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Evaluation of Prophylactic Effect of Aqueous *Liquorice* **Root Extract Against Gastric Ulcer in Rats Induced by Ethanol**



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Abstract

THE purpose of the study was to determine the effectiveness of *liquorice* root extract in preventing stomach ulcers in rats that were caused by 99.5% ethanol. The liquoric root was purchased from the Anbar Governorate local market. This study was conducted in two stages: first, plant collection, aqueous extraction of liquorice root in a Soxhlet apparatus, and phytochemical analysis for the extract; second, assessment of the extract's anti-ulcerogenic effect in comparison to omeprazole, using TNF, IL-10, ulcer index, and PH. values as parameters. Thirty rats made up the total number of animals, and they included: Group (negative) received only distilled water; Group (positive) received absolute ethanol orally to induce a gastric ulcer; Group (omeprazole) received omeprazole orally for 10 consecutive days prior to ethanol-induced ulcerogenesis; Group (liquorice 250 mg/ kg) received liquorice root extract (250mg/ kg) orally for 10 consecutive days prior to ethanol-induced ulcerogenesis; and Group (liquorice 500 mg/ kg) received liquorice root extract (500mg/ kg) orally for 10 consecutive days prior to ethanol-induced ulcerogenesis. All animals were killed one hour after the stomach ulcer was induced. Protein and coumarin were lacking from the phytochemical results, while tannin, carbohydrates, phenols, resins, flavonoids, saponins, alkaloids, terpenes, and steroids were present. Group 1 consisted of healthy animals with normal TNF, IL-10, ulcer index, and PH. values. Groups (positive) and (liquorice 500 mg/ kg) showed increasing TNF and ulcer index, decreasing PH. values and IL-10. Groups (omeprazole) and (liquorice 250 mg/ kg) showed decreasing TNF and ulcer index, while increasing in PH. values, and IL-10. Based on the aforementioned findings, we deduced that the liquorice root aqueous extract reduced the amount of absolute ethanol-induced stomach ulcers.

Keywords: liquorice root, gastric ulcer, TNF, IL-10, ulcer index, PH. values, Omeprazole.

Introduction

One of the most prevalent gastrointestinal conditions is peptic ulcer [1]. It is caused by an imbalance between the protective gastric mucosal integrity components and aggressive stomach acid secretion, which affects a significant portion of the global population [2]. An ulcer is characterized by the sloughing of inflammatory necrotic tissue, which causes the mucosal layer to erode or the tissue surface to be excavated. The goals of ulcer treatment were to reduce pain, which was important, and to stop the ulcer from happening again. However, no good treatment plan has been identified, so research is currently ongoing to develop effective ulcer remedies [3]. In gastric therapy, proton pump inhibitors (PPIs) like OME are commonly utilized [4, 5]. Inhibiting HCl secretion is another function of several PPIs, including lansoprazole, rabeprazole, pantoprazole, esomeprazole, and dexlansoprazole [6]. Botanical or phytomedicines, another name for herbal medicines, have been used for centuries as treatments for a variety of medical ailments by many different civilizations worldwide [7]. These medications are made from plants and plant components, including fruits, leaves, stalks, roots, and flowers. They are being prepared in different ways to bring out the medicinal values of the plant (Osunt in modern health care system [8].

Herbal medicine as a treatment with scientific evidences has a great promise [9]. Liquorice is the common name of Glycyrrhiza glabra L, family Leguminoceae from which a somewhat sweet flavor can be extracted [10; 11]. Liquorice is worldwide used as medicinal and therapeutic plant in addition to

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use its roots as active natural sweetening source as it is 17 - 50 time sweetest than sucrose [12]. Its important phytoconstituents were demonstrate with the presence of glycyrrhizin, glycyrrhizinic acid, glabrin A and B and isoflavones. Several phytochemical constituents in licorice extract had been identified [13]. pharmacological impacts of liquorice and its isolated active compounds on animals have been confirmed by many workers: antimicrobial, antihelicobacter, antiatherosclerotic, antioxidative, antifungal, antiviral, antiinfective, immune stimulator impacts, anti-inflammatory, antidiabetic, anti-ulcer, and antidiuretic properties [14; 15]. Liquorice root extract showed antibacterial activity because of the presence of saponin, glycosides, glucose, sucrose, resin and asparagine therein [16]. It had multiple phytochemicals with medicinal values, including the presence of tepenoids, aromatic phenolic compounds, gumarin compounds, volatile oils, which typically contains more than 80 compounds, of which it includes acetic acid, butyric acid and propnic acid. Vitamins B1, B2, B3, B6, B, C, folic acid, and biotin are also present. Apart from metallic components like cobalt, zinc, calcium, and aluminum [17]. In traditional herbal medicine, licorice was considered as a natural source of sex hormones and used to: strengthen female reproductive system, to treat cardiac conditions, increase memory function, prolong life, and improve overall health [18; 19]. Because of its flavonoids, deglycyrrhized licorice (DGL) is used to treat peptic ulcers [20]. Because of its lower mineralocorticoid action, this version is utilized to treat peptic ulcers in order to promote healing [21.

Material and Methods

Extraction and Phytochemical Screening of Plant. 750 milliliters of water were combined with 100 grams of liquorice powder, and Soxhlet equipment was used to prepare the extract. We diluted this extract to the necessary dosage and stored it in the refrigerator just before feeding the animals [22]. While the common techniques for identifying the major phytoconstituents were used in the chemical analyses performed on the extract of liquorice powder.

Experimental Animals

The experiment for this investigation used thirty rats, weighing between 200 and 300 g and roughly three months old. The rats are housed in plastic cages in a dedicated housing area for two weeks to allow for adaptation. The housing conditions were 20–25 degrees Coin air-conditioned rooms with a 14–10 light–dark cycle. The litter in the cages is changed once a week.

Induction of ulcerogenesis.

Animals were given a dose of 1 milliliter per kilogram of body weight of 99.5% ethanol orally to induce ulcerogenesis [23].

Experimental design

In this experiment 30 adult female rats were have divided equally into five groups and treatment for 10 days as follows:

Negative control group given orally distilled water.

Positive control group given orally distilled water before induction of ulcerogenesis by ethanol (99.5%).

Omeprazole group given orally (0.6 mg/kg) omeprazole for 10 consecutive days [24). Before ethanol-induced ulcerogenesis.

Liquorice root extract (250 mg/kg) group given orally Liquorice root extract. (250 mg/kg) administered for 10 consecutive days before ethanolinduced ulcerogenesis [25].

Liquorice root extract (500 mg/kg) given orally Liquorice root extract (500mg\Kg) administered for 10 consecutive days before ethanol-induced ulcerogenesis [25].

Parameters of Experiment

Estimation of gastric ulcer index

For each group, ulcer index was determined using the following equation: Ulcer Index (UI) = (nI) + (nII) 2 + (nIII) 3\Number of animals. Where: n is the number of lesions.

It was determined how many and how severe the lesions were. Based on the findings of [26], three scores were utilized: light (I) for the presence of edema, hyperemia, and a single petechial; moderate (II) for the presence of submucosal hemorrhagic lesions with minor erosions; and severe (III) for the presence of hemorrhagic lesions with severe erosions.

pH values measurement

Samples of gastric contents were analyzed for hydrogen ion concentration by pH metric titration with 0.1 N NaOH solutions using digital pH meter [27].

TNF and IL10 measurement after induction of ulcerogenesis.

Blood obtain from each animal using a sterile syringe, placed it in a gel tube, and centrifuged it for 15 minutes at 4000 rpm to extract serum both before and after induction of ulcerogenesis. The processed samples putted in Eppendorf tubes and kept them in frozen until needed.

Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference

factors in study parameters. Least significant difference–LSD test (One way, Analysis of Variation-ANOVA) was used to significant compare between means in this study.

Results and Discussion

The semisolid extract from the aqueous extraction of liquorice root had a dark brown color and an extraction ratio of 18%. We calculated the extraction ratio using the following equation: The extract's percentage yield can be calculated as follows: $(18 \times 100) \div 100 = 18\%$; or, weight of extract (g) $\times 100$) / original weight of powder (g).

These findings corroborated those of [28], who discovered that the dark brown color was a result of the beverage ice root's aqueous extraction. These outcomes could be the result of the same extraction procedures or the same plant portion [28].

Phytochemical Screening Results of Aqueous extraction of Liquorice root. Phytochemical results of aqueous extraction of liquorice root the present of tannins, carbohydrates, phenols, resins, flavonoids, saponins, alkaloids, terpenes, steroids, while the absence of protein and coumarins as in Table 1.

According to [29; 30], the extraction of liquorice root yielded alkaloids, tannins, flavonoids, phenols, resins, terpenoides, saponins, carbohydrates, and steroids. These phytochemical analysis results were consistent with their findings. These outcomes might arise from the same extraction process, but they could also stem from the same part of the plant. Prophylactic efficacy of Liquorice root extract at multiple concentrations and omeprazole on the TNF- α level in serum.

By inspecting the tables (2, 3.), it is represented the preventive effect that have a liquorice root for TNF- α serum. As you can see, the positive control group and the group that has administered with 500 mg/kg liquorice for extract has showed significant increase in level of TNF- α serum, while the significant decrease in level of TNF- α serum has completed to compare in the groups which had 50 mg/kg of omeprazole and to the group which gave beeswax has treated high dose of liquorice 250 mg/kg regard to negative control group. Its findings of prophylactic actions were congruent with those of [31], who noted licorice inhibiting prostaglandin synthesis and cyclooxygenase activity.

It also indirectly inhibits platelet aggregation and the elements of the inflammatory cascade. Licorice extract also inhibits the phosphorylation of proteins involved in macrophage intracellular signaling, including transcription factors, nuclear factor-kappa B, and activator protein (AP). These proteins are crucial in the pathways of inflammatory signaling) [31]. While for Liquorice root extract 500 mg/ kg has been associated with an increased ulcer index when consumed in large amounts due to its active component, glycyrrhizin. Glycyrrhizin has been shown to increase the secretion of gastric acid and pepsin, which can lead to gastric mucosal injury and exacerbate existing ulcers. Additionally, glycyrrhizin can inhibit prostaglandin synthesis, which is important for maintaining the integrity of the gastric mucosa and promoting mucosal defense mechanisms. [32].

Anti-inflammatory Effects of Liquorice Root Extract on The Serum Level of IL10 with different concentrations, compared to omeprazole and control

On comparing the groups receiving omeprazole and liqurice 250 mg/kg with the positive control group, a marked significant increasing of IL10 serum levels was observed. While, on comparing liqurice root extract 500 mg/kg and the positive control group with the negative (untreated) group, a marked significant reduction of IL10 serum level was observed. Preventive Results. The pattern of results obtained for the preventive effect was somewhat close to those for the curative effect as reported by [14]. After reviewing the literature, it was proposed that glycyrrhizin, a chemical found in liquorice root, may be useful in the treatment of some inflammatory diseases due to its possible anti-inflammatory properties. Many studies have supported the idea that glycyrrhizin inhibits the synthesis of interleukin-10 (IL-10), a cytokine influential in immunoregulation. This, therefore, suggests that licorice root might assist in regulating the anti-inflammatory processes, through adjusting the IL-10 levels and possibly, therefore, reducing inflammation in the body. In any case, it is important to point out that further research is needed to fully understand how liquorice root alters the levels of IL-10 and, in turn, the importance of these changes for overall health [14]. Furthermore, the study revealed that the plant phenols contained in licorice (such as flavonoids) have multiple health benefits including being anti-inflammatory and antioxidant [33].

Prophylaxis effect of Liquorice root extract with different concentrations and omeprazole on ulcer index

There is a significant difference among the severity of ulcer index for each group. The group which given 500 mg/kg of liquorice root extract have the most severity of ulcer (Table 4). Possible reason for that effects is liquorice root extract promote the level of production for prostaglandins which is needed to prevent and reduce ulcer by protecting the mucosal layer of stomach and assist stomach to repair itself. Moreover, liquorice root extract anti-inflammatory capabilities can reduce inflammation of stomach lining and reduced severity of ulcers. A recent study also found liquorice root extract inhibits the growth of Helicobacter pylori, a kind of bacteria associated with development of the ulcers [34]. In explored the gastroprotective role played by liquorice

root extract in rats who had developed ulcers in their stomach as a result of ethanol. The authors found that the administration of liquorice root extract to the group of rats who suffered understood ulcers had significantly lower ulcer index in comparison to the group that was the control group. The authors hypothesized that the activity of the digestive health 'winner': liquorice root extract could be attributed to improvement of antioxidant defense, reduction of pepsin activity, and increase in mucin production [35]. Nonetheless, high ingestion of glycyrrhizin, the active ingredient in liquorice root extract, has been linked with an elevated ulcer index of 500 mg/kg (a research study published in Physiological Research in 2002 published by the Japanese Society for Physiological Research). Literature suggests evidence for a direct or indirect action of glycyrrhizin, increasing pepsin and gastric acid secretion, thus inducing necrosis of the stomach mucosa and raising as existing peptic ulcers. Glycyrrhizin has also been speculated to stop the synthesis of prostaglandins for gastric mucosal defence and promoting its healing [32].

The Prophylaxis effect of Liquorice root extract in stomach of rats with different concentrations and omeprazole on PH

The preventative results for pH values displayed in Post-AMP group in Table (5) reveals that both groups got omeprazole and liquorice extract 250 mg/kg has significantly higher values than negative control. However, the positive control and liquorice extract 500 mg/kg have significantly lower values. The Japanese scientists [36] corroborated these findings. who found, in rats, that glycyrrhizin increased histamine secretion from enterochromaffin-like cells, which in turn increased the activity of the proton pump, and therefore gastric acid output. Rising stomach pH can be the outcome of increased acid output This represents the furthest response in the cascade we have considered so far. Hirasawa and colleagues also assessed glycyrrhizin's effects on gastric acid secretion in rats. They exposed two sets of rats to either 200 mg/kg/day or 400 mg/kg/day of glycyrrhizin for one week. They further exposed a set of control rats to a placebo. They then measured the rats' cortisol levels. The results demonstrated that, in rats, high doses of glycyrrhizin (200 mg/kg/day and 400 mg/kg/day) can boost cortisol levels. [36] acknowledge that 'The comprehensive role of glycyrrhizin with regard to the regulation of cortisol secretion and gastric acid output in human and application to health still remain to be resolved. Liquorice root extract might change pH levels in the stomach, depending on the state of the stomach. This study used liquorice root extract to treat the change in the gastric mucosa of rats with indomethacin-induced gastric ulcers. They found that the presence of liquorice root added a significant increase in pH in the indomethacin-induced gastric ulcer region, possibly protecting the stomach. the preventive results were shown in the tables below, the omeprazole and liquorice root 250 mg/kg groups showed significantly higher pH values than the negative control groups and the groups adding positive control and liquorice root 500 mg/kg showed significantly lower pH values. These results were analogous to the results found by [36] that demonstrated glycyrrhizin tonically enhanced the release of histamine from enterochromaffin-like cells and increased the activity of the proton pump, thus enhancing the output of acid in rats. The pH of the stomach might have increased due to such increased acid output. Additionally, while animal models described the effects of glycyrrhizin on gastric acid secretion and cortisol, more research will be needed to know the full implications for the health of humans [36]. Scientists have looked at potential alternatives for liquorice root extract to bring about this effect on rats with stomach ulcers related to pH changes, as well. For instance, one study investigated the impact of liquorice root products on rats experiencing stomach ulcers as a result of treatment with indomethacin. This study indicated that liquorice root extract in the presence of ulcers significantly increased pH in the part of the stomach with ulcers, thus proposing a protective effect against acids on the stomach mucosa [37].

Conclusion

When compared to omeprazole, an aqueous extract from liquorice 250 mg/ kg exhibited an antiulcerogenic effect on stomach ulcers caused by 99.5% ethanol in rats. TNF and the ulcer index also decreased, while IL-10 and pH. values increased, while liquorice 500 mg/ kg showed increasing TNF and ulcer index, decreasing pH. values and IL-10. We recommended in this researching that 250 mg/ kg extract's potential effects on induced ulcer, while the increasing in the dose will exacerbate the illness.

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Conflicts of interest

None

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The authors declare that the present study has no financial issues to disclose.

Authors contributions:

Each author made an equal contribution

TABLE 1	. Phytochemical	l analysis re	sults of <i>liq</i>	<i>uorice</i> root
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Phytoconstituents	Results
Tannins	+
Carbohydrate	+
Phenols	+
Resins	+
Flavonoids	+
Saponin	+
Alkaloid	+
Protein	-
Coumarins	-
Terpenes	+
Steroids	+
+ =	presence ,- = Absent

TABLE 2. Prophylaxis effect of Liquorice root extract with different concentrations and omeprazole on serum level of TNF-α. (pg/ml) in different groups 10 days before ethanol-induced ulcerogenesis

Group N=6	TNF -α. (pg ./ ml)
Control negative [G1) Orally distilled water	c 126.9±11.38
Control Positive [G2) Ethanol induced ulcerogenesis	a 419.0±11.01
Omeprazole [G3) [0.6 mg/kg)	c 169.5±8.96
Liquorice root extract [G4) [250 mg/kg)	c 176.4±18.16
Liquorice root extract G5 [500mg/kg)	b 248.8±23.56
LSD	32.5
Means with a different letter are significantly diff	ferent [P<0.05)

TABLE 3. Prophylaxis effect of Liquorice root extract with different concentrations and omeprazole on serum level of IL10. (pg./ml) in different groups 10 days before ethanol-induced ulcer genesis

Groups N=6	IL10 (pg./ml)
Control negative (G1) Orally distilled water	a 33.28±2.07
Control Positive (G2) Ethanol induced ulcerogenesis	b 15.26±0.7
Omeprazole (G3) (0.6 mg/kg)	ab 23.4±2.20
Liquorice root extract (G4) root extract (250 mg/kg)	a 28.1±0.83
Liquorice root extract (G5) (500 mg/kg)	b 18.31±1.79
LSD	4.88
Means with a different letter are significantly different (I	P<0.05)

TABLE 4. Prophylaxis effect of Liquorice root extract with different concentrations and omeprazole on ulcer index in different groups 10 days before ethanol-induced ulcerogenesis

Groups	N=6	Ulcer index
Control neg	ative [G1) Orally distilled water	c 0.00±0.00
Control Posi	itive [G2) Ethanol induced ulcerogenesis	a 1.40±0.21
Omeprazole	[0.6mg/kg)	b 0.90±0.20
<i>Liquorice</i> ro	ot extract G3 [250mg/kg)	b 0.80±0.
Liquorice ro	ot extract G4[500mg/kg)	a 1.30±0.10
LSD		0.38
	Means with a different letter are significantly diff	ferent [P<0.05)

Groups N=6	РН
Control negative [G1) Orally distilled water	c 4.26±0.06
Control Positive [G2) Ethanol induced ulcerogenesis	d 3.20±0.09
Omeprazole [G3) [0.6mg/kg)	a 5.88±0.18
Liquorice root extract [G4) [250mg/kg)	b 5.16±0.02
Liquorice root extract [G5) [500mg/kg)	d 3.40±0.03
LSD	0.28
Means with a different letter are significantly different [P-	<0.05)

 TABLE 5. Prophylaxis effect of Liquorice root extract in stomach of rats with different concentrations and omeprazole on PH values in different groups 10 days before ethanol-induced ulcerogenesis

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تقييم التأثير الوقائي لمستخلص جذور عرق السوس المائي ضد قرحة المعدة في الجرذان. المحدثة بالإيثانول

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الملخص

تهدف هذه الدراسة الى تحديد مدى فعالية مستخلص جذور عرق السوس ودوره في الوقاية من قرحة المعدة لدى الجرذان التي يسببها الكحول الاثيلي بنسبة 99.5% حيث تم شراء جذور عرق السوس من السوق المحلي لمحافظة الأنبار/ العراق و أجريت هذه الدراسة على مرحلتين: الأولى تم جمع النباتات والاستخلاص المائي لجذور عرق السوس في جهاز السوكسيليت والتحليل الكيميائي النباتي للمستخلص، ثانياً، تقييم تأثير المستخلص المضاد للقرحة بالمقارنة مع عقار أوميبرازول، باستخدام قيم TNF وINL10 ومؤشر القرحة وقيم الأس الهايدروجيني كمعابير. وكان عدد الحيوانات ثلاثين جرذا قسمت على خمسة مجاميع: المجموعة [1) تم اعطائها الماء المقطر فقط؛ المجموعة [2) تم اعطائها الكحول الاثيلي المطلق عن طريق الفم لاستحداث قرحة المعدة. المجموعة [3) تم تجريعها بعقار أوميبر أزول عن طريق الفم لمدة 10 أيام متتالية قبل التقرح الناجم عن الكحول الاثيلي وكذلك تم تجريع المجموعة [4) مستخلص جذر عرق السوس بجرعة [mg/ kg250] عن طريق الفم لمدة 10 أيام متتالية قبل التقرح الناجم عن الكحول الاثيلي. والمجموعة [5] تلقت مستخلُّص جذر عرق السوس بجرعة (mg/ kg500) عن طريق الفم لمدة 10 أيام متتالية قبَّل التقرح الناجم عن الكحول الاثيلي. و تم قتل جميع الحيوانات بعد ساعة واحدة من إحداث قرحة المعدة. اظهر النتائج ان البروتين والكومارين غير موجودين في الفحص الكيميائي النباتي، في حين ظهرت مجموعة من المكونات وهي التانين، الكربوهيدرات ، الفينول ، الراتنجات ، الفلافونويد ، الصابونين ، القلويدات، التربين والستيرويدات. وتألفت المجموعة الأولى من حيوانات سليمة ذات قيم TNF وINL10 ومؤشر القرحة ودرجة الحموضة طبيعية بينما أظهرت المجموعات 2، 3، 4، و 5 زيادة في عامل نخر الورم ومؤشر القرحة، وانخفاض قيم الأس الهيدروجيني. وINL10، وانخفاض عامل نخر الورم ومؤشر القرحة، على التوالي كما أظهرت المجموعة 3 أيضًا انخفاضًا في عامل نخر الورم ومؤشر القرحة، وزيادة في قيم الأس الهايدروجيني، وINL10، وأظهرت المجموعة 5 زيادة في عامل نخر الورم ومؤشر القرحة، وانخفاضًا في قيم الأس الهايدروجيني ، وINL10. بناءً على النتائج المذكورة أعلاه تستنتج الدراسة أن المستخلص المائي لجذر عرق السوس يقلل من كمية قرَّحة المعدة الناجمة عن الكحول الأثيلي في الجرذان.

الكلمات المفتاحية: قرحة المعدة، الامبر ازول، الكحول.