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Identification of Polyphenolic Compounds and Hepatoprotective Activity of Artichoke (*Cynara Scolymus L.*) Edible Part Extracts in Rats

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CHEMICAL Analysis of edible parts (fleshy receptacle, inner bracts) of artichoke (*Cynara scolymus* L., Asteraceae) are identified by using high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI-MS) method, the overall polyphenolic constituents demonstrated profiling flavonoids as, luteolin, luteolin-*O*-glycoside and luteolin-7-*O*-rutinoside , apigenin. The hydroxycinnamic derivatives detected in this work belong to mono- and di-caffeoylquinic acid compounds assigned as quinic acid and chlorogenoquinone, chlorogenic acid and neochlorogenic acids, gallic acid , dicaffeoylquinic acid , vanillic acid , syringic acid , *p*-coumaric acid , caffeic acid , cynarin , ferulic acid , tannic acid were investigated.

This study aimed to elucidate the synergistic effect and the role of artichoke extract (receptacle and bracts)for treatment of Hepatocellular carcinoma (HCC).The hepatocarcinogenesis was induced by Thioacetamide (TAA) the results showed that TAA caused liver damage as proved by significant increase in Liver enzyme markers serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase along with total protein (TP) levels activities , The levels of total bilirubin (T-Bil) activity showed significant decrease in serum content of bilirubin and marked significant increase in albumin content. Also, induced oxidative stress as pointed out an increase in Metalloproteinase (MMP-3, MMP-9 and MMP-12) activities compared with the control values. Treatment with artichoke significantly reduced the elevation in liver enzymes and oxidative stress; it also induced apoptosis by inhibition of metalloproteinase compared to HCC group.

Keywords: Artichoke (*Cynara scolymus* L.), HPLC-ESI/Mass , flavonoids, phenolic contents, hepatocellular carcinoma (HCC); Metalloproteinase.

Introduction

The globe artichoke (*Cynara cardunculus* var. scolymus), family Asteraceae **[1]** known also by French as artichoke and green artichoke in the U.S. there is a variety of a species of thistle cultivated as a food. The edible portion of the plant consists of the flower buds before the flowers come into bloom. The budding artichoke flowerhead is a cluster of many budding small flowers (an inflorescence), together with many bracts, on an edible base.

The artichoke is used as a hepatoprotective, anticarcinogenic, antioxidative, antibacterial, anti-HIV and hypocholesterolemia agent. It exhibits

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a wide range of pharmacological effects, which are provided by the synergistic roles of existing of phenolic compounds **[2].** Artichoke hearts are mostly used in the canned food industry and the remaining parts, such as the outer bracts and leaves, are referred to as byproducts (approximately 60–80% of the total plant) are used to produce herbal food supplements, dietary fiber and animal feed. The outer leaves of the artichoke could be used as a natural additive due to their rich phenolic content, which has antimicrobial and antioxidant effects **[3] .** Recent studies have demonstrated that artichoke has high medicinal benefits. It includes polyphenols such as cynarin, caffeoylquinic, chlorogenic acid, flavonoids such as luteolin or its glycosides **[4].** Other constituents are also reported such as sesquiterpenes (grosheimin, cyanoropicrin), saponins, fatty acids and others. *Cynara scolymus* receptacles contain the highest amount of chlorogenic acid. The total phenolic content of leaves of green globe was in the range of 8.76–9.56% (as chlorogenic acid) **[5]** . The *C. scolymus* bracts was reported to contain 8.82 % as chlorogenic acid **.** The edible portions of globe artichoke (receptacle and bracts) are rich in cynarin more than the leaves or roots **[6]** .They reported that the two compounds 1,3-di-*O*-caffeoylquinic acid (cynarin) and 1,5-di-*O*-caffeoylquinic acid are the major active compounds in edible portions of globe artichoke .The caffeoylquinic acid has potential health benefits as natural antioxidant which is responsible for the hepatoprotective action.

Hepatocellular carcinoma (HCC) is the 5th most common cancer worldwide and the third leading cause of cancer mortality. HCC is defined as a primary tumorigenesis in the liver, mainly in patients suffering from chronic liver cirrhosis or hepatitis B or C. **[7]**

The tumor gradually spreads to hepatocytes and in advanced stages metastasis to other organs, such as lungs and brain. HCC has become one of the very common cancers causing death, affecting more than 500,000 people in the world **[8].** The other main risk factors for HCC are alcohol and aflatoxin **[9].**

The leading cause of liver cancer is cirrhosis due to hepatitis B, hepatitis C, or alcohol. Other causes include aflatoxin, non-alcoholic fatty liver disease, and liver flukes. The most common types are hepatocellular carcinoma (HCC), which makes up 80% of cases, and cholangiocarcinoma. Less common types include mucinous cystic neoplasm and intraductal papillary biliary neoplasm **[10].**

In the light of this study which was carried out to elucidate the effective role of artichoke extract on HCC linked adverse effects, and to evaluate its activity as a chemosensitizer for HCC treatment.

Material and Method

Plant Material

The samples of (*C.scolymus* L., Asteraceae), were collected from the local market May 2017 and identified by Prof. Abd El-Halim A. Mohamed, (Desert Research Institute), the plant was hand cut into small pieces and dried at 40-45^{o} C, in an electric oven then grinded to produce 640 grams. The powder was defatted using a Soxhlet apparatus with petroleum ether (60-80). The defatted residue of plant was extracted exhaustively with ethyl acetate then by (5 × 3 liter) with methanol till exhaustion. Solvent in each case was evaporated under reduced pressure and residues obtained were kept for the study.

Chromatographic Analysis:

All standards used in the experiments were accurately weighed, dissolved in HPLC methanol, treated with ultrasonic for 15 min .The calibration curves were generated with concentrations of *p*-coumaric acid (*p*-hydroxycinnamic acid), chlorogenic acid (5-*O*-caffeoylquinic acid), ferulic acid, gallic acid, epicatechin, apigenin, luteolin, cynarin (1-3-dicaffeolylquinic acid), and rutin **[11]**. The extracts of the globe artichoke (*C.scolymus*) which previously dissolved in HPLC methanol) were filtered before analysis. The analysis of polyphenols was carried by using Zorbax Eclipse Plus C_{18} column (100 mm × 2.1 mm) **.** Chemical compounds were analyzed via reversed phase HPLC using a binary gradient consisting of solvent A: methanol and solvent B: 0.3% aqueous formic acid. A linear gradient from 8% (A) (0 min) to 48% (A) for (35 min) at a flow rate of 1 ml/min was applied, at temperature 30°C. Agilent triple quadrupole mass spectrometer equipped with a quaternary pump with anon line degasser, a thermostatic column compartment, a photo-diode array detector (DAD), an auto sampler. Mass spectra were acquired in positive ion mode at a voltage of 70 V. For all spectra manual baseline subtraction was performed. This analysis was kindly performed at institute for Environmental Studies and Research Zagazig University.

Materials for biological study

Animal management

Sixty adult male albino rats, weighting 80 -120 grams, were obtained from the Experimental Animal Care Center and were kept in metabolic cages at the experimental animal house of the faculty of Science, Zagazig University under regulated environmental conditions (25°C and a 12 h light/dark cycle) 7 days before starting the experiment. The animals were nourished on a standard diet and tap water.

Hepatocarcinogenesis model

The hepatocarcinogenesis was induced by Thioacetamide (TAA) which was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Artichoke dose selection and treatment

Outer part extract (extract 1): was taken orally in a dose of 1.5 g/kg body weight daily for 2months. Editable part extract (extract 2): was administrated orally in a dose of 1.5 g/kg daily for 2 months

Experimental Design

To accomplish the goal of this study, after the acclimatization period of 7 days with standard basal diet, a total of sixty adult male albino rats were classified into six groups with 10 animals in each group.

Group I (Negative control): Animals were given an intraperitoneal injection with 1 ml saline single dose.

Group II (Thioacetamide, TAA): Animals were injected intraperitoneally with 400 mg/kg body weight of TAA as a single dose for 3 months to induce the carcinogenic effect.

Group III : Drug1control group, outer part extract (extract I) was administrated orally with a dose of 1.5 g/kg daily for 2 months

Group IV: Drug II control group, editable part extract (extract II). was administrated orally with a dose of 1.5 g/kg daily for 2 months

Group V: (Preventive group) pre-treated with artichoke (outer part extract) for 4 weeks before HCC induction).

Group VI: (Therapeutic group) Animals were induced for HCC (as group 2). After the induction of HCC by TAA (after 4 weeks), animals were post treated with methanol extract of artichoke (edible extract) (1.5 mg/kg daily to the end of experiment

Animal and Experimental Design

Adult male albino rats, weighting 80 - 120 g, were obtained from Egyptian Organization for Biological Products and Vaccines (Agouza, Giza, Egypt) and were kept in metabolic cages under regulated environmental conditions (25°C and a 12 h light/dark cycle) 7 days before starting the experiment. The animals were nourished on a standard diet and tap water

Biochemical analysis

Estimation of serum albumin was performed according to modified Detection principle Bromocresol green (BCG) colorimetric method **[12]**. The serum activities of liver enzyme markers serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by colorimetric method of Reitman. et.al., **[13],** while the activity of Alkaline phosphatase (ALP) was performed according to the method of Belfield et.al., **[14].**

Estimation of Serum α-Fetoprotein (AFP) Concentration:

Serum α-Fetoprotein (AFP) was determined by Rat/Mouse, Immunoassay Kit, by enzyme – linked immunosorbent assay (ELISA), cloud clone corp. Company (USA).

Estimation of Metalloproteinase, (MMP-3), (MMP-9), (MMP-12)

All type of Metalloproteinase were determined by Rat/Mouse, Immunoassay Kit, by enzyme linked immunosorbent assay (ELISA), cloud clone corp. Company (USA).

Statistical analysis

The results of biochemical analysis were expressed as mean ± standard error (SE). Statistical analysis was conducted using one way analysis of variance (ANOVA). The level of statistical significance was taken at $p < 0.05$.

Experimental Section

General section:

Evaporation of the solvents by rotary evaporator, UV lamp for TLC visualization (λ_{max}254 and 366 nm). UV spectral analysis, Shimadzu UV1700 spectrophotometer (Japan). Paper chromatography was carried out on Whatman paper No.1 and preparative chromatography at 3MM, using solvent system AcOH15 % and n-butanol: acetic acid: water, (BAW, 4:1:5 upper layer)

Biochemical investigation

Collection and sampling of blood

At the final of experimental period, the animals were fasted for 12 hours, anesthetized with ether, then they were killed by cervical decapitation and blood samples were gathered in centrifuge tubes for separating the serum. The serum was prepared by collection of blood in anticoagulant–free tube, then left for 10 minutes in water bath at 37ºC until clot, then centrifuged at 2000 rpm for 10 minutes for setting apart of serum which was transferred into Eppendorf tubes and stored frozen at -20ºC until analysis.

Liver Tissue Sampling

After blood collection, after that, rats were killed by cervical decapitation and rat livers were carefully excised from animals, cleaned, dried, and weighed.

The animal liver was divided into two parts:

The first part consists of liver sections of about 5 mm thick, which were obtained from several liver lobes. They were immediately immersed in 10 % buffered formalin. After fixation overnight, ascending grades of alcohol were used for dehydration of these sections, which then cleaned and embedded in paraffin wax. 5µm thick sections were obtained from the formed wax blocks and then double-stained with Hematoxylin and Eosin and then examined microscopically. The second portion was washed by buffered saline (10% w/v) and liver frozen at -20^{o}C for further biochemical analysis.

Histopathological Analysis

Autopsy samples were gathered from the liver tissues of rats in different studied groups and fixed in 10% neutral buffered formalin solution (PH=7.4) for 24 hours, washed with tap water then serial alcohols (methyl, ethyl and absolute ethyl) were used for dehydration . Tissue samples were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 hours. Paraffin bees wax tissue blocks were sectioned at 4 microns thickness by sledge microtome. The tissue sections were deparaffinized then stained with hematoxylin and eosin (HE) dyes then examined through light electric microscope **[15].**

Results

LC-DAD-ESI-MS separation technique showing 24 chromatographic peaks belonging to various metabolite classes were derived from examined samples including phenolic acids and flavonoids, from HPLC chromatograms standard there are fingerprints of the peak of chlorogenic acid at R_t 7.6–7.9. **(Fig 1).** The extract of *C.scolymus* bracts was previously reported containing 8.82% caffeoylquinic acid which calculated as chlorogenic acid.

Identification of Polyphenolic Compounds:

The hydroxycinnamic derivatives detected in this work belong to mono- and di-caffeoylquinic acid compounds. This finding is agreed with the previously found in the literature **[16-19] .** The peaks gave the [M+H]+ ions at m/z 193.1, 353.07 with molecular formula assigned as quinic acid and chlorogenoquinone. Peaks number 9 and 20 gave the [M+H]+ ions at m/z 355.3 in accordance as chlorogenic acid and neochlorogenic acids **[20]** . Peaks number 10 and 11 with a precursor ions at [M+H]+ m/z 171.12 and 517.1188, and identical to gallic acid and dicaffeoylquinic acid (cynarin) **(Table1)**

Identification of Flavonoids Compounds

Peaks showed precursor ions at $[M+H]^+$ m/z 287.04, 449.0 and 595.15 was proposed to be luteolin, luteolin-*O*-glycoside and luteolin-7-*O*-rutinoside (17) also showed precursor ion at $[M+H]^+$ m/z 271.24 was proposed to be apigenin (**Table 2)**

Effect of Artichoke on AFP (α-Fetoprotein) Tumor Marker

Our results presented in (**Table 3)** demonstrated that treatment of rats with TAA in HCC induced rats non significantly decreased hepatic concentrations of AFP **(p>0.05)** when compared to control group. However, treatment of HCC rats with artichoke in preventive group non significantly increased hepatic concentrations of AFP **(p>0.05)** when compared to HCC group, but the induced effects were less potent in therapeutic group that caused slight increase but statistically non-significant in AFP**(p>0.05) .**

Effect of Artichoke on Metalloprotienase-3 (MMP-3)

Our results presented in **Table 4** demonstrated that treatment of rats with TAA in HCC induced rats significantly increased hepatic concentrations of MMP-3 **(p<0.05**) when compared to control group. However, treatment of HCC rats with artichoke in preventive group slightly increased hepatic concentrations of MMP-3 but statistically non-significant in MMP-3 concentration where **(p>0.05),**but the induced effects were less potent in therapeutic group that caused increase also, statistically highly significant in MMP-3 concentration **(p< 0.001).**

Effect of Artichoke on Metalloprotienase-9 (MMP-9)

Our results presented in **Table 5** demonstrated that treatment of rats with TAA in HCC induced rats slightly increased hepatic concentrations of MMP-9, but statistically non significantly **(p>0.05)** when compared to control group. However, treatment of HCC rats with artichoke in preventive group increased hepatic concentrations of MMP-9 also, statistically significant in MMP-9 concentration (**p<0.05**),when compared to HCC group, but the induced effects were less potent in therapeutic group that caused highly increase and statistically highly significant in MMP-9 concentration **(p < 0.001**).

Effect of Artichoke on Metalloprotienase-12 (MMP-12):

Our results presented in (**Table 6)** demonstrated that treatment of rats with TAA in HCC induced rats significantly increased hepatic concentrations of MMP-12 (**p<0.05**) when compared to control group. But, treatment of HCC rats with artichoke in preventive group increased hepatic concentrations of MMP-12 but, statistically non-significant in MMP-12 concentration **(p > 0.05**) ,when compared to HCC group, but the induced effects were less potent in therapeutic group that caused increase but statistically no significant in MMP-12 concentration **(p>0.05).**

Histopathological Examination of the Liver Tissue

Histopathological examination of liver sections of normal control at **Fig.2**, and Drug I control group, (outer part extract I) **at Fig .4** . Drug II control group in **Fig. 5**, editable part extract II showed that there was no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the hepatic parenchyma was recorded in respectively**.**

However**,** in HCC- induced group multi lobular liver cirrhosis with trabecular arrangement of the hepatocytes which revealed criteria of malignancy dilatation of hepatoportal blood vessel with trabecular arrangement of the hepatocytes which revealed criteria of malignancy **Fig.3.** Liver from Drug I preventive group showing stoppage of the liver cirrhosis with focal vacuolation of the hepatocytes, dilatation of hepatoportal blood vessel with regression of the

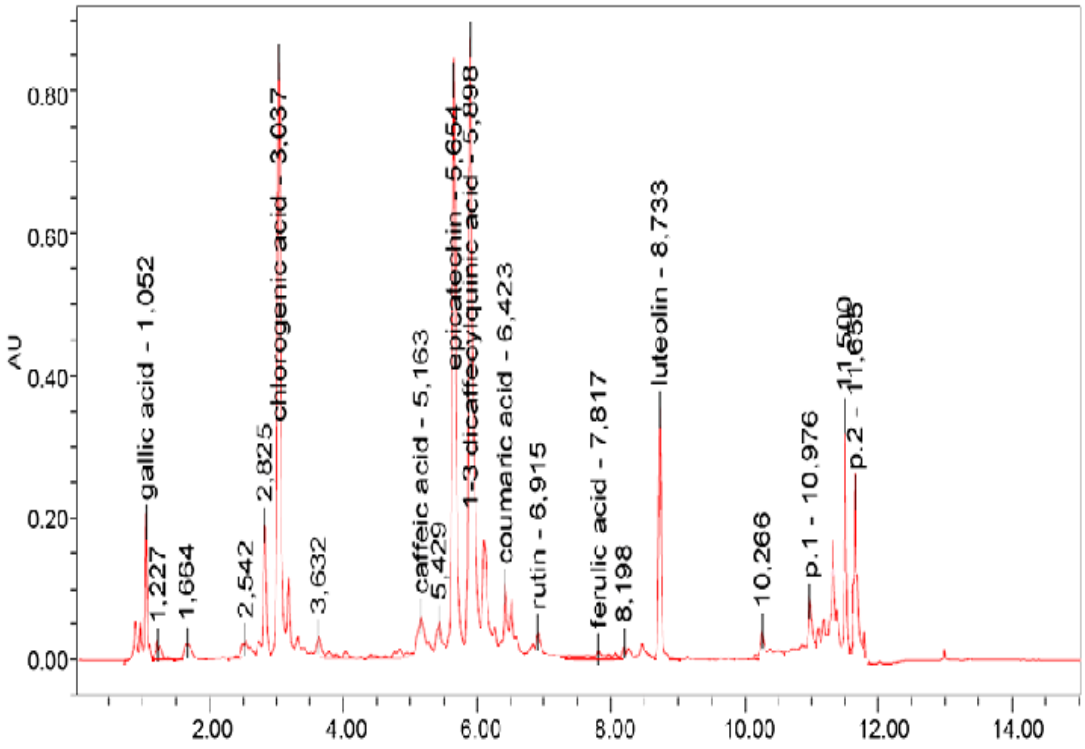


Fig. 1. High-performance liquid chromatography of Polyphenolic Compounds in A tichoke (Cynara Scolymus L.) Edible Part Extract.

TABLE 1. HPLC/ESI-MASS of edible parts (fleshy receptacle, inner bracts) of artichoke (*Cynara scolymus* L) extract.

Peak no.	Rt (min)	Name	M+	Chemical formula
1	2.1	Tyrosyl-L-leucine	295.2	$C_{15}H_{22}N_2O_4$
2	3.9	Hydroxy-octadecatrienoic acid	295.21	$C_{18}H_{29}O_3$
3	5.5	Dihydroxy-octadecatrienoic acid	311.2	$C_{18}H_{29}O_4$
4	6.9	Quinic acid	193.1	$C_7H_{12}O_6$
5	8.7	Luteolin	287.04	$C_{15}H_9O_6$
6	9.3	Chlorogenoquinone	353.07	$C_{16}H_{15}O_9$
7	10.8	Cynaroside (luteolin-7-O-glycoside)	449	$C_{21}H_{21}O_{11}$
8	12.7	3,4-Dihydroxybenzoic acid	155.02	$C_7H_6O_4$
9	13.4	Chlorogenic acid	355.3	$C_{16}H_{17}O_9$
10	16.1	Gallic acid	171.12	$C_7H_6O_5$
11	16.9	Dicaffeoylquinic acid	517.11	$C_{25}H_{23}O_{12}$
12	18	Apigenin	271.24	$C_{15}H_{10}O_5$
13	18.9	Cynarasaponin E	811.43	$C_{42}H_{65}O_{15}$
14	19.8	Luteolin-7-O-rutinoside (scolymoside)	595.15	$C_{27}H_{29}O_{15}$
15	22.4	Cynarasaponin J	943.47	$C_{47}H_{73}O_{19}$
16	23.2	Cynaropicrin	347.3	$C_{19}H_{22}O_6$
17	24	Cynarasaponin C	795.43	$C_{42}H_{65}O_{14}$
18	25.3	Hydroxy-oxo-octadecatrienoic acid	309.19	$C_{18}H_{27}O_4$
19	26.1	Grosheimin	263.3	$C_{15}H_{18}O_4$
20	27.1	Neochlorogenic acid	355.3	$C_{16}H_{18}O_9$
21	27.9	Cynarascoloside A/B	429.19	$C_{21}H_{31}O_9$
22	28.1	Cynaroscoloside C	427.18	$C_{21}H_{29}O_9$
23	29.6	Cynarasaponin A/H	927.48	$C_{47}H_{73}O_{18}$
24	32.2	Cynarasaponin F/I	781.42	$C_{41}H_{63}O_{14}$

TABLE 2. The ESI-MASS spectrometry of hydroxycinnamic derivatives and major flavonoids

Peak	MW	[M–H]–	Formula	UV λ (nm)	Tentative assignment
Hydroxycinnamic derivatives					
1	515.1418	515.1421	C 22 H 27 O 14	243, 299sh, 328	Chlorogenic acid glycoside
2	353.0899	353.0876	C 16 H 17 O 9	242, 299sh, 329	Monocaffeoylquinic acid
3	325.0917	325.0935	C 15 H 17 O 8	–	p-Coumarylglucose
4	163.0403	163.0400	C 9 H 7 O 3	282	p-Coumaric acid
5	337.0945	337.0933	C 16 H 17 O 8	312	3-p-Coumarylquinic acid
Flavone derivatives					
6	461.0737	461.0713	C 21 H 17 O 12	254, 268, 346	Luteolin 7-glucuronide
7	447.0933	447.0919	C 21 H 19 O 11	266, 346	Luteolin-7-O-glucoside (cynaroside)
8	445.0798	445.0774	C 21 H 17 O 11	277	Apigenin 7-glucuronide
9	447.0935	447.0907	C 21 H 19 O 11	–	Luteolin-7-O-glucoside (cynaroside)
10	447.0939	447.0940	C 21 H 19 O 11	–	Luteolin-7-O-glucoside (cynaroside)
11	269.0471	269.0464	C 15 H 9 O 5	267, 336	Apigenin
12	285.0406	285.0405	C 15 H 9 O 6	334	Luteolin

TABLE 3 . Effect of artichoke on AFP (α-Fetoprotein) tumor marker.

Tumor Marker	Group	Group	P-value	Significance
AFP	Group I	Group II	0.121	NS
		Group III	0.122	NS
		Group IV	0.287	NS
		Group V	0..385	NS
		Group VI	0.279	NS
	Group II	Group III	0.925	NS
		Group IV	0.298	NS
		Group V	0.119	NS
		Group VI	0.095	NS

TABLE 4 . Effect of Artichoke on Metalloprotienase-3 (MMP-3).

Metalloproteinase	Group	Group	P-value	Sig
MMP-3	Group I	Group II	0.035	*
		Group III	0.000	**
		Group IV	0.032	*
		Group V	0.000	**
		Group VI	0.000	**
	Group II	Group III	0.000	##
		Group IV	0.969	NS
		Group V	0.093	NS
		Group VI	0.000	##

TABLE 5 . Effect of Artichoke on Metalloprotienase-12 (MMP-12).

Metalloproteinase	Group	Group	P-value	Sig
MMP-9	Group I	Group II	0.193	NS
		Group III	0.462	NS
		Group IV	0.054	NS
		Group V	0.378	NS
		Group VI	0.000	**
	Group II	Group III	0.575	NS
		Group IV	0.470	NS
		Group V	0.035	#
		Group VI	0.000	##

TABLE 6. Effect of Artichoke on Metalloprotienase-12 (MMP-12).

Metalloproteinase	Group	Group	P-value	Sig
MMP-12	Group I	Group II	0.002	*
		Group III	0.005	*
		Group IV	0.002	*
		Group V	0.000	**
		Group VI	0.000	**
	Group II	Group III	0.767	NS
		Group IV	0.957	NS
		Group V	0.129	NS
		Group VI	0.115	NS

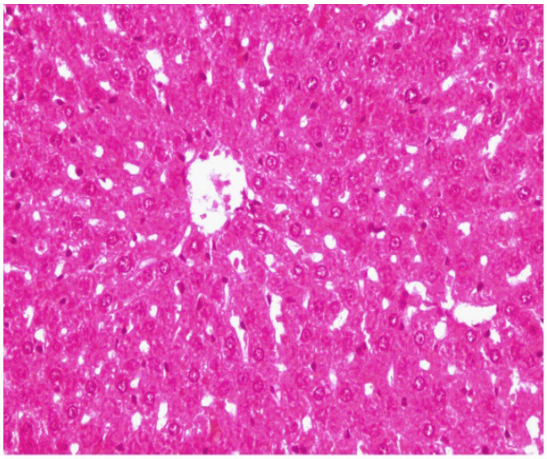
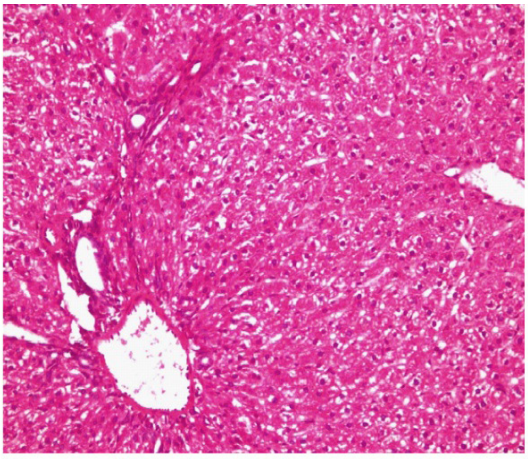


Fig2: Liver from control negative group showing normal hepatic parenchyma; note the normal hepatocytes and blood sinusoids (H&E x 400).



Liver from control negative group showing normal portal area; note the normal bile duct and hepatoportal blood vessels (H&E X 400).

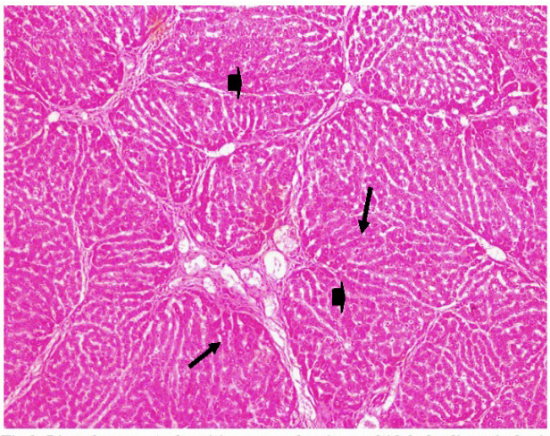
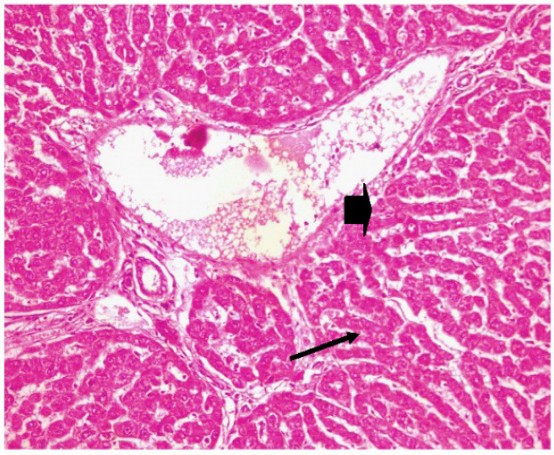


Fig.3: Liver from control positive group showing multi lobular liver cirrhosis (arrow head) with trabecular arrangement of the hepatocytes which revealed criteria of malignancy (arrows), (H&E X 100).



Liver from control positive group showing dilatation of hepatoportal blood vessel (arrow head) with trabecular arrangement of the hepatocytes which revealed criteria of malignancy (arrows), (H&E X 200).

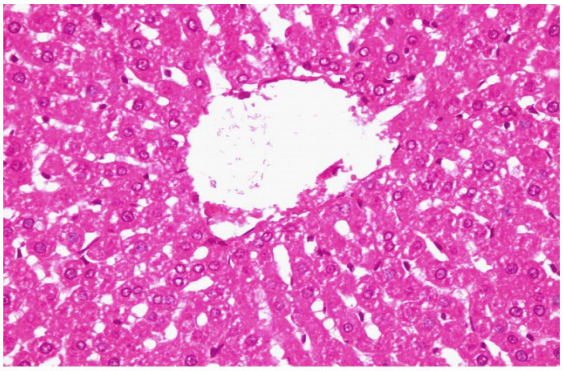
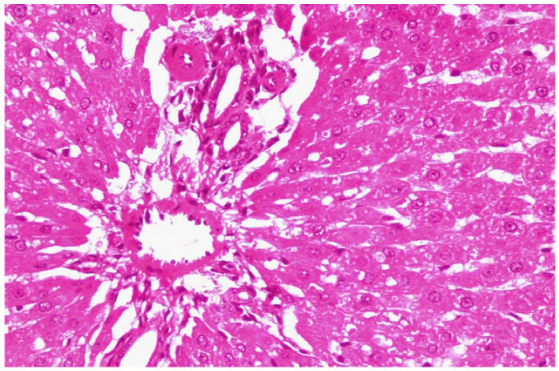


Fig.4: Liver from drug I control group showing normal hepatic parenchyma; note the normal hepatocytes and blood sinusoids, (H&E X 400).



Liver from drug I control group showing normal portal area; note the normal bile duct and hepatoportal blood vessels, (H&E X 400).

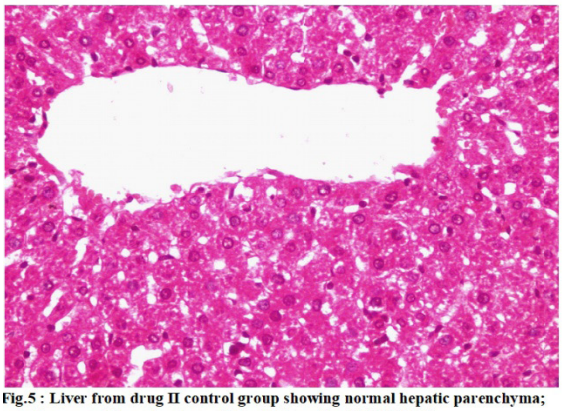
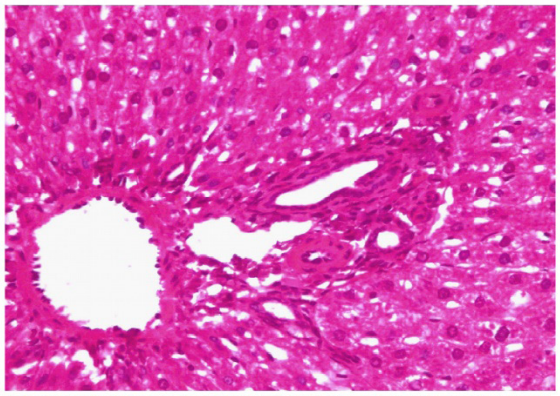


Fig.5 : Liver from drug II control group showing normal hepatic parenchyma; note the normal hepatocytes and blood sinusoids, (H&E X 400).



Liver from drug II control group showing normal portal area; note the normal bile duct and hepatoportal blood vessels, (H&E X 400).

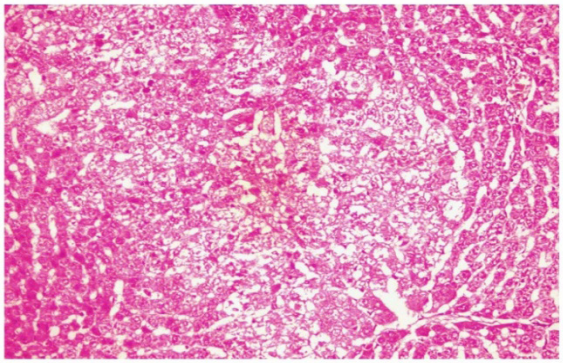
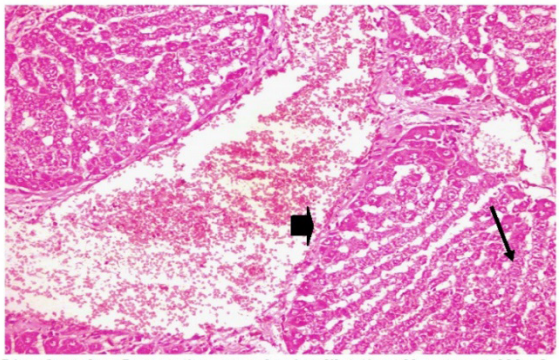


Fig.6 : Liver from drug I preventive group showing stoppage of the liver cirrhosis with focal vacuolation of the hepatocytes (arrows), (H&E X 200).



Liver from drug I preventive group showing dilatation of hepatoportal blood vessel (arrow head) with regression of the trabecular arrangement of the hepatocytes (arrows), (H&E X 200).

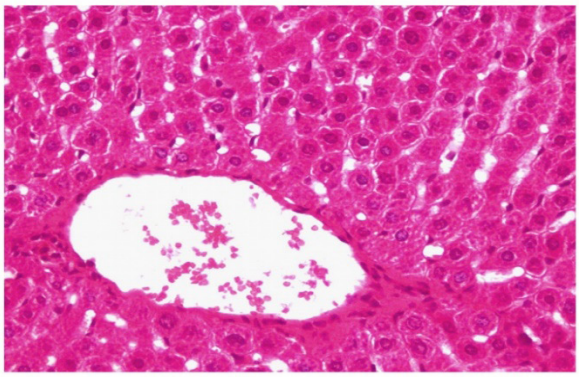
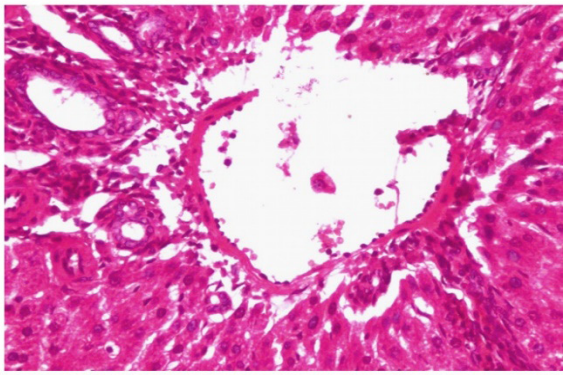


Fig. 7 : Liver from drug II treated group showing normal hepatic parenchyma; note the normal hepatocytes and blood sinusoids, (H&E X 400).



Liver from drug II treated group showing normal portal area; note the normal bile duct and hepatoportal blood vessels, (H&E X 400).

trabecular arrangement of the hepatocytes **Fig.6.** In therapeutic group normal hepatic parenchyma and the normal hepatocytes and blood sinusoids showing normal portal area; note the normal bile duct and hepatoportal blood vessels **Fig.7.**

Statistical analysis

All results were analyzed by SPSS software (SPSS, ver.16.00, USA). Data were expressed as mean ± SD. **[21].** Comparison of mean values of studied variables among different groups was done using ANOVA test. P<0.05 was significant

Discussion

Artichoke (*Cynara scolymus* L.), is an edible herbal medicine of the family Asteraceae, is a perennial herb widely studied because of its possible antioxidative and hepatoprotective effects **[22].** The extracts and derivatives from artichoke contain a variety of dicaffeoylquinic acids and many kinds of flavonoid functional compounds **[23],** which exhibits anti-microbial, anti-allergic, anti-inflammatory, and anticancer effects. One study indicated that artichoke extract had potential in reducing hypercholesterolemia through preventing lipid peroxidation and upgrading hepatic antioxidant status [24].

Another report demonstrated that artichoke aqueous leaf extract reduced serum total cholesterol (TC), triglycerides (TG), very low density lipoprotein, glucose levels, and plasma malondialdehyde (MDA) levels in streptozotocin-treated diabetic rats [25].

Artichoke showed marked anti-inflammatory effects on tissue plasminogen activator-induced inflammation and antitumor activity in an in vivo two-stage carcinogenesis test in mice[26]. Besides, it was reported that artichoke extract was very safe to the human body as no obvious side effects were observed after continuous medication for several months **[27].**

Hepatocellular carcinoma (HCC) is considered one of the famous health problems; the fifth widely spread cancer in the world **[28]**. HCC incidence and mortality have worldwide elevation over the last four decades [**29, 30**]. TAA hepatic injury is related to the disturbance in hepatocytes membrane instability and metabolism resulting in alterations of the serum levels of these enzymes. Hepatocellular injury is usually associated with an elevation in the activities of liver enzymes **[31].**

The elevation of ALT and AST activities result from leakage of these enzymes from the cell membrane of hepatocyte due to the loss of its functional integrity in response to pathological conditions as cirrhosis, adverse effects of some drugs [32].

Our data illustrated that the mean level of ALT, AST, TP, ALB serum activities are indicative for hepatic function, their increase is correlated with the hepatic injury [33].TAA hepatic injury is related to the disturbance in hepatocytes membrane instability and metabolism resulting in alterations of the serum levels of these enzymes.

Our data illustrated that the mean level of ALT activity showed to be slightly increased but statistically non-significant **(p>0.05)** for groups when compared to normal control .**Table1**

But AST, TP and ALB activities showed to be slightly increased but statistically significant**(p<0.05**) for groups when compared to normal control. **Table 2,3,4**

In TAA treated group the mean level of ALT, AST and ALB activities showed Non-significant increase **(p>0.05)** and TP activity showed slightly increase but statistically highly significant **(p < 0.001**) **.**

Another hepatic marker bilirubin which is formed as the major product result from the breakdown of heme containing proteins such as myoglobin, hemoglobin, catalase, cytochromes, tryptophan pyrrolase, and peroxidase. A large percent of daily bilirubin production (80%) from 250to 400 mg in adults is obtained from hemoglobin **[34].** The remaining percent is produced from a rapidly turning-over small pool of free heme and from other hemoproteins. The increase in bilirubin levels is usually associated with the increase in red blood cell breaking down. In addition, the increase in the level of AFP in HCC rats indicates an HCC**[35].** It was reported that elevated serum concentrations of AFP can be observed in rats due to exposure to hepatotoxicagents or hepatocarcinogens and are frequently associated with HCC. Its serum concentration can be used to confirm hepatocarcinoma and the diagnosis of tumor response therapy. More than 90% of patients with hepatitis, cirrhosis and hepatic cancer have increased serum AFP levels **[36].** The increase in the serum concentration of AFP in HCC cases is due to tumor excretion **[37].**

The current study showed that HCC rats have

slightly elevated serum AFP level compared to the normal control group. This finding is harmonizing with many studies **[38].** which reported that the serum level of AFP was significantly increased in the TAA-injected group, as compared to the control group.

Conclusion

A relative study of artichoke extracts contains the highest amount polyphenols especially the chlorogenic acid. The studies advocate using receptacle extract since it exerts most liver protection and cholesterol lowering activity comparable to silymarin.

As the histopathological study performed in rat livers also proved our finding and revealed that artichoke extract at dose level 1.5g/kg day reduced liver tissue lesions when damaged by TAA and showed almost normal histological architecture of hepatic lobule.

Bract and leaf extract approximately exert the same action, as well as more than root extract. However, higher dose of artichoke (900 mg/kg/day) is unadvised since signs of fatty degenerations and necrosis of liver cells are noticed.

So, our recommendation is the use of the edible parts of artichoke as commercial leaf preparation designed for liver protection.

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