Gastroprotective Effect of Artichoke (Cynara scolymus L.) Leaves and Pulp Extracts on Peptic Ulcer in Male Rats

By

Reham S. Ramadan

Nutrition and Food Sciences Department,

Faculty of Home Economics,

Helwan University

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Gastroprotective Effect of Artichoke (Cynara Scolymus L.) Leaves and Pulp Extracts on Peptic Ulcer in Male Rats

Reham S. Ramadan[*]

Abstract

The phytochemical screening of artichoke leaves aqueous extract (ALE) and artichoke pulp aqueous extract (APE) were undertaken and its protective effect against aspirin-induced peptic ulcer in rats was studied. Forty-two mature male rats were randomized into 6 equal groups as follows: (1) negative control rats, (2) positive control rats , Groups (3)and (4) rats pretreated with (ALE) at doses of 200 and 400 mg/kg b.wt, respectively .Groups (5) and (6) rats pretreated with (APE) at doses of 200 and 400 mg/kg b.wt, respectively for 28 days . At the last day of the experimental period all groups were given aspirin 400 mg/Kg b.wt , to induce peptic ulcer except group (1) kept as a normal rats . At the end of the experimental period rats were sacrificed and blood samples were collected for serum biochemical analyses. Stomach of the sacrificed rats was taken for determination of biomarkers of gastric ulcer and histopathological examination. The phytochemical screening revealed that both ALE and APE contains flavonoids, saponins, alkaloids, tannins, glycosides, and devoid of resins and triterpenes. Oral pretreatments with both ALE and APE at 400 mg/kg b.wt showed a promising antioxidant effect represented by significant reduction in the volume of gastric juice, total acidity of gastric juice, gastric ulcer index , concentration of pepsin enzyme ,serum interleukin-1 IL-1, serum tumor necrosis factor-alpha TNF-α and lipid peroxidation along with a significant elevation in, gastric prostaglandin PGE2 level and antioxidant enzymes compared to control positive group. Histopathological examination of the stomach showed alleviation of histological degeneration changes caused by aspirin. The study recommends that intake of artichoke in food or its use as herbal tea may be beneficial for

[*] Nutrition and Food Sciences Department, Faculty of Home Economics, Helwan University

patients who might use irritant drugs to their stomach , due to its antioxidant properties. Moreover, isolation of bioactive constituents of artichoke plant is necessary to search for safe natural agents to be developed for therapy instead of chemically synthesized drugs which are usually accompanied by deleterious side effects.

Keywords: Artichoke, Peptic ulcer, Antioxidant, Aspirin, rats and histopathological examination.

Introduction

Ulcers are deep lesions penetrating through the entire thickness of the gastrointestinal tract (GIT) mucosa and muscular mucosa **(Kaur *et al*., 2012).** Peptic ulcers are a broad term which includes ulcers of the digestive tract in the stomach or the duodenum. Recent research has shown that this ulcer developed due to aggressive factors; infection caused by bacteria Helicobacter pylori or reaction to certain medicines as nonsteroidal anti-inflammatory drugs (NSAIDs) is the causative agent of the disease **(Bandyopadhyay *et al*., 2001**). Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E2 (PGE2) content (**Laine *et al*., 2008)**.

The increasing widespread consumption of aspirin/ NSAIDs, however, is associated with an increasing incidence of their well-known gastrointestinal complications, which include dyspepsia, gastric and/or duodenal erosions and ulcers and peptic ulcer complications **(George *et al*., 2005).** Peptic ulcer complications, usually bleeding, represent the most frequent serious adverse events of the use of aspirin/NSAIDs (**Lauer, 2002**). Use of NSAIDs has also been shown to increase the risk of lower gastrointestinal bleeding (**Laine *et al*., 2003**) .The gastric mucosa has evolved to tolerate the high acidity of the stomach lumen via an intricate equilibrium of protective mechanisms. The gastric protective mechanisms (preepithelial, epithelial, and subepithelial factors) act in concert **(Aric *et al*.,2010)**.

In recent years, there has also been growing interest in alternative therapies and the use of natural products, especially those derived from

plants. Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer **(Schmeda-Hirschmann and Yesilada, 2005).**

Artichoke (Cynara scolymus L.) is one of the famous traditional medicinal plants that is widely grown in Mediterranean countries and is rich in natural antioxidants (**Joy and Haber, 2007 and Mehmetcik *et al*., 2008).** Traditionally, Artichoke leaves were used for the treatment and prevention of many diseases. Artichoke has been used to treat dyspepsia mainly because of its choleretic effect that is associated with increased bile formation **(Saénz *et al*.,2002).** Artichoke extracts have been found to exhibit hepatoprotective activity **(Mehmatcik *et al*.,2008)**; lipid lowering property **(Qiang *et al*.,2012);** antioxidant effect **(Juzyszyn *et al*.,2008)** and reduce postprandial blood glucose **(Loi *et al*.,2013)** in man and experimental animals. Artichoke extracts also produced protective effects against hepatocellular carcinoma both in vitro **(Miccadei *et al*., 2008)** and in vivo **(Metwally *et al*., 2011).**

Therefore, the present study was designed to determine the gastroprotective effect of artichoke leaves and pulp extracts on aspirin induced ulcer in male rats.

MATERIALS AND METHODS

Materials

Plant:

Fully mature Artichoke (Cynara scolymus, CV Balady, Family Asteraceae) plant was purchased from green grocery market. Both leaves and pulps (hearts) were separated, pulverized, freezing dried, and kept till preparation of aqueous extracts.

Animals:

Forty- two male albino rats, Sprague-Dawley strain weighing 160±10g, were obtained from the Laboratory Animal Colony, Helwan, Egypt.

Aspirin (Acetyl salicylic acid):

It was obtained in the form of 1 gm vial from the Ameriya Company for pharmaceutical and chemical industries, Cairo, Egypt. It was freshly prepared by dissolving one vial (1g) of aspegic in 5ml distilled water. Aspirin solution was orally given to rats on an empty stomach at a single dose of 2ml (equal to 400 mg aspirin) for induction of acute gastric ulcer.

Kits:

Kits for biochemical analysis were purchased from Gama trade for company pharmaceutical and chemicals, Dokki, Giza.

Methods:

Preparation of the basal diet

The basal diet was prepared according to the recommended dietary allowances for rats (American Institute of Nutrition, AIN) adjusted by **Reeves *et al*. (1993**). Basal diet consisted of 14 % casein , 10 % sucrose, 5 % corn oil, 0.25% choline chloride, 1% vitamin mixture **(Campbell, 1963)** , 3.5 % salt mixture **(Hegested *et al*., 1941)** , 5% fibers and the remainder was corn starch up to 100 %.

Preparation of aqueous extracts:

The leaves of artichoke were removed from the pulps (hearts or heads) and both of them were dried in shade and pulverized. The powdered plant materials 200 g of leaves and 200 g of pulps were separately soaked in 1 liter of hot water at 60 °C for 3 hours, then filtered through double layers of muslin and centrifuged at 4000 rpm for 15 minutes to remove any plant debris. The aqueous extracts were freezing dried and stored at -20°C till used. This procedure was described by **Shalaby and Hamowieh , (2010).**

Detection of active constituents of artichoke leaves and pulp aqueous extract:

The qualitative chemical determination of active constituents of artichoke leaves and pulp aqueous extract were performed to find the presence of the major chemical constituents including; alkaloids, flavonoids,

glycosides, saponins, tannins, resins and triterpenoids using standard procedures of analysis according to **Harborne ,(2007).**

Experimental design

All Animals were fed on the basal diet and water adlibitum and they were maintained under healthy conditions of humidity, temperature (20-25°C) and light (12-h light: 12-h dark cycle) for one week before starting the experiment for acclimatization .After an acclimatization period, rats were divided into six groups of equal weight and number (7 rats each).Group (I): was kept as a negative control group and Group (2): was kept as a positive control group. These two groups were fed on the basal diet and given orally saline at volume of 1.0 ml/ 100 g b.wt) for four weeks. Group(3) was fed on basal diet and orally given the aqueous extract of artichoke leaves(ALE) in a dose of 200 mg/kg b.wt, Group(4) : was fed on basal diet and orally given the aqueous extract of artichoke leaves(ALE) in a dose of 400 mg/kg b.wt, , Group(5) : was fed on basal diet and orally given the aqueous extract of Artichoke pulp (APE) in a dose of 200 mg/kg b.wt, Group(6): was fed on basal diet and orally given the aqueous extract of artichoke pulp (APE) in a dose of 400 mg/kg b.wt, for four weeks .On the last day of the experimental period (28 days), all rats were starved of food, but not of water for 24 hours and groups 2,3,4,5 and 6 orally given aspirin at a single dose of 2ml according to **Agrawal *et al.*, (2000**) . The rats were sacrificed after 4 h of administration of aspirin. Then the blood was collected under diethyl ether anesthesia and centrifuged to obtain the serum which used for biochemical analysis. Stomach was taken for determination of biomarkers of gastric ulcer and histopathological examination.

Gastric ulcer index

The method described by **Agrawal *et al.*, (2000**) was employed in the present study. In brief, after 4 hours of aspirin administration, all rats were sacrificed after using an overdose of diethyl ether and their stomachs removed and washed with saline. The gastric juice was collected in a test tube. Then stomachs were opened along the greater curvature, washed with

saline and examined under a dissecting microscope for gastric ulcers. The sum of the length of all lesion areas for each animal was measured and

served as the ulcer index. The curative ratio was calculated for each group using the following equation:

Curative ratio (CR) = (LC-LT/LC) x100

LC: The length of gastric ulcer in positive group.

LT: The length of gastric ulcer in treated group

Determination of gastric juice acidity

The total acidity was determined according to the method described in **A.O.A.C. (1995).** Percentages of the decrease in total acidity of gastric juice of the treated group compared to the positive (C+Ve) control group were calculated using the following equation:

$$\text{Percentage of the decrease} = \frac{\boldsymbol{TAC - TAT}}{\boldsymbol{TAC}} \times 100$$

Where:

TAC =Total acidity of gastric juice of the positive control group.

TAT =Total acidity of the gastric juice of the treated group.

Determination of gastric juice volume

Gastric juices from all groups were collected in test tubes, centrifuged at 5000 r.p.m. for 10 minutes and their volume of were measured by a graduated cylinder . percentages of the decrease in volume of the gastric juice of the treated groups compared to the positive (C+ve) control group were calculated according to the method described by **Agrawal *et al*., (2000)** using the following equation:

$$\text{Percentage of the decrease} = \frac{\boldsymbol{VJC - VJT}}{\boldsymbol{VJC}} \times 100$$

Where:

VJC = volume of gastric juice of the positive control group.

VJT = volume of gastric juice of the treated group.

Determination of pepsin concentration in gastric juice:

Concentrations of pepsin (a proteolytic enzyme that degrades dietary protein) in the collected gastric juices were measured chemically using spectrophotometer at 313 nm according to the method described by S**chniath (1989).**

Biochemical analysis :

Activities of antioxidant enzymes such as glutathione peroxidase, superoxidase dismutase, and catalase will be determined according to **(Paglia& Valentine, 1979, KaKKar *et al*., 1984 and Sinha, 1972, respectively)** and Determination of Malondialdehyde level (MDA) was measured by the method of **Mihara and Uchiyama, (1978)** . Prostaglandin E2 (PGE2) assay was performed with the PGE2 enzyme immunoassay kit **(R&D Systems, Inc., MN, USA)** according to the supplier's instructions. Serum tumor necrosis factor (TNF-α) was determined by enzyme-linked immunosorbent assay (ELISA) using rat TNF-α assay kit (Biosource, USA) as previously described by **Su *et al*., (2002**). Interleukin-1 (IL-1) was measured by the method of **Grassi *et al*., (1991).**

Histopathological studies:

The stomach of the rats was fixed in 10% neutral formalin solution. the fixed specimens were then trimmed , washed and dehydrated in ascending grades of alcohol .these specimens were then colored in xylene ,embedded in paraffin ,sectioned at 4-6 microns thickness and stained with hematoxylin and eosin (H&E) Then examined microscopically according to **Carleton, (1979).**

Statistical Analysis:

All data obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA) . The collected data were presented as a mean ± standard deviation (SD). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to **(Armitage and Berry, 1987).** All differences considered significant if the level of *P*-values ˙ 0.05.

Results

The phytochemical screening of artichoke leaves aqueous extract (ALE) revealed that it contains large amounts of flavonoids and saponins; moderate amounts of alkaloids and tannins and a small amount of glycosides. Otherwise artichoke pulp aqueous extract (APE) was found to contain large amounts of flavonoids, glycosides and saponins; moderate amounts of alkaloids, and a small amount of tannins, but the extracts of leaves and pulp were devoid of resins and triterpenes as depicted in Table (1).

Table (1). Phytochemical screening of active constituents of Artichoke (*Cynara scolymus*) leaves and pulp aqueous extract .

Active constituents	Alkaloids	Flavonoids	Glycosides	Saponins	Tannins	Resins	Triterpenes
Artichoke leaves (ALE)	++	+++	+	+++	++	--	--
Artichoke pulp(APE)	++	+++	+++	+++	+	--	--

+ = Small amount ++ = Moderate amount +++ = Large amount -- = Absent.

The statistical data in Table 2 showed that there was a significant increase in the volume of gastric juice of the positive control group compared to the negative control group. While, pre-treatment with aqueous extracts of both leave and pulp of artichoke in doses of 200 and 400ml/kg.B.wt., respectively, to male rats with gastric ulcer significantly decreased ($P < 0.05$) the volume of gastric juice by 41.42, 70.35, 43.97 and 69.08%, respectively as compared to the control positive group.

Table (2): The effect of aqueous extracts of Artichoke leaves (ALE) and pulp (APE) on the volume of the gastric juice against aspirin-induced gastric ulcers in rats.

Parameters / Groups	Volume of gastric juice (ml)	
	Mean ± SE	Decrease (%) Compared with C+ve
G1: Control (C-ve)	2.02± 0.26d	---
G2:Aspirin (C+ve)	7.05± 0.12 a	---
G3: ALE (200ml/kg.B.wt)	4.13 ± 0.11c	41.42
G4: ALE (400ml/kg.B.wt)	2.09± 0.21d	70.35
G5: APE (200ml/kg.B.wt)	3.95 ± 0.19c	43.97
G6: APE(400ml/kg.B.wt)	2.18± 0.13d	69.08

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at $P< 0.05$. Values with similar or partially similar superscripts are non-significant.

As shown in Table 3 aspirin administration caused a significant increase in total acidity of gastric juice of the positive control group compared to the negative control group. Pre-treatment with aqueous extracts of both leaves and pulp of artichoke at the different doses (200 and 400ml/kg.B.wt.) to rats with aspirin-induced gastric ulcer decreased the total acidity of gastric juice by 47.94, 70.73, 40.88, and 70.46%, respectively as compared the the positive control group.

Table (3): The effect of aqueous extracts of Artichoke leaves (ALE) and pulp (APE) on the total acidity of the gastric juice against aspirin-induced gastric ulcers in rats.

Parameters / Groups	Total acidity of gastric juice (mg equiv./L)	
	Mean ± SE	Decrease (%)
G1: Control (C-ve)	7.12± 0.13 c	---
G2:Aspirin (C+ve)	25.76 ± 0.51a	---
G3: ALE (200ml/kg.B.wt)	13.41± 0.23b	47.94
G4: ALE (400ml/kg.B.wt)	7.54± 0.14 c	70.73
G5: APE (200ml/kg.B.wt)	15.23± 0.13b	40.88
G6: APE(400ml/kg.B.wt)	7.61± 0.12 c	70.46

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

Data recorded in Table (4) showed that the gastric ulcer index in male rats with experimental gastric ulcer (positive control) was 11.42 ± 0.06 mm compared to zero (no ulcer) in the negative control group (normal rats). The values of gastric ulcer index were significantly decreased in all pre-treated rat groups compared with the control positive group. The ulcer healing effect was greater by use of ALE (400ml/kg.B.wt).

Table (4): The effect of aqueous extracts of Artichoke leaves (ALE) and pulp (APE) on gastric ulcer index against aspirin-induced gastric ulcers in rats.

Parameters / Groups	Gastric ulcer index Mean ± SE (mm)	Curative ratio (%) Compared with C+ve
G1: Control (C-ve)	---	---
G2:Aspirin (C+ve)	11.42 ± 0.06 a	---
G3: ALE (200ml/kg.B.wt)	8.31 ± 0.12 b	27.23
G4: ALE (400ml/kg.B.wt)	5.23± 0.13 c	54.20
G5: APE (200ml/kg.B.wt)	7.49± 0.04 b	34.41
G6: APE(400ml/kg.B.wt)	5.54± 0.24 c	51.49

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

Table 5 showed that aspirin administration caused a significant increase in gastric juice pepsin activity compared to the negative control group. Meanwhile, Oral administration of ALE and APE to male rats for 4 weeks revealed significant decreases in pepsin concentration by 21.55, 42.63, 18.89 and 41.70 % , respectively as compared to the positive control group.

Table (5): The effect of aqueous extracts of Artichoke leaves (ALE) and pulp (APE) on pepsin concentrations in gastric juice against aspirin-induced gastric ulcers in rats.

Parameters / Groups	pepsin concentration (mg/ml) Compared with C+ve	
	Mean ± SE	Decrease (%)
G1: Control (C-ve)	27.08 ± 1.07 c	---
G2:Aspirin (C+ve)	46.12 ± 2.2 a	---
G3: ALE (200ml/kg.B.wt)	36.18 ± 1.4 b	21.55
G4: ALE (400ml/kg.B.wt)	26.46 ± 1.6 c	42.63
G5: APE (200ml/kg.B.wt)	37.41 ± 1.6b	18.89
G6: APE(400ml/kg.B.wt)	26.89 ± 1.6 c	41.70

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

The statistical data in Table 6 presented that, control (+ve) rat group showed significant increase in lipid peroxide malondialdehyde (MDA) content and decrease in levels of reduced glutathione (GSH) when compared with the negative control group. Pre- treated rats with different doses of aqueous extracts of leaves (ALE) and pulp (APE) of artichoke significantly decreased MDA content and increased GSH content in liver tissue when compared with the positive control group.

Table (6): The effect of aqueous extract of aqueous extracts of Artichoke leaves (ALE) and pulp (APE) on serum malondialdehyde (MDA) and reduced glutathione (GSH) contents against aspirin-induced gastric ulcers in rats.

Parameters / Groups	MDA (μmol/dl	GSH (μmol/dl
G1: Control (C-ve)	18.09± 1.42c	31.17 ± 1.34a
G2:Aspirin (C+ve)	29.24± 2. 11a	18.02 ± 1.05d
G3: ALE (200ml/kg.B.wt)	23.02± 2. 08 b	24.14 ± 2.23c
G4: ALE (400ml/kg.B.wt)	18.89± 1. 23c	30.19 ± 1.21a
G5: APE (200ml/kg.B.wt)	23.92± 1. 03b	23.29 ± 1.21c
G6: APE(400ml/kg.B.wt)	19.07± 1. 04c	27.71 ± 2.65b

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

The results illustrated in table 7 showed that aspirin administration (control+ve group) caused a significant (p<0.05) decreases in the activity of serum superoxide dismutase (SOD), glutathione peroxidase(Gpx) and catalase (CAT) enzymes when compared with the normal control group. Oral administration of AELA and AEPA at both dosages caused a significant (p<0.05) increases in the activity of SOD, GPx, and CAT enzymes when compared with the control+ve group.

Table (7) The effect of aqueous extracts of Artichoke leaves (ALE) and pulp (APE) on activities of serum superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes against aspirin-induced gastric ulcers in rats.

Parameters / Groups	SOD (U/mg)	GPx (U/mg)	CAT (U/mg)
G1: Control (C-ve)	68.32 ± 1.12. a	76.11 ± 1.09a	7.12 ± 1.42a
G2:Aspirin (C+ve)	31.09 ± 1.28d	37. 82 ± 1.35d	2.07± 0.13c
G3: ALE (200ml/kg.B.wt)	51.53 ± 2.23b	65.20 ± 1.14b	4.56± 0.46b
G4: ALE (400ml/kg.B.wt)	67.97 ± 2.53a	75.98±0.19a	6.75± 1.25a
G5: APE (200ml/kg.B.wt)	57.81 ± 1.63c	57.28±2.16c	4.75± 0.75b
G6: APE(400ml/kg.B.wt)	66.79 ±2.13a	75.27 ± 1.12ba	6.99± 0.12a

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at P< 0.05. Values with similar or partially similar superscripts are non-significant.

Data reported in Table 8 showed that aspirin administration led to significant decreases in prostaglandin E2 while , there were significant increases in serum interleukin-1 and tumor necrosis factor-alpha levels compared to (-ve) control group. The pretreatment groups with aqueous extracts of leaves (ALE) and pulp (APE) of artichoke showed significant

decreases in interleukin-1 and tumor necrosis factor-alpha while there was a significant increase in prostaglandin E2 compared to the ulcerated positive control group.

Table (8): The effect of aqueous extracts of Artichoke leaves (ALE) and pulp (APE) on Prostaglandin E2 (PGE2), tumor necrosis factor (TNF-α) and Interleukin-1(IL-1) against aspirin-induced gastric ulcers in rats.

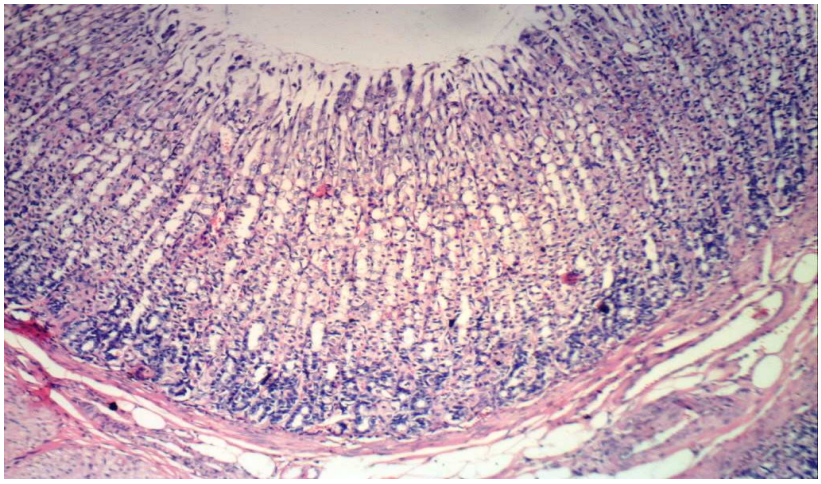
Parameters / Groups	PGE2 pg/mg	TNF-α pg/ml	IL-1 pg/ml
G1: Control (C-ve)	470.62± 1.08 a	4.52 ± 1.17c	13.45 ± 0.71c
G2:Aspirin (C+ve)	284.34 ± 0.42d	13. 43 ± 1.21a	40.32± 0.59a
G3: ALE (200ml/kg.B.wt)	422.08 ± 1.56c	7.01 ± 0.14b	22.09± 0.36b
G4: ALE (400ml/kg.B.wt)	469.62 ± 1.52a	3.99±0.26c	12.95± 0.05c
G5: APE (200ml/kg.B.wt)	393.73 ±0.78b	6.86 ± 1.36b	24.23± 1.43b
G6: APE(400ml/kg.B.wt)	466.19 ± 0.91a	5.18 ± 0.39b	12.89± 1.43c

Data are presented as means ± standard deviation, (n = 7 for each group) Values with different superscripts within the column are significantly different at $P< 0.05$. Values with similar or partially similar superscripts are non-significant.

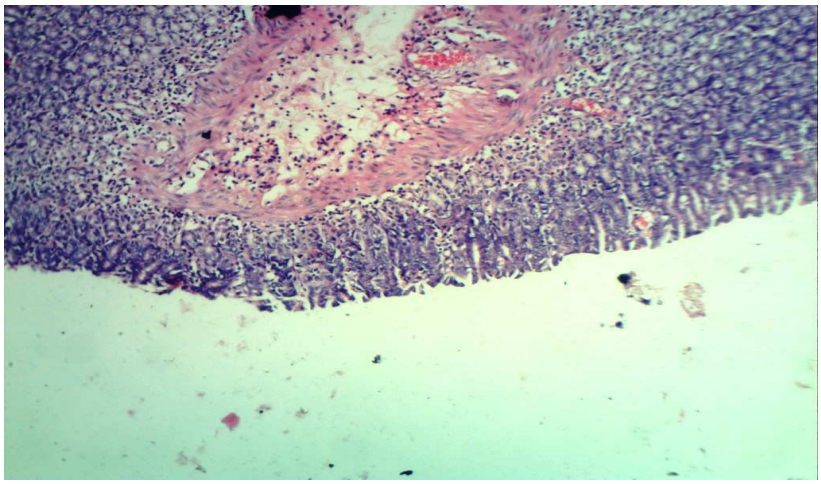
Histopathological studies:

Histopathological examination of the stomachs of the negative control (normal) rats revealed normal gastric layers (mucosa, submucosa, musculosa and serosaas shown in Photo. (1). The stomachs of the positive control rats (given aspirin) showed focal necrosis of gastric mucosa associated with inflammatory cells infiltration as well as submucosal oedema and inflammatory cells infiltration (Photo.2) . Examined sections of the stomachs of the rats orally given 200ml/kg.B.wt aqueous extracts of artichoke leaves showed congestion of mucosal blood vessels(Photo.3). Examined sections of rat orally given 400ml/kg.B.wt aqueous extracts of artichoke leaves showed no histopathological changes as shown in Photo. (4). The stomachs of rats orally given 200ml/kg.B.wt aqueous extracts of artichoke pulp showed slight submucosal oedema (Photo.5) Examined

sections of rat orally given 400ml/kg.B.wt aqueous extracts of artichoke pulp showed apparent normal mucosa as shown in Photo. (6).



photo(1): stomach of normal rat group showing normal gastric layers (mucosa, submucosa, musculosa and serosa) (H&Ex100)



Photo(2): Stomach of positive control rats (given aspirin) showing focal necrosis of gastric mucosa associated with inflammatory cells infiltration as well as submucosal oedema and inflammatory cells infiltration (H & E X 100).

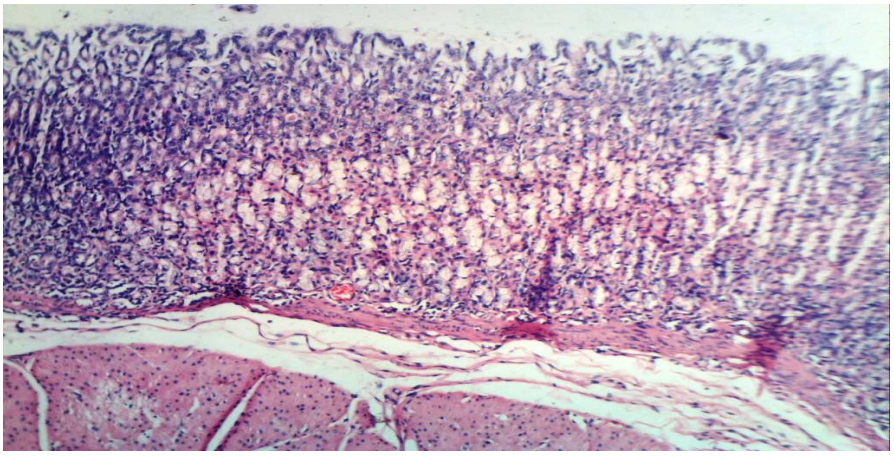


Photo (3): Stomach of rats orally given 200ml/kg.B.wt aqueous extracts of artichoke leaves showing congestion of mucosal blood vessels (H & E X 100).

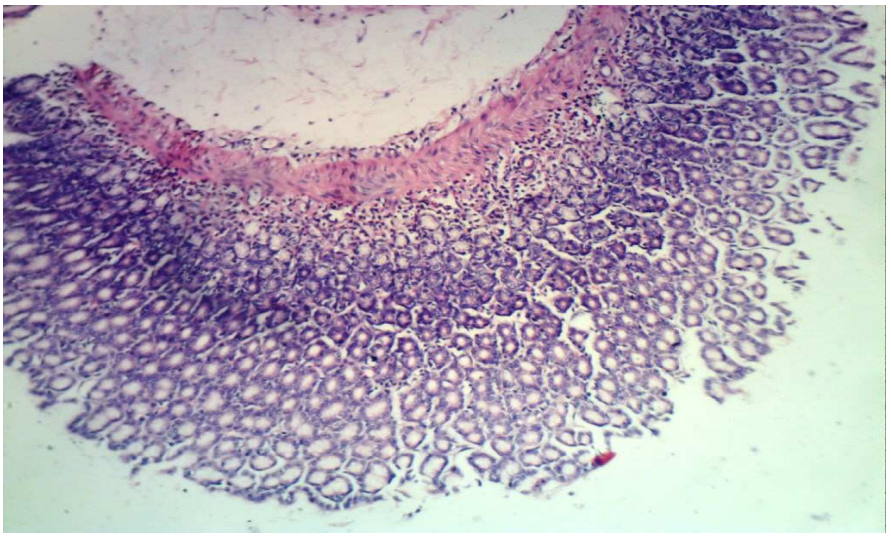


Photo (4): Stomach of rats orally given 400ml/kg.B.wt aqueous extracts of artichoke leaves showing no histopathological changes (H & E X 100).

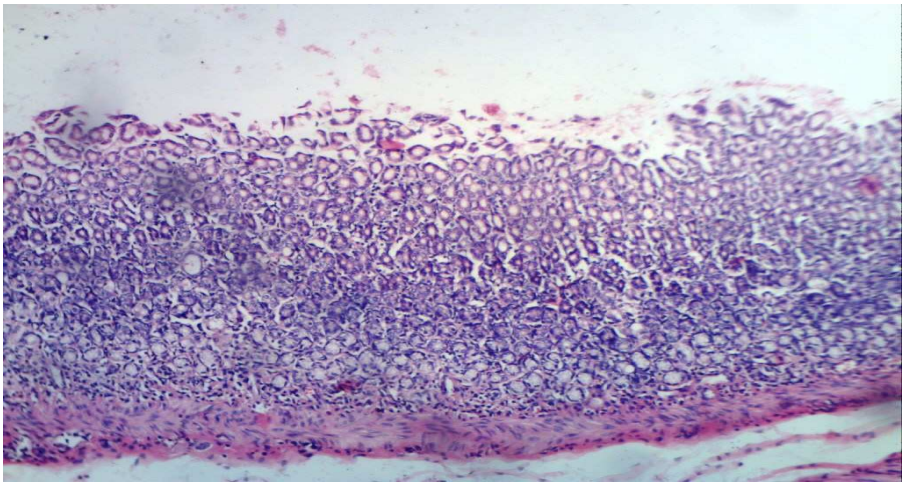


Photo (5): Stomach of rats orally given 200ml/kg.B.wt aqueous extracts of artichoke pulp showing slight submucosal oedema (H & E X 100).

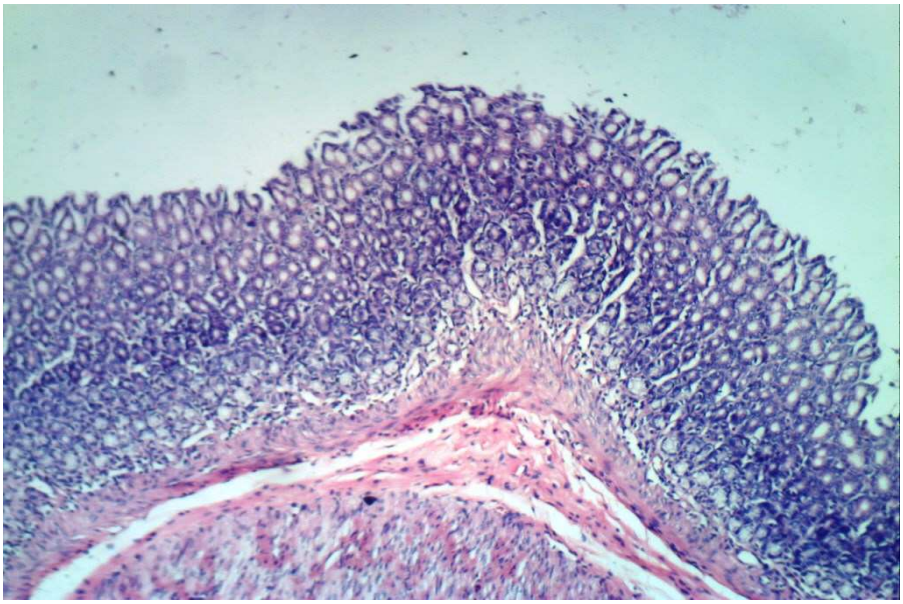


Photo (6): Stomach of rats orally given 400ml/kg.B.wt aqueous extracts of artichoke pulp showing apparent normal mucosa (H & E X 100).

Discussion

The current study was designed to determine qualitatively the chemical constituents of aqueous extracts of Artichoke (Cynara scolymus) leaves and pulp and to assess their gastroprotective effect of artichoke (Cynara scolymus L.) on Aspirin-Induced Ulcer in male Rats.

Nowadays, medicinal plants, vegetables, and fruits with gastric ulcer activity have gained much attention, especially those with low toxicity properties. The biological value of the plant materials depends on their bioactive chemical constituents such as saponins, anthocyanins, flavonoids, polyphenols, triterpenes and other phytochemicals **(Veermuthu et al., 2006 and Patel et al., 2012).**

In this study, the phytochemical screening of both Artichoke leaves and pulp aqueous extracts showed that they contain flavonoids, alkaloids, glycosides, saponins, and tannins, but they were devoid of resins and triterpenes. These results were in harmony with the previous data obtained by **(Nassar et al.,2013 , Abu-Reidah et al ., 2013 and Wu et al .,2008).** Moreover, the two later authors **(Abu-Reidah et al., 2013 and Wu et al., 2008)** isolated and characterized the bioactive phenolic constituents from fresh and canned Artichoke by HPLC. The authors concluded that Artichoke plant could be regarded as a functional food and also as a promising source of potent antioxidant polyphenolic compounds **(Wu et al., 2008).**

The present study showed that aspirin at a dose of 400mg/kg b.wt., single dose in 24hrs fasted rats caused a significant damage to the gastric mucosa. This was evidenced by a significant decrease in gastric pH, increase in acid output, ulcer index and pepsin activity as compared to normal control ($p<0.05$). The ulceration induced by Aspirin is attributed mainly to various processes, including the generation of reactive oxygen species, the initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis **(Bech et al., 2000).** Decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretions which, in turn, aggravate the

ulcer **(Miller, 1983).**Histological findings also supported the gastric mucosal damage. This finding was in accordance with **Wallace, (2008).**

The data of present study shows variable inhibitory effects of aqueous extracts of Artichoke (Cynara scolymus) leaves and pulp on total acidity, gastric volume, peptic activity, ulcer score, ulcer index and pepsin activity. These observations suggest the aqueous extracts of Artichoke (Cynara scolymus) leaves and pulp possibly have an antacid-like action. Artichoke extracts may contain biologically active substances with potential anti-ulcer properties. This gastro protective effect may be due to the high flavonoids content of Artichoke extracts **(Katrin et al.,2004).** The obtained results are consistent with those of **Kazuo et al., (2010)** who demonstrated that Artichoke possesses anti-ulcer activity against the ulceration caused by aspirin. Furthermore, **Nawal and Naglaa (2016)** reported that aqueous extracts of Artichoke (Cynara scolymus) leaves showed good gastro protective anti-ulcerogenic activity and they attributed this effect to the anti-oxidative activity of flavonoids found in the extract as reported by **Mahmoud et al.,(2013)** and these compounds may work by relaxing the smooth muscle of the gastrointestinal tract.The results suggest that some of the constituents present in the aqueous extracts of Artichoke (Cynara scolymus) leaves and pulp may have central actions, which are helpful in reducing the gastric ulcers. The reduction may be also due to local effect on gastric motility or gastric secretion.

Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer. Aspirin administration was found to increase MDA level and a decrease in GSH level, as well as SOD, CAT and GPx activities in the positive control groups compared to the negative control group, thus leading to oxidative stress. Preventive antioxidants, such as superoxide dismutase (SOD) and catalase (CAT) enzymes are the first line of defence against reactive oxygen species. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation **(Alfadda and Sallam, 2012).** Administration of aqueous extracts of Artichoke (Cynara scolymus) leaves and pulp resulted in a significant

decrease in MDA while there were significant increases in the GSH level, as well as SOD, CAT and GPx activities as compared to the positive control animals, These findings were similar to the results of the previous studies **(Juzyszyn et al., 2008).** Furthermore, **(Magielse et al., 2014)** concluded that the protective activity of Artichoke could be attributed to its constituents of many bioactive polyphenolic antioxidant compounds, mainly cynarine, luteolin, and chlorogenic acids which are abundant in both heads and leaves .

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin have the ability to cause gastroduodenal ulceration and this effect is related to the ability of these agents to suppress prostaglandin synthesis **(Lichtenberger et al., 2007 and Wang et al., 2007)** In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair. Thus, the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration **(Deore et al., 2011).** Our experimental results were in the same line with these previous data. Aspirin significantly reduced gastric mucosal prostaglandin E2 (PGE2) level compared to the negative control group. Pretreatment with aqueous extracts of Artichoke (Cynara scolymus) leaves and pulp significantly increased PGE2 levels when compared to aspirin-treated rats. This finding was explained by **Sannia, (2010)** who reported that flavonoids may protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase.

The inflammation induced in the gastric mucosa by aspirin is accompanied by increased TNF-α production **(Jainu et al., 2006),** which augments neutrophil-derived superoxide generation **(Kwiecien et al., 2002)** and stimulates IL-1 production leading to neutrophil accumulation **(Odashima et al.,2006)** Over production of TNF--α increased the risk of gastric ulcer and cancer **(Mitsushige et al.,2006)**. In the present study, the levels of TNF-α and IL-1 were increased by aspirin administration and the

pretreatment with all using aqueous extracts of artichoke (Cynara scolymus) leaves to the artichoke antioxidant activity.

The alterations of serum biochemical parameters induced by Artichoke extracts were confirmed by partial regression of histopathological degenerative changes seen in the gastric of ulcerative rats. The amelioration of histopathological lesions by oral administration of both Artichoke extracts was in accordance with that previously reported by **(Nawal and Naglaa, 2016)** The serum and tissue biochemical changes induced by pretreatment of Aspirin- ulcerative rats with Artichoke aqueous extracts, in the present study, were to some extent parallel to the histopathological findings, denoting gastroprotective activity of Artichoke extracts.

In conclusion, the present results denote that aqueous extracts of Artichoke leaves and pulp produce gastroprotective activity and antioxidant effects and partially alleviate the degenerative changes induced by Aspirin in gastric of rats. These findings affirm the traditional use of Artichoke plant for treating peptic ulcer diseases .

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التأثير الوقائي لمستخلص أوراق ولب الخرشوف علي قرحة المعدة في ذكور الفئران

ريهام شوقي رمضان متولي*

الملخص العربي

يهدف البحث لدراسة الفحص الفيتوكيميائي لكل من أوراق ولب الخرشوف وكذلك دراسة تأثيرهم الوقائي ضد القرحة التي يسببها الأسبرين في ذكور الفئران. تم توزيع اثنين واربعين من ذكور الفئران البالغة بصورة عشوائية إلى ٦ مجموعات متساوية على النحو التالي: المجموعة الأولى ضابطة سالبة (فئران غير مصابة) والمجموعة الثانية ضابطة موجبة و المجموعات الأربعة الاخرى فئران مصابة سبق إعطائها عن طريق الفم المستخلص المائى لأوراق ولب الخرشوف وتم اعطاء كل منهم بجرعتين : جرعة صغيرة (٢٠٠ مجم/كجم) وجرعة كبيرة (٤٠٠ مجم/كجم) على التوالى. لمدة ٢٨ يوما في أخر يوم من فترة التجريه أعطيت كل المجموعات الأسبرين ٤٠٠ ملجم/كجم من وزن الجسم لإحداث القرحة باستثناء المجموعة الاولي . وفي نهاية فترة التجربة تم تجميع عينات الدم لفصل المصل واستخدامه فى التحليلات البيوكيميائية، وتم اخذ معدة الفئران لقياس مؤشرات قرحة المعدة وكذلك لإجراء الفحص الهستوباثولوجى . وقد أظهرت نتائج الفحص الكيميائي النباتي أن كلا من مستخلص أوراق الخرشوف وكذلك مستخلص لب الخرشوف يحتويان على فلافونيدات، قلويدات، جليكوسيدات، ومواد صابونية وعفصية ، بينما لا يحتويان علي المواد الراتنجية والتربينات الثلاثية. كما أظهر تناول مستخلصات الأوراق واللب للخرشوف تأثير مضاد للأكسدة وظهر ذلك بإ نخفاض معنوي ملحوظ في كل من حجم العصير المعدي ودرجة الحموضة الكلية ومؤشر قرحة المعدة وتركيز انزيم الببسين وانترلوكين ١١-IL وعامل دلالة الأورام ألفا -TNF,وكذلك مؤشر الاجهاد التأكسدي جنبا إلى جنب مع ارتفاع ملحوظ في مستوى البروستاجلاندين و الإنزيمات المضادة للأكسدة مقارنة بالمجموعة الضابطة الموجبة. كما أظهر الفحص الهستوباثولوجي لأنسجة المعدة تحسن واضح في التغيرات المرضية التي أحدثها الأسبرين. ولذلك توصى الدراسة بتناول الخرشوف فى الغذاء أو فى صورة شاى عشبى وقد يكون ذلك مفيدا للمرضى الذين يتناولون ادوية قد تسبب التهاب القرحة الحاد لما للخرشوف من خصائص مضادة للاكسدة. وعلاوة على ذلك فأن عزل المكونات النشطة بيولوجيا من أوراق ولب الخرشوف ضرورى للبحث عن مواد طبيعية آمنة لإستخدامها فى العلاج بدلا من الأدوية الكيميائية التى غالبا ما يصاحبها أعراض جانبية ضارة.

الكلمات المفتاحية : الخرشوف- قرحة المعدة- مضاد للاكسدة- الاسبرين- الفئران- الفحص الهستوباثولوجى.

* قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلي – جامعة حلوان