

The Gastro-Protective Effect of *Ulmus Rubra*, *Piper Nigrum*, and *Brassica Oleracea* in Ethanol-Induced Model of Gastric Ulcer

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ABSTRACT

Background: Gastric ulcer is a state that results from an imbalance between the erosive effect of acid and pepsin and the mucosal defense mechanism of the stomach; it is correlated with the lesions induced by ethanol.

Methods: Forty-eight healthy albino rats weighing (150-200gm) were involved in the present study. The animals withholding food for 24hrs were allocated to six groups. Each group was given orally one of the following agents: distilled water for the control group, esomeprazole, aqueous extract of *Ulmus rubra* or *Piper nigrum*, or *Brassica oleracea*, then after one hour ethanol was given to all groups except group 1, which act as the control. Animals were sacrificed one hour later; the main parameters used in the present study were ulcer numbers and areas, preventive index, and changes in the trace elements (Zn and Cu) and serum electrolytes (Na⁺ and K⁺).

Results: Ethanol was found to be highly ulcerogenic at high concentrations. The preventive index of these agents was 90%, 47.7%, and 91.8% for *Ulmus rubra*, *Piper nigrum*, and *Brassica oleracea*, respectively, in comparison with esomeprazole 94.7%. These agents also produced significant changes in trace elements levels of Zn and Cu but with no significant changes in electrolytes Na⁺ and K⁺.

Conclusion: Both *Ulmus rubra* and *Brassica oleracea* extracts proved to have a potent gastroprotective effect against the ethanol-induced model of gastric ulcer and the possibility to be used in the management of peptic ulcer after assessment by clinical trials.

KEYWORDS: Aqueous extract of *Ulmus rubra*, *Piper nigrum*, *Brassica oleracea*, Peptic ulcer, Ethanol 95%, Gastroprotection.

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INTRODUCTION

A peptic ulcer is a state that results when the caustic effects of aggressive factors (acid, pepsin, and bile) overwhelm the mucosal defense mechanism of the gastrointestinal tract that, lead to discontinuity in the entire thickness of gastric and duodenal mucosa [1]. It is fully associated with *H-pylori* infection and partially with NSAIDs induced injury; the predisposing factors for peptic ulcer are stress [2], genetics, diet, regional and environmental factors, cigarette smoking, alcohol consumption, and using drugs such as reserpine, indomethacin, aspirin, and corticosteroid [3]. It had been reported that some strains of *H-pylori* have a particular mechanism for injecting the inflammatory agent peptidoglycan from their own cell wall into epithelial stomach cells [4]. *H-pylori* evades attack by the host immune

system causing chronic indolent inflammation by several mechanisms, such as damaging the mucosal defense system by reducing the thickness of the mucus gel layer, diminishing mucosal blood flow, and interacting with gastric epithelium during all stages of infection [5]. The reactive oxygen species (ROS) have been shown to play a crucial role in experimental gastric lesions induced by stress, ethanol, and NSAIDs, as documented [6]. They play an important role in inhibition of prostaglandin synthesis, infiltration of polymorphonuclear leukocytosis, induction of apoptosis, and initiation of lipid peroxidation [7]. The present study was achieved to explore the possible gastroprotective effect of medicinal plants *Ulmus rubra*, *Piper nigrum*, and *Brassica oleracea* in the ethanol-induced model of gastric ulcer. *Ulmus rubra* (Slippery elm) is a deciduous tree native to North America. The red – brown

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or orange branches grow in a downward direction and the stalkless flowers are orange in the sessile-like cluster [8]. Its constituents are mucilage (polysaccharides); the mucilage yields galactose, glucose, galacturonic acid after hydrolysis, a trace of fructose, starch, tannins, and phytosterol. It is a versatile plant used for soothing respiratory, digestive, and urinary irritation and used as a demulcent for cough, diarrhea, and skin irritation [9]. *Piper nigrum* (Black pepper) is a perennial plant cultivated in tropical Asia and the Caribbean. It is actually a liane plant with a strong woody stem, and its leaves are cordate, glossy, and pale green. Its constituents are volatile oil (sabinene, limonene, caryophyllene, betapinene, acid amides (piperine and cumaperine), glycosides, polysaccharides, and fatty oil. The plant increases the secretion of saliva and gastric mucin, which is used as a spice to stimulate appetite and to control cough, vomiting, and diarrhea [10]. *Brassica oleracea* (Cabbage) is a leafy green or purple annual or biennial or perennial plant that has a woody stem with conspicuous leaf scars found in the Mediterranean region. It is used to stimulate the digestive enzymes of the pancreas, cold flu, obesity, and eczema. Its constituents are mustard oils, 5-vinyl-oxazolidine-2-thione (goitrin), amino acids (s-methyl cysteine sulphoxide, s-methyl Methionine sulphoxide, and anthocyanins) [10].

MATERIALS AND METHODS

Chemicals

All the chemicals used in the present study were of analytical grade. Micro Labs Limited Co supplied Absolute ethanol by Gainland Chemicals Co. Glwxd U.K. Esofag® (Esomeprazole) tablets. India.

Plant extract

The test medicinal plants were purchased from the well-known herbal shop (Al-Medina) and were identified and authenticated by the Iraqi national institute for Herbs. The dried berries of black pepper, dried leaves of cabbage, and dried inner bark of slippery elm were used in the present study. The dried particles of plants were cleaned carefully, and 10gm of the well-ground dried medicinal plant was mixed with 100ml of distilled water by using an electric mixing machine for 15min. The mixture was continuously put on a hot plate magnetic stirrer for 48hrs at a temperature of 40°C. The solution was centrifuged at 6000 rpm for 30min. The sediment was discarded, and the supernatant was taken. The procedure was repeated 3 times to ensure the discharge of sediment, then the supernatant was filtered by using seitz filter (with a pore diameter of 0.45). The supernatant was collected in a dark container and concentrated using a rotary evaporator [11].

Animals

Forty-eight healthy Wistar albino rats weighing (150-200gm) were used in the present study. The animal house of Al-

Nahrain College of Medicine supplied them. They were housed in separate cages, which were provided with a wire mesh floor and at a controlled temperature of 25°C with a 12hrs light/dark cycle. They were fed standard oxido pellets and water ad libitum. The study was conducted according to the animal ethics committee of Al-Nahrain College of Medicine. The rats withholding food for 24hrs were randomly allocated to six groups (each group contains eight rats). They were given at 8:30 AM one of the following agents by stainless tube orally:

In group-1 (control group), the animals received 1.5ml of distilled water

In group-2 (induced group), the animals received 1.5ml of distilled water

In group-3 the animals received 0.04mg/kg of esomeprazole in 1.5ml of distilled water

In Group-4 the animals received 1gm/kg of aqueous extract of slippery elm in 1.5ml of distilled water

In Group-5 the animals received 1gm/kg of aqueous extract of black pepper in 1.5ml of distilled water

In Group-6 the animals received 1gm/kg of aqueous extract of cabbage in 1.5ml of distilled water

After a period of one hour at 9:30 AM, 1.5ml of ethanol 95% was given to each group except group-1. Blood samples were collected by intra-cardiac aspiration from the rats of all groups for biochemical estimation of serum Zn and Cu and electrolytes Na^+ and K^+ values by using the spectrophotometer method [12]. Later, all the rats were sacrificed under light ether anesthesia after one hour of ethanol administration.

Stomach tissue preparation

The rat's stomach was separated from the surrounding viscera, removed from the body, and then opened along gastric curvature. Washing with physiological saline and immersed in freshly prepared phosphate buffer with PH=8. Gastric tissues harvested from the sacrificed rats and the fragments from tissues were put in a 10% neutral formalin solution to fix both the inner and outer layers of the gastric wall. Embedded in paraffin, then stained with hematoxylin and eosin and examined by dissecting microscope binocular type Heerbrugy to measure the parameters of the gastric ulcer which include the number of ulcers (lesion number), the total length of ulcers (lesion length), the total area of ulcers (lesion index), and preventive index [13]. The preventive index is calculated by the following formula [13]:

$$PI = \frac{LI_{\text{ethanol}} - LI_{\text{ethanol, agent}}}{LI_{\text{ethanol}}} \times 100$$

LI = Lesion index (total area of ulcers)

PI = Preventive index

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All the data were coded and analyzed statistically by using Microsoft program (SPSS) and paired sample t-test with significant ($P < 0.05$)

RESULTS

Administration of ethanol at 95% was found to be highly effective in inducing gastric lesions with the ratio of 100% experimentally in rats. The results of test agents demonstrated a significant preventive index equal to 90%, 47.7%, and

91.8% for slippery elm, black pepper, and cabbage, respectively, compared with esomeprazole 94.7%, as in Table 1. The obtained results showed that cabbage was more potent than slippery elm and black pepper in protecting against gastric ulcers. Both serum Zn and Cu were significantly decreased in slippery elm and cabbage groups but with no significant changes in serum electrolytes Na^+ and K^+ levels as in Table 2.

Table 1: Effect of tested agents on ulcer parameters (No. of ulcers, the total length of ulcers, total area of ulcers, and preventive index).

Group	Agent	Dose	No. Of ulcers	Total length of ulcers (mm)	Total area of ulcers (mm ²)	Preventive index
2	Distilled water + ethanol	1.5ml	16.3 ± 1.2	24.8 ± 1.9	44 ± 3.5	
3	Esomeprazole + ethanol	0.04mg/kg	3.8 ± 0.6**	4.25 ± 0.5**	2.3 ± 0.3**	94.7%
4	Slippery elm + ethanol	1gm/kg	6.1 ± 0.5**	6.5 ± 0.7**	4.4 ± 0.3**	90.0%
5	Black pepper + ethanol	1gm/kg	11.2 ± 1.1	17.6 ± 1.4	23 ± 1.7*	47.7%
6	Cabbage + ethanol	1gm/kg	4.6 ± 0.6**	4.8 ± 0.4**	3.6 ± 0.5**	91.8%

* Significant at $P < 0.05$, ** highly significant at $P < 0.01$ compared with group-2

Table 2: Effect of Esomeprazole, slippery elm, black pepper, and cabbage on serum levels of zinc, copper, sodium, and potassium.

Group	Agent	Dose	Zn	Cu	Na^+	K^+
1	Distilled water	1.5ml	1.46 ± 0.03	1.08 ± 0.05	143.5 ± 0.06	5.24 ± 0.08
2	Distilled water + ethanol	1.5ml	1.40 ± 0.03	1.30 ± 0.02*	140.6 ± 0.4	5.12 ± 0.04
3	Esomeprazole + ethanol	0.04mg/kg	1.0 ± 0.05	1.0 ± 0.04	144.5 ± 0.3	5.46 ± 0.05
4	Slippery elm + ethanol	1gm/kg	0.6 ± 0.02*	0.58 ± 0.01*	142.6 ± 0.4	5.20 ± 0.05
5	Black pepper + ethanol	1gm/kg	1.36 ± 0.03	1.12 ± 0.03	145.2 ± 0.2	5.50 ± 0.06
6	Cabbage + ethanol	1gm/kg	0.86 ± 0.03*	0.8 ± 0.02*	144.1 ± 0.2	5.03 ± 0.06

*Significant at $P < 0.05$ compared with group-1

DISCUSSION

Oral administration of ethanol 95% to the rats produced multiple gastric mucosal lesions, including ulcers and erosions, similar to those occurring in peptic ulcers with extensive visible hemorrhage. Most of these lesions occurred in the glandular portion of the stomach of the rat, whereas nonglandular portion of the stomach remained intact [14, 15]. The incidence of gastro-mucosal lesions increased in animals when food was withheld for 24hrs or more [16, 17]. Using ethanol in high concentrations (group-2) may be toxic to animals causing damage to the gastric mucosa associated with the production of free radicals leading to increase lipid peroxidation and damage to the cells [18]. The ulcerogenic agent ethanol produced an increase of MDA levels and a

decrease in GSH levels in gastric tissues [19-21]. Using distilled water alone in group-1 produced no changes in ulcer parameters and serum electrolytes. Esomeprazole is a proton pump inhibitor, and it is the s-isomer of omeprazole (group-3), it acts by irreversible binding to the H^+/K^+ -ATPase pump in the parietal cells and effectively suppressing acid secretion [1]. It is a potent drug with high gastro-protective effect (94.7%) [22]. Its results are similar to the results of others when they used omeprazole against ethanol-induced gastric ulcer with a significant increase in serum Na^+ and K^+ electrolytes.

The results of the present study showed no significant changes in serum Na^+ and K^+ levels of esomeprazole (group-3) but with a significant increase in Cu level due to changing

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cytochrome enzyme levels [23-25]. The gastro-protective effect of the aqueous extract of slippery elm (group-4) was highly significant, with a preventive index equal to 90%. This effect may be related to its active constituents of mucilage that make it demulcent and emollient, and soothing to the alimentary canal [10]. The mucilage appears to act as a barrier against the damaging effect of acid; it may have anti-inflammatory and antioxidant effects locally in the stomach and intestine [23], [26, 27]. There was no available data, but it decreased significantly Zn and Cu levels may be due to the reduction of their absorption [28], with no significant changes in serum Na⁺ and K⁺ levels. The gastro-protective effect of the aqueous extract of black pepper (group-5) showed a decrease in lesion numbers and area with a moderate preventive index equal to 47.7%; this effect may be related to increasing gastric mucus secretion and antioxidant effect [29]; also it had antiinflammatory and antitumor effect inhibiting human cell proliferation, cyclo-oxygenase enzyme, and NF- κ and β [30], with no significant changes in Zn, Cu, and electrolytes Na⁺ and K⁺ levels.

The gastro-protective effect of the aqueous extract of cabbage (group-6) was highly potent with a preventive index equal to 91.8%; this effect is related to the protection of mucus membrane from gastric HCl by using the cabbage that contains anti-ulcer factor vitamin U in its juice with regeneration ability to the mucus membrane [10, 31]. The serum levels of Zn and Cu were decreased significantly, but no available data are present thus, this decrease may be related to the fact that the mechanism of absorption is thought to be homostatically controlled and influenced by prostaglandin [31, 32]. No significant changes in serum electrolytes Na⁺ and K⁺ levels.

CONCLUSION

Both slippery elm and cabbage have highly potent gastro-protective effects with moderate effect for black pepper, and the possibility to use these agents as support and protection against gastric ulceration after assessment with clinical trials.

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