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Research Article
Phytochemical Analysis and Toxicity Assessment of Artichoke By-product Extract

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Abstract

Background and Objective: Egypt produced 236,314 t of artichoke in 2016, which produce a huge amount of useless by-product, which can be used as cheaper source for many active compounds can be applied for some medical application. The objective of this study was to assess the toxicity of the artichoke by-product extract through its effect on rats' kidney, brain and liver biomarkers. **Materials and Methods:** Chemical composition of artichoke by-product (crude protein, crude fiber, crude fat and minerals) was determined. Conventional extraction (CE), microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) extraction methods were used for artichoke by-product and comparison between them were performed according to antioxidant activity using DPPH and the phenolic profile identity using HPLC technique. Chronic oral gavage of thirty adult male albino rats for 4 weeks in the concentrations of (0.1, 0.5, 1 and 5 g kg^{-1}) artichoke by-product extract was used for evaluation of its toxicity. **Results:** MAE with ethanol more suitable for extraction of the polyphenols (193.63±4.9 µg gallic acid equivalents (GAE) mg^{-1}) and showed IC_{50} = 159.7 mg mL^{-1}. Three major active phenolic compounds were identified benzoic acid, ellagic acid and caffeine. Rats administrated 5 g kg^{-1} artichoke extract have no changes in brain, liver and kidney parameters ($p<0.05$). Histology of brain and liver exhibited normal architecture. **Conclusion:** The results showed that the artichoke by-product extract had no any toxic effect on rats and considered be safe for human use even at a high level of doses (up to 5 g kg^{-1}).

Key words: Artichoke, phenolic compounds, benzoic acid, ellagic acid, caffeine, cytotoxicity, microwave-assisted extraction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Artichokes (*Cynara cardunculus* var. *scolymus*) are originated in the Mediterranean basin and have been used for centuries due to its potential medicinal properties. Artichoke extract, which contains high concentrations of bioactive compounds demonstrate several health benefits include improve digestion, lower blood sugar, support heart and liver health. Medicinal plants have long been considered as a vital source for novel drugs[1]. The artichoke also called *Cynara scolymus* L., is one of the oldest cultivated plants belongs to *Asteraceae* family[2]. It was grown in Ethiopia and then reached to southern Europe via Egypt. According to FAO, Egypt is the second top country in 2010 and 2011 for artichoke production, after Italy and it is a major component of the Mediterranean diet[3]. French scientists studied its medicinal applications in the 20th century[2]. Artichoke has been found to be rich source in polyphenolic compounds, inulin, fiber and minerals[4]. Artichoke leaves have a protective action against dyspepsia,[5] chronic Hepatitis-C,[6] and other liver complaints[7]. Artichoke leaves extract has shown antioxidant, antibacterial, bile-expelling, hepatoprotective, urinative and choleretic activities, as well as it has the ability to inhibit cholesterol biosynthesis and LDL oxidation[8]. Several reports demonstrated that artichoke showed marked anti-inflammatory and antitumor effects[9-10]. El Sohaimy[11] reported that artichoke methanolic extract has broad inhibitory activities against microorganisms such as *Proteus vulgaris* ATCC6830, *Escherichia coli* 0-143, *Staphylococcus aureus* 0006, *Klebsiella pneumonia* 8961 and *Bacillus subtilis* ATC6633 and it could be used in food industry. The identity of artichoke polyphenol content varies depending on the extraction method[12]. The main phenolic compounds are the caffeic acid derivatives which include the caffeoylquinic acid derivatives. 5-O-caffeoylquinic acid (chlorogenic acid) is the most abundant single substance (39%), followed by 1,5-O-dicaffeoylquinic acid (21%) and 3,4-O-dicaffeoylquinic acid (11%), based on total caffeoylquinic acid content[13]. Furthermore, the 1,3-O-dicaffeoylquinic acid (cynarin) content in methanolic extracts of artichoke is very low (about 1.5%)[14]. In Egypt, the artichoke industry generates large quantity of by-products contain leaves, stem and the bracts, which are not suitable for animal feed and they could be used as a source of food additive and nutraceuticals. There is no study concerning the artichoke by-products (leaves, stem and the bracts) extract (ABPE) toxicity. Therefore, the current study focuses on the assessment of chronic oral toxicity of the artichoke by-product extract through studying its effect on rats' kidney, brain and liver biomarkers. Furthermore, its phytochemical screening for the by-product itself and phenolic compounds of the extract were identified.

MATERIAL AND METHODS

The present study was carried out as a collaborative research work between the Department of Food Technology, Arid Lands Cultivation Research Institute, City of Scientific research and Technological Application and Department of Biochemistry, Faculty of Science, Alexandria University in the period of 2018 and 2019.

Chemical composition: Artichoke by-products (ABP) were cleaned, dried in a ventilated stove at 50°C, powdered by mechanical grinder and stored at -20°C. Crude protein content in dried powder was conducted using the Kjeldahl procedure according to Nelson and Sommers[15]. The crude fat and fiber contents were estimated by Soxhlet method Folch *et al.*[16], Prosky *et al.*[17], respectively. Minerals including; iron (Fe), copper (Cu), sodium (Na), potassium (K), zinc (Zn), selenium (se), chromium (Cr) and manganese (Mn) were analyzed in the dried powder by Atomic Absorption Spectrophotometer (Hitachi model 170-10), according to AOAC[18].

Solid-liquid extraction

Conventional extraction (CE): About 20 g of dried powder artichoke by-product were introduce to 200 mL of either 70% ethanol or distilled water and incubated at 37°C for 3 days. The extract was filtered, concentrated with a rotary machine and lyophilized by freeze-dryer and stored at -20°C for further analysis according to Taha *et al.*[19].

Microwave-assisted extraction (MAE): Approximately 5 g of dried powder artichoke by-product were extracted with 50 mL of 70% ethanol or 50 mL distilled water in 100 mL closed bottle for one min in household quartz browner microwave oven (MWO611/WH, supply: 230 V-50 Hz, Input: 1400 W, Frequency: 2450 Hz) 407 W. The extract was filtered, concentrated with a rotary machine and lyophilized by freeze-dryer and stored at -20°C for further analysis according to Adriana and Quan[20].

Ultrasonic-assisted extraction (UAE): Approximately 5 g of dried powder artichoke by-product were re-suspended in 50 mL of either 70% ethanol or distilled water and the extraction was performed in an ultrasonic bath (Astrason

Ultrasonic Clear, Farmingdale, NY, USA) 80% power for 10 min with a working frequency of 33 KHz. The samples were filtered, concentrated and lyophilized and stored at -20°C for further analysis according to Kong *et al.*[21].

Total phenolic content (TPC) and antioxidant activity (DPPH scavenging %): TPC of artichoke by-product extract (ABPE) was determined spectrophotometrically using Folin-Ciocalteu method,[22] the results were expressed as µg gallic acid equivalents (GAE mg^{-1}) dry extract. The DPPH assay was performed as described by Gordon *et al.*[23] with a minor modification. Briefly, 100 µL of each extract (10 mg mL^{-1} in methanol) was added to 3.5mL of DPPH (0.06 mM, in methanol) solution and incubated for 30 min in dark. The change in color (from deep violet to light yellow) was read at 516 nm. For sample blank and control, methanol was used instead of DPPH and sample, respectively. The scavenging activity (%) was calculated according to the following equation:

$$\text{Scavenging (\%)} = 1 - \frac{A_{sample} - A_{sample\ B_{lank}}}{A_{control}} \times 100$$

HPLC profile of phenolic compound: The waCE and ethMAC for ABP were applied separately for HPLC analysis. Agilent1260 (Santa Clara, CA, United States), equipped with a high pressure pump; automatic injector; a UV-visible diode array detector; controlled by Chem Station software, was used for the analysis. The samples were separated on Zorbax Eclipse plus C18) 100 mm×4.6 mm, Agilent technologies, USA) column using solvent A (0.2% H_3PO_4), B (100% methanol) and C (acetonitrile) as a mobile phase. Detector set at 284 nm. The phenolic extracts and standard compounds were analyzed under the same analysis conditions. The results of the main phenolic compounds were expressed as mg/100 g extract[24].

Chronic toxicity study of ABPE

Experimental design: Thirty adult male albino rats (100-110 g) were purchased from the National Research Center, Alexandria (Egypt). The animals were maintained on a standard laboratory diet and water *ad libitum*. After 1 week of acclimation period, the animals were housed in polypropylene cages in a temperature controlled (25°C) and artificially illuminated (12 h dark/light cycle) room, free from any source of chemical contamination. All ethical guidelines for care and use of laboratory animals were followed. The procedures and protocols were used in the present research have been approved by the Ethical Committee of Alexandria University (Protocol approval number: AU 04190727201).

Animals were divided into 5 groups 6 in each group and received artichoke 70% ethanol extract by MAE (ethMAE) at doses of (0, 0.1, 0.5, 1, 5 g lyophilized extract kg^{-1}) by gavage for 4 weeks, twice a week. All animals were anesthetized and sacrificed at the end of the study.

Sample preparation: Blood samples collected by heart puncture and centrifugation at 1000×g for 10 min at 4°C to separate serum and stored at -20°C for estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, total lipids, urea, creatinine and glucose levels as well as AChE activity; All except AChE activity were measured by standardized enzymatic procedures using commercial kits from (Diamond, Egypt). Brain and liver samples were quickly removed and cleaned with ice-cold saline. The hippocampus was immediately isolated from the whole brain and stored at -80°C for biochemical analysis. Small portion of liver and hippocampus tissues were dissected out and fixed in 10% formalin for histopathological examination.

Biochemical parameters: A 10% (w/v) homogenate of brain and liver of rats tissues were separately prepared in 0.1 M phosphate buffer saline (PBS) pH 7.4 and centrifuged at 10,000×g for 20 min at 4°C. The supernatant was used for estimation of acetylcholinesterase (AChE), glutathione transferase (GST), superoxide dismutase (SOD) and glutathione transferase (GPx) activities and malondialdehyde (MDA) level.

Acetylcholinesterase (AchE) activity was assayed as described by Ellman *et al.*[25] The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercapto thiocholine and 5-thio-2- nitrobenzoate which can be detected at 412 nm. In ELISA plate, 150 µL of phosphate buffer pH 8 was directly added in ELISA blank well and 130 µL phosphate buffer was added to ELISA activity wells. To the blank and activity wells, 5 µL of the substrate (acetylcholine thioiodide, 75 mM) was added, then 20 µL homogenate was added in ELISA activity wells only. The plate was preincubated for 15 min at 37°C before the addition of the second substrate (5,5-dithiobisnitrobenzoic acid (DTNB), 60 µL) in both the blank and activity wells. Absorbance was measured at 412 nm every 2 min. One unit of AChE activity was defined as the number of micromoles of acetylthiocholine iodide hydrolyzed min^{-1} mg^{-1} of protein. The specific activity of AChE is expressed in U g^{-1} of protein.

TBARS assay, malondialdehyde (MDA) is a major secondary product of lipid peroxidation, was

spectrophotometrically measured by the thiobarbituric acid (TBA) reaction[26]. To 500 µL of homogenate supernatant, 1 mL of 15% trichloroacetic acid was added and mixed well then the solutions were centrifuged at 3000 rpm for 10 min. One milliliter of the supernatant was added to 0.5 mL of 0.7% TBA then the mixture was heated for 60 min at 90°C. The obtained pink color was measured spectrophotometrically at 532 nm. The results were expressed as µmoles g^{-1} of protein.

The activity of GPx and GST were measured as described by Chiu *et al.*[27] and Habig *et al.*[28], respectively. The enzyme activity was expressed as units per gram of protein. Protein level was measured by the method of Bradford[29] and bovine serum albumin (BSA, 1 mg mL^{-1}) was used as a standard.

Histopathological study: Liver and hippocampus tissues from all the experimental groups were fixed in 10% formalin for 24 h. After processing (dehydrating in gradual ethanol (50-100%) and clearing in xylene), the samples were embedded in paraffin wax. Sections of 5 µm thickness were prepared using a microtome and stained by hematoxylin and eosin (H and E) for histopathological examination[30].

Statistical analysis: All quantifications for assays were repeated for three times and mean value used by taking mean of the triplicate±standard deviation (Mean±SD) for each group. The significance of differences among the groups was assessed using one-way analysis of variance (ANOVA). The p-values less than 0.05 were considered as significance.

RESULTS AND DISCUSSION

Chemical compositions of artichoke by-products: Table 1 showed the proximate composition of the artichoke by-products (ABP). Protein and fat contents were 10.25±0.55 and 1.73±0.1%, respectively, which are in agreement with Hosseinzadeh *et al.*[31] who found the protein content of artichoke leaves was in the range of 8.05-12.35% and the fat content was in the range of 1.6-2.3% on dry weight basis. The crude fiber content of ABP was 34.4±2.2%. So artichoke by-products may be considered as a great ingredient for low fat and high fiber foods that may reduce the risk of several disorders, such as obesity, cardiovascular diseases, diabetes and other diseases[32-33]. The ash content of the ABP was recorded 8.7±0.5%, that in accordance with the results of Lutz *et al.*[34]. In addition, artichoke by-products contain 115±2% sodium and 133±5% potassium, which is in agreement with other reports that, considered ABP as a rich source of minerals, in particular K and Na[35]. On the other hand, iron was 3.64±0.3% as well as zinc, copper and manganese represent 0.27±0.05, 0.12±0.01 and 0.53±0.04 ppm, respectively in the ABP, but other minerals like selenium and chromium were not detected. The obtained results emphasized the fact that the ABP might be considered as a good source of macro and micronutrients which is very useful for human health and encourage us to consider the artichoke for fortifying other food products and formulate some kinds of food supplements for human use.

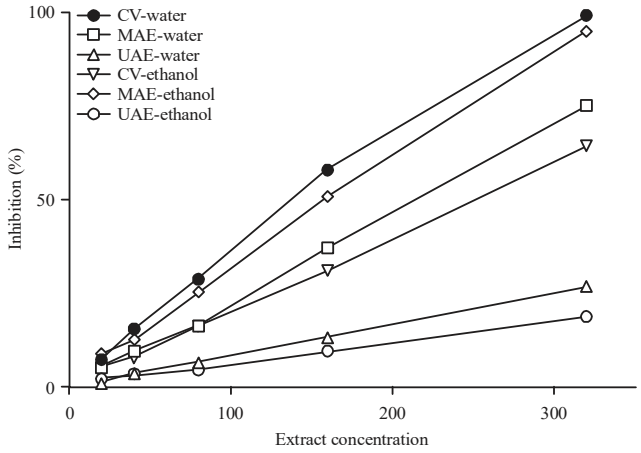

Fig. 1: Antioxidant activity of artichoke extract with different extraction techniques

Table 1: Proximate composition of the green globe artichoke (on dry weight basis)

Constituents	Powder (g/100 g)
Crude protein	10.25±0.55
Crude fat	1.73±0.1
Crude fiber	34.40±2.2
Ash	8.70±0.5
Minerals (ppm) (mg kg^{-1})	
Fe	3.64±0.3
Zn	0.27±0.05
Cu	0.12±0.01
Mn	0.53±0.04
Na	115.00±2
K	133.00±5
Se	ND
Cr	ND

Data were expressed as Mean±SD of triplicate, ND: Not detected

Total phenolic content and antioxidant activity of artichoke extract: Antioxidant activity of artichoke extract varied with the extraction techniques used (Fig. 1). Water extract by conventional method (waCE) gave the highest total phenolic contents and the lowest IC_{50} (220.38 µg GAE mg^{-1} and 130.54 µg mL^{-1}, respectively) followed by ethanol

Table 2: Effect of different extraction techniques on total phenolic contents and antioxidant activity of artichoke extract

Extraction techniques	Solvent	TPC (μg GAE mg^{-1} dry extract)	IC_{50} (μg mL^{-1})
Conventional extraction (CE)	Ethanol (70%)	156.71±2.3	247.30
	Water	220.38±1.5	130.54
Microwave-assisted extraction (MAE)	Ethanol (70%)	193.63±4.9	159.70
	Water	145.25±2.3	216.10
Ultrasonic-assisted extraction (UAE)	Ethanol (70%)	77.23±2.2	857.00
	Water	82.61±1.9	598.50

TPC data were expressed as Mean±SD of triplicate, Ascorbic acid (IC_{50} = 0.473 μg mL^{-1})

Table 3: Phenolic compounds identity in waCE and ethMAE

Phenolic acids	waCE (μg g^{-1})	ethMAE (μg g^{-1})
Gallic acid	0.48	0.45
Catechol	4.46	23.64
p-hydroxy benzoic acid	3.90	4.74
Caffeine	2.78	382.03
Vanillic acid	9.91	11.24
Caffeic acid	ND	10.48
Syringic acid	1.11	5.61
Vanillin	0.25	0.15
p-coumaric acid	0.63	4.29
Ferulic acid	0.14	25.10
Rutin	8.86	70.63
Ellagic acid	17.90	573.07
Benzoic acid	51.21	589.91
o-coumaric acid	0.55	23.62
Salicylic acid	1.18	ND
Cinnamic acid	0.13	0.13

ND: Not detected

microwave assisted extract (ethMAE) (193.63 μg GAE mg^{-1} and 159.7 μg mL^{-1}, respectively). On the other hand, ultrasonic assisted extraction (UAE) showed the lowest phenolic contents among all extraction trails (Table 2). Previous studies have reviewed the correlation between the antioxidant activity (%) and the phenolic contents[36,11]. The total phenolic contents of artichoke by-products (ABP) were higher than that of grape pomace[37], carrot peels[38], apple pomace[39], bilberry residues[40] and spent coffee grounds[41]. Therefore, artichoke by-product can be considered as a great source of polyphenols, which could be vital in prevention of several human diseases through their ability to scavenge free radicals.

HPLC polyphenols profile of artichoke by-product extracts (ABPE): Conventional water (waCE) extract and microwave-assisted ethanol (ethMAE) extract were identified sixteen phenolic compounds in HPLC profile (Fig. 2, Table 3). In the ethMAE, benzoic acid (B), ellagic acid (EA) and caffeine (C) were the predominant phenolic compounds with concentrations of 589.91, 573.07 and 382.03 mg/100 g, respectively. On the other hand, waCE showed the lowest concentration of B (51.21mg/100 g), EA (17.90 mg/100 g) and C is (2.78mg/100 g). While Ben Salem *et al.*[42] by maceration method identified verbascoside, quercetin and oleuropein (381, 192 and 180 mg/100 g, respectively) in artichoke leaf ethanol extract. Caffeic acid is not detected in waCE while was in concentration of (10.48 mg/100 g) in ethMAE. Furthermore, salicylic acid is not detecting in ethMAE while was detected in waCE in the level of (1.18 mg/100 g). This variation in phenolic profile is might be due to the extraction method[12] and ethMAE was selected for the further studies due to phenolic compounds identity and antioxidant IC_{50} value. It has been reported that organic acids, such as benzoic acid (E210), acetic acid (E260) are used as food preservatives in low pH foods. In agro food industry benzoic acid has been used as preservative and as an antioxidant. The antimicrobial activity of benzoic acid is due to its effects on the cell membrane; also it inhibits enzymes of the citric acid cycle and of the oxidative phosphorylation. It is usually applied as preservative for fruit juice and soft drinks[43]. Also, urolithins the metabolized form of EA has been reported its role in colon cancer chemo-preventive activity of foods containing this polyphenol[44]. Kaur *et al.*[45] demonstrated the neuroprotective effect of EA as a constructive herbal drug to impede cholinergic dysfunctions and oxidative stress in scopolamine induced Alzheimer's type of dementia in rats. Furthermore, Kaur *et al.*[46] reported that EA possesses anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic and cardio-protective activities. Also it has been reported that caffeine possesses antioxidant properties, anti-skin cancer by inhibition of cell proliferation, angiogenesis and has the capacity for inducing apoptosis[47]. Therefore, the present extract has the privilege to be used as a nutraceutical for several diseases applications. Other phenolic components had appeared in a good quantity in HPLC profile (Fig. 2) didn't identify until this moment but can be in great benefit as complementary food on future isolation and separation.

Biochemical toxicity markers

Effect of ethMAE on serum biochemical parameters: There were no significant changes in serum measuring parameters (ALT, AST, creatinine, glucose, lipid and AChE) observed in all treated groups compared to control group (Table 4); Whereas urea level was decreased significantly in extract treated groups in dose dependent manner. Reduced plasma/serum urea is less common and usually of less clinical significance than increased plasma/serum urea[48]. Since the balance between urea production and urea elimination in urine is depend on urea concentration in plasma or serum So,

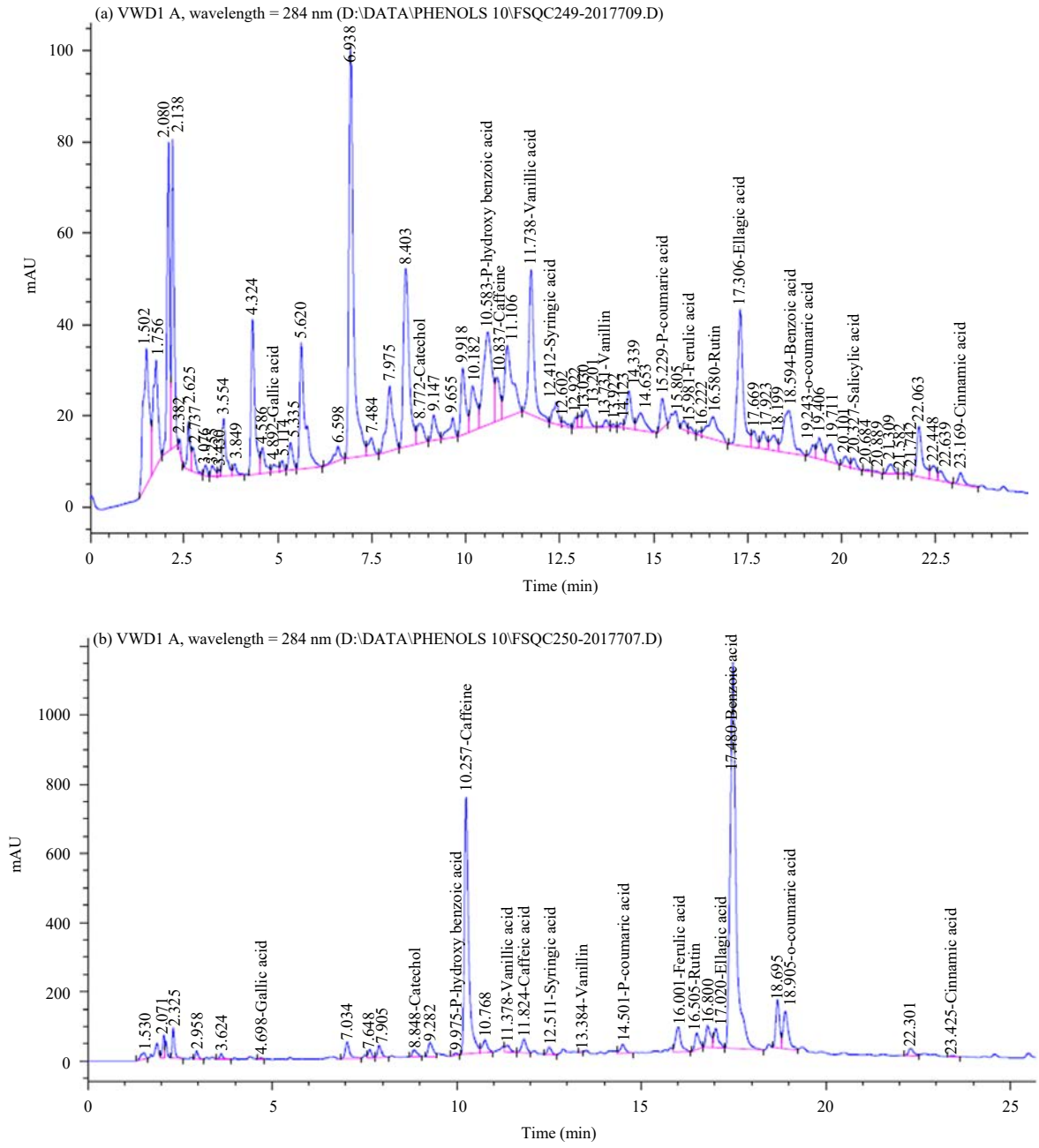

Fig. 2(a-b): HPLC chromatogram of (a) waCE and (b) ethMAE

Retention times of major peaks measured at 284 nm are indicated

Table 4: Evaluation of serum parameters

ethMAE (g kg^{-1})	ALT (U L^{-1})	AST (U L^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	Glucose (mg dL^{-1})	Lipid (mg dL^{-1})	AChE (U g^{-1})×10^{4}
0	23.60±0.95	102.14±2.47	107.56±5.3	1.33±0.09	69.3±2.8	654±30.1	2.90±0.1
0.1	24.20±1.8	94.95±3.88*	72.70±5.9	1.35±0.012	67.7±1.22	722±25.4	3.13±0.05
0.5	24.67±1.8	96.05±1.31	62.10±4.7*	1.45±0.02	67.9±1.0	663±23.4	3.00±0.91
1	24.50±1.3	96.90±2.29	58.70±4.7*	1.41±0.07	65.4±1.1	650±15.1	3.43±0.35
5	22.90±1.93	96.20±1.80	48.80±7.2*	1.37±0.15	65.9±1.02	651±24.33	2.87±0.75

Significant *p<0.05 when compared to control group

Table 5: Estimation of liver parameters

ethMAE (g kg^{-1})	SOD (U g^{-1}) protein	Glutathione peroxidase (U g^{-1}) protein	Glutathione transferase (U g^{-1}) protein	TBARS (μmol g^{-1}) $\times 10^{-3}$	AChE (U g^{-1}) $\times 10^{4}$
0	0.209±0.015	0.055±0.001	0.024±0.004	6.32±1.8	8.50±0.2
0.1	0.174±0.006	0.074±0.005	0.019±0.002	2.33±0.05*	8.60±0.34
0.5	0.154±0.008*	0.072±0.006	0.020±0.001	2.85±0.3*	7.87±0.1
1	0.151±0.0098*	0.1±0.002	0.021±0.003	3.75±0.6*	9.25±0.30
5	0.113±0.0037*	0.09±0.009	0.20±0.004	2.30±0.5*	7.60±0.25

Significant *p<0.05 when compared to control group

Table 6: Estimation of brain parameters

ethMAE (g kg^{-1})	SOD (U g^{-1}) protein	Glutathione peroxidase (U g^{-1}) protein	Glutathione transferase (U g^{-1}) protein	TBARS (μmol g^{-1}) $\times 10^{-3}$	AChE (U g^{-1}) $\times 10^{4}$
0	0.107±0.00168	1.49±0.01	0.015±0.004	11.9±1.8	12.60±0.2
0.1	0.098±0.0018	0.82±0.03	0.012±0.002	6.97±0.5*	9.80±0.24*
0.5	0.083±0.0029	1.011±0.06	0.008±0.001	7.70±0.3	12.37±0.1
1	0.076±0.0021*	0.88±0.02	0.01±0.003	7.20±0.6	11.25±0.30
5	0.011±0.0013*	0.82±0.1	0.012±0.004	8.02±0.2	14.10±0.25

Significant *p<0.05 when compared to control group

reducing blood urea can be done by decreasing urea production, increasing urea excretion, or both. Furthermore, there are two physiological factors may decrease the concentration of urea: low-protein diet and pregnancy. It has been recorded that artichoke leaves polyphenols have a protective action on the liver injury in mice induced by acute alcohol intake at 1.6 g kg^{-1} dose[7]. ALT and AST are well known enzymes used as biomarkers predicting possible toxicity[49]. The lack of bad alterations of ALT, AST, urea and creatinine is a good indicator of liver and kidney functions, respectively[50]. In general, any injury of the parenchymal liver cells will result in increase of ALT and AST[51]. In the present study transaminases (ALT and AST) were not increased at the doses of 5 g kg^{-1}. It emphasized that ingestion of ethMAE didn't cause any kind of alteration in the nature of the liver and kidneys of the rats. Heidarian and Soofiniya[52] reported that the oral administration of artichoke in the streptozotocin-treated rats (1000 and 2000 mg kg^{-1}) decrease serum glucose and total lipid levels significantly in a dose-dependent manner. From the present results and the previous studies, so ethMAE could be affecting only the metabolic markers of diabetic rats and had no more effect on normal rats. These results in agreement with the other reported data that showed feeding with artichoke leaf extract significantly improved the immune system, an antioxidant power and liver functions in cadmium (Cd) toxicity-induced oxidative organ damage in rats[53]. Due to the lack of data about the cytotoxicity effect of the artichoke by-product extract in general and on AChE activity in particular in the literatures. So, the present study will play a key role for assessment of the toxicity of artichoke extract.

Effect of ethMAE on liver biochemical parameters: The activity level of glutathione peroxidase (GPX), glutathione S-transferase (GST) and acetylcholinesterase (AChE) were determined for the toxicity effect of the extract in the liver. As shown in Table 5, detectable changes in the activity of GPX, GST and AChE for all groups compared to control group at the end of the experiment. On the other hand, SOD activity and TBARS level were decreased significantly by 23.5 and 63.6%, respectively at the end of the experimental period at the dose of 5 g kg^{-1}. According to Mittal and Kant[54], with increasing oxidative stress, SOD decreases due to irreversible inactivation by its product (H_2O_2). This reduction also increases when combined with the extracts, which might be attributed to the effect of the phenolic compounds that could be playing a crucial role in the SOD-like enzyme acting as Calderon *et al.*[55] Oliboni *et al.*[56] reported the strong correlations between the polyphenol content and the reduction of lipid peroxidation (TBARS) and protein oxidation. All the measuring parameters results confirmed the normal function of the rat's liver at the termination of the experiment.

Effect of ethMAE on brain biochemical parameters: There was no significant change on GPX and GST activities, while SOD activity was significantly decreased by 84.7% at the concentration of 5 g kg^{-1} (Table 6). TBARS level in 0.1 g kg^{-1} ethMAE feeding groups showed a significant decrease ($p<0.05$) in comparison with control group by 41.4%. While AChE activity was significantly decreased at 0.1 g kg^{-1} ethMAT dose by 22.2%. Several studies have demonstrated the clinical effects of polyphenolic compounds in showing the neuroprotective properties. They can suppress neuroinflammation and potentially increase memory, learning and cognitive functions[57-58]. Neurodegenerative diseases are multifactorial and complex disorders and monotherapy does not have significant effects. Current studies reported the great capacity of phenolic compounds to exhibit neuroprotection through a diversity of important mechanisms: (1) management the expression of antiapoptotic factors, (2) promote different signaling pathways in the cell, (3) blow

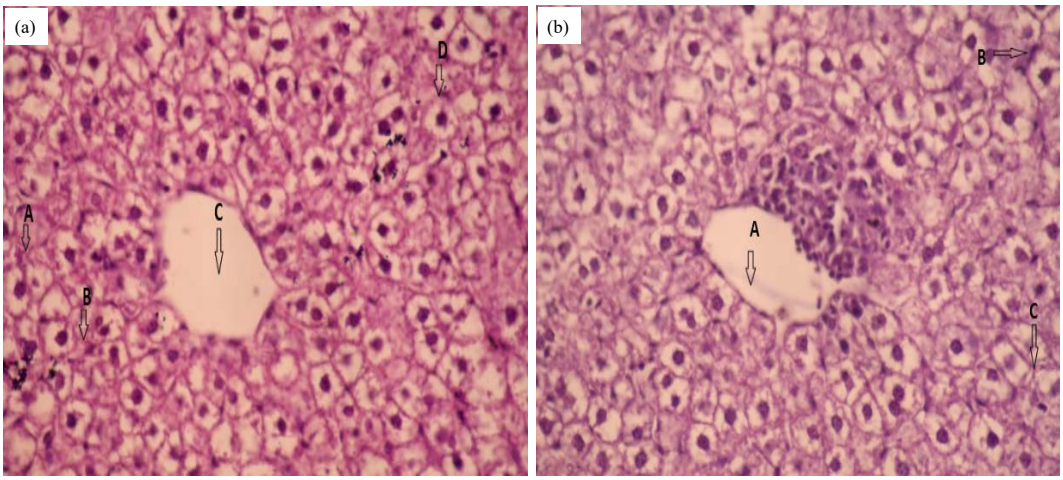

Fig. 3(a-b): Histopathological imaging of liver, (a) Control group reveals normal hepatocytes separated by sinusoidal blood vessels, A: Blood sinusoids, B: Kupffer cell, C: Central vein, D: Hepatocytes (×40) and (b) Group 5 (5 g kg^{-1}) reveals normal hepatocytes separated by sinusoidal blood vessels, A: Central vein, B: Hepatocytes, C: Blood sinusoids (×40)

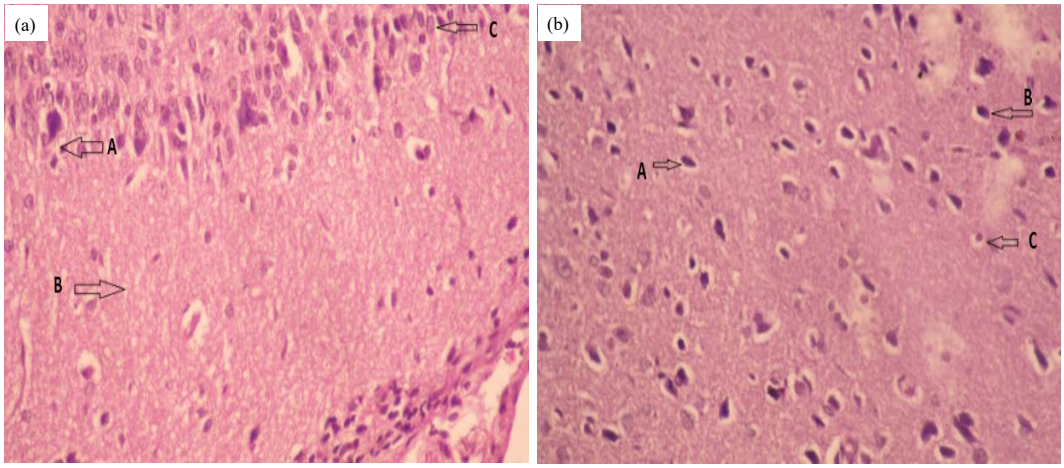

Fig. 4(a-b): Histopathological imaging of brain, (a) Control group reveals normal brain tissue formed of purkinje cell layer, molecular layer and granular cell layer, A: Purkinje cell layer, B: Molecular layer, C: Granular layer (×40) and (b) Group 5 (5 g kg^{-1}) reveals normal brain tissue in molecular layer consisting of pyramidal cells, fusiform cells and neurological cells, A: Fusiform cells, B: Pyramidal cells, C: Neurological cells (×40)

out free radical species and inhibit pro-oxidant enzymes, (4) affect mitochondrial activity, (5) chelate redox-active transition metal ions, (6) prevent protein aggregation and (7) regulate the major proteins degradation pathways[59-60]. These features make polyphenols from ethMAE will be excellent neuroprotective candidates and support their transformation from laboratory to clinical trials.

Histological imaging of ethMAE effects on rats: Histological changes were determined by using a light microscope. There was no visible alteration to histological architecture of both liver and brain after the oral administration of the ethMAE for 4 weeks with the dose levels up to 5 g kg^{-1}. The treated tissue samples of the liver showed similarities (no changes) in the histological profile compared to the control. Both samples have normal hepatocytes separated by sinusoidal blood vessels (Fig. 3). Section of the brain hippocampus showed that; the histoarchitecture of the control and treated tissues presented similar patterns without any evidence of necrosis or degenerative changes even at the highest dosage level (5 g kg^{-1}) (Fig. 4). The present investigation emphasized the ethanolic extract of artichoke is non-toxic to brain and liver via

the oral route up to maximum dose of 5 g kg^{-1}. There are no previous studies on the cytotoxicity assessment of artichoke extract. So, this study is the first study exploring the toxicity level of artichoke extract.

CONCLUSION

This study could be helpful to satisfy the main goal to use the artichoke by-product extract (as being costless food source) as food additive and adjuvant natural antioxidant and it could be useful also for the planning of future pre-clinical and clinical studies of this plant medicine, as artichoke by-product extract rich in phenolic compounds especially benzoic acid, ellagic acid and caffeine, that they can be easily recovered by an eco-friendly solvent-extraction procedure.

SIGNIFICANCE STATEMENT

This study confirmed that artichoke by-product extract is safe and has no sign of toxicity on liver, kidney and brain biomarkers and histology of rats. This study will open the door in using the artichoke extract in medical uses and other industrial application.

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