Fermentation Potentiates Antimotility Properties of Chamomile Ligulate Flower Extracts

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***Matricaria recutita* L. (Asteraceae) is one of the most ancient medical herbs, although the therapeutic uses and health benefits of chamomile are based largely on tradition. The present study aimed to investigate the effects of chamomile extract on the intestinal motility. Due to the fact that preparation techniques could significantly alter the glucoside content in the chamomile, we also explored the possible differences in the effects on gastrointestinal motility between native and fermented chamomile extract. Thirty minutes after the administration of saline or chamomile ligulate flower extracts, all animals were orally administered charcoal meal by gavage (0.6 ml emulsion–0.4 g *Carbo medicinalis* and 0.2 g *Gummi arabicum* in 10 ml of olive oil). Animals were sacrificed by cervical dislocation 30 min after charcoal meal administration. Intestinal motility was estimated according to the distance between Carbo medicinalis and pylorus in centimeters. Both native and fermented extract decreased intestinal motility compared to control. Native chamomile extract (5 mg/kg) and fermented chamomile extract (2.5 mg/kg) significantly decreased intestinal motility compared to control group (NE5=27.41±2.79, P<0.05; FE2.5=28.43±14.63, CON=40.34±5.46, P<0.05). These findings confirmed the basis of the use of chamomile extracts in traditional medicine for the treatment and management of digestive system disorders.**

Key words: Native chamomile ligulate flower extract, fermented chamomile ligulate flower extract, intestinal motility, apigenin

Matricaria recutica L. is a well-known medicinal plant species from Asteraceae family. Chamomila is one of the most common herbs used for medicinal purposes whose infusion is prepared from dried flowers for a variety of healing applications[1]. Approximately 120 secondary metabolites have been identified in chamomile. Due to its richness in therapeutically active compounds, this plant presents many beneficial health effects as antioxidant, neuroprotective, antiallergic, antiinflammatory, antimicrobial and anticancer activities[2,3]. This species is especially popular and widely used in traditional medicine for the treatment of numerous gastrointestinal disorders[4].

Like many other herbal preparations used in traditional medicine, the therapeutic uses and health benefits of chamomile are based largely on tradition rather than on scientific evidences. Hence, the present study was aimed to investigate the effects of chamomile ligulate flower extracts on the intestinal motility. Due to the fact that preparation techniques could significantly alter the glucoside content in the chamomile, we also explored the possible differences in the effects on gastrointestinal motility between native and fermented chamomile ligulate flower extracts.

Chamomile was grown at the Institute of Field and Vegetable Crops, Backi Petrovac, Serbia. Fermented and native ligulate flower extracts were prepared according to the methodology of Pekic and Zekovic[5] and content of free apigenin and apigenin-7-0-glucoside were determined by Cvetanovic *et al*.[6].

Experiment was carried out on 10 w old male NMRI-Haan mice, body weight 20-25 g, bred in the Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, Novi Sad, Serbia. Animals had free access to water and food with 12 h successive light and dark periods. Laboratory animals were under human care in accordance with guidelines in the European Union Directive of 22 September 2010 (2010/63/EU). The study was approved by the Animal Ethics Committee of the University of Novi Sad and the Ministry of Agriculture of the Republic of Serbia (No.168).

The animals were randomly divided into test and control groups and orally administered as follows (each group consisting of 6 (six) animals): 1. CON- control group (saline); 2. NE2.5 group: native chamomile extract (2.5 mg/kg); 3. NE5 group: native chamomile extract (5 mg/kg); 4. FE2.5: fermented extract of chamomile (2.5 mg/kg); 5. FE5: fermented extract of chamomile (5 mg/kg).

30 min after the administration of saline or chamomile

Accepted 26 October 2016
Revised 19 October 2016
Received 08 September 2016
Indian J Pharm Sci 2016;78(5):691-694

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extract, all animals were orally administered charcoal meal by gavage (0.6 ml emulsion-0.4 g carbomedicinalis and 0.2 g *G. arabicum* in 10 ml of olive oil). Animals were sacrificed by cervical dislocation 30 min after charcoal meal administration. Intestinal motility was estimated according to the distance between Carbo medicinalis and pylorus in centimeters. The distance travelled by the charcoal meal was expressed as a percentage of the total length of the small intestine from pylorus to caecum. The animals were without food 24 h before the beginning of the experiment.

The level of the significance was assessed with one way analyses of variance (ANOVA) followed by Fisher multiple comparisons test. All data were expressed as mean±standard deviation (SD). A value of $P<0.05$ was considered to be significant.

Although *M. recutita* L. is traditionally used in treatment of gastrointestinal disturbances, the experimental pharmacology of the inhibition of gastrointestinal motility of this herb is relatively unexplored. In the present study, we demonstrated that acute pretreatment with both native and fermented ligulate flower extracts decreased intestinal motility compared to control (Table 1).

Native chamomile extract (5 mg/kg) and fermented chamomile extract (2.5 mg/kg) significantly decreased intestinal motility compared to control group (NE5=27.41±2.79, CON=40.34±5.46, $P<0.05$; FE2.5=28.43±14.63, CON=40.34±5.46, $P<0.05$). Significant difference has also been found between the native extracts of concentration 2.5 and 5 mg/kg, as well as between native extract of concentration 2.5 mg/kg and fermented extract of concentration 2.5 mg/kg. The effect of *M. recutita* separate and in a combination with *Melissa officinalis* and *Foeniculum vulgare* on upper gastrointestinal transit was examined in mice. Group of mice treated with *M. recutita* exclusively showed a significant reduction of the motility compared to the control group, but the decrease was greater in the group simultaneously treated with the herbal formulation[7].

TABLE 1: EFFECTS ON INTESTINAL MOTILITY IN MICE

Group	Distance of the charcoal meal from the pylorus/total length of small intestine % (cm) (x±sd)
CON	40.34±5.46
NE2.5	40.03±9.61
NE5	27.41±2.79[a,b]
FE2.5	28.43±14.63[a,b]
FE5	36.25±9.73

[a]$P<0.05$ compared to control group, [b]$P<0.05$ compared to native chamomile ligulate flower extract of 2.5 mg/kg

One of the major constituents of the chamomile flowers includes several phenolic compounds, primarily the flavonoids apigenin, quercetin, patuletin as glycosides and various acetylated derivatives. Among flavonoids, apigenin is the most promising compound. It is present in very small quantities as free apigenin, but predominantly exists in the form of various glycosides[8,9]. The fermentation process activates β-glucosidase naturally present in chamomile ligulate flowers. The action of this enzyme breaks the glycoside bond between the aglycone and the sugar component, releasing a free aglycone[6]. In our survey, native chamomile extract in a dose of 5 mg/kg had similar decrease in intestinal motility as fermented extract in a dose of 2.5 mg/kg. This can be explained with different content of apigenin in these two extracts. Actually, the concentration of apigenin-7-O-glucoside was noticeably higher in fermented (0.42 mg/ml) in comparison with native chamomile extract (0.25 mg/ml), while free apigenin was much more prevalent in fermented extract (0.78 mg/ml) in comparison with native extract (0.11 mg/ml). Di Carlo *et al*., has shown that apigenin, at 12.5-50 mg/kg administered i.p. reduced both small and large intestinal transit time in mice with castor oil-induced diarrhea. On the other hand, the effect of fermented extract in a concentration of 5 mg/kg has lower decrease of intestinal transit in comparison with the same extract in smaller concentration, as well as in comparison with native extract in a concentration of 5 mg/kg. According to these results, it seems that apigenin has decreasing effect on gastrointestinal motility up to a certain concentration, after which this compound even has the opposite effect.

In conclusion, our results clearly demonstrated that the chamomile ligulate flower extracts decrease upper gastrointestinal transit in mice and that fermented extract has stronger effect on reduction of gut motility in comparison with native chamomile extract, although it refers up to certain concentrations.

Acknowledgements:

The authors are especially grateful to prof. dr Zoran Zekovic, Faculty of Technology, Novi Sad, for his support in supplying plant material.

Financial support and sponsorship:

Financial support and sponsorships: This work was supported by the Ministry of Science and Technological

development, Republic of Serbia, project No. 41012 and by the Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina (project No. 114-451-698/2015-02).

Conflicts of interest:

There are no conflicts of interest.

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