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GASTROPROTECTIVE ACTIVITY OF THE AQUEOUS EXTRACT FROM THE ROOTS OF *DAUCUS CAROTA* L IN RATS

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Received: 15-07-2010; Revised: 22-08-2010; Accepted: 17-09-2010

ABSTRACT

Daucus carota L. (Apiaceae), is used in the traditional medicine for the treatment of gastric disorders viz., acidity and gastric ulcers. The present study was undertaken to determine the gastroprotective potential of the fresh juice extract of the roots of *Daucus carota.* The juice extract of the roots of *Daucus carota* (DCE) was tested orally at the dose of 200 and 400 mg/kg body weight, on gastric ulceration experimentally induced by pylorus ligation, aspirin and ethanol induced. The parameters considered to assess the anti-ulcer activity were volume of gastric secretion, pH, free acidity, total acidity, mucus content and ulcer index. The DCE at the dose of 200 and 400 mg/kg, significantly decreased gastric volume, free acidity, total acidity and ulcer index, while it increased the pH and the mucus content as compared with control. The DCE at a dose of 400 mg/kg produced 60.45, 56.80 and 43.51 % significant inhibition when gastric ulceration were induced by pylorus ligation, aspirin and ethanol, respectively. The DCE possesses gastroprotective property and the results supported traditional uses of the roots of this plant in the treatment of gastric ulcer and acidity.

KEYWORDS*: Daucus carota*, gastroprotective, anti-ulcer.

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INTRODUCTION

Consumption of vegetables can lower the incidence and mortality rates of many ailments viz., cancer, cardiovascular and cerebrovascular diseases[1], which may be due to vegetable antioxidants. Phenolic compounds account for a major portion of the antioxidant capacity in many plants. Carrots have been ranked 10th in nutritional value among 39 fruits and vegetables[2]. The research on carrot health benefits are continues[3]. Gastric ulcer is an illness that affects a considerable number of populations worldwide. The etiological factors of this disorder includes stress, smoking, nutritional deficiencies, infections, frequent and indiscriminate use of nonsteroidal anti-inflammatory drugs (NSAIDs)[4]. For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality. The well known pathophysiology of peptic ulcer has been centralized on an imbalance between aggressive (gastric acid secretion) and protective factors (gastric mucosal integrity) in the stomach and infection with *Helicobacter pylori*[5]. The modern drugs used in the treatment of gastric ulcers is based on the inhibition of gastric acid secretion by histamine receptors blockers, proton pump inhibitors or enhancing the mucosal production like prostaglandin analogous or certain antimicrobials like metronidazole, amoxicillin etc.[6]. However, some studies have revealed that reactive oxygen species and lipid peroxidation are implicated in the pathogenesis of ethanol-induced gastric lesions and they damage many biological molecules such as prostaglandins[7-9]. Therefore, treatment with antioxidants and free radical scavengers can be effective in gastric ulcers.

Daucus carota L. belongs to family Apiaceae, is an annual or biannual herb commonly known as carrot and mostly confined to the temperate regions of Europe, Asia and Africa[10]. The roots of *Daucus carota* has been traditionally used as a local stimulant for indolent ulcer[11]. Different parts of the carrot have been used in Indian traditional medicine for the treatment of kidney dysfunction, asthma, dropsy, inflammation, leprosy, worm troubles, etc.[12-14]. A decoction of carrot root has been considered as a remedy for jaundice in Europe[14]. Pharmacological studies revealed that the roots of carrot have hepatoprotective[15] and hypoglycemic activity[16]. The *Daucus carota* has been reported high content of vitamins B_1, B_2, C, D_2, E, nicotinic acid, flavonoids[17-18], β-carotene, α-carotene, γ-carotene, lycopene, cryptoxanthin, leutenin, many partly degraded carotenoids such as abscisic acid, trisporic acid, β-apo-carotenoids, e.g., violaxanthin[19-20].

The present study was undertaken to determine the gastroprotective potential of the aqueous extract from the roots of *Daucus carota* (DCE) using three experimental gastric ulcer models viz., pylorus ligation-, aspirin- and ethanol- induced gastric lesion.

MATERIALS AND METHODS

Plant material

The roots of *Daucus carota* L. was collected from the local market of Belgaum and identified by Dr. Harsha Hegde, Taxonomist, Regional Medical Research Centre (RMRC), Indian Council of Medical Research, Belgaum. A voucher specimen (RMRC-498) of the plant has been deposited at the herbarium of RMRC.

Preparation of the juice extract

The juice extract was prepared according to Bishayee *et al*.[15] with slight modifications. The fresh roots (1 kg) of *Daucus carota* were peeled, washed, cut into small pieces and homogenized in blender without adding water. The homogenate roots were squeezed and filtered through a cheese cloth to yield a residue (DCE). The obtained juice was lyophilized to get in powdered form. The powdered extract was stored in deep freezer at -20^{o}C for experimental use.

Animals

Rats were raised in colony cages and exposed to 12 h dark/light cycle. They were fed on standard rodent pellets diet (Gold Mohur food and feeds Ltd., Vikhroli, Mumbai) with water given *ad libitum*. Experiments were carried out on overnight starved Wistar male rats, aged 10-12 weeks and weighing 150-200 g.

Before the initiation of each experiment, care was taken to avoid coprophagy. The animals were divided in 12 groups of 6 animals in each separate model (pylorus ligation, aspirin and ethanol induced

gastric lesion). Group I- served as control (vehicle treated), Group II- served as standard (omeprazole-30 mg/kg), Group III - served as test group, treated with DCE 200 and 400 mg/kg. Ethical clearance was obtained from Institutional Animal Ethical Committee (627/02/a/CPCSEA).

Experimental procedure

Pylorus ligated ulcer technique

The pyloric ligation induced gastric ulceration method was carried out according to the method of Shay et al.[21]. Pylorus ligation was made 1 h after treatment. Four hours after the ligation the animals were sacrificed and stomach was removed. The gastric contents were collected, centrifuged at 3000 rpm for 30 min and the supernatant measured. The gastric volume, pH, free acidity and total acidity were estimated. The ulcer index and mucus content of the stomach were estimated[22,23]. The percentage inhibition of ulcer was calculated as:

% inhibition of ulcer = Mean ulcer index of control-Mean ulcer index of test/Mean ulcer index of control X 100

One milliliter of the total centrifuged gastric contents from each pylorus ligated rat was analyzed for hydrogen ion concentration by titration against a 0.01 N solution of NaOH using a pH meter (Alchemie) [24]. The experiment was carried out in triplicate (**Table 1).**

Aspirin induced gastric ulcer

Gastric ulcerations were induced experimentally in male Wistar rats according to the method of Asano *et al*.[25]. One hour after drug administration, each animal received orally, 200 mg/kg of aspirin. All animals were sacrificed 4 h later by overdose of ether anesthesia. The stomach was removed and various parameters of gastric juice (gastric volume, pH, free acidity and total acidity), ulcer index and mucus content were determined as described above. The experiment was carried out in triplicate (Table 2).

Ethanol-induced ulcer

Gastric ulcerations was induced using the method of Robert et al.,[26]. One hour after drug treatment, 1 ml/100 g body weight of absolute ethanol was given per oral to each rat. The animals were sacrificed 1 h later using an over dose of ether anesthesia followed by removal of stomach and various parameters of gastric juice (gastric volume, pH, free acidity and total acidity), ulcer index and mucus content were determined as described above. The experiment was carried out in triplicate (Table 3).

Statistical Analysis

Statistical analysis was performed using ANOVA followed by Dunnet's post hoc test and significance of difference between treatments was accepted at $P<0.01$, $P<0.05$. Data are expressed as Mean± SEM.

RESULTS

Pylorus ligation induced gastric ulceration

The results of gastric ulceration provoked by pylorus ligation are shown in Table 1. The DCE produced a significant dose dependent decreases in gastric volume with a maximum percentage of inhibition were 24.22 and 60.45 % at dose of 200 and 400 mg/kg, respectively. At the dose of 200 and 400 mg/kg of the DCE significantly and dose dependently increase the pH and mucus content of the gastric mucosa while decrease free acidity, total acidity and ulcer index.

Aspirin induced ulcer

As shown in Table 2, rats treated with the DCE at the doses of 200 and 400 mg/kg, exhibited inhibition percentage of 46.80 and 56.80 %, respectively. At the same above dose a significant dose dependent decrease in gastric volume, free acidity, total acidity, ulcer index, while increase the pH and mucus content.

Ethanol induced ulcer

Oral administration of ethanol produced gastric ulceration in rats treatment with DCE (200 and 400 mg/kg) produced a dose-dependent inhibition of gastric ulceration were 18.78 and 43.51 % while, animal treated with omeperazole at a dose of 30 mg/kg showed 58.38 % inhibition. The DCE produced a significant dose-dependent decrease in gastric volume, free acidity, total acidity and ulcer index while increase pH and mucus content.

DISCUSSION

There are several factors that may induce ulcer in human beings, such as stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion. Although in most cases the etiology of ulcer is unknown, it is accepted that it is result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism. There are many products in the market for the treatment of gastric ulcer, including antacids, proton pump inhibitors, anticholinergics and histamine H_2-antagonists. Most of these drugs produce several adverse reactions, such as gynecomastia, hematopoietic changes, acute interstitial nephritis[27], thrombocytopenia[28], anaphylaxis reactions[29], nephrotoxicity and hepatotoxicity[30]. The world health organization in 1980 has recommended the evaluation of the effectiveness of the plants in conditions where there is lack of safe synthetic drugs[31]. Thus, it is a need for more effective, less toxic and less expensive antiulcer agents. Medicinal plants are amongst the most attractive sources of new therapeutic agents, and have been shown to give promising results in the treatment of gastric ulcers[32]. The candidate for an effective drug against peptic ulcer should basically act either by reducing the aggressive factors on gastrointestinal mucosa or by increasing mucosal resistance against them[33]. Antisecretory activity was done by pylorus ligation. The causes of gastric damage after pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and /or stasis of acid. The increased accumulation of gastric acid and pepsin leads to auto digestion of the gastric mucosa[5]. Nonsteroidal anti-inflammatory drugs (NSAIDs), like aspirin and indomethacin, are known to induce ulcers during the course of anti-inflammatory therapy, by inhibiting prostaglandin synthesis through the cyclooxygenase pathway. In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and maintaining the mucosal cell turnover and repair. Thus, the suppression of prostaglandin (PG) synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastric ulceration. It was observed that *Daucus carota* L. displayed significant reduction in mucosal damage against aspirin induced ulceration rat model.

Ethanol produces mucosal damage by severe gastric hemorrhagic erosions. The genesis of ethanol induced gastric lesion is multifactorial with the depletion of gastric or mucus content as one of the involved factors and this damage induced by ethanol may be due to mucosal leukotriene release. Mucosal blood flow has also been attributed to be an important factor in the damage caused by alcohol and is modulated by PG. Submucosal venular constriction by ethanol and eventual injury is caused due to perturbation of superficial mucosal cells. Ethanol induced damage to the gastric mucosa is associated with a significant production of free radicals leading to an increased lipid peroxidation and damage to the cell and cell membranes. Accumulation of activated neutrophils in the gastric mucosa may be a source of free radicals. It was observed that *Daucus carota* L. displayed significant reduction in mucosal damage of ethanol induced ulcer in rat model.

Many plants containing flavonoids have been reported to possess anti-ulcerogenic activity. Several mechanisms have been proposed to explain their biological effects; including increase of mucosal PG content, decrease of histamine secretion from mast cells, inhibition of acid secretion and inhibition of *H. pylori* growth**.** In addition, flavonoids are free radical scavengers that are known to play an important role in ulcerative and erosive lesion of the gastrointestinal tract[34,35].

Therefore, as reported the presence of antioxidant, flavonoids and other bioactive compounds in *Daucus carota* may be associated with the gastroprotective effect. The *Daucus carota* possess

antioxidants could play a protective role of gastric mucosa from free radical induced damage and acts as antiulcer action.

CONCLUSION

In conclusion the results obtained from the present study demonstrated that *Daucus carota* roots extract has antiulcer and gastroprotective activity. This supports the traditional use of *Daucus carota* roots in the treatment of gastric ulcer. *Daucus carota* roots may be a new alternative remedy for clinical management of gastric ulcer diseases. However, it is difficult to explain the exact mechanism of action underlying. Therefore, further study is needed to explain the anti-ulcer activity of the *Daucus carota* roots.

ACKNOWLEDGEMENT

The authors are thankful to the Principal Dr. F.V. Manvi and Vice Principal Prof. A. D. Taranalli, K L E College of pharmacy Belgaum India, for the support and constant encouragement.

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Table 1: Effect of treatments on various parameters of pylorus ligated rat model

Treatment	Dose	Gastric volume (ml)	pH	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)	Mucous content (abs/g of tissue)	Ulcer index	% Inhibition
Control	-	10.72±0.22	2.50±2.22	77.50±2.79	154.3±5.97	0.112±0.004	4.83±0.33	-
Omeprazole	30(mg/kg)	6.93±0.23**	4.46±0.19**	33.33±2.44**	69.17±4.30**	0.255±0.011**	0.75±0.17**	84.47
DCE	200 (mg/kg)	9.06±0.19	2.70±0.06	53.00±1.23**	108.8±3.04**	0.162±0.004**	3.66±0.27**	24.22
	400 (mg/kg)	8.51±0.09**	3.30±0.08**	45.67±0.76**	88.33±1.82**	0.198±0.007	1.91±0.30**	60.45

All values are expressed as mean ± S.E.M. for six animals in each group ** P<0.01, * P<0.05.

Table 2: Effect of treatments on various parameters of aspirin induced rat model

Treatment	Dose	Gastric volume (ml)	pH	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)	Mucous content (abs/g of tissue)	Ulcer index	% Inhibition
Control	-	1.51±0.11	2.63±0.07	71.33±1.35	147.0±3.86	0.141±0.002	5.00±0.28	-
Omeprazole	30(mg/kg)	0.80±0.12**	3.59±0.15**	54.17±1.85**	117.8±2.86**	0.302±0.012**	1.00±0.18**	80
DCE	200 (mg/kg)	1.11±0.04	2.74±0.03	67.33±0.88	143.2±1.07	0.176±0.003	2.66±0.27**	46.80
	400 (mg/kg)	0.98±0.06	2.90±0.04	63.33±1.28**	136.3±0.71	0.222±0.006**	2.16±0.24**	56.80

All values are expressed as mean ± S.E.M. for six animals in each group ** P<0.01, * P<0.05.

Table 3: Effect of treatments on various parameters of ethanol induced rat model

Treatment	Dose	Gastric volume (ml)	pH	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)	Mucous content (abs/g of tissue)	Ulcer index	% Inhibition
Control	-	2.45±0.09**	1.89±0.12	74.83±2.12	1.56±5.30	0.152±0.006	8.41±0.32	-
Omeprazole	30(mg/kg)	1.73±0.10**	3.43±0.10**	58.33±2.31**	119.8±3.42**	0.342±0.016**	3.50±0.28**	58.38
DCE	200 (mg/kg)	1.90±0.07	2.15±0.05	72.17±1.04	149.3±2.47	0.208±0.007	6.83±0.33	18.78
	400 (mg/kg)	1.83±0.04**	2.83±0.05**	68.67±0.84	139.0±1.18	0.256±0.006**	4.75±0.21**	43.51

All values are expressed as mean ± S.E.M. for six animals in each group ** P<0.01, * P<0.05.

Source of support: Nil, Conflict of interest: None Declared