

STUDY THE EFFECT OF ETHANOLIC EXTRACT OF (*MATRICARIA RECUTITA* AND *GLYCYRRHIZA GLABRA*) COMPARED WITH CIMETIDINE IN TREATING GASTRIC ULCERATION IN RABBITS INDUCED BY ASPIRIN

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ABSTRACT

This study aimed to evaluate the effect of the drenching ethanolic extract of Chamomile and licorice at a dose of (100 mg/kg and 250 mg/kg) respectively in treating gastric ulcer induced by aspirin in rabbits. The study included the effect of drenching the ethanolic extract of both plants on the gastric ulcer index ,gastric juice pH, and volume of gastric fluid as well as curative ratio. Results revealed that treatment with cimetidine and Chamomile or licorice or their combination was associated with significant ($p < 0.05$) increase in pH values compared to the respective value of the untreated (positive) group. Chamomile or licorice was superior to that of cimetidine and to combination of the extracts. Groups treated with cimetidine and drenching of Chamomile or Licorice were associated with significant ($p < 0.05$) reduction in the volume of gastric juice compared to positive and control groups, a group treated with a combination of extracts caused significant ($p < 0.05$) reduction in gastric juice volume compared to positive group. A curative ratio of gastric ulcer was better in groups given chamomile or licorice over those given cimetidine or combination of chamomile and licorice. Furthermore, chamomile was superior over licorice in its curative ratios of gastric ulcer.

In view of above results we can conclude that a drenching ethanolic extract of Chamomile and licorice in the doses used in this study caused increased gastric ulcer healing compared with treatment by 200mg/kg of cimetidine in treatment gastric induced by Aspirin in rabbits.

INTRODUCTION

Peptic ulcers are open sores in the mucous lining of the stomach and duodenum. Gastric ulcer is among the most serious diseases in the world(1). The etiology of gastro duodenal ulcers is influenced by various aggressive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermal growth factors(2).

Some other factors, Some of the causes of these disorders are stress, smoking, nutritional deficiencies and ingestion of non-steroidal anti-inflammatory drugs (3,4).

Nowadays, drugs are expensive and have many side effects during treatment of any disorders. Therefore, the potential of the health promoting and disease preventing properties of plant-derived compounds have received increased attention from researchers in recent years(3). Plants that have curative properties are used to improve

symptoms or prevent diseases such as diarrhea, stomach disorder, asthma, hypertension, etc.(5). Licorice (*Glycyrrhiza glabra*) is known for its anti-inflammatory and anti-allergic effects, It had been shown that licorice – derived compounds can raise the concentration of prostaglandins in the digestive system that promote mucus secretion from the stomach(5).It was also reported that licorice prolongs the life span of surface cell in the stomach and has an anti pepsin effect, The combined effect may lead to the healing of ulcer(6).

Matricaria recutita or chamomile flowers & bisabolol (Terpenoid) inhibited stomach ulcers caused by stressful stimuli, alcohol, and NSAIDs (7,8).

Healing times for ulcers induced by chemical stress or heat coagulation were reduced by a bisabolol. Extracts of the flowers of German chamomile had an inhibitory effect on gastric acid secretion(9).

The aim of the present work was to study the possible anti-inflammatory activities of both *Glycyrrhiza glabra* and *Matricaria recutita* and their combination in comparison with cimetidine on the healing properties of aspirin induced gastric ulcer of rabbits .

MATERIALS AND METHODS

Animals; Seventy-two (72) female rabbits ,weighting 1-1.5 kg of domestic strain was used for the study. The animals were kept for one week in the animal house before the experiment to be acclimatized, and they were maintained on unrestricted supplies of food and water. Animals were randomly divided into Six study groups : two control groups and four treated groups (12 rabbits of each).

Preparation of ethanolic extract;

The plant materials the licorice roots and chamomile flowers were purchased from the local Basrah market and shade dried at room temperature, made into a coarse powder by electrical grinder to get a uniform particle size and then used for extraction, the powder was relaxed with (96%) ethanol for 12 hours by soxhlet (10).

Induction of ulcer: After 48 hours of fasting, aspirin 500mg suspended in 3ml of (10%) ethanol has been orally administered for (3days) using modified insulin syringes to all groups except the control group .

Experimental design:

1. Negative control group (NC) : given normal saline 3ml/kg for 3days, then subdivided into subgroup (1) sacrificed after 10 days (n=6),and subgroup (2) sacrificed after 20 days (n=6) .
2. Positive control group(PC): given aspirin 500mg/ 3ml of (10%) ethanol/ kg.bw, for (3days) ,then subdivided into subgroup (1) sacrificed after 10 days (n=6),and subgroup (2) sacrificed after 20 days (n=6).

Treated groups : for each group 12 rabbits. Each group was subdivided into subgroup (1) treated for 10 days (n=6); subgroup (2) treated for 20 days (n=6) .

3. Chamomile treated group A(A1,A2): was treated by given chamomile Extract (100mg/kg bw), twice daily.

4. Licorice treated group B(B1,B2) : was treated by given licorice Extract (250mg/kg bw), twice daily.
5. Cimetidine treated group C(C1,C2): was treated with cimetidine 200mg diluted in normal saline give 3ml/ kg bw. once daily.
6. Chamomile and licorice treated group D(D1,D2): was treated with a combination of ethanolic extract of chamomile and licorice (100 mg/kg and 250 mg/kg respectively) daily.

After ending the experiment, the animals were sacrificed, The stomach was opened a long of the greater curvature; Gastric fluid was collected and its volume and pH were determined, and gastric lesions induced by aspirin served as ulcer index (U.I) were calculated.

Gastric ulcer index:

The method described by (11), was employed in the present study stomachs opened along the greater curvature, washed with saline and examined by magnifying glass for gastric ulcers observation .The sum of length for all lesions area for each animal was measured and served as the ulcer index(11). The curative ratio was calculated for each group using following equation:

Curative ratio (CR) = $(LC-LT/LC) \times 100$.

LC: The length of gastric ulcer in positive group.

LT: The length of gastric ulcer in treated group.

Statistical analysis

Data were expressed as Mean $\pm$ SD.and evaluated statistically using two-way analysis of variance (ANOVA). Significant difference between means was estimated at($p < 0.05$) (12).

RESULTS

Grossly Examination:

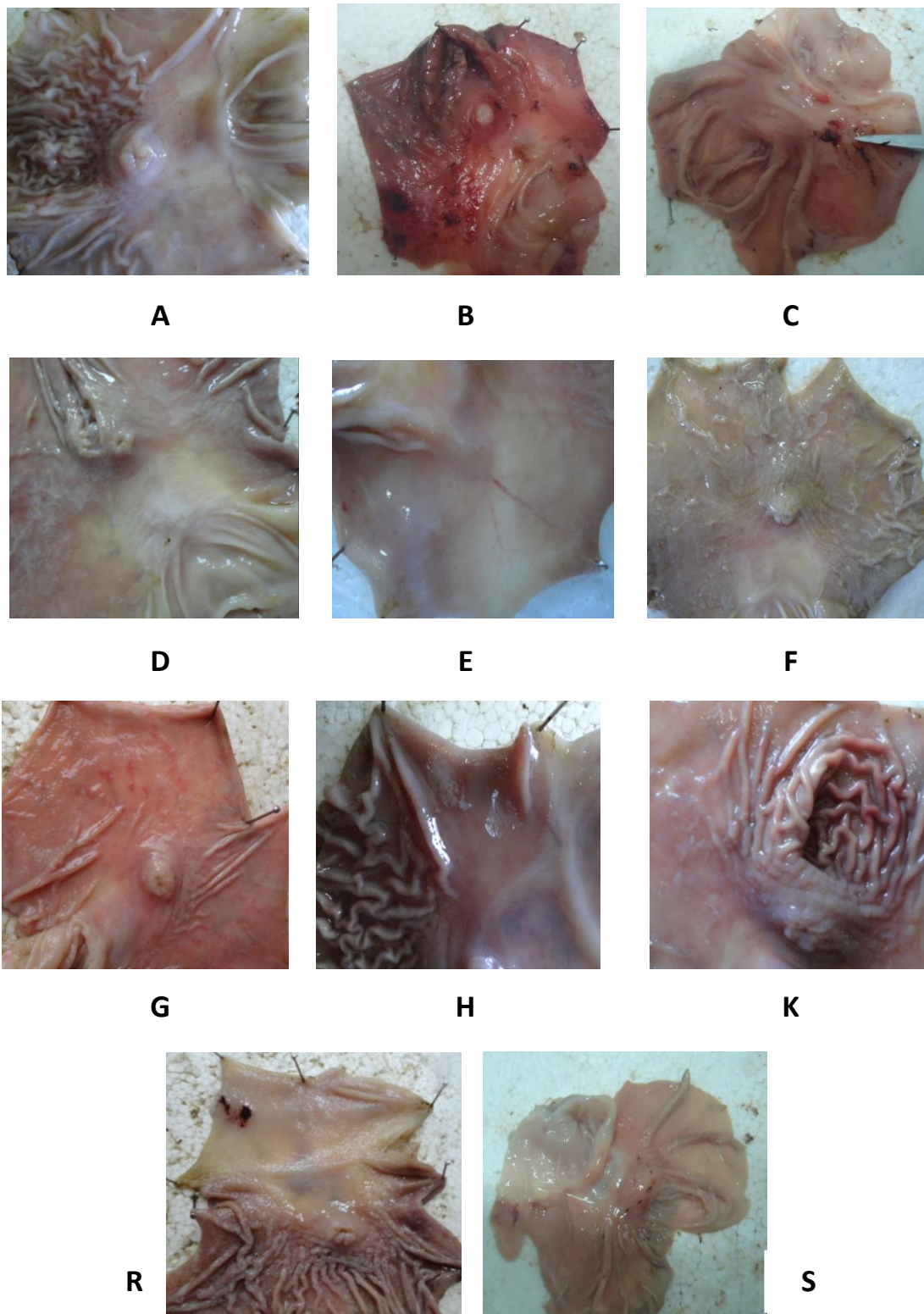
Aspirin induced gastric damage showed marked gross mucosal lesion, including hemorrhagic lesions and petechial lesion.

On gross examination these hemorrhagic bands were characterized by different sizes along the longitudinal axis of the glandular part of stomach (Fig.1;B,C).

Animals treated with Chamomile showed very mild lesions with interstitial hemorrhage and sometimes no lesion at all (Fig.1;D,E).

Fig.1;F,G reveals the degree of preventing Aspirin-induced ulcerations when the animals were treated with Licorice, There was a profound inhibition of ulcer formation than cimetidine treated which showing also necrosis and more hyperemic areas(Fig.1;H,K).In the group treated with a combination extracts, there was a period-dependent increase of the ulcer prevention ability of a combination , and has lowest acceptability healing results (Fig.1;R,S)

Results showed that all treated groups had significant increase in pH value of gastric juice at ($p < 0.05$) as compared to positive group. The mean pH value in the groups of rabbits which received chamomile and licorice and combination of plants were not significantly different from the value of the control group. Groups treated with Chamomile and Licorice (20days treatment) had higher mean values of gastric fluid pH compared to those given cimetidine and combination of extracts, These data are presented in (figure 2).



Figure(1): Photograph showing: A: Negative control, B: positive control 10days, C: positive control 20days, D: Treated by chamomile 10days, E: Treated by chamomile 20days, F: Treated by Licorice 10days, G: Treated by Licorice 10days, H: Treated by cimetidinee 10days, K: Treated by cimetidinee 20days, R: Treated by Combination of extracts 10days, S: Treated by Combination of extracts 20days.

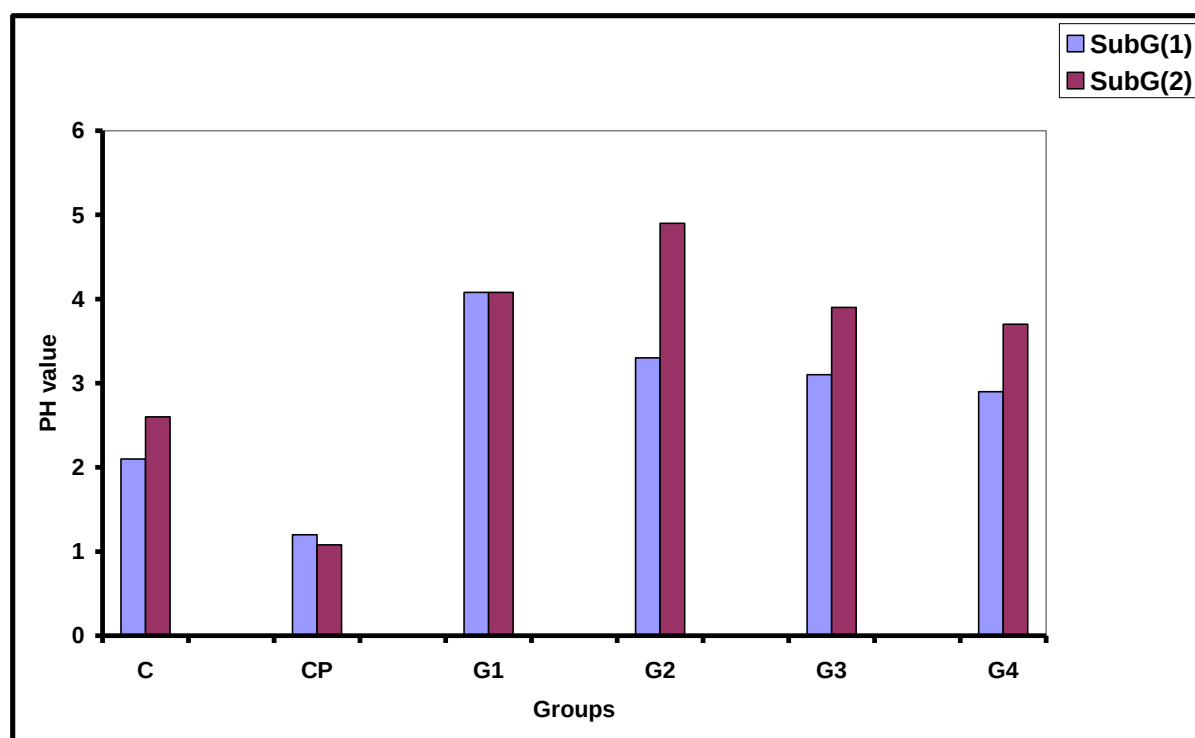


Figure (2) ; Effect of ethanolic extract of Chamomile, Licorice, Cimetidinee and a combination of extracts on gastric juice pH in ulcerated rabbits by Aspirin.
(C=control, CP= control(positive) group, G=Group, SubG.=Subgroup).

Volume of gastric juice (ml) in rabbits treated with different treatments at different doses is shown in (figure 3). Data was obvious that volume of gastric juice (ml) as mean $\pm$ SD of groups treated with cimetidinee and groups treated with chamomile and licorice and 20days treatment of combination were significant decrease at ($p < 0.05$) as compared to control and positive groups. ten days treatment of combination group had no significant decrease in volume of gastric juice compared to control group.

The length of gastric ulcer (mm^2) in rabbits as result of different treatments effect is recorded in (Figure 4). Tabulated results revealed that the gastric ulcer index (mm^2) as mean $\pm$ SD , 10days treatment of Groups given orally chamomile ,licorice &cimetidinee had significant decrease in the length of gastric ulcer at ($p < 0.05$) as compared to positive group.

20days treatment of groups treated with chamomile ,Licorice were significantly decrease at ($p < 0.05$) compared to untreated group (positive group). Group of combination caused no significant decrease in the length of gastric ulcer as compared to cimetidinee &positive groups.

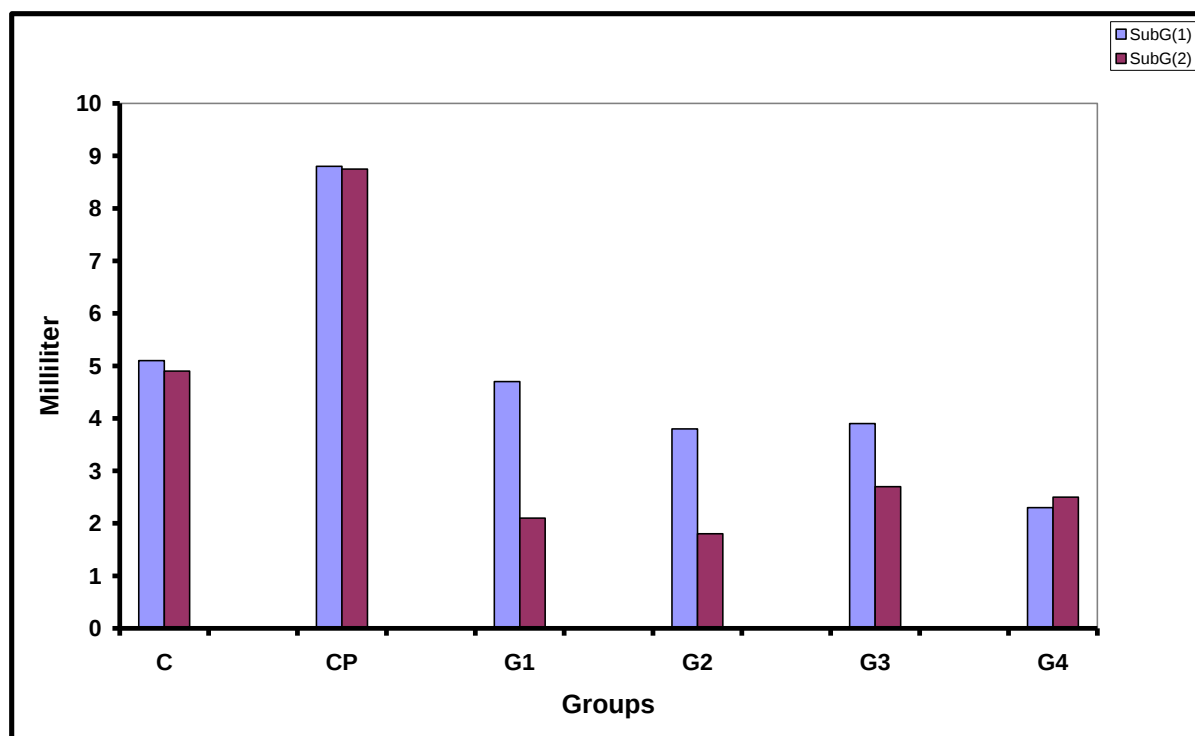


Figure (3) ; Effect of ethanolic extract of Chamomile, Licorice, Cimetidinee and a combination of extracts on gastric juice volume(ml) in ulcerated rabbits by Aspirin.

(C=control, CP= control(positive) group, G=Group, SubG.=Subgroup).

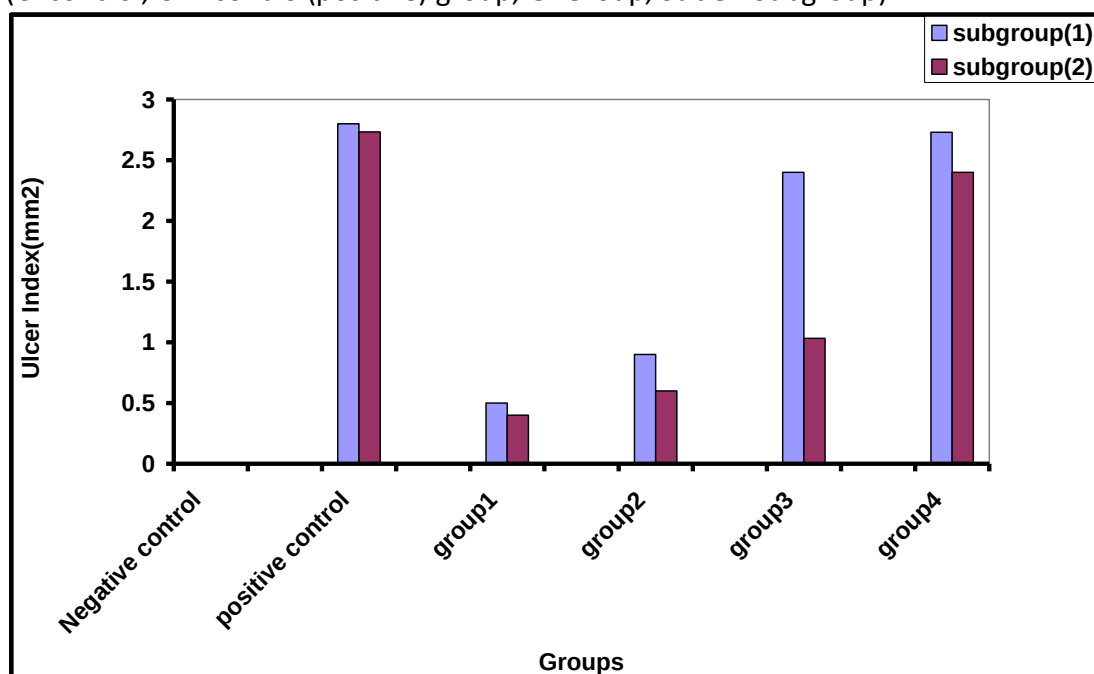


Figure (4) ; Effect of ethanolic extract of Chamomile, Licorice, Cimetidinee and a combination of extracts on ulcer index (mm²) in ulcerated rabbits by Aspirin.

The ethanolic extract of chamomile was found to possess remarkable ulcer-curative properties at 10 and 20 days when compare to other treatment. The maximum effect of ulcer healing (85.19%), (77.78%)& (61.85 %) were produced at 20 days treatment for chamomile, Licorice extracts and cimetidine, and 10 days treatment for chamomile, Licorice extracts gave (82.14%& 67.86%) of ulcer healing (figure 5).

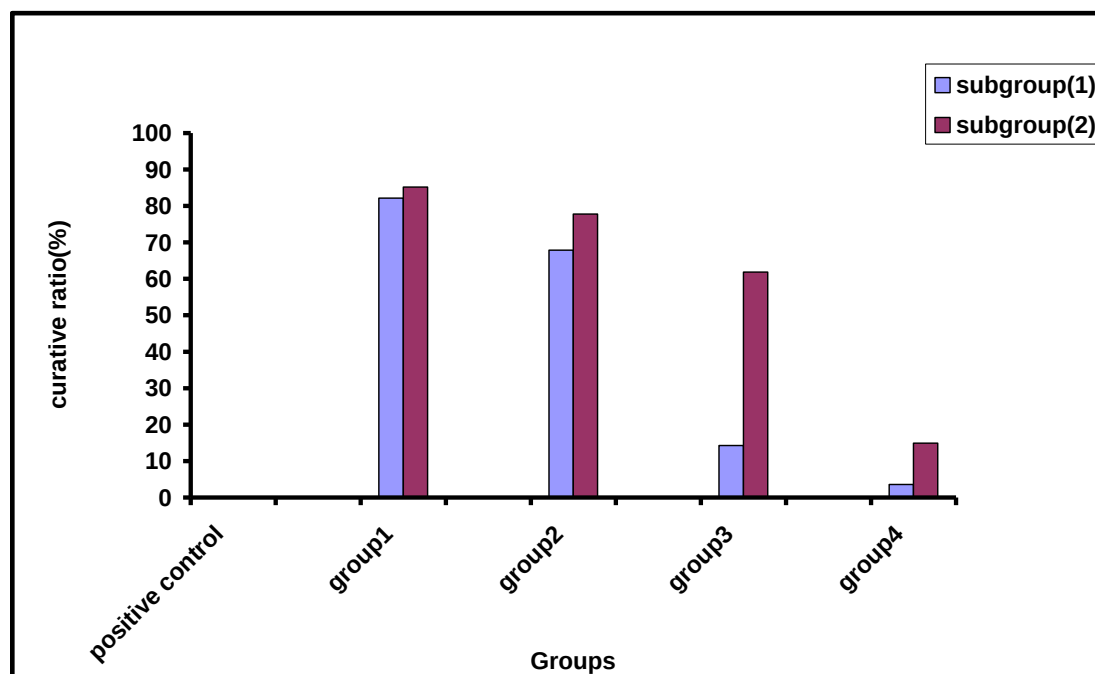


Figure (5) ; Effect of ethanolic extract of Chamomile, Licorice, Cimetidinee and a combination of extracts on ulcer index (mm²) in ulcerated rabbits by Aspirin

DISCUSSION

The present study showed the gastro treatmental effects of chamomile flower & Licorice root extract on acute experimental gastric ulcer in rabbits. measurement of the ulcer indices of the rabbits. The healing potency of the plants extracts led to faster ulcer healing compared to Cimetidine. The healing potency of the drug was different, This was also corroborated by the macroscopic gastric tissue .

Aspirin has been reported to reduce the gastric juice pH and increase the volume of gastric juice (3), or decrease the volume of gastric juice and its acid output (3,4). Our finding showed that chamomile and licorice ethanolic extracts at the different test periods (10,20days) had treatmental effects on acute experimental gastric ulcer in rabbits. Treatmental effect of chamomile and licorice ethanolic extract was higher than of antiulcer drug (cimetidine). ethanolic extract of chamomile had much more favorable healing effect, compared to ethanolic extract of licorice alone or in a combination with chamomile.

Peptic ulcer disease is a problem of the gastrointestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion (13). Therefore, the major mechanism action of chamomile and Licorice ethanolic extracts as treatment appears may be due to its effect in the decrease of acid, pepsin secretion and volume of gastric juice, and the promotion of mucosal protection by gastric mucin activity(14). Glycyrrhizin and its metabolites inhibit hepatic metabolism of

aldosterone and suppress 5- β reductase, properties responsible for the well-documented pseudoaldosterone syndrome(5). The similarity in structure of glycyrrhetic acid to the structure of hormones secreted by the adrenal cortex accounts for the mineralocorticoid and glucocorticoid activity of glycyrrhizic acid(15).

About 19 Invitro research has also demonstrated glycyrrhizic acid inhibits cyclooxygenase activity and prostaglandin formation (specifically prostaglandin E2), as well as indirectly inhibiting platelet aggregation, all factors in the inflammatory process(16,17). On the other hand, the anti-spasmodic and antipeptic actions of chamomile extract may be due to chamomile flavonoid constituents, apigenin (18). In addition to, similar results were observed with alpha-bisabolol and the cis-spiroethers and the small amount of coumarins contribute to smooth muscle relaxation(19). The effect of chamomile extract agreed with(7), who reported that chamomile had anti-inflammatory and spasmolytic effects on the stomach and duodenum, Therefore, it is thought to heal ulcers. Previous study reported that Chamomile flower extract has a direct effect on acid secretion, and increases mucosal resistance against damaging agents such as ethanol and aspirin(20). Following the combination of Chamomile and licorice show a significant increase($p < 0.05$) in both ulcer index and gastric fluid volume more than that of the Chamomile alone or licorice alone; this result indicates that there may be a antagonism effect between Chamomile and licorice and that effect may occur at the site of action of both. In comparison, cimetidine 200mg/kg treated group produced significant decrease ($p < 0.05$) in gastric secretion parameters. These results could be explained by that prostaglandins normally protect the gastrointestinal mucosa from damage by maintaining blood flow and increasing mucosal secretion of mucous and bicarbonate(21). Synthetic non-steroidal anti-inflammatory (NSAIDS) like aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and block diffusion of H^+ (22). Aspirin blockade of cyclooxygenase-1 (Cox-1) and (Cox-II) results in reduction of prostaglandin synthesis(21). The interruption of prostaglandin synthesis results in impairment of mucosal damage repair, thus facilitating mucosal injury, aspirin and related non-steroidal anti-inflammatory drugs and alcohol can aggravate or interfere with the healing of peptic ulcers(23).

تأثير المستخلص الكحولي لأزهار نبات البابونج و عرق السوس *Matricaria recutita* and *Glycyrrhrza glabra* مقارنة بالسمتين في معالجة قرحة المعدة المستحدثة بالأسبرين في الأرانب.

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الخلاصة

تناولت هذه الدراسة تأثير المستخلص الكحولي للبابونج وعرق السوس بجرعة (100 و 250 مغم/كغم) على التوالي في معالجة قرحة المعدة المستحدثة بالأسبرين في الأرانب ، اذ تم دراسة تأثير تجريب المستخلص الكحولي للنباتين المذكورين على حجم القرحة المعدية ، معامل الحموضة للعصارة المعدية ، حجم السائل المعدي وكذلك نسبة شفاء القرحة المعدية . أوضحت النتائج ان المجاميع المعالجة بكل من السميتين أو البابونج أو عرق السوس وكذلك المجموعة المعالجة بكلا المستخلصين سببت زيادة معنوية عند مستوى ($p < 0.05$) في معامل الحموضة مقارنة بمجموعة السيطرة الإيجابية ، وان المجموعة المعالجة بكل من البابونج و عرق السوس كانت نسبة الشفاء أعلى مقارنة مع مجموعة السمينتين والمجموعة المعالجة بكلا المستخلصين . اما بالنسبة الى المجموعة المعالجة بالسميتين او المجموعة المعالجة بالبابونج او عرق السوس سببت نقصان معنوي عند

مستوى ($p < 0.05$) في حجم السائل المعدي مقارنة بمجموعي السيطرة، أما المجموعة المعالجة بـ كلا المستخلصين فقد سببت نقصان معنوي عند مستوى ($p < 0.05$) في حجم السائل المعدي مقارنة بمجموعة السيطرة الايجابية. نسبة شفاء القرحة المعدية كانت الأعلى بمجموعي المعاملة بالبابونج و عرق السوس مقارنة مع المجموعتين المعاملة بالسمندين و المعاملة بكلا المستخلصين، وان المجموعة المعالجة بالبابونج كانت نسبة الشفاء فيها أعلى من المجموعة المعالجة بعرق السوس. في ضوء النتائج أعلاه نستنتج بان المعالجة بتجريع المستخلص الكحولي لعرق السوس وأزهار نبات البابونج بالجرع المستعملة ومقارنة بالمعالجة بعقار السيمتدين (200مغم/كغم)، سبب تحسن في نسبة شفاء قرحة المعدة المستحدثة بالأسبرين في الأرانب.

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