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 (CCl₄) free radicals, in liver and kidney, was assessed in rats. Relative to the CCl₄-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 CCl₄ 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% AlCl₃ 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 GF₂₅₄. All solvents were of analytical reagent grade. 1H and 13C NMR spectra were recorded on a Bruker AM500 instrument at 500 MHz for 1H and 125 MHz for 13C in CDCl₃ or DMSO-d₆ with TMS as an internal standard (chemical shifts in δ, ppm). Ce(SO₄)₂ reagent (1%) was used for detection of compounds, 5% FeCl₃ 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.
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°C), CHCl3, 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). Two successive fractions eluted with 10% MeOH in CHCl3 afforded compound 3 (12.0 mg) and 5 (140 mg), respectively. FC was separated by Si gel CC using CHCl3 MeOH (100:0 to 0:100, gradient) to give compound 3 (10 mg). FE (25 g) was further separated over Si gel CC using light petrol – EtOAc (100:0 to 0:100, gradient) to 6 subfractions FE7.1– FE7.5. Among these fractions, FE7.2 and FE7.5 were subjected to Si gel column chromatography (CC), using light petrol EtOAc (100:0 to 0:100, gradient) as protocatechuic acid (He et al., 2012), 6 (Rf = 0.34 g) eluted by CHCl3 - MeOH (95:5) was purified by light petrol (40-60°C), CHCl3, and EtOAc to yield compound 8 (45 mg). FE7.5.1– FE7.5.6. Among these fractions, FE7.5.2– EtOAc (100:0 to 0:100, gradient) to 6 subfractions FE7.4.1– FE7.4.6. Fraction FE7.4.3 eluted with 1% MeOH in CHCl3 was further separated over Si gel CC using light petrol – EtOAc (100:0 to 0:100, gradient) to give 15 fractions (FE1 to FE15). FE4 and FE6 eluted with 2% and 4% MeOH in CHCl3, yielded compounds 1 (20 mg) and 2 (15 mg), respectively. FC was separated by Si gel CC using CHCl3 MeOH (100:0 to 0:100, gradient) to 12 subfractions (FE7.1 to FE7.12). Fraction FE7.2 eluted by 100% CHCl3 yielded another crop of compound 3 (8.0 mg). Fraction FE7.4 eluted with 1% MeOH in CHCl3 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 CHCl3 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 CHCl3, 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, 1H & 13C NMR spectral data, and spectral comparison with literature data, compounds 1 (Rf = 0.43 [Si gel TLC, light petrol – EtOAc, 8.5:1.5]) and 2 (Rf = 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 (Rf = 0.90 [Si gel TLC, CHCl3 - MeOH – AcOH, 8.3:1:7:0.2]) as methyl 2,4,6-trihydroxybenzoate (Lee et al., 2008), 4 (Rf = 0.85 [Si gel TLC, CHCl3-MeOH – AcOH, 8.3:1:7:0.2]) as methyl protocatechuate or methyl 3,4-dihydroxy benzoate (Miyazawa et al., 2003), 5 (Rf = 0.59 [Si gel TLC, CHCl3 - MeOH - AcOH, 8.3:1:7:0.2]) as quercetin (Kyriakou et al., 2012), 6 (Rf = 0.88 [Si gel TLC, CHCl3-MeOH - AcOH, 8.7:1:3:0.2]) as isorhamnetin (Lee et al., 2008), and 7 (Rf = 0.62 [Si gel TLC, CHCl3-MeOH - AcOH (8.3:1:7:0.2)] as protocatechic acid (He et al., 2009).

Estimation of total phenolic content and antioxidant activity of ACPE
Folin-Ciocalteau Assay
Different concentrations of ACPE or a pure phenolic compound (quercetin or gallic acid) in MeOH were mixed with 0.2 N Folin-Ciocalteau 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 30 min of incubation, the absorbance “A” at 517 nm was measured and DPPH radical scavenging % (table 1) was calculated from the equation: [(Ablank – Asample) / Ablank] x 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

$\text{CCl}_4$-induced hepatorenal toxicity and treatment experiment

After 1-week acclimatization period, the rats were randomly divided into 4 groups (I-V, 6 animals/group). Groups I and II received vehicle (3% w/v gum acacia in distilled water, p.o.) and $\text{CCl}_4$ (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 $\text{CCl}_4$ 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), $\gamma$-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 $\text{TP} = (A_{\text{sample}} / A_{\text{standard}}) \times \text{concentration of the standard.}$

![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.](image-url)
carrageenan injection. The volume of paws was measured prior to carrageenan injection then 1 and 2 h afterwards using a Hydro-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.

**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, CHCl3, 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 13C NMR spectrum of ACPE (fig. 1) indicated the presence of high intensity sp3 carbon signals of phenolic (δC 145-165 ppm) and carbonyl moieties (δC 166-176 ppm) of extract rich in flavonol and phenolic acid derivatives. The sp3 carbon signals appearing in the region of (δC 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 IC50 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 CCl4**

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 CCl4-induced toxicity model by measuring the liver (ALT, AST, GGT, ALP, albumin, and components of lipid profile)-linked and kidney (creatine, 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.

**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% FeCl3 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...
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 (Rf = 0.15) relative to compound 5 (Rf = 0.59) on Si gel TLC, CHCl3-MeOH - AcOH (8.3:1.7:0.2). However, compound 8, relative to 5, exhibited six additional oxygenated sp² carbons in the ¹³C NMR spectrum which were found correlated in the HSQC spectrum to five oxymethine and one oxymethylene protons at δH 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 (ΔδC + 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’ (δH 4.86) to C-4’ of ring B (δC 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 ¹³C NMR spectral comparison (fig. 1), it was revealed that ACPE is composed mainly of phenolic constituents as

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Fig. 3:** The effect of ACPE on the biochemical parameters of CCl₄-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 CCl₄ only group.
A heptonephro-protective phenolic-rich extract from red onion (Allium Cepa L) peels

depicted from their dense sp² 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

<table>
<thead>
<tr>
<th>Index</th>
<th>Value</th>
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<tbody>
<tr>
<td>Phenol contenta</td>
<td></td>
</tr>
<tr>
<td>mg GAE/g</td>
<td>72.33±5.30</td>
</tr>
<tr>
<td>mg QUE/g</td>
<td>49.25±5.83</td>
</tr>
<tr>
<td>Antioxidant activity (IC50 µg/mL)b</td>
<td>13.8 [5.2]</td>
</tr>
</tbody>
</table>

Indices were measured by *Folin-Ciocalteu, ‘DPHH radical scavenging assays. The linear regression equations were *y* = 0.0212x + 0.0548, r²=0.998 and *y* =0.0193x + 0.1714, r²=0.993. *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 CCl⁴ and other activities

After intoxication, CCl⁴ 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 CCl⁴ 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 CCl⁴ 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 CCl⁴-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, ameliorated 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 CCl⁴ play a key role in initiation of oxidative stress. The production of trichloromethyl and peroxy trichloromethyl radicals from CCl⁴ 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

1976
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 and its 3-O-glycoside was induced oxidative liver injury (Cui et al., 2013). 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 2: The effect of ACPE on serum concentrations of creatinine, uric acid, and calcium from control and different treated groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal control</th>
<th>CCl4</th>
<th>CCl4 + ACPE (50 mg/kg)</th>
<th>CCl4 + ACPE (100 mg/kg)</th>
<th>CCl4 + silymarin (10 mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.90±0.05</td>
<td>7.07±0.11 ***a</td>
<td>6.53±0.60 b</td>
<td>5.45±0.20 ***b</td>
<td>6.38±0.14 b</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>1.20±0.09</td>
<td>6.42±0.42 ***a</td>
<td>4.86±0.20 b</td>
<td>3.29±0.21 ***b</td>
<td>3.18±0.21 ***b</td>
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<tr>
<td>Urea (mg/dL)</td>
<td>64.78±3.77</td>
<td>189.66±2.09 **a</td>
<td>185.16±5.74 a</td>
<td>167.66±3.48 b</td>
<td>134.50±0.22 b</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>5.07±0.43</td>
<td>29.52±0.84 ***a</td>
<td>27.74±0.86 b</td>
<td>22.15±0.58 b</td>
<td>9.20±0.45 ***b</td>
</tr>
</tbody>
</table>

All values represent mean ± SEM (n = 6). *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test. As compared with normal group. As compared with CCl4 only group.

### 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.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal control</th>
<th>CCl4</th>
<th>CCl4 + ACPE (50 mg/kg)</th>
<th>CCl4 + ACPE (100 mg/kg)</th>
<th>CCl4 + silymarin (10 mg/kg)</th>
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<tr>
<td>Liver</td>
<td></td>
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<tr>
<td>TP (g/L)</td>
<td>110.97±7.21</td>
<td>51.09±2.52 ***a</td>
<td>59.08±2.81 b</td>
<td>67.08±4.34 b</td>
<td>82.63±4.04 ***b</td>
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<tr>
<td>MDA (nmol/g)</td>
<td>0.85±0.15</td>
<td>8.39±0.39 ***a</td>
<td>7.96±0.89 b</td>
<td>5.52±0.31 ***b</td>
<td>2.89±0.39 ***b</td>
</tr>
<tr>
<td>NP-SH (nmol/g)</td>
<td>7.24±0.69</td>
<td>3.36±0.46 ***a</td>
<td>5.41±0.54 a</td>
<td>7.35±0.63 ***b</td>
<td>7.03±0.52 ***b</td>
</tr>
<tr>
<td>Kidney</td>
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<tr>
<td>TP (g/L)</td>
<td>96.20±3.68</td>
<td>36.32±2.42 ***a</td>
<td>41.91±3.94 b</td>
<td>53.89±2.11 ***b</td>
<td>71.05±3.99 ***b</td>
</tr>
<tr>
<td>MDA (nmol/g)</td>
<td>0.65±0.13</td>
<td>9.08±0.58 ***a</td>
<td>6.08±0.60 b</td>
<td>4.52±0.49 ***b</td>
<td>2.27±0.22 ***b</td>
</tr>
<tr>
<td>NP-SH (nmol/g)</td>
<td>9.61±0.41</td>
<td>4.95±0.40 ***a</td>
<td>5.47±0.34 b</td>
<td>6.55±0.36 b</td>
<td>8.63±0.37 b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Net edema in mL (% Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal control CCl4</td>
</tr>
<tr>
<td></td>
<td>After 1 h</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>0.36</td>
</tr>
<tr>
<td>ACPE (500 mg/kg)</td>
<td>0.23 (36.11%)</td>
</tr>
<tr>
<td>QU (100 mg/kg)</td>
<td>0.34 (05.55%)</td>
</tr>
</tbody>
</table>

### 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 hepat- and nephro-protective activity against xenobiotic-producing free radicals such as CCl4 with the consequent recovery of the physiological function of the susceptible organs. Moreover, ACPE...
demonstrated anti-inflammatory and analgesic properties. The extract possesses many 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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