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Evaluation of Antiulcer Activity of *Picrasma quassioides* (D. Don) Bennett Aqueous Extract in Rodents

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ABSTRACT

The present study was to evaluate the protective activity of the *Picrasma quassioides* (PQ) whole plant aqueous extract using three methods. With Aspirin-pylorus ligation induced method PQ extract produced 64.63% ulcer inhibition at 400mg/kg body weight to that of standard (ranitidine, 50mg/kg) which produced 68.52% inhibition. In HCl-ethanol induced ulcer model 400mg/kg body weight of PQ produced 72.24% ulcer inhibition to that of standard (sucralfate, 100mg/kg) which produced 75.01% inhibition. In water immersion-stress induced ulcer model 400mg/kg body weight of PQ produced 84.11% ulcer inhibition compared to that of standard (omeprazole, 20mg/kg) which produced 82.17% ulcer inhibition. PQ showed a significant percentage of ulcer inhibition in the three models tested.

INTRODUCTION

Peptic ulcer is the most common problem in the stomach region resulting due to excessive acid secretions associated with *Helicobacter pylori*. Ulcers can also be caused or worsened by non-steroidal antiinflammatory drugs. Although allopathic antiulcer agents brought about remarkable changes in ulcer therapy, the incidences of relapses and adverse effects and possible drug interactions are still in debate. Hence, the search towards achieving better therapy has been extended to herbal drugs which afford better protection and decrease the incidence of relapse.

About 75% of world population is still depending on indigenous plants for primary health care because of the lesser side effects [1]. Since ancient times, herbs have been used to treat various gastrointestinal illnesses including peptic ulcers [2]. Even though the etiology of gastric ulcers is still debated, it is accepted that ulcers are caused due to net imbalances in mucosal offensive and defensive factors [3]. Research is now mainly focused on the search safer medications especially herbal drugs, which protect the gastric mucosa from damaging agents without influencing acid secretion. Numerous medicinal plants were found to exhibit antiulcer activity and found useful in the treatment of peptic ulcer.

Picrasma quassioides (PQ) (D. Don) Bennett (family Simaroubaceae) is a species of *Picrasma* native to temperate regions of southern Asia, from the northeast of Pakistan east along the Himalaya and through southern, central and eastern China to Taiwan and Japan. It is a deciduous large shrub or small tree, up to 10 m high, almost glabrous except the young inflorescence and branches. Leaves unequally pinnate, scarcely pubescent, 19-37 cm long; leaflets 4-15 pairs, ovate-lanceolate, acuminate, serrate, membranous, 5.0-7.5 × 2.0-3.5 cm and lower leaflets small, petiolules small, 1-2 mm long or sometimes longer. Flowers c. 7 mm in diameter and pedicel 7-8 mm long. Calyx: sepals 5, much smaller than petals, c. 1 mm Fong, acuminate, glabrous. Corolla: petals (4-) 5(-6), glabrous, ovate-obovate or rarely lanceolate, valvate. Stamens (4-) 5, arising from a thick disc. Ovary with 3-5 free carpels. Fruit a drupe, black. [4] The wood contains a number of medicinal compounds and has

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been shown to be anthelmintic, antiamoebal, antiviral, bitter, hypotensive and stomachic. It increases the flow of gastric juices. It is used in the treatment of digestive problems, especially chronic dyspepsia. A decoction of the stem bark is bitter, febrifuge and tonic. The leaves have been used to treat itchy skins. The bark is used as an insecticide. It has been used as a parasiticide to get rid of lice, fleas, etc. [5].

The main chemical constituents include quassin, neoquassin and 18-hydroxyquassin. Other alkaloids such as 4,5-dimethoxy-10-hydroxycanthin-6-one; canthin-6-one alkaloids; 8-hydroxycanthin-6-one; 4,5-dimethoxycanthin-6-one; 5-hydroxy-4-methoxycanthin-6-one and 3-methylcanthin-5,6-dione are found in the stem of PQ and their cytotoxic activity was evaluated [6]. Recently, beta-carboline alkaloids were identified and their antiinflammatory activity was tested from the stems of PQ [7, 8] Some quassins have been reported to have important antileukemic properties [9].

Though this herb is being used in Ayurveda and folklore medicine for centuries, there is scanty scientific evidence for its antiulcer activity [10]. In the present investigation, the aqueous extract of PQ was tested for its antiulcer activity in albino rats using three models.

Material and Methods

Identification of the plant

The plant was authenticated by Prof. V. Jaya, Head, Botany Department, Hindu College, Guntur, Andhra Pradesh, India. The plant specimen was deposited in the College herbarium at Hindu College of Pharmacy, Guntur-522001, Andhra Pradesh, India.

Preparation of aqueous extract

The whole plant was collected, shade dried and powdered coarsely with a multi-mill. The decoction of the powder was collected in purified boiling water in the ratio of 1:16. The decoction was filtered; the dry weight of the extract was estimated and used for the present study.

Animals

Adult albino rats of Wistar strain and sprague dawley (SD) albino rats were employed to study acute toxicity and antiulcer activity respectively. SD albino rats weighing 100-200 g and albino mice weighing 24-30 g of either sex were used for the present study. The animals were housed in groups of four in standard laboratory conditions (25 ±1 ^{0}C, relative humidity 55 ±5% and 12.00:12.00 h of dark:light cycles), fed with standard pellet diet and water *ad libitum*. Healthy adult albino rats of Wistar strain (procured from the national Institute of Nutrition, Hyderabad, registered with CPCSEA) were taken and maintained at 22 ±3 ^{0}C, 50-60% relative humidity, 12.00:12.00 h cycle of light and dark and were housed individually with standard pellet diet and water *ad libitum*. The animals were fasted overnight with water *ad libitum* prior to the test. The experiments were performed on the animals as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The Institutional Animal Ethics Committee approved the study protocol (IAEC/HCOP/01/2009).

Acute toxicity studies

Healthy adult albino rats of Wistar strain were divided into five groups each containing six animals. Group-I animals were treated with distilled water (2 ml/kg/p.o.). Group II to V received 1, 2, 4 and 8 g/kg/p.o. respectively, of the plant extract by gastric intubation using a soft catheter [11]. The animals were observed continuously after 2 h at an interval of 1 h up to 48 h for their behavioral, neurological and autonomic profiles blindly by two qualified observers during acute toxicity studies.

Experimental design

Aspirin-pylorus ligation induced gastric ulcer in albino rats:

The SD albino rats of either sex were divided into four groups each consisted of six animals. All the animals received 200 mg/kg p.o. of aspirin once daily for three days. Group I served as control and received water. Group II was treated with 50 mg/kg, p.o. of ranitidine as standard. Group III and IV were treated with aqueous plant extract of 200 and 400 mg/kg p.o. respectively. The pylorus part was ligated following 36 h fasting [12]. The animals were sacrificed by cervical dislocation after 4 h after the pyloric ligation. The stomach was opened and the ulcer index was determined [13]. The gastric content was titrated against 0.01 N NaOH to determine the free acid and total acidity [14]. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers was measured by micrometer. The following arbitrary scoring system (0-50 points) was used to grade the incidence and severity of lesions: (i) 10 = denuded epithelium; (ii) 20 = petechial and flank hemorrhages; (iii) 30 = one or 2 ulcers; (iv) 40 = multiple ulcers and (v) 50 = perforated ulcers [15].

Ulcer index (UI) was then calculated using the formula:

$$UI = UN + Us + Up \times 10 - 1$$

where, UN is average number of ulcers/animal, Us is mean severity of ulcer score and Up is percentage of animals with ulcer incidence.

The percentage inhibition was calculated by :

$$\% \text{ inhibition} = \frac{\text{UI control} - \text{UI treated}}{\text{UI control}} \times 100$$

HCl-ethanol induced ulcer in albino mice:

Albino mice weighing 24-30 g of either sex were divided into four groups, each containing six animals. Group I served as a control and received water. Group II received 100 mg/kg, p.o. sucralfate as standard. Group III and IV have received aqueous plant extract of 200 and 400 mg/kg p.o. respectively. After 1 h all the animals were treated with 0.2 ml of HCl-ethanol mixture p.o. (0.3 M HCl and ethanol 60%) to induce gastric ulcers. The

animals were sacrificed by cervical dislocation at 1 h after administration of HCl-ethanol. The stomach was excised and lesion index was determined by measuring each lesion in mm along its greater length [16].

Water immersion stress induced ulcer in albino rats:

Stress ulcers were induced by forced swimming in the glass cylinder (45 cm height and 25 cm diameter) containing water to the height of 35 cm maintained at 25 °C for 3 h. The animals were fasted for 24 h prior to the experiment and divided into four groups each consisting of six animals [17]. The group I received water and served as control and group II treated with 20 mg/kg, p.o. omeprazole as standard. Group III and IV have received aqueous plant extracts of 200 and 400 mg/kg p.o. respectively. The animals were allowed to swim in water for 3 h after the treatment. The stomach of each animal was removed and the extent of gastric damage was assessed as described by Alphine and Word [18].

Statistical analysis

The statistical analysis of the results was carried out using one-way ANOVA followed by post hoc Dunnet's multiple comparisons and the results were compared to those of the control group.

Results

The acute toxicity studies revealed that aqueous extracts of PQ were safe even at the dose of 8 g/kg body weight. The animals did not show any significant gross behavioral changes except an increase in urination. From the acute toxicity studies, the doses were chosen in a logarithmic progression of 200 and 400 mg/Kg body weight as suggested by Turner for further investigation [11].

The effect of all the aqueous extract on gastric secretion, acidity, pH, ulcer index and % ulcer inhibition were screened. In the present study aspirin-pylorus ligation induced gastric ulcer was adopted to screen the antisecretory activity of plant extract. PQ extract produced 64.63% ulcer inhibition at 400mg/kg body weight that that of standard (ranitidine 50mg/kg body weight) which produced 68.52%. The results are shown in Table 1 and Fig.1. The effect of the aqueous extract on gastric lesions and % inhibition were screened by using HCl-ethanol induced ulcer in albino mice. In this model 400mg/kg body weight of PQ has produced 72.24% ulcer inhibition than that of standard (sucralfate 100mg/kg body weight) which produced 75.01% ulcer inhibition. The results are summarized in Table 2 and Fig. 2. The effect of all the aqueous extract on the mean ulcer score and % inhibition were screened by water immersion stress induced ulcer in albino rats. The results are shown in Table 3 and Fig. 3. In this model 400mg/kg body weight of PQ has produced 84.11% ulcer inhibition than that of standard (omeperazole 20mg/kg body weight) that produced 82.17% inhibition. PQ showed significant % ulcer inhibition in all the models.

Discussion

Peptic ulcer therapy is now mainly focused on limiting the deleterious effects of offensive acid secretion. Although conventional treatment is available for the management of gastric ulcers, natural medicine is gaining importance due to its less possible adverse effects and patient acceptance. In our study aqueous extract of PQ was studied for its potential antiulcer activity in albino rats.

The genesis of ethanol-induced gastric lesions is multifactorial with the depletion of gastric wall mucous content as one of the involved factors. It is also associated with significant production of free radicals, leading to an increased oxidative stress and damage to the cell and cell membrane [19]. Aspirin causes a dose-dependent reduction in mucosal prostaglandin E2 and prostaglandin I2 biosynthesis accompanied by an increase in the mean of gastric ulceration. It is therefore reasonable to assume that the observed gastric mucosal lesion induced by aspirin is due to deficiency of mucosal prostaglandin [20].

Pylorus ligation induced ulcer is one of the most widely used

Table 1. Effect of aqueous extract of *Picrasma quassioides* on gastric secretion, acidity, pH, ulcer index and ulcer % inhibition.

Groups	Volume of gastric secretion (ml/100g)	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)	pH	Ulcer index	% ulcer inhibition
Control	5.750 ± 0.187	55.78 ± 0.327	97.82 ± 0.698	2.167 ± 0.218	32.18 ± 0.177	-
Ranitidine (50 mg/kg)	1.600 ± 0.057	25.22 ± 0.411	41.83 ± 0.138	4.483 ± 0.135	10.13 ± 0.197	68.52%
P.Q (200 mg/Kg)	3.400 ± 0.136	41.55 ± 0.092	51.73 ± 0.049	3.550 ± 0.117	22.55 ± 0.147	29.92%
P.Q (400 mg/Kg)	2.483 ± 0.116	28.15 ± 0.232	45.63 ± 0.098	4.100 ± 0.057	11.38 ± 0.070	64.63%

***P<0.001 Vs control group analyzed by one way ANOVA followed by post hoc Dunnet's test ; P.Q - *Picrasma quassioides*

methods for studying the effect of drugs on gastric secretion. Agents that decrease gastric acid secretion and/or increase mucus secretion are effective in preventing the ulcers induced by this method. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach, leading to the development of ulcers in the stomach. The original Shay rat model involves fasting of rats for 72 h, followed by ligation of the pyloric end of the stomach for 19 h [21]. In the present study, the modification of the Shay rat model described by Kulkarni was followed, which involves fasting of the animals for 36 h and pyloric ligation only for six hours. Ranitidine significantly decreases the secretion of gastric aggressive factors, free acidity, total acidity and pepsin content, and increases the mucus secretion, which is cytoprotective.

Ethanol and HCl induced gastric ulcer were employed to study the cytoprotective effect of plant extract. It rapidly penetrates the gastric mucosa, causing cell and plasma membrane damage, leading to increased membrane permeability to sodium and water. It also produces a massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [22, 23].

The pathophysiology of stress-induced ulcers is complex; the ulcers are produced due to the release of histamine, leading to an increase in acid secretion and a reduction in mucus production [24, 25]. Stress also causes an increase in gastrointestinal motility, which causes folds in the gastrointestinal tract. The folds in the stomach are more susceptible to damage, when they come in contact with acid. Agents that decrease GI motility, gastric secretion or those having central actions are helpful in reducing ulcers due to stress.

In the present study aspirin-pylorus ligation induced gastric ulcer was adopted to screen the antisecretory activity of plant extract. At a dose of 400 mg/kg, the PQ extract produced similar ulcer inhibition comparable to that of the standard drug ranitidine (50 mg/kg body weight) which indicates its effectiveness as standard drug. Similarly the same dose was as effective to that of sucralfate (100mg/kg) in HCl-ethanol induced ulcer model which indicates that the extract is showing cytoprotective activity. Further this dose of PQ was efficient as proton pump inhibitor comparable to that of omeperazole (20 mg/kg) in water immersion stress induced ulcers. In all the three models PQ has showed significant % ulcer inhibition.

Conclusion

The present study confirms the antiulcer activity of PQ as it produced significant antiulcer property by their antisecretory, cytoprotective and proton pump inhibitory properties. Future studies are needed to isolate the chemical moieties responsible for the antiulcer activity of this extract.

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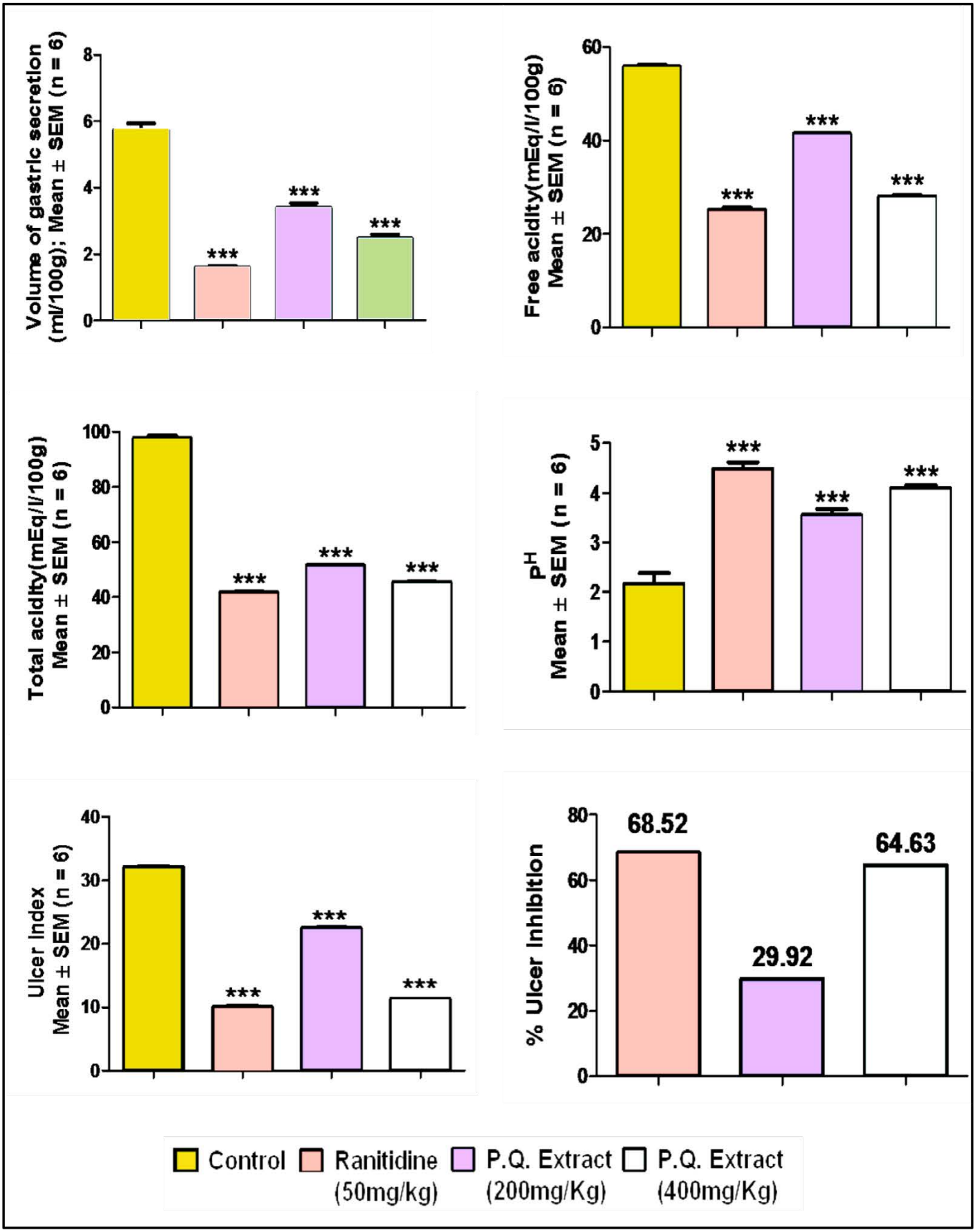
Table 2. Effect of aqueous extract of *Picrasma quassioides* against HCl - ethanol induced.

Groups	Gastric lesions	% Ulcer inhibition
Control	22.28 ± 0.358	-
Sucralfate (100 mg/kg)	5.567 ± 0.098	75.01%
P.Q (200 mg/kg)	11.65 ± 0.346	47.71%
P.Q (400 mg/kg)	6.183 ± 0.104	72.24%

***P<0..001 Vs control group analyzed by one way ANOVA followed by post hoc Dunnet's test ; P.Q -*Picrasma quassioides*

Figure 1. Effect of aqueous extract of *Picrasma quassioides* on gastric secretion, acidity, pH, ulcer index and ulcer % inhibition.



***P<0.001 Vs control group analyzed by one way ANOVA followed by post hoc Dunnet's test ; P.Q - *Picrasma quassioides*

Table 3. Effect of aqueous extract of *Picrasma quassioides* against water immersion stress induced ulcer in albino rats.

Groups	Mean ulcer score	% Ulcer inhibition
Control	18.68 ± 0.110	-
Omeprazole (20 mg/kg)	3.33 ± 0.080	82.17%
P.Q (200 mg/kg)	6.750 ± 0.076	63.86%
P.Q (400 mg/kg)	2.967 ± 0.140	84.11%

***P<0.001 Vs control group analyzed by one way ANOVA followed by post hoc Dunnet's test ; P.Q -*Picrasma quassioides*

Figure 2. Effect of aqueous extract of *Picrasma quassioides* against HCl-ethanol induced ulcer in mice.

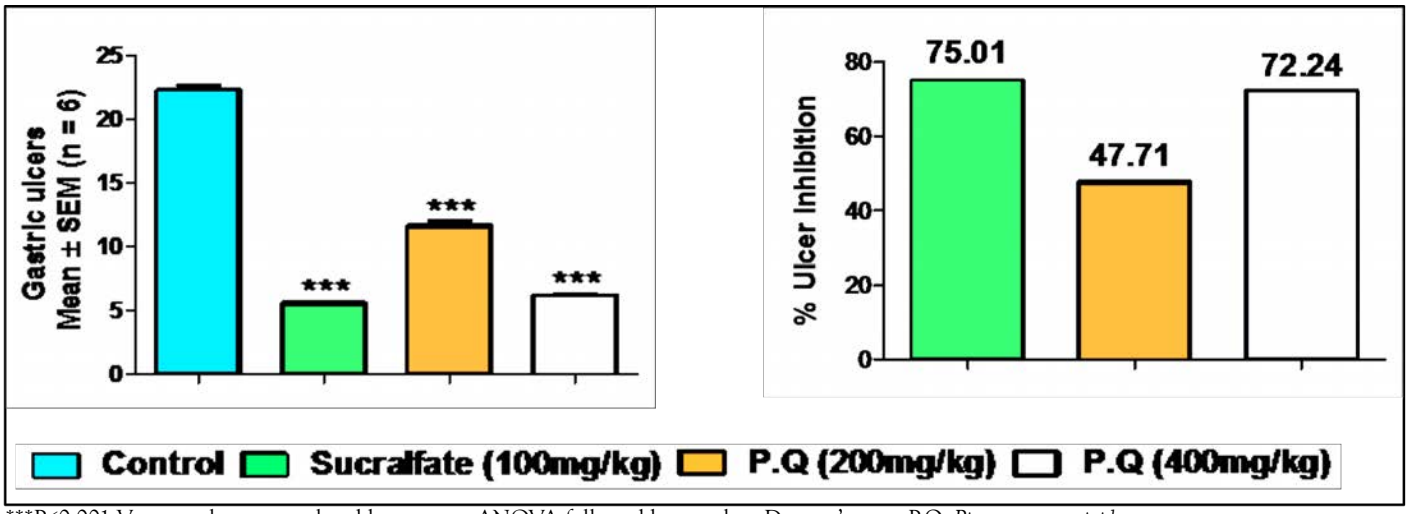


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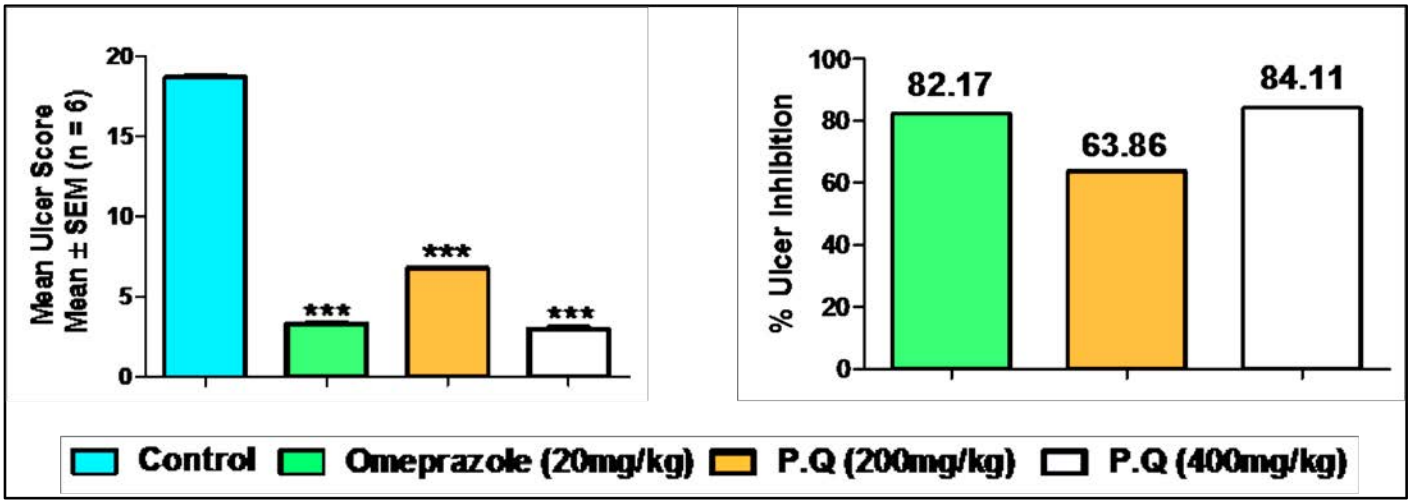
Figure 3. Effect of aqueous extract of *Picrasma quassioides* against water immersion stress induced ulcer.



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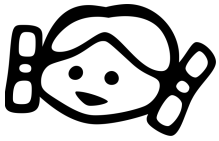

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