ANTIULCER ACTIVITY OF TRADITIONAL FORMULATION IN WISTAR RATS

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Summary

A formulation of *Glycine max* L. and Drakshasava, widely used by traditional healers for the treatment of peptic ulcer in rural northern Karnataka in India, appears to be effective, as assessed by patients. The present study was undertaken to evaluate the safety and efficacy of the formulation. The study, approved by IAEC was carried out in male Wistar rats after assessing its toxicity in mice. Three groups of rats (N=6) were treated with aspirin200mg/kg oral. In addition to aspirin control group received 2% gum acacia, standard group received ranitidine 50 mg/kg and third group received test formulation 40mg/kg. All the treatments were administered orally every 24hrs for 7days .After 24 hrs fasting, on eighth day under anesthesia stomach contents were aspirated to estimate free & total acidity. Ulcer scoring in stomachs opened along the greater curvature was done to calculate ulcer index. The results were analyzed by one way ANOVA followed by Dunnets post hoc test. $P \leq 0.05$ was considered as significant. The test formulation found to be effective against Aspirin induced ulcers.

Key words: Aspirin, Gastric acidity, Gastric ulcer, *Glycine max, Vitis vinifera*.

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. (Fabaceae) and Drakshasava is found to be widely used for the treatments of peptic ulcer in rural northern Karnataka. The chemical contents of *Glycine max* are flavonoids, alkaloids, saponins and phenols[1]. *Glycine max* reported for its trypsin inhibitory effect[2]. Ethanolic extract of *Glycine max* has also shown the antinociceptive and anti-inflammatory activity[3]. *Glycine max* seeds are also reported to be useful in arthritis[4]. Petroleum ether and alcohol extract of *Glycine max* seeds were reported to possess antihyperglycemic activity[5].

Second component of the formulation is 'Drakshasava'. It is a fermented liquid preparation, with main ingredient being 'Draksha' (*Vitis vinifera* L.-Vitaceae) API (Active Pharmaceutical Ingredient). Chemical constituents of *Vitis vinifera* include flavonoids, glucose, fructose, glycosides and polyphenols[6]. The antioxidant[7], spasmolytic[8], bronchodilator[9] and antidiabetic[10]activities were also reported for the same. *Woodfordia fruticosa* Kurz*., Cicca acida* (L.) Merr*., Santalum album* L. and *Cinnamomum zeylanica* L. are the other minor ingredients of 'Drakshasava'[11]. Though, the feed back from the treated patients indicate that the formulation is quite effective, there is scanty information regarding the antiulcer activity of constituents of the formulation. Moreover other reported actions of *Glycine max* indicate its ulcerogenic potential. The present study was therefore undertaken to evaluate the safety and efficacy of the formulation.

Materials and Methods

Preparation of Formulation

The formulation was prepared by following the exact procedures of traditional practitioners. Twenty five gram over night soaked grains of *Glycine max* was triturated with 10ml (two tea spoon) of Drakshasava. Human adult dose of the formulation prescribed by traditional healers was converted to animal equivalent dose as per conversion table devised by Paget & Barnes[12].

Animals

Healthy, adult, Wistar rats of either sex weighing between 100-120g; healthy, female Swiss mice weighing 15-20g were procured from Shree Venkteshwara Traders, Bangalore, India. They were housed in the laboratory for about a week for acclimatization at room temperature (25 ± 3 ºc) with 12:12 hr light & dark cycle and were fed with standard rat chow and tap water ad libitum. The study was approved by (IAEC), constituted as per CPCSEA Guidelines.

Drugs and Chemicals

Ranitidine and Aspirin were purchased from SIGMA Chemicals co (St Louis MO). Phenolphthalein and NaOH (Sodium Hydroxide Pellets) were purchased from Fischer Scientific Co (Pittsburg, PA) Topfers reagent was purchased from NICE Chemicals Cochin. Drakshasava was purchased from local medical shop, MFG by Shree Baidyanath Aurvedic Bhavan Pvt. Ltd, Nagpur.

Acute toxicity studies
Swiss mice weighing 15-20 g were used in the study. The animals were fasted over night, to receive a single dose (2000mg/kg BW) of herbal formulation next day and were observed as per OECD guideline 423-2002[13].

Aspirin-induced gastric ulcer studies
Aspirin and standard antiulcer drug ranitidine were prepared in 2% gum acacia suspension as vehicle. Eighteen animals were divided in three groups (N=6). All three group were treated with aspirin 200mg/kg. In addition to aspirin group I (control) received 2% gum acacia 10ml/kg, group II (standard) received ranitidine 50mg/kg and group III received test formulation 40mg/kg. All the treatments were administered orally and repeated every 24 hrs for seven days. On 8th day animals in the entire group were fasted for 18 hrs after the respective assigned treatment. Animals were sacrificed with halothane over anesthesia. Abdomen was opened by midline incision to aspirate the gastric contents in to a measuring cylinder. The gastric secretions were expressed as ml/100g bw. Supernatants taken after centrifuge at 3000 RPM for 10 minute, were individually assayed for the acidity by titration to pH 3.5 with 0.01N NaOH using Topfers reagent as indicator and total acidity by titration to pH 8.0 with 0.01N NaOH using phenolphthalein as indicator[14]. The free acidity and total acidity were expressed in µeq/100g. The stomachs were opened along with greater curvature to observe mucosa for ulcers under dissecting microscope and ulcer index was calculated[15].

Statistical analysis
The results were expressed as Mean ± SEM and the data were analyzed by ANOVA followed by Dunnett's post hoc test. $P \leq 0.05$ was considered as significant.

Results

Acute toxicity studies
There was no mortality over a period of observation for 14 days in animals treated with a single over dose of 2000mg/kg. There were no other signs of toxicity and LD_{50} was considered to be more than 2000mg/kg.

Aspirin induced ulcer
The severity of aspirin induced ulceration was significantly ($P<0.05$) decreased in herbal formulation treated group as compare to that of control group and was comparable to that of ranitidine treated group (Table 1).

Table 1. Antiulser activity of Test Formulation in comparison with Control and Standard.

Parameters	Groups		
	Control	Ranitidine	Test Formulation
Gastric juice ml/100g	1.26 ± 0.18	0.9 ± 0.23*	0.9 ± 0.06*
Free acidity µ eqv/100g	217.5 ± 0.52	117.5 ± 0.11**	111.66 ± 0.07**
Total acidity µ eqv/100g	890 ± 0.32	610.83 ± 1.02**	665± 0.38**
Gastric Ulcer Score	16.5 ± 1.02	1.33 ± 0.33**	1.83 ±0.74**

* = $P<0.05$, ** = $P<0.01$

Both ranitidine and test formulation significantly (P<0.05) decreased the gastric volume, total and free acidity, as compared to that of control group. Free acidity in vehicle, ranitidine and test formulation treated group was found respectively 217.5±0.52, 117.5±0.11, 111.66±0.07, while corresponding total acidity was found to be 890 ± 1.02, 610± 1.02 and 665 ± 0.38 (Table 1). Mean ulcer score of vehicle, ranitidine and test formulation treated group was found to be 16.5 ± 1.02, 1.33 ± 0.33 and 1.83 ± 0.74 respectively. In both the ranitidine and test formulation treated animals there was significant (P<0.05) decrease in ulcer index as compared to that of controls (Table 1).

Discussion

Gastric ulcer is the common condition encountered in clinical practice. Ulcers are produced because of imbalance between aggressive and protective factor of the mucosal layer. Plenty of therapeutic agents are available to maintain the balance between aggressive and protective factor, as a treatment. They may be proton pump inhibitors, histamine H_2 antagonists, antacids and anticholinergics[16]. Most of these product are reported to have adverse effects such as gynecomastia, acute interstitial nephritis[17], thrombocytopenia[18], nephrotoxicity and hepatotoxicity[19]. Several herbal formulations are used frequently in the traditional medical system to treat peptic and duodenal ulcers, which are believed to be effective and have lesser side effects. Hence, one such traditional formulation was selected to evaluate the safety and efficacy by aspirin induced ulceration model.

The test formulation provided significant gastro protection against aspirin induced gastric ulcer and the protection was almost comparable to that of ranitidine, a commonly used drug for peptic ulcer. There is paucity of information regarding antiulcer activity of components of the test formulation (*Glycine max* and *Vitis vinifera*) used in the present study. The reported anti-inflammatory activity of *Glycine max,* on the contrary, suggests its ulcerogenic potential, since most of the anti-inflammatory agents are known to be gastro toxic. Gastroprotective activity of the formulation could be attributed to flavonides present in *Glycine max*, as flavonides being antioxidants, could protect from the injury due to oxygen free radicals and its trypsin inhibitory effect might also be contributing. Gastroprotective activity of *Glycine max* could be further enhanced by addition antioxidant effect of *Vitis vinifera* (Drakshasava), the other component of the formulation. It is desirable to elucidate the antiulcer mechanism of the formulation, prior to establish its efficacy in large number of patients suffering from acid peptic ulcer disorders.

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