economic Co-operation and Development OECD. No death was recorded in the rats treated orally with varying doses (250; 500; 1000; 2000 and 4000 mg/kg) of the extract of *Mucuna pruriens*. The extract was well tolerated by the rats without any overt signs of toxicity.

Experimental design

Ulcerative index: Testing for antiulcer activity
Group 1: Aspirin control (150 mg/kg)
Group 2: Aspirin + Standard drug (Pantoprazole 40 mg/kg/per oral)
Group 3: Aspirin + *Mucuna pruriens* aqueous extract (200mg/kg body weight)
Group 4: Aspirin + *Mucuna pruriens* aqueous extract (400mg/kg body weight)

Both male and female wistar rats were fasted for 36 h but allowed free access to drinking water. Twenty-four rats were randomly allocated to 4 groups of 6 rats each. The rats in the all 4 groups (group 1-5) were treated with 200 mg/kg body weight aspirin as ulcer inducing agent. Group 1 rat (aspirin control) served as disease control and was not given any treatment. Group 2 (standard drug treated group) was administered with a standard antiulcer drug (Pantoprazole 40 mg/kg/per oral), Group 3 and group 4 rats were pre-dosed orally with 200 & 400 mg/kg body wt. solution of *Mucuna pruriens* extract. The rats were euthanized by ether, 4 hours after administration of acidified ethanol. The stomachs of rats were removed, incised and open along the greater curvature, washed with normal saline and observed for the mucosal edema, severity of ulcers and necrosis by histopathological studies. The degree of ulceration (Ulcer index) was graded according to a previously mentioned method, described by Shay and colleagues [14] as follows:

0 = no lesions (normal stomach)
0.5 = hyperemia (red coloration)
1 = hemorrhagic spots
2 = 1–5 small ulcers
3 = many small ulcers
4 = many small and large ulcers
5 = stomach full of ulcers along with perforations. Percentage protection was calculated by comparison with the untreated control group.
Protection percentage = 100 − Mean ulcer index of treated group/Mean ulcer index of control group X 100.

Histopathological analysis

The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and were embedded in paraffin wax. Serial sections were obtained using a microtome. This was followed by staining with hematoxylin and eosin. Parameters including mucosal congestion, edema, desquamation and necrosis were observed and recorded.

Statistical analysis

The results of mean ulcer index were compared using the non-parametric Kruskal-Wallis test. The results are expressed as mean ± standard deviation (SD).

RESULTS

Twenty-four rats were randomly allocated to 4 groups of 6 rats each and all were analyzed to get the result of the study. Effect of plant extract on acute gastric lesions induced by NSAID.
The primary gross appearance of gastric damage pattern observed in positive group, aspirin alone induced group showed shallow and linear shape severe ulceration. The gross damage looks to be greater in group two and was significantly reduced in standard drug treatment and test drug treatment.

Antiulcer activity

The aqueous extract of *Mucuna pruriens* demonstrated a dose-dependent protection against Aspirin induced gastric ulceration. Table 1 shows that the extract protected the rat stomach from ulceration by 58.3% at a dose of 200 mg/kg body wt. ($P \leq 0.001$) and this increased to 86.8 ($P \leq 0.001$) at 400 mg/kg body wt. as compared to solvent treated rats, respectively. These results showed same percentage protection as 40 mg/kg body wt. of the proton pump inhibitor, pantoprazole. (Table 1) .

Table 1: Antiulcer activity of aqueous extract of *M. pruriens* seeds

Group	Extract Dose	Mean ulcer index (n=6)	% protection
1	Solvent Control	6	0
2	40 mg/kg (Pantoprazole)	0.8 ± 0.6	86.7
3	200 mg/kg	2.8 ± 0.9	58.3
4	400 mg/kg	0.7 ± 0.6	86.8

Histopathology

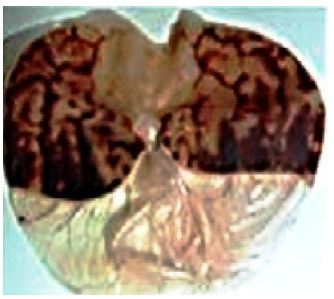

Figure 1: Aspirin Control

Figure 2: Ulcer + Pantoprazole 40 mg/kg

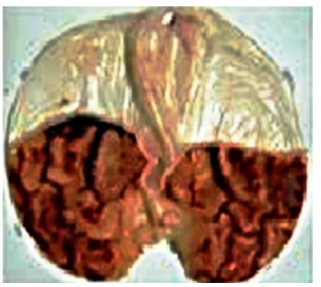

Figure 3: Ulcer + Extract 200 mg/kg

Figure 4: Ulcer + Extract 400 mg/kg

Group A (Negative Control): Rats were given only distilled water orally prior to stomach ulcer induction with aspirin (p.o.). The histopathologic findings revealed numerous severe erosions with marked disorientation of the surface epithelium (Figure 1). In some of the areas, the damage extended into the muscularis mucosa.

Group B (Positive Control): Animals in this group were given Pantoprazole (40 mg/kg), orally before ulcer induction. The mucosa was fairly protected even though; few areas of disorganization of the villi and crypts were visible (Figure 2).

Group C: The histopathologic effect of a low dose (200 mg/kg) of *Mucuna pruriens* extract on aspirin -induced gastric ulceration in rats showed that the epithelium of the gastric mucosa had considerable levels of disorganization.

Group D: Animals in this group were given oral treatment of *Mucuna pruriens* extract (400 mg/kg) prior to administration of aspirin (1 ml) per os. The gastric epithelium was fairly protected.

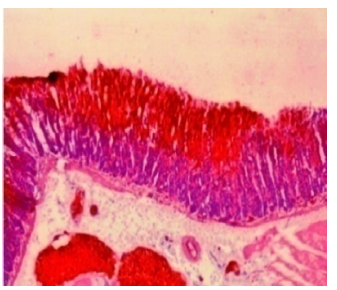

Figure 5: Micrograph of Control rat stomach showing severe ulcer lesions and desquamation of the surface epithelium in aspirin induced gastric ulcers.

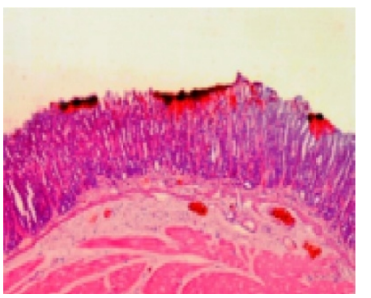

Figure 6: Micrograph of rat stomach fairly protected with pantoprazole (40 mg/kg) in aspirin-induced ulceration.

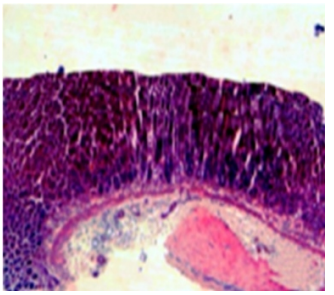

Figure 7: Micrograph of rat stomach showing a protected epithelium due to *M. pruriens* (200 mg/kg) in aspirin-induced gastric ulceration.

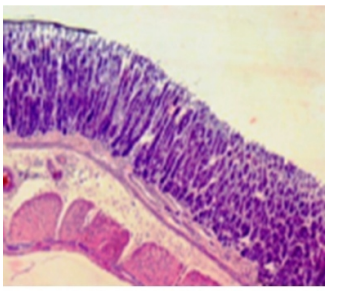

Figure 8: Micrograph of rat stomach showing a protected epithelium due to *M. pruriens* (400 mg/kg) in aspirin-induced gastric ulceration.

DISCUSSION

Physico-chemical parameters like, ash value of a drug provides a view of the earthy matter and inorganic composition of the drug**.** The total evaluated ash value was 3.6% w/w, acid insoluble ash was 0.5% w/w, acid soluble ash values 3**.**4% w/w of the powdered *Mucuna pruriens* seeds shows high concentration of total ash**.** Loss on drying of the powdered *Mucuna pruriens* seeds revealed the presence of 7.5% of moisture in a drug**.** Extractive values are primarily useful for the determination of exhausted or adulterated drugs**.** The extracted value of *Mucuna pruriens* in petroleum ether was 6.5% w/w, in alcohol was 6.4% w/w and water extractive values was 24**.**1% w/w**.** These studies help in authentication of the plant since there is no work reported on their Pharmacognostical investigation previously. Preliminary phytochemical screening of the alcoholic extract of seeds of *Mucuna pruriens* seeds showed the presence of alkaloids, glycosides, terpenoids, tannins and reducing sugars.

This study reveals that pretreatment with *Mucuna pruriens* improve the ulcer index, biochemical and histological changes of Aspirin induced gastric ulceration in rats. Induction of aspirin produces severe gastric hemorrhagic erosions and markedly decreases the gastric output because of back diffusion of HCl through the broken barrier, acute inflammation and inhibition of mucosal blood flow that is consistent with the present report. *Mucuna pruriens* confirmed significant antiulcer activity by decreasing the volume of gastric juice, acid output and pepsin output, ulcer lesions and rise in pH. The *Mucuna pruriens* reduces the volume of gastric secretion with marked decreased of total acid output and pepsin concentration indicative of its anti-secretory effect. Mucus secretion is considered as a critical factor in the protection of gastric mucosa from the gastric lesions hence observed as an important defensive factor in the gastric mucus barrier.

The reports illustrate increased levels of mucus content of gastric tissue pretreated with *Mucuna pruriens* signifies its cytoprotective action on aspirin induced gastric ulcers. *Mucuna pruriens* treatment demonstrated maintenance as well as regeneration of gastric mucosa in the ulcerative regions. The antiulcer activity of *Mucuna pruriens* might be attributed to the presence of biological compounds such as triterpenoids, glycosides, saponins, tannins, beta-sitosterol and amino acids in the extract.

CONCLUSION

In conclusion, *Mucuna pruriens* demonstrated noteworthy antiulcer activity in aspirin induced ulcers in rat model by diminishing the gastric secretions and by improving glycoprotein levels. Furthermore, studies should be done to identify the active principle involved in the antiulcer activity of *Mucuna pruriens*.

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