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PHYTOCHEMICAL SCREENING AND IN-VIVO SCREENING OF ANTIULCER ACTIVITY ON *FOENICULUM VULGARE* SEEDS

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Abstract

Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a major burden for health care resources. Although potent anti-ulcer drugs are available, most of them produce several toxicities, thus emphasizing the need to search for new alternatives. It is universally known as *Fennel* and is known by more than 100 names. It is a traditional and popular herb with a long history of use as a medicine. A series of studies showed that *F. vulgare* effectively controls numerous infectious disorders of bacterial, fungal, viral, mycobacterium, and protozoal origin. It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic activities. Some of the publications stated that *F. vulgare* has a special kind of memory-enhancing effect and can reduce stress. The aim of present study was to evaluate antiulcer effect of *F. vulgare.* In the present study, different doses of the extract (100, 200, and 400 mg/kg) were evaluated for their effect on volume of gastric secretion, pH, total acidity, ulcer score, and ulcer index along with the standard drug ranitidine (50 mg/kg). In the single-dose study of the pylori ligation model, HEFV 100 mg/kg did not show any better activity when compared to the negative control. This indicates that the low dose of the extract is not an adequate dose to produce ulcer healing. This model showed that the highest dose of the plant extract (HEFV 400 mg/kg) has got better antisecretory activity as evidenced by reduction in the mean volume of gastric secretion, rise in pH, and reduction in total acidity ($P<0.01$) compared to the negative control. Significant reduction in ulcer index (measure of ulcerated area) was noted for HEFV 200 mg/kg ($P<0.05$) and HEFV 400 mg/kg ($P<0.001$) as compared to the negative control.

Key words: Peptic ulcer, *F. vulgare,* antiulcer effect, Pylori ligation model

Introduction

Phytochemical screening and *in-vivo* screening of antiulcer activity on *Foeniculum vulgare* seeds. Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality. Epidemiological data for this disease and its complications have shown striking geographical variations in incidence and prevalence. Development of ulcer disease and death from it has been associated with the birth of urbanisation and was interpreted as a birth-cohort event with the peak of disease in those born during the late 19th century [1-2].

Our understanding of the disease changed greatly with the discovery of Campylobacter pyloridis (renamed Helicobacter pylori in 1989 because of a revised taxonomic classification) in 1982 by Warren and Marshall [3-4].

This discovery switched the notion from an acid-driven disease to an infectious disease, opening a huge area for intensive research that resulted in the reconciliation of previously suggested mechanisms of pathogenesis. The fall of the acid dogma in peptic ulcer disease, which had found its undisputed acceptance during and after the introduction of histamine H2-receptor antagonists, led to the present therapeutic principle. Maintenance acid suppressive therapy for duodenal ulcer, which followed decades of dominance of surgical interventions (subtotal gastric resections, several forms of vagotomy), was replaced with a short-term antibiotic regimen targeting eradication of *H. pylori* infection [5-6].

H. pylori eradication as cure of peptic ulcer received its full recognition when the Nobel Prize for Medicine and Physiology was awarded to Warren and Marshall in 2005. This recognition has not, however, closed the chapter on peptic ulcers. The management of ulcer disease and its complications remains a clinical challenge. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin are an increasingly important cause of ulcers and their complications even in *H. pylori*-negative patients. Other rare causes of ulcer disease in the absence of *H. pylori*, NSAIDs, and aspirin also exist.

NSAIDs, including low-dose aspirin, are the most important cause of ulcer complications in developed countries where prevalence of *H. pylori* infection is falling.

In patients who develop uncomplicated peptic ulcers while on NSAIDs, more than 90% of gastric or duodenal ulcers heal with 8 weeks of standard-dose H2-receptor antagonists (eg, ranitidine 150 mg twice a day), provided that NSAIDs are discontinued. [7] However, healing of gastric ulcers will be greatly impaired if patients continue to take NSAIDs. A descriptive review of head-to-head trials suggested that PPIs might be better than a standard dose of ranitidine in healing gastric ulcers in patients receiving continuous NSAIDs. [8]

Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a major burden for health care resources. Although potent anti-ulcer drugs are available, most of them produce several toxicities, thus emphasizing the need to search for new alternatives. As high as 80% of the world population depends on plant-derived medicines for the first line of primary health care reinforcing the theory that plant extracts can be good sources of new drugs. Ethiopia is a country characterized by a wide range of climatic and ecological conditions possessing enormous diversity of flora and fauna, including a wide range of potentially useful medicinal plants.

It is universally known as *Fennel* and is known by more than 100 names. It is a traditional and popular herb with a long history of use as a medicine. A series of studies showed that *F. vulgare* effectively controls numerous infectious disorders of bacterial, fungal, viral, mycobacterium, and protozoal origin. It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic activities. Some of the publications stated that *F. vulgare* has a special kind of memory-enhancing effect and can reduce stress. The aim of present study was to evaluate antiulcer effect of *F. vulgare.*

Extraction by maceration process

Defatted powdered of *Foeniculum vulgare* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C[9].

Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the

quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} x100$$

Phytochemical Screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods [10].

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [11].

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2%

$AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [11].

In vivo antiulcer activity of hydroalcoholic extract

Healthy adult Wistar albino rats of either sex were selected randomly for the study. Rats of 12–16 weeks, weighing 160–200 g, were used for the experiment. Each rat was housed in a plastic box cage under standard conditions at 19–25°C and was kept under 12/12 h light/dark cycle. The rats were allowed free access to standard pellet feed and water ad libitum. The study was carried out according to the CPCSEA and Organization of Economic Co-operation and Development (OECD) guidelines. Approval from Institutional animal ethical committee (IAEC) was also obtained[12].

Acute toxicity test

Acute toxicity study was carried out using the limit test dose of 2000 mg/kg as described by OECD 425 guideline [76]. Three female albino rats were fasted for 24 hours but allowed free access to water. A limit dose of 2000 mg/kg of Hydroalcoholic extract of *Foeniculum vulgare* was administered sequentially and animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after dosing continuously for 2 hours, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days[13].

Grouping and dosing of animals

Animals were randomly assigned to different groups each consisting of six rats. All treatments were given orally 1 hour before the experiment by oral gavage. Doses were determined based on the acute toxicity studies as per OECD guidelines.

Pylorus ligation model

For the single-dose study: Group 1 (negative control [NC], received distilled water), Group 2 (STD ie, positive control and received ranitidine 50 mg/kg), Group 3 (HEFV 100

ie, received HEFV 100 mg/kg), Group 4 (HEFV 200 ie, received HEFV 200 mg/kg), and Group 5 (HEFV 400 ie, received HEFV 400 mg/kg) [14].

Statistical analysis

Data were expressed as mean ± standard error of mean and statistically evaluated using one-way analysis of variance, followed by Tukey's multiple comparison tests. $P<0.05$ was considered to be significant.

Results and Discussion

Pyloric ligation-induced ulcer model is an important method for the measurement of mean ulcer index in ulcerogenesis. Gastric ulceration in this method may be the stressinduced secretion of HCl in excess amounts from the parietal cells and autodigestion of mucosa by the gastric juice. Free radicals may also be associated since studies have shown changes in the antioxidant status following pylorus ligationinduced ulceration in rats.

In the present study, different doses of the extract (100, 200, and 400 mg/kg) were evaluated for their effect on volume of gastric secretion, pH, total acidity, ulcer score, and ulcer index along with the standard drug ranitidine (50 mg/kg). In the single-dose study of the pylori ligation model, HEFV 100 mg/kg did not show any better activity when compared to the negative control.

This indicates that the low dose of the extract is not an adequate dose to produce ulcer healing. This model showed that the highest dose of the plant extract (HEFV 400 mg/kg) has got better antisecretory activity as evidenced by reduction in the mean volume of gastric secretion, rise in pH, and reduction in total acidity ($P<0.01$) compared to the negative control. Significant reduction in ulcer index (measure of ulcerated area) was noted for HEFV 200 mg/kg ($P<0.05$) and HEFV 400 mg/kg ($P<0.001$) as compared to the negative control.

While Ranitidine 50mg/kg has more antisecretory effect than the extract, HEFV 400mg/kg amazingly exhibited a better reduction of ulcer index than the standard drug (2.35±0.25 vs 2.72±0.66), which might be due to more combined cytoprotective and antisecretory activity effect of the extract than of the standard drug.

Table 1: % Yield of hydroalcoholic extract of seeds of *Foeniculum vulgare*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	2.84%
2.	Hydroalcoholic	7.52%

Table 2: Phytochemical screening of extract of *Foeniculum vulgare*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	+ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	**Glycosides**	
	Modified Borntrager's Test	-ve
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam Test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	-ve

Table 3: Estimation of total phenolic and flavonoids content of *Foeniculum vulgare*

S. No.	Hydroalcoholic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	***Foeniculum vulgare***	0.562	0.745

Table 4: Effect of HEFV and standard drug on gastric secretion in pylorus ligation-induced ulcer in rats

Groups	Volume of gastric secretion
NC	3.8±0.31
STD	2±0.31
HEFV100 mg/kg	3.84±0.86
HEFV200 mg/kg	2.83±0.22
HEFV400 mg/kg	2.24±0.16

Table 5: Effect of HEFV and standard drug on gastricacid pH in pylorus ligation-induced ulcer in rats

Groups	pH
NC	3.41±0.50
STD	5.72±0.76
HEFV100 mg/kg	3.21±0.30
HEFV200 mg/kg	4.01±0.27
HEFV400 mg/kg	5.08±0.76

Table 6: Effect of HEFV and standard drug on total acidity in pylorus ligation-induced ulcer in rats

Groups	Total acidity
NC	84.82±3.94
STD	52.91±8.12
HEFV100 mg/kg	85.07±3.32
HEFV200 mg/kg	72.16±4.55
HEFV400 mg/kg	55.15±3.35

Table 7: Effects of HEFV and standard drug in pylorus ligation-induced ulcer in rats

Groups	Ulcer score	Reduction in ulcer score (%)	Ulcer index	% Inhibition of ulceration
NC	5.75±0.955	-	5.32±0.62	-
STD	3.0±0.70	47.83	2.72±0.66	48.87
HEFV100 mg/kg	4.83±0.90	16.00	5.03±0.26	5.45
HEFV200 mg/kg	3.67±1.01	36.17	3.15±0.18	40.79
HEFV400 mg/kg	2.08±0.39	63.83	2.35±0.25	55.82

Notes: Each value represents the mean ± SEM for each group (n=6)

Conclusion

The findings in this study confirm the absence of oral acute toxicity at the doses employed, and presence of anti-ulcer pharmacologic activity of *Foeniculum vulgare*. Its efficacy is comparable to the standard drugs. Anti-ulcer effects may be related to antisecretory as well as cytoprotective activities of one or more of the identified phytochemicals. Thus, the present work validates the use of *Foeniculum vulgare* for

gastric ulcer in the Indian folk medicine, and further studies shall focus on isolation of specific phytochemicals and elucidating mechanisms of action.

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