Hepatoprotective and Antioxidant Effects of Artichoke against Carbon Tetrachloride- Toxicity in Rats

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Abstract:Background and Objectives: The present study aimed to evaluate the Hepatoprotective and antioxidant effects of Artichoke (*Cynara scolymus*) against carbon tetrachloride (CCl_4) induced hepatocellular damage in rats .**Material and Methods:** Sixty rats were divided into 6 groups. Group I: control (control –ve). Group II: treated orally with CCl_4 (control +ve) at a dose 1ml/kg body weight (BW) twice weekly for 4 weeks. Groups III & IV orally treated with different doses 100 & 200 mg/kg BW/day of artichoke powder respectively. Groups V& VI treated orally with artichoke powder 100 & 200 mg/kg BW/day respectively plus CCl_4 1ml/kg body weight (BW) twice a week for 4 weeks. **Results:** The treatment with Artichoke significantly increased RBCs count, PCV, and hemoglobin in (Gp V&VI) comparing with CCl_4 treated group (Gp.II). Also artichoke treatment significantly decreased ALT, AST & ALP and improved the reduced levels of total protein and total antioxidant capacity (T-AOC) in the serum; meanwhile, the reduced levels of SOD, glutathione and GPx were markedly increased and the MDA and NO formation was significantly inhibited in liver tissue. **Conclusions**: Overall results proved the hepatoprotective action of Artichoke, which is related to its antioxidant activity and support the use of Artichoke as a hepatoprotective and antioxidant agent .

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1. Introduction

Liver is the largest and most complex internal organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse functions. (Wang *et al.,* 2008). Liver is considered the key organ in the metabolism, detoxification, and secretory functions in the body, and its disorders are numerous with no effective remedies (Luper, 1999).

Medicinal plants and their derivatives are widely used all over the world as medicinal, salutistic or functional food (Block *et al.,* 1992). Some medicinal plants are promising natural source of hepatoprotective and antioxidant compounds and are valuable in the treatment of liver and gallbladder disorders and in the protection against poisoning from chemical and environmental toxins (Muriel and Mourelle, 1990). Artichoke (*Cynara scolymus* L.) is one of the world's oldest medicinal plants. It is an important crop of ancient Greece, grows in Egypt, Mediterranean area and other countries .It is belonging to the family (*Asteraceae*). It has medical properties and used in traditional folk medicine. It is a good source of natural antioxidants such as vitamin C , hydroxycinnamic acids (Jimenez *et al.,* 2003) and caffeoylquinic acid derivatives (cynarin and chlorogenic acid) (Joy and Haber, 2007). Artichoke is rich in flavonoids (luteolin, apigenin) which it's potential protective effect as antioxidant have been demonstrated for the extracts of this vegetable in reducing reactive oxygen species from stimulated human neutrophil and in protection of hepatocyte from t-butyl hydrogen peroxide induced cytotoxicity (Gebhardt, 1997). The artichoke extracts were assessed for their protective role in the control of oxidative damage to biological molecules (proteins, lipids and DNA), caused by free radicals such as RCOO and/or OH, using the b-carotene/linoleate assay, the deoxyribose assay and the metmyoglobin assay. Artichoke by-products are rich in phenolic compounds, especially chlorogenic acid and 1,5-O-dicaffeoylquinic, 3,5-O-dicaffeoylquinic and 3,4-O-dicaffeoylquinic acids. When the biological activity of artichoke extracts is considered, the presence of luteolin-7- glucoside and hydrolysable tannins, besides caffeoylquinic derivatives, in the phenolic fraction of these extracts must be taken into account: all these phenolics possess a good antioxidant activity against peroxyl and hydroxyl radicals when assessed using the b-carotene/linoleate assay and the metmyoglobin assay (Lattanzio et *al.,* 1994). Artichoke leaf extracts (ALE) is known to have antioxidant potential. Several *in vitro* studies have shown that the antioxidant potential of ALE is dependent on radical scavenging and metal ion chelating effect of its constituents such ascynarin, chlorogenic acid and flavonoids (Brown and Rice-Evans, 1998; Perez-Garcia *et al.,* 2000). Pure constituents of ALE have also been shown to produce less inhibitory activity on free radical production than the extract itself (Gebhardt, 1997). Moreover the

artichoke reported to have hypoglycemic effect (Kim *et al.,* 2003), hypocholesterolemic, antifungal, immunomodulatory, antimicrobial and anticarcinogenic (Ghada and Tamer, 2009).

Carbon tetrachloride (CCl_4) is a widely used as a free radical generator to induce experimentally liver injury. While acute CCl_4 application results in hepatitis, chronic CCl_4 application has been shown to cause liver cirrhosis Oxidative stress has been reported to play an important role in the pathogenesis of liver injury due to CCl_4 application (Natarajan *et al.,* 2006). CCl_4characterized to be bioactivated by cytochrome P450 (CYP450), causes liver damage following the cleavage by CYP450 to form the trichloromethyl free radical. This radical quickly adds molecular oxygen to form the trichloromethyl peroxyl radical , that initiate lipid peroxidation and protein oxidation (Lin *et al.,* 1998 and Alalmalki ,2010).

This study aimed to study, the protective effect of artichoke in rats intoxicated with CCl4, by evaluation its antioxidant activities, some selective biochemical parameters as well as the hematological picture.

2. Material and Methods

2.1. Experimental animal

Sixty male albino rats of 1-2 month old (average body weight 140-150 gm) were obtained from Helwan farm of laboratory Animals (Ministry of Public health) . The animals were acclimatized under standard laboratory conditions for 2weeks prior to dosing .They had free access to standard diet and water *ad-libitum* .They were maintained under standard condition of temperature(30ºC) with an alternating 12h light / dark cycles .All the experimental studies were conducted inconformity with the guidance for care and standard experimental animals of our collage ethical protocols.

2.2. Chemicals

Artichoke powder (dried extract) manufactured by Western Pharmaceutical Industries for International Medicinal Guide under name super artichoke with Reg.No.:779/2008. EDTA, phosphate-buffered saline (PBS), 0.9% NaCl solution for preparation of liver homogenate. Carbon tetrachloride obtained from ADWIA Company, Egypt.

2.3. Experimental design

The animals were divided into six groups, consisting of ten rats in each, and they were treated as follows**:**

Group I Normal control (Rats treated orally with Physiological saline 1ml /kg BW orally twice a week.

Group II Toxic control (Rats treated orally with 1ml/kg BW of CCl_4 through olive oil (1ml CCl4:1ml olive oil) twice a week (Huseini *et al.,* 2011).

Group III Rats were treated orally with 100 mg/kg BW of artichoke powder suspended in 2ml distilled water daily (Michel and Remscheid, 2002).

Group IV Rats treated orally with 200 mg/kg BW of artichoke powder suspended in 4ml distilled water daily (Heidarian and Soofiniya, 2011).

Group V Rats treated orally with 1 ml/kg of 1:1 carbon tetrachloride diluted in olive twice a week plus 100 mg/ kg BW of artichoke powder suspended in 2 ml distilled water daily.

Group VI Rats treated orally with 1 ml/kg of 1:1 carbon tetrachloride in olive oil twice a week plus 200 mg/kg BW of artichoke powder suspended in 4 ml distilled water daily.

2.4. Collection of blood samples

At the end of 2nd and 4th week post treatment two blood samples were withdrawn from medial canthus of the eye, the first sample in Epipendorff tubes with EDTA for hematological examination and the second blood samples were collected in clean test tubes and allowed to clot, then centrifuged for ten minutes at 3000 r.p.m. Serum was separated and stored into Epipendorff tubes at – 20°C to be used for biochemical analyses.

2.5. Measurement of hematological parameters

Whole blood used for the determination of erythrocyte count, hemoglobin content and haematocrit value according to Feldman *et al.* (2000).

2.6. Measurement of liver function markers

Serum ALT and AST were determined using diagnostic kits obtained from (Colorimetric Randox) UK according to Reitman and Frankel(1957). ALP using diagnostic kits obtained from (Teco diagnostics) USA according to Roy (1970). Total protein and albumin, were determined using diagnostic kits obtained from (Stanbio laboratory) USA according to Burtis and Ashwood (1999) and Dumas and Biggs (1972) respectively .

2.7. Measurement of kidney function parameters

Serum urea and creatinine were determined using diagnostic kits obtained from(Diamond) Egypt and (Human) Germany according to Fawcett and Soctt (1960) and Szasz *et al.*(1979), respectively.

2.8. Measurement of antioxidant and oxidative stress markers

2.8.1 Preparation of liver homogenate

At the end of 2nd and 4th week post treatment one gram of liver tissues was collected from each rat . Liver tissue was washed by ice-cold 0.9% NaCl solution and homogenized in 9ml ice-cold PBS (PH 7.5) using homogenizer instrument .The homogenate was cold centrifuged for 15 minutes at 3000 r.p.m and the supernatant was collected used directly, or stored into Epipendorff tubes and kept at – 80°C for further use (Ferdandez-Botran *et al.,* 2002).

All the antioxidant and oxidative stress markers were determined using diagnostic kits obtained from (Biodiagnostic) Egypt.

2.8.2 Measurement of liver Malondialdehyde (MDA)

MDA level was determined according the method of Satoh (1978). Briefly, 0.2mL supernatant of liver homogenate was mixed with 0.67% 2- thiobarbituric acid (TBA) and 20% trichloroacetic acid solution, and heated in a boiling water bath for 30 min. The pink colored chromogen formed by the reaction of TBA with MDA was measured spectrophotometrically at 532 nm. The results were expressed as MDA mmol/gm tissue.

2.8.3 Measurement of liver Nitric oxide(NO)

NO level in liver tissue homogenates was determined according to the method of Ignarro *et al.* (1987). The assay is based on the diazotization of sulfanilic acid with nitric oxide at acidic pH and subsequent coupling with N-(10-naphthyl)-ethylenediamine to yield an intensely pink colored product that is measured spectrophotometrically at 540 nm. The results were expressed as NO mmol/gm tissue.

2.8.4. Measurement of liver Glutathione (GSH)

Levels of GSH in liver homogenates was determined according to the method of Beutler *et al.* (1963). Briefly, the deproteinzation of liver homogenate was made by 10% trichloroacetic acid and centrifuged at 3500 rpm for 10 min. 50µl supernatant was mixed with 0.32 mol/l disodium hydrogen phosphate and 0.04% 5,5'- dithiobis 2-nitrobenzoic acid (DTNB) solution. The yellow-colored substance formed by the reaction of GSH and DTNB was measured spectrophotometrically at 412 nm. The results were expressed as GSH mmol/gm tissue.

2.8.5 Measurement of liver Glutathione peroxidase (GPx)

GPx level was determined in liver homogenate according to the method of Lawrence and Burk (1976). This method is based on measuring the oxidation of NADPH using hydrogen peroxide as the substrate. Absorbance was measured at 340 nm for 5 minutes, and an extinction coefficient of 6.22×10-3 was used for calculation. The results were expressed as mmol /gm tissue. The changes in the absorbance at 340 nm were recorded spectrophotometrically at 1-min interval for 5min.

2.8.6. Measurement of liver Superoxide dismutase (SOD)

SOD activity in liver homogenate was determined according to the method of Minami and Yoshikawa (1979). This method is based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan color development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan color development). The increase in the absorbance measured spectrophotometrically at 560 nm for 5min. The results were expressed as U/gm tissue

2.8.7. Measurement of serum total antioxidant capacity(T-AOC)

Serum T-AOC was determined diagnostic kits obtained from (Biodiagnostic) Egypt according to the method of Koracevic *et al.* (2001)**.** The determination of T-AOC is performed by the action of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H2O2).The antioxidants in the sample eliminate a certain amount of the provided H2O2.The residual H2O2 is determined calorimetrically by an enzymatic reaction with involves the conversion of 3,5,dichloro-2-hydroxy benzensulphonate to a colored product The absorbance were recorded spectrophotometrically immediately at 505 nm. The results were expressed as T-AOC mM/L.

3. Statistical analysis

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 20, USA). Means and standard error for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column show a significance (P<0.05)**.**

4. Results:

4.1. The effect of Artichoke and CCl4 on some hematological parameters

As shown in Table (1), CCl4 induced macrocytic hypochromic anemia which reflected by significantly decrease in RBCs , Hb, PCV and MCHC (p < 0.05), Meanwhile significantly increase in MCV. Treatment with artichoke reduced CCL*4* –induced anemia and resulted in significant increase in RBCs , Hb and PCV in groups (V and VI) compared by CCl4 treated group (Gp II) at the end of 4th week of the experiment.

4.2. The effect of Artichoke on CCl4 –induced hepatic damage on serum biochemical parameters

As presented in Table (2), CCl4 induced hepatic damage as reflected by significantly elevated serum ALT, AST, and ALP activities (p < 0.05) and significantly reduced serum total protein and albumin. No significant changes were observed in serum ALT, AST, and ALP after Artichoke treatment with both doses (groups III and IV) compared to control group . On the other hand, rats treated with Artichoke and CCl4 together (groups V and VI) had no significantly change after two weeks from the beginning of the experiment , meanwhile had significantly lower serum ALT , AST and ALP, and increase serum total protein and albumin levels compared by CCl4 treated group after four weeks of treatment. In spite of these

results, serum ALT , AST and ALP, in groups (V and VI) still significantly elevated compared to groups (I, III &IV). As shown in Table-2. The present study showed that administration of either CCl4 or artichoke not induced any significant changes on renal parameters as there is no significant changes in serum levels of urea and creatinine in all groups in compared to control one.

4.4. The effect of Artichoke on CCl4 –induced oxidative stress

As presented in Figs 1& 2, MDA and NO level was increased in CCl_4 treated rats ($p < 0.05$) as compared to normal control **.** Treatment with artichoke inhibited CCl_4 -induced lipid peroxidation and resulted in a significant decrease in MDA level ($p < 0.05$) in groups V and VI and NO level ($p < 0.05$) in groups VI as compared to CCl4 group alone (Gp II), Although these results, the level of MDA and NO in these groups (V and VI) still significantly elevated compared to groups III, IV and control group , after four weeks of treatment.

Treatment with artichoke alone, in groups IV resulted in significant decreased in MDA and NO level in comparison to control group after four weeks of treatment.

4.5. The effect of Artichoke on liver antioxidant markers

As demonstrated in (Fig. 3), GPx activity was reduced in CCl_4 treated rats ($p < 0.05$) as compared to normal control rats. Artichoke alone enhanced GPx activity in group IV in compare to group III and control group after four weeks of treatment and group III in compare to control group. Treatment with artichoke reduced CCl_4 -induced GPx inhibition and resulted in a significant increase in GPx activity ($p < 0.05$) in group VI as compared to group V and CCl_4 treated group a (Gp. II) but still significantly lower than groups III , group IV and control group after four weeks of treatment.

As given in (Fig.4), CCl_4 produced a depletion of hepatic GSH ($p < 0.05$) content as compared to normal control group. Artichoke alone induced a remarkable increase in its level in group IV in compare to group III and control group after four weeks of treatment and group III in compare to control group. Treatment with artichoke reduced CCl_4 -induced GSH depletion and resulted in a significant increase in GSH level ($p < 0.05$) in group VI as compared to group V and CCl_4 group a (group II) but still significantly lower than groups III , group IV and control group after four weeks of treatment.

As shown in Fig. 5, CCl_4 significant decrease in serum T-AOC ($p < 0.05$) content as compared to normal control group. Artichoke alone induced a significant increase in its level in groups III& IV in compare to control group after four weeks. . Treatment with artichoke reduced CCl_4 -induced T-AOC depletion and resulted in a significant increase in T-AOC level ($p < 0.05$) in group VI as compared to group V and CCl_4 treated group but still significantly lower than groups III , group IV and control group after four weeks of treatment.

SOD activity was reduced in CCl_4 treated rats ($p< 0.05$) as compared to normal control rats. Artichoke alone enhanced SOD activity in group IV in compare to group III and control group. Treatment with artichoke reduced CCl_4 -induced SOD inhibition and resulted in a significant increase in SOD activity ($p < 0.05$) in group VI as compared to groups II & V but still significantly lower than groups III , group IV and control group after four weeks of treatment (Fig. 6).

Table (1): Some selective hematological parameters at the end 2nd and 4th week post treatment with CCl_4 and artichoke(Mean ± SE) ($P < 0.05$).

	Group	RBCs $10^6/\mu l$	Hb gm/dL	PCV %	MCV fL	MCH Pg	MCHC %
2nd week	GroupI	7.51 ± .41[a]	14.48 ±.56[a]	47.8 ±1.49[a]	64.08 ±1.36[a]	19.38 ±.53[a]	30.31 ±.82[a]
	GroupII	7.07 ± .67[a]	13.11 ±.49[a]	45.8 ±1.49[a]	63.36 ± 1.18[a]	18.18 ±1.26[a]	29.49 ±1.45[a]
	GroupIII	7.77 ± .44[a]	14.61 ±1.15[a]	48.00 ±.70[a]	64.17 ±1.14[a]	19.54 ±.58[a]	30.6 ±1.99[a]
	GroupIV	7.9 ±.22[a]	14.86 ±1.09[a]	48.2 ±1.20[a]	64.24 ±1.81[a]	19.82 ±1.38[a]	30.90 ±2.18[a]
	GroupV	7.21 ±.49[a]	13.37 ±1.8[la]	46.4±1.29[a]	63.58 ±1.25[a]	18.45 ±1.05[a]	29.72 ±1.5[a]
	GroupVI	7.4 ±.22[a]	13.87 ±1.54[a]	47.00 ±1.0[a]	63.81 ±1.25[a]	18.78 ±1.16[a]	29.99 ±2.6[a]
4th week	GroupI	8.01±.46[a]	14.20±.83[a]	47.40±.93[a]	57.27±1.07[b]	17.80±.20[a]	31.24±1.39[a]
	GroupII	4.72±.41[c]	8.06±.43[c]	37.60±1.21[c]	81.18±1.99[a]	17.20±.58[a]	21.40±1.65[b]
	GroupIII	9.38±.51[a]	14.46±.74[a]	47.80±.80[a]	59.02±1.22[b]	15.20±.37[a]	29.21±1.75[a]
	GroupIV	9.6±.47[a]	14.54±1.24[a]	48.20±1.53[a]	55.45±1.25[b]	16.40±.60[a]	30.83±1.95[a]
	GroupV	6.48±.38[b]	10.46±.57[b]	40.6±.71[b]	66.24±1.65[b]	17.20±.37[a]	26.87±1.05[a]
	GroupVI	6.92±.27[b]	11.62±.63[b]	41.7±.68[b]	55.81±1.23[b]	15.80±.97[a]	28.51±1.24[a]

Table (2): Some selective serum biochemical parameters at the end 2nd and 4th week post treatment with CCl_4 and artichoke (Mean ± SE) ($P < 0.05$).

	Group	Total Protein gm/dL	Albumin gm/dL	Globulin gm/dL	A/G ratio	ALT U/L	AST U/L	ALP U/L	Urea mg/dL	Creatinine mg/dL
2nd week	GroupI	7.22±.33^{a}	3.21±.25^{a}	3.04±.33^{a}	.8±.23^{a}	32.06±1.03^{b}	49.37±2.2^{b}	181.47±2.38^{b}	57.4±2.78^{a}	.51±.06^{a}
	GroupII	5.54±.16^{b}	2.59±.15^{b}	2.85±.14^{b}	.89±.12^{a}	44.87±1.15^{a}	70.60±4.9^{a}	198.26±2.55^{a}	60.6±2.87^{a}	.57±.04^{a}
	GroupIII	7.09±.52^{a}	3.48±.34^{a}	3.60±.68^{a}	.96±.27^{a}	32.31±.93^{b}	46.45±1.31^{b}	181.46±1.94^{b}	59.2±3.73^{a}	.53±.03^{a}
	GroupIV	7.57±.41^{a}	3.51±.12^{a}	4.06±.47^{a}	.92±.11^{a}	32.45±.99^{b}	49.38±1.23^{b}	181.53±3.52^{b}	58.6±2.78^{a}	.52±.01^{a}
	GroupV	5.89±.24^{b}	2.39±.24^{b}	3.5±.07^{a}	.68±.08^{b}	41.62±2.03^{a}	67.26±1.78^{a}	199.68±1.47^{a}	61.8±2.58^{a}	.52±.03^{a}
	GroupVI	6.01±.25^{b}	2.41±.19^{b}	3.61±.38^{a}	.66±.12^{b}	40.38±1.51^{a}	63.46±1.33^{a}	197.30±1.63^{a}	64±2.14^{a}	.58±.02^{a}
4th week	GroupI	6.95±.47^{b}	3.91±.27^{a}	3.04±.50^{b}	1.47±.29^{a}	34.95±.82^{c}	57.47±1.49^{c}	184.6±2.29^{c}	59.6±3.58^{a}	.54±.027^{a}
	GroupII	4.95±.30^{d}	2.01±.18^{c}	2.94±.45^{b}	.68±1.48^{b}	95.68±2.03^{a}	103.12±1.58^{a}	209.4±1.5^{a}	56±.527^{a}	.55±.024^{a}
	GroupIII	8.28±.70^{a}	3.78±.18^{a}	4.5±.67^{a}	.92±.13^{ab}	35.37±1.25^{c}	54.98±2.52^{c}	184±2.86^{c}	57.4±3.88^{a}	.53±.060^{a}
	GroupIV	9.13±.3^{a}	3.87±.15^{a}	5.26±.31^{a}	.74±.05^{b}	36.75±1.19^{c}	56.02±2.84^{c}	179.6±1.32^{c}	54.2±2.92^{a}	.5±.030^{a}
	GroupV	5.13±.48^{bc}	2.91±.21^{b}	2.22±.61^{c}	1.34±.70^{a}	87.32±1.51^{b}	91.54±1.64^{b}	203±1.93^{a}	60.6±3.61^{a}	.52±.054^{a}
	GroupVI	6.33±.16^{c}	3.14±.19^{b}	3.17±.18^{b}	.99±.16^{ab}	83.45±1.44^{b}	84.93±2.14^{b}	193.6±.98^{b}	62.2±3.44^{a}	.56±.018^{a}

Table (3): Liver tissue antioxidant parameters the end 2nd and 4th week post treatment with CCl_4 and artichoke (Mean ± SE) ($P < 0.05$).

	Group	MDA mmol/g.tissue	NO mmol/g.tissue	GPX mmol/g.tissue	GSH mmol/g.tissue	T-AOC mM/L	SOD U/gm tissue
2nd week	GroupI	50.54±2.65^{b}	16.32±.63^{b}	2.22±.35^{a}	6.22±.47^{a}	.29±.024^{b}	287.51±5.09^{c}
	GroupII	65.72±2.8^{a}	21.11±.91^{a}	1.73±.15^{a}	4.05±.50^{b}	.22±.019^{c}	270.64±5.03^{e}
	GroupIII	43.48±4.09^{b}	15.98±.84^{b}	2.11±.27^{a}	6.18±.53^{a}	.33±.016^{b}	291.54±4.85^{b}
	GroupIV	49.47±1.86^{b}	14.08±.12^{c}	2.37±.14^{a}	7.32±.29^{a}	.43±.025^{a}	295.54±4.95^{a}
	GroupV	64.2±1.83^{a}	19.6±1.58^{a}	1.80±.14^{a}	4.17±.38^{b}	.17±.023^{c}	272.45±5.01^{e}
	GroupVI	60.44±1.41^{a}	19.87±1.15^{a}	2.17±.15^{a}	4.4±.49^{b}	.22±.032^{c}	280.6±5.02^{d}
4th week	GroupI	49.22±3.85^{c}	18.85±.41^{c}	2.28±.08^{c}	7.65±.41^{c}	.34±.019^{c}	288.11±4.93^{c}
	GroupII	80.81±2.31^{a}	31.79±1.01^{a}	1.03±.05^{e}	2.87±.31^{e}	.12±.017^{e}	242.06±4.88^{f}
	GroupIII	42.56±3.45^{cd}	18.74±.62^{c}	3.11±.29^{b}	8.81±.21^{b}	.56±.035^{b}	301.13±.499^{b}
	GroupIV	37.88±1.95^{d}	16.55±.81^{d}	4.63±.61^{a}	10.33±.33^{a}	.74±.023^{a}	311.84±.498^{a}
	GroupV	68.03±4.53^{b}	30.31±.68^{a}	1.42±.8^{ed}	3.34±.46^{e}	.19±.024^{ed}	264.03±5.04^{d}
	GroupVI	62.26±1.61^{b}	27.47±.62^{b}	1.89±.26^{d}	4.93±.27^{d}	.26±.037^{d}	273.37±5.03^{e}

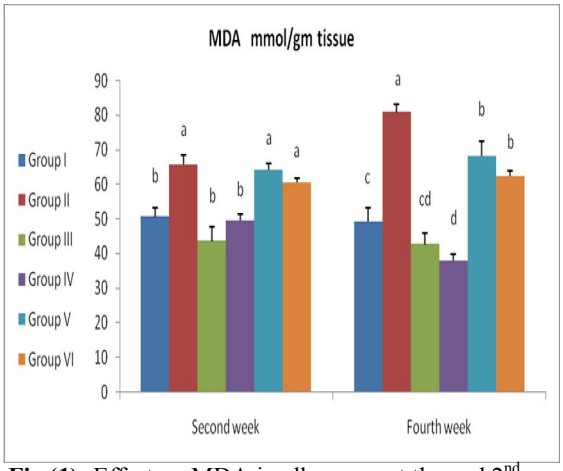

Fig (1): Effect on MDA in all groups at the end 2nd and 4th week post treatment (Mean ± SE) .

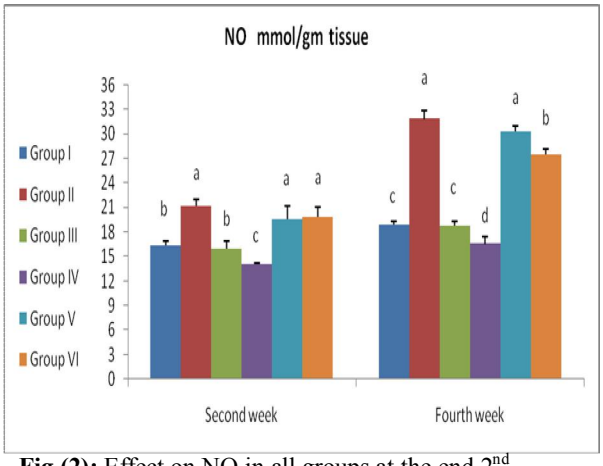

Fig (2): Effect on NO in all groups at the end 2nd and 4th week post treatment (Mean ± SE) .

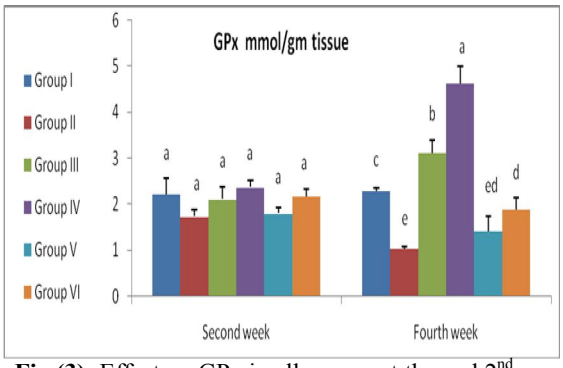

Fig (3): Effect on GPx in all groups at the end 2nd and 4th week post treatment (Mean ± SE) .

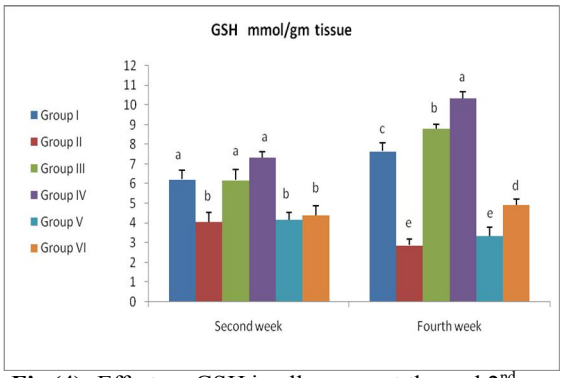

Fig (4): Effect on GSH in all groups at the end 2nd and 4th week post treatment (Mean ± SE).

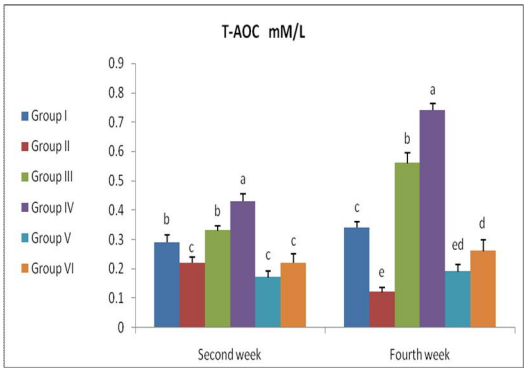

Fig (5): Effect on T-AOC in all groups at the end 2nd and 4th week post treatment (Mean ± SE) .

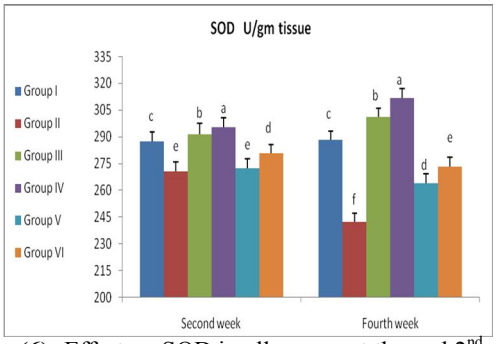

Fig (6): Effect on SOD in all groups at the end 2nd and 4th week post treatment (Mean ± SE) .

5. Discussion

The present study addresses the hepatoprotective and antioxidant effect of artichoke treatment in CCl_4 induced experimental liver injury. In liver CCl_4 is biotransformed by cytochrome P450 to produce its active metabolite trichloromethyl free radical (CCl3*) , which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide which intern gives toxic aldehyde that causes damage to liver (Kotsanis and Metcalfe, 1991). Secondary mechanisms for carbon tetrachloride metabolism that The possible involvement of toxic intermediates radical species are such as trichloromethyl (CCl3*), trichloromethylperoxy (OOCCl3*) and chlorine (Cl*) free radicals as well as phosgene and aldehydic products of lipid peroxidation. This radical can also react with oxygen to form a highly reactive specie, trichloromethylperoxy radical (CCl3OO*). CCl3OO* initiates the chain reaction of lipid peroxidation which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. This affects the permeability of mitochondrial, endoplasmic reticulum and plasma membranes resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage represented by hepatocellular necrosis, fatty vacuole and microvesicular fatty changes (Koneri *et al.,* 2008).

In the present study, oral administration of CCl_4 greatly affected all hematological parameters, as decrease in RBCs count, Hb, PCV (Mandal *et al.,* 1998). The depression in RBCs count and Hb content recorded in the present work could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation (Tung *et al.,* 1975). Moreover, administration of CCl_4 induced macrocytic hypochromic anemia as CCl_4 caused a significant increase lipid peroxidation , degradation of membrane proteins, alterations of membrane-bound enzymes as well as erythrocyte osmotic fragility (Makni *et al.,* 2012). The destruction of red cells may be assumed that the free radicals resulting from CCl_4 metabolism caused liver injury and a proportion of these free radicals librated from the liver into the blood (Sherlock and Dooley, 1993). Artichoke treatment alone ,actually have no significant effect on all hematological parameters (Michel and Remscheid, 2002).The protective effect of artichoke in CCl_4 treated group may be due to its role to decrease the production of reactive oxygen species (Perez-Garcia *et al.,* 2000), the oxidation of low-density, lipid peroxidation and protein oxidation (Jimenez-Escrig *et al.,* 2003).

As presented in Table (2), CCl_4-induced hepatotoxicity characterized by significant increases in serum ALT, AST, and ALP activities as CCl_4 lead to increase permeability of cell membranes and the enzymes were released and their activities were considered as classical indicators of liver or hepatocytes injury (Recknagel *et al.,* 1989). The antioxidant properties of caffeoylquinic acids present in artichoke leaf extracts are considered to be mainly responsible for the hepatoprotective action (Speroni *et al.,* 2003) Treatment of rats with artichoke significantly reduced the activities of ALT, AST, and ALP activities . Our results were consistent with previous reports that artichoke prevented the enzymes release from rat hepatocytes induced by CCl4 (Huseini *et al.,* 2011).

Albumin synthesis is extremely sensitive to CCl_4 probably secondary to the alterations in the cytoplasmic protein-synthesizing system (Rui *et al.,* 2012). The liver loss its ability to synthesize albumin, as albumin is produced on a polysome bound to the endoplasmic reticulum (Kaneko, 2008). The significant depression of serum albumin levels due to CCl_4 intoxication was possibly associated with the apparent disassociation of albumin protein into smaller subunits (Folmar *et al.,* 1993). In the present study, CCl_4 caused reduction in serum total protein and albumin and treatment with Artichoke significantly increased the reduced levels of serum total protein and albumin which was in agreement with previous study (Abd El-Aleem *et al.,* 2009).

Oxidative stress is defined as an imbalance between local ROS production (chiefly superoxide anions, hydrogen peroxide, hydroxyl radicals) and ant oxidative mechanisms (Frei, 1994). The protective effect of flavonoids against oxidative injury may depend not only on their antioxidant activity, but also on their affinity to and permeability through plasma membranes.

Moreover, Lipid peroxidation is a chemical mechanism capable of disrupting the structure and the function of the biological membranes that occurs as a result of free radical attack on lipids, which was usually reflected by levels of MDA (Schinella *et al.,* 2002; Abdel-Wahhab and Ahmed, 2004).

In this study, MDA levels in the liver of CCl_4-treated rats were significantly elevated, indicating increased lipid peroxidation and oxidative stress in liver. Treatment with Artichoke significantly suppressed the elevation of MDA caused by CCl_4 at the dose of (100 and 200mg/kg BW). The elevated level of NO in CCl_4-treated group could be attributed to lipid peroxidation that stimulates host cells, mainly monocytes/ macrophages, to produce and release NO by induction of inducible nitric oxide synthase (iNOS) protein, resulting in cytotoxicity and DNA damage, therefore NO levels in the liver of CCl_4-treated rats were significantly elevated (Raso *et al.,* 2001). The elevation of MDA and NO level in liver caused by CCl_4 has also been reported to be suppressed by Artichoke in rats (Heidarian and Soofiniya., 2011). These results suggest that Artichoke may play an important role in hepatic cellular protection against oxidative stress-induced injury by free radicals.

The coordinate actions of various cellular antioxidants in mammalian cells are critical for the effective detoxification of free radicals (Yang et al., 2010). According to Waters et al.,(2001) in vitro and in vivo studies, several classical antioxidants (SOD, GSH, GPx ,and T-AOC have been shown to protect hepatocytes against lipid peroxidation or inflammation, therefore preventing the occurrence of hepatic necrosis. SOD and GSH are major antioxidants, which involve in defense mechanism against lipid peroxidation in biological system and convert active oxygen molecules into non-toxic compounds (Rui *et al.,* 2012). A reduction in SOD and GSH is associated with the accumulation of high-living free radical, leading to injury of cell function (Okamoto and Colepicolo, 1998).

In the present study, treatment of the rats with CCl_4 decreased the antioxidant capacity of liver as evidenced by the decreased GSH level and activities of SOD, GPx and T-AOC. Artichoke treatment prevented the reduction in GSH, SOD, GPx and T-AOC and the consequent oxidative damage to the liver. The antioxidant effects of artichoke have also been reported in rat (Mehmetcik *et al.,* 2008: Abd El-Aleem *et al.,* 2009).

6. Conclusion:

We concluded that: the imbalance between production of oxygen free radicals and the endogenous antioxidant defense system, as a result of the effect of CCl_4, is the main mechanism responsible for peroxide accumulation and hepatotoxicity. Artichoke reduces the oxidative stress through the inhibition of lipid peroxidation and increasing of the activity of cellular antioxidant enzymes. Base on this study artichoke at these doses are safe and effective antioxidant agent.

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