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Acute toxicity and anti-ulcerative potential of caralluma flava N.E.Br methanolic extract against ethanol-induced gastric ulcers in rats

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Abstract
The study was performed to assess the anti-ulcer activity of *Caralluma flava* methanolic extract against ethanol induced gastric ulcer in rats model. A acute toxicity and antioxidant activities of *Caralluma flava* extract was evaluated. Acute toxicity of the *Caralluma flava* extract was evaluated with a single dose administered orally to rats in a dosage of 3g/kg for 2 weeks. Result showed that the extract was effective antioxidant towards DPPH radical and the scavenging activity was approximately nearly from the ascorbic acid. Acute toxicity test up to 3 g/kg of this extract did not display any toxicological effect.. The treated rats with *Caralluma flava* at 500 mg/kg, produced antiulcer effect in rats with a preventive index of 68.37 %. In conclusion, this study shows that of *Caralluma flava* extract was nontoxic up to 3g/kg and showed antiulcer properties. The antiulcer activity is exerted, possibly, via its high antioxidant activity.

Keywords: anti-ulcer; *Caralluma flava N.E.Br*; antioxidant; toxicity

1. Introduction
The peptic ulcer is disease characterized by mucosal damage, caused by Helicobacter pylori, [1], anti-inflammatory drugs (NSAIDs) such as oral bisphosphonates, potassium chloride, immunosuppressive medications [2, 3], alcohol consumption, and cigarette smoking [4] Secretion of gastric acid is still recognized as a central component of this disease. Therefore, the main therapeutic target is to control acid secretion using antacids. The therapy of gastric ulcer faces a major drawback due to the side effects of the long-term use of commercially available drugs.
It was reported that toxic oxygen radicals play an important role in the ethiopathogenesis of gastric damage [5]. It was observed in parallel to tissue damage, there is a decrease in antioxidants such as glutathione and superoxide dismutase and an increase in oxidants [5]. Therefore, the search is still on to find drug possessing antioxidant and antiulcer properties, which will serve as a powerful therapeutic agent to cure gastric ulceration, and the search extends to the systematic development of natural products [6].
Many herbal remedies have been employed in various medicinal systems for the treatment and management of various diseases. Some of medicinal plants with gastroprotective properties have been reported by gastric ulcer researchers [7-8]. Many species of the genus *Caralluma* are edible and used as the traditional medicine in many countries. *Caralluma flava* (CF) is a leafless, succulent and angular plant, which grows wildly in many regions of Yemen In addition; it is consumed freshly by many people in Yemen in the treatment of rheumatism, diabetes, leprosy, gastric ulcer and as a antipyretic [9]. In traditional Yemeni folk medicine, CF is used in the case of diabetes and peptic ulcers, and its juice as drops for ear inflammation [10]. The present study was undertaken with the aim to assess the antiulcer properties of CF leaves methanolic extract. Acute toxicity test was performed in this study to evaluate of the toxic characteristics of the methanolic extract of the CF leaves and thereby select the treatment dose for antiulcer activity.

2. Materials and methods
2.1 Chemicals
1, 1-diphenyl-2-picrylhydrazyl (DPPH), was purchased from Sigma-Aldrich (St. Louis, MO). Ascorbic acid was purchased from Fisher Scientific (Loughborough, UK). Methanol and

ethanol were purchased from Fisher Scientific (Fisher Scientific Co Ltd., Ottawa, ON). Omeprazole was purchased from Ibn Hyan Pharmcy in local market in Sana`a.

2.2 Plant collection.

Caralluma flava plant leaves were collected from Rada, Albyda and from Bani Matr, Sana`a Yemen, the plant was identified and authenticated by a plant taxonomist at Department of Botany, Faculty of Agriculture, Sana`a University, Yemen. The plant was dried and protected from the light; the dried plant was stored at 4ºC and protected from light prior to further use.

2.2 Preparation of CF methanolic extract

The dried CF leaves was finely ground using an electritical blinder (IKA LABORTECHNIK M 20 Brand) and ground samples of each plant was extracted with methanol (LC-MC grade JJ-Baker, USA). For methanol extraction, powder plant was subjected to extraction with methanol at 1:5 (w/v) ratio for 48h on stirrer and dark conditions. Mixture then was filtered using filter paper. The combined filtrate was concentrated by rotary evaporation at 40ºC. The extract was transferred into glass dark bottles and then stored at 4°C for subsequent analyses. The CF leaves extract showed yield of 18%.

2.3 Scavenging Activity of DPPH Radical

The antioxidant activity of the CF extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, were determined by the method described by Benzie and Strain (11). L ascorbic acid was used as standard antioxidants and methanol was used as the control. An aliquot of 0.5 ml of a methanolic solution of DPPH (50 mg DPPH/100 mL MeOH) was added into the different concentration (1, 0.5, 0.25 mg/ml) of each extract and ascorbic acid as long as control samples (both extract and ascorbic acid were dissolved in methanol). All samples were incubated in the dark at room temperature for 30 min before absorbance values were read at 517 nm (Amersham 2100Pro, UV-vis spectrophotometer, UK). The decrease in absorbance was calculated as an IC_{50} and expressed as µg/ml, which is the concentration of sample required for 50% scavenging of DPPH radicals in the specified time period.The radical scavenging effect was calculated as follows:

Radical scavenging effect (%) = Ac – As/Ac × 100

Ac = absorbance of control and As = absorbance of test sample.

Where control is the absorbance of the DPPH radical+ methanol

2.4 Acute Toxicity test

Healthy male *Sprague Dawley* rats (8-12 weeks old, weighed between 180 and 220 g) were used in this experiment. The animals were given standard rat pellets and tap water *ad libitum*, individually caged. The acute toxicity study was used to determine the safe dose for the *CF methanolic* extract. Ten male rats were assigned equally into 2 groups; control group was given 3 mL of distilled water, and treated group was given 3 g/kg of the leaf extract for 14 days. Rats were observed daily for signs of toxicity (behavioural changes and mortality), for 14 days. The animals were sacrificed then 15th day. Liver enzymes, namely alanine aminotransferase (ALT), Gamma-glutamyltransferase (GGT), total albumin, creatinine and urea were determined using their serum blood. All the blood analysis were carried out in Alulaqi Specials Med. Lab in Sana`a Yemen.

2.5 Animal study for gastric ulcer

Twenty healthy adult *Sprague Dawley* rats, weighed between150 and 200 g, were used in this study. The animals were maintained at Animal House, School of Agriculture, Sana`a University prior to study. The animals were fed with standard diet and water libitum during the maintenance period. The experimental protocols were approved by the Animal Ethics Committee of Agriculture Faculty, University of Sana`a. The rats were deprived of food for 48 h before the experiment, but were allowed free access to drinking water (bottled tap water) up till 2 h before the experiment. Gastric ulcer in male rats was induced by 96 % ethanol at 1ml. Experimental rats were divided into 4 groups, first group was not induced with gastric ulcer with methanol and received 3ml of distilled water and serve as negative control, second group was induced with gastric ulcer with 1ml methanol and 2 hours later, the animals received 2ml of distilled water and serve as control, the third group were induced with gastric ulcer with 1 ml of methanol and 2 hours later treated with 2ml of CF methanolic extract at 500mg/kg and serve as treated group. The forth group was induced with gastric ulcer with 1 ml methanol and treated with 2ml of omeprazole orally (20 mg/kg) dissolved in distilled water. Two hours later the animals were anesthetized and the stomach was removed. Estimation of ulcer area was to see if the ulcer lesions are present or not. All stomachs were removed and the intact stomachs were put in sample container in normal saline 0.9% to keep them undamaged. The stomach was then observed for ulcer lesion and stomach content was removed into centrifuge tube. All samples were cut along the greater curvature. Content of stomachs were used in PH evolution. The stomachs were washed with normal saline to clean the surface. Gastric lesions were measured and the opened stomach were photographed.

The ulcerated area was calculated and determined as ulcerative index (UA) and the inhibition percentage was estimate by the following formula.

Inhibition %=[(UA control- UA treated) / UA control] x 100

2.5.1 pH examination

During the stomach dissection, their content was taken and put into centrifuge tube and the tubes were centrifuged at 1000 rpm for 10 minutes. The aim of centrifuging was to separate the mucosa and stomach's acid. The supernatant part was used in pH examination before. The pH examination was done to know the acidity of the stomach's acidity of collected supernatant was determined using a pH meter.

3. Results

3.1 Antioxidant activity

Table.1 shows DPPH scavenging activity of *CF* extracts and L- ascorbic acid. Result from this assay clearly showed that both three samples exhibited high antiradical activity towards DPPH radical and the activity was approximately similar to the pure antioxidant standard, ascorbic acid. After 30 minutes of the reaction, the *extract*, at concentration of 0.25 mg/ml scavenged more than 59% of the total radicals in the reaction system. Subsequently, the scavenging activity of *CF extract* were gradually increased to more than 85% of the total radicals, at higher concentration (1mg/ml). As shown in Table 2 shows the IC^{50} values of *CF methanolic extract* was 150±1.5 and for L- ascorbic acid was 120±2.0.

Table 1: The DPPH scavenging activity of the methanolic extract of selected samples

Samples	Radical scavenging effect (%)			
	Concentration (mg/ml)			
	1.00	0.5	0.25	0.1
L- ascorbic acid	90±4	85±5	79±6	47±5
CF methanolic extract	86±6	66±6	59±2	36±8

The values represent means ± SD for three different experiments.

Table 2: Antioxidant activities of the *CF* leaves extract and positive control using the (DPPH) free radical-scavenging assay

Samples	Antioxidant activity IC^{50}/ DPPH (µg/ml)
L- ascorbic acid	120±8.0
CF methanolic extract	150±5.5

The values represent means ± SD for three different experiments.

3.2 Acute toxicity test

The acute toxicity test was performed by treating animals with single dose of *CF* methanolic extract (3 g/kg) for 14 days. All animals were under observation for 14 days. All rats survived and in the observational duration of 2 weeks, there were no remarkable toxicological symptoms, signs of abnormalities such as alteration in body weight and behavioral changes. The obtained results from present study showed no different in the body weight, food and water intake, organ weight and also for the kidney and liver tested parameters as shown in Tables 3,4 and 5. In this effort, the effect of CF plant extract on kidney and liver parameters like urea and creatinine as renal parameters along with total albumin, ALT, AST and GGT as liver parameters in administered rats were considered and displayed no noticeable changes in comparison with normal control rats as shown in table 6. The obtained results proved the safety of *CF* methanolic extract at the determined dose without any acute toxicity effect.

Table 3: Effect of *CF* methanolic extract (3g/kg) on body weight of male SD rats

Body Weight (g)	Day		
	Day 0	Day 7	Day 14
Normal Control (D.Water)	179.54 ± 17.09	208.40 ± 15.66	222.74 ± 13.53a
Treated rats with 3g/kg	193.33 ± 18.93	210.63 ± 18.67	237.90 ± 20.33

The values represent means ± SD for 5 animals in each group.

Table 4: Water intake and feed consumed by controls and rats treated with *CF* methanolic extract during the acute toxicity study

Water Intake (ml)	Week 1	Week 2
Normal Control (D.Water)	189.5	190.7
Treated rats with 3g/kg	204.3	191.3
Feed Consumed (g)	Week 1	Week 2
Normal Control (D. Water)	135.4	117.5
Treated rats with 3g/kg	147.0	124.2

The values represent means ± SD for 5 animals in each group.

Table 5: The relative organ weight per 100g body weight recorded at the end of the study

Relative Organ Weight	Normal Control (D. Water)	Treated rats with 3g/kg *CF* extract
Liver	3.4 ± 0.3004	3.1± 0.2960
Kidney	0.4 ±0.0676	0.4 ±0.418
Heart	0.5 ±0.0004	0.4.4 ±0.0224
Lung	0.7 ±0.1591	0.7 ±0.0805

The values represent means ± SD for 5 animals in each group.

Table 6: Clinical biochemistry values of controls and rats treated with *CF* methanolic extract during the acute toxicity study

Parameters	Control	Treated rats with 3g/kg of CF methanolic extract
Albumin (g/L)	49.16 ±1.97	42.93 ±2.82
ALT (U/L)	55.34 ±21.38	51.03 ±7.41
ALP (U/L)	89.00 ±17.36	113.67 ±22.23
AST (U/L)	208.00 ±73.97	216.60 ±8.98
Urea (mmol/L)	7.04±0.10	6.63 ±0.61
Creatinine (umol/L)	68.60 ±7.23	61.00 ±2.64
GGT (U/L)	< 3	< 3

3.3 Anti-ulcer activity

In the present study, *CF* methanolic extract was evaluated for its anti-ulcer activity against ethanol induced gastric ulceration in rats. Oral administration of ethanol produces ulceration. As shown in Figure 1. The CF methanolic extract reduces the incidence and severity of ulceration in ethanol induce ulcer model by 68.39% protection whereas the reference drug omeprazole exhibited 47.11% protection as shown in Table 7. The pH examination was done to know the acidity of the stomach's of the experimental rats. Normal group of the rats shows pH of 2.95 ± 0.4 which refer to the normal pH of the rats stomach. Ethanol inducing more acidity compared to the normal rats where the pH was 1.56±0.04. The pH of treated group with *CF* methanolic extract show less acidity compared to control rats where the pH was 3.74±0.42, which reveal protection against gastric ulcer. The pH of treated group with omeprazole show less acidity compared to control rats where the pH was 5.60 ± 0.5, which reveal some protection against gastric ulcer.

Table 7: Anti-ulcer effects of CF methanolic extract in experimental rats

Groups	Ulcer index mm mean ± STD	Percentage of inhibition %	pH measurment
Normal rats	-	-	2.95 ± 0.4
Control (Ethanol + Distilled water)	13.67± 1.09	-	1.56±0.04
Treated (Ethanol + CF extract at 500mg/kg)	4.32± 1.09	68.39	3.74±0.42
Reference Drug (Ethanol+ omeprazole at 20 mg/kg)	7.23± 0.89	47.11	5.60±0.5

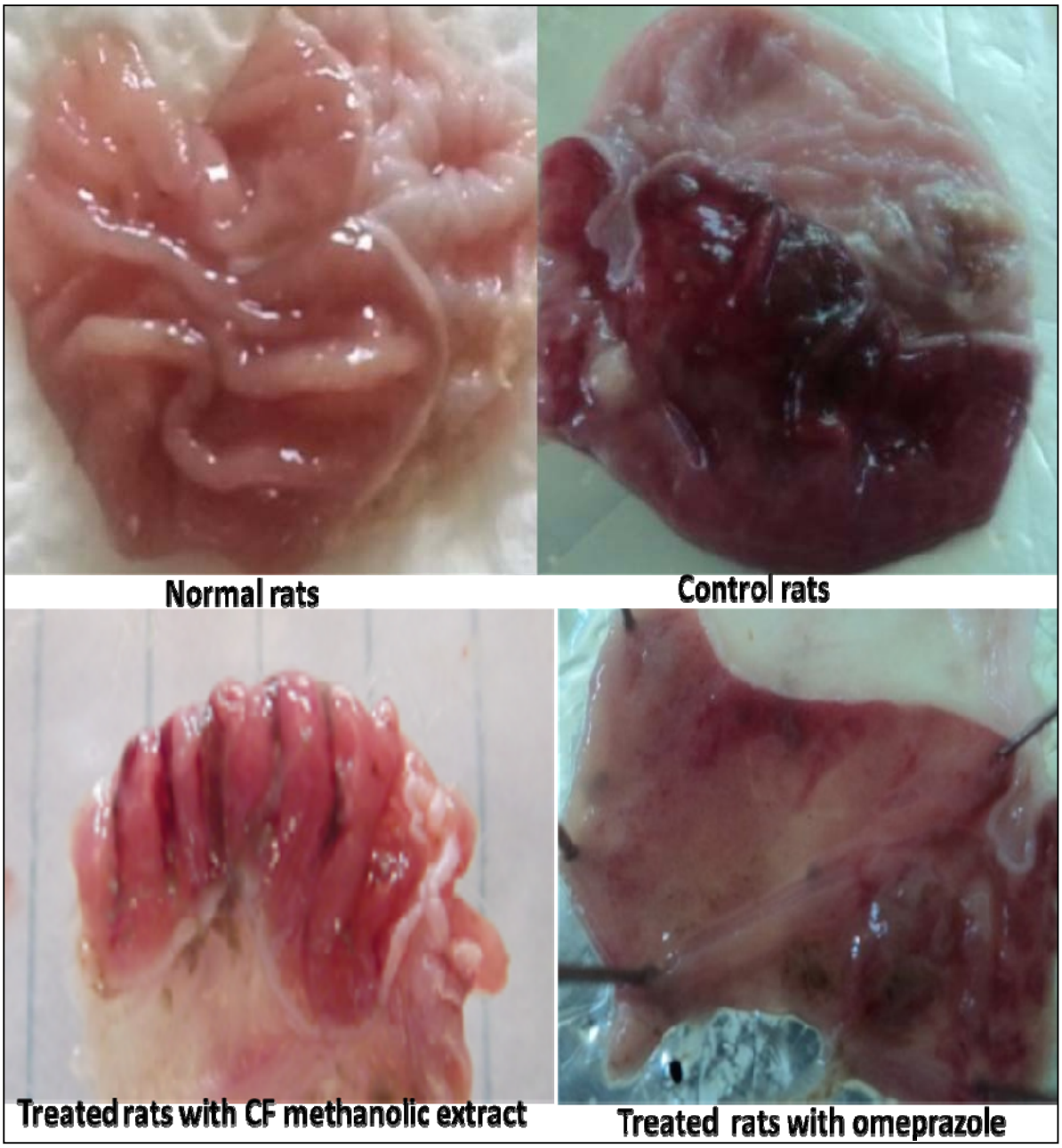

Fig 1: Photographs of gastric mucosa in stomach of experimental rats

4. Discussion

Gastric ulcer is one of the most important concerns as a result of many factors. Because of poorly understanding the pathophysiology of this disease [12], studies investigating new active compounds are needed. As well, various pharmaceutical products currently used for treatment of gastric ulcers are not completely efficient and cause many adverse side effects [13]. Consequently, it is necessary to develop more effective agents that are also less toxic, with medicinal plants being an attractive source for the development of new drugs because of their wide array of active ingredients [14]. The aim of this study was to ascertain the acute toxicity and anti-ulcer, antioxidant effect of CF methanolic extract. In the present study ethanol induced ulcerations in the rats where, rats in the control group suffered from severe lesions, as shown in representative dissected stomach sections (Figure 1).

Antioxidants are important in maintaining good health and there is a growing interest in the investigation of antioxidant activity from medicinal plants with higher potency and lower toxicities than the synthetic ones currently available [15]. Our attention has been focused, in particular, on the CF grown in Yemen. The antioxidant activity using scavenging activity of DPPH radical method for the methanolic leaves extract of CF plant was determined in this study. The DPPH method is based on the reaction that is characterized as a preformed stable free radical with a deep violet colour and any substance that can donate a hydrogen atom to DPPH reduces it to a stable diamagnetic molecule [16]. The CF methanolic leaves extract reduced the DPPH radical. It was observed that activity increased with increasing concentration of the extract in the assay. This extract showed 86±6, 66±6, 59±2and 36±8% at 1, o.5, 0.25, 0.1mg/ml respectively. In accordance to the result of this effort, CF can be considered as a natural source with antioxidant activity.

Although the usage of different plants in medicine as curative agents, not many scientific studies have been performed to investigate and support their safety and effectiveness.

Although, *Caralluma flava* plant is consumed freshly by many people in Yemen as a traditional [9], no research was done on the toxicity of this plant extract. The results of this research indicated the safety of the *CF* plant extract according to absence of any behavioral changes, clinical symptoms and mortality after 14 days of observation. In accordance to the obtained results of this effort, oral dose of CF methanolic extract is 3 g/kg.

Anti-ulcer effect of *CF* extract was evaluated to know the efficacy of this plant in preventing ulcer formation. It was done by assessing the ulcer formation, which then was used to estimate the inhibition percentage, and the pH of stomach content. The methanolic extract was used in a dose of 500 mg/kg. Omeprazole was used as standard drug showed anti-ulcer effect while it inhibited the gastric ulcer formation up to 47%. Anti-ulcer activity of *CF* extract against ethanol-induced gastric ulcer in experimental rats indicated that the *CF* plant extract showed higher inhibition percentage, comparing to control untreated group and treated group with omeprazole. The obtained results of this study indicated that the methanolic extract of *CF* leaves considerably shows anti-ulcer in rats through inhibition percentage and lesser ulcer area with lower range of ulcer index, increasing pH.

5. Conclusion

To conclude, the methanolic extract of *CF* has been found to have antiulcer effect, which could be related to its antioxidant potential. More work is required for a clear understanding of the mechanism of action with chemically identified active principle

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