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Research Article

EFFECT OF PHARMACOLOGICAL DOSES OF GARLIC AND OMEGA 3 ON GASTRIC LESIONS INDUCED BY ETHANOL IN MICE

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ABSTRACT

Objective: This work was designed to investigate and compare the possible protective effect of pharmacological doses of garlic and omega 3 against gastric lesions induced by ethanol in mice.

Methods: A total of 30 mice involved in the study were divided into five groups with 6 mice for each. Groups 1 (ulcer control) and 2 (normal control), Groups 3 (positive ulcer control treated with ranitidine 50 mg/kg), Groups 4 and 5 treated with garlic oil and omega 3 oil at doses 200 and 150 mg/kg, respectively. All groups treated orally by gastric gavage once daily for 14 days before starting gastric ulcer (GU) induction process by absolute ethanol administration.

Results: A treatment with garlic and omega 3 ameliorated the severity of gastric ulceration evidenced by reduced ulcer index area, increase % gastroprotection, increase mucus content, reduced erosions, and necrosis.

Conclusion: Oral administration of pharmacological doses of garlic oil and omega 3 oil shows significant gastroprotection against GU models induced by absolute ethanol confirmed by biochemical and histological data.

Keywords: Gastric ulcer, Garlic, Omega 3, Gastroprotection.

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INTRODUCTION

Gastric ulcers (GU) and gastric lesions are serious diseases affecting a wide variety of individuals worldwide [1]. Imbalance between defensive mechanisms versus aggressive factors in the mucosa is the major etiology of GU and recently modulation of such imbalance may be the target of many researchers [2]. The risk of developing open sores in mucous lining of the stomach depends on several endogenous and exogenous factors including HCl, pepsin, refluxed bile, Cytokines, free radicals, stress, non-steroidal anti-inflammatory drugs, alcohol, and *Helicobacter pylori* [3]. Indeed cytokines such as tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10 play a key roles in the development and maintenance of gastric ulceration [4]. On the other hand, one of the common causes for the genesis of GU is the involvement of free radicals that play an important role in the pathogenesis of GU by lipid peroxidation and tissue lesions [5]. Normally, the gastric epithelium is exposed to high level of reactive oxygen species (ROS) derived from different microbiological, physical and chemical factors that normally eradicated by the action of endogenous antioxidant defense system [6]. Shifting of balance toward oxidative stress leads to apoptosis and necrosis of gastric epithelium. In general, there are a wide variety of antioxidative compounds play an essential role in ulcer treatment based on it is role in oxidative stress [7]. In this respect, garlic could be the most promising compound for the prevention and treatment of GU diseases for it is strong antioxidant action by powerful free radical scavenging capacity, enhancing cellular antioxidant enzymes activity like catalase, glutathione peroxidase, and superoxide dismutase. Add to garlic has some anti *H. pylori* activity [8]. Furthermore, there have been many literature, reports and researches indicating the pharmacological importance of garlic as anticancer, in cardiovascular disorders, neurological diseases, liver diseases, allergy and arthritis for their antioxidant and anti-inflammatory properties [9]. Another compound largely known for their antioxidant property was omega 3 [10]. The clinical importance of omega 3 (polyunsaturated fatty acid) in cardiovascular, cancers and inflammatory diseases, including arthritis, ulcerative colitis, and psoriasis are well documented [11]. It also exhibits promising effects in the treatment of fatty liver disease and has displayed protective roles against gastric lesions caused by several stimuli [12,13].For our information, this is the first article studied the effect of pharmacological doses of omega 3 and garlic oils for treatment of GU in mice.

This study was designed to evaluate and compare the possible protective effect of pharmacological doses of garlic and omega 3 against gastric lesions induced by ethanol in mice.

METHODS

Chemicals

Garlic (500 mg softgel) and omega 3 (1000 mg softgel) were purchased from Vitane's Nature Pharmaceutical Company, USA. Ranitidine was purchased from SDI Company Samarra, Iraq. Diethyl ether was purchased from May and Baker, England while absolute obtained from BDH chemicals, Ltd., Poole, England, respectively.

Animals

A total of 30 albino mice (30-35 g) of both sexes were involved in the study. The mice were obtained from College of science, Basrah University and housed in the animal house of College of Pharmacy, Basrah University, Iraq. The mice were housed under a 12-hrs light-dark cycle and controlled condition (around 25°C) and (air volume change 20 times/hrs) with two mice per cage. The mice were allowed to acclimatize for 7 days before the experiment and received standard diet and water *ad libitum*. Animal experiments were approved by the Animal Ethics Committee of Pharmacy College, Al-Basrah University.

Experiment and mice treatment design

The mice were divided into five groups consisting of 6 mice each: Groups 1 (ulcer control), Group 2 (normal control) received distilled water at a dose of (3 ml/kg), the other three groups are treated with one of the followings: Ranitidine (50 mg/kg), garlic oil 200 mg/kg, and omega 3 oil 150 mg/kg. Pharmacological doses of garlic and omega 3 were precisely calculated based on the previous papers in which such

compounds used as anti-inflammatory or antioxidants [14,15]. All groups treated orally once daily for 14 days using gavage needle before GU induction. Subsequently in the 15th day of treatment and after 24 hrs fasting with free access to water, all mice received regular calculated doses of drugs and vehicle just 1 hr before GU induction.

Induction of GU

In this GUs mice model, absolute ethanol was administered according to a method of Palacios-Espinosa *et al*. with slight modification [16]. 7 ml/kg absolute ethanol was administrated orally to all mice except in normal control group. Then 3 hrs later, the animals in all groups were sacrificed by inhalation of a very high dose of diethyl ether. Subsequently, abdomen was opened and the stomach was excised and examined for the ulcers in stomach, it has been found that stomach of ulcer mouse was inflated may be due to oversecretion of gastric acidity as shown in Fig. 1.

Evaluation of ulcer area and gastroprotection

The stomach's ulcerative lesions were examined by the aid of digital pictures under a dissection microscope, measurement of ulcer area done using software UTHSCSA image tool 3.0 program for calculation ulcer area [17,18]. The summation of total ulcer areas (mm^2) divided by 6 (total mice number in each group) to achieve the mean ulcer area or what is called ulcer index (UI). Meanwhile, gastroprotection percent was measured using the equation [19]: % Gastroprotection=([UA control-UA treated] × 100)/UA control.

Evaluation of gastric mucus content

The amount of gastric mucus was measured using a piece of glass slide were gently scraped the mucus for its evaluation. The mucus obtained was weighed using sensitive electronic weighing balance.

Histopathological assessment

At first, the collected gastric tissue samples fixed in 10% formalin solution for 4 days, sectioned, and then subsequently processed for routine paraffin blocking. Sections were cut at 4-5 μm and stained with hematoxylin and eosin [20], then observed under a dissection microscope by a special histopathologist for evaluation for histopathological change such as congestion, edema, erosions, ulcerations, and necrosis.

Statistical analysis

The results of this study expressed as mean±standard deviation; the data were analyzed using one-way analysis of variance. Values with $p<0.05$ were considered significantly different. Analysis was performed using GraphPad Prism software version 5.0.

RESULTS

Macroscopic evaluation of stomach

The stomach's ulcerative areas were examined by a 10·magnifier lens under dissection microscope to assess ulcers areas. The numbers of ulcers were counted and scored. Ethanol lead to deep ulcer lesions characterized by inflammation combined with hemorrhage and edematous lesions observed in ulcer control group in contrast to normal control group no lesions were observed, as seen in Figs. 2 and 3.

Effect of garlic and omega 3 on ethanol-induced gastric lesions

The treatment with garlic and omega 3 ameliorated the severity of gastric ulceration. The positive control ranitidine significantly reduced the gastric lesions compared with treated groups as shown in Figs. 4-6.

Histopathological investigation

Histopathological investigation further confirmed the results that absolute ethanol-induced severe hemorrhage, edema, necrosis, and congestion in stomach mice sections. Such effect markedly ameliorated

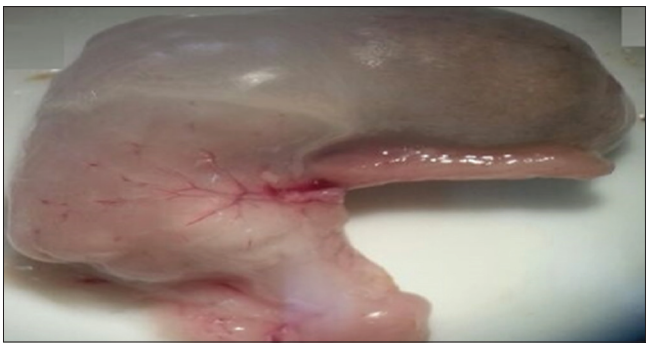

Fig. 1: Inflated stomach with acute ulcer due to absolute ethanol administration

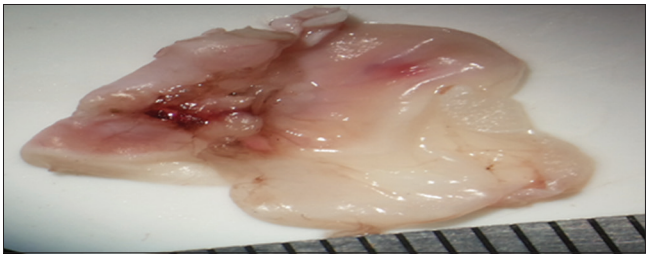

Fig. 2: Photograph under a dissection microscope of gastric mucosa in ulcer group, associated with hemorrhage and inflammation

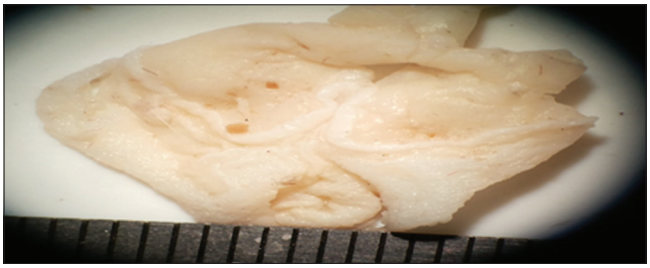

Fig. 3: Photograph of gastric mucosa in normal control group

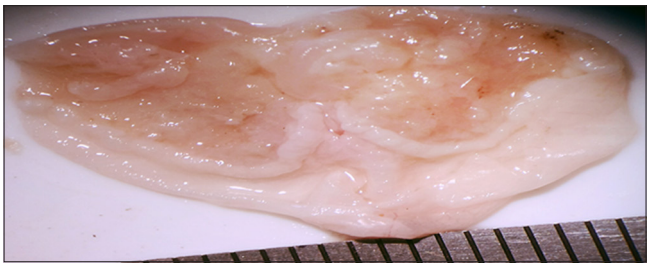

Fig. 4: Photograph of gastric mucosa under a dissection microscope in ranitidine treated group

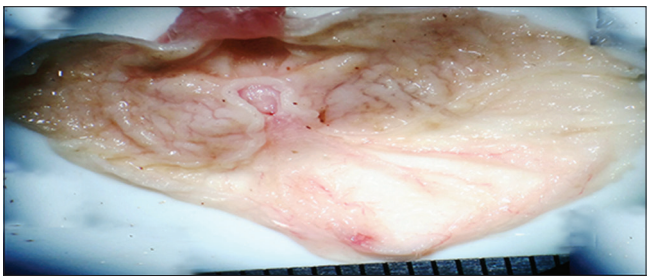

Fig. 5 Photograph of gastric mucosa under a dissection microscope in garlic treated group

by ranitidine pretreatment, and to a lesser extent by omega 3 and garlic as shown in Fig. 7.

UI and % gastroprotection

Oral administration of absolute ethanol-induced extensive gastric ulcerations in the gastric mucosa of the stomach (lesion area=24.5±0.85); actually, pretreatment with garlic and omega 3 reduced ethanol-induced mucosal damage significantly with a mean difference 16.3 reduction in omega 3 treated group and 16.61 in garlic treated group in the ulcer size compared with ulcer control group. Furthermore, the gastroprotective effect of garlic and omega 3 had been shown to be comparable with significantly different from that observed in positive control and ulcer control groups, highest gastroprotection percent observed in the positive control group as seen in Figs. 8 and 9.

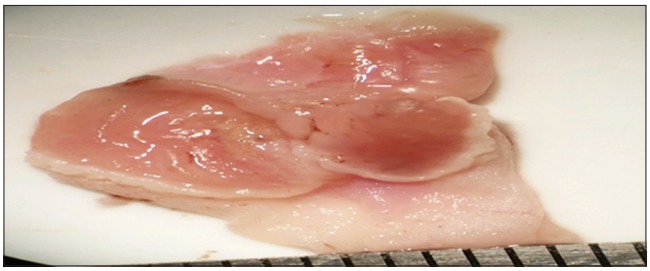

Fig. 6: Gastric mucosa photograph under dissection microscope in omega 3 treated group

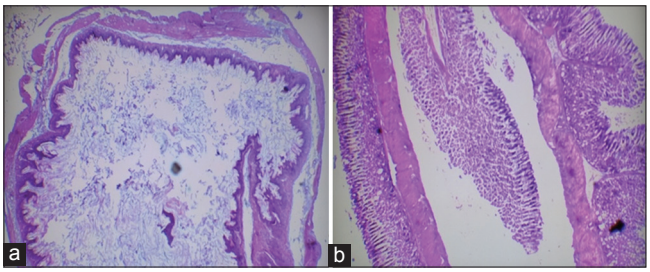

Fig. 7: Photomicrographs of gastric mucosa stained with hematoxylin and eosin (×100). (a) Ulcer control; (b) normal control

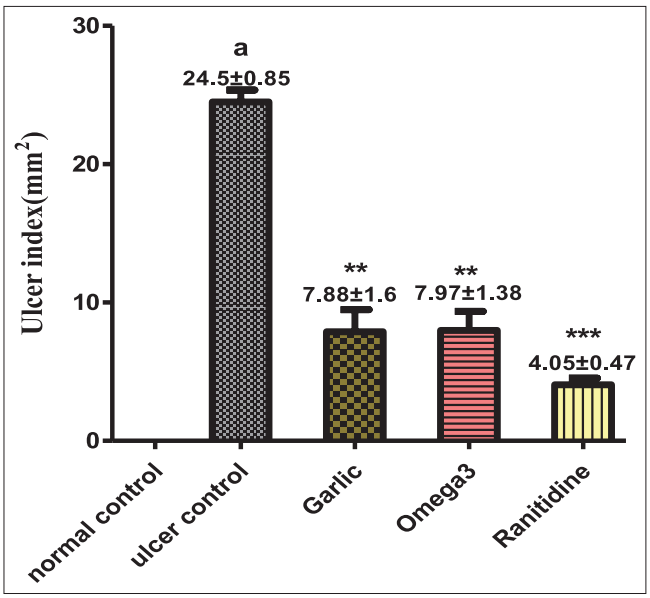

Fig. 8: Gastric ulcer index of gastric lesions induced by absolute ethanol in mice (n=6), analysis of variance: **p<0.01, ***p<0.001 compared to normal and ulcer control group, [a]p<0.001 compared to normal control and all treated groups

Evaluation of gastric mucus content

There was a significant increase in viscosity of gastric mucus content in both garlic and omega 3 treated groups, tested compound antagonize mucus depletion by absolute ethanol but such effect less significant compared with ranitidine treated group (positive control as shown in Fig. 10.

DISCUSSION

Ethanol-induced GU was widely used as an experimental model to determine the gastric healing effect of different compounds because it is closely resembles chronic ulcers in humans, particularly in the healing process. Furthermore such model is linked to the increase release of free radicals (mainly oxygen species), production of inflammatory mediators, stasis of blood flow and microvascular damage lead to hemorrhage and necrotic lesion [21,22]. In the stomach, H+ ion secretion is an oxidant-dependent process and in the presence of

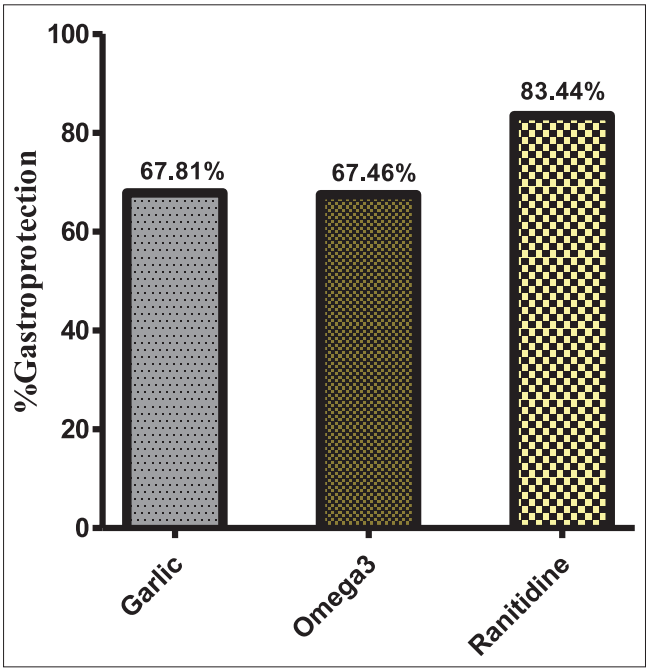

Fig. 9: Gastroprotection % of both garlic and omega 3 oils against acute gastric lesions induced by absolute ethanol in mice (n=6)

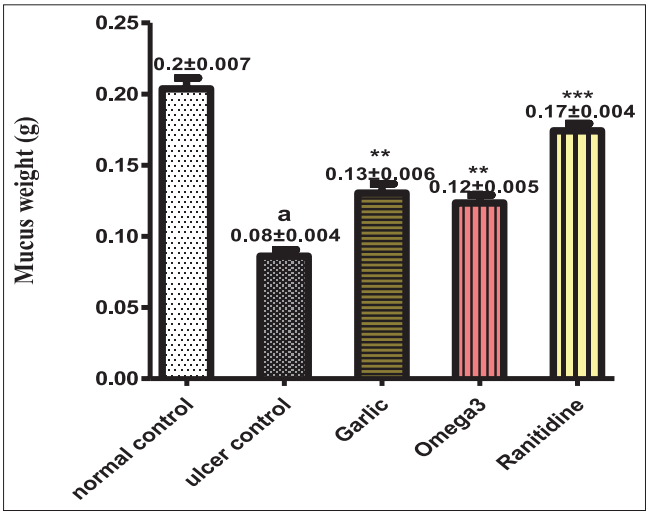

Fig. 10: Mucus content in the stomach of control and all treated groups in mice (n=6), analysis of variance: **p<0.01, ***p<0.001 compared to normal and ulcer control group, [a]p<0.001 compared to normal control and all treated groups

Cl- and H_2O_2, hydrochloric acid will be formed and these are very toxic oxidant that leads to mucosal lesion and lipid peroxidation, and this is the major pathogenesis of ulcer [23]. The endogenous anti-oxidant enzymes catalase, superoxide dismutase and glutathione in the gastric mucus are the key components of cellular defense system against stress induced ulcer and shifting the balance toward endogenous antioxidant is the target [24]. Furthermore, elevated ROS is a critical factor in the onset and development of various gastric disorders [25]. Hence, the general approach is to reduce ROS production that can provide a beneficial effect in combat gastric diseases. In this study, both garlic and omega 3 provide a promising gastroprotective effect on ethanol-induced GU explained by a significant reduction in ulcer area and significant increase in mucus content as compared with ulcer group. An important explanation of such results is scavenging capacity of the studied compounds lead to free radicals eradications [26]. Garlic is good traditional medicines have been widely used in prevention or management of GUs for a long time [27]. It seems that garlic protect gastric mucosa from absolute ethanol injury through neutralization of released free radicals and increase mucus content. This came in concurs with Borek (2001) who reported that garlic inhibits lipid peroxidation and reduces free radical induced transcription factor and nuclear factor kappa B, thus protecting mucosa from injury [28]. In addition, garlic contains antioxidant phytochemicals that prevent oxidants damage and protect gastric mucosa against oxidative stress [26]. In contrast for such finding, Oboh (2005) reported that garlic induced oxidative stress and produced hepatotoxicity [29]. The another compound used in this study was omega 3 or what is called fish oil is rich in polyunsaturated fatty acids like eicosapentaenoic acid and docosahexaenoic acid and such compounds considered gastroprotective after exposure to several insults [30]. Several studies [31,32] indicate the gastroprotective effect of fish oil against oxidative stress, this study demonstrates that administration of omega 3 in supraphysiological or pharmacological doses induced gastroprotection in an absolute ethanol-induced gastric injury in mice model. It has been found that omega 3 save and protect gastric mucosa against aspirin, cold restraint stress and alcohol by acting on both aggressive (decrease significantly acid-pepsin secretion) and defensive (mucin and gastric mucus secretion) gastric mucosal factors [31]. Another explanation of such result, omega 3 exert a range of anti-inflammatory effects including reduced inflammatory cytokine, adhesion molecule production, platelet activating factor, IL-6 also decreased leukocyte-adhesive interactions [33,34]. The comparable efficacy of garlic and omega 3 against gastric lesions in the present study found is consistent with the similarity of the mechanisms whereby that support their efficacy on this model as free radical scavenger [28,32]. From this omega, 3 intake is associated with a decreased risk of different inflammatory diseases such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, and asthma by modulation of inflammatory events [34].

CONCLUSION

Supraphysiologic or pharmacological oral doses of both garlic and omega 3 oils show significant gastroprotective effects against absolute ethanol-induced GU confirmed by cytoprotective, histological, anti-secretory and biochemical data.

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