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Antiulcer property of Libyan *Helianthemum kahiricum* plant

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Abstract

Objective: although there are many treatment regimens for a gastric ulcer disease, it is difficult to find cost-effective treatment regimen that meets all needed goals. Therefore, there has been significant interest to find a suitable treatment from natural product sources. *Helianthemum kahiricum* is one of Libyan medicinal plants belongs to family Cistaceae. This plant distributed in western mountain region of Libya and used traditionally in Libyan folk medicine as remedy of gastric ulcer. Therefore, this study was carried out to prove its antiulcer property.

Methods: The methanolic extract of *Helianthemum kahiricum* was studied to evaluate its antioxidant and antiulcer properties. The antiulcer property was evaluated by using ethanol induced ulcer model; efficacy was assessed by determination of mean ulcer size, ulcer number, ulcer index, protective percent and pH of gastric juice. Omeprazole was used as standard. The antioxidant property was evaluated using DPPH assay Quercetin and Ascorbic acid were used as standard.

Keywords: Ethanol induced ulcer model, *Helianthemum kahiricum*, Cistaceae, antioxidant property, antiulcer property.

1. Introduction

Ulcer is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. About 19 out of 20 peptic ulcers are duodenal. Gastric ulcers, found in the stomach wall, are less common. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [1].

A large number of spices and herbs have been evaluated by various researchers for their antiulcer effects to achieve a favorable outcome. Large numbers of medicinal plants and dietary nutrients have been shown to possess gastro-protective activities such as Aloe, *Terminalia chebula*, *Vetiveria zizanioides*, Ginseng, etc.

The genus *Helianthemum* belongs to the Cistaceae family, it is native mainly in the Mediterranean and includes ten species. This plant distributed in western mountain region of Libya and used traditionally in Libyan folk medicine as remedy of gastric ulcer [2].

2. Materials & Methods

i. Chemicals and Standards

Methanol of analytical grade, 2,2, Diphenyl-1-picryhydrazyl (DPPH), Tween 80, Quercetin, Ascorbic acid and Omeprazole were purchased from Sigma, UK.

ii. Plant material

The whole aerial part of *Helianthemum kahiricum* (*H. kahiricum*) plant belongs to Cistaceae family was collected from area of western mountain, Libya in spring 2010. It was identified and authenticated by department of Botany, Faculty of science, Tripoli University, Tripoli, Libya and a voucher specimen deposited at the National Medical Research Centre, Zawia, Libya under code (PNS20).

iii. Preparation of extract:
The plant material was dried on shade, powdered by a mechanical grinder; and then extracted with methanol organic solvent using Soxhlet apparatus for 72 hr. The crude extract was dried using rotary evaporator and stored in -20 °C.

iv. Preliminary phytochemical screening:
The phytochemical screening of the methanolic extract of *H. kahiricum* was performed according to the standard procedures Mayer's and Dragendorff's tests for alkaloids, Fehling's test for free reducing sugars, fehling's test for glycosides, Liebermann - burchard's test for triterpenoids, liebermann-burchard's test for steroids, frothy test for saponins, shinoda's and sodium hydroxide tests for flavonoids, ferric chloride test for tannins and borntrager's test for free anthraquinones [3].

v. Antioxidant activity:
The antioxidant activity of *H. kahiricum* methanolic extract was evaluated by using 2,2,Diphenyl-1-picryhydrazyl (DPPH•) according to a method described [4]., a stock solution of the extract (1 mg/ml) was prepared. DPPH solution (400 µl of 0.1 µM) was added to 1 ml cuvet; then the extract solution was added in different doses (1–50 µg). Absolute ethanol (600 µl) was added, the mixture was shaken vigorously and allowed to stand in dark place at room temperature for 5 min. The absorbance was measured at 517 nm in U.V-Visible-NIR spectrophotometer (Varian Cary 5000-U.S.A). The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation [5].

$$\text{Percent of DPPH inhibition} = [(A_A - A_B)/A_B] \times 100$$

Where $\mathbf{A_A}$ and $\mathbf{A_B}$ are the absorbance values of the test and the blank sample respectively, a percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value. All the results presented as means of three independent experiments.

vi. Animals
In this study, male, wistar albino rats obtained from the animal house of Pharmacology and Toxicology Department, National Medical Research Center, Zawia, Libya were used. The animals were housed in cages and maintained at 22±1 °C under 12 h: 12 h light/ dark cycle, and they were fed with standard laboratory chow and had free access to water. The study was conducted in accordance with the nationally accepted guidelines for laboratory animal use and care (NMRC35/2009).

vii. Antiulcer activity
Fifty fasted Male wistar albino rats (150-200 gm) with freely access to water were used. The ethanol-induced ulcer model was used to investigate the antiulcer effect of *H. kahiricum* methanol extract [6,7]. The animals were divided into five groups, 10 rats each. All the groups were treated by oral route one hour before ulcer induction with different types of treatment. The first group treated with 1ml/kg tween 80 (vehicle) (negative control). The second group treated with Omeprazole 20 mg/kg (positive control). The third, fourth and fifth groups treated with the plant extract dose 125 mg/kg, 250 mg/kg and 500 mg/kg, respectively. Oral administration of absolute ethanol (1 ml/animal) was used to induce ulcer. after One hour of ethanol administration, animals were killed by suffocation with chloroform, and stomachs were incised along the greater curvature; stomach contents were collected to measure gastric pH (HANA pH meter); Ulcers found in the gastric mucosal layer, appeared as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Each specimen of gastric mucosa was thus examined for damage. The length (mm) and width (mm) of the ulcer on the gastric mucosa were measured by micrometer tool; and ulceration was scored. Mean ulcer score for each animal will be expressed as ulcer index (U.I). The ulcer index was determined using the following formula [8].

$$\textbf{Ulcer index} = 10/X$$

Where X = Total mucosal area / Total ulcerated area. The ulcers were given scores based on their intensity as follows:
0=Normal stomach, 0.5=Red coloration, 1=Spot ulcer, 1.5=Hemorrhagic streak, 2=Ulcers, 3=Perforation.

The percentage of ulcer protection was determined as follows

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index - Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

viii. Histopathological evaluation
Specimens of the gastric walls from each rat were kept in 10% buffered formalin for 24 h; and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 µm using a rotary microtome; and stained with hematoxylin and eosin for histological evaluation [9].

ix. Statistical analysis
The results of this study were analyzed by using one way ANOVA using 13.00 version of SPSS computer software. *P* values <0.05 were considered significant. Results are presented as mean ± SE.

3. Results

i. Phytochemical investigations for the main active constituents
The preliminary phytochemical tests revealed presence of tannins, flavonoids, simple phenolic, glycosides, free reducing sugar, saponine, steroids and terpenoids in *H. kahiricum* methanolic extract. While they revealed the absence of free anthraquinones, and alkaloids.

ii. Antioxidant activity
H. kahiricum methanolic extract showed a good antioxidant activity, where (22.34 µg/ml) showed 50% inhibition on DPPH Scavenging activity, in comparing with Quercetin (7.82 µg/ml) and ascorbic acid (13.59 µg/ml) as standards.

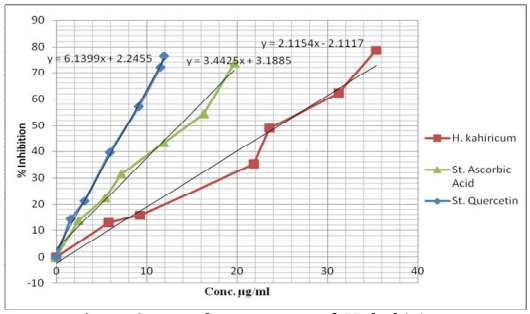

Fig 1: Antioxidant activity of *H. kahiricum*

iii. Antiulcer activity
Ethanol administration produced gastric lesions of mucosal layer

in the glandular portion of rat stomach; these lesions appeared as elongated bands of thick, black and dark red color (figure 2a). *H. kahiricum* Pre-treatment and standard ranitidine were found to protect the mucosal layer from injury that induced by ethanol in rats. The protection activity of *H. kahiricum* methanol extract was in a dose dependent manner. The percent of protection were 32.48%, 45.97%, 66.66% of 125, 250 and 500 mg/kg respectively compared to control (Figure 2c, 2d and 2e). There was a statistical significant difference between the effects of *H. kahiricum* methanol extract doses compared to the control ($P<0.05$). Omeprazole also significantly inhibited the ethanol-induced gastric lesion; the percent of inhibition was 71.26% compared to the control (figure 2b). The effect of *H. kahiricum* methanol extract at dose 500 mg/kg were comparable to that of omeprazole effect (P>0.05). (Table 1).

Table 1: Anti-ulcer activity of *H. kahiricum* methanolic extract

Group	Treatment	Ulcer index	%Protection	pH of gastric juice
I	Control (1ml/kg)	8.6±.31	----------	2.4±.15
II	Omeprazole (20mg/kg)	2.5±.24	71.26	5.71±.15
III	Extract (500mg/kg)	2.93±.22	66.66	4.43±.18
IV	Extract (250mg/kg)	3.7±.07	45.97	3.37±.16
V	Extract (125mg/kg)	4.82±.39	32.48	3.01±.19

Results are expressed as means ± S.E. and data are evaluated by using one-way analysis of variance *p<0.05 n=10

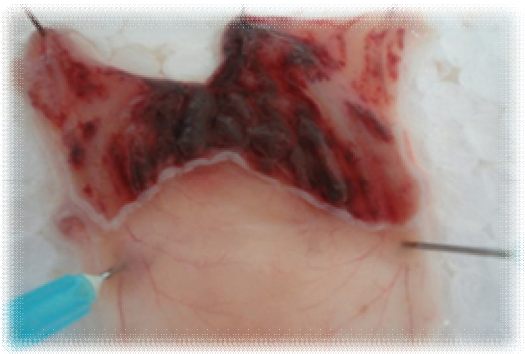

Fig 2a: Gross appearance of the gastric mucosal layer in negative control group (1 ml) of 1% tween 80 shows severe injuries are seen in the mucosal layer.

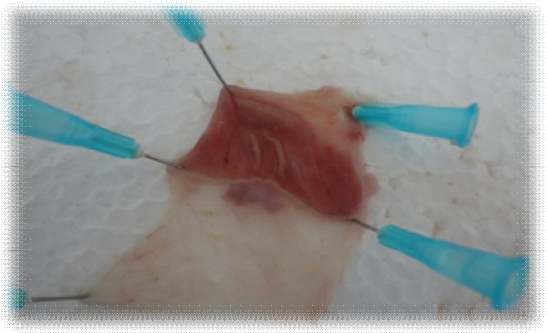

Fig 2b: Gross appearance of the gastric mucosal in positive control group treated with standard Omeprazole (20 mg/kg) shows no significant injuries in the mucosal layer compared to negative control group.

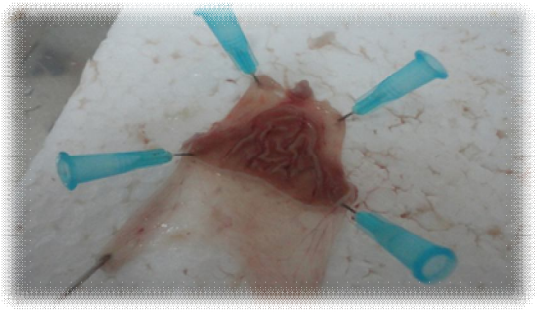

Fig 2c: Gross appearance of the gastric mucosal layer in group treated with. *H. kahiricum* (500 mg/kg) shows no injuries to the mucosal layer, and flattening of mucosal layer compared to negative control group.

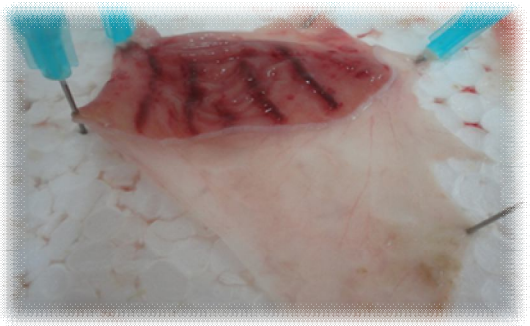

Fig 2d: Gross appearance of the gastric mucosal layer in group treated with. *H. kahiricum* (250 mg/kg) shows moderate injuries to the mucosal layer compared to negative control group.

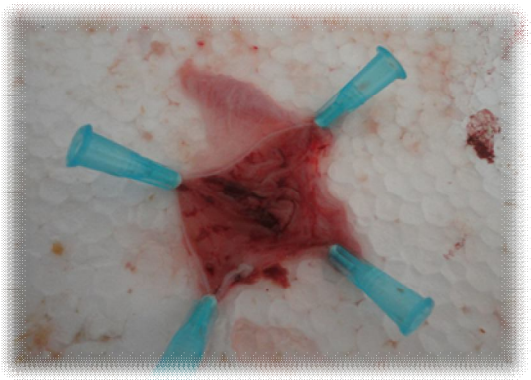

Fig 2e: Gross appearance of the gastric mucosal layer in group treated with. *H. kahiricum* (125 mg/kg) shows moderate injuries to the mucosal layer compared to negative control group.

iv. Histopathological evaluation

The evaluation of ethanol induced gastric lesions histological in ulcer control group pretreated with only tween80 (vehicle) showed severe injury and discontinues of villii in the mucosal, in addition to the inflammation that appear in the submucosal layer (figure 3a). Animals that pre-treated with *H. kahiricum* at doses 125 mg/kg 250, 500 mg/kg had comparatively better protection of the gastric mucosa as seen in (figure 3c, 3d and 3e) by absence or reduce of extensive damage, continue of villii in mucosal layer, reduced or absence of submucosal inflammation in comparing with control group. Group pre-treated with *H. kahiricum* 500 mg/kg showed a higher protection activity comparatively similar to the positive control group pre-treated with Omeprazole 20 mg/kg. (Fig 3b) The *H. kahiricum* has been shown to exert the cytoprotective effects in a dose-dependent manner.

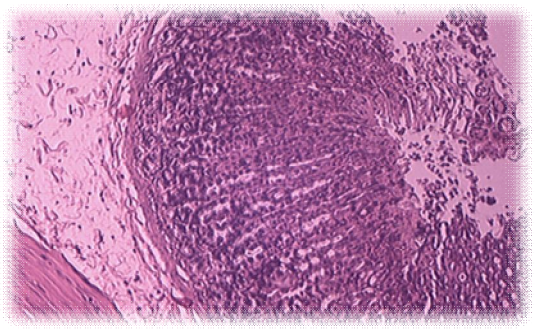

Fig 3a: Histological section of gastric mucosa in a rat pre-treated with tween 80 (1 ml/kg). There is severe disruption to the surface epithelium, and edema of the submucosal layer with leucocytes infiltration (H&E stain, 10x).

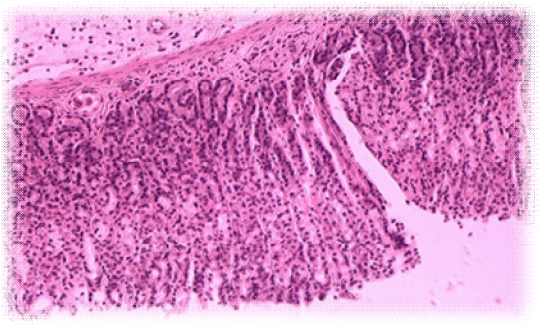

Fig 3b: Section of mucosal layer in positive control group (Omeprazole 20 ml/kg). There is no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer (H and E stain 10x).

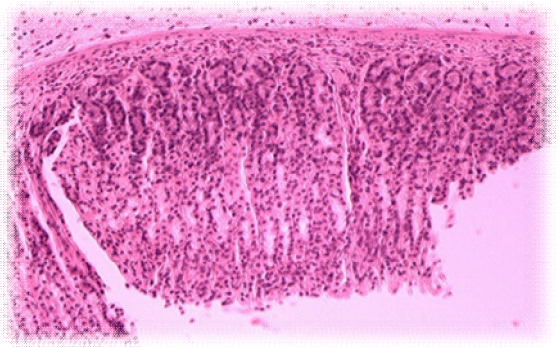

Fig 3c: Section of gastric mucosa in group pre-treated with 500 mg/kg of *H. kahiricum* methanolic extract. There is mild disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer (H and E stain10x).

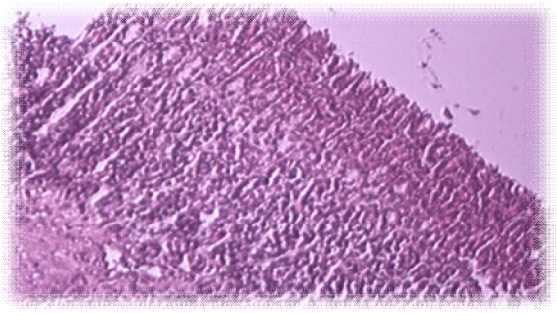

Fig 3d: Section of gastric mucosa in group pre-treated with 250 mg/kg of *H. kahiricum* methanolic extract. There is mild disruption to the surface epithelium with no edema and leucocytes infiltration of the submucosal layer (H and E stain 10x).

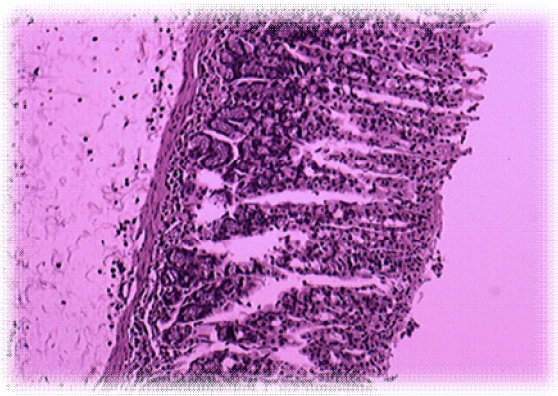

Fig 3e: Section of gastric mucosa in group pre-treated with 125 mg/kg of *H. kahiricum* methanolic extract. There is moderate disruption to the surface epithelium with edema and leucocytes infiltration of the submucosal layer (H and E stain 10x).

4. Discussion

The preliminary phytochemical tests revealed presence of tannins, flavonoids, simple phenolic, glycosides, free reducing sugar, saponine, steroids and terpenoids in *H. kahiricum* methanolic extract. It is well know that these phytochemicals are with potentially beneficial effects on human health [10-13]. Yet over the years they have been found to be are important part of the human diet and are considered to be active principles in some medicinal plants, the antioxidant activity of these compounds (flavonoids) is efficient in trapping superoxide. Anion (O_2^-), hydroxyl(OH^-), peroxyl (ROO^-) and alcohoxyl (RO^*) radicals [14]. The anti-ulcer pharmacological effects of this plant are related to its flavonoid content [15]. The active compound of this plant including flavonoid, flavonoids, simple phenolic, glycosides, free reducing sugar, saponins, steroids and terpenoids may be regarded as possible active compounds against gastric lesions by acting as protective factors or increasing antioxidant activity [16-17]. The methanolic extract of *H. kahiricum* possesses significant free radical scavenging and antioxidant activities in vitro, the therapeutic action of *H. kahiricum* methanolic extract could be due to, in part, to their ability to scavenging oxygen free radicals, which may be involved in ulcer and inflammatory diseases.

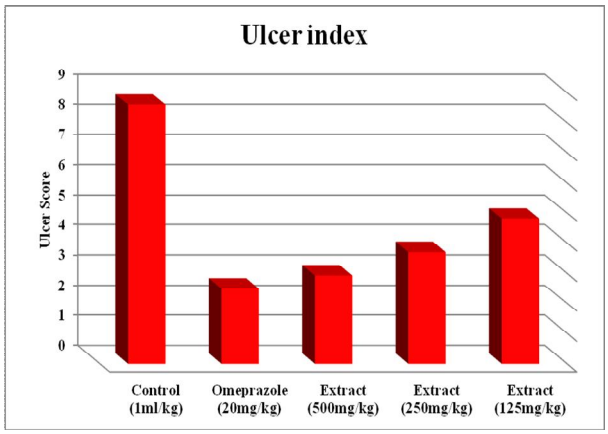

Fig: 4(a)

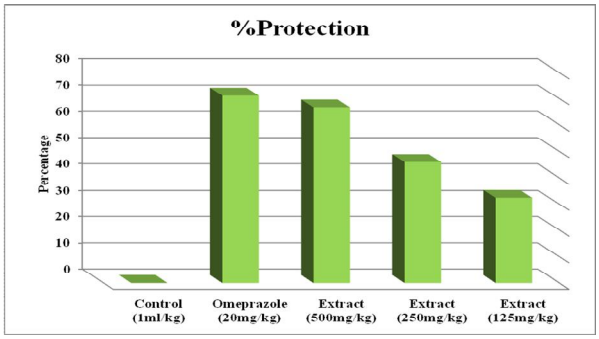

Fig: 4(b)

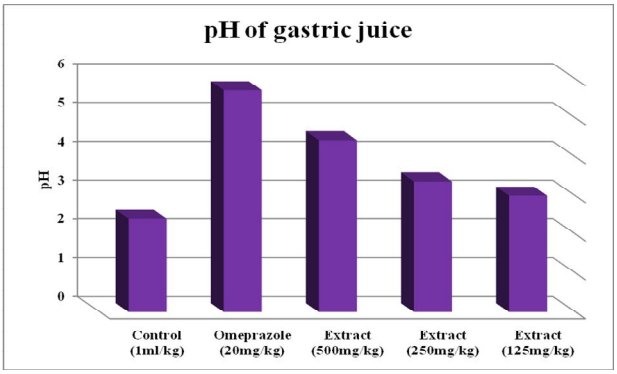

Fig: 4(c)

Figure 4(a), 4(b), 4(c): Effect of *H. kahiricum* extract on various parameters in ethanol induced gastric ulcer.

5. Conclusion

The plant and phytomedicine are important choice to treat the peptic ulcer. Most of the phyto-constituents showed better result than the modern medicine [18]. Various phyto-chemicals like flavanoids, tannins, saponins, terpinoids showed their antiulcer activity due to their cytoprotection, antisecretory and antioxidant property. The plants are a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis [19,20]. Thus plant and phyto-medicine can be our source of drug with less toxic effect and better result to treat peptic ulcer in future.

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