Studying Protective Effect of Ethanolic Extract of Artichoke (*Cynara scolymus*) Leaves and Vitamin E against Oxidative Stress Induced in Rats

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Abstract

The present study was conduct to evaluate the protective effect of Artichoke leaves extract (ALE) and vitamin E administration against oxidative stress induced by antitumor drug cyclophosphamide (CP) in rats**.** Forty five male rats were randomized into 5 equal groups. Group (1) was normal rats (negative control), while the other four groups were intraperitoneally injected by a single daily dose of CP (50 mg/kg BW) for 3 days to induce oxidative stress. Group (2) was used as positive control (intoxicated) and groups (3), (4) and (5) received orally daily doses of ALE (400 mg/kg/BW); vitamin E (100 mg/kg/BW) and both ALE and vitamin E for six weeks, respectively. Blood samples were collected for separation of the serum which used for biochemical analyses. Halve of livers of the sacrificed rats was used for preparing liver homogenates for biochemical analyses. The other halve was used for histopathological examination. The results showed that oral administration of ALE increased body weight gain in CP- administered rats. ALE decreased the high serum levels of liver enzymes; normalized serum levels of total protein, total bilirubin, urea, creatinine, total cholesterol, triglycerides, malondialdehyde (MDA) and reduced glutathione (GSH). In hepatic tissues, there were a significant decrease in MDA and increases in GSH levels and activities of antioxidant enzymes (SOD, GPx and CAT). These biochemical alterations were accompanied with mitigation of histopathological lesions (fatty degeneration and necrosis) seen in the liver of CP-intoxicated rats. Concurrent administration of ALE and vitamin E exhibited the best effects when compared to their administration alone. In conclusion, concomitant administration of ALE and vitamin E has hepatoprotective, nephroprotective, hypolipidemic and antioxidant activities in CP-intoxicated rats. The study recommends that intake of edible parts of artichoke leaves in food or as an herbal tea with vitamin E as a food supplement may be beneficial for patients suffering from liver and kidney diseases due to oxidative stress. Isolation of bioactive constituents of artichoke leaves is necessary also to search for safe natural antioxidant agents to be developed for therapy of liver and kidney diseases.

Keywords: *Cynara scolymus*, vitamin E, Cyclophosphamide, hepato-renal functions, serum lipid profile, antioxidant, histopathology.

Introduction

Oxidative stress has an important role in the development and manifestations of many critical diseases. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses of the body against them, so cellular damage results. The antioxidant defenses enable the body system to scavenge ROS, restore the prevailing reducing environment and repair the tissue damage **(Agarwal *et al.*, 2012)**. Free radicals such as nitric oxide (NO) and superoxide ions are produced as second messengers, particularly by immune cells. Superoxide ions react rapidly with nitric oxide by nitric oxide synthase enzyme to produce peroxynitrite. Hydrogen peroxide (H_2O_2) slowly decomposes to the highly reactive hydroxyl radical. Both peroxynitrite and hydroxyl radicals are highly reactive oxidizing radicals, capable of damaging proteins, lipids, and DNA **(Abd El-Ghany *et al.*, 2011)**. Oxidative stress plays an important role in the etiology and pathogenesis of several chronic diseases (hypertension, atherosclerosis, diabetes mellitus and cancers (**Krajcovicova-Kudlackova *et al.*, 2012**).

Cyclophosphamide (CP), is a widely used antitumor and immunosuppressant drug. It is used for the treatment of acute and chronic leukemia, breast cancer, multiple myeloma and lymphomas (**Jain and Jain, 2012**). In spite of its therapeutic importance owing to its broad spectrum efficacy, its metabolites exhibit severe undesired toxicities on normal cells including oxidative stress, nephrotoxicity, hepatotoxicity, lung and cardiac injuries, genotoxicity, and induction of tissue lipid peroxidation (**Nitharwal *et al.*, 2013**). Intraperitoneal injection of CP in a dose of 50 mg/kg for 3 days to rats significantly elevated endogenous reactive oxygen species (ROS) and induced oxidation of lipids and proteins which are biomarkers of oxidative stress **(Ince *et al.*, 2014**).

Dietary intake of antioxidants can inhibit or delay the oxidation of susceptible cellular substrates so prevent oxidative stress. Phenolic compounds from medicinal plants such as vegetables and fruits have been reported on their effective antioxidants, anticancer, antibacteria, cardioprotective agents, anti-inflammation, and immune system promoting (**Meng *et al.*, 2018).** Therefore, it is important to enrich our diet with antioxidants to protect our bodies against many chronic diseases. Moreover, antioxidants play an important role in food

quality preservation due to their ability to prevent oxidative deterioration of lipids **(Erukainure *et al*., 2012).**

Artichoke (*Cynara scolymus*, Family *Asteraceae*) is an important component of the Mediterranean diet (**Pagnotta *et al.*, 2017).** Several studies have confirmed the high bioactive compounds content and antioxidant potency of the edible part **(Kollia *et al.*, 2016**). It was reported that the aqueous artichoke leaves extract contains caffeoylquinic acid with chlorogenic acid being the most abundant (0.30%), and luteolin-7-O-glucoside (0.15%) as a major flavonoid (**Magielse *et al*., 2014**). Artichoke has been used traditionally to treat a variety of conditions, including hepatic diseases, jaundice, dyspepsia, and chronic albuminuria. Artichoke leaves have been used as a diuretic and as a choleretic to stimulate bile flow from the liver and gallbladder (**Ben Salem *et al.*, 2015)**.

The present study was designed to evaluate the effect of concurrent administration of Artichoke leaves extract (ALE) and vitamin E against oxidative stress induced by antitumor drug cyclophosphamide (CP) in rats**.**

Materials and Methods

Plant Materials: Fully mature Artichoke (*Cynara scolymus*, CV Balady, Family *Asteraceae*) plant was purchased from green grocery market. The leaves were separated, pulverized; freezing dried and kept in a refrigerator till preparation of the alcohol extract.

Cyclophosphamide and biochemical kits: Cyclophosphamide (Indoxan®) was purchased from a local pharmacy in the form of 500 mg vials. It is manufactured by Baxter BioPharma Company for Pharmaceuticals, USA. It is dispensed as crystalline white powder and freshly prepared in 0.9% sodium chloride saline solution. It was injected in a single daily dose of 50mg /kg/BW in the peritoneal cavity for 3 days for induction of oxidative stress (**Nitharwal *et al*., 2013**). Biochemical kits for the determination of serum liver enzymes (AST, ALT and ALP), total protein, total bilirubin, urea nitrogen, uric acid, creatinine, total cholesterol and triglycerides were purchased from Alkan Company for Chemicals and Biodiagnostics, Dokki, Cairo, Egypt.

Preparation of artichoke leaves extract (ALE): ALE was prepared by soaking 200 g of the dried leaves powder in 1 liter of 90% ethyl alcohol and kept in a refrigerator with daily shaking for 5 days. The ethanol was evaporated using

a rotatory evaporator apparatus (manufactured in West Germany) attached with a vacuum pump. Twenty grams of the obtained semisolid extract were suspended by 2 ml Tween 80 (suspending agent) and 80 ml of distilled water were gradually added to prepare a 20% liquid extract. This procedure was described by **Shalaby and Hamowieh (2010)**.

Rats: Forty five mature Sprague Dawley male rats weighing 200 -205 g each and 10-11 weeks of age were purchased from the Laboratory Animals Colony, Ministry of Health and Population, Helwan,. The rats were housed at a controlled room temperature of 23 ± 1ºC, 55 % humidity and under 12-hr light / 12-hr dark schedule. The animals were fed on basal diet and water was provided *ad libitum*.

Preparation of basal diet: The dietary supply of protein, fat, carbohydrates, vitamins and minerals was in accordance with the recommended dietary allowances for rats **(Reeves *et al.*, 1993)**. Basal diet was consisted of 14 % protein (casein), 10 % sucrose, 4 % soybean oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers (cellulose). The remainder was corn starch up to 100 %.

Experiment Design: The rats used in this study were randomized into 5 equal groups, of 9 rats each. Group (1) was fed on basal diet, given orally 1 ml /rat of 0.9% sodium chloride saline solution (vehicle) and used as negative control.

The other four groups were injected by a daily single intraperitoneal dose of cyclophosphamide (50 mg/kg BW) for 3 days to induce oxidative stress as mentioned by **Nitharwal *et al.*, (2013).** Group (2) was left positive control without treatment and groups (3), (4) and (5) were given orally Artichoke leaves extract (ALE) in a dose 400 mg/kg/BW; vitamin E in a dose 100 mg/kg/BW and ALE concomitantly with vitamin E for 6 weeks, respectively.

The food intake was calculated daily and the body weight gain was recorded weekly. At the end of experiment, the rats were anesthetized by ether anesthetic and blood samples were collected into clean centrifuge tubes and centrifuged at 4000 rpm for 15 minutes to obtain the serum which used for biochemical analyses. Halve of livers of the sacrificed rats was immediately excised, rinsed with saline solution, blotted on filter paper and stored frozen at -70^{o}C till used for the preparation of liver homogenates for biochemical analysis.

The other halve was preserved in 10% neural formalin solution till processed for histological examination.

Biochemical analyses: Activities of serum liver enzymes aspartate and alanine aminotransferases and alkaline phosphatase were determined using colorimetric methods as described in the kits instruction (Diamond Co, Hannover, Germany). The absorption of the test samples were read at 505nm for AST and ALT and 510 nm for ALP, respectively. Serum concentrations of total cholesterol and triglycerides were determined using enzymatic methods as described in the instructions provided with the kits (Analyticon® Biotechnologies AG, Germany). The absorbance of the testes samples were read using spectrophotometer adjusted at 546 nm for TG and TC. Serum total protein and total bilirubin concentrations were determined using colorimetric methods as described in the kits instruction (Diamond Co, Hannover, Germany). The absorption of the test samples were read at 546 nm for TP, 578 nm for total bilirubin respectively. Serum urea nitrogen and uric acid concentrations were determined by enzymatic colorimetric method and using colorimetric kinetic as described by **Young (2001).** Serum creatinine concentrations were determined using colorimetric kinetic as described by **Waiker and Bonventre (2005)**. Serum lipid peroxide malondialdehyde (MDA) and reduced glutathione (GSH) contents were estimated according to methods described by **Draper and Hadley, (1990)** and **Afzal *et al.*, (2002),** respectively.

Preparation of liver homogenate: One gram of frozen liver tissue was washed in ice-cold 0.9% NaCl and homogenized in ice-cold 1.15% solution of potassium chloride and 50 mM potassium phosphate buffer solution (pH 7.4) to yield 10% homogenate (W/V). The homogenate was then centrifuged at 4000 rpm for 15 minutes at 4°C and the supernatant was collected for further use.

Lipid peroxidation and tissue antioxidant enzymes: Lipid peroxidation (LPO) was determined by quantifying malondialdehyde (MDA) that formed in terms of thiobarbituric acid reactive substances (TBARS). Liver homogenate was used for determination of tissue lipid peroxide malondialdehyde (MDA), enzymatic (GPx, SOD and CAT enzymes) and non enzymatic reduced glutathione (GSH) antioxidants. The reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method modified by **Bulaj *et al.*, (1998)**. Lipid peroxide (MDA) was determined according to **Ohkawa *et al.*, (1979)**. Activities of hepatic antioxidant enzymes glutathione peroxidase

(GPx), superoxide dismutase (SOD) and catalase (CAT) were determined chemically according to **Paglia and Valentaine (1979), Spitz and Oberley (1989) and Sinha (1972)**, respectively.

Histological examination: Halve of livers of the sacrificed rats was taken and 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 **(Bancroft and Gamble, 2002)**.

Statistical analysis: Statistical analysis was performed by using computer program statistical package for social science (SPSS. All obtained results were tabulated as means ± SD). Statistical analysis has been achieved using IMB-P-C computer by SPSS, program version 20.0. Universal analysis was conducted using one way analysis of variance (ANOVA) test**.**

Results

Intraperitoneally injection of cyclophosphamide in a daily single dose 50 mg/kg BW for 3 days to rats significantly decreased food intake and body weight gain when compared to negative control rats. Rats with oxidative stress when orally given Artichoke leaves extract (ALE) alone gained more weight as compared with the positive control group. Coadministration of ALE and vitamin E produced the best effect on body weight gain when compared with their administration of alone as depicted in Table (1).

Table (1): Effect of ALE and vitamin E on body weight gain and feed intake in rats with oxidative stress

Groups / Parameters	Control (-ve)	Control (+ve)	ALE (400mg/kg)	Vitamin E (100mg/kg)	ALE + Vitamin E
Initial weight (g)	200.00 ±8.56^{a}	202.00 ±7.38^{a}	205.00 ± 6.48^{a}	203.00 ± 5.43^{a}	201.00 ± 6.22^{a}
Final weight (g)	270.50 ±11.16^{a}	248.00 ±12.22^{c}**	258.00 ±13.53^{b}**	259.00 ±12.44^{b}**	267.50 ±11.38^{a}***
Weight gain (g)	70.50 ± 6.55^{a}	46.00 ± 3.53^{d}***	53.00 ± 4.97^{c}**	56.00 ± 4.23^{c}**	66.50 ± 5.22^{b}***
Feed intake (g/day)	24.20 ± 1.76^{a}	19.74 ± 1.34^{b}*	19.25 ± 1.54^{b}*	19.95 ± 1.43^{b}*	19.88 ± 1.55^{b}*

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significant when compared with both control groups at * P<0.05, ** P<0.01, *** P<0.001

Rats injected intraperitoneally by a single daily dose (50 mg/kg BW) for 3 days of cyclophosphamide had significant increases in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC) and triglycerides (TG) when compared with the negative control group. Oral administration of Artichoke leaves extract (ALE) for 6 weeks to rats with oxidative stress had significant decreases in the elevated serum levels of AST, ALT, TC and TG when compared with the positive control group. The best lowering effect on the elevated serum levels of the abovementioned parameters was reported by concomitant administration of ALE and vitamin E when compared with their administration alone as recorded in Table (2).

Table (2): Effect of ALE and vitamin E on serum AST, ALT, ALP liver enzymes, total cholesterol (TC) and triglycerides (TG) in rats with oxidative stress

Groups / Parameters	Control (-ve)	Control (+ve)	ALE (400mg/kg)	Vitamin E (100 mg/kg)	ALE + Vitamin E
AST (U/dL)	45.36 ± 3.55^{d}	75.62 ± 5.95^{a}***	69.21 ± 5.61^{b}**	64.31 ± 5.98^{b}**	59.45 ± 4.98^{c}***
ALT (U/dL)	37.22 ± 2.11^{d}	58.66 ± 3.71^{a}***	49.66 ± 3.71^{b}**	46.19 ± 5.66^{b}**	39.49 ± 4.28^{c}***
ALP (U/dL)	94.50 ± 4.15^{d}	114.00 ± 2.37^{a}***	105.00 ± 3.22^{b}*	102.00 ± 2.27^{b}*	98.00 ± 3.17^{c}**
TC (mg/dL)	98.50 ± 3.32^{d}	133.50 ± 6.44^{a}***	118.43 ± 7.15^{b}***	112.33 ± 5.13^{b}***	99.45 ± 4.16^{c}***
TG (mg/dL)	95.00 ± 2.32^{d}	122.50 ± 3.14^{a}***	117.42 ± 4.52^{b}**	115.75 ± 3.41^{b}**	102.35 ± 4.90^{c}***

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significant when compared to the control groups at * P<0.05, ** P<0.01, *** P<0.001.

Intraperitoneally injection of CP to rats induced significant decreases in serum levels of total proteins (TP), albumin (Alb) and globulin (Glb), and an increase in total bilirubin (TBil) when compared with normal control rats. Administration of Artichoke leaves extract (ALE) to CP- administered rats normalized serum levels of TP, Alb, Glb and TBil when compared with the positive control group. Concurrent administration of both ALE and vitamin E produced the best effect on the abovementioned serum parameters when compared with their administration alone (Table 3).

Table (3): Effect of Artichoke leaves extract (ALE) and vitamin E on serum total protein (TP), albumin, globulin and total bilirubin (TBil) in rats with oxidative stress

Groups / Parameters	Control (-ve)	Control (+ve)	(ALE) (400mg/kg)	vitamin E (100 mg/kg)	(ALE) + vitamin E
TP (g/dL)	7.45 ± 1.04^{a}	2.88 ± 0.32^{d**}	4.75 ± 1.11^{c**}	5.88 ± 1.13^{c**}	6.85 ±1.11^{b**}
Albumin (g/dL)	2.95 ± 0.02^{a}	1.15 ± 0.07^{d**}	1.90 ± 0.05^{c*}	2.05 ± 0.03^{c*}	2.40 ± 0.06^{b**}
Globulin (g/dL)	3.90 ± 0.02^{a}	1.90 ± 0.04^{d**}	2.70 ± 0.02^{c*}	2.85 ± 0.01^{c*}	3.60 ± 0.01^{b**}
TBil (mg/dL)	0.19 ± 0.02^{d}	0.63 ± 0.04^{a**}	0.40 ± 0.02^{b*}	0.35 ± 0.01^{b*}	0.22 ± 0.01^{c**}

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significant when compared to the control groups at * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Rats inflicted with oxidative stress induced by cyclophosphamide (CP) had significant increases in serum levels of urea nitrogen (UN) and creatinine (Cr) when compared with negative control rats. Oral administration of Artichoke leaves extract (ALE) to CP- intoxicated rats for 6 weeks induced significant decreases in the high serum UN and Cr. levels when compared with the positive control group. No significant changes in serum uric acid levels were reported between the control and treated groups. Coadministration of ALE and vitamin E produced the best lowering effect on serum UN and Cr levels when compared with their administration alone (Table 4).

Table (4): Effect of ALE and vitamin E on urea nitrogen, uric acid and creatinine concentrations in rats with oxidative stress.

Groups / Parameters	Control (-ve)	Control (+ve)	(ALE) (400mg/kg)	vitamin E (100 mg/kg)	(ALE) + vitamin E
Urea nitrogen (mg/dL)	31.00 ± 2.35^{d}	59.56 ± 5.14^{a}***	41.65 ± 3.11^{b}**	39.88 ± 3.13^{b}**	33.14 ± 2.11^{c}***
Uric acid (mg/dL)	1.35 ± 0.02^{a}	1.36 ± 0.04^{a}	1.33 ± 0.02^{a}	1.35 ± 0.03^{a}	1.34 ± 0.03^{a}
Creatinine (mg/dL)	0.57 ± 0.01^{d}	0.99 ± 0.04^{a}**	0.68 ± 0.02 ^{c}**	0.71 ± 0.01^{c}**	0.86 ± 0.01^{b}**

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significant when compared to the control groups at * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Intraperitoneal injection of cyclophosphamide to rats in a single daily dose of 50 mg/kg BW for 3 days increased serum level of lipid peroxide malondialdehyde (MDA) and decreased reduced glutathione (GSH) level when compared with negative control rats. Artichoke leaves extract (ALE) when given orally to rats inflicted with oxidative stress induced a significant decrease in MDA and increase in GSH serum levels when compared with the positive control group. Concomitant administration of ALE and vitamin E exhibited the best effect on MDA and GSH serum levels when compared with their administration alone as recorded in Table (5).

Table (5): Effect of ALE and vitamin E on serum malondialdehyde (MDA) and reduced glutathione (GSH) in rats with oxidative stress

Groups / Parameters	Control (-ve)	Control (+ve)	(ALE) (400mg/kg)	Vitamin E (100 mg/kg)	(ALE) + Vitamin E
MDA (mmol/ml)	33.50 ± 2.11^{d}	52.43 ± 4.21^{a}***	42.54 ± 2.23^{b}**	40.43 ± 2.66^{b}**	36.55 ± 2.28^{c}***
GSH (mmol/ml)	75.36 ± 4.71^{b}	49.11 ± 3.91^{d}***	60.21 ± 4.11^{c}**	62.31 ± 3.18^{b}**	72.31 ± 3.12^{a}***

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significant when compared to the control groups at * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Rats with oxidative stress induced by cyclophosphamide had significantly higher in hepatic MDA levels and lower GSH levels when compared with the negative control group. Oral administration of Artichoke leaves extract (ALE) significantly reduced hepatic MDA levels and increased GSH levels when compared with the positive control group. Concurrent administration of ALE with vitamin E amplified the effect on hepatic MDA GSH levels and as compared to their administration alone (Table 6).

Table (6): Effect of ALE and vitamin E on liver malondialdehyde (MDA) and reduced glutathione (GSH) in rats with oxidative stress

Groups / Parameters	Control (-ve)	Control (+ve)	(ALE) (400mg/kg)	Vitamin E (100 mg/kg)	(ALE) + Vitamin E
MDA (nmol/min /mg protein)	0.32 ± 0.003^{d}	0.78 ± 0.002^{a}***	0.45 ± 0.001^{b}**	0.42 ± 0.003^{b}**	0.37 ± 0.002^{c}***
GSH (nmol/min /mg protein)	24.1 ± 2.11^{a}	12.6 ± 4.15^{d}***	19.22 ± 3.31^{c}**	20.19 ± 2.16^{c}**	23.17 ± 3.18^{b}***

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significant when compared to the control groups at * P<0.05, ** P<0.01, *** P<0.001

Regarding the activity of antioxidant enzymes, the rats inflicted with oxidative stress by cyclophosphamide had significant decreases in activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes in liver tissues when compared with the negative control group. Oral administration of Artichoke leaves extract (ALE) caused significant increases in the activity of hepatic GPx, SOD, CAT antioxidant enzymes when compared with the positive control group. Concurrent administration of ALE with vitamin E showed the best effect on the activity of hepatic antioxidant enzymes when compared with their administration alone as depicted in Table (7).

Table (7): Effect of Artichoke leaves extract (ALE) and vitamin E on the activity of hepatic antioxidant enzymes in rats with oxidative stress

Groups / Parameters	Control (-ve)	Control (+ve)	(ALE) (200mg/kg)	vitamin E (100 mg/kg)	(ALE) + vitamin E
GPx (nmol/min /mg protein)	0.81 ± 0.002^{a}	0.26 ± 0.001^{d}***	0.58 ± 0.003^{c}**	0.60 ± 0.002^{c}**	0.78 ± 0.001^{b}***
SOD (U/mg protein)	61.73 ± 3.52^{a}	46.82 ± 3.75^{d}***	52.36 ± 4.11^{c}**	53.19 ± 4.16^{c}**	59.49 ± 4.28^{b}***
CAT (nmol/min /mg protein)	0.188 ± 0.003^{a}	0.136 ± 0.002^{d}***	0.158 ± 0.001^{c}**	0.162 ± 0.002^{c}**	0.172 ± 0.001^{b}***

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significant when compared to the control groups at * P<0.05, ** P<0.01, *** P<0.001

Histopathological examination on liver sections on normal control rats showed normal histological structure of hepatic lobule with normal hepatocytes and hepatic sinusoids (Fig.1-a). Intraperitoneal injection of cyclophosphamide (CP) to rats in a dose of 50 mg/kg/ day for 3 days induced fatty degeneration and necrosis (Fig. 1-b) associated with proliferation of fibrous connective tissue (Fig. 1-c). The microscopical examination of liver sections of rats pretreated with Artichoke leaves extract (400 mg/kg BW) showing mild fatty degeneration and necrosis (Fig. 1-d). Examination of liver of rats pretreated with vitamin E (100 mg/kg BW) showed only mild fatty degeneration and sporadic necrosis of hepatocytes (Fig. 1.e). Liver sections of rats pretreated by concurrent administration of Artichoke leaves extract and vitamin E

showed upon examination almost normal histological structure of hepatic lobule (Fig. 1-f).

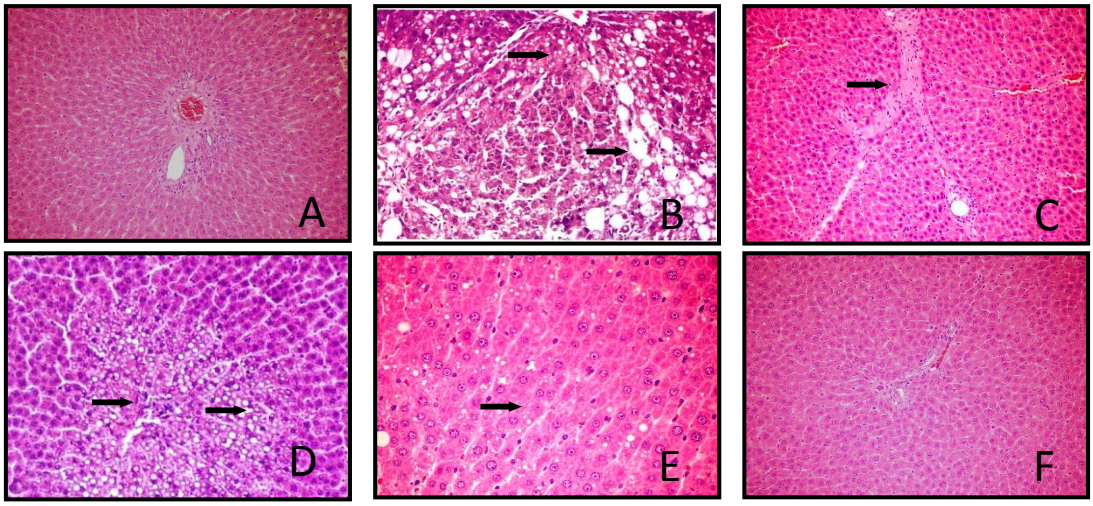

Fig. (1): Effect of ALE and vitamin E on liver histopathology in rats with oxidative stress (H&E X 200).

A) Liver section of a normal control rat showing normal architecture with normal central vein, portal tract, hepatocytes and sinusoids. (H&E X 200). **B)** Liver section of a rat injected with cyclophosphamide (+ve control) showing severe fatty degeneration (Arrow) and necrosis (Arrow), **C)** Liver section of a rat injected with cyclophosphamide (+ve control) showing proliferation of fibrous connective tissue (Arrow), **D)** Liver section of a rat pretreated with Artichoke leaves extract (400 mg/kg BW) showing mild fatty degeneration and sporadic necrosis (Arrow) of hepatocytes. **E)** Liver section of a rat pretreated with vitamin E (100 mg/kg BW) showing mild fatty degeneration (Arrow) and necrosis (Arrow), **)** Liver section of a rat pretreated with Artichoke leaves extract vitamin E showing mild almost normal histological structure of hepatic lobule.

Discussion

The present study aimed to evaluate the effect of concurrent administration of ALE and vitamin E (Vit. E) against oxidative stress induced by antitumor drug cyclophosphamide (CP) in rats**.** Cyclophosphamide (CP) is a widely used antitumor drug for the treatment of acute and chronic leukemia, breast cancer, multiple myeloma, and lymphomas (**Jain and Jain, 2012**). It was reported that intraperitoneal injection of CP to rats significantly elevated endogenous reactive

oxygen species (ROS) and induced oxidation of lipids and proteins which are biomarkers of oxidative stress (**Ince *et al.*, 2014**).

Results of the present study showed that intraperitoneal injection of CP in rats induced oxidative stress characterized by reduction in food intake and body weight gain; increases in serum biomarkers of liver and kidney function; increases in lipid peroxidation marker (MDA) and decreases in antioxidant capacity in hepatic tissue. These biochemical changes were linked with histopathological lesions (fatty degeneration and necrosis with sporadic fibrosis) seen in hepatic tissue of CP-intoxicated rats. These findings were in accordance with those reported by **Nitharwal *et al.*, (2013)** and **Ince *et al.*, (2014)**. The previous authors concluded that the toxic effects of cyclophosphamide (CP) include oxidative stress and organ toxicity such as hepatotoxicity, nephrotoxicity, and lung and heart injuries as well as genotoxicity in rats and mice. The toxic effect of CP could be attributed to generation of reactive oxygen radicals (ROS) by its metabolites which cause oxidative stress (**Nitharwal *et al.*, 2013)**. In addition, oral administration of small dose (5 mg/kg BW) of CP to male rats for a long period (6 weeks) induced testicular injury in rats (**Jalali *et al.*, 2012**).

Lipid peroxidation is a complex process that damages the cell structure and function. Peroxidation of cell membrane lipids initiates a loss of membrane integrity; membrane bound enzyme activity and cell lyses. Oxidative damage in tissues can be limited by the defense system of the host. These defenses appear to be inducible by nutrient and non nutrient antioxidants. Low levels of tissue antioxidant enzymes are likely to result in tissue damage by lipid peroxides mainly malondialdehyde (MDA) or protein carbonyls (**Prabu *et al.*, 2010**).

Results of the present study showed that oral administration of ALE to cyclophosphamide (CP) - intoxicated rats increased body weight gain, improved serum biomarkers of hepatorenal function and normalized lipid profile. ALE significantly prevented the elevation of plasma and hepatic malondialdehyde formation and increased activities of hepatic antioxidant enzymes. The increase in body weight gain of rats pretreated by Artichoke leaves extract that recorded in this study was similar to that reported by **Saénz-Rodriguez, *et al.* (2002)** who reported that rats given orally Artichoke leaves extract gained more than control rats. The previous authors attributed the increase in body weight gain to the increase in food intake and improvement in food digestion by Artichoke because

of its choleretic effect that is associated with increased bile formation.

Many medicinal plants were found to produce high antioxidant activity and prevent oxidation of lipids. Various phenolic compounds such as flavonoids, phenolic acids, diterpenes, saponins and tannins possess diverse biological activities and are thought to be beneficial for reducing cell damage induced by oxidative stress. The activity of phenolic compounds might be related to their ability to scavenge the free radicals by presence of hydroxyl groups in these compounds (**Djeridane *et al.*, 2006**).

The hepatoprotective action of ALE reported in the present study was similar to that demonstrated by **Miccadei *et al.*, (2008).** The hypocholesterolemic and hypolipidemic effects of ALE were in accordance with those reported by **Wider *et al.*, (2013)** and **Heidarian and Rafieian-Kopaei, (2013)**. These effects might due to inhibition of cholesterol via inhibiting the enzyme responsible for its synthesis or through inhibition of intestinal absorption of cholesterol by the high fiber content of ALE. The antioxidant effect of ALE, reported in this study, agreed with that previously reported by **Magielse *et al.*, (2114)**. The hepatoprotective and nephroprotective effects of ALE could be attributed to the high antioxidant protection activity of bioactive constituents of Artichoke leaves. The present results also showed that the antioxidant effect of ALE was amplified by its concomitant administration with vitamin E. This could be attributed to the addition effect of both Artichoke leaves extract and the antioxidant vitamin E.

In conclusion, the present results denote that Artichoke leaves extract has hepatoprotective, nephroprotective, hypolipidemic and antioxidant properties in cyclophosphamide-intoxicated rats. These effects are amplified by concurrent administration of Artichoke leaves extract with vitamin E. The study recommends that intake of edible parts of Artichoke leaves in food or as an herbal tea together with vitamin E as a food supplement may be beneficial for patients suffering from liver and kidney diseases due to oxidative stress Moreover, the use of artichoke leaf extract for the prevention of arteriosclerosis can be expected owing to it lipid lowering property. Isolation of bioactive constituents of Artichoke leaves is necessary to search for safe natural agents to be developed for therapy instead of chemically synthesized drugs which are usually associated with deleterious side effects.

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دراسة التأثير الوقائي للمستخلص الإيثانولي لأوراق الخرشوف وفيتامين هـ ضد الإجهاد التأكسدي في ذكور الفئران

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المستخلص

استهدف هذا البحث دراسة التأثير الواقى لخلاصة أوراق الخرشوف وفيتامين هـ منفردين ومتلازمين معا ضد الإجهاد التأكسدى الناجم عن إعطاء دواء سيكلوفوسفاميد (دواء مضاد للسرطان) فى ذكور الفئران. واستخدم فى الدراسة خمس مجموعات من ذكورالفئران كل منها تسعة فئران. كانت المجموعة الأولى ضابطة سالبة (فئران غيرمصابة)، وتم حقن فئران المجموعات الأربعة الاخرى فى التجويف البريتونى بجرعة مفردة هى 50 مجم /كجم من دواء سيكلوفوسفاميد يوميا لمدة 3 أيام لإحداث الإجهاد التأكسدى. وظلت المجموعة الثانية ضابطة موجبة (فئران مصابة وغيرمعالجة). وتم إعطاء فئران المجموعات الثالثة والرابعة والخامسة خلاصة أوراق الخرشوف بجرعة 400مجم/كجم أو فيتامين هـ بجرعة 100 مجم /كجم أوالخلاصة وفيتامين هـ متلازمين معا على التوالى، عن طريق الفم يوميا لمدة ستة أسابيع. وتم وزن الفئران فى بداية ونهاية التجربة وحساب وزن الجسم المكتسب. وفى نهاية فترة التجربة تم سحب عينات من الدم لفصل المصل لاستخدامه فى التحليلات البيوكيميائية. وتم أخذ الكبد لإجراء التحليلات البيوكيميائية فى نسيج الكبد، وكذلك تم الفحص الهستوباثولوجى للكبد. وأوضحت النتائج أن إعطاء خلاصة أوراق الخرشوف عن طريق الفم للفئران المصابة بالاجهاد التأكسدى أدى إلى زيادة وزن الجسم المكتسب. كما أدى إلى نقص معنوى فى المستويات المرتفعة من إنزيمات الكبد (اسبرتات امينو ترانسفيريز، الانين امينو ترانسفيريز والكالين فوسفاتيز) وتركيزات البليروبين الكلى ، اليوريا، الكرياتنين، المالونديالدهيد، الكوليسترول الكلى والجلسريدات الثلاثية بينما أدى الى زيادة بروتينات الدم وتركير الجلوتاثيون المختزل مقارنة بالمجموعة الضابطة الموجبة. و أدى ايضا إلى نقص أكسدة الدهون وارتفاع مستوي الإنزيمات المضادة للأكسدة بأنسجة الكبد. وأظهر الفحص الهستوباثولوجى للكبد اختفاء التغيرات الهستوباثولوجية التى سببها دواء سيكلوفوسفاميد بنسيج الكبد. وأظهرت النتائج أن الإعطاء المتلازم لخلاصة أوراق الخرشوف مع فيتامين هـ كان هو الأفضل فى

احداث التغيرات البيوكيميائية والهستوباثولوجية السابقة . وتدل هذه النتائج أن الأعطاء المتلازم لخلاصة أوراق الخرشوف مع فيتامين ه كان له تأثير واقى للكبد وواقى للكلى ومخفض لدهون الدم ومضاد للأكسدة فى الفئران المصابة بالإجهاد التأكسدى الناجم عن اعطاء دواء سيكلوفوسفاميد. ولذا توصى الدراسة أن تناول خلاصة أورق الخرشوف فى الغذاء أو كمشروب عشبى مع فيتامين ه كمكمل غذائى قد يكون مفيدا للمرضى الذين يعانون أمراض الكبد والكلى الناتجة عن الإجهاد التأكسدى .وقد يكون مفيدا ايضا فى الوقاية من تصلب الشرايين بسبب تأثيره المخفض لدهون الدم. وأن عزل المواد الفعالة من أورق الخرشوف ضرورى للبحث عن مواد طبيعية آمنة تستخدم كمضدات للأكسدة فعالة فى علاج أمراض الكبد والكلى الناجمة عن الاجهاد التأكسدى بدلا من الأدوية الكيميائية المستخدمة والتى غالبا ما يصاحبها أعراض جانبية ضارة.

الكلمات المفتاحية : الخرشوف، وفيتامين ه، سيكلوفوسفاميد، وظائف الكبد والكلى، صورة دهون الدم، مضاد الأكسدة، هستوباثولوجى.