

The Therapeutic Effect of *Carum copticum* Seed Aqueous Extract on Peptic Ulcers Induced by Ibuprofen in Rat

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Article information	Abstract
<p>Article history: Received: 13 Mar 2011 Accepted: 23 Apr 2011 Available online: 17 Aug 2011</p> <p>Keywords: Carum copticum Gastric Ulcer Rat Ibuprofen</p> <p>*Corresponding author at: Department of Physiology, Zahedan University of Medical Sciences, Zahedan, Iran. E-mail: komeili@zaums.ac.ir</p>	<p>Background: Non-steroidal anti-inflammatory medicines are widely used today and of their side effects is gastric ulcer. This study is aimed at examining the effect of <i>Carum copticum</i> aquatic extract on healing gastric ulcers induced using ibuprofen in an animal model.</p> <p>Materials and Methods: Thirty heads of adult Wistar female rats were used in this empirical study. Gastric ulcer was induced using ibuprofen. The animals were randomly divided into 5 groups. Then omeprazole or <i>Carum copticum</i> plant extract was administered (125, 250 and 500mg/kg doses) twice a day for two weeks. In the end, the number and area of the animals' gastric ulcers were assessed. To examine the side effects of the medicine on liver, the amount of liver enzymes Aspartate transferase (AST) and Alanine transferase (ALT) was measured in the animals' serum.</p> <p>Results: <i>Carum copticum</i> plant aqueous extract had a significant effect on healing gastric ulcers comparing to the control group ($p<0.05$). The mean number and area of the gastric ulcers in the groups receiving the extract were significantly less than negative-control group ($p<0.01$). The amount of liver enzymes had also significantly ($p<0.05$) increased in the groups receiving the extract (250 and 500 mg/kg doses).</p> <p>Conclusion: <i>Carum copticum</i> plant seed extract is effective in healing gastric ulcers and its effect is comparable with omeprazole. So, recognition of the extent and mechanism of the effect requires further studies.</p> <p>Copyright © 2012 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

The ulcers of alimentary tract and in particular gastric ulcers can be induced by acid secretion increase for different reasons like taking non-steroidal anti-inflammatory medicines, alcohol abuse, long term starvations, bad nutritive habits and severe permanent stresses [1]. According to the World Healthcare Institute, one out of 10 individuals living in the U.S.A will be a sufferer of the gastric ulcer and 15000 deaths happen annually as a result of the disease. Economic effects of the disease are considerable (\$10 milliards annually in the U.S.A) [2, 3].

Non-steroidal anti-inflammatory medicines are of the most widely used drugs across the world and many studies show that there a relationship between taking them and emergence of gastric ulcers in Western communities [4]. Bleeding and mucus impairments of the stomach are among the commonest side effects of long term consumption of the drugs and of the major challenges of the medicinal science so that non-steroidal anti-inflammatory medicines have been recognized as the second reason for optic ulcer after *Helicobacter pylori* [5].

Gastric ulcer treatment using chemical medicines such as Omeprazole, metronidazole, and ranitidine are expensive and has many side effects and issues like

autoimmunity phenomenon and the probability of the impairments return after not taking the drugs anymore. So there has been an extensive attempt to find out natural and herbal effective compounds existing for treating gastric ulcers [6].

Carum copticum of Apiaceae line originating from Asia and growing naturally or planted in India, Iran, Afghanistan, Egypt [7,8]. Eastern Iran and Baluchistan are *Carum copticum*-rich. The usable section of the plant is its fruit containing high levels of thymol. The fruit is small, oval in yellowish brown with a smell like thymol [9]. Also, extract of the plant has been used as antihistamine in traditional medicine [10]. Regarding the antioxidant and anti-inflammatory effects of *carum copticum*[7,9], it is likely that the extract of the plant is effective in treating gastric ulcer, as well. According to the abovementioned and side effects of non-steroidal anti-inflammatory medicines, further studies are required for finding appropriate alternatives in treating gastric ulcers. So, considering outspread of taking ibuprofen and also its increasing consumption as well as mucosal impairments induced by excessive ibuprofen use, we decided to study the healing effect of *carum copticum* aquatic seed extract on the gastric ulcer induced by ibuprofen in rats.

Materials and Methods

The study was carried out empirically on rats. First, 30 heads of female Wistar rats (in body weight range 150 ± 30 g) were selected and kept separately in single cages to adapt themselves to new conditions. During the experiments temperature, they were kept in 12:12-h light: dark cycle and temperature range of $22\pm 3^\circ\text{C}$. Then, the animals were randomly divided into five sub-groups: negative control, positive control, and three groups of receiving the extracts (125, 250 and 500 mg/kg doses).

To induce gastric ulcers, fasting animals were kept in specific cages with a mesh floor for 48h. In the meantime, they were fed by sodium chloride (0.2%) and sucrose (2%) to prevent from the body water loss. Then, they received ibuprofen (Iran Pastor Inst.) with dose 400mg/kg using gavage form. In the negative control group, no intervention was done after ulcer induction and they were assessed like other groups at the end of two weeks regarding the number and area of the gastric ulcers. Water and food intake of all groups were carried out and primary and final weights of the groups were recorded. The animals orally received about 0.5ml normal saline a day. In the end of the two weeks, the stomach of the animals were dislodged and microscopically examined concerning gastric ulcers.

In positive control sub-group, the animals received omeprazole (Iran Pastor Inst.) twice a day (200 mg/kg) and in gavage form. In the end of the two weeks, the stomach of the animals were dislodged and microscopically examined concerning gastric ulcers. In the groups under study, after inducing the ulcers, the animals received *carum copticum* aqueous extract (125, 250 and 500 mg/kg doses) twice a day for two weeks in gavage form. In the end of the two weeks, the stomachs of the animals were dislodged and microscopically examined concerning gastric ulcers. Computer software was employed to calculate ED50 (Effective Dose 50%). The amount of liver enzymes in the serum of animals was assessed using laboratory routine assays.

Extract preparation assay: Plant seeds, purchased from grocery, were identified first by Herbarium expert of Department of Biology of Sistan and Baluchestan University, Zahedan, Iran. Then, it was dried in shadow, after being ground, each time the resulting powder (20g) was solved in distilled water (100ml) and then placed in Soxhlet device for 8h. The extract was collected and filtered using Whatman filter paper No.1 and poured into glass containers. The solvent evaporated at 37°C . The residue of dried extract was poured into a glass container and weighted. When required, the resulting powder was weighted based on the used dose, solved in normal saline solution and then fed into the animals in gavage form.

Gastric ulcer examination assay: after dislodging the stomach, it was incised from its large curve and, after being washed by normal saline, fixed on anatomy tray and microscopically examined using magnifier. Then, it was photographed using fixed position camera and the photos were transferred to computer and the area of ulcers assessed. The ulcers with an area $\geq 1\text{mm}$ were detected in

each stomach and the area of the stomach with ulcers was calculated [11]. Measurement of the number and area of the ulcers was conducted using Blind technique. Using the following formula, Therapeutic Index was calculated for each of the animals.

TI= the number of ulcer in control group – the number of ulcers in test group/the number of ulcer in control group $\times 100$

Data was analyzed using SPSS-17 Software and statistical tests of quantitative variables (One-Way ANOVA and then Tukey test). And, the statistical difference was considered $p < 0.05$. The study is carried out in 2009 and in laboratory of Physiology Department, Medical School of Zahedan and approved by the Medical Ethics Committee of Zahedan University of Medical Sciences.

Results

The value of ED50 was determined as 48.2mg/kg for *carum copticum* aquatic extract in treating ibuprofen-induced gastric ulcer in rat. At the end of treatment course, the number and percentage of the animals with ulcers were determined in each group. In negative and positive control and the receiving (125 mg/kg) groups, all animals had ulcers, whereas in the groups receiving the extract (250, 500 mg/kg) respectively 50% and 30% of the animals had ulcers.

Mean number and area of the ulcers in different groups were also determined and results are represented in Figure 1. Furthermore, therapeutic index of different test groups is calculated comparing to negative control group and showed in table 1. It increases as the medication dose increases and it is comparable with therapeutic effects of omeprazole even in minimum doses and has significantly ($p < 0.01$) reduced the number and area of the ulcers with all doses of the extract comparing to negative control group.

Table 1. Determining mean number and area of the gastric ulcers (in mm^2) and therapeutic index in the groups under study

Groups	Ulcer number (Mean \pm SD)	Ulcer dimension (Mean \pm SD)	Therapeutic index(TI)
Negative control	9.2 \pm 2.1	37.2 \pm 11.8	0
Positive control	3.5 \pm 1.1	13.5 \pm 4.4	62%
Extract 125 mg/kg	2 \pm 0.89*	5.2 \pm 2.6*	76.5%
Extract 250 mg/kg	0.8 \pm 0.67*	1.7 \pm 1.5*	92.7%
Extract 500 mg/kg	0.5 \pm 0. *34	1.5 \pm 1*	96.7%

* $p < 0.01$ comparing to negative control group

Liver enzymes levels in different groups comparing to negative control group showed that taking the extract (250, 500mg/kg doses) has established a significant increase ($p < 0.05$) in AST and ALT enzymes, but dose

125mg/kg did not make any significant changes (Figure 2).

Table 2. Mean AST and ALT enzymes of liver in the groups under study

Group	Enzyme	AST(IU/L)	ALT(IU/L)
		Mean ±SD	Mean ±SD
Negative control		150.5±39.9	69.8±15.8
Omeprazole (200 mg/kg)		135.7±28.2	66.7±20.3
Extract 125 mg/kg		131.8±15.6	76.7±9.8
Extract 250 mg/kg		180.6± 21.5*	91.2±10.6*
Extract 500 mg/kg		220.8±18.3*	118±13.9*

* $p < 0.05$ comparing to negative control group

Discussion

The study results showed that consumption of *carum copticum seed* extract improves peptic ulcers induced by Ibuprofen in the rat stomach, and this effect is dose dependent i.e. the more is the dose, the more is the cure. The results showed that the healing effect of *carum copticum seed* extract was comparable with omeprazole drug and even the therapeutic effects with different doses of *carum copticum seed* extract were more than that of Omeprazole (Table 2).

The results indicated that the effect of extract is dose dependent and the doses much lower than omeprazole had almost similar therapeutic effect on gastric ulcer. This could be because of the strength of the extract used; it is clear that whatever the medication dose is less, there are likely less side effects.

Extracts used in doses of 250 and 500 mg per kilogram of body weight caused to reduce significantly the number of ulcers and percentage of animals that had stomach ulcers (Table 1). Using the extract in each three doses led to significant and meaningful decrease in number and area of the wounds created when compared to the negative controls, in addition, doses of 250, and 500 mg/kg body weight showed significant effect of improvement compared to positive control group and also considerably better effects had been shown in reducing the number of wounds, and area of ulcers (Table 1). The therapeutic index can be seen in table 2.

Carum copticum seed extract therapeutic mechanism in the treatment of gastric ulcers is not clear and the aim of this study was not about its mechanism. But to compare

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its therapeutic effects with popularly known drug Omeprazole and thus it can be commented that this extract perhaps had the similar effect as that of Omeprazole in affecting the stomach acid secretion pump. However, to confirm this theory, future studies should be held to measure the amount of secretion of gastric acid after consumption of *carum copticum seed* extract, and thus to allow definitive comment on these mechanisms.

Also, according to the mechanisms involved in the pathogenesis of oxidative stress ulcers created by non-steroidal anti-inflammatory drugs [4] may be that *carum copticum seed* extract through its antioxidant effects [7] played an effective role in gastric wound healing.

Using extract with a dose of 125 mg per kg did not have any significant effect on the amount of liver enzymes, but at doses 250 and 500 mg/kg the amount of ALT enzyme was significantly elevated. Left behind significantly an increase in the amount of AST enzymes, and this elevation of enzyme level may be due to adverse effect of high dose of the extract in the liver tissue (Figure 3). There are not many reports regarding the effect of the seed extract on liver enzymes, and it is difficult to compare the results of this study with those of previous researches. Only one of these studies showed that the *carum copticum seed* extract reduced the toxicity effect caused by Carbon Tetra Chloride on the liver in the rats, and this effect of reducing the amount of AST and ALT makes the study results in a dose 500 mg/kg inconsistent and this may be related to differences in extract dosage [12]. According to the study, the effect of *carum copticum seed* extract is useful in treating ulcers caused by Ibuprofen, and future studies are needed to approve its action of mechanisms. Because the therapeutic effect of the extract in doses of 250 and 500 did not have significant differences compared to 125mg dose and, on the other hand, the therapeutic effect of doses of 125 mg compared to Omeprazole is acceptable, consumption of doses over 125 mg/kg due to hepatic adverse effects is not recommended.

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