Ulcer healing activity of *Mumijo* aqueous extract against acetic acid induced gastric ulcer in rats

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ABSTRACT

Objective: Gastric ulcer is an important clinical problem, chiefly due to extensive use of some drugs. The aim was to assess the activity of *Mumijo* extract (which is used in traditional medicine) against acetic acid induced gastric ulcer in rats. Materials and Methods: The aqueous extract of *Mumijo* was prepared. Animals were randomly (n = 10) divided into four groups: Control, sham-operated group (received 0.2 ml of acetic acid to induce gastric ulcer), *Mumijo* (100 mg/kg/daily) were given for 4 days postacetic acid administration, and ranitidine group (20 mg/kg). The assessed parameters were pH and pepsin levels (by Anson method) of gastric contents and gastric histopathology. Ranitidine was used as reference anti-ulcer drug. Results: The extract (100 mg/kg/daily, p.o.) inhibited acid acetic-induced gastric ulceration by elevating its pH versus sham group (P < 0.01) and decreasing the pepsin levels compared to standard drug, ranitidine (P < 0.05). The histopathology data showed that the treatment with *Mumijo* extract had a significant protection against all mucosal damages. Conclusion: *Mumijo* extract has potent antulcer activity. Its anti-ulcer property probably acts via a reduction in gastric acid secretion and pepsin levels. The obtained results support the use of this herbal material in folk medicine.

KEY WORDS: Acetic acid, gastric acidity, *Mumijo*, pepsin, ulcer

Pepitic ulcer being one of the most uncontrolled gastrointestinal problems representing a chief health hazards in terms of morbidity and mortality. The etiology of gastroduodenal ulcers is influenced by diverse aggressive and defensive factors for example acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, and endogenous protective agents.[1,2] Mucosal injury may happen when noxious factors “overwhelm” an intact mucosal protection or when the mucosal defense is somehow disrupted.[3]

A number of chemical drugs is accessible for treatment of peptic ulcer, but some side effects and drug interactions make them difficult to use. This is a reason for the development of new anti-ulcer drugs, and the search for novel molecules has been extended to herbal drugs that would offer better protection and decreased relapse.[1] Iran has unique meteorological situation that contributed to the variety of medicinal plants.[4]

In the developed countries, there are several lines of studies about *Mumiju* and its importance in the treatment of different diseases by health experts and pharmacological organizations. Some have reported the beneficial effects of *Mumiju* in the treatment of gastrointestinal disorders,[5,6] bone pains, and fractures.[7,8] Asian *Mumiju* includes 20% minerals, 15% protein, 5% lipids, 5% steroids, and also some carbohydrates, alkaloids, and amino acids.[9,10] Few therapeutic effects of this substance are as follows: Memory improving, neuroprotective, anti-inflammatory, and anti-oxidant roles.[11,12] The biological effect of *Mumiju* has been attributed to di-benzo-alpha-pyrone, hunic acid, and folic acid contents.[13,14]

With regard to the several beneficial effects mentioned for *Mumiju*, we hypothesized that the administration of this substance can be effective in the improvement of gastric ulcer. Therefore, the aim of this study was to investigate the
gastroprotective effects of this substance by measuring gastric acid and pepsin levels.

Materials and Methods

Animals

Male Wistar rats (200-250 g) obtained from the animal room of the Kerman University of Medical Sciences. They were kept in a temperature-controlled environment on a 12:12 h light/dark cycle with free access to food and water. The procedures were in accordance with the guidelines for the care and use of laboratory animal of Kerman University Medical Science (IAEC No.: 01/027/04).

Study design

This experimental study was carried out on 40 male Wister rats to study therapeutic effects of Mumijo extract on acetic acid-induced gastric ulcer. Animals were randomly (n = 10 in each group) divided into four groups: (I) Control received normal saline (2 ml/kg). (II) sham-operated group received 0.2 ml of acetic acid to induce gastric ulcer, (III) Mumijo (100 mg/kg/daily) was given for 4 days postacetic acid administration, (IV) ranitidine group received the standard drug, ranitidine (20mg/kg), dissolved in normal saline. Twenty-four hours before each experiment, animals were deprived of food, but free to drink water.

Plant material and extraction

Mumijo was prepared from the local residents of Sardoieyeh in Jiroft/Kerman/Iran. Then it was recognized by botanist from Botanical Survey of Kerman, Iran. Immediately, it was washed thoroughly with running tap water and cut into small pieces. Then the plant material was shade dried at temperature 21-24°C and ground mechanically into a coarse powder and stored in an airtight container. Powdered plant material (150 g) was macerated with 400 ml of distilled water at 21-24°C temperature for 3 days with frequent shaking. After 3 days, the extracts were filtered and to the marc part 300 ml of the solvent was added and allowed to stand for next 2 days at same temperature for second time maceration (re-maceration) and after two days, again filtered similarly. The combined filtrates (macerates) were evaporated in vacuo at 40°C and the dry extract obtained was stored in a vacuum desiccator for future use. All the test samples were administered by oral lavage in a volume of 1 ml/100 g body weight once a day to each rat.

Acetic acid-induced ulcer model

The ulcers were induced by the local application of acetic acid to serosal surface of the stomach as described earlier. Under anesthesia, the midline incision was made, and the stomach was taken out. On the serosal surface of the glandular portion of the stomach, 0.2 ml of 100% acetic acid (anterior gastric wall) was injected. After the treatment, the rats were sacrificed, and stomachs were removed and weighed, fixed in 10% neutral buffered formalin, embedded in the paraffin wax, sectioned at 5 µm, stained with hematoxylin and eosin and then examined by the light microscopy.

Evaluation of pepsin

Pepsin levels in the gastric effluent were determined as Anson method. Briefly, 2 ml of 2.5% bovine hemoglobin plus 0.5 ml of 0.3 N HCl and 0.5 ml of gastric effluent were maintained in separate tubes at 37°C for 10 min and then mixed. Mixtures were incubated for 10 min at 37°C, and the reaction was stopped by the addition of 5 ml 0.3 N trichloroacetic acid. After agitation and filtration, optical density was measured at 280 nm by using a spectrophotometer (Unikon 950, Kontron Instrument, Italy). The results were compared to a standard curve, which was generated in an identical manner using known amounts of porcine pepsin (1 µg = 3 peptic units), and were expressed as micrograms of pepsin.

Measurement of intragastric pH

In order to know whether or not the intragastric pH is contributing to the mucosal lesions, intragastric pH was measured 4 days after administration of Mumijo and ranitidine. The pH measurement was performed as previously reported. Laparotomy and pylorus ligation were performed under ether anesthesia. The pylorus of the stomach and esophagocardiac junction were immediately ligated, and the stomach was then removed from each rat, and the gastric contents were collected, centrifuged, and the supernatants were used for pH measurement using pH meter (Ø 50 pH Meter, Beckman, Fullerton, CA, USA).

Ethical considerations

This study protocol was approved by the Ethics Review Committee, Kerman University of Medical Sciences, Kerman, Iran.

Statistical analysis

The results were expressed as mean ± standard error of mean, and statistical significance was evaluated using one-way ANOVA, followed by a Tukey’s post-hoc test. Significance of difference was accepted as P < 0.05.

Results

pH of gastric contents

pH data are shown in Figure 1. The pH was 3.43 ± 0.32 and 3.93 ± 0.54, respectively, in sham and ranitidine-treated animals and this difference was not significant. However, when the extract was administered to the acid acetic-treated group, there was a significant increase in pH of gastric contents to 4.54 ± 0.78 (P < 0.01).

Pepsin levels of gastric contents

Gastric pepsin levels in Mumijo group were significantly lower than ranitidine group (P < 0.05). However, there was no
difference between ranitidine or sham groups compared to control [Figure 2].

**Histopathological findings of gastric tissue**

Acid acetic caused histopathological lesions including ulcer with transmural necrosis and degeneration of the gastric tissue. Treatment with *Mumijo* extract offered significant protection against all damages to mucosa by acid acetic [Figure 3].

**Discussion**

Gastric ulcers are due to inequality between aggressive (acid-pepsin secretion, *Helicobacter pylori*, bile, increased free radicals, and decreased antioxidants) and defensive factors (mucus, bicarbonate secretion, prostaglandins, blood flow and the process of restitution, and regeneration after cellular injury) of the gastric mucosa. In the present study, it was recognized that the *Mumijo* had protective effects against the gastric ulcer induced by acetic acid. Protective effects of *Mumijo* on the stomach may be related to its anti-secretary action as our results showed that the *Mumijo* reduced the amount of gastric acidity and pepsin levels in the damaged stomach.

According to the some reports, *Mumijo* has significant repairing effects. It was shown that the presence of some polyphenols compounds such as fulvic acids (FAs), 4-methoxy-6-carbomethoxybi-phenyl, tirucallane-type triterpenoids, and benzoic acid in *Mumijo* samples have very important role in decreasing of acid-pepsin secretion, cell shedding, and gastric ulcer index. Also antioxidant activity, cellular repairing, and regenerative functions reported. It also can increase the mucin secretion and carbohydrate/protein ratio which have very important role as anti-oxidant and anti-inflammatory effects as reported by other researchers, so they stated its use in gastric ulcer and wound healing. In general, *Mumijo* has an expected broad biochemical and pharmacological activities due to its contents of FA and other antioxidant materials. FAs have been taken orally as a therapy for gastritis, stomach ulcers, and colitis.

In our study, it was also recognized that *Mumijo* has regenerative and repairing effects as shown in the histopathological findings. It was reported that *Mumijo* pretreatment at the dose of 100mg/kg orally reduced ulcer index in immobilization and aspirin induced gastric ulcers. In duodenal ulcers also, *Mumijo* pretreatment significantly reduced the incidence of ulcers induced by cysteamine in rats and histamine in guinea pigs. It may be partly related to its role in decreasing of acid-pepsin secretion as mentioned above but the other functions of this plant is also important. In some studies, the antioxidant and anti-inflammatory effects of this plant are declared. It was shown, that alterations in the antioxidant status following ulceration, implying that free radicals may be associated with gastric mucosal damage in rats.

The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells may contribute to gastric mucosal damage. Therefore, by scavenging of free radicals, it may be useful for protecting the gastric mucosa from oxidative damage. *Mumijo* as antioxidant, inhibit lipid peroxidation and other free radical-mediated process, and therefore, they protect the
human body from several diseases attributed to the reactions of radicals.[27]

Additionally, Mumijo has significant anti-inflammatory effects in chronic inflammation. There are some evidence showing the effects of Mumijo on increasing superoxide dismutase, catalase, and glutathione peroxidase activities in rats. [28] Mumijo can significantly decrease carrageenan-induced edema in rat paw.[29] In some studies, it has also been reported that Mumijo has anti-allergic effects on histamine release and causes mast cells degranulation.[30] Antioxidant properties of Mumijo extract can be attributed to the presence of dibenzo-pyrones and FA.[24]

On the other hand, anti-anxiety activity and anti-stress effects of Mumijo have roles in healing of gastric ulcer as stated by Frawley and Lad, who indicated that Mumijo had significant anxiolytic and anti-stress activity.[31] Furthermore, due to bacteriostatic and anti-inflammatory action, Mumijo extract facilitates the process of wound cleaning from necrotic tissues, granulation, and epithelization and decreases the period of wound healing.[32]

Overall, the results of the present study showed that Mumijo administration has gastroprotective effects in acetic acid-induced ulcers through decreasing gastric acid and pepsin. Furthermore, the antioxidant and anti-inflammatory effects of this extract can be mentioned as probable mechanisms to improve gastric tissues. However, further studies are required to determine other functional mechanisms of Mumijo.

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References
