A hepatonephro-protective phenolic-rich extract from red onion (*Allium cepa* L.) peels

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Abstract: Onion peel is a common bio-waste, occasionally used in traditional medicine in treatment of liver ailment and inflammation. However, a phytochemical and biological study is further required to provide the scientific evidence for this use. A phenolic-rich extract of red onion peels (coded as ACPE) was primarily prepared and then subjected to chromatographic separation. From the extract, six phenolic antioxidant compounds along with two phytosterols were isolated and identified by means of spectroscopic (NMR and MS) analyses. The *in vivo* protective activity of the ACPE against the oxidative stress induced by carbon tetrachloride (CCl_4) free radicals, in liver and kidney, was assessed in rats. Relative to the CCl_4-challenged animals, pre-treatment with ACPE could significantly ameliorate the hepatonephro-linked serum and tissue markers in a dose-dependent response. The flavonol- and phenolic acid-based nature of constituents, the high phenolic content (72.33±5.30 mg gallic acid equivalent per one gram) and the significant antioxidant capacity (>1/3 potency of rutin) of ACPE may be thus attributed strongly to the hepatonephro-protective and anti-inflammatory effect of ACPE. The results suggest that red onion peels can serve as a convenient and cost-effective source of high-value antioxidant nutraceuticals for protection against oxidative stress-related disorders.

Keywords: *Allium cepa,* antioxidant, hepatoprotective, nephroprotective, anti-inflammatory.

INTRODUCTION

Worldwide consumption of onion (*Allium cepa* L.) bulbs results in about 15 MMT of peels and skins as a waste product (Santana-Méridas *et al.,* 2012). Although onion bulbs has been phytochemically studied extensively (Pérez-Gregorio *et al.,* 2014) and reported to exhibit various medicinal effects and bioactivities, the non-edible part (onion peels) has been not yet subjected to a similar evaluation as a beneficial health material. In Middle East, onion peel is occasionally used in traditional medicine in the treatment of liver ailment. A previous HPLC analysis on onion peels of different cultivars indicated the presence of flavonoids and phenolic acid derivatives (Kim *et al.,* 2013, Singh *et al.,* 2009) but without confirmation through isolation and NMR identification. This study aimed to measure the phenolic content and antioxidant capacity, and to phytochemically investigate, and to evaluate the protective potential of ACPE against oxidative stress induced by CCl_4 in liver and kidney. The anti-inflammatory and analgesic effect of ACPE is also reported. The results of this study could be the first step in developing red onion peels as a dietary supplement to combat oxidative stress-based disorders.

MATERIAL AND METHODS

General

EI- and ESI-MS spectra were obtained on a Shimadzu QP-2010 plus and an Agilent 6320 ion trap mass spectrometers, respectively. UV spectra were obtained by Shimadzu UV-160/PC spectrophotometer in methanol (λ_{max} in nm). Melting points were determined using a Mettler FP 81 MBC Cell apparatus and were uncorrected. All UV-shift reagents, NaOMe, 5% $AlCl_3$ in MeOH (w/v), 18% HCl aqueous solution (v/v) and anhydrous NaOAc were prepared according to (Harborne 1984). TLC was performed with Si gel GF_{254}. All solvents were of analytical reagent grade. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM500 instrument at 500 MHz for ^1H and 125 MHz for ^{13}C in $CDCl_3$ or DMSO-d_6 with TMS as an internal standard (chemical shifts in d, ppm). $Ce(SO_4)_2$ reagent (1%) was used for detection of compounds, 5% $FeCl_3$ reagent was used for detection of phenolic compounds (Stahl 1969), and 0.1% 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used for detection of antioxidant compounds (Moon and Shibamoto 2009) on TLC. DPPH and deuterated solvents were purchased from Sigma-Aldrich Co. (MO, USA). Spectroscopic grade solvents were used for spectroscopic analyses. Evaporation of the solvents was done at 40°C under vacuum using a Buchi® rotary evaporator, model 011, Switzerland.

Plant materials

A Ha'il red cultivar of onion (*A. cepa* L.) was purchased in spring from a local market at Riyadh, Saudi Arabia. A voucher specimen (ATA32-1) was kept at the department herbarium. The outer dry scales (1kg) were only removed, washed, air-dried, and then powdered.

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Extraction and isolation

Powdered red onion peel (1 kg) was thoroughly extracted with acetone - distilled water (7:3) mixture at RT every 48 h (2 L x 6) until exhaustion. The combined extracts were evaporated to produce a dark brown gum (156.2 g, 15.62% w/w, ACPE). ACPE was dissolved in 30% MeOH in distilled water (0.5 L) and successively fractionated with light petrol (40-60^{o}C), $CHCl_3$, and EtOAc to yield FP (1.2 g), FC (3.0 g), and FE (80.0 g), respectively. FP was subjected to Si gel column chromatography (CC), using light petrol EtOAc (100:0 to 0:100, gradient) as a mobile phase. Two successive fractions eluted with 10% EtOAc in light petrol yielded compounds 1 (20 mg) and 2 (15 mg), respectively. FC was separated by Si gel CC using $CHCl_3$ MeOH (100:0 to 0:100, gradient). Fraction (0.34 g) eluted by $CHCl_3$ - MeOH (95:5) was purified by Si gel CC using light petrol EtOAc (100:0 to 0:100, gradient) to give compound 3 (10 mg). FE (25 g) was further fractionated over Si gel CC and eluted, in a gradient mode, with MeOH in $CHCl_3$ to give 15 fractions (FE1 to FE15). FE4 and FE6 eluted with 2% and 4% MeOH in $CHCl_3$ yielded compounds 4 (12.0 mg) and 5 (140 mg), respectively. Fraction FE7 eluted with 5% MeOH in $CHCl_3$ was further purified on Si gel CC using $CHCl_3$ MeOH (100:0 to 0:100, gradient) to afford 12 subfractions (FE7.1 to FE7.12). Fraction FE7.2 eluted by 100% $CHCl_3$ yielded another crop of compound 3 (8.0 mg). Fraction FE7.4 eluted with 1% MeOH in $CHCl_3$ was further separated over Si CC using light petrol – EtOAc (100:0 to 0:100, gradient) to give 8 subfractions FE7.4.1 to FE7.4.8. Material 6 (25 mg) was deposited as yellow solid from FE7.4.2. FE7.5 eluted with 2% MeOH in $CHCl_3$ was further isolated on Si gel CC using light petrol – EtOAc (100:0 to 0:100, gradient) to 6 subfractions FE7.5.1– FE7.5.6. Among these fractions, FE7.5.2 afforded compound 7 (25 mg). Fraction FE8 eluted by 7% MeOH in $CHCl_3$ afforded compound 8 (45 mg). Structures of the isolated compounds 3 - 8 are shown in fig. 1.

Characterization of the isolated compounds

On the basis of UV, MS, ^{1}H & ^{13}C NMR spectral data, and spectral comparison with literature data, compounds 1 (R_f= 0.43 [Si gel TLC, light petrol – EtOAc, 8.5:1.5]) and 2 (R_f = 0.40 [Si gel TLC, light petrol-EtOAc, 8.5:1.5]) were identified as *β*-sitosterol and stigmasterol (Cayme and Ragasa 2004), respectively, 3 (R_f = 0.90 [Si gel TLC, $CHCl_3$ – MeOH – AcOH, 8.3:1.7:0.2]) as methyl 2,4,6-trihydroxybenzoate (Lee and Wang 2007), 4 (R_f = 0.85 [Si gel TLC, $CHCl_3$ -MeOH – AcOH, 8.3:1.7:0.2]) as methyl protocatechuate or methyl 3,4-dihydroxy benzoate (Miyazawa *et al.,* 2003), 5 (R_f = 0.59 [Si gel TLC, $CHCl_3$ - MeOH - AcOH, 8.3:1.7:0.2]) as quercetin (Kyriakou *et al.,* 2012), 6 (R_f = 0.88 [Si gel TLC, $CHCl_3$-MeOH - AcOH, 8.7:1.3:0.2]) as isorhamnetin (Lee *et al.,* 2008), and 7 (R_f = 0.62 [Si gel TLC, $CHCl_3$ – MeOH - AcOH (8.3:1.7:0.2]) as protocatechuic acid (He *et al.,* 2009).

Compound 8

It is obtained as fine yellow powder; m.p. 209-211 °C; R_f = 0.15 [Si gel TLC, $CHCl_3$ – MeOH – AcOH (8.3:1.7:0.2)]; It gives positive $FeCl_3$ test and decolorizes DPPH reagent on TLC; ^{1}H NMR (DMSO-d_6, 500 MHz) δ_H 12.43 (1H, s, 5-OH), 10.80, 9.54, 9.02 (each 1H, *br s*, 3-OH, 7-OH, and 3'-OH), 7.71 (1H, *d*, *J*=1.5 Hz, H-2'), 7.64 (1H, *dd*, *J*= 8.5, 1.5 Hz, H-6'), 7.26 (1H, *d*, *J*=8.5 Hz, H-5'), 6.46 (1H, *br s*, H-8), 6.21 (1H, *br s*, H-6), 4.86 (1H, *d*, *J*=6.5 Hz, H-1''), 3.74 (1H, *m*, H-6''a), 3.51 (1H, *m*, H-5''), 3.41 (1H, *m*, H-4''), 3.35 (1H, *m*, H-3''), 3.23 (1H, *m*, H-6''b), 3.21 (1H, *m*, H-2''); ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C 176.0 (qC, C-4), 164.0 (qC, C-7), 160.7 (qC, C-5), 156.2 (qC, C-9), 146.7 (qC, C-4'), 146.3 (qC, C-2), 145.9 (qC, C-3'), 136.4 (qC, C-3), 125.1 (qC, C-1'), 119.5 (CH, C-6'), 115.8 (CH, C-5'), 115.1 CH, (C-2'), 103.0 (qC, C-10), 101.3 (CH, C-1"), 98.3 (CH, C-6), 93.5 (CH, C-8), , 77.2 (CH, C-3"), 75.9 (CH, C-5"), 73.2 (CH, C-2"), 69.7 (CH, C-4"), 60.7 (CH_2, C-6").

Estimation of total phenolic content and antioxidant activity of ACPE

Folin-Ciocalteu Assay

Different concentrations of ACPE or a pure phenolic compound (quercetin or gallic acid) in MeOH were mixed with 0.2 N Folin-Ciocalteu reagent in a ratio of 1:2.5 mL and processed according to a procedure of Singleton and Rossi (1965). The results are presented in table 1.

Estimation of 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The method of Brand-Williams *et al.,* (1995) was applied to measure the antioxidant activity of ACPE at different concentrations (250 to 0.4 μg/mL). The methanolic solution (1 mL) of ACPE or standard (rutin) was mixed with DPPH in MeOH (1 μM/125 μL) and MeOH (375 μL). After 30min of incubation, the absorbance "A" at 517 nm was measured and DPPH radical scavenging % (table 1) was calculated from the equation: $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$.

Biological Assays

Animals

Healthy male adult Wistar albino rats (150-170 g) were obtained from Animal Care Centre, College of Pharmacy, King Saud University. They were housed, handled, and fed according to the regular procedure (Al-Yahya *et al.,* 2013). The experiment conduct and the sacrifice procedure (under ether anaesthesia) were accepted by Ethics Committee of the Experimental Animal Care Society, King Saud University.

Acute toxicity test

The acute toxicity of ACPE was performed on rats (150-170 g) according to the OECD guideline No. 420.(OECD 2001).

Hepatorenal protective activity

CCl_4-induced hepatorenal toxicity and treatment experiment

After 1-week acclimatization period, the rats were randomly divided into 4 groups (I-V, 6animals/group). Groups I and II received vehicle (3% w/v gum acacia in distilled water, p.o.) and CCl_4 (i.p.) and kept as an untreated control and an intoxicated control, respectively. Groups III and IV were pretreated with ACPE at doses of 50 and 100 mg/kg/rat p.o., respectively, while group V was given silymarin at a dose of 10 mg/kg/rat p.o., for 17 days. Groups II-V were challenged with CCl_4 in liquid paraffin (1:1, 1.25 mL/kg/rat i.p.) At the 19th day, the blood was collected, serum was separated, and biochemical markers were analyzed as previously described (Al-Yahya *et al.*, 2013).

Assessment of serum marker enzymes and bilirubin

The biochemical parameters in serum samples were measured by the following colorimetric methods. Serum alanine and aspartate aminotransferases (ALT & AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), and bilirubin were determined as described by Reitman and Frankel (1957), King and Armstrong (1988), Fiala *et al.,* (1972), and Stiehl (1982), respectively.

Estimation of serum lipid profile

Triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were estimated by as previously described by Foster and Dunn (1973), Demacher and Hijamaus (1980), and Burstein and Scholnick (1973), respectively. Low- and very low-density lipoprotein cholesterol (LDL-C and VLDL-C), were derived from the equations of Friedward *et al.* (1972): LDL-C = TC - HDL-C -VLDL-C and VLDL-C = TG/5

Determination of serum creatinine, calcium, uric acid, and urea

Serum creatinine and calcium were determined by the methods described by Fabiny and Erthinghausen (1971) and Gitelman (1967) using kits CS604 and CE500 supplied by Crescent Diagnostics, Jeddah, Saudi Arabia, respectively. Uric acid and urea were estimated using the methods of Fossati *et al.* (1980) and Munan *et al.* (1978), respectively.

Determination of lipid peroxidation in liver and kidney tissues

A modified method (Al-Yahya *et al.,* 2013) of Utley *et al.* (1967) was used. The final reaction solution was measured at 535 nm and the lipid peroxidation was assessed in terms of nmol malondialdehyde (MDA) per gram of wet tissue.

Estimation of non-protein sulfhydryl (NP-SH) groups in liver and kidney tissues

NP-SH groups were measured in the liver or kidney tissue by the method described by Sedlak and Lindsay (1968). After homogenization of the organ in cold 0.02% EDTA solution, addition of 50% TCA, and centrifugation, an aliquot of supernatant was mixed with Tris buffer and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.1 mL) and then measured within 5 min against a reagent blank without homogenate at 412 nm.

Determination of total protein (TP) and albumin

TP and albumin were estimated according to the method reported by Doumas (1975) by kits (CS610 and CS600, respectively) supplied by Crescent Diagnostics. The measured absorbance (A) of the color at 546 nm is equivalent to the concentration of protein which was then calculated as TP = (A_{sample} /$A_{standard}$) x concentration of the standard.

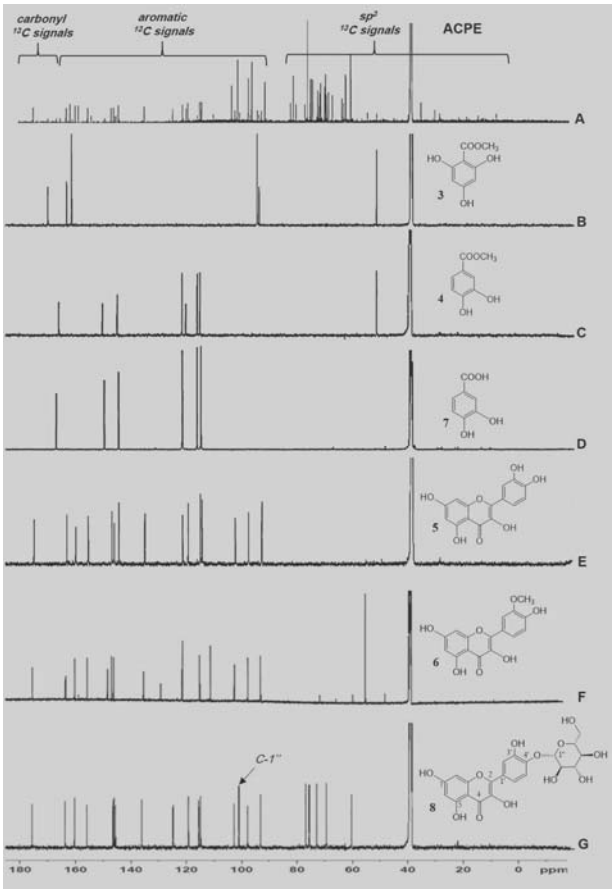

Fig. 1: ^{13}C NMR spectrum of ACPE (A) and ^{13}C NMR spectra of compounds 3 (B), 4 (C), 7 (D), 5 (E), 6 (F), and 8 (G) with the structures of compounds 3 - 8.

Anti-inflammatory activity

Carrageenan-induced rat hind paw edema assay is carried out according to the method of Winter *et al.,* (1962). The animals were divided into three groups (3 rats/group). A dose of carrageenan (1%, 0.2 ml) was inoculated into the rat paw. The 1st group was kept as a negative control. The 2nd and 3rd groups were treated with 500 mg/kg of aqueous suspension of ACPE and pure quercetin (isolated from ACPE, 100 mg/kg), i.p., separately, 1 hr before

carrageenan injection. The volume of paws was measured prior to carrageenan injection then 1 and 2 h afterwards using a Hydo-Plethysmograph (Model 7150, Ugo Basile, Italy) and the results was expressed as % inhibition of edema relative to control. The following equation: % inhibition = (PC- PT) x 100 / PC was used, where PC= increase in paw thickness of control group and PT= increase in paw thickness of the treatment group.

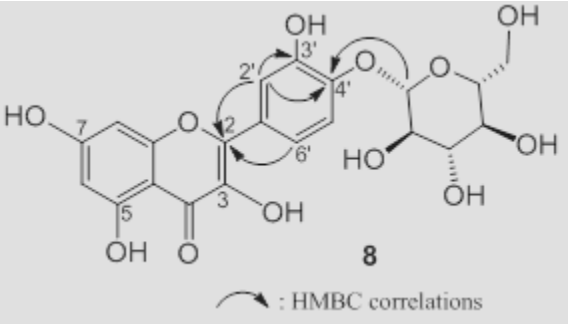

Fig. 2: Key HMBC correlations of compound 8.

Hot plate test

The analgesic activity of ACPE (500 mg/kg, p.o., group II) and quercetin (100 mg/kg, p.o., group III) were assessed separately in male albino mice, as previously described by Eddy and Leimbach (1953), in comparison with control animals (saline, p.o., group I). The animals (6/group) were placed in Perspex cylinder on a hot plate (Ugo Basile, Italy) with a temperature kept at 55±0.5 °C. The time elapsed till appearance of the signs of acute distress, was recorded as response latency. The response latency was measured at 0, 1, 2, 3 and 5 h after the drug intake with 30 sec cut-off time.

STATISTICAL ANALYSIS

Values are presented as means± S.E.M. (standard error of the mean). Data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test. The P value less than 0.05 was taken as a criterion for a statistically significant difference.

RESULTS

Extraction and isolation of ACPE constituents

Powdered red onion peel was thoroughly extracted with acetone-distilled water (7:3) mixture at RT and the solvent-free extract (ACPE) was successively fractionated with light petrol, $CHCl_3$, and EtOAc to yield FP, FC, and FE, respectively. Each fraction was separately subjected to series of chromatographic fractionation and purification to yield compounds 1 and 2 from FP; 3 from FC; and 4 - 8 from FE (fig. 1). The ^{13}C NMR spectrum of ACPE (fig. 1) indicated the presence of high intensity sp^2 carbon signals of phenolic (δ_C 145-165 ppm) and carbonyl moieties (δ_C 166-176 ppm) of extract rich in flavonol and phenolic acid derivatives. The sp^3 carbon signals appearing in the region of (δ_C 60.5-101.5) indicated that some of the phenolic/flavonoid constituents are in glycosidal form. Compound 8 was identified as quercetin 4`-O-*β*-D-glucopyranoside on the basis of 2D NMR correlation analysis (fig. 2) and spectroscopic comparison with quercetin.

Phenolic content and antioxidant capacity of ACPE

By using Folin-Ciocalteu and DPPH radical scavenging assays, ACPE showed a substantial phenolic content of 72.33±5.30 mg gallic acid equivalent (GAE)/g or 49.25±5.83 mg quercetin equivalent (QUE)/g and an antioxidant activity of IC_{50} 13.8 mg/mL (of more than 1/3 potency of the pure flavonoid rutin), respectively (table 1).

Assessment of the protective activity ACPE on liver and Kidney challenged with CCl_4

Prior to evaluation, an acute toxicity test was done and indicated the safety of ACPE at the level of twenty-fold higher (2 g/kg) than the therapeutic dose in rats. The hepatonephro-protective potentiality of the extract was assessed against an *in vivo* CCl_4-induced toxicity model by measuring the liver (ALT, AST, GGT, ALP, albumin, and components of lipid profile)-linked and kidney (creatinine, uric acid, urea, and calcium)-linked serum and tissue (TP, MDA, and NP-SH) markers. Pretreatment with ACPE has ameliorated most of the serum and tissue levels significantly in a dose dependent manner as illustrated in fig. 3 and tables 2 and 3.

The anti-inflammatory and analgesic effect of ACPE

ACPE and its major constituent quercetin significantly reduce the net carrageenan-induced edema in the paw of rats, after 2h (68.25% inhibition at 500 mg/kg and 42.86% inhibition at 100 mg/kg, respectively) (table 4). In another experiment, ACPE (500 mg/kg) and quercetin (100 mg/kg) when given to animals in the hot plate test for analgesia, separately, it was found that quercetin increased the pain threshold by only 4.9%. However, ACPE augmented the pain threshold by 15.5%.

DISCUSSION

Phenolic content and antioxidant capacity of ACPE

Unlike the steroidal nature of compounds 1 and 2, compounds 3 - 8 are UV active at 254 nm and further disclosed a phenolic and an antioxidant nature as revealed from the positive reactions given with 5% $FeCl_3$ and 0.1% DPPH spray reagents on Si gel TLC, respectively. The isolated compounds (fig. 1) were identified by physical, spectroscopic (including MS and NMR), and spectral comparison with literature data as β-sitosterol (1) and stigmasterol (2), methyl 2,4,6-trihydroxybenzoate (3), 3,4-dihydroxy benzoic acid methyl ester (methyl protocatechuate (4), quercetin (5), isorhamnetin (6), and

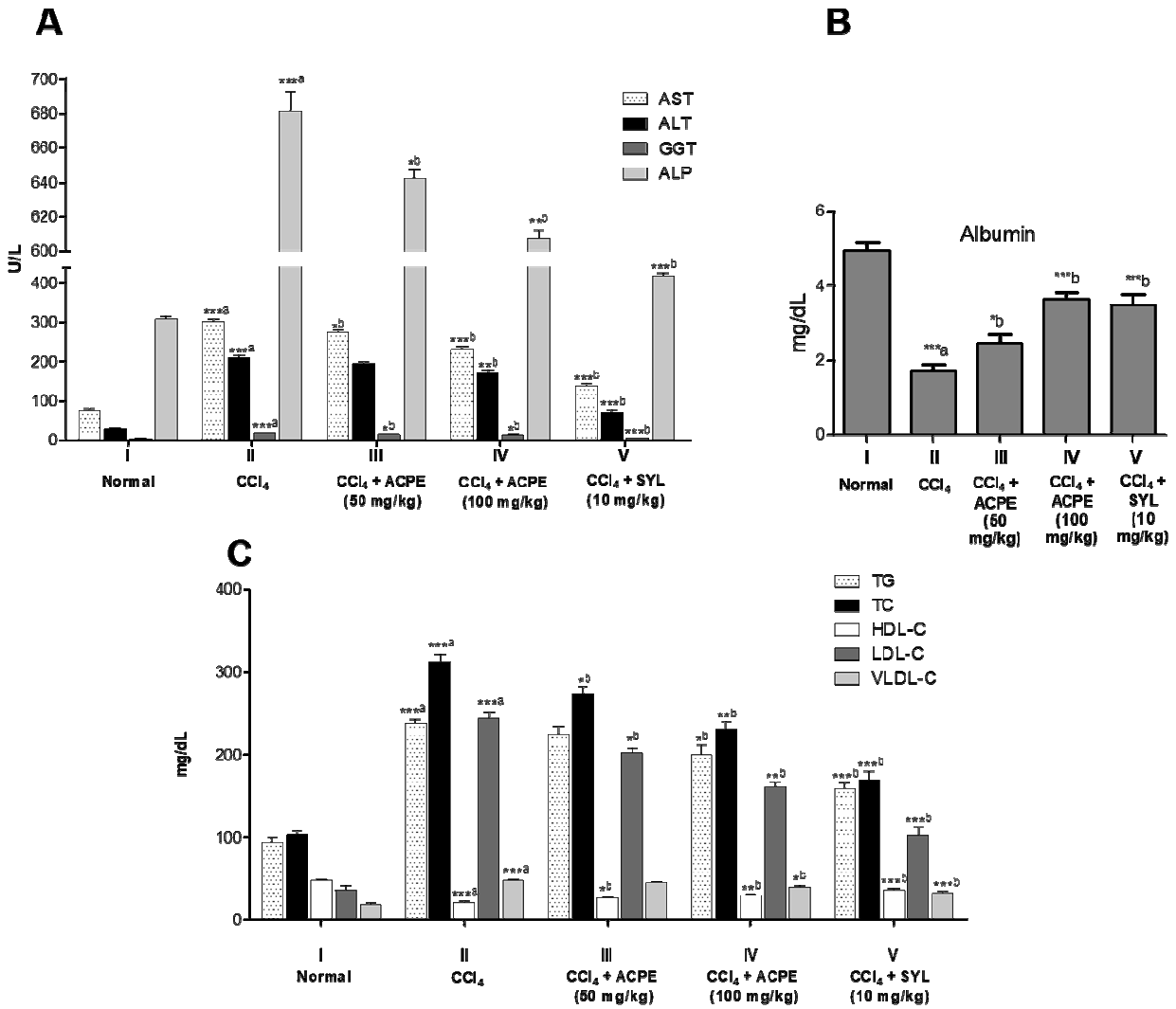

Fig. 3: The effect of ACPE on the biochemical parameters of CCl_4-intoxicated livers of rats. The effect of ACPE on concentrations of serum (A) liver-linked serum enzymes (AST, ALT, GGT, and ALP); (B) albumin; and (C) lipid profile (TG, TC, HDL-C, LDL-C, and VLDL-C); All values represent mean ± SEM (n = 6). *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. ^{a}As compared with normal group. ^{b}As compared with CCl_4 only group.

protocatechuic acid (7). Compound 8 was isolated as yellow powders, showed antioxidant property with DPPH reagent while its methanolic solution exhibited two UV absorption bands at 300-380 nm correspond to cinnamoyl (Band I) and at 240-280 nm correspond to benzoyl (Band II) moieties characteristic for flavonoids. It possessed a higher polarity (R_f = 0.15) relative to compound 5 (R_f = 0.59) on Si gel TLC, $CHCl_3$ -MeOH - AcOH (8.3:1.7:0.2). However, compound 8, relative to 5, exhibited six additional oxygenated sp^3 carbons in the ^{13}C NMR spectrum which were found correlated in the HSQC spectrum to five oxymethine and one oxymethylene protons at δ_H 3.21-4.86 of a β-D-glucopyranoside moiety. This finding together with NMR spectral comparison with compounds 5 suggested compound 8 as a quercetin glucoside. Since the quaternary carbons C-1` and H-5` of compounds 8 showed significant downfield shifts ($\Delta\delta_C$ + 3.2 and + 0.39 ppm, respectively) relative to those of compounds 5 isolated herein, thus the 4`-location of glucose moiety was suggested. The 4-linkage of the sugar moiety was finally confirmed by the HMBC correlation observed from the anomeric proton H-1`` (δ_H 4.86) to C-4` of ring B ($\delta_C$ 146.7) of the aglycone moiety (fig. 2). Compounds 8 was thus identified as quercetin 4`-O-β-D-glucopyranoside (spiraeoside) (Kajjout and Rolando 2011). It is noteworthy to mention that compounds 1 - 8 have been isolated herein for the first time from the peels of a Saudi cultivar of red onion. However, flavonoids 5, 6, and 8 were previously reported but in the inner scales of red onion var. Red Baron (Fossen *et al.,* 1998). Based on the ^{13}C NMR spectral comparison (fig. 1), it was revealed that ACPE is composed mainly of phenolic constituents as

depicted from their dense sp^2 aromatic and carbonyl carbon signals characteristic for flavonol and phenolic acid derivatives e.g. compounds 3 - 8. Therefore, it was concluded from table 1 that the considerable antioxidant activity of ACPE can be linked to its high phenolic content. Therefore, it was predictable that the extract would demonstrate a significant protection effect against the oxidative stress induced by free radicals as in cases of some hepatitis, nephritis, and inflammation.

Table 1: Total phenol content and antioxidant activity of ACPE

Index		Value
Phenol content[a]		
	mg GAE/g[b]	72.33±5.30
	mg QUE/g[c]	49.25±5.83
Antioxidant activity (IC_{50} µg/mL)[d]		13.8 [5.2][e]

Indices were measured by [a]Folin-Ciocalteau, [d]DPPH radical scavenging assays. The linear regression equations were [b]y = 0.0212x + 0.0548, r2=0.998 and [c]y=0.0193x + 0.1714, r2= 0.993). [e]A value obtained by a reference antioxidant flavonoid [rutin]. GAE=gallic acid equivalent, QUE=Quercetin equivalent, CT = (+) Catechin.

The protective activity ACPE on liver & Kidney challenged with CCl_4 and other activities

After intoxication, CCl_4 considerably elevate the level of ALT, AST, ALP and GGT and decreased the level of albumin (group II) relative to the normal control (group I). However, pre-treatment with ACPE succeeded to reduce the elevated levels of these enzymes (groups III and IV) in a dose-dependent manner (fig. 3A). Moreover, the extract strongly and significantly (p<0.001) enhanced the level of albumin (fig. 3B) at a dose of 100 mg/kg (group IV), similar to that observed by silymarin at 10 mg/kg (group V). ACPE has also ameliorated the levels of TG, total TC, HDL-C, LDL-C, and VLDL-C in a dose-dependent manner in comparison to those of the lipid profile of CCl_4 intoxicated control (fig. 3C). The effect was found to be much pronounced on the serum levels of TC, HDL-C, and LDL-C on pre-treatment with ACPE at 100 mg/kg. This finding is going similar to that exhibited by certain flavonoid-containing plant extracts on lipid profile relative to the intoxicated control (Al-Yahya *et al*., 2013). The intoxication with CCl_4 also resulted in impaired renal function associated with high level of serum creatinine, urea, uric acid, and calcium (table 2). Nevertheless, pretreatment with ACPE could, dose-dependently, attenuate these renal parameters in serum (groups III and IV) (table 2). ACPE achieved a high corrective effect against the nephrotoxicity-induced high levels of creatinine and uric acid at a dose of 100 mg/kg, as that exerted by silymarin (table 2). Moreover, as shown in table 3, the CCl_4-induced toxicity dramatically increased the level MDA (an index of lipid peroxidation) and decreased the tissue levels of NP-SH (non-protein sulfhydryl) and TP (an index of protein synthesis capacity). However, ACPE could, dose dependently, ameloriated the NP-SH level toward normal, particularly that of the liver tissue. Moreover, ACPE markedly reduced MDA level in liver and kidney relative to that of intoxicated control, being much pronounced in kidney tissues. ACPE also specifically and significantly increased kidney TP synthesis (table 3).

Since plant phenolics can limit the inflammatory responses through inhibiting oxidative stress (Li *et al.,* 2001) as that induced by carrageenan (Chaudhary *et al.,* 2015), ACPE was tested in this study as anti-inflammatory agent *in vivo*. ACPE could significantly reduce (68.25% inhibition) the net carrageenan-induced edema (an inflammation marker) in the paw of rats, after 2 h. However, the potency of ACPE is thought to be partially attributed to the high content of quercetin (table 4) which has been previously reported to possess an anti-inflammatory effect (Stewart et al., 2008). Moreover, when ACPE and quercetin were tested in animals as analgesics, it was found that the pure flavonoid increased the pain threshold by 1/3 of that exerted by ACPE. This indicated that the combination of quercetin and other constituents of the extract is superior to quercetin in the analgesic effect.

It is well known that free radical-induced xenobiotics such as CCl_4 play a key role in initiation of oxidative stress. The production of trichloromethyl and peroxy trichloromethyl radicals from CCl_4 by the metabolizing function of CYP2E1 has been shown liver (Lin *et al.,* 2008) and kidney (Khan *et al.,* 2010) injuries. These free radicals initiate lipid peroxidation and protein deterioration which alter cell permeability and function with a subsequent outflow of intercellular enzymes into serum (Nakamura *et al.,* 1985), change lipid and cholesterol metabolism, and induce oxidative DNA damage (Chen *et al.,* 2014). The tissue NP-SH, the non-enzymatic part of the antioxidant defense system (e.g. glutathione) may be also consumed (Dai *et al.,* 2014) in progression of the oxidative stress. So, scavenging of generated free radicals by antioxidant natural products would be a vital step in combatting liver and kidney ailments. Natural polyphenols which include flavan-3-ols, flavonols (e.g. quercetin), and phenolic acid derivatives are of abundant occurrence in food. The phenolic OH groups act as hydrogen or electron donors to stop the free radical chain reaction and thus natural polyphenols could be considered as a protective therapy against oxidative stress-related diseases. In this study, the hydro-acetone soluble phenolic-rich extract (ACPE) prepared from a Saudi cultivar red onion peel was found to mainly contain flavonol (5, 6, and 8) and phenolic acid (3, 4 and 7) derivatives as identified by phytochemical and NMR spectroscopic analyses (fig. 1). On the basis of its high phenolic content and strong DPPH free radical scavenging capacity (table 1), the protection of ACPE

Table 2: The effect of ACPE on serum concentrations of creatinine, uric acid, and calcium from control and different treated groups

Variables	Normal control	CCl_4	CCl_4 + ACPE (50 mg/kg)	CCl_4 + ACPE (100 mg/kg)	CCl_4 + silymarin (10 mg/kg)
Creatinine (mg/dL)	1.90±0.05	7.07±0.11^{***a}	6.53±0.60^{*b}	5.45±0.20^{***b}	6.38±0.14^{**b}
Uric acid (mg/dL)	1.20±0.09	6.42±0.42^{***a}	4.86±0.20^{**b}	3.29±0.21^{***b}	3.18±0.21^{***b}
Urea (mg/dL)	64.78±3.77	189.66±2.09^{***a}	185.16±5.74^{b}	167.66±3.48^{*b}	134.50±0.22^{**b}
Calcium (mg/dL)	7.17±0.43	29.52±0.84^{***a}	27.74±0.86^{*b}	22.15±0.58^{**b}	9.20±0.45^{***b}

Table 3: The effect of ACPE on tissue concentrations of TP, MDA, and NP-SH from liver and kidney of control and different treated groups.

Variables		Normal control	CCl_4	CCl_4 + ACPE (50 mg/kg)	CCl_4 + ACPE (100 mg/kg)	CCl_4 + silymarin (10 mg/kg)
Liver						
	TP (g/L)	110.97±7.21	51.09±2.52^{***a}	59.08±2.81^{b}	67.08±4.34^{*b}	82.63±4.04^{***b}
	MDA (nmol/g)	0.85±0.15	8.39±0.39^{***a}	7.96±0.89^{b}	5.52±0.31^{**b}	2.89±0.39^{***b}
	NP-SH (nmol/g)	7.24±0.69	3.36±0.46^{***a}	5.41±0.54*^{b}	7.35±0.63^{***b}	7.03±0.52^{***b}
Kidney						
	TP (g/L)	96.20±3.68	36.32±2.42^{***a}	41.91±3.94^{b}	53.89±2.11^{***b}	71.05±3.99^{***b}
	MDA (nmol/g)	0.65±0.13	9.08±0.58^{**a}	6.08±0.60^{**b}	4.52±0.49^{***b}	2.27±0.22^{***b}
	NP-SH (nmol/g)	9.61±0.41	4.95±0.40^{***a}	5.47±4.34^{b}	6.55±0.36^{*b}	8.63±0.37^{***b}

All values represent mean ± SEM (n = 6). *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. ^{a}As compared with normal group. ^{b}As compared with CCl_4 only group.

against free radical-induced hepatonephro-toxicity has been thus evidenced. The steroid, flavonoid, and phenolic acid derivatives exist in ACPE were proposed to play a major role in combatting the free radical-induced oxidative stress as following. Phytosterols such as β-sitosterol and stigmasterol has been found to act as a modest radical scavenger and as a biomembranes stabilizer (Yoshida and Niki 2003). The high amount of quercetin in ACPE is partially linked to the observed protective effect of the extract against chemically-induced hepatonephro-toxicity. This was substantiated by a protective effect exhibited by this flavonol against CCl_4-induced oxidative liver injury (Cui *et al.,* 2013). Mechanistically, quercetin and its 3-O-glycoside was proposed to ameliorate CCl_4-induced liver injury through acting as free radical scavenger, nuclear factor-Kappa B (NF-κB) activation inhibitor, antifibrotic, and nuclear factor E2–related factor 2/heme oxygenase-1 (Nrf2/HO-1) inducer (Domitrovic *et al.,* 2012). Moreover, in EtOH-induced liver injury, quercetin exhibited its protective effect by enhancing the antioxidant system, by induction of EtOH metabolizing enzymes, and by lowering the expressions of several pro-inflammatory cytokines (Chen 2010). The possible role of quercetin and its 3'-O-methylated derivative (isorhamnetin) in lowering liver thiobarbituric reactive substances (TBARS, e.g. MDA) has been also evidenced based on their antioxidative and superoxide anion suppressing activities (Igarashi and Ohmuma 1995). Furthermore, isorhamnetin protected against oxidative stress through activation of Nrf2 and induction of its genes expression (Yang *et al.,* 2014). Isorhamnetin also protected against arachidonic acid/iron-induced hepatotoxicity through inhibition of reactive oxygen species generation and glutathione reduction, and activation of AMP-activated protein kinase (AMPK) pathway (Dong *et al.,* 2014). Moreover, protocatechuic acid has been previously reported to possess potential antioxidant, anti-inflammatory, analgesic, and hepatoprotective activity (Kakkar and Bais 2014). However, the role of other constituents of ACPE in the hepatonephro-protective and anti-inflammatory activity in the extract cannot be ruled out.

Table 4: Effect of ACPE on carrageenan-induced inflammation in rat paw in comparison with quercetin (QU).

Treatment	Net edema in mL (% Inhibition) Normal control CCl_4	
	After 1 h	After 2 h
Control (saline)	0.36	0.63
ACPE (500 mg/kg)	0.23 (36.11%)	0.20 (68.25%)
QU (100 mg/kg)	0.34 (05.55%)	0.36 (42.86%)

CONCLUSION

The hydro-acetone extract of the red onion peels possesses high phenolic content and antioxidant activity and yielded six antioxidant compounds of flavonol and phenolic acid-based nature. ACPE demonstrated to be safe *in vivo* and exhibited hepato- and nephro-protective activity against xenbiotic-producing free radicals such as CCl_4 with the consequent recovery of the physiological function of the susceptible organs. Moreover, ACPE

demonstrated anti-inflammatory and analgesic properties. The extract possesses mainly various phenolic phytochemicals which can diminish the free radicals-induced oxidative stress. The outcomes of this work suggest that red onion peels can serve as a highly available and invaluable source of antioxidants, dietary supplements, or food additives or for improving the quality of human life.

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