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Original Article

Anti-ulcer effect of *Tripleurospermum disciforme* (C.A. Mey) Shultz Bip on pylorus ligated (Shay) rats

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Abstract

Gastric ulcer is one of the most prevalent gastrointestinal (GI) disorders, which affects approximately 5-10% of people during their life. In recent years, plentiful works have been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. *Tripleurospermum disciforme* is one of the indigenous plants that is readily available and has been traditionally used to improve GI disorders. We decided to study its anti-ulcer effects in pylorus-ligated rats. Hydroalcoholic extract of flowers (125, 500, 2000 mg/kg), vehicle and ranitidine (50 mg/kg) were administered orally (p.o.) to separated groups of Wistar rats of either sex (n=8). Other groups received extract (500 mg/kg), ranitidine and vehicle intraperitoneally (i.p.). Volume of contents, pH, ulcer number, scoring, incidence, area and finally ulcer index were assessed and compared with control groups. Volume of gastric contents as well as pH (in reverse with acidity) increased in extract groups but the difference was not significant. In treatment groups, regardless of the changes in ulcer number and scores, the differences were not significant for both parameters compared to control groups. Both the extract and reference drug (ranitidine) resulted in significant reduction in ulcer area and ulcer index and for latter the range of reduction was 21.8-39.1%. The least dose of extract (125 mg/kg) was not effective. We conclude that hydroalcoholic extract of *T. disciforme* was effective to protect against ulcer formation in pylorus-ligated rats and the action is not likely to be mediated through acid reduction.

Key words: Gastric ulcer, Shay method, *Tripleurospermum disciforme*

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly for the population of non-industrialized countries (1).

Several factors are implicated in the pathogenesis of gastric ulcer including: increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer (2, 3). In recent years, a powerful association between peptic ulcers and infection of *Helicobacter pylori* has been adopted (4). At least 70-90% of patients with gastric ulcers and 80-95% with duodenal ulcers are infected by *H. pylori* and eradication of this micro-organism seems to be curative for the disease (4).

There is a balance between the aggressive (i.e. acid, pepsin, active oxidants, *H. pylori*) and the mucosal protective (*i.e.*

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mucus, bicarbonate, prostaglandins) factors in stomach. Thus, drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating defensive ones (5).

Despite the progress in conventional chemistry and pharmacology in producing highly effective drugs, some of them are expensive (especially for poor patients) and have different adverse effects (6, 7), however, screening plants for active drugs is still important and might provide a useful source of new anti-ulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies (8).

Tripleurospermum disciforme (C.A. Mey) Shultz Bip (Asteraceae) is one of the traditional indigenous plants of Iran which has some similarities to the main species; *Matricaria recutita*. It is distributed widely in Iran and is commonly used instead of main species for respected therapeutic uses (e.g. anti-inflammatory, anti-spasmodic, anti-septic, carminative and as a hair color) (9, 10). On the contrary, the flavonoids that have potentially anti-ulcerative properties (rutin, apigenin and apiin) could not be found in *T. disciforme* but a number of flavons and flavonols, tannins and essential oils (especially trans-beta-farnesene) are among active materials, which are found in significant amounts in aerial parts of the plant (10). This experiment was undertaken to study the potential anti-ulcer or ulcer protective efficacy of hydroalcoholic extract of *T. disciforme* in pylorus-ligated rats.

MATERIALS AND METHODS

Plant collection

The plant aerial parts were collected from Filabad, Shahrekord in Chaharmahal-va-Bakhtiari province in June 2004. The plant materials authenticated in Biology Department, School of Science, Isfahan University, Isfahan, Iran. A voucher specimen was deposited in the herbarium of pharmacy school by the number of 1273.

Preparation of hydroalcoholic extract

Air-dried and finely powdered aerial parts of plant (100 g) were percolated with 600 ml of ethanol/water (70/30) for 48 hours at laboratory temperature. Then it was filtered and evaporated to dryness in an evaporator under reduced pressure at low temperature (11). The extract yield was 35% (w/w).

Animals

Wistar rats of either sex (weighting 150-200 g) purchased from the Pasteur Institute (Tehran, Iran). The animals were fed with standard pelleted diet and water *ad libitum* and were left 48 hours for acclimatization to animal room condition.

The food was withdrawn 18 hours before the experiment but allowed free access of water. To avoid corpophagy and fighting, the rats were fasted in wire-bottomed cages.

Grouping

The following groups of animals were used

1, 2: Sham groups; vehicle (1 ml, p.o. & i.p.) without ulcer induction

3, 4: Control groups; vehicle (1 ml, p.o. & i.p.) with ulcer induction

5, 6: Standard groups; ranitidine (50 mg/kg, p.o. & i.p.) with ulcer induction

7, 8, 9, and 10: Treatment groups; hydro-alcoholic extract (150, 500, 2000 mg/kg p.o. and 500 mg/kg i.p., respectively) with ulcer induction. For each group 8 rats were used.

Experimental procedure

The test samples were administered to animals in 5 ml/kg as a suspension in 0.5% Tween 80/saline. Oral treatments and i.p. injections were carried out 1 and 0.5 hour before pyloric ligature, respectively. After 18 hours of fasting, ulcer induction was undertaken according to Shay et al. (12).

The rats were quickly and mildly anaes-

thetized with ether and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the animal were allowed to recover from anesthesia. After ligation of the pylorus, drinking water was withheld and the gastric examinations were under-taken 19 hours after pylorus ligation.

The animals were sacrificed with an overdose of ether and the stomachs were removed after clamping the esophagus. The gastric contents were collected through the esophagus. The gastric juice was centri-fuged and volume was recorded.

Total acidity of gastric juice

An aliquot of 1ml of gastric juice was taken in to a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was established. The volume of 0.01N NaOH consumed was noted. The total acidity was expressed as meq/l by the following formula:

$N \times 0.01 \times 36.45 \times 1000$; which N is volume of NaOH consumed, 40.0 is molecular weight of NaOH, 0.01 is normality of NaOH and 1000 is the factor to be respected in liter (13).

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a ×5 magnifier lens to assess the formation of ulcer. The number of ulcers was counted. Ulcer scoring was undertaken according to Vogel et al. (14). The scores were: 0 = no ulcer, 1= superficial ulcer, 2= deep ulcer, 3= perforation.

Ulcer area was assessed by using 3 M scaled surgical transpore tapes, which was fixed on a light and transparent sheet. Each cell on the tape was $1mm^2$ in area, so the number of cells was counted and the ulcer area was measured for each stomach (15).
Ulcer index was measured by using follow-ing formula according to Vogel et al. (14).

$UI = U_N + U_S + U_P \times 10^{-1}$ which UI = ulcer index, U_N = mean of ulcer number, U_S = mean of ulcer score, U_P = ulcer probability (incidence %) for each group.

Statistical analysis

The data were analyzed by a one-way ANOVA, followed by Post hoc Scheffe test. The results are expressed as mean ± SEM.

RESULTS

In vivo results have been shown in tables 1 to 4 and figure 1.

Table 1. Effects of oral administration of *Tripleuro-spermum disciforme* (C.A.Mey) Shultz Bip extract on volume, acidity, and pH of gastric contents in pylorus ligated (Shay) rats (Data are means ± S.E.M., n= 8).

Treatment	Dose (mg/kg)	Volume (ml)	Acidity (meq/L)	pH
Vehicle	1 ml	4.9 ± 0.4	0.071 ± 0.002	1.14 ± 0.01
Extract	125	7.2 ± 0.9	0.059 ± 0.004	1.23 ± 0.03
Extract	500	6.4 ± 0.6	0.071 ± 0.005	1.15 ± 0.03
Extract	2000	6.5 ± 0.4	0.066 ± 0.003	1.18 ± 0.02
Ranitidine	50	5.1 ± 0.6	0.052 ± 0.003*	1.29 ± 0.02*

*P<0.05, significant difference from control group (Scheffe test).

Table 2. Effects of intraperitoneal administration of *Tripleurospermum disciforme* (C.A. Mey) Shultz Bip extract on volume, acidity, and pH of gastric contents in pylorus ligated (Shay) rats (Data are means ± S.E.M., n= 8).

Treatment	Dose (mg/kg)	Volume (ml)	Acidity (meq/L)	pH
Vehicle	1 ml	5.0 ± 0.3	0.069 ± 0.004	1.14 ± 0.01
Extract	500	6.3 ± 0.4	0.076 ± 0.004	1.12 ± 0.03
Ranitidine	50	6.1 ± 0.4	0.051 ± 0.003*	1.30 ± 0.02*

*P<0.05, significant difference from control group (Scheffe test).

Table 3. Effects of oral administration of *Tripleurospermum disciforme* (C.A. Mey) Shultz Bip extract on gastric lesions in pylorus ligated (Shay) rats (Data are means ± S.E.M., n=8)

Treatment	Dose (mg/kg)	Number	Scoring	Incidence (%)	Index	Inhibition (%)
Vehicle	1 ml	5.25 ± 1.5	2.00 ± 0.3	100.0	17.2	–
Extract	125	5.37 ± 0.9	1.62 ± 0.3	100.0	16.7	1.5
Extract	500	3.37 ± 0.6	1.38 ± 0.3	87.5	13.5	21.8
Extract	2000	3.25± 0.9	1.00 ± 0.3	62.5	10.5	39.1
Ranitidine	50	3.90 ± 1.1	1.25 ± 0.3	75.0	12.6	26.6

No ulcer or erosion was observed in rats of sham-operated groups indicating that the surgical procedure had no interference with experimental outputs. In control groups, the ulcer parameters were evident and indicate that the Shay method was effective enough to produce gastric ulcers. Volume of gastric contents (ml) was the first parameter noted. An increase was observed in all of the treatment groups but the difference was not significant ($p>0.05$) comparing to control groups (Tables 1 and 2). Total acidity and respected pH were two other factors which were measured. The results showed that no significant changes were observed in acidity (or pH) in extract treated groups compared to respected control groups however, the reference drug ranitidine (p.o. and i.p.) resulted in a significant reduction ($p<0.05$) in acidity (tables 1 and 2). Number and scoring were other two parameters compared to respected control groups and the differences were not significant for all of the treatment groups ($p>0.05$, Tables 3 and 4). Ulcer incidence decreased from 100 % for control and lowest dose of extract to 50 % for ranitidine (i.p.). Range of incidence reduction was between 12.5-37.5% for effective doses of plant extract. The index value for treatment groups are shown in Table 3 and 4. Reduction range for ulcer index was between 21.8-39.1% for effective doses of extract. Ulcer area was the main parameter reduced significantly ($p<0.05$, $p<0.01$) after treatment with plant extract as well as reference ranitidine. The exception was for the lowest dose of extract (Figure 1).

Table 4. Effects of intraperitoneal administration of *Tripleurospermum disciforme* (C.A.Mey) Shultz. Bip extract on gastric lesions in pylorus ligated (Shay) rats (Data are means ± S.E.M., n= 8).

Treatment	Dose (mg/kg)	Number	Scoring	Incidence (%)	Index	Inhibition (%)
Vehicle	1 ml	5.11± 1.1	2.0 ± 0.2	100.0	17.1	–
Extract	500	4.75 ± 1.4	1.1 ± 0.4	62.5	12.1	29.2
Ranitidine	50	3.37 ± 2.3	1.0 ± 0.3	50.0	9.4	45.2

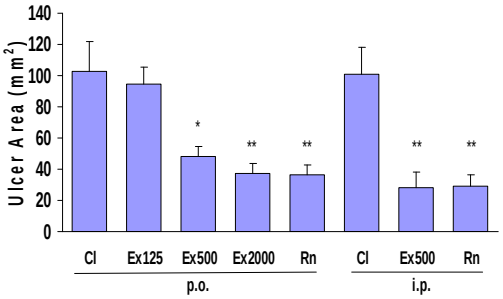

Figure 1. Effects of oral and intraperitoneal administration of *Tripleurospermum disciforme* (C.A. Mey) Shultz Bip on gastric ulcer area in pylorus ligated (Shay) rats (Data are mean ± S.E.M., n= 8)
Cl (control, 1ml of vehicle), Ex (hydroalcoholic extract with doses of 125, 500, 2000 mg/kg). Rn (ranitidine 50mg/kg). Oral (p.o.) and intraperitoneal (i.p.) treatments carried out 0.5 and 1 hour respectively prior to pylorus ligation. *$p<0.05$, **$p<0.01$, significant difference from control groups (Scheffe test).

DISCUSSION

Although in most of the cases the etiology of the ulcers is unknown, it is generally accepted that they are resulted from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defensive mechanisms (16). To regain the balance, different therapeutic agents including plant extracts may be used (17, 18). *T. disciforme* extract is one of such herbal drugs used in the present study primarily to evaluate the anti ulcerogenic or ulcer preventive potency. The total plant extract didn't have significant effect on properties of stomach in acid secretion, so the gastric juice volume and total acidity remained unchanged. Conversely, ranitidine as an acid reducing agent was effective to reduce the acidity and in a reverse manner changed the gastric pH. These results are in accordance with those were obtained in the studies of Sairam et al. (19), Muniappan (20) and Rao et al. (21) on different plant extracts. Regarding the mechanisms likely involved, our findings about the anti ulcer properties of the plant may be explained. Indeed, the ability of plant extract to protect the stomach against ulcer without influencing acid secretion and neutralizing intra-gastric acidity can lead the plant to be classified as a cytoprotective agent (19, 21-23). It is worthy to be remembered that the

Shay-rat has been proven to be a valuable tool to evaluate anti-ulcer drugs with various mechanisms of actions (14).

Ulcer number and scores are two other parameters which may help to assess the anti-ulcerogenic efficacy of the treatments. Unexpectedly both the number and the scores did not reduce significantly ($p>0.05$) even for the reference ranitidine groups from the control groups (tables 3 and 4). A number of reasons may be implicated. Ulcer number alone cannot present an accurate and sensitive index, because the number of ulcers may increase while their dimensions actually became smaller (15). On the other hand ulcer score is a reliable and relevant parameter used by several investigators as a marker for ulcer severity (24, 25). Respected results in our study showed that a mean score reduction was occurred in treatment groups especially for greatest dose of extract and ranitidine but the differences were not significant comparing to control groups. Lack of histopathological evaluation, scoring assessment by one observer and limited ulcer scoring range could be accounted for our unusual scoring results. Ulcer incidence was the first parameter suggesting the plant effectiveness as an anti-ulcer drug (Tables 3 and 4). Plant extract with the greatest dose (2000 mg/kg) was more effective to reduce ulcer incidence rather than orally administered ranitidine (Table 3). The same effects were observed when the extract was injected intraperitone-ally (500 mg/kg), whereas in this case, ranitidine (i.p.) was more effective suggesting an important role for route of administration.

Ulcer index measurements indicated that the index value was rather smaller for all of the treatments than control groups by the way of exception for ext. 125 mg/kg. The reduction was dose dependent and when the same doses were used, the efficacy was greater for i.p. injections (Tables 3 and 4). These results are in accordance with previous findings and those obtained by Khayyal et al. (26) suggesting that higher doses of extracts were likely to protect against ulcer formation and the efficacy was both dose and route dependent. In latter experiment, for all extracts were used including *M. recutita* extract, the anti-ulcer activity was associated with a reduced acid output and an increased mucin secretion, an increase in prostaglandin E2 release and a decrease in leukotrienes. The effectiveness may actually attributed to different active constituents which exist the hydroalcoholic extract. Although the exact chemical composition and their identities have not been fully known, it seems that flavonoids like flavons and flavonols, tannins and essential oils have been partly associated with pharmacological findings in our study. Flavonoids are among the cytoprotective active materials for which anti-ulcerogenic efficacy has been extensively confirmed (8, 26, 27). It is suggested that these active compounds will be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen (23, 28, 29). Several extracts containing flavonoids have been found to exert gastroprotective activity however, the precise chemical composition in many cases is unknown (26). Tannins are other active compounds for which antiulcerogenic properties have been delineated in several studies (8). Tannins are known to “tan” the outermost layer of the mucusa and to render it less permeable and more resistant to chemical and mechanical injuries (30, 31). It is not so clear whether these natural compounds are the only contributing components of this plant or not. By attention to ulcer area it is evident that the ulcer protective efficacy of treatments had been grossly reflected in this parameter. Extracts with doses of 500 mg/kg and greater were effective to reduce ulcerated region and the effectiveness was comparable with those obtained after ranitidine usage. According to Szabo et al. (32) and Wallace et al. (33), ulcer area is

one of the most reliable and accurate factors for which the properties of ulcerated region regardless of its size and shape could be assessed. Regarding the obtained results, we concluded that hydroalcoholic extract of *T. disciforme* was effective to protect against ulcer formation in pylorus-ligated rats through the mechanism(s) other than acid reducing activity. In addition, the efficacy was greater for higher doses and when it was used after parenteral injection.

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