

SCRUTINIZING THE AQUEOUS EXTRACT OF LEAVES OF PEDALIMUM MUREX FOR THE ANTIULCER ACTIVITY IN RATS

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ABSTRACT

Peptic ulcer is manifested largely due to an alteration in lifestyle and diet. The antiulcer efficacy of the aqueous extract of leaves of *Pedaliium murex* on ethanol induced gastric lesions was investigated in our studies. This has been substantiated by ascertaining the content of total acid, acid volume, total protein, ulcer index and glutathione. Ulceration was induced in 36 hours fasted rats by the administration of 80% ethanol (1ml/kg) orally. The reference standard (famotidine, 3mg/kg) and aqueous extract of leaves of *Pedaliium murex* in doses of 50, 100, 200mg/kg was given to different groups, one hour before the administration of ethanol. Marked gastric mucosal lesions were observed with ethanol. A perceptible elevation in ulcer index, total acidity, acid volume, total protein and diminution of glutathione was observed. Pretreatment with aqueous extract of leaves of *Pedaliium murex* particularly at a dose of 200mg/kg in a single schedule and 100mg/kg for 15 and 30 days treatment annihilated these alterations and elevated the level of glutathione. Therefore the aqueous extract of leaves of *Pedaliium murex* could be regarded as a favorable antiulcerogen which could be attributed to its content of flavonoids and mucilage.

Keywords: Antiulcer pedaliium murex, ulcer index, estimation of glutathione, estimation of total protein.

INTRODUCTION

Lifestyle and dietary changes are the most prominent causes of peptic ulcer and related acid peptic disease (Chaturvedi *et al.*, 2007). Although, these disorder appear simple, they require prompt medical attention (Burks, 1995). Peptic ulcer is a major health hazard both in terms of morbidity and mortality (Chaturvedi *et al.*, 2007). Untreated peptic ulcer is capable of inducing upper gastrointestinal bleeding (Gerard, 2003). Peptic ulcer can ensue due to an imbalance between offensive (acid-pepsin secretion, H. Pylori, bile) versus defensive factors (mucus, bicarbonates secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury).

The high degree of efficacy and safety with herbal medicines make them more acceptable compared to other therapeutic intervention (Chaturvedi *et al.*, 2007). *Pedaliium murex* is a useful plant in many ways. Traditionally, the juice of the leaves have healing potential when applied to ulcers (Nadkarni, 1976), it serves as an emmenagogue and is effective in puerperal disease. Splenic enlargement is diminished with leaves and gonorrhoea can be prevented (Kirtikar *et al.*, 1987). *Pedaliium murex* is rich in mucilage (Kirtikar *et al.*, 1987), flavanoids (Jeffrey *et al.*, 2002), saponin glycosides (Bhakuni *et al.*, 1992). The inquistiveness to determine the antiulcer activity of *Pedaliium murex* was propelled by the presence of its active constituents and to corroborate its traditional claim.

MATERIALS AND METHODS

Drugs

DTNB solution (5-5 dithiobis-2 nitrobenzoic acid) was procured from M/s. Sisco research laboratories Pvt. Ltd, Mumbai-400099, India.

Plant material

The fresh leaves of *Pedaliium murex* was collected from the outskirts of Jodhpur in June 2008. The plant was identified by a Botanist and a voucher specimen is maintained in our herbarium bearing number PM/5.

Extraction and preparation of extract

The leaves of the plant were coarsely powdered and 50g of leaf powder was weighed. Extraction was then carried out with 200mL of water under reflux by maintaining the temperature between 40°C-50°C for 32 hours. The extract was then evaporated under vacuum and stored in a refrigerator. The required quantity of extract was suspended in 1.0% aqueous solution of tragacanth and used.

Animals

Albino rats weighing 180-200 gm of either sex were used in this study. They were divided into 5 groups, with each group containing 6 animals. Clearance to carry out the work was obtained from the Institutional animal ethical committee bearing No.IAEC/Clear/51/2007-08 dated 22/09/2007.

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Safety evaluation testing

The safety of the aqueous extract of leaves of *Pedaliu murex* was ascertained by the up and down method. Sequential dosing of animals was done with 50mg/kg, 200mg/kg, 500mg/kg, 1250mg/kg, 3000mg/kg and observing the animals for gross behavioral changes for 24hours (Ghosh, 1984).

Ethanol induced gastric ulcer

Ulceration was induced in 36 hours fasted rats by the administration of 80% ethanol orally at a dose of 1mL/rat. Group-1 received aqueous tragacanth (1ml/kg) and served as control, group-2 received famotidine (3mg/kg) orally and served as standard. Groups-3, 4 and 5 received 50, 100 and 200mg/kg doses of aqueous extract of leaves of *Pedaliu murex* (AELPM) respectively, 1 hour before the administration of ethanol in the dose dependent studies. In the time dependent profile, AELPM was administered for 15 and 30 days in a dose of 100mg/kg. 1 hour before the last dose of AELPM on day 15 and day 30, ethanol (1ml/rat) was administered per os. After 2 hours of ethanol administration, animals were sacrificed by an overdose of ether. Stomach was dissected out, small nick was made along the greater curvature, contents were drained into a graduated centrifuge tube and the acid volume was determined (Robert, 1979). The contents were centrifuged at 3000 rpm for 10 minutes. Total acidity and pH was analyzed from the decanted supernatant. pH was determined using digital pH meter (Type DPH – 100- Data instruments) (Parmar *et al.*, 1993; Susan *et al.*, 1990). Total acidity was ascertained by titrating with 0.1N NaOH using phenolphthalein as indicator and expressed as mEq/L (Mukherjee, 1989).

Ulcer index

The stomach was opened along the greater curvature and fixed on a cork plate. The number and severity of ulcers was registered using the following scores (Kulkarni, 2002).

Severity Score:

- 0 = Normal colored stomach
- 0.5 = Red coloration
- 1 = Spot ulcer
- 1.5 = Hemorrhagic streaks
- 2 = Ulcers ≥ 3 but ≤ 5
- 3 = Ulcers > 5 .

Ulcer index was calculated as: $UI = UN + US + UP \times 10^{-1}$
Where,

UI = ulcer index, UN= average of number of ulcers per animal, US= average of severity score and UP = percentage of animals with ulcer.

Biochemical estimation of Glutathione (GSH)

The mucosa of glandular layer of stomach was removed by scraping with a blunt knife and 10% homogenate was

prepared. The homogenate was precipitated with 25% trichloroacetic acid (TCA) and centrifuged. The supernatant was taken for GSH estimation using freshly prepared DTNB solution (5-5 dithiobis-2 nitrobenzoic acid). The intensity of the yellow color formed was read at 412 nm. A parallel blank for each sample without reagent was run (Moron *et al.*, 1979).

Estimation of Total Protein

It was estimated according to method described by Lowry *et al* (Lowry *et al.*, 1951).

STATISTICAL ANALYSIS

The result are expressed as mean $\pm$ SEM and analyzed using unpaired student 't' test. P values < 0.05 was considered statistically significant.

RESULTS

Control group exhibited profound increase in acid volume, total acidity, ulcer index and total protein. A decline in gastric pH to 2.6 ± 0.06 was obtained following ethanol treatment (fig. 1). Glutathione content was diminished considerably to 0.89 ± 0.012 mol/g wet weight.

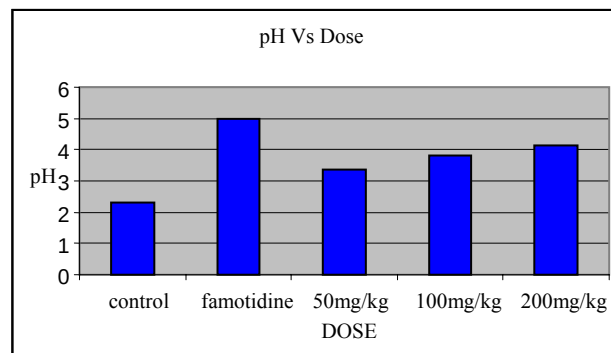


Fig. 1: Effect of aqueous extract of leaves of *Pedaliu murex* on gastric pH in dose dependent studies.

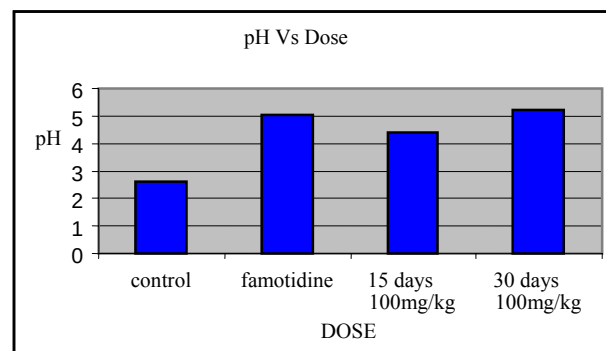


Fig. 2: Effect of aqueous extract of leaves of *Pedaliu murex* on gastric pH following 15 and 30 days treatment.

Table 1: Dose dependent studies of the aqueous extract of leaves of *Pedaliium murex* (AELPM) rats in ethanol induced ulcer model.

S. No.	Treatment	Dose (mg/kg)	Ulcer index (UI)	Total acidity (mEq/L)	Acid volume (mL)	Glutathione (Mol/g wet weight)	Total Protein (gm/dL)
1	Control	-----	11.6±1.07	114.1±1.13	8.61±0.21	0.89±0.012	0.806±0.02
2	Famotidine	3	4.33±0.42**	59.6±0.84**	4.75±0.33**	1.45±0.19*	0.699±0.03*
3	AELPM	50	9.00±0.28	90.5±0.22*	6.75±0.11*	0.86±0.065	0.714±0.09
4	AELPM	100	6.16±0.21*	77.0±0.516*	5.73±0.09*	0.95±0.094	0.657±0.05*
5	AELPM	200	1.65±0.11**	67.3±0.21**	5.10±0.04**	1.01±0.024*	0.571±0.02*

n=6, Values are seen mean±SEM, **P<0.001, *P<0.05 compared with control.

Table 2: Time dependent studies of the aqueous extract of leaves of *Pedaliium murex* (AELPM) using rats in ethanol induced ulcer model.

S. No.	Treatment	Dose (mg/kg)	Duration (Days)	Ulcer index (UI)	Total acidity (mEq/L)	Acid volume (mL)	Glutathione (Mol/g wet weight)	Total Protein (gm/dL)
1	Control	-----	15	11.6±0.42	108.8±3.13	8.25±0.42	0.89±0.012	0.806±0.08
2	Famotidine	3	15	4.1±0.48**	56.4±0.95**	4.50±0.22**	1.65±0.66**	0.61±0.06*
3	AELPM	100	15	1.25±0.11**	64±0.365**	4.23±0.08**	1.03±0.03*	0.550±0.02*
4	AELPM	100	30	0.00	55.5±0.22**	4.05±0.02**	1.08±0.09*	0.505±0.002*

n=6, Values are seen mean±SEM,

In the single dose study, a significant effect ($p<0.001$) was observed following treatment with 200mg/kg in terms of reduction of acid volume to 5.10 ± 0.04 ml, total acidity to 67.3 ± 0.21 mEq/L, ulcer index to 1.65 ± 0.11 , total protein to 0.571 ± 0.02 g/dl compared with the control (table 1). pH of gastric contents was enhanced with this dose of AELPM compared with the control ($p<0.05$) (fig. 1). A significant difference in the volume of acid generated, ulcer index, total acidity, total protein was seen in groups pretreated with AELPM for 15 days compared with the control ($p<0.05$) (table 2).

An abundant reduction in the incidence of ulcer was observed following treatment with AELPM for 30 days. The ulcer index was reduced to zero. A conspicuous decrease in acid volume, total acidity, ulcer index and total protein was observed compared with the control ($p<0.05$). An elevation in the level of glutathione was facilitated with AELPM to 1.03 ± 0.03 and 1.08 ± 0.09 mol/g wet weight following treatment for 15 and 30 days compared with the control (table 2).

DISCUSSION

An intricate system is involved in maintaining integrity of the gastroduodenal mucosa by providing mucosal defense and repair. This intricate biologic system consists of

mucus/bicarbonate layer, surface epithelial cells and a rich submucosal micro-circulatory bed which provides bicarbonate ions to neutralize the acid generated by the parietal cells. Moreover, this micro-circulatory bed provides an adequate supply of micronutrients and oxygen while removing toxic metabolic by products. Disruption of mucosal integrity of the stomach and duodenum is facilitated by a host of noxious and aggressive factors like acid, pepsin, bile acids, pancreatic enzymes, drugs and bacteria (Chaturvedi *et al.*, 2007). Ethanol serves as a common and potential ulcerogenic agent as it induces gastric haemorrhagic erosions when administered intragastrically (Shetty *et al.*, 2000). The genesis of ethanol induced gastric lesions is multifactorial with the depletion of gastric wall mucus (Al-Harbi *et al.*, 1997) and this may be attributed to mucosal leukotriene release. Ethanol induced damage to the gastric mucosa is associated with a significant production of free radicals due to enhanced lipid peroxidation leading to damage of the cell and cell membranes (Vanisree *et al.*, 1996). It produces mucosal mast cell degranulation leading to the release of vasoactive mediators including histamine (Miller *et al.*, 1984). Activation of adenylyl cyclase by histamine stimulates the production of cyclic AMP which in turn activates the gastric proton pump and releases hydrogen ions (Tripathi, 2003).

The volume of acid present in gastric secretion which encompasses HCl, pepsinogen, mucus, bicarbonates, intrinsic factor and protein reflects acid volume. Exposure of unprotected lumen of the stomach to accumulating acid could facilitate ulceration (Olsen, 1988). Another major aggressive factor responsible for ulcers is the content of acid present in gastric juice. Over secretion of histamine contributes to increased secretion of gastric juice (Grossman, 1978). When the concentration of hydrogen ions in gastric juice decreases, it is reflective of high pH. The genesis of ulcer and gastric damage is facilitated by hydrogen ions which serve as another aggressive factor (Lüllmann *et al.*, 2000).

Gastric acid decimation by famotidine is attributed to its ability to antagonize the binding of histamine to the H₂ receptor on the parietal cells. Famotidine can therefore counter the effect of ethanol on acid secretion.

It has been documented that flavonoids are capable of reducing gastric histamine content (Ebadi, 2002). A decline in the acid volume, total acidity and elevation in pH of gastric secretion is exhibited by AELPM particularly at a dose of 200mg/kg in a single schedule and following treatment for 15 and 30 days as observed from our studies. The protective efficacy of *Pedaliium murex* might also be related to its H₂ receptor antagonizing action as it has ample content of flavonoids.

Leakage of plasma protein into the gastric juice can cause weakening of the gastric mucosal barrier (Mizushima *et al.*, 1967). Enhancement of total protein of gastric juice is commonly seen in ulcerated tissue. AELPM in a dose of 200mg/kg as a single schedule and when administered for 30 days was capable of decreasing protein content of gastric juice implying its ability to strengthen the mucosal barrier and increase its resistance to the damaging effects of aggressive factor.

The free radicals generated due to ethanol exposure could decrease the formation of glutathione by reducing formation of cysteine (Body *et al.*, 1981; Loguercio *et al.*, 1993). Consistency of the gastric mucosa is maintained by glutathione which serves as a free radical scavenger, as it protects the thiol protein groups (Stein *et al.*, 1989). AELPM enhances the glutathione content when treatment was continued for 30 days and with 200mg/kg in a single dose implying that it also functions by countering the free radicals generated by ethanol.

The rich content of mucilage in *Pedaliium murex* may carpet the gastroduodenal lining, thereby abrogating the impact of ulcerogens such as ethanol.

In conclusion, it can be stated the AELPM can serve as a competent antiulcer agent.

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