



STUDIES ON ANTI-ULCER ACTIVITY OF ROOT TUBERS OF *CHLOROPHYTUM BORIVILIANUM* SANTAPAU & FERNANDES

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ABSTRACT

The anti-ulcer activity of the root tubers of *Chlorophytum borivillianum* Sant. & Fern. (Fam. Liliaceae) was studied on albino rats. The anti-ulcer activity of the methanolic extracts of *Chlorophytum borivillianum* Sant. & Fern. root tubers (MECB) in dose of 200 & 400 mg/kg b.w. were evaluated by using Pyloric ligation and aspirin induced ulceration models. All the test samples revealed significant anti-ulcer activity and dose dependent. The results indicated that the root tubers of *Chlorophytum borivillianum* are endowed with effective anti-ulcer activity. The antiulcer activity of the drug can be attributed to free radical scavenging property, inhibition of acid secretory parameters and strengthening of gastric mucosa barrier.

Keywords: Safed Musli, *Chlorophytum borivillianum*, Liliaceae, Anti-ulcer, Pyloric ligation, Aspirin.

INTRODUCTION

Peptic ulcer is an inflamed break in the lining of the stomach or the duodenum caused due to either increased acid production or damaged to the mucous lining of the stomach. In most condition the event of peptic ulcer is due to an imbalance taking place because of increased HCl secretion and decreased cytoprotective activity of the mucosal barrier¹.

Peptic ulcer disease is a common GIT disorders and includes gastro-esophageal reflux, peptic ulcer and Zollinger Ellison Syndrome. Many herbal drugs are used for the management of peptic ulcer disease. Peptic ulcer, including both gastric and duodenal is a common GIT disorder affecting millions of people. It is rarely fatal, however is troublesome for many. Gastric ulcers can be attributed either *H. pylori* or NSAIDS- induced mucosal damaged² besides, imbalance between aggressive factors and defensive factors³. Many herbal drugs are being used as Ayurvedic preparations or in Indian Traditional Medicinal Systems for the management of the peptic ulcers^{4,5}.

Chlorophytum borivillianum Sant. & Fern. (Safed Musli) belongs to the family Liliaceae is a native Indian plant and widely distributed in western Madhya Pradesh, South Rajasthan and North Gujarat⁶. It is an age old and popular constituent in Indian folklore medicine, it is also a key ingredient in several ayurvedic, unani and siddha medicinal preparations⁷. The tubers of these herbs are used as tonic for general debility and also as aphrodisiac⁸. The steroidal sapogenine is the main active principle of this herb. The tuber of Safed musli fetches good market value and has good export potential.

The present work is aimed to investigate the anti-ulcer activity of *Chlorophytum borivillianum* Sant. & Fern. root tubers on experimental animal models.

MATERIALS AND METHODS

Plant materials

Authenticated planting materials were collected through NBPGR (ICAR) from NRC for M & AP, Anand, Gujarat bearing DS no. 413 dated 5th July 2004 and planted by following standard method of cultivation in herbal garden. *C. borivillianum* root tubers were harvested in time and voucher specimen preserved for future references.

Root tubers were made free from aerial parts & wiry rootlets and thoroughly washed, peeled & shade dried. They were powdered to 40 meshes and stored in airtight glass container for further work.

Preparation of Extract

The powder root tubes were subjected to successive extraction with Pet. Ether (40^o – 60^oC), chloroform, ethyl acetate and methanol in a soxhlet apparatus. Solvent from the methanolic extract was removed by vacuum distillation and a brown colour viscous residue was obtained (Yield- 9.78%w/w with regard to the dry weight basis of plant material) and stored in a desiccator. The preliminary phytochemical analysis, of the extract showed positive test and for the presence of alkaloid, glycoside, steroids, saponin and flavonoids.

The methanolic extract of *Chlorophytum borivillianum* Sant. & Fern. root tubers (MECB) in different doses were suspended in 2% w/v gum acacia in distilled water and used as test drug for anti-ulcer activity studies.



Animals

Healthy adult Wister albino rats weighing between 150-200g of both sexes in equal number were used for the study. The animals were housed in standard polypropylene cages at room temperature and provided with standard diet with water *ad libitum*. They were fasted 24 hrs prior to commencement of the experimental procedure. The animals were deprived of food and water during the experimental period. The study was permitted by the Institutional Animal Ethical committee and all efforts were made to minimize animal sufferings and to reduce the number of animals.

Preparation of standard and test drug

2% gum acacia in distilled water was used as vehicle. Ranitidine at dose of 20 mg /kg body weight and Sucralfate at dose of 250mg./Kg. body weight suspended in 2% gum acacia in distilled water were used as standard drugs. Methanolic extract at dose of 200 & 400 gm / kg body weight suspended in 2% gum acacia in distilled water were used as test drugs.

Evaluation of anti-ulcer activity

Pyloric ligated ulceration method (1)

This test was performed as suggested by Shay⁹ et al, 1945. The selected animals were divided into four groups of six animals each. Each group of animals received one of the following test samples through oral route. The control group (Gr-I) received 2% w/v gum acacia in distilled water (2ml/kg), the standard group (Gr-II) received Ranitidine (20mg/kg), the test drug groups (Gr-III & IV) received methanolic extracts in two dosages of 200mg/kg & 400mg/kg of body weight respectively. After one hour of respective drug administration, Pylorus ligation was done under ether anesthesia. The animals were then returned to the observation chamber. After four hours, the animals were sacrificed by decapitation, the abdomen of each animal was opened and stomach was isolated after suturing the lower oesophageal end. The gastric juice was collected by giving a small cut to the pyloric region just above the knot in a measuring cylinder and stomach was opened along the greater curvature. The mucosal layer was washed with 1ml distilled water and the washings were added to the gastric secretions. The gastric contents were centrifuged at 2000rpm for 10 min. The supernatant fluid (1ml) was diluted with 9 ml of distilled water and then titrated against 0.01N NaOH solution using Topfer's reagent till the solution turns to orange in colour. The volume of NaOH required corresponds to free acidity. The solution was further titrated till the solution regained pink colour. The volume of NaOH required corresponded to total acidity as suggested by Gupta¹⁰ et al 1992. Each stomach was then examined carefully for characterizing severity of ulcer. The ulcers were graded as suggested by Kunchandy¹¹ et al, 1985 and Kulkarni¹², 1993.

O = Normal coloured Stomach

0.5 = Red coloration

1 = Spot ulcer

1.5 = Hemorrhagic streaks

2 = Ulcer ≥ 3 but ≤ 5

3 = Ulcer > 5

The mean ulcer score of each animal was expressed as ulcer index. The results are presented in Table 1 & Fig 1.

Aspirin induced ulceration method (2)

This test was performed as suggested by samant¹³ et. al. 1998. The selected animals were divided in to 4 groups of six in each. Each group of animals received the test sample as earlier through oral route. Sucralfate (250 mg/kg) was used as reference standard. After 30 min, each animal was administered 200 mg/kg aspirin through oral route. After 1 hr, pylorus ligation was made as per previous procedure. The animals were killed after 4 hrs; the stomach was opened through along the greater curvature and carefully observed for severity of ulceration as described earlier. The results were presented in Table 2 & Figure 2. A comparative ulcer index study of both methods was done and depicted in Fig 3.

Statistical Analysis

Results were expressed as one way analysis of variance (ANOVA) followed by Dunnett's test and $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The findings of the studies revealed that the methanolic extract of *Chlorophytum borivilianun* root tubers possesses significant anti ulcer activity at higher dose of 400 mg/kg. In pyloric ligated ulceration (Shay model), all the test samples were found to reduced the volume of gastric acid to a significant extent ($P < 0.05$), where as Ranitidine reduced the volume significantly. The total acidity and the free acidity also registered significant decrease in a similar manner. The ulcer index was significantly reduced with all test samples. The order of reduction of ulcer score observed was Ranitidine < 400 mg/kg extract < 200 mg/kg extract. (Table 1 & fig 1)

In Aspirin induced ulceration model, the extracts reduced the ulcer index significantly; but the stomach mucosal layer was found to be normal. The available literature information on possible mechanism of action of sucralfate reveals that it accelerate ulcer healing by forming ulcer adherent complex with proteinaceous exudate, as a results of which pepsin activity is inhibited, where as H₂ antagonist protect experimental animals from gastric ulceration induced by stress, pyloric ligation, aspirin, H₂ receptor agonist or cholinomimetics as suggested by Laurence¹⁴, 1996. (Table 2 & fig 2).

The more ulcerogenic effect by NSAIDS induced mucosal damaged was found and proved by comparison study of Pyloric ligated ulceration and Aspirin induced ulceration methods. (fig 3).



Table 1: Effect of MECB on pyloric ligated ulceration in rats

Group	Treatment	Dose	Volume of Gastric juice	Total acidity (meg/lit)	Free acidity (Meg /lit)	Ulcer index
Gr-I (Control)	Vehicle (2 % w/v gum acacia)	2 ml/kg	4.16±0.47	16.66±0.84	3.16±0.40	3.83±0.47
Gr-II (Standard)	Ranitidine	20 mg/kg	2.16±0.16*	6.5±0.56*	0.68±0.11*	0.71±0.1*
Gr-III (Test drug – I)	(MECB)-I	200 mg/kg	3.5±0.22	10.33±1.42*	0.96±0.03*	1.33±0.33*
Gr-IV (Test drug - II)	(MECB)-II	400 mg/kg	3.16±0.31*	9.66±1.05*	0.83±0.08*	1.16±0.16*

MECB - Methanolic extract of *Chlorophytum borivilianum*; Each value represents the mean ± SEM of six animals in each group; *P<0.05 as compared to control (ANOVA followed by Dunnett's test)

Table 2: Effect of MECB on aspirin induced ulceration in rats

Group	Treatment	Dose	Ulcer index
Gr-I (Control)	Vehicle (2 % w/v gum acacia	2 ml/kg	4.33±0.33
Gr-II (Standard)	Sucralfate	250 mg/kg	0.83±0.16
Gr-III (Test drug – I)	MECB -I	200 mg/kg	1.66±0.21
Gr-IV (Test drug - II)	MECB -II	400 mg/kg	1.33±0.21

MECB- Methanolic extract of *Chlorophytum borivilianum*; Each value represents the mean ± SEM of six animals in each group; *P<0.05 as compared to control (ANOVA followed by Dunnett's test)

Figure 1: Effect of MECB on Aspirin induced ulceration in albino rats (method -1)

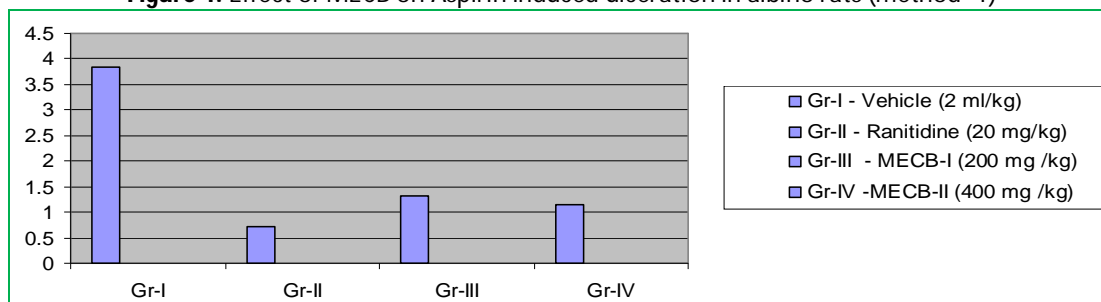


Figure 2: Effect of MECB on Aspirin induced ulceration in albino rats (method -2)

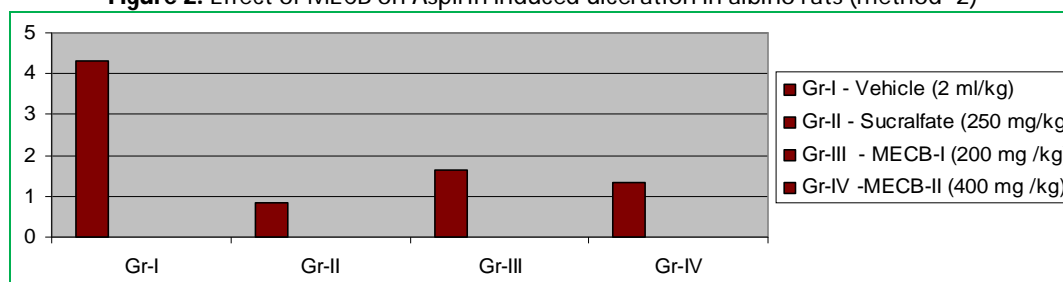
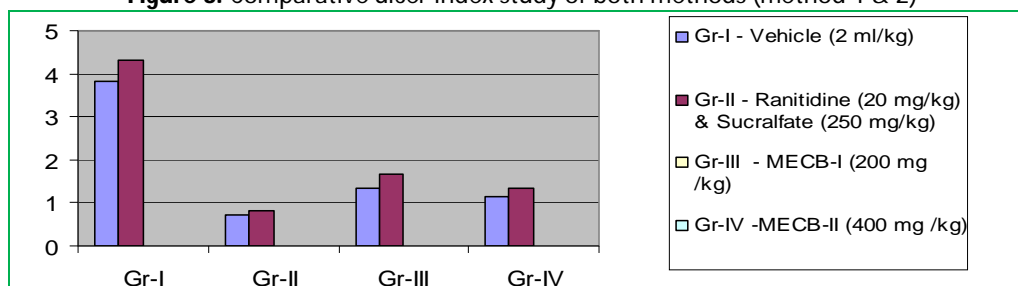


Figure 3: Comparative ulcer index study of both methods (method 1 & 2)



CONCLUSION

The present investigation therefore indicates that the tubers of *Chlorophytum borivillianum* exerts anti-ulcer activity which may be due to anticipated inhabitation of H₂ receptors resulting in inhibition of gastric acid secretion elicited by histamine and gastrin.

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