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Original Article

Artichoke edible parts are hepatoprotective as commercial leaf preparation

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ABSTRACT

Chemical profile analyses of artichoke (*Cynara scolymus* L., Asteraceae) edible parts (fleshy receptacle, inner bracts) as well as roots are compared with the commercially usable leaf extract using HPLC-DAD-ESI-MS *via* chlorogenicacid as a marker. Overall polyphenolic constituents demonstrated by means of LC/MS profiling. The nutritional values and inulin contents of different assessed parts were investigated. The present study was designed to determine the effect of artichoke: leaves, bracts, receptacles and roots alcoholic extracts against CCl_4-induced acute hepatotoxicity and hyperlipidemia in rats by means of histopathological and biochemical parameters. Serum liver enzymes levels of aspartate amino transferase, alanine amino transferase, alkaline phosphatase and lipid peroxidase content (malondialdehyde MDA) were estimated. Blood glutathione, total cholesterol, triacylglycerides and high density lipid level were estimated in plasma. The ethanol extract of roots, leaves, bracts and receptacles were standardized to (0.82 ± 0.02, 1.6 ± 0.06, 2.02 ± 0.16 and 2.4 ± 0.27 mg chlorogenic acid/100 mg extract), respectively. The receptacle showed the highest content of polyphenols and exhibits the highest antioxidant activity. HPLC analysis of inulin in the receptacles of globe artichoke revealed high content of inulin (41.47 mg/g) dry extract. All artichoke parts contain comparable vitamins and minerals. Artichokes receptacles extract when taken in dose of (500 mg/kg/day) reduce the lesion caused by CCl_4 alone more than groups receiving silymarin. Bracts and leaves extract exert nearly the same effect.

Introduction

Developing countries generally have greater share of burden liver diseases (Elizabeth, 2008). The load of liver diseases in Egypt is exceptionally high maintaining the highest prevalence of hepatitis C virus worldwide, as well as rising rates of hepato-cellular carcinoma (Strickland, 2006). Egypt has the highest serological prevalence of hepatitis C virus in the world ranging from 6 to 28% with average approximately 15% in the general population (Saad et al., 2011). The above serious problem in Egypt demonstrates the urgency of finding a hepatoprotective drug from natural source in order to face this intense ongoing endemic outbreak.

Globe artichoke (*Cynara scolymus* L.) is a perennial plant belonging to Asteraceae. It is cultivated worldwide for its immature edible flower heads consisting of fleshy leaves (bracts) and receptacles (Jonne et al., 2007). Globe artichoke was known by the ancient Egyptians as food and medicinal entity and known by Greeks and Romans. It was an important menu item at feasts until the fifteenth century. It is popular for its pleasant bitter taste which is mostly attributed to bioactive material called cynarin (Rottenberg and Zohary, 1996).

The plant is recognized in herbal medicine where foliage leaves are utilized for production of commercial extracts used as hepatoprotective and choleretic in food supplements (Gebhardt, 2005). These important activities have been attributed to several metabolites including polyphenols such as cynarin, caffeoylquinic, chlorogenic acid, flavonoids such as luteolin or its glycosides (Lattanzioa et al., 2009; Farag et al., 2014). Other constituents are Q3 also reported such as sesquiterpenes (grosheimin, cyanoropicrin), saponins, fatty acids and others. Metabolite variability are reported among the different cultivars of *C. scolymus* (Farag et al., 2013) as determined by analysis of principle components. Variation of constituents has been documented depending on genotype, harvest time, part used, soil, climate *etc.* (Farag et al., 2014). Globe artichoke

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has been the subject of study by many investigators (Gebhardt, 2005; Wagenbreth and Eich, 2005; Jonne et al., 2007). These investigations focused mainly on using the artichoke leaf extract for liver protection against chemical pollutant and viral infections. Artichoke plant is traditionally used for treating liver diseases as well as hyperlipidemia (Gebhardt and Fauseel, 1997).

The aim of this work is: (i) to evaluate and set up standard measures for *C. scolymus* cultivar balady growing at Nahia region at Giza; (ii) to reveal the characteristic profile of major phenolics and structurally related ingredients in the extracts of different organs, especially the edible parts (receptacle and bracts) using optimized HPLC conditions; (iii) to quantify the contents of dietary constituents; (iv) to compare the therapeutic value of this plant as a hepatoprotective drug *versus* the well-established silymarin obtained from milk thistle.

Experimental

Plant material

The samples of *Cynara scolymus* L., Asteraceae, were collected from Nahia region at Giza, Egypt, May 2014. The cultivar is known as Balady. A voucher specimen (numbered 8.5.2014) has been located at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt. Three kilogram of air-dried powdered different parts (leaves, receptecals, bracts and roots) of *C. scolymus* was extracted by cold percolation (5 × 3 l) with ethanol (70%) till exhaustion. Greenish brown residues were collected after evaporation of the solvent. Solvent in each case was totally evaporated below reduced pressure and residues obtained were kept for the study.

Chemical measures

Solvent: ethanol 70% (commercial grade). Reagants: Folin-Ciocalteu reagent (Sigma-Aldrich). The DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent (Sigma-Aldrich). Authentic reference materials: chlorogenic acid, benzoic acid, gallic acid, caffeic acid, cinnamic acid, ellagic acid and inulin (Sigma-Aldrich).

HPLC standardization of artichoke extracts

Artichoke particularly the edible organs namely receptacle and bracts were reported to contain chlorogenic acid. Hence, it deemed necessary to shed light on its concentration indifferent plant extracts. The artichoke extracts of leaves, bracts, receptacles and roots were standardized according to their chlorogenic acid content (European Pharmacopoeia, 2006). An Agilent 1200 series HPLC was used, equipped with a quaternary pump and anauto sampler. Samples were dissolved in methanol, filtered through PTFE 0.45 μm syringe filter (Macherey-Nagel, Germany) and injected into Zorbax C8 (5 μm, 4.6 × 250 mm) column. The mobile phase was methanol (solvent A) and 0.3% phosphoric acid in water (solvent B). Gradient elution was carried out at a flow rate of 1 ml/min. Measurements were made with an injection volume of 20 μl and UV detection at 330 nm.

Preparation of standard for HPLC

Standard stock solution of chlorogenic acid was prepared by dissolving 10 mg chlorogenic acid in 50 ml methanol. Transfer 5 ml of this solution to a volumetric flask, add 5 ml of methanol and dilute to 20 ml with water. Then concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mg/ml were prepared.

Preparation of test solution

To 50 mg extract (leaves, bracts, receptacles and roots) 5 ml methanol added then filtered, 5 ml of filtrate was taken in volumetric flask and 5 ml methanol was added then completed to 20 ml with water. These concentrations were separately subjected to HPLC analysis according to condition mentioned before then calibration curve were established. Another sample of Super Artichoke capsules® (Western Medical) which is a commercially available drug, consisting of cynarin at a concentration of 320 mg, was analyzed under the same conditions for comparison with four artichoke extracts.

LC-DAD-ESI-MS separation technique

Separation of the sample was carried by a bounded silica C18 column (4.6 × 150 mm 3 μm). The sample filtrate by Teflon 0.45 membrane filter, then 10 μm of sample dissolved in 1 ml of MeOH to carry out analysis (Fritsche et al., 2002). Chemical compounds were analyzed *via* reversed phase HPLC using a binary gradient consisting of solvent A: isopropanol/acetonitrile/methanol/0.3% aqueous formic acid 18:30:12:40 (v/v) and solvent B: 0.3% aqueous formic acid. A linear gradient from 8% (A) (0 min) to 48%(A) (35 min) at a flow rate of 1 ml/min was applied, at temperature 25 °C. The column is interfaced with electrospray (Bruker daltonic Esquire-LC mass spectrometer (Bremen, Germany), and Dionex Ultimate 300 (Germany) equipped with a quaternary pump with an on line degasser, a thermostatted column compartment, a photodiode array detector (DAD), an auto sampler, and HyStar software. Mass spectra were acquired in positive ion mode at a voltage of 70 V. Nitrogen was used as drying gas at a flow rate of 10 l/min and 300 °C. Nebulizer pressure was set to 60 psi. The capillary voltage was optimized to 3500 V. For all spectra manual baseline subtraction was performed. This analysis was kindly performed at institute for Environmental Studies and Research.

Spectrophotometric determination of total polyphenols

Determination of total phenolics according to Singleton and Rossi (1965).

Preparation of standard solution

The standard stock solution of gallic acid was prepared with different concentrations (0.025, 0.05, 0.1, 0.2, 0.3 and 0.4 mg/ml) in ethanol.

Preparation of test samples

Extract (0.1 g) was dissolved in 100 ml distilled water, 0.8 ml was taken to which 0.4 ml Folin-Ciocalteu and 4 ml distilled water were added, then diluted to 10 ml with sodium carbonate (290 g/l). The solution was thoroughly mixed and incubated for 30 min, then total phenols were spectrophotometrically estimated at 760 nm. The concentration of total phenolics was expressed as gallic acid equivalent (GAE) per 1 g of fresh sample. All experiments were carried out in triplicates.

HPLC analysis and identification of phenolic compounds

The phenolic compound analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with a UV-Visible multi wavelength detector. The separation was carried out on a 250 × 4.6 mm, 4 μm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase

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