IN VIVO ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *PASSIFLORA EDULIS SIMS* FOR ANTIULCER ACTIVITY

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Abstract

Now a day, traditional medicines are used for the most types of diseases. In this study, Ethanolic extraction of *Passiflora edulis sims* extract is used for anti-ulcer activity. This plant extract is also used in treating different types of diseases. It is mainly used for gastric ulcer. This Extract showed 85.58% ulcer protection in ethanol induced model, 89.97% in pylorus ligation and 74.40% in swim stress model. *P. edulis sims* treatment increases the gastric pH and may reduce the ulcer index in both ethanol and aspirin induced rats. It also decreases the lipid peroxidation and reduces the glutathione level.

KEYWORDS: - Traditional medicines (*Passiflora edulis sims*), Gastric ulcer, albino rats.

1. INTRODUCTION

Ulcer may define as a breakdown of tissue into the stomach. In some cases, peptic ulcer may be formed. It may penetrate the performance, hemorrhage and obstruction.[1,2,3] It may manifest the gastric mucosa by acid and pepsin. Many NSAID drugs is been used for the healing of ulcer, type C gastritis, treatment of small and large intestinal injury.[4,5,6] For the evaluation of gastroprotective activity, Pylorus ligation method, Ethanol induced model and Swim stress. Ethanol when use for the treatment of acute it may increase the oxidative stress, DNA damage etc.[7,8,9] Aspirin induced ulcer may damage the free radicals. It may produce the hydroxy fatty acids from hydroperoxyl. For protection from ulceration oxygen derived free radicals are used. It may protect from acute and chronic ulceration.[10,11] Now a day, Herbal medicines are used. It plays most important role in ulceration. Plant and plant extract are safer medicines due the presence of natural ingredient. It has not any side effect on any part of the body.[12,13] Identification of new antiulcer drugs from natural sources are been renewed. *Passiflora edulis sims* is obtained from vines and passion flower. It belongs to the family *Passifloraceae*. It is used for the gastrointestinal conditions, neurological, cardiovascular, inflammation and anxiety. All part of this plant is used for medicinal purpose. In this study, extract is used to perform the *in-vitro* and *in-vivo* process. For this process, animal models are used.[14,15]

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The plant were collected from local farm behind NCP, Erode District, Tamil nadu. The plant material was identified as *Passiflora edulis sims* (family Passifloraceae). The leaves were washed and air dried for five days and pulverized into coarse powder. The coarse powder was packed tightly in the Soxhlet apparatus and extracted with 500 ml 95% ethanol at 55 ˚C for 72 hours by continuous hot percolation method.

2.2 EXPERIMENTAL ANIMAL

All experimental animals used were male albino rats (*Rattus novergicus*) weighing between 180-250g and were obtained from the animal house of Nandha College of pharmacy and Research Institute. They were fed using standard laboratory rodent chow diet feed and given water *ad libitum.*

2.3 EXPERIMENTAL DESIGN FOR ANIMAL STUDY

Twenty-four (24) albino rats were used for every model of ulcer induction for the experiment; four groups of rats were used for antiulcer activity study and the following treatments were taken, namely; control, standard, EEPE200 mg/kg extract, and EEPE 400 mg/kg extract. Four (4) groups of rats were used for anti-diarrhoeal study namely; group 1 (treated with: 0.5%w/v CMC vehicle control), group 2 (treated with loperamide (5 mg/kg,) as standard drug), group 3 (treated with EEPE 200 mg /kg), group 4 (treated with EEPE 400 mg/kg). All groups consisted of six animals each. The extract was administered orally. Anthelmintic activity was carried out on earthworm, *Pherithema*

posthuma. 7 groups used for study, group 1 for control and 3-3 group used for standard and extract, under doses of 10, 30 and 50 µg/ml.

2.4 ETHANOL INDUCED ULCERATION

After 12 h of fasting, Wistar Albino rats were divided into 4 Groups of six animals each. Group 1 served as Normal control (vehicle) received 0.5% CMC, and the Group 2 was treated with Sucralfate (100 mg/kg). The remaining two Groups received 200 and 400 mg/kg of extract of *Passiflora edulis sims.* All are administered orally. One hour after treatment, all rats received 1ml of absolute ethanol to induce gastric ulcer After 5 h, the animals were killed and lesions in the gastric mucosa were scored. After identification of ulcer areas, the length of the ulcer was measured along the greater diameter and the mean ulcer index was calculated. The ulcer were graded as follows,
(0= normal coloured stomach, 0.5= red colouration, 1= spot ulcers, 1.5= haemorrhagic streaks, 2=ulcers $\geq$ 3 but $\leq$ 5, 3=ulcers > 5)

2.5 PYLORUS LIGATION INDUCED ULCERATION

In this model Wistar Albino rats of either sex weighing about 180-250g (pregnancy was excluded) were selected and divided into four groups containing 6 animals in each group. Group 1 served as vehicle control 0.5% CMC (p.o.), Group 2 received omeprazole 20 mg/kg (p.o.). Group 3 and group 4 received 200 mg/kg and 400 mg /kg (p.o.), of extract of *Passiflora edulis sims* respectively daily for 3 days. The rats were taken in the individual animal cages and fasted (water allowed) for 48 hours prior to pyloric ligation, care being taken to avoid coprophagy. Under light anaesthesia (ketamine hydrochloride 50 mg (IP) the abdomen was opened by a small midline incision below the xiphoid process; pyloric portion of the stomach is slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully, and the abdominal wall was closed interrupted sutures. The drugs are administered orally two hours prior to pyloric ligation. They are deprived of both food and water during the postoperative period, and are sacrificed at the end of 6 hr. after operation. Stomach is dissected out and the contents are drained into the tube and this is subjected to analysis for pH and for free and total acidity . The stomach is then open along the greater curvature and is examined for any ulceration. The degree of ulceration is graded from zero to five depending on the size and severity of ulcers.

2.6 SWIM STRESS INDUCED ULCERATION

The swim Stress ulcers were induced by forced swimming in glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm and maintained at 25 ^{0}C for 5 hr. the animals were fasted for 24 hr. prior to the experiment and divided into 4 groups of 6 animals in each group. Group 1 served as vehicle control and received 0.5% CMC (p.o), and group 2 received 20 mg per kg (p.o), of ranitidine as standard. Group 3 and 4 received extract of *Passiflora edulis sims* (200 mg per kg and 400 mg per kg, p.o) respectively. After the drug treatment animals were allowed to swim in water for 5 hr. then animals were sacrificed and the stomach was opened. The ulcer index and percentage inhibition of ulcer was determined.

3. RESULT AND DISCUSSION

The antiulcer activity of ethanolic extract of *passiflora eduli sims* was carried out by Ethanol induced ulcer model (Fig.1). *passiflora edulis sims* showed 200mg/kg and 400mg/kg dose produced an ulcer index of (5.96±0.23 & 3.56±0.29) and percentage protection (64.93 & 85.58). (table 2). This shows the significant decreased in ulcer index and increased in % ulcer protection compared to standard drug Sucralfate (100mg/kg) ulcer index (2.99±0.27) and % ulcer protection (86.01) (table 1).

The antiulcer activity of ethanolic extract of *Passiflora edulis sims* was carried out by Pylorus ligation induced ulcer model (fig4). *Passiflora edulis sims* showed doses of 200 mg/kg and 400 mg/kg produced an ulcer index of (3.13±0.0744 & 2.39±0.198) and % protection (84.10 & 89.97) (table 3). This shows the significant ($p<0.01$) decreased in ulcer index and increased in % ulcer protection compared to standard drug Omeprazole 20mg/kg ulcer index (1.84±0.0838) and % ulcer protection (91.04) (table 2).

The concentration of total hexoses at the dose of 200 mg/kg and 400 mg/kg group was found to be 323.50±2.19 µg/ml and 349.80±5.01 µg/ml which shows significant ($p<0.01$) increase when compared with Omeprazole treated group (374.58± 5.22 µg/ml) (table 3).

The concentration of hexosamine at the dose of 200 mg/kg and 400 mg/kg group was found to be (417.64±2.71) µg/ml and (466.05±4.56) µg/ml. It shows the significant ($p<0.01$) increase of hexosamine content when compared to Omeprazole treated group (470.19±7.27) µg/ml (table 3).

The concentration of fucose at the dose of 200 mg/kg and 400 mg/kg group was found to be (130.26±3.70) µg/ml and (72.47±4.02) µg/ml. This shows the significant ($p<0.01$) increase of fucose content when compared to Omeprazole treated group (185.70±3.11) µg/ml (table 3).

The volume of acid secretion at the dose of 200 mg/kg and 400 mg/kg group was increased (3.58±0.18&2.59±0.18).and total acidity (34.25±0.61&27.77±0.8) and free acidity (18.54±1.2&24.17±0.915) was decreased and pH (3.47±0.166&4.76±0.348) of the gastric juice was increased. This shows the significant ($P<0.01$) compared to ulcer control group.

The antiulcer activity of ethanolic extract of *Passiflora edulis sims* was carried out by Swim stress induced ulcer model (fig.10). *Passiflora edulis sims* showed doses of 200 mg/kg and 400 mg/kg produced an ulcer index of (5.14±0.30) and (3.44±0.17) and % protection (60.79) and (74.40). This shows the significant ($p<0.01$) decreased in

ulcer index and increased in % ulcer protection when compared to standard drug Ranitidine 20 mg/kg ulcer index (2.7±0.18) and % ulcer protection (79.40) (table 4).

3.1 Ethanol induced Ulcer Model

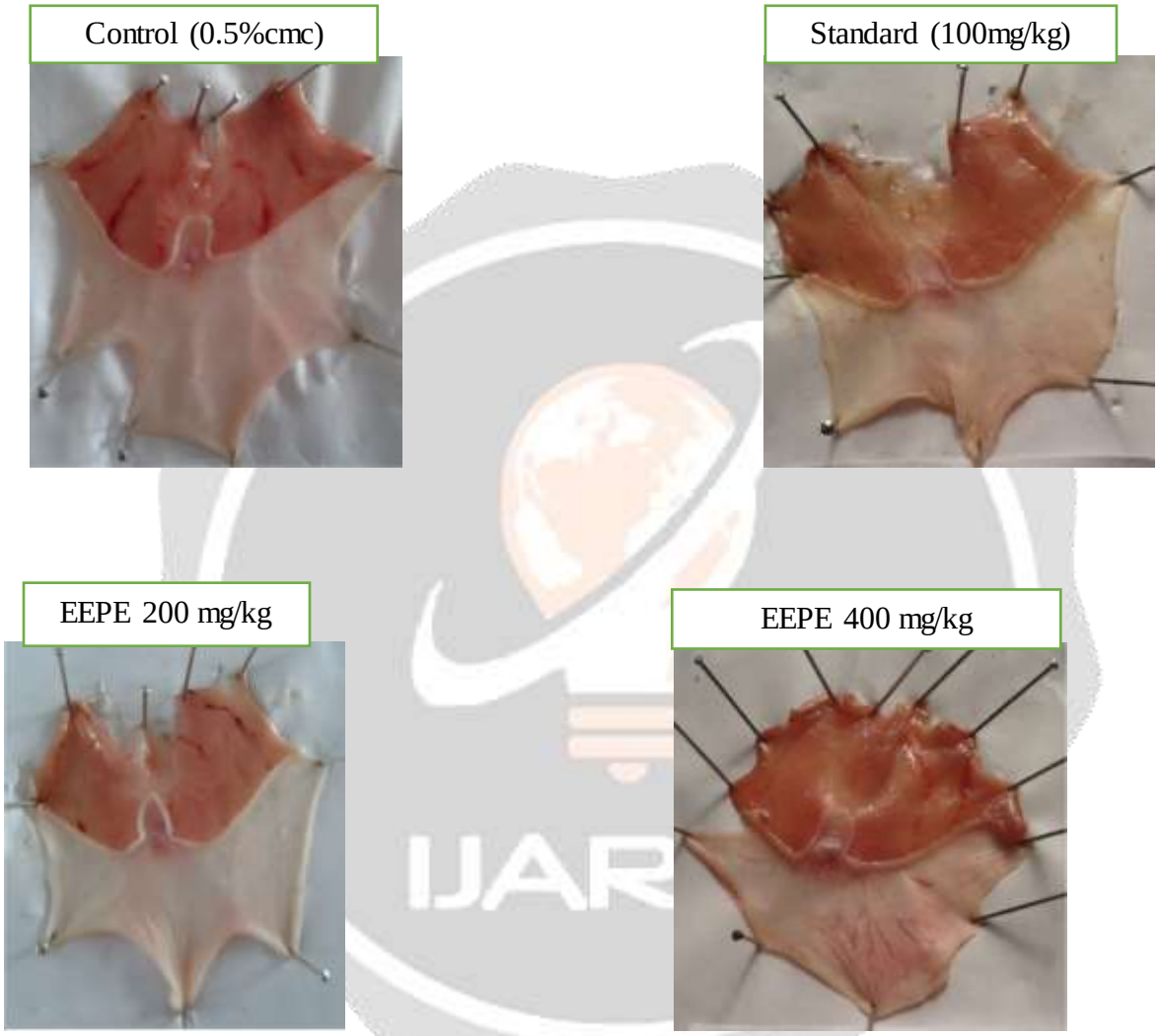


Figure 1. Effect of passiflora edulis sims on Ethanol induced Ulcer Model.

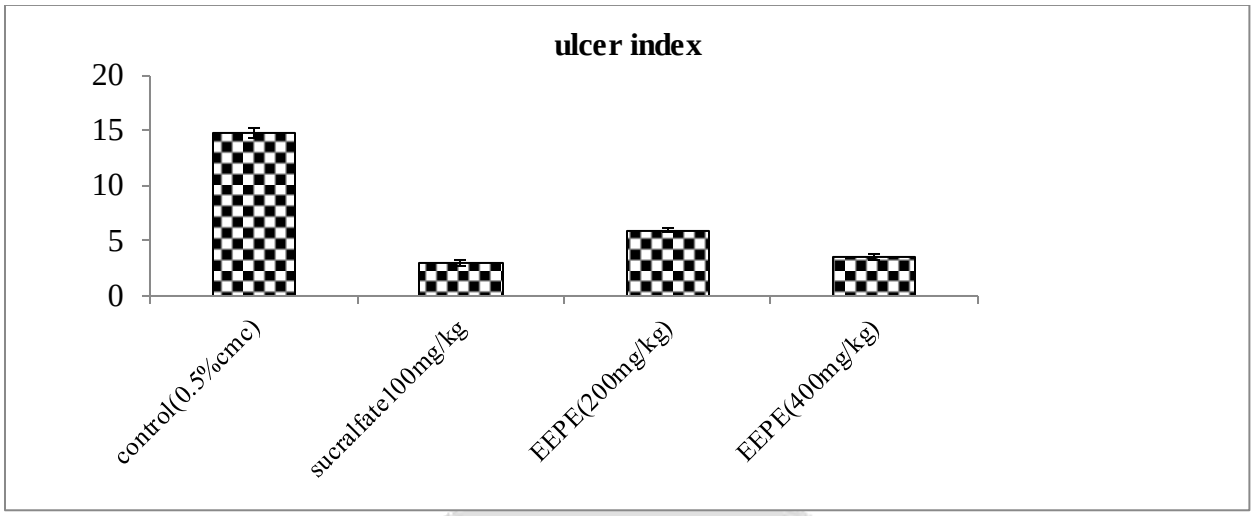


Figure 2.Effect of ethanolic extract of passiflora edulis sims on Ethanol Induced Ulcer Model Indicating Ulcer index

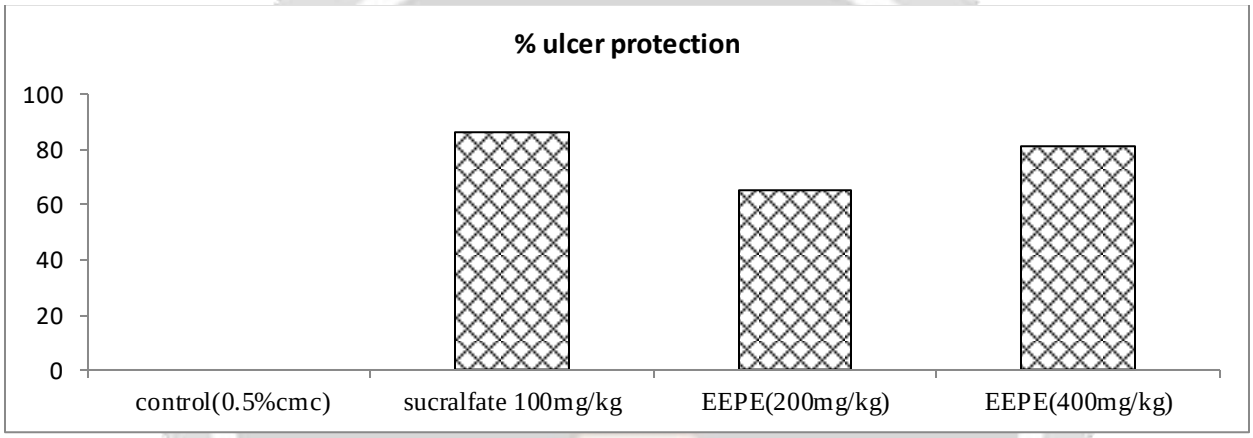


Figure 3. Effect of ethanolic extract on percentage (%) of ulcer protection in Ethanol Induced Ulcer Model

Sl no	treatment	Ulcer index	% of ulcer protection
1	Control 0.5% (1ml/kg)	14.83±0.43	
2	Sucralfate 100mg/kg	2.99±0.27**	86.01
3	EEPE 200mg/kg	5.96±0.23**	64.93
4	EEPE 400 mg/kg	3.56±0.29**	85.58

Values are mean ± SEM; No. of animals in each group = 6 ** P value <0.01 compared with the corresponding control

Table 1. Effect of ethanolic extract of passiflora edulis sims on Ethanol Induced Ulcer Model Indicating Ulcer index & Percentage Ulcer Protection

3.2 Pylorus Ligated (SHAY) Rat Model

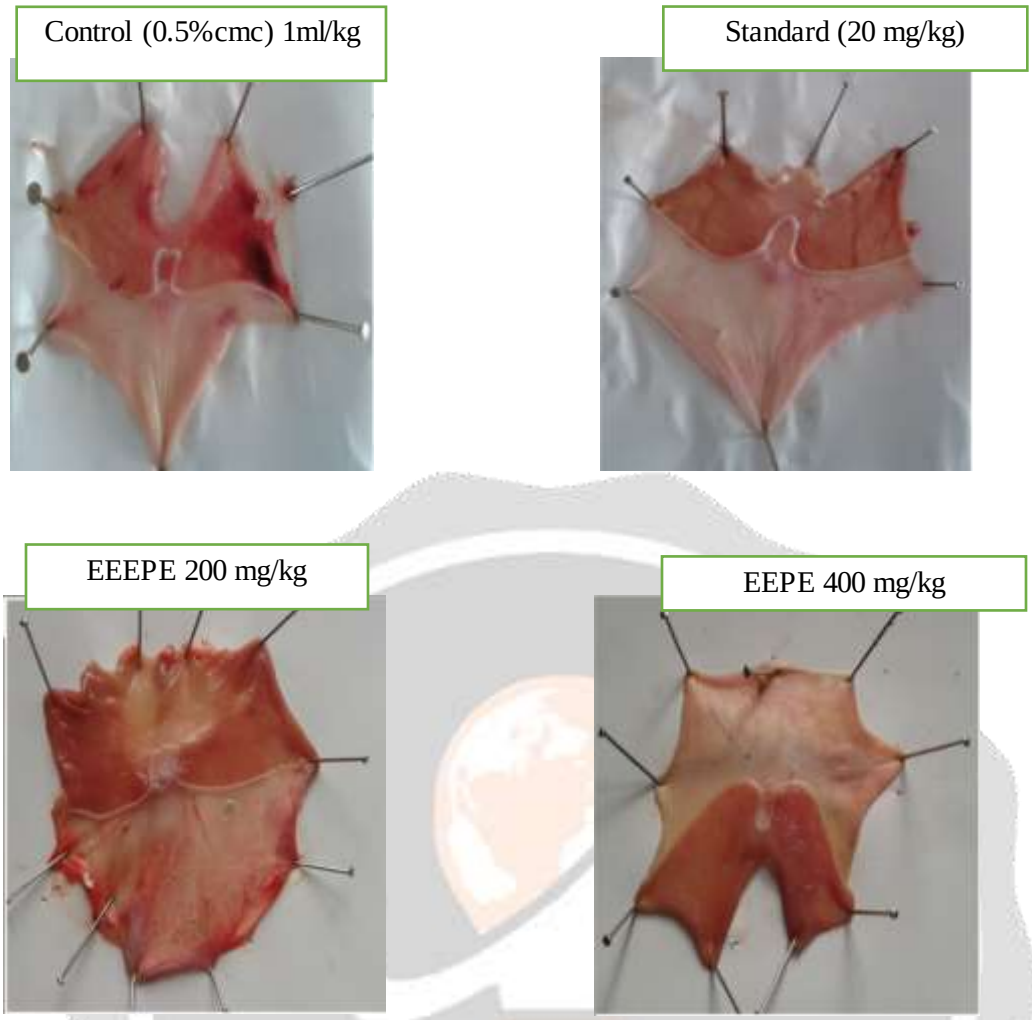


Figure 4. Effect of passiflora edulis sims on the Ulcer induction by Pylorus Ligated (SHAY) Rat Model.

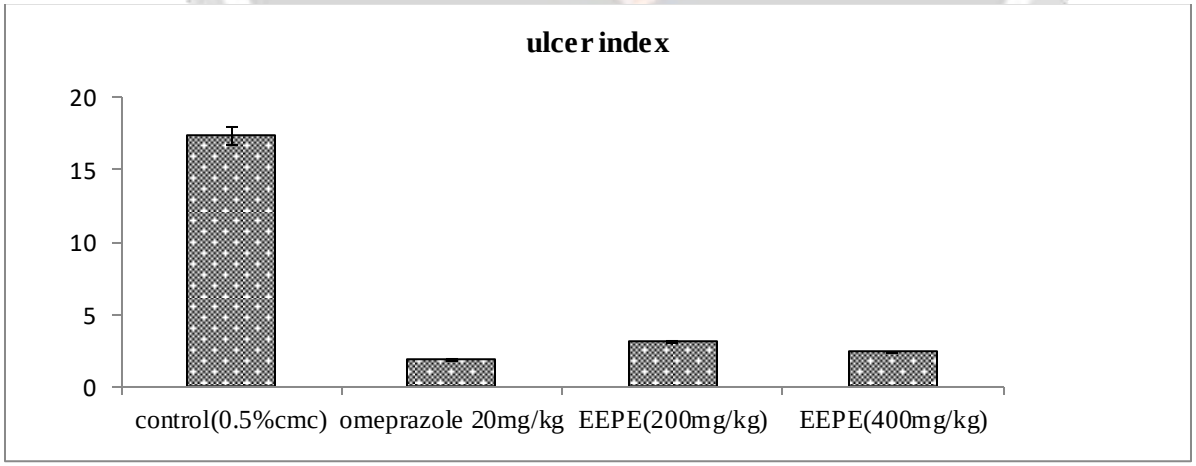


Figure 5.Effect of Passiflora edulis sims on Pylorus Ligated (Shay) Rat Model Indicating Ulcer index

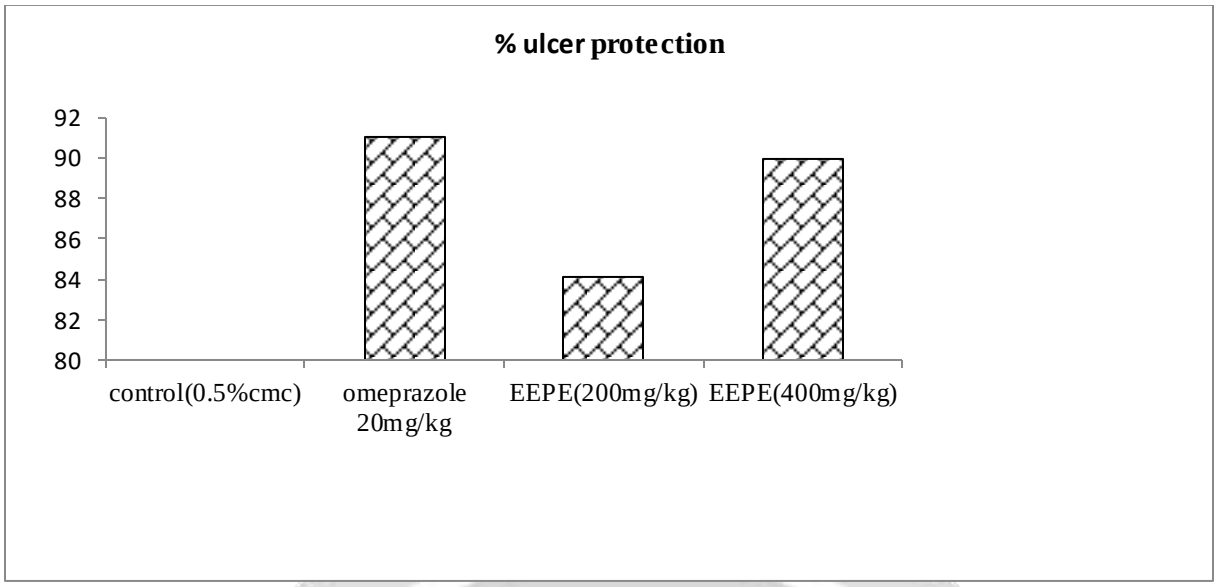


Figure 6. Effect of Passiflora edulis sims on percentage (%) of ulcer protection in Pylorus Ligated (Shay) Rat Model.

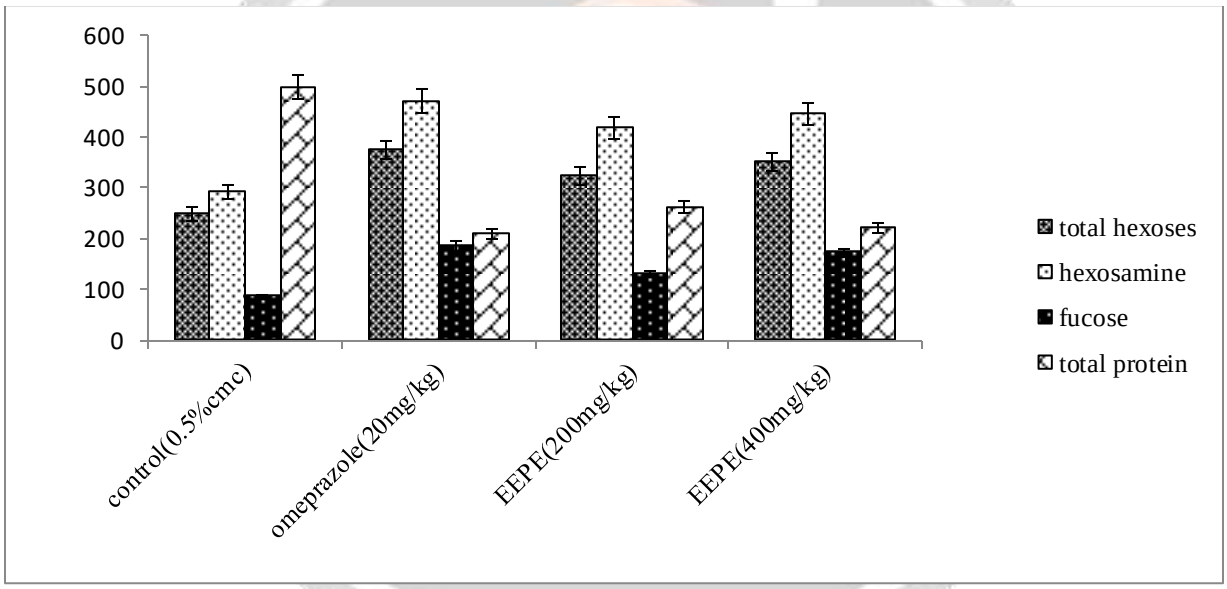


Figure 7. Effect of Passiflora edulis sims on Pylorus Ligated (Shay) Rat Model indicating Total Hexose, Hexosamine, Fucose and Protein

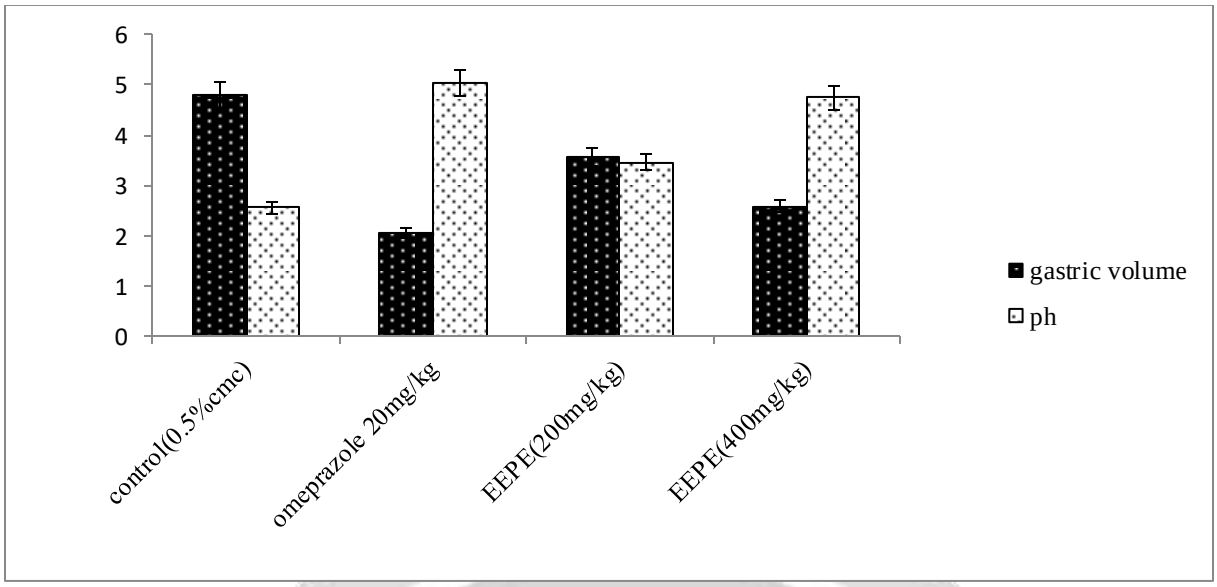


Figure 8. Effect of Passiflora edulis sims on Pylorus Ligated (Shay) Rat Model Indicating gastric volume, and pH

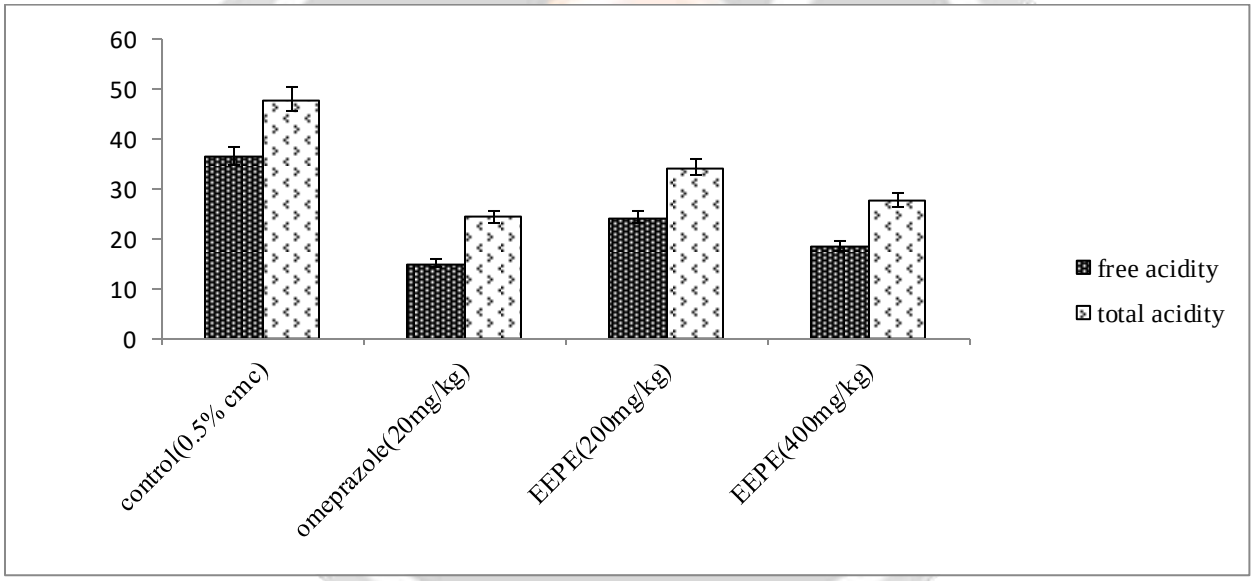


Figure 9. Effect of P.edulis sims on Pylorus Ligated (Shay) Rat Model indicating free acidity and Total acidity of gastric juice

Sl no	**treatment**	**Ulcer index**	**% of ulcer protection**
1	Control 0.5% (1ml/kg)	17.32±0.6120	
2	omeprazole 20mg/kg	1.84±0.0838**	91.04
3	EEPE 200mg/kg	3.13±0.0744**	84.10
4	EEPE 400 mg/kg	2.39±0.0198**	89.97

Values are mean ± SEM; No. of animals in each group = 6 ** P value <0.01 compared with the corresponding control

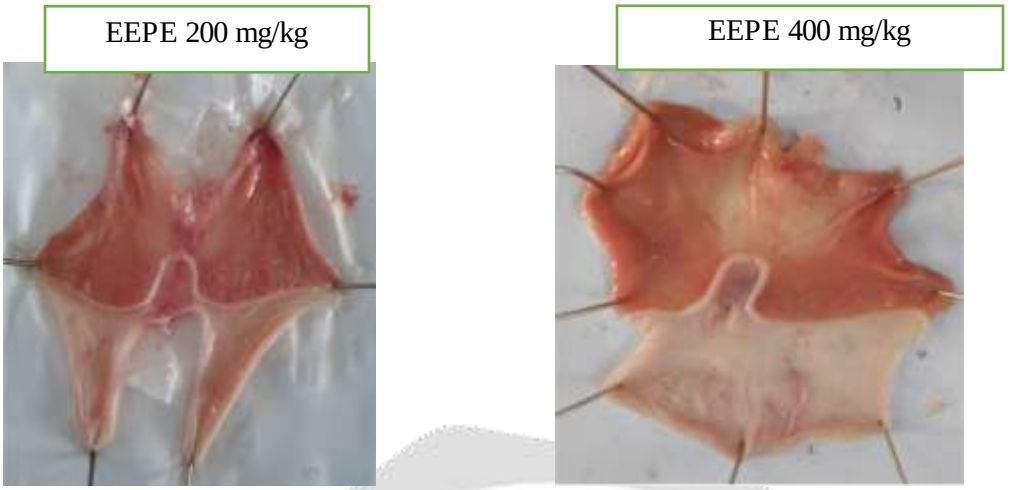
Table 2. Effect of Passiflora edulis sims on Pylorus Ligated (Shay) Rat Model Indicating Ulcer index & Percentage Ulcer Protection

sn	treatment	Total hexose	Total protein	fucose	hexosamine
1	Control 0.5% cmc	248.10±3.36	148.62±2.15	85.88±3.27	292.08±2.15
2	Omeprazole 20mg/kg	374.58±5.22**	564.92±4.93**	185.70±3.11**	470.19±7.27**
3	EEPE 200 mg/kg	323.50±2.19**	523.79±3.38**	130.26±3.70**	417.64±2.71**
4	EEPE 400 mg/kg	349.80±5.01**	553.87±3.91**	72.47±4.02**	466±4.56**
	Treatment	Gastric volume	pH	Free acidity	Total acidity
5	Control 0.5% cmc	4.8±0.262	2.56±0.93	36.97±0.51	47.80±1.13
6	Omeprazole 20 mg/kg	2.05±0.133**	5.05±0.122**	14.95±0.72**	24.32±0.59**
7	EEPE 200 mg/kg	3.58± 0.18**	3.47±0.166**	24.17±0.915**	34.25±0.61**
8	EEPE 400 mg/kg	2.59±0.18**	4.76±0.348**	18.54±1.25**	27.77±0.85**

Values are mean ± SEM; No. of animals in each group = 6.** P value <0.01 compared with the corresponding control.

Table 3. Effect of Passiflora edulis sims on Pylorus Ligated Rat Model Indicating Total Hexose, Hexosamine, Fucose & Total Protein gastric volume, pH, free acidity and total acidity.

3.4 SWIM STRESS (SS) INDUCED ULCER MODEL



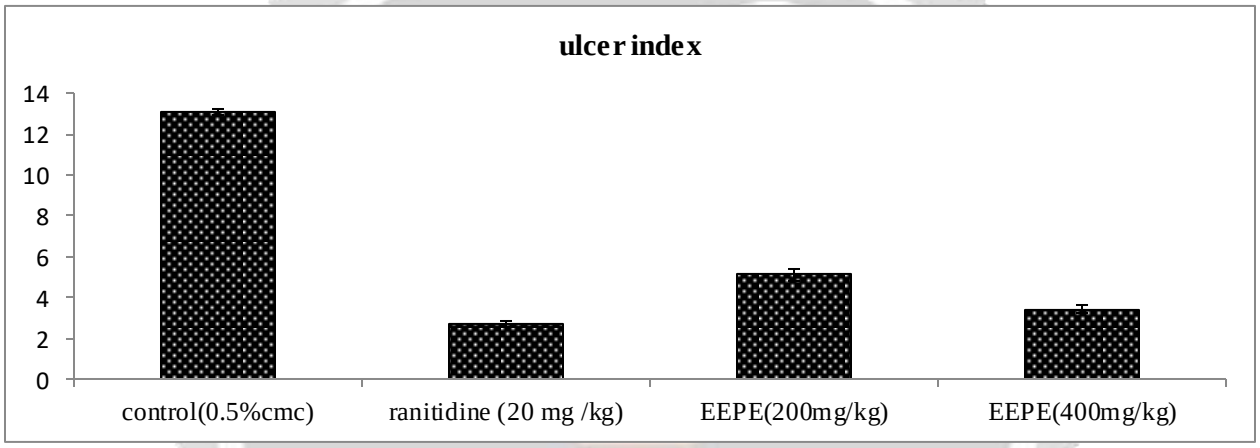


Figure 10. The Effect of Passiflora edulis sims on Swim stress Induced Ulcer Model Indicating Ulcer index

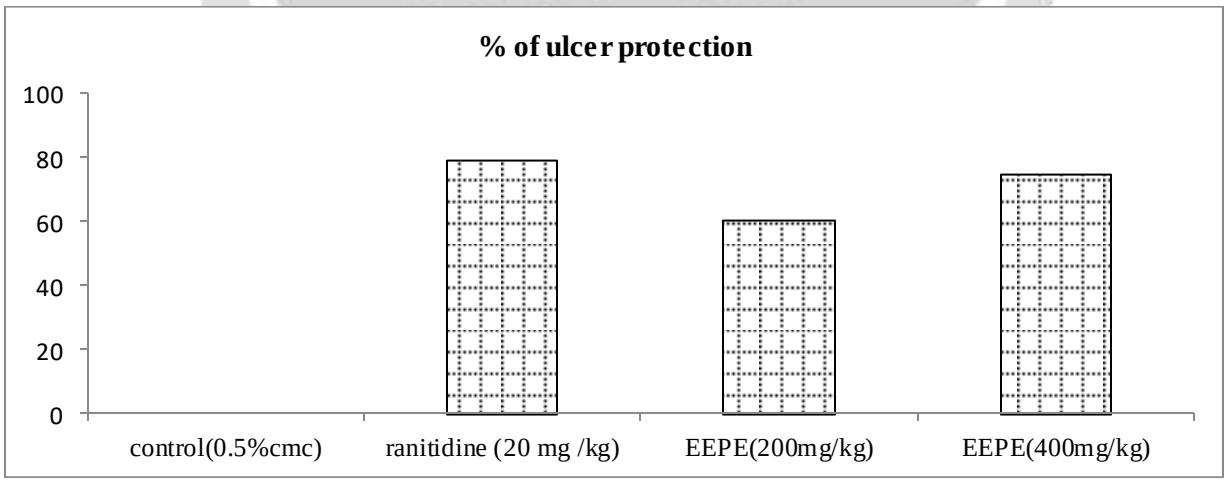


Figure 11. The Effect of Passiflora edulis sims on Swim stress Induced Ulcer Model Indicating % of ulcer protection

Sn	Treatment	Ulcer index	% of ulcer protection
1	Control 0.5 % cmc	13.11±0.17	
2	Ranitidine	2.7±0.18**	79.40
3	EEPE 200 mg/kg	5.14±0.30**	60.79

4	EEPE 400 mg/kg	3.44±0.17**	74.40

Values are mean ± SEM; No. of animals in each group = 6** P value <0.01 compared with the corresponding control

Table 4. The Effect of Passiflora edulis sims on Swim stress Induced Ulcer Model Indicating Ulcer index & Percentage Ulcer Protection

4. DISCUSSION:

The present study is to explain the anti-ulcer activities of the ethanolic extract of leaves of *Passiflora edulis sims*.

Ethanol induced gastric damage ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions, which is attributed mainly to the inhibition of biosynthesis of cytoprotective PG resulting in overproduction of leukotrienes and other products. These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H + and Na + ions reducing the transmucosal potential difference and induce formation of erosions and ulcers. In this model *Passiflora edulis sims* extract was able to produce a dose dependent significant reduction of the gastric mucosal damage, indicating a probable local increase in PG synthesis. The ulcer protective action of 400mg/kg dose was nearly closer to that of standard drug sucralfate.

In aspirin plus pylorus ligation model, ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In vehicle control group animals pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in higher ulcer index. The extract of *Passiflora edulis sims* reduced the gastric volume, free acidity, total acidity and hence ulcer index showing the anti-secretary mechanism.

Water immersion stress model provides both emotional stress as well as physiological stress to the animal. The extract showed significant (P<0.001) ulcer inhibition. The anti-ulcer effect observed in the present study might be due to a possible relationship between protection of mucosal injury, inhibition of acid secretion and the antioxidant nature of *Passiflora edulis sims*.

The extract possesses antisecretory, cytoprotective and proton pump inhibition mechanism. This study indicates that *Passiflora edulis sims* extract has a potential antiulcer activity. The secondary metabolites identified may also have been flavonoids, tannins and triterpenoids are the possible constituents behind the anti-ulcer activity of *passiflora edulis sims*. as flavonoids, have been reported to possess antiulcer activity in activity in various experimental models of ulcers.

5. CONCLUSION:

The result of this study confirms the use of the ethanolic extract of leaves of *passiflora edulis sims* in traditional management of peptic ulcer effect. Further study is required to isolate the active phytochemical constituents present in the extract and pharmacological studies on the healing action of drug on chronic ulcer as well as on the possible side effects. The investigation on mode of action may pave way for establishment of new anti-ulcer therapy regimen.

6. REFERENCE:

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