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Pharmacological Studies on 'Clove' *Eugenia caryophyllata*

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Abstract

An aqueous suspension of clove, *Eugenia caryophyllata* (Spreng.) (Myrtaceae) was tested for its ability to inhibit basal gastric secretion and to protect gastric mucosa against the injuries caused by pyloric ligation, indomethacin and cytodestructive agents (80% ethanol, 0.2 M NaOH and 25% NaCl) in rats. The suspension in doses of 250 and 500 mg/kg body weight had a significant antiulcerogenic activity on the models used. Besides, ethanol-induced depleted non-protein sulfhydryl (NP-SH) and gastric wall mucus contents were replenished by pretreatment with clove suspension. No significant histopathological changes are noted. In acute, chronic and genotoxicity tests clove suspension showed a large margin of safety in the animals and did not induce any significant changes in the micronucleated polychromatic erythrocytes (PCE) and the ratio of PCE and normochromatic erythrocytes (NCE) at both doses used, as compared to control group, suggesting lack of any significant clastogenic and/or cytotoxic potentials.

Keywords : *Eugenia caryophyllata*, anti-ulcerogenic, Genotoxicity, micronucleated polychromatic erythrocytes, clastogenic, cytotoxic.

Introduction

Clove consists of buds of *Eugenia caryophyllata* (Spreng.) Sprague, locally known as Quranful, family Myrtaceae. They are used as a spice, condiments, relishes and gravies [1-3]. In Pharmaceuticals, clove bud oil is used for its symptomatic relief of toothache [2]. In cosmetics, clove bud oil are used extensively as fragrance components in detergents, soaps, tooth pastes, creams, lotions and perfumes [4]. It is also an important ingredient of 'Arabian Gahwa' (Arabian coffee).

In traditional medicine of many countries, clove has been used as carminative and occasionally used in the treatment of flatulent colic, anti-emetic, toothache remedy and as a counter irritant. Clove has many medicinal virtues, they are stimulant, and useful in counteracting spasmodic disorders and in relieving flatulence, promote digestion and in dyspepsia. In Arabian and Indian traditional medicine, it is used in the form of a powder or a decoction made from them for the treatment of gastric ailments. Clove oil, applied externally, has stimulating effects on the skin, producing heat and redness [5].

The principal constituents of clove are volatile oils, which contains eugenol, eugenol acetate and caryophellene [2,3]. Earlier the presence of myrietin, gallic acid, ellagic acid, kaempferol were reported in the clove [6,7]. Clove oil has been found to be effective in growth inhibition against gram-negative anaerobic pathogens and said to possess anthelmentic and larvicidal properties [3,8]. Therefore, the present investigation was carried out to evaluate the effect of an aqueous suspension of clove on gastric acid secretion, necrotizing agents and indomethacin-induced gastric ulcers and the effects on its acute and prolong consumption in experimental animals, besides genotoxic potential in mice.

Materials and methods

Plant material

Dried fruits of Clove were purchased from local market in Riyadh, identified by our taxonomist Dr. Atiqur Rahman (College of pharmacy, King Saud University). A voucher specimen was preserved at the herbarium of College of Pharmacy for future reference. The dried fruits were ground to a very fine powder in a Sanyo electric grinder and the suspension was made with water. The aqueous suspension was used for treatment in different experiments.

Animals

Male Wistar albino rats, aged 8-10 weeks, weighing about 150-200 g. were obtained from the Experimental Animal Care Centre, King Saud University, Saudi Arabia. The animals were maintained under standard conditions of temperature (24 ± 2), humidity (60%) and light (12 hr dark, 12 hr light). They were provided with Purina chow and free access to water. Before testing, the animals were fasted for 36 hours with access to water ad libitum. The experimental protocols were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, in accordance with the guide to the care and use of exzperimental animals (Canadian Council) (9).

Dose selection and route of administration

The doses (250 and 500 mg/kg., body weight) selected for the conduct of the experiments were based on preliminary experiments conducted .on the pharmacological activity of Clove. The route of administration of the aqueous (water) suspension was oral (gastric intubation) in all the experiments.

Gastric lesions induced by necrotizing agents

The animals in the test groups were orally administered 1 ml per rat of different necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl), which are known to produce gastric lesions (10). NaCl (25%) and NaOH (0.2 M) were used only in cytoprotection studies. Based on the gastric emptying in fasted rats, Clove was given 30 min before the necrotizing agents. Animals were sacrificed under ether anesthesia 1 hr after treatment with ulcerogenic agents. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a binocular magnifier. The ulcers were scored according to the method of Valcavi et al., (11) and assessed on the basis of their cirumference: Deep circular more than 8 mm = 9-10; 7-8 mm = 8; 6-7 mm = 7; (if a ulcer is 6 mm then it get a score of 7 or 6) 5-6 mm = 6; 4-5 mm = 5; 3-4 mm = 4; 2-3 mm = 3; 1-2 mm = 2 and 0-1 mm = 1. The deep linear ulcer more than 10 mm in length = 6 and linear ulcer less than 10 mm in length = 3. The score for each single lesion were than summed up for the determination of ulcer index.

Gastric wall mucus determination

The modified procedure of Corne et al. (12) was used to determine gastric-wall mucus. The glandular segments from the stomach was removed and weighed. Each segment was transferred immediately to 1% Alcian blue solution (in 0.25 M sucrose solution), buffered with sodium acetate pH 5), and the excess dye was removed by rinsing with sucrose solution. The dye complexed with the gastric wall mucus was extracted with magnesium chloride solution (0.5 M). A 4-ml aliquot of blue extract was then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 RPM for 10 minutes and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted/g (net) of glandular tissue was then calculated.

Estimation of Nonprotein Sulfhydryl groups

Gastric mucosal (NP-SH) was measured according to the method of Sedlak and Lindsay, (13) to analyze the oxidant/antioxidant balance. The glandular stomach was removed and homogenized in ice-cold 0.02M ethylenediaminetetraacetic acid. The homogenate was

mixed with distilled water and 50% (w/v) aqueous TCA and centrifuged at 3000 RPM for 15 minutes; the supernatant was mixed with 0.1 ml of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) (pH 8) was added and the sample was vortexed (speed of 2) at room temperature. The absorbance was read within 5 min of addition of DTNB, at 412 nm, against a reagent blank with no homogenates.

Histopathological assessment

The gastric tissue samples were fixed in neutral buffered formalin. The fixed tissues were subjected to treatment in a VIP automatic processing machine. This processing includes (i) fixation in 10% neutral buffered formalin (ii) dehydratiuon through graded alcohol (70%, 95% and absolute) (iii) clearing through xylene (iv) impregnation in paraffin wax and finally (v) embedding in paraffin blocks. After these procedures are completed in the VIP processor, the tissues in wax blocks are taken for section cutting in an American optical rotary microtome. The chosen section size was of thickness 5 μm. These sections were stained with haematoxylin and eosin using standard procedures (14). The slides were then examined under a microscope for morphological changes, such as congestion, hemorrhage, edema and erosions using an arbitrary scale (- = Normal; + = Moderate effect; ++ = Severe effect; +++ = Intensely severe effect) for the assessment of severity of these changes.

Determination of Anti-secretary activity

The method of Shay et al. (15) was used to determine the anti-secretory activity. The animals were fasted for 36 hr with free access to water. Ligation of the pylorus was done under light ether anaesthesia. Care was taken not to bleed or occlude the blood vessels. Aqueous suspension of Clove was administered intraduodenally, immediately after pylorus ligation. The animals were sacrificed, 6 hour after the pylorus ligation. The stomach was removed, contents collected, volume of contents measured, centrifuged at a speed of 2500 RPM for 10 minutes. The pH of supernatant was adjusted to 7 by addition of 0.01 N sodium hydroxide. The titrable acidity is calculated by the following formula:

Titrable acidity = Sodium hydroxide consumed x 100 ÷ Volume of gastric juice

Gastric lesions induced by indomethacin

Indomethacin was suspended in 1% carboxy-methylcellulose in water (6 mg/ml) and administered orally to the fasted rats at a dose of 30 mg/kg. body weight (0.5 ml/100 g). Control rats were treated similarly with an equivalent volume of the vehicle (16). The animals were sacrificed 5 hr after the treatment (11). The stomach of the animals were removed, rinsed with normal saline, and studied.

Statistical Analysis

The results are expressed as mean ± SEM. The mean determination of treatment groups were statistically analysed by One way ANOVA and Post hoc Tukey-Kramer multiple comparisons.

Results

An aqueous suspension of clove significantly inhibited gastric secretary volume, acidity and the intensity of shay ulcers in both the doses groups (Table 1). Administration of an aqueous suspension of clove resulted in significant reduction of gastric ulceration induced by indomethacin in dose-dependent manner (Table 2).

A significant depletion of gastric wall mucus was observed in animals treated with 80% ethanol alone as compared to control rats. Pretreatment of rats with clove suspension significantly increase the wall mucus content in high dose (500 mg/kg. Body weight) group (Table 3).

Table 4 shows that the aqueous suspension of clove has a protective effect on gastric mucosal damage induced by various necrotizing agents.

Animals treated with 80% ethanol alone showed a significantly lowered level of (Non-protein sulphydryl) NP-SH content in the stomach. Pretreatment of rats with clove significantly replenish the NP-SH content in higher dose (500 mg/kg.) group (Table 5).

On isolated guinea pig ileum preparation, the suspension of clove produced no direct effect on the preparation. However, the clove suspensionantagonized the acetylcholine induced contraction of the muscle.

Discussion

The findings of the present study demonstrate that aqueous suspension of clove possesses gastric antisecretory, as well as mucosal protective activity.

In pylorus-ligated rats, the intraperitoneal administration of aqueous suspension of clove, at doses used, produced a significant decrease of basal gastric secretion volume titratable acidity and ulceration. The reduced acid output, measured after pylorus ligation, suggest that the protective mechanism of the clove suspension on gastric mucosa might involve an inhibition of gastric secretion [17].

A role of gastric acid secretion in indomethacin-induced gastric injury was proposed [18]; they observed that administration of antisecretory drugs like cholernergic and histamine H_2 receptor blocking agents markedly inhibited the formation of indomethacin-induced gastric lesions. In an earlier investigation [19], indomethacin potentiated the stress-, 2-deoxy-D-glucose-, and vagal-stimulated gastric secretion, and produced severe gastric lesions. Later, it was also found that indomethacin caused an increase in stress-induced ulceration and gastric acid secretion [20]. These findings suggested that indomethacin-induced gastric lesions are related to a significant increase in the acidity of the gastric juice. In the present study, the oral administration of clove suspension significantly protected the gastric mucosa of rats against

Table 1: *Effect of aqueous suspension of clove on the volume of gastric secretion, titratable acidity and the degree of ulceration in 6-hr pylorus ligated (Shay) rats.*

Treatment	Dose (mg/kg, i.p.)	Mean ± .S.E.		
		Volume of gastric content (ml)	Titratable acid (mEq/l)	Ulcer index
Control	–	8.41±0.40	122.77±3.98	0.83±0.16
Clove	250	3.66±0.77***	93.88±3.59***	00***
Clove	500	2.08±0.24***	72.77±5.92***	00***

(n=6) , ***P < 0.001. Student's *t*-test.

Table 2: *Effect of aqueous suspension of clove on the gastric ulcers induced by indomethacin in rats.*

Treatment	Dose (mg/kg, p.o.)	Ulcer index (Mean ± S.E.)
Control (Indo. only)	–	41.00±1.65
Clove + Indo.	250	29.83±3.07**
Clove + Indo.	500	26.00±1.37***

(n=6), **P < 0.01; ***P < 0.001. Student's *t*-test.

Table 3: *Effect of aqueous suspension of clove on 80% ethanol-induced gastric wall mucus changes in rats.*

Group	Dose (mg/kg, p.o.)	Gastric wall mucus µg Alcian blue of wet glandular tissue
Control	–	490.49±19.88
80% ethanol only.	–	289.05±7.58***[a]
Clove + 80% ethanol	250	321.58±17.70[b]
	500	367.16±18.85***[b]

[a]As compared to the control group. [b]As compared to the 80% ethanol-treated group.
(n=6), ***P < 0.001. Student's *t*-test.

Table 4: *Effect of aqueous suspension of clove on the gastric lesions induced by various necrotizing agents in rats.*

Treatment	Dose (mg/kg, p.o.)	Ulcer index (Mean ± S.E.)		
		80% EtOH	0.2M NaOH	25% NaCl
Control	–	7.33±0.33	7.83±0.16	7.50±0.34
Clove	250	1.50±0.22***	1.00±0.25***	3.00±0.89***
Clove	500	1.00±0.81***	0.33±0.21***	1.33±0.33***

(n= 6) ***P < 0.001. Student's *t*-test.

Table 5: *Effect of aqueous suspension of clove on nonprotein-sulfhydryl (NP-SH) concentration in gastric tissue of rats treated with 80% ethanol.*

Sl. No.	Treatment and dose (mg/kg, body weight)	NP-SH concentration (µmol/100 mg wet tissue) Mean ± S.E.
1	Control (distilled water, 1 ml/rat).	8.59±0.33
2	Control (80% ethanol, 1 ml/rat)	6.27±0.51[a]*
3	Clove (250) + 80% ethanol (1 ml/rat)	7.13±0.63[b]
4	Clove (500) + 80% ethanol (1 ml/rat)	10.03±0.49[b]**

(n=6), [a] = as compared to control (distilled water) group., [b] = as compared to control (80% ethanol) treated group., *P < 0.05; **P < 0.01. Student's *t*-test.

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Table 6: *Effect of aqueous suspension of clove on 80% ethanol-induced histopathological lesions in gastric mucosa of rats.*

Groups	Treatment and dose (mg/kg body weight)	Histopathological Lesions							
		Congestion	Haemorrhage	Edema	Necrosis	Inflammatory changes	Dysplastic changes	Erosions	Ulceration
1	Control (distilled water) (1 ml/rat).	–	–	–	–	–	–	–	–
2	Ethanol, 80% (1 ml/rat)	+++	++	+	+	+	+	++	++
3	Clove + 80% ethanol	–	–	–	–	–	–	–	–
4	Clove+ 80% ethanol	–	–	–	–	–	–	–	–

– = Normal; + = moderate effect; ++ = severe effect; +++ = intensely severe effect.

indomethacin-induced damage. These results suggest that the antiulcer activity of the suspension against indomethacin injury might be related to its antisecretory effect, as an antisecretory drug ranitidine has been reported to protect gastric mucosa, injured by indomethacin [21]. Moreover, the pathogenesis of gastric damage induced by non-steroidal anti-inflammatory drugs (NSAIDs) involve multiple elements, such as deficiency in prostaglandins (PGE_2), gastric hypermotility and acid secretion [22]. The observed gastroprotective activity of clove suspension can not be strictly related to inhibition of gastric secretion, but can be partly explained through a prostaglandin-dependent mechanism [23].

In our study, the aqueous suspension of clove significantly reduced the formation of gastric ulcer induced by ethanol, sodium hydroxide and sodium chloride in rats. These noxious chemical-induced ulcer model is commonly used for screening of antiulcer agents, but although extensive studies on the pathophysiology of the acute gastric mucosal lesions have been carried out, the pathogenesis of the mucosal lesions is not fully understood [24]. The possible mechanism(s) for antiulcer effects suggested are antisecretory activity on pepsin and acid, mucosal protection by increased mucus synthesis, prostaglandin level, protective coating and radical scavenging [25,26]. Furthermore, ethanol caused a significant reduction in non-protein sulfhydryl concentration and depletion of gastric wall mucus contents. Pretreatment of rats with clove suspension showed a significant replenishing effect on the NP-SH concentration and preventing the decreased level of wall mucus contents. Thus, NPSH seemed to be involved in possible gastroprotection mechanism [27,28].

Clove contains a number of chemical components, including eugenol which is the principal constituent of its oil [2,3]. The clove suspension shown to increase the gastric NP-SH level; it is well established that eugenol increases the level of glutathione in the liver [29]. Furthermore, eugenol was also found to inhibit lipid peroxidation [30]; and some other reports also suggest its free radical scavenging as antioxidant potential [31]. Eugenol has also been shown to have antioxidant activity in human beings [32].

The results on histopathological investigation on the gastric mucosa of rats revealed the pretreatment with clove suspension absolutely inhibited the ethanol-induced congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulceration. Our results are in corroboration with the anti-gastric ulcer activities of the aqueous suspension observed under the studies on pharmacological and biochemical evaluation. On acute toxicity, the clove suspension in low dose exerted mild excitation in mice. A typical feature observed in the animals of LD_{50} (4.5 g/kg body weight) group was the induction of comatose condition in mice. The prolonged (12 weeks) treatment with clove suspension was found to increase significantly the body weight in both doses used (250 and 500 mg/kg). The rate of mortality was not affected; only 10 percent mortality was recorded in male mice. The effect of clove on body weight and its lack of effects on mortality appear to be related to its antioxidant property [33].

In conclusion, it appears that clove suspension possesses antisecretory and antiulcerogenic principles which protect gastric mucosa by various mechanisms. Further studies are required to identify the antiulcer component(s) to elucidate their mechanism(s) of action to substantiate its use in Indian and Arabian traditional medicine for various gastric ailments.

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