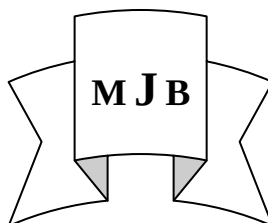


Effect of Liquirice and Chamomile Extracts in the Management of Gastric Ulcer in Rats

Shaima Ali Abid Nisreen J. Mohammed Haider Abid Al-Ridha Al-Kaffaji
College of Medicine, University of Babylon, Hilla, Iraq.



Abstract

Back ground: Gastric hyperacidity and peptic ulcer are very common causes of human suffering. Although there are different types of antiulcer drugs still they are expensive and have many side effects.

Objective: To investigate the antiulcer activity of watery extracts of (liquirice and chamomile) and chamomile oil in rats models.

Material and methods: Liquirice (*glycyrrhiza glabra*) roots extraction by crushing and boiling, while chamomile extraction by hot water maceration and the yield were used after cooling. Antiulcer effects were evaluated in rats using ethanol and salicylic acid ulcer induced method.

Results: The aqueous extract of chamomile and liquirice and chamomile oil exhibited significant ($p < 0.05$) anti-ulcer activity in this ulcer induced models compared with omeprazole as standard drug.

Discussion: The protection effects of these plants extracts may be due to its antioxidant contain and protective effect on the lining mucosa by enhancing the protection factors.

تأثير مستخلص نبات عرق السوس والبابونج في علاج القرحة المعدية في الجرذان

الخلاصة

ارتفاع حامضية المعدة والقرحة المعوية من أكثر أسباب المعاناة عند الإنسان، وعلى الرغم من كثرة أنواع العلاجات المستعملة إلا أن هنالك الكثير من الأضرار الجانبية لهذه العقارات بالإضافة إلى غلاء تكلفتها. الهدف من الدراسة: للتحري عن مضادات التقرح الموجودة في مستخلصات نبات عرق السوس ونبات البابونج. المواد وطرق العمل: تم تحضير المستخلص المائي لنبات عرق السوس والبابونج بواسطة الهرس والغليان ثم التصفية والتبريد، أما زيت البابونج فقد تم شراؤه مستخلصاً من الأسواق. النتائج: أظهرت النتائج أن هنالك تأثير كبير لمستخلص نبات عرق السوس المائي ومستخلص البابونج المائي وزيت البابونج في حماية بطانة المعدة ضد تأثير الأذى الناتج عن استعمال الكحول الأيثيلي وحامض الساليسلك، هذه النتائج صاحبت وجود تغيرات هامة في مستويات مضادات الأكسدة في الدم والذي يحتمل أن يكون سبب الحماية الناتجة عن استعمال مستخلص عرق السوس ونبات البابونج.

Introduction

The exact pathogenesis of ulcer continues to elude scientists and medical researchers. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in

the stomach [1,2]. Such factors could range from infections (*H. pylori*), drugs (non steroidal anti-inflammatory agents and lifestyle (stress, alcohol and cigarette smoking)[3,4]. Nowadays, some of antiulcer drugs are expensive and have many side effects during treatment of peptic ulcer. Therefore,

the potential of the health promoting and disease preventing properties of plant-derived compounds has received increased attention from researchers in recent years. Many tropical herbs have been scientifically reported to possess potent antiulcer activity [5-7]. There is growing evidence that oxygen derived free radicals such as $\text{OH}\cdot$, $\text{O}_2\cdot^-$, $\text{RO}\cdot$, and $\text{ROO}\cdot$ play a role in the pathogenesis of various disorders of the digestive system including gastric ulcer[8].

Liquorice is a wild plant. It grows in tropical and subtropical areas[9]. Liquorice is very stable in the gastrointestinal tract, from where it is slowly absorbed. Its Leaves, roots and seeds are used for medicinal purposes. It is used for the treatment of conjunctivitis, epilepsy and externally, it is applied to treat abscesses and stomatitis[10]. It is also traditionally used in the treatment of ulcer and tumor [11]. The saponin components of liquorice root, such as liquiritoside, has shown in-vitro anti-inflammatory activity [12].

Chamomile is widely used throughout the world. Its primary uses are as a sedative, anxiolytic and antispasmodic, it was used as a treatment for mild skin irritation and inflammation. Chamomile's main active constituents are chamazulene, apigenin, and bisabolol. Other component of chamomile includes amino acids, polysaccharides, fatty acids, essential oils, mineral elements, flavonoids, and other phenolic compounds [13]. Chamomile used in modern medicine primarily for their spasmolytic, antiphlogistic, antibacterial properties, and as a multipurpose digestive to treat gastrointestinal disturbances including flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea, and vomiting[14]. German chamomile (*Matricaria chamomilla*) is also used

to healing wound [15], treat various diseases including diarrhea [16], and inflammation, cancer[17]. Its extract blocks aggregation of *Helicobacter pylori* and various strains of *Escherichia coli*[18]. Chamazulene, alpha-bisabolol, flavonoids, and umbelliferone display antifungal properties against *Trichophyton mentagrophytes* and *Trichophyton rubrum*[19]. Apigenin, alpha-bisabolol, and the cisspiroethers appear to provide the most significant antispasmodic effects. Other flavonoids and coumarins contribute to smooth muscle relaxation [20].

Aim of the Study

To investigate the gastroprotective effect and antioxidant effect of liquorice watery extract 0.5 gm/kg body weight, chamomile watery extract 0.25gm/kg body weight and chamomile oil 10 ml/Kg and compare these extracts with omeprazole as standard antiulcer drug. Reliable parameter was presented for comparing the data including histopathological examination of gastric ulcer and detection of total antioxidant level in the serum of the rats.

Materials and Methods

Animals: Forty two healthy Wister albino rats were used in this study. Their weight was between (200-250 gm). Rats had free access to drink water ad libitum and normal pellet diet.

Drugs: Anti-ulcer agent (Gasec TM) (Omeprazole) was obtained in the form of capsule from Mepha Company Switzerland.

Preparation of plants

Extraction of liquorice: The plants were purchased from local market; the aqueous extracts of liquorice were prepared using 20 gm of powdered plant with 100 ml of distilled water and boiled at 100°C then 0.5 gm/kg

(equivalent to 2.5ml) of clear solution was given orally every day throughout the duration of the experiment to liquorice treated group [21].

Extraction of chamomile:

Chamomile was prepared using 10 g dried material in 100 ml distilled water and boiling for 5 min at 100°C [22], then 0.25 gm/Kg (equivalent to 2.5 ml).

Chamomile oil: Chamomile oil was used already prepared by steam distillation in a dose of 10 ml/Kg body weight.

Design of the study

After two weeks of adaptation the rats were randomly divided into six groups (seven rats in each group) as follows:

Group 1: Negative control group, this group was maintained on normal pellet diet throughout the duration of experiment with distilled water (10 ml/kg).

Group 2: Ulcer induced group, after fasting overnight, salicylic acid (0.2 g/kg) with 1 ml of 80 % ethylene alcohol as single daily doses were given for three consecutive days.

Group 3: After fasting overnight, the rats were received 20 mg/kg of omeprazole in 2 ml of distilled water (one hour prior to administration of salicylic acid and ethylene alcohol).

Group 4: After fasting overnight the animals were received (20 gm/kg) of aqueous extract of liquorice one hour prior to administration of salicylic acid and ethylene alcohol.

Group 5: After fasting overnight, the animals were received (10 gm/kg) of aqueous extract of chamomile one hour prior to administration of salicylic acid and ethylene alcohol.

Group 6: After fasting overnight, the animals were received 10 ml/kg of Chamomile oil one hour prior to administration of salicylic acid and ethylene alcohol.

The blood samples were aspirated directly from the hearts of the rats before and after giving the extracts of the oral chemicals and herbs, then the serum were used to measure level of the total anti-oxidant.

Sample preparation

Serum sample preparation

After overnight fasting 2 ml of blood was drawn directly from the heart of the rats then after receiving medication and ethanol with salicylic acid for three consecutive days another 2 ml of blood were aspirated. Fresh blood was placed in plane test tube and left to stand for 30 minutes at 37 °C in incubator to allow clotting. The serum was prepared by centrifugation at 3000 RPM for 10 minutes and 1 ml of serum was obtained to determine the levels of total serum antioxidant.

Tissue sample preparation:

Rats had been killed using overdose of ether, the abdominal wall was incised longitudinally, the stomach was then isolated and separated from the surrounding viscera by means of two cuts; the first was done one centimeter proximal to the cardiac sphincter and the second was done two centimeter distal to the pyloric sphincter of the stomach, then the stomach was removed and slightly inflated by injecting of formalin through esophageal opening, then the inflated stomach was immersed in 10% neutral formalin for 10 mints, this formalin used to fix both the inner and outer layers of gastric wall. The stomach was opened along the greater curvature rinsed briefly under running tap water then the mucosa was examined to determine the ulcer parameters by means of dissecting microscope. The stomach then preserved in 10% of neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol,

embedded in paraffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin stain and mounted in DPX, to be examination under the light microscope [23].

Measurement of different parameters

Gastric lesion parameters

The method described by Agrawal [24] was employed in the present study. In brief, after 1 hour of administrated ethanol and salicylic acid, all rats were sacrificed after using an overdose of diethyl ether and their stomachs removed and washed by saline. The stomachs opened along the greater curvature, washed with saline and examined under dissecting microscope for gastric ulcers. The sum

of length for all lesions area for each animal was measured and served as the ulcer index.

The mucosa of glandular portion of stomach was examined grossly by means of Olympus binocular dissecting microscope supplied with micrometer squares standardized by a ruler under a magnification power of (10 times) to calculate lesions parameters which constitute the followings:

- 1- Number of lesions per glandular stomach (L.N).
- 2- Total lesion length in (mm) present per glandular stomach (L.L).
- 3- Total surface area of the lesion in (mm²) present per glandular stomach as lesion Index (L.I).

$$P.I. = \frac{LI_{ethanol\&salicylic\ acid} - LI_{ethanol\&salicylic\ acid\ \&\left(\frac{Drug}{agent}\right)}}{LI_{ethanol\&salicylic\ acid}} \times 100$$

Measuring of serum total antioxidant level:

Principle of antioxidant assay

The combined action of the antioxidants provided by the sample or standard acts to reduce Cu⁺⁺ to Cu⁺. This reduced form of copper will selectively form a 2:1 complex with the chromogenic reagent. This complex is stable and has an absorption maximum at ~ 450 nm[25]. A known concentration of Trolox is used to create a reference curve to compare those readings obtained by the samples. Data expressed as mM Trolox, as presented in Figure (1).

Results obtained for the Total Antioxidant Power for a series of serum samples were also compared to the resistance to oxidation of the serum lipids in these samples.

Dilution buffer, copper solution and stop solution should be allowed to equilibrate to room temperature for 30 minutes prior to running the assay.

Dilute both sample and standards 1:40 in the provided dilution buffer (e.g. 15 µl serum + 585 µl buffer).

Procedure:

1. Place 200 µl of diluted samples or standards in each well.
2. Reagent blanks should be dilution buffer provided in the absence of standard or sample.
3. The plate was measured at 450 nm as a reference measurement.
4. Then 50 µl of Cu solution was added to each well and incubate 3 minutes at room temperature.
5. 50 µl of stop solution was added, the absorbance of the samples were measured at 450 nm.

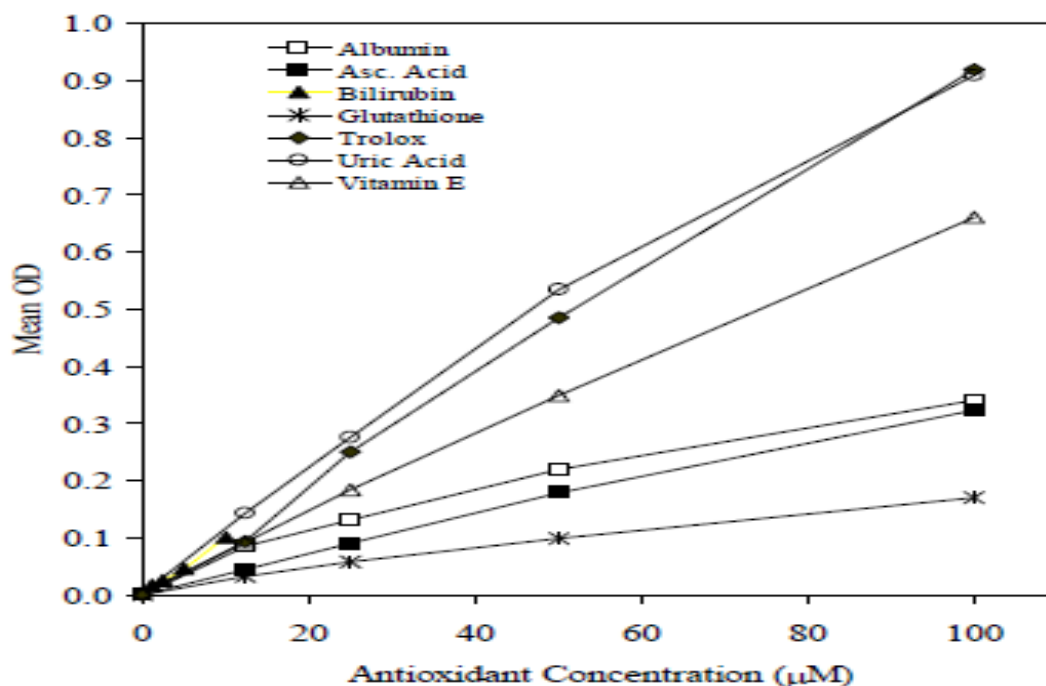


Figure 1 Correlation between antioxidant concentration and reduction from Cu^{++} to Cu^{+} using this method.

Statistical analysis: The obtained results were expressed as Mean $\pm$ SE. Data were evaluated statistically using one-way analysis of variance (ANOVA) using SPSS 18. Significant difference between means was estimated at $p < 0.05$ considered the least significance level [26].

Results

Gastric lesions

Gross pathological finding

Control negative group: The stomachs from negative control group show no gastric mucosa lesions (figure 2).

Ulcer induced group: Severe injury was found in the gastric mucosa.(Figure 3).

Omeprazole treated group: The degree of the lesions of the gastric mucosa in omeprazole treated group showed a less degree of total mucosa ulceration, with less severe mucosal necrosis and hemorrhage than that occur in induced group with significant protection $p < 0.05$.

Liquirice treated group: Liquirice pretreated group show few mild lesions with maintenance of normal architectures of the gastric mucosa

Chamomile treated group: Chamomile pretreated group, both CHAE and CHO show significant protection to the lining mucosa $P < 0.05$ with numerous small lesions affect the gastric mucosa but less severe than that lesion occur in induced group and more than that occur with liquirice pretreated group.

Multiple comparisons between groups

The length of gastric ulcer (mm) in rats as a result of ethanol and salicylic acid administration with and without antiulcer drug or agents (liquirice watery extract, chamomile aqueous extract and chamomile oil effect is recorded in Table (1). Tabulated results revealed that the length of gastric ulcer (mm) as mean $\pm$ SE of liquirice (0.5 gm/kg) treated group with antiulcer drug (control positive group) had significant decrease at $p < 0.05$ compared to

untreated group (ulcer induced group). Groups given orally adequate doses of CHAE (0.25gm/kg of b.wt) also had significant decrease in the length of gastric ulcer at $p < 0.05$ as compared to positive and control groups but less protection than that with liquorice.

Table 1 The effect of oral administration of omeprazole, liquorice, chamomile aqueous extract and chamomile oil on number and length of gastric ulcer (mm) in rats.

Groups	Mean $\pm$ SE Number of gastric ulcers	Mean $\pm$ SE Length of gastric ulcer(mm)
Induced	15 $\pm$ 2.4	12.42 $\pm$ 0.61
Omeprazole 20 mg/kg	3.8 $\pm$ 0.59	1.28 $\pm$ 0.04
Liquorice aqueous extracts 0.5g/kg	4.2 $\pm$ 0.56	1.8 $\pm$ 0.06
Chamomile aqueous extracts 0.25g/kg	8.7 $\pm$ 0.61	4.42 $\pm$ 0.61
Chamomile oil 10ml/kg	10.7 $\pm$ 1.37	4.28 $\pm$ 0.56

The differences in the length of gastric ulcer in rats given orally CHO (10 ml/kg) were significantly differing from that of induced group and less than CHAE treated group.



Figure 2 The stomach of the rat show normal lining mucosa in negative control group.



Figure 3 The stomach of the rat from ulcer induced group show multiple lesions (arrows) caused by salicylic acid and alcohol without any medication.

Table 2 The effects of omeprazole and medicinal plants used in this study on the lesion index and the preventive index of gastric lesions in rats:

Groups	Total lesion index	Preventive index
Water	0	0
Induced	51.0±0.2	0
Omeprazole 20 mg/kg.	8.7±0.1	84.3
Liquirice 0.5gm/kg	10.5±0.4	80.3
Chamomile aqueous 0.25g/Kg	29.1±0.8	43.1
Chamomile oil 10 ml/kg	32.8±1.2	37.2

The ability to prevent ulcer induction by ethanol and salicylic acid were tested during this study. The preventive index was then calculated using the equation mentioned in chapter two and the results were indicated in table (2).

Microscopical finding

The histological differences between different treated groups were observed at the cellular level as the following:

Control group:

The histological study show normal gastric mucosal lining and submucosal tissue (figure 4), there is intact mucosal lining and there is no significant pathological changes.

Ulcer induced group:

The stomachs of rats from ulcer induced group showing hemorrhagic mucosal erosions with inflammatory cell infiltrations developed in the glandular stomach with necrosis of gastric mucosa, associated with congestion of submucosal blood vessels (figure 5).

Liquirice treated group

The administration of liquirice watery extracts at dose of 0.5 gm/kg markedly reduced these changes (Figure 6).

Chamomile treated group

The administration of CHAE at dose of 0.25 gm/kg and CHO 10ml/kg also reduced these lesions but less than liquirice Figure (7) and Figure (8).

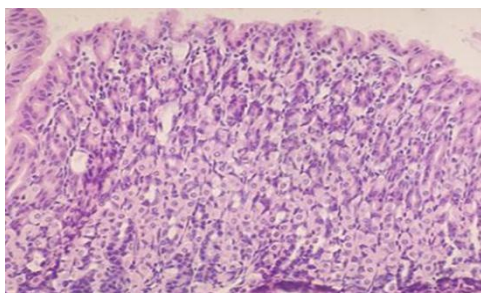


Figure 4 Microscopic view of normal stomach mucosa of the rat H&E x 400.

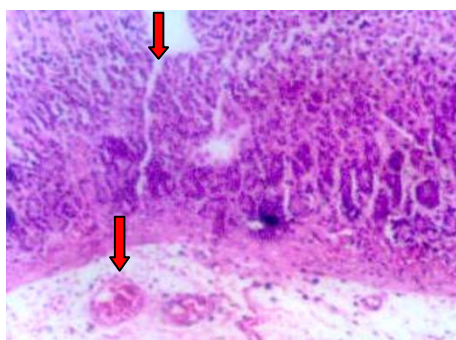


Figure 5 Stomach of rat from ulcer induced group showing necrosis of gastric mucosa, congestion of submucosal blood vessels associated with submucosal edema and hemorrhage (arrows) (H and E x 100).

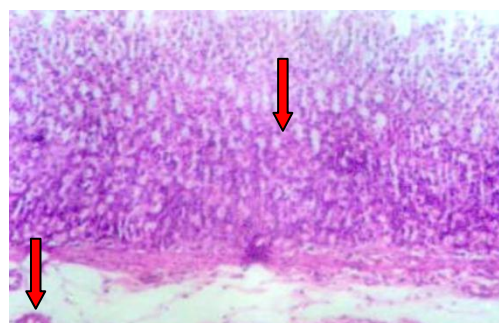


Figure 6 Gastric mucosa of rats given orally liquorice watery extract showing mild edema and vascular congestion (H and E x 100).

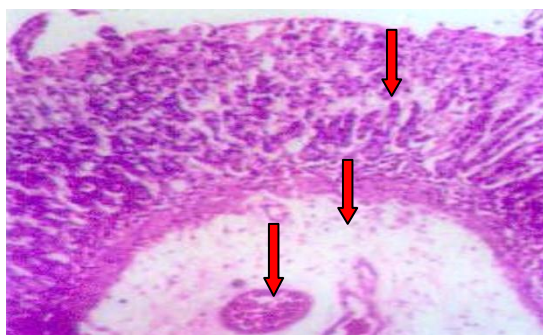


Figure 7 Gastric mucosa of rats given orally CHAE in a dose of 0.25 g/kg of b.wt showing mild edema with congestion of submucosal blood vessels associated with edema (arrow) (H and E x 100).

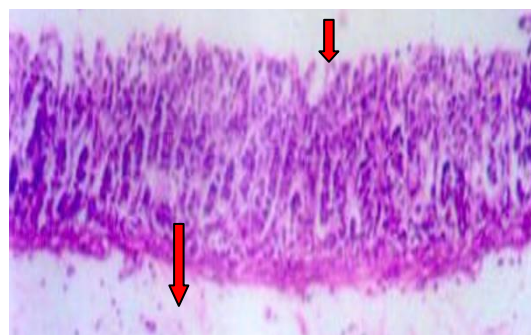


Figure 9 Stomach of rats given orally CHO in a dose of 10 ml/kg of body weight showing edema with mild ulceration (arrow) (H and E x 100).

Liquirice watery extract pretreated group

The total serum antioxidant was significantly increased in this

group compared to the control group ($p < 0.05$) as shown in figure (10).

CHAE and CHO effect on total serum antioxidant level

The total serum antioxidant was significantly increased in chamomile treated group compared to the control group ($p < 0.05$) as shown in figure (11).

Multiple comparisons between groups

There was significant decreased in the serum concentration of total AO in ulcer induced group as compared with normal control group ($P < 0.05$). At these times, there is

significant increased in the serum concentration of total AO concentration in liquorice watery extract treated group as compared with negative control group with greater reduction in total AO concentration in ethanol and salicylic acid ulcer induced group ($P < 0.05$).

There is a significant difference in serum concentration of total AO between liquorice watery extract treated groups and chamomile treated group (watery extract and chamomile oil) and control group ($P < 0.05$) (Figure 12).

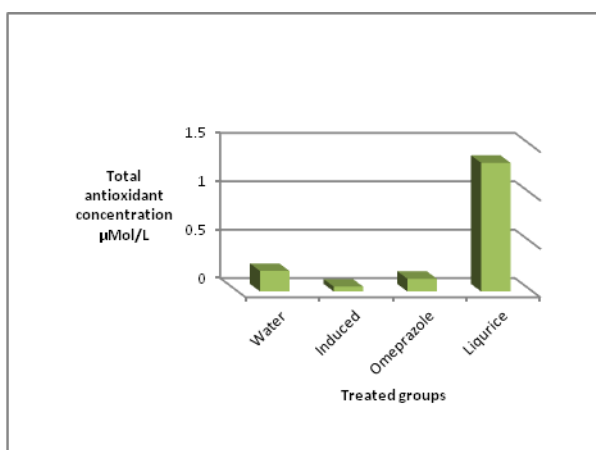


Figure 10 Total antioxidant concentration (μmol/L) in Omeprazole, Liqurice and induced groups.

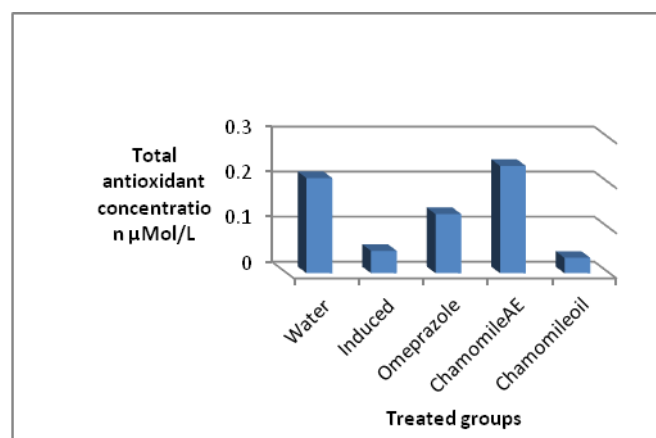


Figure 11 Total antioxidant concentration (μmol/L) in induced, Omeprazole and chamomile treated groups.

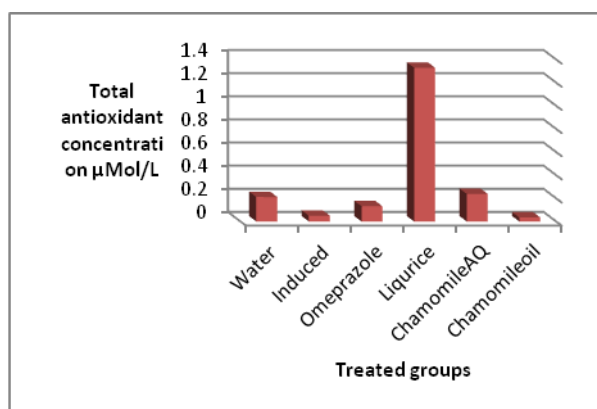


Figure 12 Total antioxidant concentration (μmol/L) in different treated groups.

Discussion

The gastric hyperacidity and ulceration of the stomach mucosa due to various agents are serious health problems of global concern. Moreover, there is growing evidence that oxygen derived free radicals such as $\text{OH}^\cdot$, $\text{O}_2^\cdot$, $\text{RO}^\cdot$, and $\text{ROO}^\cdot$ play a role in the pathogenesis of various disorders of the digestive system including gastric ulcer [8].

A number of antiulcer drugs developed over the years, have proven useful in controlling hyperacidity and ulceration though their long-term use is associated with various side effects. The search for new anti-ulcer preparations from non-toxic medicinal plants is currently developed in order to obtain alternative sources of medicine for the management of gastric hypersecretion and gastroduodenal ulcers.

Ethanol and salicylic acid serves as the most common ulcerogenic agents when given intragastrically to rats [27].

The chronic use of aspirin with ethanol can produce lesions that appear as blackish lesions grouped in patches of varying size usually parallel to major axis of the stomach. This study reveals the difference in the severity of mucosal lesions that ranged from superficial lesions to that extending through the mucosa associated with alteration in the concentration of total serum antioxidant after exposure to salicylic acid with ethanol and after omeprazole and herbs administration.

Gastric lesions

Control negative group

In this group seven rats were given 2 ml of distilled water orally without any additional medication or herbs in this group we get no lesion in the stomach.

Ulcer induced group

In this group seven rats were given 2 ml of DW orally one hour

before oral dose of SA (0.2 gm/kg) with 1 ml 80% ethylene alcohol the same dose repeated for three consecutive days. By this mean we get 100% induction of acute ulceration. The parameters of control group were 15.5 ± 2.4 for lesion number, 3.42 ± 0.6 for lesion length. The total lesion index was 51.0 ± 0.2 .

The genesis of ethanol induced gastric lesions is multifactorial with the depletion of gastric wall mucus content as one of the involved factors [28]. Oral administration of high concentration ethanol in rats is in fact noxious for the stomach, affecting the gastric mucosa topically by disrupting its barrier and provoking pronounced microvascular changes in few minutes after its application. Thus, rapid and strong vasoconstriction is accompanied by rapid and vigorous arteriolar dilatation and this combination of microvascular events induces damage in mucosal capillaries [29]. In addition to the direct mucosal injury free-radical production like hydroxyl radicals are well known pathogenic effects of ethanol [30]. The effects of alcohol bring about depression in gastric defensive mechanisms leading to the formation of gastric mucosal lesions with severe gastric hemorrhagic erosions [31].

Not only alcohol causes direct mucosal damage, its abuse is also associated with development of gastric diseases such as gastritis and even gastric cancer [29]. Acute gastritis caused by direct mucosal damages are usually further aggravated by other important risk factors such as non steroidal Anti-inflammatory drugs (NSAIDs), acid, H. pylori infection and physiological stress [32].

Ethanol induces damage to the gastric mucosa which is associated with a production of free radicals [33] leading to increase lipid peroxidation

this cause damage to cell and cell membrane.

Another explanation for ethanol induced gastric mucosal injury by increases the release of histamine, the influx of calcium ions and the generation of free radicals [34]. Recently, much attention has been focused on the role of ROS, including O_2^- , OH^- and H_2O_2 in mediating alcoholic tissue damage. Preventive endogenous antioxidants, such as SOD and catalase enzymes are the first line of defense against ROS. Reduced glutathione is a major scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation.

Control positive group

In this group, the animals were given omeprazole (20 mg) in 2 ml of DW orally. It is a potent proton pump inhibitor that showed a drop in lesions parameters, as lesions number was 3.8 ± 0.59 , lesions length was 1.28 ± 0.04 mm, and lesions index was 8.7 ± 0.1 . These results were significant at $P < 0.05$ and its preventive index (P.I) was equal to 84.3 and so it is very effective in the prevention of aspirin and ethanol induced gastric lesion. This result is agrees with Ohara et al (1988). This effect is related to its mechanism of action by binding to the H^+/K^+ ATPase enzyme system of the parietal cells, suppressing the secretion of hydrogen ion into gastric lumen and these results agree other study [35].

Liquirice treated group

Our finding showed that aqueous extracts of liquirice root at the tested dose (0.5gm/kg of b.wt) had gastroprotective effects on experimental induced acute gastric ulcer in rats. The lesion number was 4.2 ± 0.56 , lesion length was 1.8 ± 0.06 mm, and lesion index was 10.5 ± 0.4 . These results were significant at $P < 0.05$ and its preventive index (P.I)

was equal to 80.3 and so it is very effective in the protection against salicylic acid and ethanol induced gastric lesion. It is possible that the mechanism by which liquorice prevents its gastric mucosa damage may be due to increased mucus production or prevention of mucus depletion on exposure to noxious agent. In the current study, statistical analysis indicated that the liquorice inhibition of ethanol-induced gastric ulceration was significant in the stomach this finding agree with other study [9].

Chamomile watery extract treated group

The animals received 2 ml of aqueous extract of chamomile which reduced the lesion parameters to 8.7 ± 0.61 for lesion number and 4.42 ± 0.61 for lesion length, and 29.1 ± 0.8 for lesion index and 43.1 for preventive index. These results were significant at $P < 0.05$. The results may be related to the anti-inflammatory effect caused by chamomile flavones. Alpha-bisabolol decreases the proteolytic activity of pepsin by 50%. Previous study reported that chamomile flower extract has a complex effect on the luminal and mucosal environment of the stomach and duodenum. Some of these actions are important in healing the ulcers and others are important in preventing subsequent ulcer relapse. Chamomile aqueous extract has a direct effect on acid secretion, and increases mucosal resistance against damaging agents such as ethanol and aspirin [36]. Other research revealed that chamomile aqueous extract, singly or combined with other plants have antiulcerogenic activity [37]. The anti ulcer action of CHAE may be related to a variety of mineral elements including manganese and magnesium and 1-2% volatile oils including α - bisabolol, α - bisabolol oxides A and B, matricine presented in

the chamomile flowers[38]. Many components that may exert antiulcer effects like phenolic and flavonoids compounds, apigenin, quercetin, patuletin, luteolin and their glycosides are the major flavonoids present in the flower. The presence of large amounts of cinnamic acid derivatives, folic and caffeic acid and all of the constituents, may have therapeutic effects. Polysaccharides, amino acids and fatty acids are some of its constituents[39].

The present study showed that the gastroprotective effects of chamomile aqueous extract on acute experimental gastric ulcer in rat. Chamomile aqueous extract contains many components that may exert antiulcer effects. The results are consistent with Khayyal and co-workers[37]. The cytoprotective effect of the CHAE extracts could be partly caused by their flavonoid content and to their free radical scavenging properties[37].

Chamomile oil treated group

The animals received 2 ml of chamomile oil which slightly reduced the lesion parameters to 10.7 ± 1.37 for lesion number and to 4.28 ± 0.56 for lesion length and 32.8 ± 1.2 for lesion index. These results were significant at $P < 0.05$. The preventive index was 37.2. The results may be related to the mild anti-inflammatory effect caused by chamomile flavones.

The antipeptic actions of chamomile extract may be due to chamomile flavonoid constituents, apigenin. Similar results were observed with alpha-bisabolol and the cis-spiroethers and the small amount of coumarins contribute to smooth muscle relaxation[40]. The antiulcer effect of chamomile extract agreed with other study which reported that chamomile had anti-inflammatory and spasmolytic effects on the stomach and duodenum [41]. Therefore, it is thought to heal ulcers.

Histopathology

The histopathological observations in this study showed that there is no lesion in the stomach in control negative groups in contrast to the stomach from ulcer induced group that show severe ulceration and hemorrhage, upon liquorice pretreatment, the mucosal epithelium had near normal architecture and it had less hemorrhage as against the ethanol and salicylic acid induced damages in the mucosal epithelium of the positive control. These observations on the cytoprotective nature of liquorice against ethanol and salicylic acid induced gastric ulcers prove its antiulcer activity. Also ethanol treatment caused a significant increase in the ulcer index whereas liquorice pretreated rats showed a significant reduction in the ethanol and salicylic acid effect. This further supports Glavin and Szabo study[42] which revealed that liquorice protect the gastric mucosa damage.

Regarding CHAE and CHO, the lesion of gastric mucosa show less severe effects with protection of the glandular stomach from the effects of ethanol and salicylic acid but less than the effects of liquorice. This may be explained by various constituent of chamomile that have protection of lining epithelium against injury by ethanol and salicylic acid.

Total Antioxidant Concentration

- The total antioxidant in control negative group shows increased in its concentration that can be explained by the presence of antioxidant component from the pellet diet.
- In ethanol induced group, our findings demonstrated that ethanol decreased level of total antioxidant than in non-treated control rats, but no significant differences were found in liquorice treated with respect to the control. These results

might be due to the accumulation of free radicals, as free radicals induce lipid peroxidation damage to the tissues.

- Omeprazole treated group show some reduction in total antioxidant concentration that may explain by stress effect that have no counter effect by antioxidant supplementation.
- Liquorice pre-treatment offered protection against the action of ethanol and aspirin on gastric ulceration showing that the presence of some antioxidant phytoconstituents might have protected the gastric mucosa from free radical induced damage. Some of which include; gallic acid[45]. This supports the fact that antioxidants reduce oxidative damage in tissues. These finding agree with Valenzuela results [44] how found that antioxidants, given prior to ethanol, abolished both hepatic oxidized glutathione accumulation and the increase in lipid breakdown products.

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