

## EFFECT OF THE AQUEOUS EXTRACT OF MATRICARIA CHAMOMILLA ON STRESS-ETHANOL INDUCED ACUTE GASTRIC ULCERATION IN RABBITS

Nadheera F. Naema, Nabeel A. Ali, Jawad H. Ahmed

### ABSTRACT

The anti-ulcer effect of the aqueous extract of *Matricaria chamomilla* flowers (chamomile extract, CE) had been tested on stress- ethanol induced gastric ulceration in rabbits. The first part of the study was to induce acute gastric ulceration by stress or stress-ethanol combinations. Stress alone was unable to produce gastric ulcerations while ulcer was induced in the group of rabbits exposed to stress in the presence of ethanol (5ml / kg ) with a mean value of ulcer index of  $108.3\text{mm} \pm 29$  (n=8). Histopathological examination was done to confirm the effect of stress-ethanol combination on gastric mucosa. The second part of the study was performed to investigate the pharmacological effect of various concentrations of the aqueous extract of chamomile (1%, 4% and 8%) on gastric ulceration. The extract was given 15 minutes before ethanol administration. The results have shown a significant anti ulcer effect of chamomile extract with mean values of ulcer index of  $76.4\text{mm} \pm 14.2$  ( $P < 0.01$ );  $11\text{mm} \pm 14.2$  ( $P < 0.005$ ) and  $53\text{mm} \pm 27.9$  ( $P < 0.01$ ) for the concentrations 1%, 4% and 8% respectively as compared to the control value of  $133.25\text{mm} \pm 46.2$  (n=8). It is concluded that the extract of *Matricaria chamomilla* flowers has anti ulcer effect.

### INTRODUCTION

Aqueous extract of chamomile has been used in the symptomatic relief of dyspepsia and impaired digestion<sup>[1]</sup>. It was traditionally used also in symptomatic relief of irritations of respiratory tract due to common cold<sup>[2,3]</sup>. In Iraqi folk medicine chamomile has been used as analgesic, anti-microbial, diuretic and in treatment of peptic ulceration<sup>[4]</sup>. The latter use was based on observational impression rather than on clinical or experimental studies. Therefore, the aim of the present study was to induce gastric ulceration in rabbits and to investigate the anti-ulcer effect of the aqueous extract of chamomile.

### MATERIALS AND METHODS

#### 1. Preparation of the aqueous extract of chamomile

Dried flowers of chamomile were purchased from the center of herbal medicine, Baghdad. Voucher specimens were kept at the Department of Pharmacology. Aqueous extract was prepared by adding 16 grams of the flowers material to 100 ml of boiling distilled water and was kept boiling for another 3 minutes after adding the flowers of chamomile. The average volume of the resulted solution was 10.8 ml. The solution was filtered, then dried by evaporation, and the solid materials were obtained. It was estimated that each one milliliter of the watery extract contains 160 mg of the solid material. The

strength of such solution is 16%. Accordingly one milliliter solution of 8%, 4% and 1% contains 80mg, 40mg and 10mg of the solid material respectively<sup>[5,6]</sup>.

#### 2. Preparation of laboratory animals

Many pilot studies had been done to standardise the model. Experiments were carried out on local breed, male rabbits, and average body weight of 1-1.5 kg. The rabbits were maintained on a low fiber diet (less than 10% fibers<sup>[7]</sup>) for at least one week before the study day with a free access to ordinary water. The rabbits were then divided into groups, 6-8 each. Forty-eight hours before the study day each rabbit was kept in a separate cage with a free access to water only. To prevent direct coprophagy and to ensure complete fasting condition, a modified restraint cage was used to prevent free movement and made the rabbit unable to withdraw its head back. Restraint is considered as a specific stressful condition<sup>[8]</sup>. On the day of the experiment, extract of chamomile (5ml/kg) or distilled water (5ml/kg) were administered randomly by a separate person through a fine stomach tube introduced through a hole in an ordinary clinical wood tongue depressor to control jaw movement and to prevent the rabbit from chewing the tube<sup>[9]</sup>. To study the anti ulcer effect of chamomile extract, absolute ethanol (99.86%, James Burroughs, England) was

administered in a dose of 5ml /kg body weight 15 minutes after the administration of chamomile and was given to the animals through a gastric tube. Three hours after the administration of ethanol, animals were killed by a single sharp blow on the back of the head.

### **3. Preparation of the stomach**

The abdomen was opened, and both ends of the stomach were ligated. The stomach was then, separated from other viscera and opened along the greater curvature. The content of the stomach was aspirated by an ordinary syringe, inspected, and the volume was measured. Then the stomach was immersed in normal saline to remove food particles and examined macroscopically to determine the length of the affected areas. Finally the stomach was kept in 10% formalin for 24 hours for histopathological examination<sup>[5]</sup>.

### **4. Measurement of gastric lesions**

Ulcer index is the sum of length (in millimeters) of ulcers measured by an ordinary ruler. Haemorrhagic lesions and necrotic areas were measured in the same way<sup>[5,10]</sup>.

### **5. Measurement of gastric acidity**

Acidity of gastric contents was measured by a standard titration method<sup>[5]</sup>.

### **6. Histopathological examination**

Histopathological examination was performed at the Department of Pathology by a qualified pathologist.

### **7. Statistical analysis**

Unpaired t-test was used to test differences between the control and active treatment. P-value <0.05 is considered significant. The data are presented as mean±SD.

## **RESULTS**

The first part of the study was designed to investigate the effect of orally administered absolute ethanol in the presence of stress on gastric mucosa of the rabbits. It was found that stress-ethanol in a dose of 5ml/kg had induced gastric ulceration in all rabbits (n=8). Macroscopical examination using dissecting microscope revealed severe damage of gastric mucosa with signs of congestion, necrosis, and

ulceration. The mean value of ulcer index in this group was (108.3±29mm) and for the congested area was (73.3±56mm). Areas of necrosis were also observed with a mean value of (28.8±42mm). In the control group of rabbits which were given 5ml/kg of distilled water under the same stressful condition (n=7), no ulcer or hyperemia were observed but there were areas of necrosis. The mean value of necrosis in this group was 117.2±56 mm which was significantly higher than 28.8±42mm in the ethanol treated group (P<0.01). Gastric acidity was increased in the ethanol treated group. There was a significant reduction in the pH of stomach contents from (1.67±0.07) in the control group to (1.19±0.2) in ethanol treated group (P<0.05). The volume of stomach contents in the ethanol treated group was 15.5±7.3 ml which was significantly higher than (5.16±2.4 ml) in the control group (P<0.05). Histopathological examination showed massive tissue destruction with various lesions: ulcers, necrosis, and congestion, in addition to the appearance of inflammatory cells and oedema (Figure-1).

The second part of the study was designed to investigate the effect of chamomile on the ethanol-induced gastric ulceration. The aqueous extract of chamomile produced significant anti-ulcer activity. Those rabbits (n=7) which received 5ml/kg body weight of 1% of the extract 15 minutes before ethanol administration had a significant reduction in ulcer index from (133.25±46.2mm) in the control to (76.4±14.2 mm) in the treated group (P<0.01). When the concentration of the extract was increased to 4% further reduction in the ulcer index to 11±14.2mm has been observed in this group of rabbits which is significantly different from the control (P<0.001) (Figure-2). Two rabbits in this group had shown complete inhibition of ulceration. It was noted that when the concentration of chamomile was increased to 8% the ulcer index was found to be 53±27 mm which was slightly but significantly (P<0.01) lower than 76.4±14.2 obtained by the 1% concentration of chamomile but it was higher than the value obtained by the 4% concentration. The effect of chamomile extract on the volume of stomach contents was also studied. There was a small reduction in the volume of stomach content in the treated group

compared to the control (data is not presented) and statistically significant reduction was achieved when the concentration of chamomile

extract was 1%. There were no differences in the pH of the stomach between the control and the group treated with chamomile.

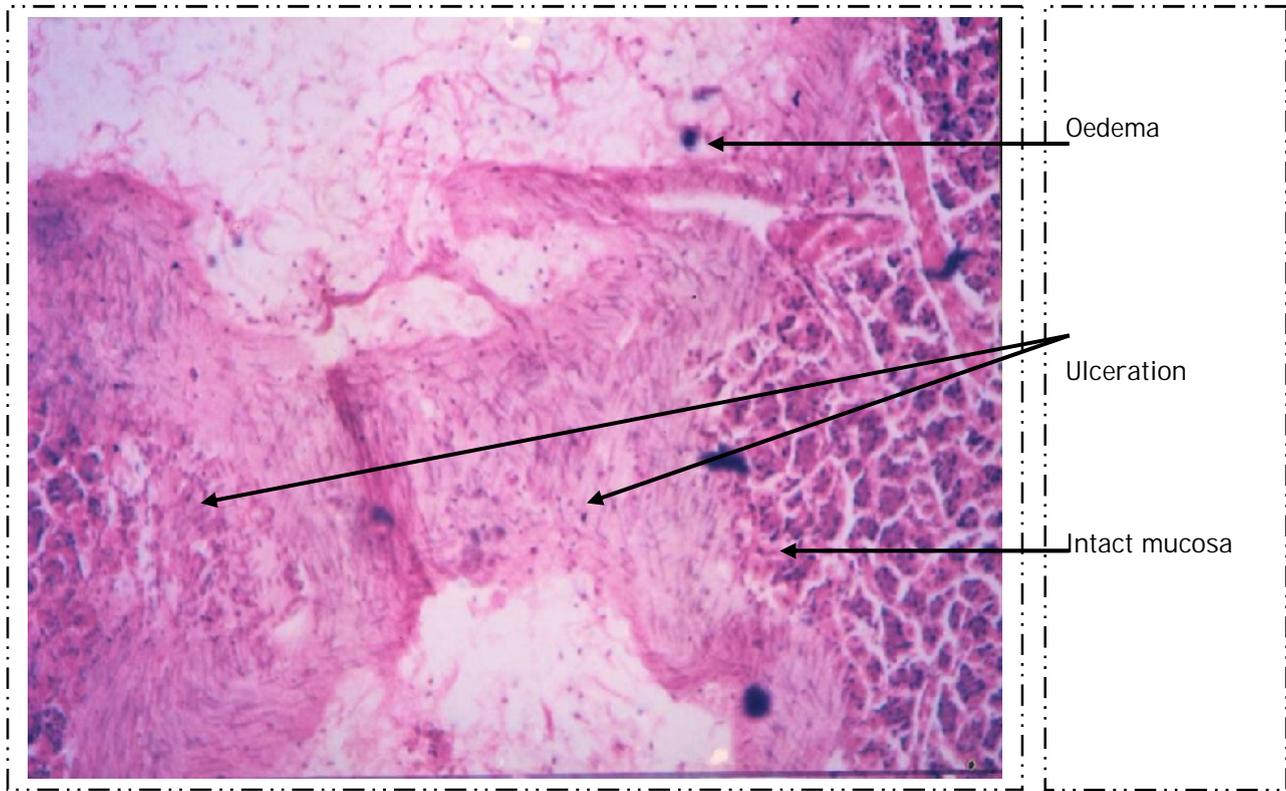


Fig 1. *Histopathology of rabbit stomach: absolute ethanol causes complete destruction of gastric mucosa.*

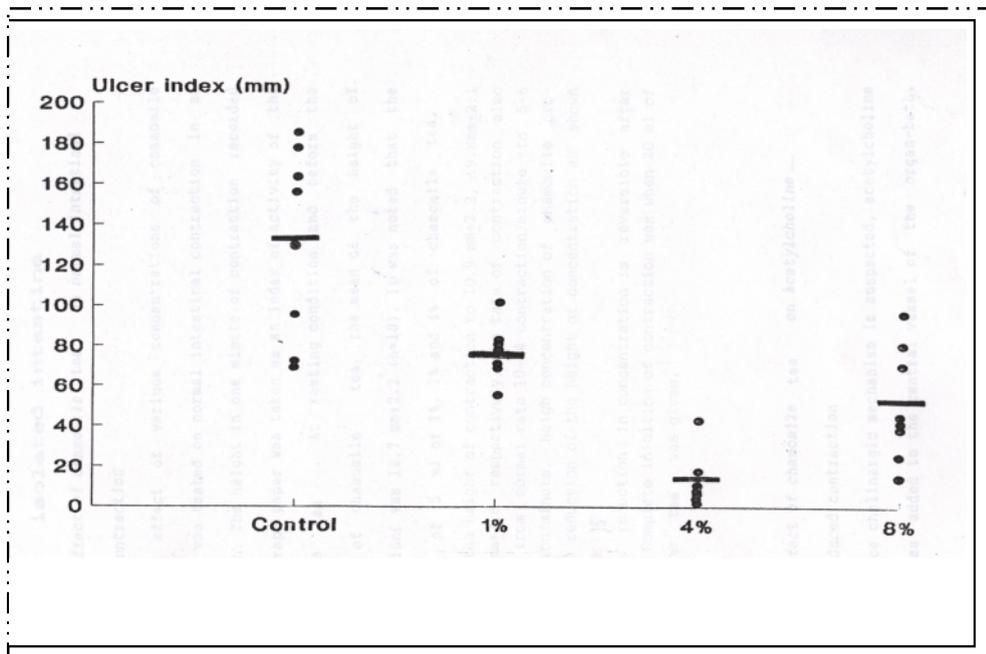


Fig 2. *The effect of different concentrations of chamomile extract on ulcer index. Ulcer index was significantly reduced at 1%, 4% and 8% concentration of chamomile extract ( $p < 0.01$ ,  $p < 0.001$  and  $p < 0.01$  respectively)*

## DISCUSSION

In the present study the effect of oral administration of absolute ethanol to the rabbits were studied after exposing the animals to stressful condition (prolonged fasting for 48 hours and restraint). The results of the first part of the study showed that stress alone produced necrosis of gastric mucosa which could be due to reduction in gastric blood flow and therefore might act as a predisposing factor for gastric ulceration<sup>[11]</sup>. In the group of rabbits that received absolute ethanol after similar stressful conditions, further disturbances in gastric mucosal microcirculation could have taken place. These disturbances were thought to include increased then decreased in gastric blood flow<sup>[11]</sup>; which results from increased blood viscosity. These alterations could result in haemorrhagic band-like lesions of various sizes parallel to the major axis of the stomach<sup>[8,12,13]</sup>. In this group, ulcers were induced and the main site of which was in the corpus region. Ethanol, due to direct irritation, results in a significant decrease in gastric pH and an increase in the volume of stomach contents which includes gastric secretions, blood, mucus and sloughed mucosa<sup>[14,15]</sup>. In the part of the study which was concerned with the evaluation of the effect of chamomile, the extract was given 15 minutes prior to ethanol administrations in order to reduce direct chemical interaction and to avoid ethanol dilution<sup>[16]</sup>. All concentrations of chamomile extract used in this study resulted in significant reduction in the ulcer index. The most effective concentration of chamomile extract was 4%. This concentration resulted in maximum inhibition of ulcer index. In two rabbits complete prevention of ulcers was observed. Chamomile extract in a concentration of 8% resulted in a significant reduction of ulcer index but this effect was less than that observed at the 4% concentration of the extract. The mechanism behind this concentration-independent behavior of chamomile is not clear. Chamomile extract reduces ethanol induced gastric damage by decreasing the damaged area and reducing the haemorrhagic lesions. Similarly, chamomile extract in a concentration of 4% resulted in complete disappearance of haemorrhagic lesion while treatment with 1% and 8% produced less effect on haemorrhagic lesions. It seems that the effect of chamomile

extract is dose-dependent up to a concentration of 4%. Above this concentration the protective effect on gastric mucosa tend to be reduced. This observation could have a practical importance since in traditional medicine people believe that the effect of herbs increased with increasing the amount consumed and therefore, can be used freely and relatively in unlimited amount. In conclusion the extract of *Matricaria chamomilla* flowers has anti-ulcer effect particularly at lower concentrations.

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