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ANTI ULCER, ANTI SECRETORY AND CYTOPROTECTIVE EFFECTS OF *TRIANTHEMA DECANDRA* IN RATS

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ABSTRACT

The herb Trianthema decandra. L (Aizoceae family) mentioned in Siddha Literatures had been studied in rats for its ability to inhibit gastric secretion and to protect the gastric mucosa against injuries caused by pyloric ligation, swim stress, acetic acid and cytodestructive agent ethanol. The extracts derived successively by using solvents of increasing polarity from the root powder of TD (Petroleum Ether Extract, Ethyl Acetate Extract, Alcoholic Extract, and Aqueous Extract) at the dose of 200 mg/Kg, p.o, and Crude powder of its root at the dose of 2g/Kg, p.o, were used in all experiments. Famotidine (20mg/Kg, p.o) was used as a standard anti ulcer drug for comparison. Eth.Ace.Ext and CP only showed significant anti ulcer and anti secretory effects in pyloric ligation, swim stress models and all the four extracts and CP showed pronounced cytoprotective effect in ethanol induced gastric ulcer in rats. Significant effect exhibited by Eth.Ace.Ext in acetic acid induced ulcer study reveals its efficacy in chronic ulcer.

KEYWORDS

Anti ulcer, Anti secretory, chronic ulcer, Cytoprotection, Trianthema decandra.

ABBREVIATIONS

PU- Peptic Ulcer, TD- Trianthema decandra, Pet.Eth.Ext- Petroleum Ether Extract, Eth.Ace.Ext- Ethyl Acetate Extract, Alc.Ext- Alcoholic Extract, Aq.Ext- Aqueous Extract, CP- Crude Powder, p.o- per oral, HCl- Hydrochloric acid, FA- Free acid, TA- Total acid, UI-Ulcer index, sc- subcutaneous, PG-Prostaglandin, NPSH- Non Protein Sulfhydryls, NP- Neuropeptides.

INTRODUCTION

Peptic ulcer is caused when the natural balances between the aggressive factors (acid and pepsin) and defensive factors (cytoprotection) are disturbed[1]. No acid- no ulcer- is the old dictum. In the past, drugs used in Peptic ulcer have been directed mainly against luminal agent HCl and drugs like antacids, anti- cholinergics, H_2 receptor antagonists, Proton pump inhibitors etc., have flooded in the market. Recent researches have added more concentration and consideration in cytoprotection and mucosal protective mechanisms and their role in the recovery of upper gastro intestinal tract from damage. In the present study, it has been planned to evaluate the efficacy of TD on these two factors i.e. aggressive factors and defensive factors. Among the various herbs which had been used in Siddha tradition for treating peptic ulcer (in Siddha, Vali gunmam and Eri gunmam), the root of *Trianthema decandra* was selected for this research based on wide-ranging literary evidences in Siddha manuscripts [2-10].

In Siddha system of medicine, since ancient times, several herbs have been used to treat peptic ulcer. From indigenous drug *Glycyrrhiza glabra*[11], the first systemically effective drug carbenoxolone has been formulated against gastric ulcer. Later, research on cabbage[11] previously employed as an oral anti ulcer agent in folk medicine, had led to the development of gefarnate which acts as an anti ulcer agent by enhancing the gastric mucosubstances and

gastric mucosal strength. Raw banana[11] fruit has also found to inhibit peptic ulceration by promoting the regeneration of gastric mucosa. *Melia azadarach*[11] has been observed to inhibit stress induced ulcer formation in rats. Konturek *et al,* have reported the effectiveness of solon [11], a plant flavonoid against ulcers in experimental animals and recent studies on flavonoids[12] supported its efficacy on gastro protection. Protection of mast cells against degranulation and anti secretory effect are the mechanisms involved in anti ulcer activity of *Leucas aspera*[13]. *Wedelia calendulacea*[14], Ethanolic extract of *Eruca* sativa[15], Celery (Apium graveolens)[16] have been reported of having anti ulcer, anti secretory and cytoprotective activities against experimentally induced gastric lesions. Ganoderma lucidum polysaccharide fractions were found to be effective in the acceleration of healing of ulcer in acetic acid induced ulcer studies in rats[17]. Likewise the experiments on herbal drugs against peptic ulcer are still continuing with modern parameters to establish the scientific background of traditional knowledge and practice.

The phytochemical and pharmacological profile of Trianthema decandra species are reviewed by *Geethalakshmi et al* [18] and justified the anti ulcer property of Trianthema pendendra, flavonoid content and anti oxidant properties of Trianthema species.

MATERIALS AND METHODS

Animals

Adult albino male rats (100-130 g) were divided into groups of 6 each and they were deprived of food for 24 hours before the commencement of experiments but water was allowed adlibitum.

Drugs

Plant root of TD from Aizoceae family were collected around Gobichettipalayam area, Erode District, Tamil Nadu and plant specimen was identified and confirmed at BSI Herbarium, Coimbatore, Tamil Nadu. The root powder of TD was extracted successively by using solvents of increasing polarity; Petroleum ether, Ethyl acetate, Ethanol and water. The dose level used in the study: Pet.Eth.Ext, Eth.Ace.Ext, Alc.Ext, Aq.Ext- 200 mg/Kg, p.o, Crude powder- 2g/Kg, p.o, Famotidine (20mg/Kg, p.o), vehicle- 5% w/v acacia mucilage at the dose of 1ml/Kg. The above mentioned dose levels had been fixed based on the results of LD 50 determination experiments[19] conducted in mice to find out the tolerable dose and preliminary study conducted on various dose levels to assess its efficacy.

Pylorus ligated (Shay) rats

The drugs were given orally 2 hours prior to pyloric ligation which was carried out according to the technique of Shay *et al* [20]. The animals were sacrified 6 hours after pyloric ligation for observation of gastric lesions described by Gupta *et a l* [21]**.** The gastric juice was collected, centrifuged and its volume and pH was measured. FA and TA were estimated titrimetically[22] with 0.01 N NaOH using methyl orange and phenolphthalein as indicators. The data concerning the pH, volume, FA, TA and UI were analysed by student's t test.

Stress induced gastric ulcers in rats

The rats were forced to swim for 5 hours [23] in a steel water tank (60 x 90 cm with 60 cm water level) at a constant temperature **$33^0 \pm 1^0$C**. The drugs with specific doses were given orally 2 hours before forcing the rats to swim. The animals were sacrified after the period of swimming. The stomach were removed and examined for the severity of lesions [21] and were analysed by student's t test.

Ethanol induced gastric lesions in rats (Cytoprotective study)

The rats (175-225 g) were deprived food for 36 hours and water for 24 hours before commencement of the experiment. The animals were pre-treated orally with the drugs. 30

minutes later 1 ml of absolute ethanol was given orally to induce gastric lesions[24]. The animals were killed 1 hour later and stomachs were examined for linear lesions. The length (mm) was summed per stomach and analysed by student's t test.

Adaptive cytoprotective study in rats [25]

The drugs (Pet.Eth.Ext and Aq.Ext) were given orally 30 minutes before the necrotizing agent absolute ethanol. Indomethacin (10mg/Kg, sc) was given 1 hour before the necrotizing agent. One hour after administration of ethanol the animals were killed and examined for total area of lesions and were analysed by student's t test.

Acetic acid induced chronic ulcers in rats

Gastric ulcers were produced by the subserosal injection of 0.05 ml of 10% acetic acid[26]. Eth.Ace.Ext and Famotidine were given orally twice daily for 9 days from second to tenth day after ulcer production. After the last dose the animals were fasted until next day when they were sacrified and examined for the total area of lesion in each stomach and were analysed by student's t test.

RESULTS AND DISCUSSION

Table 1 shows the effect of TD on gastric ulceration and acidity induced by pyloric ligation. Only Eth.Ace.Ext (200mg/Kg, p.o) and CP (2g/Kg, p.o.) reduced gastric secretory volume, FA, TA ($p<0.001$), raised pH of the gastric juice ($p<0.001$) and afford significant protection ($p<0.01$) against the ulcer produced by pylorus ligation similar to Famotidine (20mg/Kg, p.o.). Other extracts were found to be ineffective.

In stress induced ulcer (forced swimming for 5 hours at $33^0 \pm 1^0$ C) in rats. Only Eth.Ace.Ext (200mg/Kg, p.o) and CP (2g/Kg, p.o.) produced significant inhibitory effect ($p<0.01$) against stress induced ulcers as like as Famotidine (20mg/Kg, p.o.) and data shown in **Table 2**.

Table 3 shows the cytoprotective effects of the extracts, CP and Famotidine in lesions induced by ethanol. All four extracts (200mg/Kg, p.o.), CP (2g/Kg, p.o.) and Famotidine (20mg/Kg, p.o.) significantly reduced ($p<0.001$) the extent of mucosal lesions and afford cytoprotection.

Table 1. Effect of *Trianthema decandra* in pylorus ligated rats (mean ± SE, n=6)

Group	Dose mg/Kg	Gastric secretory volume	pH	Total acidity (meq/L)	Free acidity (meq/L)	Ulcer index	Percentage Reduction in ulcer index
Control	------	3.02±0.18	1.1±0.25	92±2.21	70±7.42	33.3±4.22	----------
Pet.Eth.Ext	200	2.65±0.24	1.4±0.12	86.17±3.02	63.66±2.96	28.3±3.33	15.22
Eth.Ace.Ext	200	1.05±0.13**	4.1±0.16**	29±0.41**	16±1.05**	11.66±3.20*	64.99
Alc.Ext	200	2.92±0.48	1.6±0.09	88.16±2.21	66.50±2.92	30±3.65	9.91
Aq.Ext	200	3.05±0.14	1.0±0.09	90.50±2.63	67.33±2.96	31.6±3.07	5.11
Crude powder	2000	1.07±0.26**	3.8±0.15**	34±2.22**	19±1.86**	13.33±3.33*	59.97
Famotidine	20	1.05±0.13**	4.3±0.12**	27±1.96**	17±1.39**	10±3.65*	69.97

***p<0.01, **p<0.001 vs control**

Table 2. Effect of *Trianthema decandra* on stress induced gastric ulcer in rats (mean ± SE, n=6)

Group	Dose (mg/Kg)	Ulcer index	Percentage Reduction in ulcer index
Control	-------	28.33±3.07	----------
Pet.Eth.Ext	200	21.66±1.67	23.55
Eth.Ace.Ext	200	13.33±3.33*	52.95
Alc.Ext	200	23.33±3.33	17.65
Aq.Ext	200	20±2.58	29.41
Crude powder	2000	15±2.24*	47.06
Famotidine	20	11.67±3.07*	58.81

***p<0.01 vs control**

Table 3. Cytoprotective effect of *Trianthema decandra* on ethanol induced gastric ulcer in rats (Mean ± SE, n=6)

Group	Dose (mg/Kg)	Mean length of gastric **lesions (mm)**	% reduction in lesions
Control	-------	28.83±2.80	----------
Pet.Eth.Ext	200	4.5±0.42**	84.12
Eth.Ace.Ext	200	6.33±0.75**	77.66
Alc.Ext	200	4.33±0.99**	84.72
Aq.Ext	200	4.16±0.70**	85.32
Crude powder	2000	5.67±0.66**	80.64
Famotidine	20	8.33±1.28**	70.60

****p<0.001 vs control**

Table 4. Adaptive cytoprotective study in indomethacin pre-treated rats (mean ± SE, n=6)

Group	Dose (mg/Kg)	Total lesion area (mm^2)	Percentage reduction in lesions
Control	----------	26.60±3.25	----------
Pet.Eth.Ext	200	7±0.73**	73.69
Aq.Ext	200	8.66±0.88**	67.45

****p<0.001 vs control**

Table 5. Effect of *Trianthema decandra* on the healing of acetic acid induced chronic gastric ulcer in rats (mean ± SE, n=6)

Group	Dose (mg/Kg)	Total lesion area (mm^2)	Percentage reduction in lesions
Control	----------	29.83±3.26	----------
Eth.Ace.Ext	200	6.33±0.61**	78.78
Famotidine	20	9.3±0.71**	68.83

****p<0.001 vs control**

Pet.Eth.Ext and Aq. Ext of TD which were found to be effective in ethanol induced gastric lesion study were selected for adaptive cytoprotective study. **Table 4** presents the effect of Pet.Eth.Ext (200mg/Kg, p.o.) and Aq. Ext (200mg/Kg, p.o.) in Indomethacin(10mg/Kg, sc) pre-treated adaptive cytoprotective study and they produced significant cytoprotection (p<0.001). Eth.Ace.Ext (200mg/Kg, p.o) was found to be very effective in all previous acute models had been selected for this acetic acid induced ulceration in rats. Eth.Ace.Ext produced significant effect (p<0.001) in healing of chronic

ulcer like that of Famotidine (20mg/Kg, p.o.). The size indices and percentage decrease of the ulcer size is shown in **Table 5**.

In pylorus ligated rats Eth.Ace.Ext and CP only significantly reduced FA, TA, UI and raised pH of gastric juice similar to Famotidine, H_2 receptor antagonist. H_2 receptors possess anti secretory [27]activity and protect animals from gastric ulceration induced by pylorus ligation, stress and aspirin related compounds[28]. Cimetidine antagonised penta gastrin, histamine and carbachol induced hyperacidity in gastric fistula in rats[29]. These observations lend support to the view that anti-secretory and anti ulcer activities of Eth.Ace.Ext and CP might be due to its reduced cholinergic, reduced gastrin and reduced histaminergic activities like that of cimetidine by its H_2 receptor blockade mechanism.

Increased mast cell degeneration due to vagal over activity and increased production of histamine are considered[30, 31] as the main factors for stress induced ulceration via gastric hyper secretion of acid. Only Eth.Ace.Ext and CP showed significant anti ulcer effect similar to Famotidine and it appears that the anti secretory property of these drugs might be the possible mechanism involved in protection against stress induced ulcers.

All the four extracts and CP showed significant cytoprotective effect in ethanol induced gastric lesions in rats. Many of the orally effective cytoprotective drugs acts by increasing the generation of endogenous PGs due to mild irritation action described as adaptive cytoprotection [32]. Szabo [33] proposed later that gastric cytoprotection might be mediated through at least two mechanisms i.e. PGs and NPSH of the gastric mucosa. After Szabo, Jacobson [34] classified into three i.e. PGs, NPSH and NP. Hence both Pet.Eth.Ext and Aq.Ext showed significant cytoprotective effect in indomethacin (a known PG biosynthesis inhibitor) pre-treated adaptive cytoprotective study, it has been confirmed that the cytoprotective effect of TD was not mediated through PGs, it might be due to some other factors like NPSH, NP etc.

The unique nature of PU is chronicity, remission and relapse. Beside acute ulcer models, acetic acid induced ulcer model was selected to evaluate ulcer healing property of the trial drug and its efficacy in chronic ulcer. Eth.Ace.Ext is the drug produced significant effect in all acute ulcer models was used in this research and exhibited significant effect. The curative effects of aluminium hydroxide, atropine and cimetidine suggest that the reduction in gastric acidity may be greatly involved in the healing of acetic acid induced chronic ulcers [35]. Thus, it has been confirmed that the anti secretory effect of Eth.Ace.Ext was evidenced in pylorus ligated rats study, might be the possible mechanism in the ulcer healing effect in this model.

Among the four extracts of TD used in this study, Eth.Ace.Ext only showed significant effect in all experiments in controlling aggressive factors, promoting defensive factors (cytoprotection) and enhancing ulcer healing (chronic ulcer) i.e. it showed significant anti ulcer, anti secretory, cytoprotective and ulcer healing properties. In connection with this study, qualitative chemical analysis [19] had been done on all four extracts. Eth. Ace.Ext, Alc.Ext and Aq.Ext were found to be having flavonoids. Presence of Flavonoids in Eth.Ace.Ext might be the added value for its efficacy in all experiments. Flavonoids have been reported to possess anti ulcer and anti inflammatory effects [36, 37]. Flavonoids found to be effective in pylorus ligated[38], stress[38], cytoprotective[39] experimental models and duodenal ulcer studies[36]. Flavonoids produces cytoprotective activity by increased mucus production, glycoprotein content [40] and its gastro protective effects was partly explained through non PG dependent

mechanism[41] and the same has been established in adaptive cytoprotection study.

CONCLUSION

An ideal anti ulcer drug is one which not only checks acid secretion (aggressive factors) and improves mucosal resistance (cytoprotection / defensive factors) but also should promote cell regeneration (ulcer healing) [42]. All these findings of the present study indicate that TD may offer another approach to the therapy of Peptic ulcer (acute and chronic), but extensive clinical trial could be conducted further to establish its potency in human beings.

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