

# Antiulcer activity of water soaked *Glycine max L.* grains in aspirin induced model of gastric ulcer in Wistar rats

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## ABSTRACT

**Introduction:** *Glycine max L.* with *Drakshasava*, widely used by traditional healers as a formulation for the treatment of peptic ulcer in rural northern Karnataka in India, appears to be effective as assessed by patients and in our previously published research work of traditionally used formulation. **Aim:** The present study was undertaken to evaluate the safety and efficacy of the overnight water soaked *G. max* grains. This is one of the components of traditional formulation. The study, approved by Institutional Animal Ethics Committee was carried out in male Wistar rats after assessing its toxicity in mice. **Materials and Methods:** Four groups of rats ( $n = 6$  in each group) were treated with aspirin 200 mg/kg oral. In addition to aspirin control group received normal saline, standard group received 20 mg/kg omeprazole and 3<sup>rd</sup> and 4<sup>th</sup> group received *G. max* 250 and 500 mg/kg, respectively. All treatments were administered orally every 24 h for 7 days. After 24 hours fasting, on the 8<sup>th</sup> day stomach contents were aspirated under anesthesia to estimate free and total acidity. Stomachs were opened along the greater curvature to calculate ulcer index and subjected to histopathology studies. **Statistics:** The results were analyzed by one-way analysis of variance followed by Dunnett's *post hoc* test.  $P \leq 0.05$  was considered as significant. **Results:** The severity of aspirin induced ulceration was found significantly ( $P < 0.05$ ) decreased in test groups compared with the control group. Free and total acidity was significantly reduced in 500 mg/kg treated group, compared with the control group and was inferior to omeprazole treated group. **Conclusion:** The grain of *G. max* was found to be effective against aspirin induced ulcers.

**Key words:** Aspirin, free acidity, gastric ulcer, *Glycine max*, total acidity

## INTRODUCTION

Traditional healers still play a significant role in health-care delivery system, particularly in rural parts of India. A formulation of *Glycine max L.* and *Drakshasava* is found to be widely used for the treatments of peptic ulcer in rural Northern Karnataka. The formulation was investigated for its anti-ulcer activity on experimental animals and was found

effective to provide significant protection against aspirin induced gastric ulcers.<sup>[1]</sup> Therefore, the present study was planned to explore antiulcer activity of *G. max* grains against aspirin induced gastric ulcers in Wistar rats. *G. max* common name is soyabean, belonging to *Fabaceae* family, perineal herb, has been reported to contain flavonoids, alkaloids, saponins and phenols.<sup>[2]</sup> There is scanty information on antiulcer activity of *G. max* grains. Therefore, the present study was undertaken to evaluate the safety and efficacy of the *G. max*.

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## MATERIALS AND METHODS

### Source of crude drug

Grains of *G. max* were procured from KLE Ayurveda Pharmacy, Belgaum, Karnataka. The material was authenticated by RMRC (Regional Medical Research Centre, an ICMR institute) Belgaum, Karnataka. A sample of the material (Voucher Specimen Number RMRC-930) has been preserved in the institute.

### Animals

Healthy adult Wistar rats of either sex weighing between

100 and 120 g, female healthy Swiss mice weighing 15-20 g were procured from Shree Venkateshwara Traders, Bangalore, India. They were housed in the laboratory for about a week for acclimatization in standard polyacrylic cages at room temperature, under natural light: Dark cycle, were fed with standard rat chow and clean tap water *ad libitum*. The study was approved by Institutional Animal Ethics Committee, constituted as per Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

### Drugs and chemicals

Omeprazole and aspirin were purchased from SIGMA Chemicals co (St Louis, MO, USA). Phenolphthalein and NaOH (sodium hydroxide pellets) were purchased from Fisher Scientific Co (Pittsburg, PA, USA). Topfers reagent was purchased from NICE Chemicals Cochin, India.

### Acute toxicity studies

Swiss mice weighing 15-20 g were used in the study after acclimatization for a week under laboratory conditions at room temperature with standard rat chow and tap water *ad libitum* in polyacrylic cages. The animals were fasted overnight, to receive a single dose (2000 mg/kg BW) of *G. max* grains paste next day and observation were carried out as per Organisation for Economic Co-operation and Development (OECD) guideline 423-2002.<sup>[3]</sup> The animals were observed for first 24 hours (h) with special attention during the first 4 h and intermittent observation for next 14 days. On the 3<sup>rd</sup> day, the experiment was repeated in two more animals and observations were carried out as described earlier.

### Aspirin-induced gastric ulcer studies

Aspirin and standard antiulcer drug omeprazole were prepared in 2% gum acacia suspension as vehicle. A total of 24 Wistar rats were divided into four groups ( $n = 6$  in each group). All four groups were treated with 200 mg/kg body weight aspirin. In addition to aspirin, group I (control) received 10 ml/kg 2% gum acacia, group II (standard) received 20 mg/kg omeprazole and group III and IV received test formulation at 250 and 500 mg/kg, respectively. All treatments were administered orally and repeated every 24 h for 7 days and 30 min of interval was maintained between interventions and aspirin. On the 8<sup>th</sup> day, animals in each group were fasted for 18 h after their respective assigned treatment. Animals were sacrificed with over anesthesia and abdomen was opened by midline incision to aspirate the gastric contents in to a measuring cylinder. The gastric secretion volumes were measured and expressed as ml/100 g body weight. Supernatants were taken after centrifugation at 3000 RPM for 10 min and individually assayed for the acidity by titration to pH 3.5 with 0.01N (NaOH) using Topfers reagent as

indicator and total acidity was estimated by titration to pH 8.0 with 0.01N (NaOH) using phenolphthalein as indicator.<sup>[4]</sup> The free acidity and total acidity were expressed in  $\mu$  equivalent/100 g. The stomachs were opened along with greater curvature and mucosa was observed for ulcers under a dissecting microscope. Thereafter, stomachs were examined for mucosal edema, necrosis and ulcer depth by histopathological studies. Ulcer index was calculated as described by Gupta *et al.*<sup>[5]</sup>

### Histological study

The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thickness were obtained using a microtome. The deparaffinized sections were stained with hematoxylin and eosin. Mucosal congestion, edema, desquamation and necrosis were observed.

### Statistical analysis

The results were expressed as Mean  $\pm$  SD and data were analyzed by one way analysis of variance followed-by Dunnett's *post hoc* test for parametric, Kruskal-Wallis *post hoc* test for non-parametric test here and  $P \leq 0.05$  was considered as significant.

## RESULTS

### Acute toxicity studies

There was no mortality over the observation period of 14 days in animals treated with a single over dose of 2000 mg/kg. There was no visible sign of toxicity and LD50 was therefore, considered to be more than 2000 mg/kg.

### Aspirin induced ulcer

The severity of aspirin induced ulceration was found significantly ( $P < 0.05$ ) decreased in test groups compare with the control group and the extent of reduction was comparable with that of omeprazole treated group [Table 1].

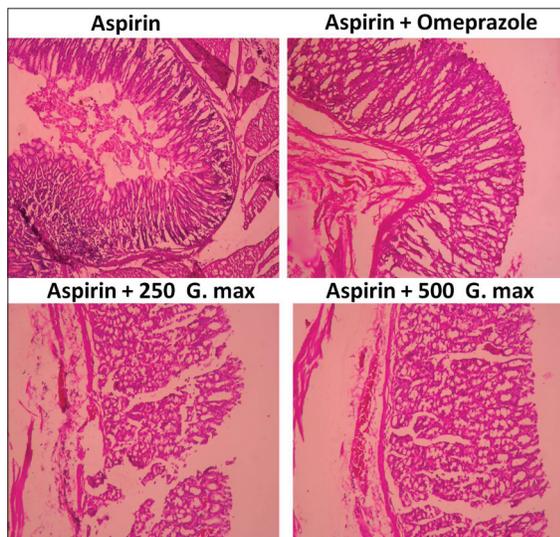
### Histological study

Histopathology results [Figure 1] revealed mucosal congestion, edema, necrosis and desquamation in (aspirin and vehicle treated control group).

Aspirin and omeprazole treated group showed mild congestion, whereas aspirin and *G. max* 250 mg/kg treated group showed the presence of mucosal necrosis, edema, congestion while aspirin and *G. max* 500 mg/kg treated group showed mild congestion and desquamation, which were almost comparable that in omeprazole treated group. *G. max* in the higher dose showed marginal, but significant reduction in gastric acid. Both dose of *G. max* significantly

**Table 1: Free, total acidity and ulcer index in experimental groups**

Assesment parameters	Group I control	Group II omeprazole 20 mg/kg	Group III <i>G. max</i> 250 mg/kg	Group IV <i>G. max</i> 500 mg/kg
Free acidity $\mu$ eqv/100 g	49.22 $\pm$ 4.95	25.15 $\pm$ 3.97**	46.78 $\pm$ 4.54	44.19 $\pm$ 3.54*
Total acidity $\mu$ eqv/100 g	169.49 $\pm$ 4.72	118.74 $\pm$ 5.03**	166.49 $\pm$ 4.08	159.66 $\pm$ 5.60*
Gastric ulcer score	20.83 $\pm$ 2.13	1.0 $\pm$ 0.63**	15.33 $\pm$ 2.33	11.66 $\pm$ 2.73*

\* $P < 0.05$ , \*\* $P < 0.01$ **Figure 1:** Histopathology photomicrograph of various treated groups ( $\times 40$ )

reduced ulcer scores. Though, their effect was almost 10 times lesser than that of omeprazole. It is interesting to note that a lower dose of *G. max* without affecting the gastric acid content significantly reduced the ulcer score, indicating the antiulcer mechanism of *G. max* is probably by increasing gastro protective substance rather than by suppressing acid output, which mucoprotective indicates the antiulcer activity of *G. max* need to be explored. The role of *Drakshasava* is clearly reflected, it helps in decreasing acid secretion and ulcer score.

## DISCUSSION

Gastric ulcer is the common condition encountered in clinical practice. Ulcers are produced because of the inequity between aggressive and protective factor of the mucosal layer. To maintain the imbalance between aggressive and protective factor plenty of therapeutic agents are available. The antiulcer products available in the market such as proton pump inhibitors, histamine H<sub>2</sub> antagonists, antacids and anticholinergics reduced HCl an aggressive factor while drugs like sucralfate might prevent ulcerogenic potential of HCl.<sup>[6]</sup> Most of these products produce several adverse effects such as gynecomastia, acute interstitial nephritis,<sup>[7]</sup> thrombocytopenia,<sup>[8]</sup> nephrotoxicity and hepatotoxicity.<sup>[9]</sup> Traditionally used herbal formulations are a source of new drug and have been used frequently to treat peptic and

duodenal ulcers. Hence, the traditional formulation take to evaluate the safety and efficacy by aspirin induced ulceration model. The *G. max* grains provided significant gastro protection against aspirin induced gastric ulcer and the protection was almost comparable with that of omeprazole commonly used drug for peptic ulcer. There is a paucity of information regarding antiulcer activity of the *G. max* grains, which is a component of traditional herbal formulation, used in North Karnataka. The reported anti-inflammatory activity of *G. max* on the contrary suggests its ulcerogenic potential since most of the anti-inflammatory agents are known to be gastrototoxic. Gastro protective activity of the formulation could be attributed due to the presence of flavonoids in *G. max*, as flavonoids have been reported to decrease the gastric acid secretion by its antihistaminic properties, chalcones are reported to increase the mucosal blood flow, stimulate the synthesis of mucosubstances and their antioxidant properties. Quercetin and naringenin also have been reported to accelerate the healing of gastric ulcers.<sup>[10]</sup> In addition, the primary metabolites such as protein, fat and carbohydrates are abundant in the seeds, which may have their role, apart from their nutritional value, in healing gastric ulcer.<sup>[11]</sup> Our earlier studies showed that the traditional formulation (water soaked *G. max* grains in *Drakshasava*) showed 89% reduction in ulcers and 50% inhibition in acid secretion, whereas *G. max* grains showed only 44% decrease in ulcers and 10% inhibition in acid secretion. *G. max* grains found less effective compared with traditional formulation in terms of the ulcer index and acidity decrease.<sup>[1]</sup> The results of the present study clearly indicate that *Drakshasava* help in further decreasing of acid secretion and ulcer score. However, it is desirable to elucidate the antiulcer mechanism of protection by *G. max* grains, prior to establish its efficacy in the large number of patients suffering from peptic ulcer disorders.

## CONCLUSION

Based on the results, it can be concluded that overnight soaked *G. max* grains have limited antiulcer activity, when compared to that of traditional formulation containing *G. max* grains and *Drakshasava*.

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