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Anti Ulcerogenic Effect of the Rhizomes of *Zingiber officinale* Against Ethanol Induced Gastric Ulcers in Rats

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Abstract: The effects of methanolic extract of the rhizomes of *Zingiber officinale* were studied in rats for their ability to inhibit gastric lesions induced by ethanol. Animals pre-treated with ginger root extract significantly inhibited gastric lesions compared to control rats. The root extract at a dose of 1000 mg kg^{-1} orally exert highly significant cytoprotection against ethanol-induced gastric lesions compared to 500 mg kg^{-1}. This cytoprotection was accompanied with increase in mucus synthesis by gastric mucosa grossly when compared with the control rats. These observations strongly suggest cytoprotective effect of the ginger extract against ethanol-induced gastric ulcer in rats.

Key words: Gastric ulcer, *Zingiber officnale*, cimetidine

INTRODUCTION

Zingiber officinale Roscoe (ginger, Zingiberaceae) is one of the most widely used spices and it is a common additive in a large number of compounded foods and beverages due to its flavor and pungency. Ginger root, the rhizome of *Z. officinale*, is one of the most commonly used medicinal herbs as well as one of the most commonly used condiments in Chinese cuisine. Folk people have long used the soup of ginger root to warm the human body in winter. Though spicy and hot in nature, the rhizome of *Z. officinale*, has been used to treat symptoms and signs including pale feature, cold extremities, weak and tardy pulse and weak physical status after blood loss in some Chinese medicinal regimens. Several pharmacological effects of the Zingiber plant had been reported, such as anti-ulcer effect[1,2], analgesic effect[3], hypoglycemic effect[4] inhibitory effect on cholesterol biosynthesis[5], antioxidant effect[6], apoptosis effect[7], potential chemoprotective properties[8], potent antibacterial activity[9], anti-inflammatory[10], potent anti-fungal activity[11] anti-platelet activity[12] and broad spectrum antiemitic[13]. In the present study, the anti-ulcer activity of ginger was investigated using methanol rhizome extract of *Z. officinale* on ethanol-induced ulcer in rats.

MATERIALS AND METHODS

Collection of plant materials: The rhizome of *Z. officinale* were purchased from the local market and identified by comparison with specimens available at the Herbarium of the Forest Research Institute, Kepong, Malaysia. Voucher specimens of the ginger roots are deposited in the Department of Pharmacy, University of Malaya, Malaysia. The roots were cut, washed with distilled water and dried in an oven at 50°C for 5- 7 days until fully dried. The dried roots were ground into powder by using a grinder and stored at 4°C.

Preparation of plants extracts: The dried powdered ginger rhizomes were extracted by maceration in methanol (100 g/1500 mL) in a conical flask for 5 days at 37°C. Afterwards, the solvents were filtered using filter paper and the solvents were dried under reduced pressure in an EYELA rotary evaporator. The extract was then submitted to lyophilization using a freeze-dryer to produce powdered forms of the extract. 500 mg and 1000 mg extract were suspended in vehicle (Tween 80, 10% v/v) at a concentration of 100mg mL^{-1} and 200 mg mL^{-1}, respectively.

Cimetidine: The reference anti-ulcer drug, cimetidine, was obtained from University Malaya Medical Centre (UMMC). Each tablet weighed 200 mg. The tablet was ground to powder and suspended in vehicle (Tween 80, 10% v/v) at a concentration of 20mg mL^{-1}, thoroughly mixed and administered to each animal (100 mg kg^{-1} body weight) at an amount of 1 mL/animal orally. Tween 80, 10% v/v in distilled water was used as vehicle for dosing in all the experimental animals.

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Table 1: Effects of *Z. officinale* root extracts on ethanol-induced gastric lesions in rats

Groups	Pre-treatment	Oral dosage mL kg^{-1}	Ulcer area (mm2) (Mean±S.E. M)	Protection %
1	Tween 80 (control)	5 ml kg^{-1}	885.00±25.38	-
2	*Z. officinale* 500 mg kg^{-1}	5 ml kg^{-1}	187±4.16*	78.87%
3	*Z. officinale* 1000 mg kg^{-1}	5 ml kg^{-1}	85.5±6.48**	90.33%
4	Cimetidine 100 mg kg^{-1}	5 ml kg^{-1}	355?14.94***	59.89%

*p<0.05 significantly from control (Group 1) **p<0.05 significantly from control (Group 1) and Group 2 ***p<0.05 significantly from control (Group 1) Group 2 and Group 3

Experimental animals: *Sprague Dawley* adult male rats were obtained from the animal house, Faculty of Medicine, University of Malaya. The rats were divided randomly into 4 groups of 6 rats each. Each rat weighed between 180-220 gm. The animals were placed individually in cages (Bollman cages) with wide-mesh wire bottoms to prevent coprophagy. The animals were left for 48 h to acclimatize to the animal room conditions and were maintained on standard pellet diet and tap water.

Effect of extract on gastric ulcer induced by ethanol in rats: All rats were fasted for 48 h before the experiment but excess water was allowed and just two hours before starting the experiment the water also were removed. Group I was fed with the vehicle (Tween 80, 10% v/v) at a volume of 5 mL kg^{-1} by orogastric intubations whereas treated animals in Groups II and III received extract at doses of 500 mg kg^{-1} and 1000 mg kg^{-1}, respectively by orogastric intubations (suspended in vehicle) in the same volume. Group IV received 100 mg kg^{-1} cimetidine by same rout (suspended in vehicle) in the same volume. Thirty minutes after their pretreatment, all animals were fed with absolute ethanol (5 mL kg^{-1}). They were sacrificed 30 min later by exposing to diethyl ether and their stomachs rapidly removed and fixed in 10% buffered formalin.

Gross gastric lesions evaluation: Each stomach was opened along the greater curvature, rinsed in ice-cold PBS and fixed with 10% formalin and examined macroscopically for gastric damage. The length (mm) and the width (mm) of the ulcer on the gastric mucosa were measured by planmeter square (10 X 10 mm) under a dissecting microscope (20x). The Ulcer Area (UA) was calculated as described by Kauffman and Grossman[14]. The total ulcer area (mm^2) of each stomach was recorded and the % protection was calculated as follow.

% Protective = UA control –(UA treatment)/UA control X 100

Histological examination : Stomach biopsies were processed and assessed for damage by taking a 5µm section, stained with Hematoxylin and Eosin were analyzed under light microscopy.

Statistical analysis of data: Results were expressed as mean±M.S.E. The statistical difference between the groups in the term of the mean rate of wound healing was calculated by using Student's t-test

RESULTS

Grossly, the results of the current study showed that pretreated rats with root extracts significantly reduced the formation of gastric ulcer induced by absolute ethanol compared to animals pretreated with vehicle and administered absolute ethanol (Table 1, Fig. 1 and 2). Also animals pretreated with root extract significantly reduced the gastric lesion compared to rats pretreated with cimetidine (Table 1). Cytoprotection were significantly higher in animals pretreated with 1000 mg kg^{-1} root extract than 500 mg kg^{-1}. Histologically, rats pretreated with root extracts also significantly inhibited the gastric lesions formation and submucosal edema, induced by absolute ethanol compared to control animals. Animals pretreated with root extract significantly inhibit the formation of gastric lesions and submucosal edema compared to animals pretreated with cimetidine (Table 1).

DISCUSSION

The present results demonstrate that the methanolic extract of *Z. officinale* protect the rat gastric

Fig. 1: Sever macscopic necrosis of gasric mucosa Gastric mucosal damage caused by absolute ethanol. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa in control group

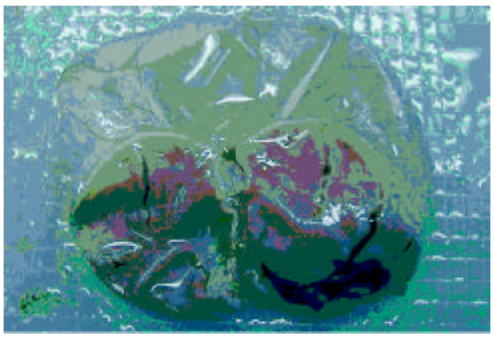

Fig. 2: Mild macroscopic necrosis of gastric mucosa Cytoprotection of gastric mucosa against absolute ethanol. Methanolic extract (1000 mg kg^{-1}) reduce the formation of gastric lesions induced by absolute ethanol

mucosa against hemorrhagic lesions produced by absolute ethanol compared with control. Grossly this cytoprotective effect was associated with increase mucus secretion adheres to gastric mucosa. Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Ethanol-induced gastric ulcer has been widely used for the experimental evaluation of anti-ulcer activity. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus[15]. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol[16]. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury[17]. Absolute ethanol produces a marked contraction of the circular muscle of rats fundus strip. Such contraction can lead to mucosal compression at the site of the greatest mechanical stress, i.e. at the crest of mucosal folds leading to necrosis and ulceration[18].

As ginger contains a number of coactive constituents, which might be potentially useful in the treatment of various diseases including gastric ulcer[13,19]. Similarly, oral acetone extract of zingiberene and 6-gingerol significantly inhibited HCl/ethanol-induced gastric lesions[20]. Ginger extract prevented the occurrence of gastric ulcers induced by non-steroidal anti-inflammatory drugs and hypothermic restraint stress and exerted highly significant cytoprotection against 80% ethanol and 25% NaCl[21].

Ginger, a pungent stomachic natural medicine and condiment, contain 6-gingerol, a pungent principle, which has already been, reported to increase the secretion of bile as one of its effects on digestive tract function[21]. Methanolic root extract protect gastric damage. It is likely that antioxidant compound present in this root extract, may be responsible for gastroprotection[6]. It has been shown that drugs, which are effective against ethanol-induced gastric lesions, can possess gastric mucosal membrane protective actions. Gefarnate, which are terpenoids, are known as anti-ulcer drugs. There are relatively large amounts of volatile oils belonging to the terpenoids, such as zingiberine, found in the species of ginger, one of the pungent stomachics. Ginger terpenoids may be regarded as important protective against gastric lesions, thus supporting the use of ginger as natural stomachic medicine[20].

Grossly, the result of the present study showed that animals pretreated with methanolic extract the gastric mucosa secrete a layer of mucus that adheres to its surface and protect them from necrotizing agent. Similarly, the gastrodudenal mucosa secret a layer of water-insoluble mucus gel that adheres to its surface. This adherent mucus layer is considered to act as a protective barrier against the endogenous and exogenous damaging agents[23]. Gastric mucus also provides protection by scavenging oxidants produced in the gastric lumen[24]. Substances that increase the synthesis and secretion of gastric mucus or enhance the mucus gel qualities have been demonstrated to have the effect of cytoprotection [23]. In this study, we find the methanolic extract of ginger significantly inhibit gastric lesion, which is associated with increase in mucus layer in the gastric mucosa. That indicates the enhancement of the mucus modulation by ethanolic ginger extract play significant role in its potentiating effect on gastric cytoprotection. In conclusion, the anti-ulcer effects of methanolic extract of *Z. officinale* appeared to have several important properties that make it useful ideal as a remedy for anti-ulcer. We can suggest that it may be possible to use the root extract as remedy to prevent ulcers. However, further investigations are required to elucidate their exact mechanism (s) of anti-ulcer activity.

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