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Original Article

Antiulcerogenic Effects of Matricaria Chamomilla Extract in Experimental Gastric Ulcer in Mice

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Abstract

Background: There is extensive variety of chemical compounds with antiulcer activity, which are isolated from medicinal plants. Matricaria chamomilla or Matricaria recutita or German chamomile, also spelled chamomile (MC), is one of the most widely used medicinal plants. In the present study, the extract of MC flowers was evaluated for antiulcerogenic activity and acute toxicity profile.

Methods: To evaluate antiulcer effect of MC extract, 15 female bulb-c mice were divided into three groups (five mice in each group). The first and second groups received 400 mg/kg sucralfate and 400 mg/kg MC extract respectively by the intragastric route. The control group received 1.0 ml distilled water. After 30 min, gastric ulceration was induced by oral administration of 1.0 ml of a 0.3 M solution of HCl in 60% ethanol in all animals. One hour later, the area of the gastric lesions and hemorrhage was measured by stereological method. To evaluate the toxicity of MC extract, 10 male and 10 female mice were divided into control and experimental groups (5 mice in each group). The experimental and control groups received by the intragastric route a single dose of 5000 mg/kg MC extract and water respectively. After 14 days the mice's liver, kidneys, lung, and heart were examined macroscopically and the relative weights (organ/body) were determined. Statistical comparisons between the groups were performed by Mann-Whitney U test.

Results: Oral administration of MC extract at 400 mg/kg can be effective in preventing gastric ulceration in mice and does not produce toxic effects in doses up to 5000 mg/kg.

Conclusion: Matricaria chamomilla can prevent experimental gastric ulcer in mice.

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Keywords ● Matricaria chamomilla ●sucralfate ●gastric ulcer

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Introduction

For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality.[1] The pathophysiology of peptic ulcer is related to an imbalance between aggressive and protective factors in the stomach such as acid–pepsin secretion, mucosal barrier, mucus secretion,

blood flow, cellular regeneration, prostaglandins, and epidermal growth factors.[1] Although hospital admissions for uncomplicated peptic ulcers in some countries have started to decline, there is a striking rise in admissions for ulcer hemorrhage and perforation among the elder. This increase has been attributed to the increased use of non-steroidal anti-inflammatory drugs, alcoholic beverages, cigarettes, and infections with *Helicobacter pylori*.[1] Borrelli and Izzo revealed an extensive variety of chemical compounds isolated from medicinal plants with antiulcer activity.[2] This is an important reason to investigate antiulcer effects in medicinal plants with traditional use in gastric diseases.

Matricaria Chamomilla (MC) *or* Matricaria recutita or German chamomile, also spelled chamomile, is one of the most widely used and well-documented medicinal plants. It has been included in the pharmacopoeia of 26 countries. It has been shown that amino acids, polysaccharides, fatty acids, essential oils, mineral elements, flavonoids, and other phenolic compounds are the main constituents of matricaria chamomilla.[3]

Preparations (e.g. ointments, inhalations, tinctures, teas) of MC are using in modern medicine primarily for their spasmolytic, antiphlogistic, and antibacterial properties.[4-7] One of the chamomile's main roles is its use as a multipurpose digestive aid to treat gastrointestinal disturbances including flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea, and vomiting. Chamomile is thought to heal ulcers and acts as an herbal bitter to stimulate the liver.[5] It has been shown that the extracts from the plants matricaria recutita, singly or combined with other plants have antiulcerogenic activity.[6] But the methodology of ulcer estimation received less attention. Most of ulcer estimations are based on this assumption that the ulcers' shapes are pointed, narrow, or extensive and to obtain a scale for comparison of data, researchers have counted the ulcer numbers. However, it has to be noted that this method is not completely reliable and precise. The aim of the present study was to investigate and compare the gastroprotective effect of chamomile with sucralfate, as a reference antiulcer drug with a precise stereological method. In this study, ulcer area was estimated and a reliable parameter was presented for comparing the data.[8]

Material and Methods

Plant Material

MC flowers were collected in spring of 2008 from Yasuj province (Yasuj, Iran) and verified by Dr. M.R. Panjeh-Shahin (Department of Pharmacology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran) and voucher specimen (No. 1387-1) was deposited in the central herbarium of Shiraz University of Medical Sciences.

Preparation of Aqueous Extract

The flowers were air-dried while protected from direct sunlight and then powdered. The powder was kept in a closed container at 10 °C. For the preparation of the extracts, the collected plant materials were pulverized and extracted with distilled water for 5 min at 100°C. Then they were filtrated, concentrated at 50 °C under reduced pressure using a rotavapor, and lyophilized. The extract was kept at -15 °C until it was used in the experiments. The yield of final extract in terms of starting crude materials was determined to be 10% for this aqueous extract.[7]

Animals

All animal experiments were approved by the Animal Ethics Committees of the Shiraz University of Medical Sciences. To evaluate gastroprotective effects of MC extract, 15 female bulb-c mice weighing between 33-36 g were selected from the laboratory animal center of Shiraz University of Medical Sciences. To evaluate the toxicity of the extract, 10 male and 10 female mice were selected. The animals were housed in cages where humidity and temperature were kept constant. The animals had free access to food and water, and treated according to the standard directive as recommended by the research authorities of Shiraz University of Medical Sciences.

Extract, Drug and Acute Gastric Ulcer Induction

The method described by Markman was employed in the present study. The mice were divided into three groups each including five mice.[8] The first and second groups received by the intragastric route 400 mg/kg sucralfate (reference drug) and 400 mg/kg MC extract, respectively. The positive control group received 1.0 ml distilled water. After 30 min, ulceration was induced by oral administration of 1.0 ml of a 0.3 M solution of HCl in 60% (v/v) ethanol in all animals. The mice were sacrificed by ether inhalation 1 hour later. The stomachs were removed, opened along the greater curvature, and gently rinsed with 0.9% saline solution. Mouse stomach has two main parts: the non-glandular and glandular. Ulceration in this method was induced at the glandular part. The area of the gastric lesions and hemorrhage was measured.

Acute Toxicity

Acute toxicity studies were performed on mice according to the method of Markman.[8] Ten male and 10 female mice were divided into control and experimental groups. Each group included five male and five female mice. The experimental group received MC extract and the control group received water by gavage with the aid of a metal gastric needle at a single dose of 5000 mg/kg of the animal weight. The animals were observed carefully every 2 days to record toxic manifestations, and to measure body mass and water and ration consumption. After 14 days, the mice were sacrificed. The livers, kidneys, lungs, and hearts were observed macroscopically and the relative weights (organ/body) were determined.

Stereological Study

The lesions were divided into erosions and hemorrhagic areas. Erosion was limited to the superficial mucous layer without injury to the vessels but hemorrhagic ulcer deepened to the submucosal layer and injured the vessels and caused bleeding. The lesions were mainly at the lesser curvature of the stomach but it was possible to detect them randomly at the other parts. Lesion areas (erosions and hemorrhage) were estimated using a video-microscopy system made up of a stereomicroscope (CETI, Belgium) linked to a video camera, a computer, and a monitor to determine the lesion relative area at a final magnification of 19 by means of a stereology software designed at our laboratory (Stereological Research Laboratory, Shiraz University of Medical Sciences, Shiraz, Iran).

A point probe consists of a number of crosses (figure 1). As the figure shows right upper corner of each cross is considered as point. If this point lies on the ulcer it will be estimated in final summation. The area associated with point (a/p) was calculated by multiplying d1 by d2, where the “d1” and “d2” are the distance with the neighbor point. The stereological probe of points was superimposed upon the images of the stomach viewed on the monitor. The total area of the stomach or ulcer was calculated by the following formula: Area= ΣP×(a/p), where the "Σp" was the total number points hitting the normal or ulcerative surfaces and "a/p" was the area associated with each point after correction for magnification.

The number of points hitting the lesions and also total gastric surface were counted and multiplied by the (a/p) to achieve the total lesions and gastric area (figure 2).

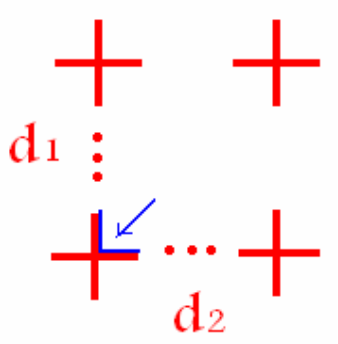

Figure 1: A point probe consists of a number of crosses. Right upper corner of each cross is considered as point (the arrow). The area associated with point (a/p) is calculated by multiplying d1 by d2 where the “d1” and “d2” are the distance with the neighbor point.

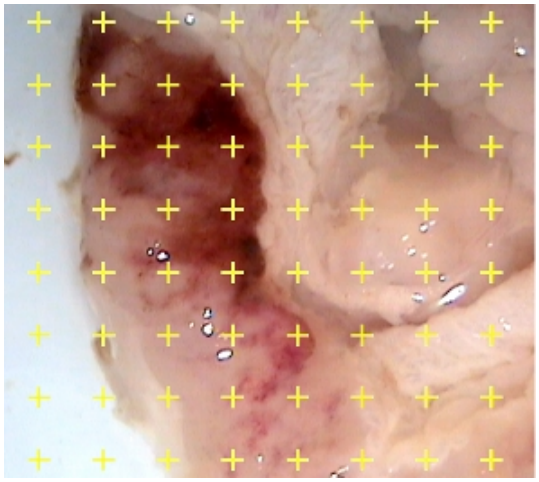

Figure 2: The number of points hitting the lesions and total gastric surface were counted and multiplied by the area associated with point to achieve the total lesions and gastric areas.

The lesion relative area (area of erosion or hemorrhage/total stomach area) was obtained using a point-counting method and the following formula:[9]

Lesion relative area = $\Sigma P_{lesion}/\Sigma P_{stomach} = \Sigma A_{lesion}/\Sigma A_{stomach}$

‘ΣP_{lesion}’ and ‘$\Sigma P_{stomach}$’ are the number of test points located in the lesion area and the stomach surface, respectively. ΣA_{lesion} and $\Sigma A_{stomach}$ are the total area of lesion area and the stomach surface, respectively. The mean numbers of 445 points were laid on the live image of the stomach mucosal surface in each mouse.[9]

Statistical Analysis

The data was reported as mean ± standard deviation. Statistical comparison between the groups was performed by Mann-Whitney U test. $P < 0.05$ was considered as significant.

Results

Gastro Protective Evaluation of MC Extract

Comparison of the relative area (%) of gastric erosion and hemorrhage (lesion area/stomach area) in control, sucralfate and MC extract treated mice showed that MC possesses gastroprotective effects. No hemorrhage was observed in MC extract and sucralfate groups (table 1). Figure 3 shows the stomach of the positive control mice (no treatment), and those treated with sucralfate and MC extract.

Toxicity Evaluation of MC

The acute toxicity test after oral administration of 5000 mg/kg of MC extract revealed no toxicity at this dose. There were no significant alterations in water or food consumption, or body weight during the experiment. The body weights, relative weights of the kidneys, livers, lungs, and hearts were not statistically different from those of the control group (tables 2 and 3).

Table 1: Mean (standard deviation) relative area (%) of gastric erosion and hemorrhage (lesion area/stomach area) in positive control, sucralfate, and matricaria chamomilla treated mice.

	Positive control	Sucralfate	Matricaria Chamomilla	P value
Erosion	0.13 ±0.03	0.07 ± 0.02 *	0.04 ± 0.03 *	<0.02
Hemorrhage	0.01 ±0.02	0.0	0.0	

* p<0.02 control *vs*. sucralfate or matricaria chamomilla treated mice

Table 2: Mean of body weight (g) and relative organ weight [(organ weight/ body weight) ×100] of the liver, kidney, lung and heart of the male control and experimental groups to evaluate the acute toxicity of matricaria chamomilla after oral administration of the extract (5000 mg/kg). The values are mean ± standard deviation.

	Male Control	Male Experimental	P value
Body weight	36.2 ± 2.3	34.8 ± 2.9	0.54
Liver	7.4 ± 2.0	8.6 ± 1.9	0.31
Kidney	0.83± 0.09	0.95 ± 0.08	0.06
Lung	1.4 ± 0.17	1.3 ± 0.23	0.84
Heart	0.55± 0.06	0.62 ±0.77	0.09

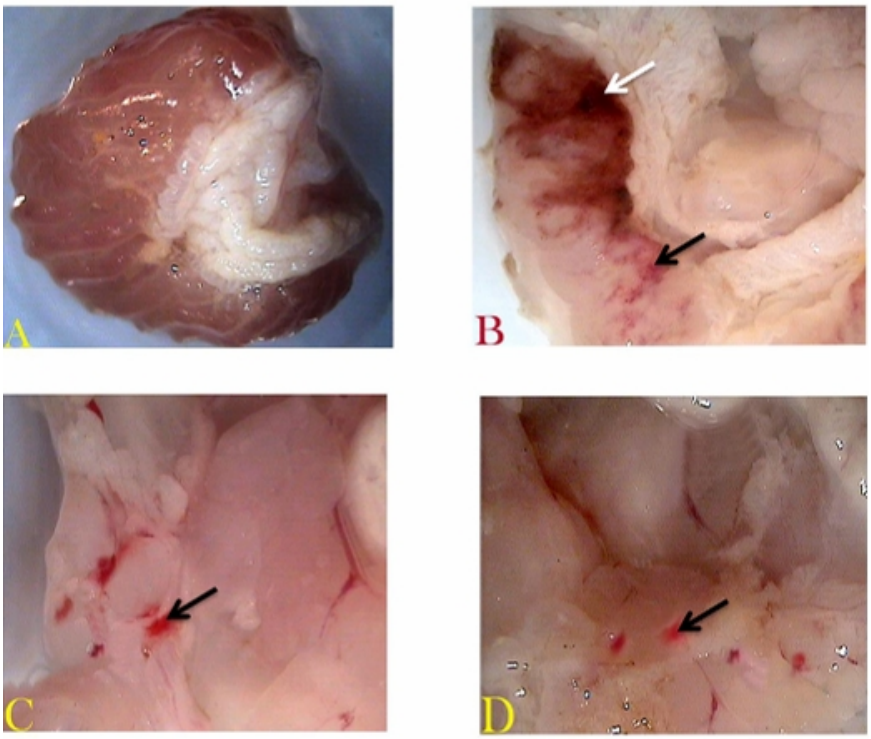

Figure 3: The normal stomach of mouse (A), ulcerated stomach without any treatment (B), ulceration treated by sucralfate (C) and ulceration treated by MC extract (D). The white structure in the "A" shows the non-glandular part of mouse stomach. White and black arrows indicate hemorrhagic and erosive areas, respectively.

Table 3. Mean of body weight (g) and relative organ weight [(organ weight/ body weight) ×100] of the liver, kidney, lung and heart of the female control and experimental groups to evaluate the acute toxicity of matricaria chamomilla after oral administration of the extract (5000 mg/kg). The values are mean ± standard deviation.

	Female Control	Female Experimental	P value
Body weight	33.0 ± 2.7	33.6 ± 2.3	0.69
Liver	7.4 ± 1.0	7.5 ± 0.90	0.84
Kidney	0.78 ± 0.13	0.81 ± 0.17	0.31
Lung	1.5 ± 0.18	1.4 ± 0.05	0.84
Heart	0.44± 0.11	0.49± 0.11	0.69

Discussion

The present study showed the gastroprotective effects of chamomile flower extract on acute experimental gastric ulcer in mice. In addition, the toxic effects of the extract were evaluated.

MC extract contains many components that may exert antiulcer effects. Amino acids, polysaccharides, and fatty acids are some of its constituents. The flowers of chamomile contain 1-2% volatile oils including α-bisabolol, α-bisabolol oxides A & B, matricine, a variety of mineral elements including manganese and magnesium.[3] Flavonoids and other phenolic compounds have been identified in various parts of the chamomile flower head. Apigenin, quercetin, patuletin, luteolin and their glucosides are the major flavonoids present in the flower. The presence of large amounts of cinnamic acid derivatives, frolic and caffeic acid, as well as other unidentified phenolic derivatives of the total flower has been investigated. All of the constituents, which have also been found in other plants, may have therapeutic effects.[10-16]

Here we reported the gastroprotective effects of chamomile flower extract on acute experimental gastric ulcer in mice. Our results are consistent with Khayyal and co-workers.[6] They have shown that the extracts from the plants Iberis amara, Melissa officinalis, Matricaria recutita, Carum carvi, Mentha x piperita, Glycyrrhiza glabra, Angelica archangelica, Silybum marianum, and Chelidonium majus, alone or in combination have antiulcer activity.[6] They have reported that the cytoprotective effect of the herbal extracts could be partly caused by their flavonoid content and to their free radical scavenging properties.[6]

MC extract may be able to eradicate *Helicobacter pylori*. Stamatis and colleagues have studied the anti-*Helicobacter pylori* effect of some plant extracts including Chamomilla recutita and have proved that it has been effective against one standard strain and 15 clinical isolates of *H. pylori*.[17] MC extract has been used in the treatment of oral aphtus ulcer. Ramos-e-Silva and others showed that water extract of Chamomilla recutita had analgesic effect in oral aphtus ulcer.[18]

Our results showed that antiulcer effect of chamomile flower extract was similar to that of sucralfate. Sucralfate has a complex effect on the luminal and mucosal environment of the stomach and duodenum. Some of these actions are important in healing the ulcers whilst others are important in preventing subsequent ulcer relapse. Although sucralfate has little direct effect on acid secretion, it has been shown that it increases mucosal resistance against damaging agents such as ethanol and aspirin.[19] Some studies have shown that this protective action may be related to the drug's effect on various protective zones such as the 'mucous-bicarbonate' barrier, mucosal hydrophobicity, epithelial cell function and morphology, and mucosal blood flow.[19] These multiple actions of sucralfate are in part related to direct interaction between the drug or its components and gastroduodenal tissues, and also to the effects on various mediators of tissue injury and repair.[19]

Conclusion

Oral administration of Matricaria chamomilla extract at 400 mg/kg can be as effective as sucralfate in preventing experimental gastric ulcer and dose not produce toxic effects in mice, in doses up to 5000 mg/kg.

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Conflict of Interest: None declared

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