Original Article: The Protective Role of Menthone Against Indomethacin-Induced Gastric Ulcers in Rat Model

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ABSTRACT

Background: The use of novel agents has been considered for management of different diseases. Gastric ulcer is a common disease. This study aimed to evaluate the effects of Menthone, as a novel agent, on gastric ulcers in Indomethacin-induced model rat.

Materials and Methods: Animals were divided into 3 groups: 1. Control group that received only distilled water; 2. Animals were treated with 40 mg/kg bw omeprazole (Omeprazole); 3. Animals were treated with 40 mg/kg bw Menthone (Menthone). A single dose of indomethacin (30 mg/kg body weight) was administrated to per animal. Gastric secretions and antioxidant parameters were evaluated in the end of trial.

Results: It was observed that an increased ulcer index, gastric volume, malondialdehyde level and decreased super oxide dismutase and pepsin activity in control group in comparison to other groups ($P<0.05$). The use of Menthone and Omeprazole could alleviate the effects of indomethacin.

Conclusion: Menthone may not have positive effects similar to Omeprazole, but it could attenuate effects of indomethacin. We recommend using of Menthone, as an efficient agent, for treatment of gastric ulcer.

Introduction

Gastric ulcer has been known as one benign lesion in the mucosalepithelium that depends to high production of acids and aggressive pepsin activity [1]. It is estimated that it as one of most common gastrointestinal disorder in all over the world [2, 3]. Structure of the stomach mucosal barrier is protected by a balance between irritation and defensive factors [4]. The different factors including non-steroid anti-inflammatory drugs, nutritional deficiencies, smoking, stress, etc can

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provide condition for gastric ulcer [5, 6]. The excessive production of neutrophils in the site of injury increases concentrations of Reactive Oxygen Species (ROS) and other mediators of inflammation, which causes oxidative damage [7]. The usual treatments have been used to treat the gastric ulcer including antacids, histamine H_2 receptor antagonists and proton pump preventers. These drugs included cimetidine, misoprostol, ranitidine, omeprazole and esomeprazole, but the drugs have side effects. It has been reported that antiulcer compounds must prevent gastric secretion but may have multiple effects.

An antiulcer drug must have antioxidant, anti-apoptotic and anti-inflammatory properties [8]. Phenolic compounds as safe compounds can have profit effects on gastric ulcers that could be attributed to its activity such as antioxidant and anti-inflammatory. Peppermint has been known to have gastric antiulcer effects that could be attributed to its compound. Peppermint compounds have been reported to have antioxidant and anti-inflammatory effects [9]. Menthone, as one of main compound in peppermint, seems to have an anti-inflammation and antioxidant compound that help to improve the gastric ulcers. This study was conducted to evaluate the Menthone, as a novel compound for management of gastric ulcers in Indomethacin-induced gastric ulcers in rat model.

Materials and Methods

Materials

Menthone with purity of >97% was purchased from Sigma-Aldrich. Omeprazole was purchased from Darou-Pakhsh Pharmaceutical Company (Alborz-Iran). Indomethacin was also prepared from Caspian Taamin Pharmaceutical Company.

Animals

A total number of 72 Albino Wistar rats (6 weeks-age, 170±10 g) were adapted for one week before of trial and kept on basis the animal welfare laws. All the animals were maintained in an optimal temperature (25±1ºC), and humidity (55±5%) and illumination period (12 h light and 12 h dark) were kept in the experimental period. Before of trial, all the rats were fasted for 16 hours and grouped in cages with increased floors of a wide mesh to prevent coprophagy. There was no anesthetic procedure before the oral administrations. All the efforts were conducted to decrease animal injuries.

Induction of ulcer and grouping

Induction of gastric ulcer was conducted as reported by Sayanti et al. [10]. Summary, a single dose of indomethacin (30 mg/kg body weight) was administrated to per animal. Animals were divided into 3 groups: 1. Control group that received only distilled water; 2. Animals were treated with 40 mg/kg bw omeprazole (Omeprazole); and 3. Animals were treated with 40 mg/kg bw Menthone (Menthone). Pre-treatment with Omeprazole and Menthone was lasted for 3 weeks prior to administration of indomethacin. The agents were applied once/day by an oral incubator. The different degree of ulceration was considered 4 h after indomethacin administration.

Isolation of stomach and collection of gastric juice

To isolate the stomach, it was isolated in end of trial and gastric juice was collected, 3 h after induction of ulcer. Rats were then killed by cervical dislocation. Following isolation of stomach, it was opened and gastric contents were separated in a centrifuge tube. Then, 5ml of distilled water was included it and t centrifuged in 2500 rpm for 15 min. The resulted supernatants were used to analyse the biochemical parameters. Macroscopic investigation and homogenization was conducted before the cleaning stomach.

Investigation of gastric secretion variables

Gastric acid output (volume) was investigated by using titration and Toepfer's reagent. Pepsin activity, mucin concentration and gastric pH were evaluated as reported by previous studies [11, 12].

Quantification of ulceration

The degree for quantification of ulceration was conducted as reported by others [13]. A scale of 0-5 was considered, so that, 0=almost normal mucosa, 1=vascular congestions, 2=one or two lesions, 3=severe lesions, 4=very severe lesions and 5=mucosa full of lesions.

Providing of stomach homogenate and evaluation of antioxidant parameters

Following induction of ulcer, the prepared tissues were ground with liquid nitrogen in a mortar. The tissue samples were homogenized and the homogenized samples were centrifuged in 3000 rpm for 10 min. The obtained supernatants were frozen in -20ºC. The levels of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) were evaluated as reported by Marklund and Marklund [14] and Devasagayam and Tarachand [15].

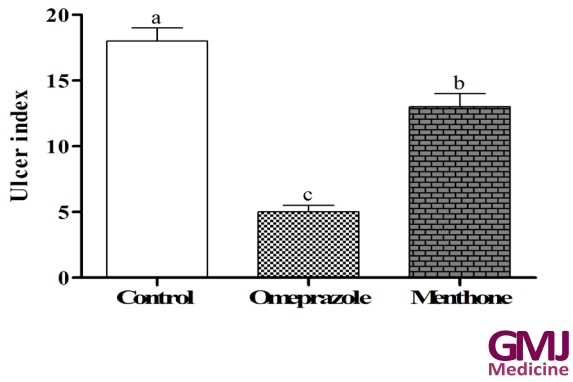

Figure 1. Effects of menthone on ulcer index in the Indomethacin-induced gastric ulcers

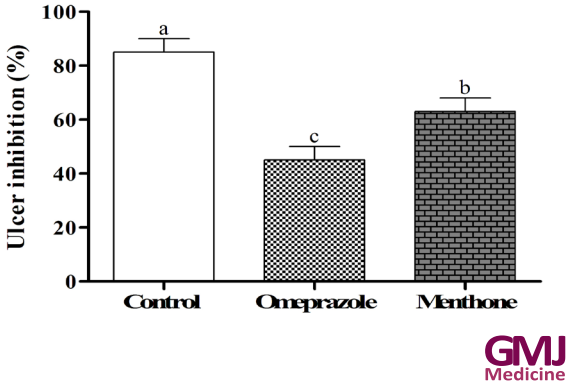

Figure 2. Effects of menthone on ulcer index in the Indomethacin-induced gastric ulcers

Statistical analysis

The data were reported as mean±SD and analyzed by one-way Analysis of Variance (ANOVA) procedure. Tukey post-hoc test was used to compare the groups. A level of $P<0.05$ was considered as significant. The figures were illustrated by Prism, version 7 (GraphPad Software, Inc., San Diego, CA, USA).

Results

Ulcer index and ulcer inhibition

The effects of experimental treatments on ulcer index and ulcer inhibition are presented in Figures 1 and 2. Our findings showed that ulcer index was significantly higher in control group in comparison to other groups ($P<0.05$). Administration of Omeprazole and Menthone could significantly decrease ulcer index ($P<0.05$). The best response was observed in Omeprazole group. With regards to ulcer inhibition, the highest value was observed in control group, Menthone and Omeprazole, respectively ($P<0.05$). The lowest ulcer inhibition was seen in Omeprazole group ($P<0.05$) but Menthone also had significant effect ($P<0.05$).

Gastric volume and pH

Our findings for gastric volume and pH are shown in Figure 3. Results showed that gastric volume and pH were significantly higher and smaller in control group ($P<0.05$). Administration of Omeprazole and Menthone reversed responses, so that animals in Omeprazole and Menthone groups showed lower and higher gastric volume and pH compared with control ($P<0.05$).

Figure 3 Effects of Menthone on pepsin activity and mucin content (microgram/ml) in the Indomethacin-induced gastric ulcers

Antioxidant status

The parameters for antioxidant status are shown in Figures 4 and 5. The MDA (Figure 4) concentration and SOD levels (Figure 5) were respectively higher and lower in control group in comparison to other groups ($P<0.05$). The use of Omeprazole and Menthone decreased levels of MDA and increased level of antioxidant enzyme ($P<0.05$).

Discussion

In the current study Menthone could have positive effects on alleviation of effects of Indomethacin. Phytotherapy has been considered for human health and also for preventionof some diseases such as gastric ulcer obtained from drugtoxicity [16, 17]. It has been accepted ability of phytonutrients due to antioxidant properties that play major roles in management of toxicity related disorders. Biochemical analysis for gastric secretions (pH and gastric volume) and mucosal integrity for stomach has been used employed to evaluate the pharmacological agents [18].

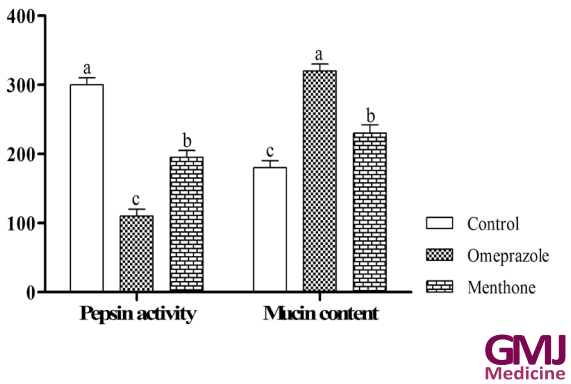

Figure 3. Effects of menthone on pepsin activity and mucin content (microgram/ml) in the Indomethacin-induced gastric ulcers

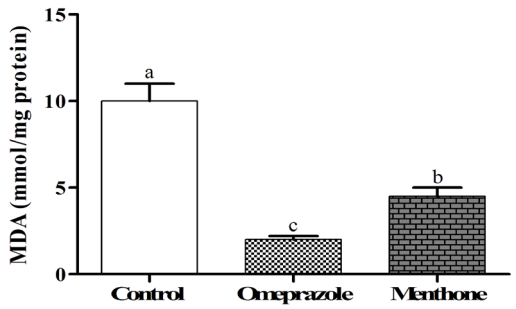

Figure 4. Effects of Menthone on MDA in the Indomethacin-induced gastric ulcers

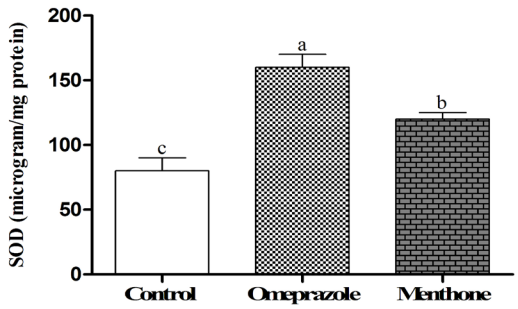

Figure 5. Effects of Menthone on SOD in the Indomethacin-induced gastric ulcers

The pH index provides an idea of the level of acidity and also gastric secretions volume. It has been reported a relation between pH index and ulcer and gastric damage [17]. Gastric damages could be attributed to production of oxidants in gastric lumen. Previous studies have shown that Indomethacin has negative effects on gastric secretions [18, 19]. As mentioned oxidants could have negative effects on ulcers. It seems that Menthone by antioxidant capacity could improve ulcer parameters. Our findings for antioxidant capacity confirm such claim. Other mechanisms modulating included buffering capacity for the neutralization of luminal acid and also protection effects against endogenous and exogenous toxins, i.e. indomethacin, that increases the rate of local healing process [20].

In this study, increased pepsin activity was corresponded with decrease in mucin secretion; it means that pepsin activity decreases mucin secretion. It could be argued that Menthone reduces pepsin activity and increases mucin secretion. It has been generally accepted that agents increasing gastric mucus could accelerate gastric ulcer. It has been accepted that imbalance between oxidants and antioxidants stress cause cellular activities that result in different pathological conditions [21]. Increased concentration MDA and decreased activity of SOD in the stomach of indomethacin-ulcerated rats cause to stimulate the lipid peroxidation and over production of free radicals which finally result in gastric damages. Antioxidant enzymes activities reduces with increased oxidants.

MDA, as index for lipid peroxidation, has been considered as one important index for toxicity mechanism of indomethacin [22]. It has been reported decreased antioxidant enzymes activity in ulcerated stomach [23]. Our observations showed that Menthone increased antioxidant activity and decreased MDA. In the current study, Omeprazole could improve ulcer parameters and the best response was observed in omeprazole group. Results showed that Omeprazole has powerful antioxidant properties that help to improve the ulcer. Other mechanism was attributed to its inhibiting effects on proton pump [24, 25]. Menthone may act in a similar way that needs further future investigations.

Conclusion

In summary, Menthone, as a novel agent, could help to improve the gastric ulcers due to its antioxidant properties. It did not have ability similar to Omeprazole but could partly improve ulcer. It could be advised to use the Menthone as an agent for treatment of gastric ulcers.

Ethical Considerations

Compliance with ethical guidelines

Approval for this study was obtained from Lorestan University of Medical Sciences Research Committee.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of interest

The authors declared no conflict of interest.

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