

Antiulcer Activities of *Commiphora molmol* (Myrrh) Extract in Male Rats

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Abstract

Medicinal plants used in folk medicine contain a wide range of substances that can prevent and treat many diseases. The Antiulcer Activities effects of myrrh or *Commiphora molmol* extract (CME) were assessed in rats. In this experiment, thirty rats were divided into 6 groups as follows: G1) normal control (vehicle); G2) received aspirin without any treatment; G3) pretreated with Omeprazole (antiulcer drug) and groups (4), (5) and (6) were pretreated with CME at 125, 250 and 500 mg/kg b.wt, respectively for 2 weeks. At the end of experiment the volume, pH and total acidity of gastric juices; mucus content and ulcer lengths were measured and protection percentages were calculated. Gross and histopathological examinations of stomachs were also performed. CME induced an antiulcer effect manifested by decreased volume and total acidity of gastric juice and increased mucus content and percentages of protection from ulcer as well as partial amelioration of gross and histopathological lesions seen in stomach of ulcerated rats. In conclusion, the results denote that *Commiphora molmol* extract possess antiulcer effects in rats. These results affirm the traditional use of Myrrh extract for the treatment of gastric ulcer.

Keywords

Commiphora molmol, Myrrh Extract, Antiulcer, Gastric Juices, Omeprazole

1. Introduction

Peptic (gastric) ulcer is the most common gastrointestinal disorder in clinical practice and it arises due to imbalance between aggressive and protective factors in the stomach. Several factors are involved in the etiology of gastric ulcers such as imbalance between

HCl acid and pepsin secretion, irritant drugs as NSAIDs (aspirin), stress factors, smoking, and *H. pylori* infection [1]. A number of drugs including proton pump inhibitors (Omeprazole) and histamine (H₂) receptor antagonists (Ranitidine) are available for the treatment of peptic ulcer, but these drugs have shown incidence of relapses, side effects, and drug interactions. Thus the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse [2]. The long term use of chemical drugs for treating gastric ulcer leads to an ineffectiveness of different drug regimens and even resistance to drugs is emerged. Therefore, there is an urgent need to identify more effective and safe antiulcer agents from medicinal plants [3].

Medicinal plants used in folk medicine contain a wide range of substances that can prevent and treat many diseases. The medical value of these plants is due to presence of some bioactive constituents such as flavonoids, phytosterols, diterpenes, triterpenes, and polyphenolic compounds [4] [5].

Commiphora molmol (Family *Burseraceae*) is small perennial tropical plant tree that grown in arid and semiarid regions in East Africa, Saudi Arabia and India [6]. Myrrh is a resinous exudate obtained from the stem of the plant trees. Myrrh has been approved in USA by the FDA as a safe flavoring agent in foods and beverages and as fragrance (Balsam of Mecca from *Commiphora gileadensis*) in cosmetics [7] [8]. For many years, myrrh of *Commiphora molmol* is used for healing wound injuries [6] [9]. The benefits of using myrrh in medicine have been proven in many scientific studies [7] [10] [11]. Previous studies showed that *Commiphora* species produced anti-inflammatory [12]; analgesic [12] [13]; antiulcer [6] [14] [15]; antioxidant [16] and hypolipidemic [17] [18] effects. *Commiphora* tree also induced antibacterial [19] [20] and antischistosomal [21] [22] activities.

The present study was designed to investigate (CME) extract on healing of peptic ulcer in rats inflicted with aspirin-induced gastric ulcer.

2. Materials and Methods

2.1. *Commiphora molmol* Resin

Dried oleo-gum resin of *Commiphora molmol* (myrrh), Family *Burseraceae*, was purchased from the Agricultural Seeds, Herbs and Medicinal Plants Company, Cairo, Egypt. Myrrh resin is present in the form of yellowish masses as illustrated **Figure 1**. It



Figure 1. *Commiphora molmol* (myrrh) resinous exudate.

has an aromatic odour and bitter taste. Myrrh resin was grinded using a coffee grinder into a fine powder that kept till used for alcohol extraction.

2.2. Drugs

Omeprazole was used as reference antiulcer drug, and it was obtained in form of capsules (Epirazole[®] 20 mg capsule, EIPICO, Egypt). The drug was dissolved in distilled water and given orally to the rats in a dose of 20 mg/kg b.wt. Aspirin (acetyl salicylic acid, Aspegic[®], 1 g vials) was purchased from Amriya Company for Pharmaceutical industries, Cairo, Egypt. It was given orally in a dose of 200 mg /kg b.wt for induction of gastric ulcer.

2.3. Rats

A total of 30 adult male rats of Sprague Dawley strain (150 - 155 g b.wt. and 8 - 9 weeks old) were used in this study. The animals were purchased from the Laboratory Animal Colony, Helwan, Egypt. Basal diet and water were offered to rats *adlibitum*.

2.4. Diet

The rats were fed on basal ration composed of wheat bran, soya bean powder 44%, fish meal, molasses, fibers 3.3%, sodium chloride, calcium carbonate, calcium phosphate, methionine and ash with net protein 22% and fats 4.7% [23].

2.5. Preparation of Plant Extract

Two hundred grams of myrrhresin powder were soaked in one liter of 90% ethanol and kept in a refrigerator with daily shaking for 3 days. The ethanol extract was filtrated using a double layer of musl into get rid of debris. The liquid extract was concentrated under reduced pressure using Rotatory evaporator adjusted at 50°C and connected to a vacuum pump. The prepared ethanol extract was kept in refrigerator till further use. This procedure was described by Shalaby and Hamowieh [24].

2.6. Experiment Design

For this experiment, thirty rats were randomized into 6 equal groups, of 5 rats each. Group (1) was kept normal (negative) control and given 1 ml distilled water (vehicle), while the other 5 groups were given aspirin solution in a dose of 200 mg/kg b.wt for induction of gastric ulcer [25]. Group (2) was kept positive (ulcer group) control and group (3) was orally pretreated with Omeprazole (antiulcer drug) in a dose 20 mg/kg [26]. Groups (4), (5), and (6) pretreated by *Commiphora molmol* extract at 125, 250 and 500 mg/kg, respectively for 2 weeks. The rats were fasted for 48 hours, but were allowed free access to drinking water, up till 4 hours before the experiment. The rats were euthanized, the pyloric and cardiac openings were legated and gastric juices were collected and measured. The pH of juices was measured using a pH meter and the total acidity was measured by titration of 1 ml of gastric juice against 0.01 N sodium hydroxide using phenolphthalein as indicator [27]. The stomachs were immediately ex-

cised and each stomach was opened along the greater curvature, washed with distilled water and grossly examined for gastric lesions. The lengths of ulcer were measured and percentages of protection from ulcer were calculated [26]. Mucosal scrapings were collected and weighed and incubated with 1% Alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The tubes were then centrifuged at 1400 rpm for 10 min and the absorbance of the supernatant was measured at 489 nm to determine the total adherent mucus content as described by Corne *et al.*, (1974) [28]. The stomachs were then preserved in 10% neutral formalin solution till processed for histological examination.

2.7. Histological Procedure

Stomachs of the sacrificed rats were preserved in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. The specimens were then cleared in xylene, embedded in paraffin boxes, sectioned at 4 - 6 microns thickness, stained with Hematoxylen and Eosin (H & E) and then examined under microscope as described by Bancroft and Gamble, (2008) [29].

2.8. Statistical Analysis

Data were expressed as mean \pm standard error (SE). Differences between the control and treated groups were tested for significance using Student "t" test according to Snedecor and Cochran (1986) [30].

3. Results

The results showed that oral administration of aspirin (200 mg/kg) to normal rats significantly increased the volume of gastric juice and titratable total acidity and decreased pH (increased acidity) and mucus content in gastric mucosa. Oral pretreatment with Omeprazole (20 mg/kg) and *Commiphora molmol* extract (CME) in doses of 250 and 500 mg/kg significantly decreased the volume of gastric juice and total acidity and increased pH (reduced acidity) and mucus content. The small dose of CME revealed no significant changes in volume, pH and total acidity of gastric juice and mucus content when compared to the positive control (ulcer) group as depicted in **Table 1**.

Table 1. Effect of *Commiphora molmol* extract (CME) on volume, pH and total acidity of gastric juice and mucus content of gastric mucosa in rats (n = 5 rats).

Parameters Groups	Volume (ml)	pH	Total acidity (mEq/ml)	Mucus content (mg/g tissue)
Group (1) Negative vehicle	4.12 \pm 0.02	3.77 \pm 0.01	18.10 \pm 0.1	195.5 \pm 6.2
Group (2) Positive Ulcer	6.20 \pm 0.04***	1.67 \pm 0.04**	29.10 \pm 2.6**	179.3 \pm 4.3***
Group (3) CME (20 mg/kg)	2.55 \pm 0.01**	3.60 \pm 0.02**	14.70 \pm 0.3***	218.6 \pm 7.4***
Group (4) CME (125 mg/kg)	4.22 \pm 0.03	1.90 \pm 0.01	27.80 \pm 0.5	181.0 \pm 4.6
Group (5) CME (250 mg/kg)	4.40 \pm 0.02**	3.40 \pm 0.03**	16.30 \pm 0.3***	204 \pm 5.6 ***
Group (6) CME (500 mg/kg)	4.90 \pm 0.01**	3.50 \pm 0.02**	15.40 \pm 0.2***	210.5 \pm 6.2***

*Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$.

Oral administration of aspirin (200 mg/kg) to normal rats induced gastric ulcer with ulcer length of 0.72 ± 0.05 ml. Pretreatment with Omeprazole (20 mg/kg) and CME decreased the lengths of gastric ulcer when compared to the negative control (ulcer) group. The protection percentages from ulcer by CME in doses of 125, 250 and 500 mg/kg were 5.55%, 38.88% and 51.38% versus to 65.27% induced by Omeprazole as recorded in **Table 2**.

The gross examination of stomachs of rats inflicted by aspirin induced-gastric ulcer showed severe streaks of hemorrhage and gastric ulcerations (**Figure 2(a)**). The stomachs of rats pretreated with Omeprazole (antiulcer drug) showed mild streaks of hemorrhage and ulcerations (**Figure 2(b)**). In rats pretreated with CME by a dose 500 mg/kg, the gross examination of stomachs revealed moderate streaks of hemorrhage and ulcerations (**Figure 2(c)**).

The histopathological examination of stomachs of normal (negative) control rats showed normal histological structure of gastric layers (**Figure 3**). In rats given orally aspirin, the examination revealed severe gastric ulcerations, sub-mucosal edema and leukocyte inflammatory cells infiltration (**Figure 4**). The pretreatment of ulcerated rats with Omeprazole revealed mild ulcerations and sub-mucosal edema as shown in **Figure 5**. In ulcerated rats pretreated with *Commiphora molmol* extract in a dose 500 mg/kg, the microscopic examination showed moderate ulcerations, sub-mucosal edema and leukocyte inflammatory cells infiltration (**Figure 6**).

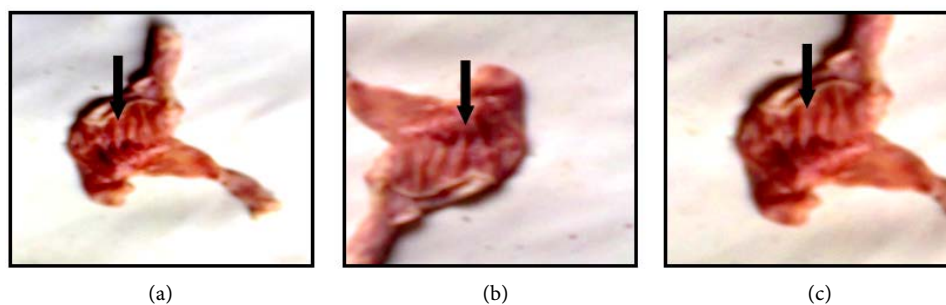


Figure 2. Gross examination of stomachs of rats showing: (a) severe streaks of hemorrhage (+++) and ulcerations in positive control rat given aspirin; (b) mild streaks of hemorrhage (+) in omeprazole-pretreated rat and (c) moderate streaks of hemorrhage (++) and small sized ulcerations in rat pretreated with 500 mg/kg of *Commiphora molmol* extract.

Table 2. Effect of *Commiphora molmol* extract (CME) on gastric ulcer length (UL) and percentages of protection from ulcer in rats (n = 5 rats).

Parameters Groups	UL (ml)	Protection (%)
Group (1) Negative vehicle	No ulcer	-
Group (2) Positive Ulcer	7.2 ± 0.15	-
Group (3) CME (20 mg/kg)	$2.5 \pm 0.11^{***}$	65.27
Group (4) CME (125 mg/kg)	6.8 ± 0.23	5.55
Group (5) CME (250 mg/kg)	$4.4 \pm 0.21^{**}$	38.88
Group (6) CME (500 mg/kg)	$3.5 \pm 0.14^{***}$	51.38

*Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$.

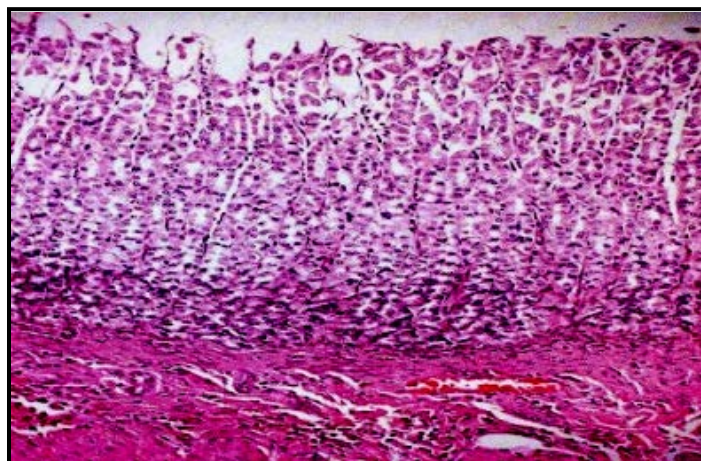


Figure 3. Cross section in stomach of a normal (negative) control rat showing normal histological structure of gastric layers (H & E stain 200 \times).

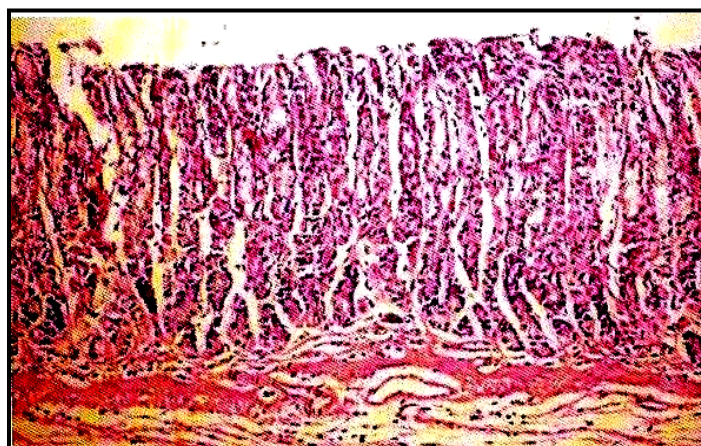


Figure 4. Cross section in stomach of a positive control rat given orally aspirin showing severe gastric ulcerations, sub-mucosal edema and leukocyte cells infiltration (H & E stain 200 \times).

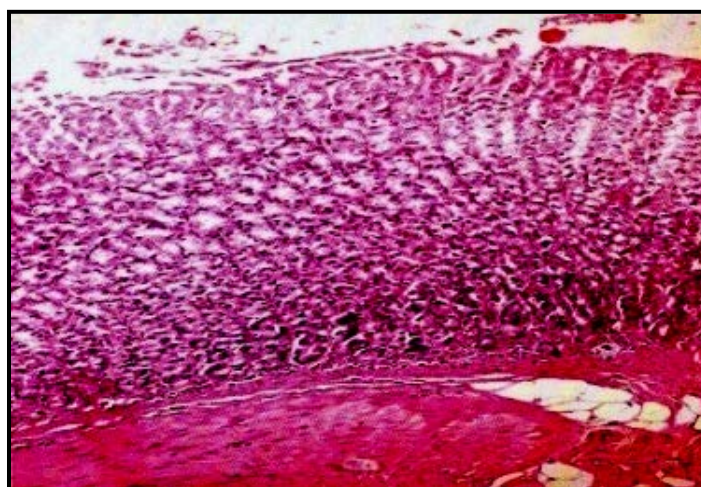


Figure 5. Cross section in stomach of a rat given orally aspirin and pretreated with Omeprazole showing mild ulcerations and sub-mucosal edema (H & E stain 200 \times).

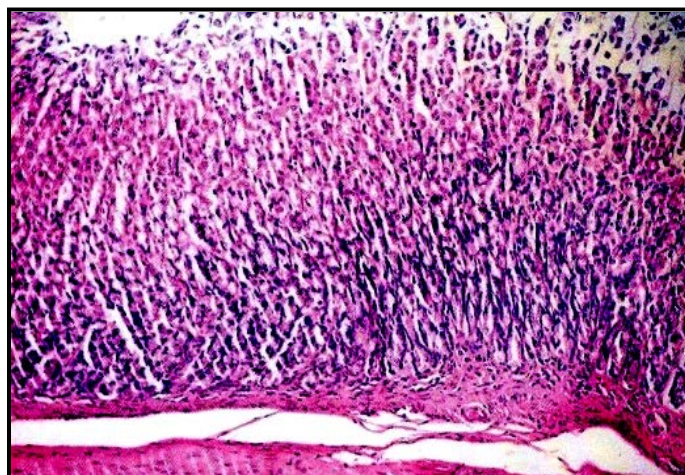


Figure 6. Cross section in stomach of a rat given orally aspirin and pretreated with CME in a dose 500 mg/kg showing moderate ulcerations, sub-mucosal edema and leukocyte inflammatory cells infiltration (H & E stain 200×).

4. Discussion

The present study aimed to assess the antiulcer activities of *Commiphora molmol* extract (CME) in rats inflicted with aspirin-induced gastric ulcer.

Gastritis is the inflammation of the mucosal surface of the stomach. It can range from a mild, asymptomatic form to severe ulceration, which, if untreated, may lead to perforation [31]. Peptic ulcer disease (PUD) refers to painful sores or ulcers in the mucosal lining of the stomach or the first part of the small intestine, the duodenum, secondary to pepsin and gastric acid secretion [32]. Although in most of the cases the etiology of ulcers is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity via endogenous defense mechanisms. The increases in gastric acid secretion, pepsin activity and oxidative stress in the gastric mucosa, and decreases in mucous and bicarbonate secretion are factors implicated in the pathogenesis of gastric ulcers [33] [34].

In this study, *Commiphora molmol* extract (CME) exhibited a significant protective effect on gastric mucosa against aspirin-induced ulcer. The protective effect of CME was evident by the decreased volume and total acidity of gastric juice and length of ulcer. The cytoprotective effect of CME was confirmed in the present study by gross and histopathological examinations as severity of hemorrhage, ulcerations, inflammatory leukocyte cells infiltration and submucosal edema were decreased. The antiulcer effect of CME reported herein was in agreement with that previously demonstrated by Haffor, (2010); Su *et al.*, (2011) and Su *et al.*, (2012) [6] [12] [35].

The antiulcer activity of *Commiphora molmol* extract could be attributed to its anti-inflammatory effect that was previously reported by Tariq *et al.* (1986); Haffor, (2010); Su *et al.* (2011) and Su *et al.* (2012) [6] [10] [12] [35]. The authors concluded that healing of gastric ulcer induced by ethanol in rats is enhanced by *Commiphora molmol* extract via decreasing serum level of the inflammatory mediator prostaglandin E₂ (PGE₂). In addition, the gastroprotective property of *Commiphora molmol* extract was perhaps as a

result of the interplay between its anti-secretory, cytoprotective, anti-inflammatory, and antioxidant effects [16].

In conclusion, the results denote that *Commiphora molmol* (Myrrh) extract produces an anti-ulcer activity in aspirin-induced ulcer rat model. These data confirm its traditional use for the treatment of gastric ulcer.

5. Conclusion

The results denote that *Commiphora molmol* extract possess antiulcer effects in rats. Therefore, *Commiphora molmol* (myrrh) resinous exudate may be beneficial for patients suffering from gastric ulcer.

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