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Original Article

Antioxidant and hepatoprotective effects of *Capparis spinosa* L. fractions and Quercetin on tert-butyl hydroperoxide- induced acute liver damage in mice

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ABSTRACT

The present study investigates the antioxidant and hepatoprotective effects of *Capparis spinosa* L. and Quercetin in tert-butyl hydroperoxide (t-BHP) induced acute liver damage. Different fractions of *C. spinosa* were examined for total phenolic content and antioxidant property. Among these fractions, hydroalcoholic extract was used to assess the hepatoprotective effect in tert-butyl hydroperoxide (t-BHP) induced hepatotoxicity model by determining serum biochemical markers, sleeping time and antioxidant assay such as reduced glutathione (GSH) as well as histopathological examination of liver tissues. The total phenolic and Quercetin contents of hydroalcoholic fraction were significantly higher than other fractions. It also showed high antioxidant activity. Pretreatment with hydroalcoholic fraction at the dose of 400 mg/kg and Quercetin at the dose of 20 mg/kg showed liver protection against t-BHP induced hepatic injury, as it was evident by a significant decrease in serum enzymes marker, sleeping time and MDA and an increase in the GSH, SOD and CAT activities confirmed by pathology tests. The final results ascertained the hepatoprotective and antioxidant effects of *C. spinosa* and Quercetin in a dose-dependent manner. Moreover, this study suggests that possible mechanism of this protection may be associated with its property of scavenging free radicals which may be due to the presence of phenolic compounds.

1. Introduction

Liver is the largest internal organ in the human body that plays a major role in metabolism and detoxification of various chemicals, drugs and other toxic compounds.[1] Oxidative stress is a biochemical condition that occurs in the body producing several types of reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).[2] Oxidative stress is regarded as one of the pathological mechanisms that causes initiation and progression of liver damage through inducing irreversible alteration of lipid membranes, proteins and DNA and, more importantly, through modulating pathways that control biological function.[3] A number of pro-oxidants are implicated in the oxidative stress and cell injury that result from their intracellular metabolism to free radical intermediates.[4] tert-butyl hydroperoxide (t-BHP) is an organic lipid hydroperoxide analogue which is often used as a model compound to induce oxidative stress during *in vitro* and *in vivo* studies. t-BHP can be metabolized into free radical intermediates by cytochrome P-450 which subsequently initiates lipid peroxidation and depletes cellular reduced glutathione (GSH) content. t-BHP caused leakage of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and formation of malondialdehyde (MDA) in hepatocyte. It also mediated DNA base damage in mammalian cells.[5–7]

The genus *Capparis* belonging to the Capparaceae family includes nearly 250 species; many of which are distributed in the

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mediterranean regions.[8] *Capparis spinosa* L. has been used for pharmaceutical, cosmetic, and nutritional purposes. In many countries, the flower buds, fruits, roots, and seeds of the *C. spinosa* have been used in folk medicine as an anti-rheumatic, tonic, expectorant, anti-spasmodic, diuretic and analgesic agents.[9] They are very good sources of glucosinolates (glucocapparin, glucoiberin, sinigrin and glucobrassicin), flavonoids, phenolic acids and alkaloids; all of which are known to provide health-improving benefits due to their various biological activities such as antioxidant, anticancerogenic, antimicrobial and antimutagenic.[10] Furthermore, leaves of *C. spinosa* is used to prepare Liv-52 which is employed to treat alcoholic liver disease, viral hepatitis, and liver cirrhosis.[11]

Quercetin is a flavonoid found in many fruits and vegetables including green apple, onion, green tea, lemon as well as in medicinal botanic plants. In addition, *C. spinosa* is one of the rich sources of Quercetin. It is one of the most active antioxidants owing to its high ability to scavenge free radicals.[12]

The present study was conducted to investigate the antioxidant activity and phenolic content of *C. spinosa* fractions. We have also examined the mechanism of *C. spinosa* and Quercetin as hepatoprotective agents by studying their effects on serum liver function, oxidative stress biomarkers and hepatic histopathology of mice subjected to t-BHP-induced hepatotoxicity.

2. Materials and methods

2.1. Chemicals

Quercetin, chloroform, ethyl acetate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), trichloro acetic acid (TCA), thiobarbituric acid (TBA), bovine serum albumin (BSA) and bradford reagent were purchased from Sigma–Aldrich chemical company (St. Louis, MO), USA. All chemicals and reagents used were analytical grade. Tert-butyl hydroperoxide (t-BHP) was also purchased from Roche chemical company (Germany).

2.2. Plant extraction

Plant was collected from the Iranian province of Khuzestan during spring 2015. Plant materials were taxonomically identified by central herbarium of Ahvaz Jundishapur University of Medical Sciences, Iran. The leaves of *C. spinosa* were shade-dried, ground and soaked in 80% aqueous-ethanol, ethyl acetate and chloroform in separate containers for three days with occasional shaking.

Each solvent was filtered through a filter paper (Whatman No. 2) and then was removed under vacuum in a rotary evaporator until dryness. The percentage yields of extracts in different solvents were 15.75% (w/w) for dried hydroalcoholic, 4.51% for ethyl acetate and 3.9% for chloroformic fraction, respectively.

2.3. HPLC analysis

A high performance liquid chromatography system (HPLC) (Shimadzu-02-0600, Japan) was used for quantitative and qualitative analysis of Quercetin. Analyses were conducted using a C18 column (4.6 mm × 150 mm, 5 μm diameter particle sizes, Waters, Symmetry, Milford, USA). The mobile phase used was methanol: acetonitrile: water (10:10:80) with a flow rate of 1 ml/min and injection volume was 20 μl. An amount of 0.5 g of each extracts were dissolved in 10 ml of solution (methanol–acetic acid–water (1:2:1)) for 1 h on a shaker at laboratory temperature. 2 ml of the solutions were centrifuged for 10 min at 2000 × g. Then, the solutions were filtered through a micro filter with regenerated cellulose membranes of the pore size 0.22 μm. The filtrate was applied for HPLC. Standard solutions of Quercetin were also prepared by dissolving different amount in methanol. Suitable concentrations were injected to find the RT (retention time) values and check the linearity between concentration and peak areas. The spectrum was detected at 370 nm using a UV detector.

2.4. Total phenolic content (TPC)

Total phenolic contents of crude extracts were determined by a spectrophotometer using the Folin-Ciocalteu's reagent.[13] Different dilutions of each dry extract (3.75, 6.25, 12.5 and 25 mg/ml) were prepared in 10 ml of their own solvent. Moreover, different concentrations of tannic acid (0.02, 04, 06 and 08 mg/ml) were prepared as standards. Then, 0.5 ml of extracts or standards was transferred into a 5 ml volumetric flask and was swirled with 2.5 ml of Folin-Ciocalteu's reagent (diluted 1:10, v/v). After 5 min, 2 ml of Na_2CO_3 (7.5%, v/v) solution was added and mixed. The solution was thoroughly mixed and placed at ambient temperature for 2 h until the characteristic blue color was developed. The absorbance was measured at 765 nm after 30 min. Quantification of TPC was based on a standard curve generated with tannic acid (TAC) at 765 nm.

All tests were conducted in triplicate and averaged. Results were expressed as tannic acid equivalent (mg tannic acid/g dried extract). Additional dilution was done in case the absorbance value measured was over the linear range of the standard curve.

2.5. In-vitro free radical scavenging activity

2.5.1. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of the methanol solution of DPPH.[14,15] Tests were carried out in triplicate. A methanol solution (0.1 ml) of the test sample at various concentrations was added to 3.9 ml of a DPPH solution (25 mg/lit). The decrease in absorbance at 515 nm was ceaselessly determined every 1 min with a UV–Vis spectrophotometer for 30 min. The percentage inhibition (I %) of DPPH radical was calculated in the following way:

$$\text{Inhibition of DPPH\%} = \left[1 - \frac{A\ sample}{A\ blank}\right] \times 100$$

A blank is the absorbance of the control reaction (containing all reagents except the test compound) while A sample is the absorbance of the test compound. The percentage of inhibited DPPH radical was calculated by the above equation; then, it was plotted against the sample/standard concentration to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50% (IC50). Based on the parameter IC50, the result was expressed in terms of mg dry matter of sample/standard equivalent g^{-1} DPPH in the reaction medium.

2.5.2. Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was performed according to Benzie and Strain method.[16] The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 ml of a 10 mmol/L TPTZ (2,4,6-tripyridy-*s*-triazine, sigma) solution in 40 mmol/L HCl plus 2.5 ml of 20 mmol/L $FeCl_3 \cdot 6H_2O$ and 25 ml of 0.3 mol/L acetate buffer, pH 3.6 and was prepared freshly and warmed at 37 °C. First, absorption of fresh FRAP reagent were measured at the wavelength of 593 nm. Then, 100 μl sample or standards were mixed with 300 μl distilled water and 3 ml FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically. Finally, the absorbance was

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