

Gastroprotective effects of aqueous extract of *Chamomilla recutita* against ethanol-induced gastric ulcers

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ABSTRACT

الأهداف: اختبار فعالية خلاصة نبتة الشومال ريكروتيتا (المعروفه شعبياً باسم البابونج) في حماية المعدة من حصول القرحة التي يحفزها تناول كحول الإيثانول لدى مجموعة من الجرذان الذكور من النوع ألبينو ويستر.

الطريقة: تمت هذه الدراسة في معامل قسم علم وظائف الأعضاء بكلية الطب في جامعة الملك خالد، أبها، المملكة العربية السعودية وذلك خلال الفترة من يناير إلى فبراير 2009م. شملت هذه الدراسة 60 جرذ أبيض من النوع ألبينو ويستر حيث تم تقسيمها إلى 5 مجموعات وهي كالتالي: مجموعة التحكم (المجموعة التي لم تُصَب بالقرحة) وتم إعطاؤها الماء المقطر لمدة 28 يوماً، أما المجموعات من 2 إلى 5 فتم علاجها بجرعات مختلفة من نبتة البابونج على النحو التالي: صفر، و 0.5، و 1، و 2 جم/كجم على التوالي لمدة 27 يوماً. لقد جرى تخفيف قرحة المعدة في المجموعات 2 إلى 5 بإعطائهم ما يعادل 70% من كحول الإيثانول عن طريق الفم مرة واحدة يومياً في اليوم 28 من التجربة. لقد تم تشريح معدة الجرذان وفحصها تحت المجهر من أجل تحديد الآفات التي حصلت في الغشاء المخاطي وكذلك حساب مؤشر التقرح المعدي وتقدير معدل المادة المضادة للتأكسد والمعروفة باسم الفلوتاتوين لدى الحيوانات التي تضمنتهم الدراسة.

النتائج: أشارت النتائج إلى أن مؤشر تقرح المعدة قد انخفض عند الجرذان التي تم علاجها بخلاصة البابونج، وكان هذا المؤشر يتناسب عكسياً مع كمية الجرعة المعطاة. لقد انخفض معدل مادة الفلوتاتوين عند الجرذان المصابة بالقرحة بفعل مادة الإيثانول، غير أنه تم تفادي هذا النقص بإعطاء الحيوانات من خلاصة هذه النبتة. وأخيراً بين الفحص المجهرى لأنسجة المعدة أن علاج الجرذان بخلاصة البابونج قام بتقليل أو القضاء كلياً على التغيرات السلبية التي أحدثها الإيثانول بما فيها تغير ترتيب الخلايا المعدية، وتغير شكل الغدد مع تآكل الغشاء المعدي المخاطي وكذلك اختراق الغشاء المخاطي العضلي المعدي.

خاتمة: تؤكد هذه الدراسة على قدرة نبتة الشومال ريكروتيتا (البابونج) في حماية المعدة من القرحة التي يحفزها كحول الإيثانول وذلك عن طريق المادة المضادة للتأكسد والمعروفة باسم الفلوتاتوين.

Objectives: To investigate the gastroprotective effects of an orally administered aqueous extract of *Chamomilla recutita* (ACE) against ethanol-induced gastric ulcers in male Wistar rats.

Methods: This study was performed during January and February 2009, in the Research Labs in the Department of Physiology at the Medical School, King Khalid University, Abha, Kingdom of Saudi Arabia. Sixty white albino rats were divided into 5 groups. Group 1 (control group) was treated with deionized water for 28 days; animals in group 2 to group 5 received zero, 0.5, 1, or 2 gm/kg ACE for 27 days. Stomach ulcerations were induced by orally administering a single dose of 70% ethanol on day 28. Lesions in the gastric mucosa were examined macroscopically to calculate the ulcer index (UI) and estimated glutathione (GSH) for each animal.

Results: Compared to non-ACE treated rats, the UI decreased significantly in a dose-dependent manner in treated animals. Furthermore, GSH levels fell significantly after ethanol treatment; this decrease was prevented by ACE treatment. However, daily treatment of rats with the maximum ACE dose actually led to an increase in GSH levels. Histological examination revealed that ACE treatment alleviated, or completely resolved ethanol-induced degenerative alterations, including disorganization of cell nuclei and gland morphology with erosion in the gastric mucosa and interrupted muscularis mucosa.

Conclusion: This study provides evidence for the regulation of ACE-mediated gastroprotection against ethanol-induced ulceration by GSH.

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Gastric ulcers are benign lesions that develop at sites, in which the mucosal epithelium is exposed to acidic gastric juice. It is now considered a modern-age epidemic, affecting approximately 10% of the global population.¹ Gastric ulcers are induced by many factors, such as stress, smoking, alcohol, nutritional deficiencies, and noxious agents. Most gastroprotective drugs, such as antacids, H₂ receptor blockers, anticholinergics, or proton pump inhibitors, act on the offensive factors to neutralize acid secretion.¹ Clinical reports on these drugs have demonstrated that adverse effects and drug interactions occur during ulcer therapy.² Thus, there is a need for more effective and less toxic antiulcer agents. Plants are an alternative source of new drugs. There is a rich abundance of plants that are reputed in traditional medicine to have antiulcer properties.³ Several plant extracts that have been used traditionally to treat gastric ulcers have also been tested for their cytoprotective effects in experimental animals; they include *Zingiber officinalis*,⁴ *Punica granatum*, *Trigonella foecum*,⁵ *Matricaria chamomilla*, *Matricaria recutita*⁶ and *Althaea officinalis*.⁷ Thus, plants are likely to continue to be a valuable source of new molecules that, after chemical manipulation, provides novel and improved antiulcer drugs.⁸ *Chamomilla recutita* is one of the most popular and well-documented herbal medicines in the Arabian area, where it is called Pabonege. Its flower heads are used internally, and topically to alleviate or cure many ailments, particularly those that are related to inflammatory conditions.⁹ Although it is used in different pharmaceutical preparations,¹⁰ *Chamomilla recutita* is primarily consumed as an infusion for sedative, and anxiolytic purposes;^{11,12} as a digestive aid to treat gastrointestinal conditions, especially in babies, and small children;¹³ and in domestic medicine in moist cotton pads for topical application to heal skin wounds and cuts.¹³ Few articles have studied the protective effect of *Chamomile* against gastric ulcer.¹⁴ To determine whether *Chamomilla recutita* has antiulcer effects, we examined it in a rat model of gastric ulcer. Thus, the aim of our study was to investigate the protective effect of aqueous extracts of *Chamomilla recutita* (ACE) on ethanol-induced gastric mucosal lesions in male Wistar rats and to evaluate the antioxidant potential of *Chamomilla recutita* with regard to reduced glutathione (GSH) levels.

Methods. *Preparation of the extract.* This study was performed from January to February 2009 in the Research Labs of the Department of Physiology at the Medical School, King Khalid University, Abha, Kingdom of Saudi Arabia. *Chamomilla recutita* flowers were purchased from a local market in Abha region, southwest of the Kingdom of Saudi Arabia. The plant was identified by the Department of Pharmacognosy staff at the College of Pharmacy of King Khalid University. The

flowers were washed with distilled water and dried. The dried flowers were ground to a powder and extracted by maceration in distilled water (200 g/1500 mL, w/v) for 2 days at 37°C. The extract was filtered and the excess water was evaporated under reduced pressure in a rotary evaporator, producing a dry extract (18 g of solid residue). The extract was dissolved in distilled water to a final stock concentration of 1 g/ml for further use.

Acute toxicity test. To measure acute toxicity, we used a modified method of Seth et al.¹⁵ Rats were divided into 7 groups. The control group received 1 ml of distilled water, and other groups received a solution of 1 ml of distilled water that contained 0.25, 0.5, 1, 2, 4, or 8 gm of the aqueous extract per kg body weight. Immediately after administration, the animals were observed for 4 hours and twice daily for 7 days for signs of behavioral changes and mortality.

Animals and experimental design. Rats were housed in 5 plastic cages (6 rats per cage) in a climatically controlled room temperature 22°C, and humidity, 55%, with 12 hours light/12 hour dark cycles. Water and standard pellet diet were available ad libitum throughout the experimental period. The rats were acclimatized for 10 days prior to the experiment. All studies were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The design of the experiments was approved by the Physiology Department Research Committee of King Khalid University. Albino rats aged between 14-16 weeks each weighing approximately 200 g, were obtained from the Animal House at the College of Medicine, King Khalid University in Saudi Arabia and used in this study. Rats were separated into 5 groups. Group 1 (control group) received 1 ml of distilled water for 28 days. The rats from group 2 to group 5 received zero, 0.5, 1 and 2 gm/kg of ACE for 27 days and on the 28th day ulceration was induced in their stomach by administering a single dose of 2 ml/kg 70% ethanol.³ All treatments were administered orally using a stainless steel cannula. At the end of each experiment, rats were humanly killed and their stomachs were removed for histopathological examination. The lesions in the gastric mucosa were examined macroscopically to calculate the ulcer index (UI) and GSH.

Gastric UI. To quantitatively measure the protective effect of ACE on gastric induced ulcers, UI were calculated. The stomach was cut open along the greater curvature. The lesions were examined macroscopically using hand lens and UI was calculated by using the following equation:¹⁶

$$UI = \frac{\text{Total ulcer score (mm}^2\text{) in a group of rats}}{\text{Number of ulcerated animals in the same group}}$$

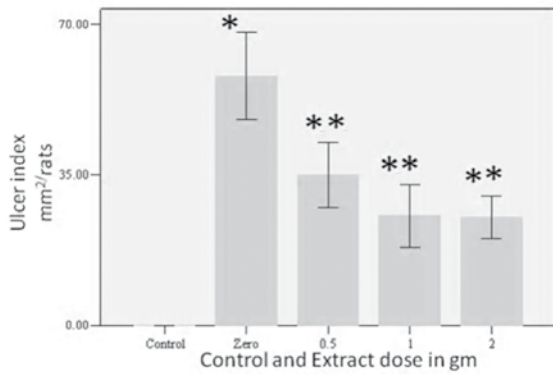


Figure 1 - Gastric ulcer index in control (normal rats) and 4 experimental rat groups that were treated daily with different doses of aqueous *Chamomilla recutita* extract (ACE) for 27 days; on the 28th day, a single dose of 70% ethanol was given to induce gastric ulcers. Values are given as mean ± SD and are statistically significant ($p < 0.05$). *Significant compared with the control group. **Significant compared with ulcerative rats that received a zero dose of ACE. mm² - square millimeter, gm - grams.

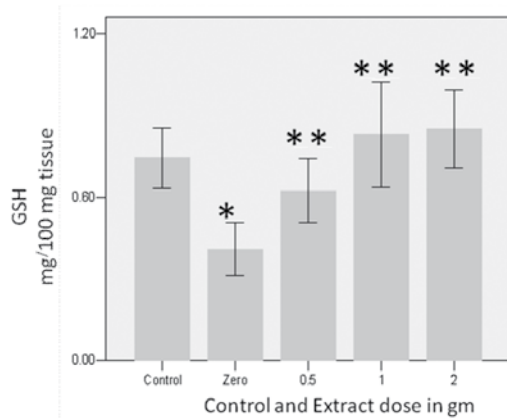


Figure 2 - Gastric reduced glutathione (GSH) levels in control rats and 4 experimental rat groups that were treated daily with different doses of aqueous *Chamomilla recutita* extract (ACE) for 27 days; on the 28th day, a single dose of 70% ethanol was given to induce gastric ulcers. Values are given as mean ± SD and are statistically significant ($p < 0.05$). *Significant compared with the control group. **significant compared with ulcerative rats that received a zero dose of ACE. gm - grams.

Estimation of GSH level. To estimate gastric GSH, stomachs were washed in ice-cold isotonic saline and blotted individually on ash-free filter paper. The tissues were then homogenized separately in 0.1 M Tris-HCl buffer, pH 7.4 at 4°C using a Potter-Elvehjem homogenizer at a dilution factor of 4, and the crude tissue homogenate was centrifuged at 9000 rpm for 15 minutes in a cold centrifuge. The supernatant was kept at -20°C to estimate GSH levels. The homogenate was precipitated with 50% trichloroacetic acid and centrifuged at 1000 rpm for 5 minutes. The reaction mixture contained 0.5 mL of supernatant, 2.0 mL of

Tris-EDTA buffer (0.2 M; pH 8.9), and 0.1 mL of 0.01 M 5,5'-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 minutes and was read at 412 nm on a spectrophotometer. The GSH content of the stomach homogenate was measured at 412 nm using a method that was developed by Sedlak and Lindsay.¹⁷ The values were expressed as mg/100 mg of tissue.

Histological studies. Histological examination of stomach samples was carried out by routine histological procedures. Tissue fixation was carried out, immediately after the experimental procedure, with 10% neutral buffered formaldehyde solution (pH 7.0). Processing was carried out as per the schedule for dehydration, clearing and paraffin infiltration, and then the collected tissue was embedded in paraffin. Finally, 5 µm sections were cut and mounted on clean glass slides coated with Mayer's egg albumin.¹⁸

Statistical analysis. At different stages, the data were compiled and fed to a computer. The Statistical Package for Social Sciences version 10 (SPSS Inc, Chicago, IL, USA) was used for standard statistical analysis. Data are given as the mean ± SD. Student's t-test was used to determine the difference between groups, and $p < 0.05$ denoted statistical significance.

Results. Figure 1 shows that the ACE reduced UI in a dose-dependent manner; the maximum dose was 2 gm/kg. No gastric ulcer was observed in control group rats to which distilled water was given. Single-dose administration of 70% ethanol, however, induced gastric ulcers in all rats. The severity of induced-gastric ulcer, as estimated by UI, was inversely proportionally to the dose of ACE that was given daily for 27 days. In the zero-dose groups, the UI was 58.0 ± 5.05 mm/rats which decreased significantly to 35 ± 3.79, 25.5 ± 3.61, and 25.1 ± 2.48 mm/rats in rats that were pretreated with 0.5, 1, and 2 gm/kg of ACE, translating into a 39.6%, 56.0%, and 56.6% decrease.

Figure 2 shows gastric GSH levels in the control and experimental groups. The GSH level in the control group was 0.74 ± 0.06 mg/100 mg, and it decreased significantly to 0.41 ± 0.05 mg/100 mg in the second group. Gastric GSH rose to 0.62 ± 0.06 mg/100 mg, however, in rats that received 0.5 gm/kg of ACE daily; this increase was significant compared with the second group. Gastric GSH also increased to 0.83 ± 0.1 and 0.85 ± 0.1 mg/100 mg in rats that received 1 and 2 gm/kg of extract daily; these increases were also significant relative to the second group.

Figure 3 demonstrates the gastric histopathological study, which shows disorganization of cell nuclei and gland morphology with erosion in the gastric mucosa, which is completely lost in some areas with interrupted muscularis mucosa in ethanol treated rats (received zero ACE). On the other hand, rats treated with 0.5 gm/kg of ACE extract showed better organization of

cell nuclei and gland morphology with some erosion in the gastric mucosa. Moreover, these erosions completely disappeared in the group of rats treated with 1 or 2 gm/ kg of ACE.

Discussion. Our results showed that ethanol administered to rats induced ulcers in 100% of recipients, generating a mean ulcer score of 58.0 ± 5.05 , which was accompanied by a reduction in GSH levels. These lesions were prevented or minimized by pre-treating rats with aqueous ACE extracts for 27 days as demonstrated by reducing UI as well as abolishing histopathological changes of gastric mucus lesions and the good organization of cell nuclei, gland morphology, and intact muscularis mucosa in treated rats. Also, ACE prevents the reduction of gastric GSH levels and all these prophylactic changes were observed in a dose-dependent manner. These findings support the local folk-based medicinal claim that ACE may have beneficial effects for human gastric ulcers.¹⁴ In acute toxicity studies, animals that were treated with the extract (up to 8 g/kg

body weight) did not manifest any significant clinical or macroscopic signs of toxicity.

Induction of gastric mucosal damage in rats by using high concentrations of ethanol has been used widely to investigate the gastroprotective effects of medicinal plants, because ethanol induces petechial lesions quickly.¹⁹ Ethanol induces necrotic lesions in the gastric mucosa through its toxic effects by reducing bicarbonate production, decreasing mucus secretion²⁰ and increasing free radical formation.²¹ Also, ethanol-induced gastric lesions might be due to stasis in gastric blood flow, which contributes to the development of the hemorrhage, and necrotic aspects of tissue injury.²⁰ Concentrated ethanol causes a marked contraction of circular muscles of the fundus strip in rats. Such contractions can lead to mucosal compression at the site of greatest mechanical stress, for example, at the crest of mucosal fold-leading to necrosis, and ulceration.²²

Although the etiology of gastric ulcers is unknown in most cases, it is generally accepted to result from

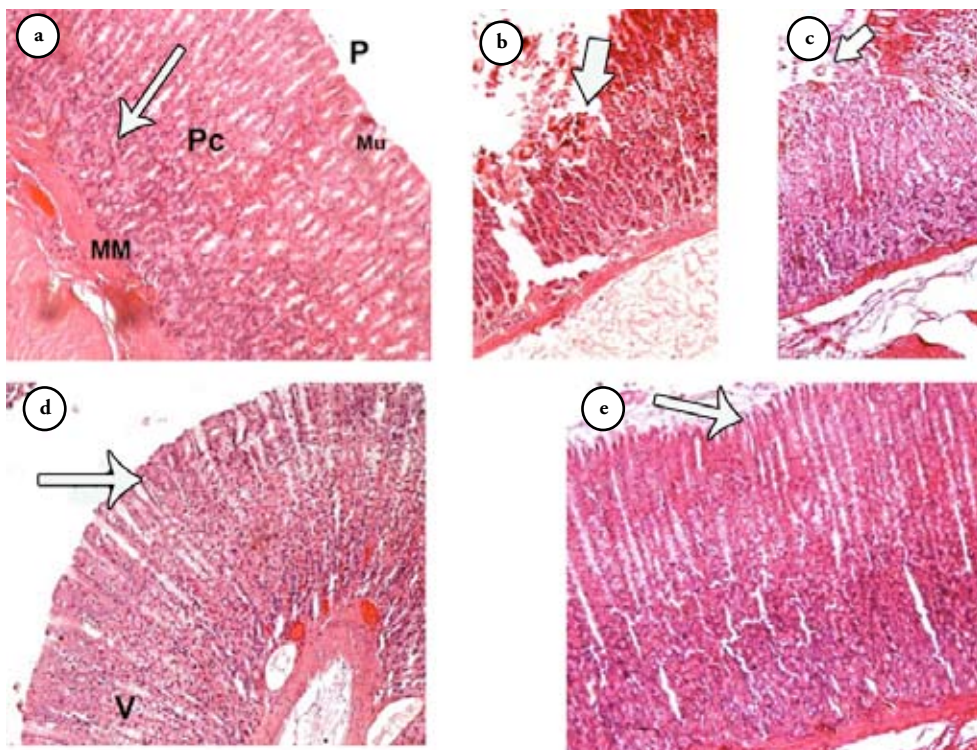


Figure 3 - Photomicrographs of the control and experimental groups of rats. a) Control showing the gastric pits (P) are lined by pale stained surface mucous pits (Mu). The base of the glands show presence of parietal cells (Pc) and peptic (chief) cells (arrow), the muscularis mucosa is also present and intact. b) Ethanol treated rats, which did not received any aqueous *Chamomilla recutita* extract (ACE) showing disorganization of cell nuclei and gland morphology (arrow) with erosion in the gastric mucosa, which are completely lost in some areas with interrupted muscularis mucosa. c) 0.5 gm/kg ACE treated rats showing good organization of cell nuclei and gland morphology with some erosion in the gastric mucosa (arrow). The gastric glands are scattered and regular. The muscularis mucosa is also present and still intact. d) 1 gm/kg *Chamomile* treated rats showing good organization of cell nuclei and gland morphology (arrow) with intact muscularis mucosa. The gastric glands are scattered and regular with engorgement of blood vessels. Some vacuoles (V) are present in between the glands. e) 2 gm/kg ACE treated rats showing good organization of cell nuclei and gland morphology (arrow). The gastric glands are scattered and regular. The muscularis mucosa is also present and still intact. (hematoxylin and eosin stain, 200X).

an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms.²³ Gastric ulceration may also be related to oxidation, increased lipid peroxidation, and generation of free radicals.²⁴ To this end, antioxidants have been reported to prevent gastric ulcers. Antioxidants might be an important component of gastric mucosal protection.²⁵ Studies have shown that antioxidants strengthen gastric walls significantly and protect tissue from oxidative damage. In 1979, Body et al²⁶ found that the gastric mucosa contains high concentrations of GSH, which are the chief constituent of the endogenous nonprotein sulfhydryl (NP-SH) pool. The NP-SH pool might effect the scavenging of oxygen-derived free radicals and influence the production and character of mucus.²⁷⁻²⁹ We observed a significant decrease in gastric mucosal GSH levels in rats in which ulceration was induced by ethanol, compared with control rats, suggesting that GSH regulates ethanol-induced ulcerogenesis. One notable feature of ACE-induced reversal of ethanol is the significant increase in GSH levels in gastric mucosa of rats that have been ulcerated with ethanol. Higher GSH levels were observed at 2 gm/kg rat body weight.

Prostaglandins (PGs) protect the gastric mucosa against different types of gastric lesions. Notably, antisecretory PGs protect the mucosa at non-antisecretory doses. Moreover, non-antisecretory PGs, such as PGF₂, are also protective.³⁰ The GSH is a cofactor in some steps of PG synthesis, aiding in the conversion of PGG₂ to PGH₂ and the subsequent conversion to PGE₂.^{31,32} These steps lead to the formation of protective PGs. Prostaglandin synthetase is nearly incapable of synthesizing PGE₂ after GSH depletion from the medium,³³ shedding light on possible antiulcer mechanisms of *Chamomilla recutita* via enhancing GSH levels in rat stomachs.

One hundred and twenty chemical constituents have been identified in *Chamomilla recutita*, including terpenoids, chamazulene, tannins, flavonoids (apigenin, luteolin), and coumarins (umbelliferone, alpha-bisabolol),^{34,35} The flavonoids apigenin, and luteolin have anti-inflammatory, carminative, and antispasmodic properties.³⁴ The anti-inflammatory, wound-healing, and antimicrobial effects of German chamomile are attributed to a blue essential oil, that contains sesquiterpene alcohol, alpha-bisabolol, chamazulene, and flavonoids.³⁶ The anti-inflammatory activity prevents gastric ulcers and is a possible mechanism of this plant.

The inhibitory effect of the extract might be due, at least in part, to the presence of terpenes and flavonoids in *Chamomilla recutita*.^{34,35} Terpenes are associated with anti-ulcerogenic activity in other plants.^{37,38} Some triterpenes are antiulcer drugs, and their activity has

been suggested to be due to a reduction in mucosal prostaglandin metabolism, cytoprotective actions, and reduction in gastric vascular permeability.³⁹ Moreover, flavonoids have antiulcer, and gastroprotective properties.⁴⁰ Tannins and polyphenols share physical and chemical properties that underlie their physiological and pharmacological actions, such as their antioxidant (radical-scavenging activities) and their ability to complex with other molecules, such as proteins, and polysaccharides.⁴¹ An in vitro study,⁴² showed that the vegetable polyphenols also inhibit lipid peroxidation; also, there is an evidence of their ability to scavenge radicals. Tannins could prevent ulcer development either via vasoconstricting effects,⁴³ or due to their protein-precipitating where it promotes precipitation of microproteins at the ulcer site, forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from irritants.⁴³

In conclusion, the ACE has potent protective, and effective antiulcer activity against ethanol-induced gastric ulcers when administered orally to rats. Further, studies are needed to disclose the potent components of this action and also to understand the exact prophylactic mechanisms.

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