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Prunus avium lyophilized extract ameliorates renal oxidative injury: *in cellulo* and *in vivo* study

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ABSTRACT: *Prunus avium* (sweet cherry) fruits are rich in bioactive phytochemicals that exhibit potent antioxidant properties, as well as anti-inflammatory, anti-tumor, hypoglycemic, and anti-obesity activities. In this study, first, the lyophilized sweet cherry extract (LCE; 250 mg/ml) showed *in vitro* antioxidant potential comparable to that of ascorbic acid and rutin in DPPH free-radical scavenging and β-carotene-linoleic acid bleaching assays. Further, *in cellulo* assessment of LCE (200 µg/ml) showed restoration of cell viability in HEK293 (transformed kidney cells) and HUVEC (primary umbilical endothelial cells) by ∼19% ($p < 0.01$) by attenuating oxidative stress and reducing apoptotic cell death via caspase-3/7 activity by ∼18% against DCF-induced cytotoxicity. Further, in the CCl_4-treated rats, LCE (500 mg/kg) significantly lowered the serum levels of urea ($p < 0.001$), uric acid ($p < 0.001$), and creatinine ($p < 0.001$) to levels close to those observed with silymarin (standard) treatment. In addition, LCE significantly reduced renal malondialdehyde level and increased nonprotein sulfhydryl and total protein contents, indicating its *in vivo* antioxidative effects. Moreover, LCE also improved CCl_4-induced histological lesions by reducing necrosis and inflammation in kidney architecture. Further, the quantitative HPTLC validation showed presence of antioxidant phytoconstituents, β-sitosterol (1.45 µg/mg) and β-amyrin (0.61 µg/mg), in the extract. In conclusion, sweet cherry fruit significantly and potently protected against chemical-induced renal oxidative injury in both cell culture and a rat model, attributed to its antioxidant constituents.

KEYWORDS: *Prunus avium*, hepato-renal protection, carbon tetrachloride, antioxidant, cell culture, rat model

INTRODUCTION

A wide range of dietary and environmental factors contributes to metabolic diseases, including liver and kidney disorders. Despite breakthroughs and advances in modern therapeutics for the management of kidney diseases, there is a continuing effort to discover safer and alternative medicines, such as natural or plant-derived nutraceuticals [1]. In recent decades, several dietary supplements and natural xenobiotic molecules have been investigated for their nephroprotective efficacies.

Fruits of sweet cherry (*Prunus avium* L.; family: Rosaceae) are consumed in a variety of food preparations worldwide [2, 3]. Sweet cherry requires favorable cold season or low winter temperature to grow where different temperature conditions (heating and chilling) result in variation in flowering patterns. Because sweet cherry fruits are rapidly perishable postharvest, the preharvest methodologies and cold storage are required for immediate transport to markets. Several classes of phytochemicals with robust antioxidant properties, which are attributed to various pharmacological effects, have been isolated from sweet cherry fruits. The sweet cherry antioxidant phenolic compounds include polyphenols (ascorbic acid, *p*-coumoroquinic acid, and chlorogenic acid), flavonoids (quercetin and kaempferol, and their derivatives), catechins, and triterpenes (ursolic and oleanolic acids) [4, 5]. Notably, in sweet cherries, anthocyanins are the major phenolic compounds, mainly derivatives of cyanidins, pelargonidins, and peonidins, a major proportion of which exhibit hitherto identified bioactivities [6]. In addition, sweet cherries are also rich in melatonin, an antioxidant hormone [7], and volatile aromatic compounds such as hexanals [8].

Several health-protective benefits of sweet cherry consumption have been reported [9, 10]. Administration of sweet cherry extracts was shown to have anti-obesity [11] and anti-diabetic [12] effects in animal models. Other pharmacological activities of cherry include anticancer [13], antifungal [14] and anti-inflammatory [15] effects. In addition, cherry extracts were documented to possess modulatory effects on the cardiovascular system [16]. Recently, though the *in vitro* hepatoprotective effect of sweet cherry extract has been demonstrated in a liver cell (HepG2) culture model via antioxidative and free-radical scavenging activities [17], its *in vivo* hepatoprotective and renal protective effects remain elusive. In this study, we have

assessed the nephroprotective efficacy of sweet cherry fruits against chemical toxicity in cultured cells as well as in a rat model.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals (Sigma-Aldrich, Schnelldorf, Germany) and commercial diagnostic kits (Roche Diagnostics GmbH, Mannheim, Germany) were used for biochemical analyses using a Reflotron Plus Analyzer (Roche Diagnostics GmbH), and MDA, NP-SHb, and proteins were estimated using a Shimadzu UVmini-1240 spectrophotometer (Shimadzu Europe, Milano, Italy).

Experimental animals

Wistar albino rats (male; 3 months old; 170–180 g; $n = 6$ per group) were housed under controlled temperature (22–24 °C) and a 12-h dark/light cycle and received standard food and water according to CPCSEA (New Delhi) guidelines. The study was approved by the Institutional Animal Ethics Committee (Ref. no. 837/PO/Re/S/04/CPCSEA), IFTM University, Moradabad, India.

Collection of plant material and sample preparation

The fresh sweet cherry fruits (organically grown; size: large, dark red; cultivar: 0900 Ziraat; harvested in the Anatolia region; Supplier: Fresh Fruit Co., Muratpasa/Antalya, Türkiye) were purchased from a local supermarket in Riyadh and identified by the plant taxonomist Dr. Mohammad Yusuf (Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh). The fruits were washed with clean water, chopped into small pieces, and deseeded, prior to juice preparation in an electric juicer-blender. The juice was sterile-filtered and transferred into a glass container partially stoppered to allow vapor to escape. Lyophilization was performed at −40 °C using a freeze-drying system (Lyophilizer; Labconco, Kansas City, USA) until completely dry. The shelf temperature was increased further to remove unfrozen bound water or residual moisture, prior to complete sealing and removal of the glass container under vacuum from the lyophilizer. The dry powder (lyophilized sweet cherry extract; LCE) was then stored until it was tested in cultured cells or animals, while its methanolic extract was used in other assays.

Human cell culture

The human embryonic kidney (HEK293; stably transformed) and primary human umbilical vein endothelial (HUVEC 16549) cells (ATCC, Manassas, USA) were maintained in T75 flasks in DMEM culture medium (Gibco, Waltham, USA), supplemented with heat-inactivated fetal calf serum (10%; Gibco) and a penicillin-streptomycin mixture (1x; Gibco) at 37 °C in a 5% CO_2 incubator.

In cellulo nephroprotective and anti-apoptotic assays

HEK293 and HUVEC cells were seeded at a density of 0.5×10^5/100 µl/well (in triplicate) in a 96-well flat-bottom plate (Becton-Dickinson Labware, New Jersey, USA) a day prior to the assay. Cell protection or viability restoration efficacy of LCE was determined against 2,7-dichlorofluorescein (DCF; Sigma) in HEK293 (IC_{50}: 120 µg/ml) and HUVEC (IC_{50}: 32.5 µg/ml) cells, using the MTT assay (MTT-Cell Proliferation Assay Kit, Tervigen, USA) as previously described [18]. Four different concentrations of LCE (25, 50, 100, and 200 µg/ml) were first prepared in DMSO (>0.1%, final) and then diluted in DMEM to obtain the working concentrations, including untreated and DCFH-only-treated controls. After 48 h of incubation at 37 °C, cultures were subjected to an MTT assay according to the manufacturer's instructions.

Based on the nephroprotective activity, the two optimal doses (100 and 200 µg/ml) of LCE were assessed for caspase-3/7 activity (Apo-ONE Homogeneous Caspase 3/7 Assay Kit; Promega, Madison, USA) according to the manufacturer's instructions. In brief, HEK293 and HUVEC cells were exposed to DCFH to induce toxicity/apoptosis as mentioned above and then treated with the optimal doses of LCE. Following 48 h of incubation, caspase-3/7 reagent was added (100 µl/well) to the cultures and further incubated for 5 h in the dark at room temperature (RT), and the absorbance was recorded.

For both assays, absorbance at 570 nm was recorded using a microplate reader (ELx800; BioTek, Vermont, USA), and cell survival (%) was estimated relative to the untreated control using the equation: $[(A - B)/A] \times 100$, where A and B represented the absorbances of untreated and treated cultures, respectively. Data were analyzed (nonlinear regression; Excel 10.0, Microsoft, Redmond, USA) and presented relative to the untreated control.

In vitro DPPH free-radical scavenging assay

Free-radical scavenging activity of LCE using DPPH was estimated as described elsewhere with slight modifications [19]. Briefly, LCE was reconstituted in water to obtain different concentrations (10, 50, 100, 500, and 1000 µg/ml), with ascorbic acid as the standard, and 25 µl of DPPH (1 mM in methanol) and 375 µl of methanol were added. Following incubation for 30 min at 25 °C, absorbance at 517 nm was measured, and the percentage of scavenging activity was calculated using the following equation: [(Absorbance of control − Absorbance of sample)/Absorbance of control] × 100.

In vitro β-carotene-linoleic acid assay

The antioxidant activity of LCE was assessed using the β-carotene bleaching method as described elsewhere

[20], where the flavonoid rutin (1 mg/ml) was used as a standard. Absorbance at 470 nm at 15 min intervals was recorded, using a UV-visible spectrophotometer (UVmini-1240, Shimadzu Europe, Milano, Italy), and the percentage antioxidant activity was calculated using the following equation: 1 − [(Absorbance of sample at 0 min − Absorbance of sample at 120 min)/(Absorbance of control at 0 min − Absorbance of control at 120 min)] × 100.

Pre-treatment and carbon tetrachloride (CCL_4)-induced renal toxicity

The lyophilized cherry extract (LSE) was dissolved in distilled water for further experiments. Animals were divided into five groups (I–V). Group I received distilled water (normal control), while group II received distilled water and CCL_4 (1.25 ml/kg b.w.) in liquid paraffin (1:1) and served as the toxin control. The test groups III and IV were orally pretreated with LCE at doses of 250 and 500 mg/kg/b.w., respectively, for 21 days until sacrificed. Likewise, group V animals (standard control) were orally pretreated with silymarin (10 mg/kg b.w.) for 21 days until sacrificed. On day 16, groups II–V received CCL_4 as described for group II. After 72 h, 3 days (total treatment time: 21 days) rats were euthanized with pentobarbital sodium (50 mg/kg; Sigma-Aldrich, Schnelldorf, Germany) and sacrificed by cervical dislocation. Following death confirmation, blood was withdrawn via cardiac puncture, and serum was prepared (1000 × *g*, 20 min, 4 °C) and stored at −20 °C until analysis. The kidney was quickly dissected for biochemical and histopathological assessments.

Analysis of serum creatinine, urea, and uric acid

Serum creatinine levels were measured using the Jaffe reaction method described elsewhere [21], whereas serum levels of urea and uric acid were determined according to previously described methods [22] using Roche kits (Roche Diagnostics GmbH).

Estimation of tissue malondialdehyde (MDA)

Kidney tissue MDA levels were estimated by the method described elsewhere [22]. Briefly, dissected tissues were homogenized in ice-cold 0.15 M KCl (Potter-Elvehjem Type C Homogenizer; Thomas Fischer Scientific), and the absorbance of the homogenate at 532 nm was recorded. The MDA content (nmol/g wet tissue) was calculated using an MDA standard curve.

Estimation of nonprotein sulfhydryl (NP-SH) groups

The NP-SH content was measured following the method described elsewhere [23]. Briefly, tissue homogenization was performed in ice-cold 0.02 mM EDTA using Potter-Elvehjem Type-C Homogenizer. Following the addition of Ellman's reagent (DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); 0.02 ml), absorbance at 412 nm was recorded against a blank. NP-SH content (nmol/g wet tissue) was determined as follows: [Corrected absorbance × dilution factor/weight in g].

Determination of total protein

Tissue total protein was determined using a commercial kit (Crescent Diagnostics Kit, Jeddah, Saudi Arabia). The absorbance at 556 nm was recorded, and total protein content was calculated using the formula: Total protein = (Absorbance of sample/Absorbance of standard) × concentration of standard.

Histopathological evaluation

The dissected kidney specimen was washed with 1x PBS and fixed in 10% neutral buffered formalin for 48 h at RT, then processed overnight for dehydration and clearing, followed by embedding in paraffin blocks (Automatic Tissue Processor; Sakura Finetek Europe BV, Netherlands) and sectioning into 4-µm-thick sections (Rotary Microtome; RM2245, Leica Microsystems GmbH, Germany). Specimen sections were subjected to hematoxylin and eosin staining (2–3 min, RT), using a standard routine method [24]. The specimen was analyzed using a light microscope (Nikon OMX1200C, Japan) and photographed (×200 and ×400) with a mounted digital camera.

Bioactivity validation by high-performance thin layer chromatography (HPTLC)

A quantitative HPTLC method was applied to standardize the bioactivity of LCE using an HPTLC plate (10 × 10 cm; precoated silica gel F254) with two phytoconstituents, β-sitosterol and β-amyrin, as standard biomarkers, as described elsewhere [25]. Briefly, among several mobile phases optimized for good resolution and clear separation of different phytoconstituents present in the sample, hexane and ethyl acetate (7.5:2.5 v/v) were selected as a suitable mobile phase to carry out HPTLC. The two standards along with the test sample (prepared in methanol) were applied to the plate using a CAMAG Automatic TLC Sampler 4 (Basel, Switzerland), developed under controlled conditions using a CAMAG Automated Developing Chamber 2, and scanned at 540 nm using a CAMAG TLC Scanner 3.

Statistical analysis

All data were analysed using one-way analysis of variance (ANOVA; SPSS 20.0, Chicago, USA) and expressed as the mean ± SEM. Mean values among the groups were compared using Dunnett's multiple comparison test ($^{*}\,p < 0.05$; $^{**}\,p < 0.01$; $^{***}\,p < 0.001$).

RESULTS

In vitro antioxidant activity of LCE

The strong antioxidative efficacy of LCE was demonstrated by its DPPH free-radical scavenging and linoleic acid oxidation inhibition activities. LCE significantly reduced the stable DPPH to a yellow color at low concentrations (125 and 250 mg/ml), which was comparable to the ascorbic acid (Table 1). In addition, in the β-carotene/linoleic acid assay system, LCE also inhibited the bleaching of β-carotene at 125 mg/ml. Overall, the calculated total antioxidant value was 90.8%, which was comparable to that of rutin (Table 1).

In cellulo nephroprotective and anti-apoptotic effects of LCE

LCE treatment at 200 µg/ml restored the HEK293 and HUVEC cell viability by ∼17% ($p < 0.01$) and 19% ($p < 0.01$), respectively, compared with ∼6% and 8% activity by 100 µg/ml dose in relation to DCF-treated control (Fig. 1; left panel). Further, the anti-apoptotic marker analysis showed inhibition of caspase-3/7 activity by DCF in both cells. LCE treatment at 200 µg/ml enhanced caspase-3/7 activity by ∼27% ($p < 0.01$) in HEK293 and ∼28% ($p < 0.01$) in HUVEC cells compared with DCF-treated cells (Fig. 1; right panel). In comparison, at 100 µg/ml, the activity was ∼13.5% and ∼17%, respectively.

In vivo renoprotective effect of LCE

CCl_4 administration significantly increased the levels of serum urea, uric acid, and creatinine in rats, which were blunted by LCE (250 and 500 mg/kg b.w.) in a dose-dependent manner. Notably, the 500 mg/kg dose significantly lowered the levels of urea ($p < 0.001$), uric acid ($p < 0.001$), and creatinine ($p < 0.001$) to levels close to those observed with silymarin (positive control) (Table 2).

The modulatory effect of LCE on CCl_4-elicited redox imbalance and renal injury was further assessed in rat kidney tissues. In the experimental animals, CCl_4 treatment significantly increased renal MDA levels and decreased NP-SH and total protein (TP) levels compared with the control group (Fig. 2–Fig. 4). Compared with 250 mg/kg LCE, the 500 mg/kg dose significantly decreased CCl_4-induced MDA levels ($p < 0.001$), with an effect close to that of silymarin treatment (Fig. 2). Although there was a notable improvement in NP-SH levels in LCE-treated animals compared with CCl_4-treated group, the effects of both doses were almost similar ($p < 0.05$) (Fig. 3). Moreover, the higher dose significantly increased TP levels ($p < 0.01$) compared with the lower dose relative to the CCl_4-treated group (Fig. 4).

In addition, the histopathological analysis of kidney tissues from control (untreated) rats revealed normal glomeruli, interstitial tubules, and blood vessels, whereas the CCl_4-treated rats exhibited interstitial inflammation, focal tubular atrophy, glomerular congestion with vacuolization of the tuft, as well as sloughing of the renal tubular linings (Fig. 5). Notably, in rats treated with 500 mg/kg LCE, kidney tissues showed moderate interstitial nephritis, including some eosinophils, mild focal tubular atrophy, and tubular necrosis, as well as vacuolization of renal tubules (Fig. 5). Taken together, LCE improved CCl_4-induced tissue lesions by curtailing necrosis and inflammation in kidney architecture.

Identification of β-sitosterol and β-amyrin in LCE

The quantitative HPTLC analysis revealed the presence of β-sitosterol and β-amyrin in the extract (Fig. 6). Using the optimized mobile phase, compact spots of clearly separated β-sitosterol and β-amyrin were identified (Fig. S1; left panel), along with other unidentified phytoconstituents in the extract (Fig. S1; right panel). The estimated contents of β-sitosterol and β-amyrin were 1.45 µg/mg and 0.61 µg/mg, respectively, in the dried extract.

DISCUSSION

Chemical intoxication in animals causes pathological lesions of the kidney, mainly through reactive oxygen species (ROS)-induced oxidative stress triggered by the accumulation of unstable, oxygen-containing molecules in healthy tissues, resulting in organ toxicity, dysfunction, carcinoma, or failure [1]. Several medicinal plants with pharmacologically active secondary metabolites are capable of ROS scavenging and renal protection [26].

In recent decades, the production and consumption of sweet cherry fruits have increased due to growing consumer awareness of their nutraceutical value. The United States, Iran, and Türkiye are the major producers of sweet cherry. In this study, we comprehensively assessed the poorly investigated protective effect of sweet cherry extract against toxin-induced oxidative renal damage in a rat model. First, in the *in vitro* DPPH free-radical scavenging and β-carotene/linoleic acid bleaching assays, LCE exhibited strong antioxidant activity comparable to that of ascorbic acid and rutin used as standards. In experimental or *in vitro* settings, DCF is generally used to measure free radicals generated by oxidative stress via its oxidation [27]. In addition, DCFH has also been found to induce ROS-dependent cytotoxicity in cultured hepatic (HepG2), cardiac (H9C2), and renal (HEK293) cell lines [28–31]. *In cellulo* or cell culture assays provide the first-line biological evidence for testing therapeutics, including plant extracts or isolated phytochemicals. Therefore, we used DCF to induce cytotoxicity in cultured HEK cells, along with HUVEC cells as a primary cell control, and demonstrated the protective effect of LCE against DCF-induced cytotoxicity. Activation of the cellular cysteine proteases, i.e., caspase-3/7, is a major indicator of the early stage of apoptosis

Table 1 Free-radical scavenging and total antioxidant activities of LCE.

Sample	Radical scavenging activity (%)					Total antioxidant activity (%)
	10 (µg/ml)	50 (µg/ml)	100 (µg/ml)	500 (µg/ml)	1000 (µg/ml)	1000 (µg/ml)
LCE	5.1	20.6	41.2	50.2	62.4	71.8
Ascorbic acid*	16.8	80.2	90.1	94.8	94.9	–
Rutin*	–	–	–	–	–	90.9

LCE, lyophilized sweet cherry extract; * Standards.

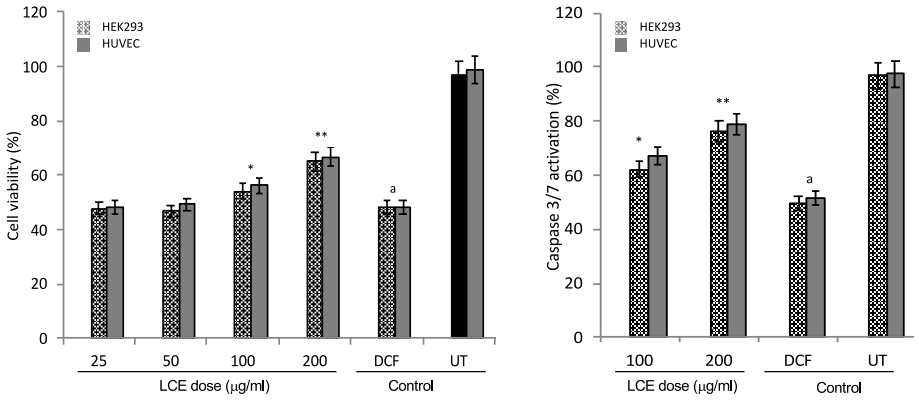

Fig. 1 The protective effect of LCE against oxidative cell damage (LCE: 25, 50, 100, and 200 µg/ml; left panel) and apoptotic cell death (LCE: 100 and 200 µg/ml; right panel) in 2,7-dichlorofluorescein (DCF) -treated HEK293 and HUVEC cells. All values are expressed as the mean ± SEM of triplicate measurements ($^{*}p < 0.05$, $^{**}p < 0.01$). [a] Compared with the untreated control (UT).

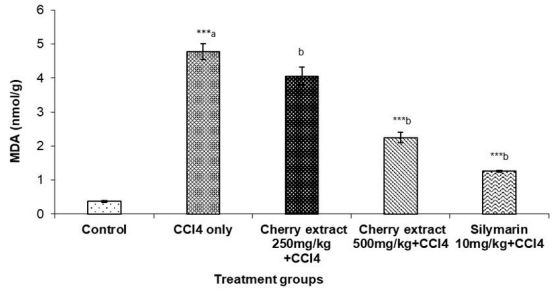

Fig. 2 Effect of LCE on kidney tissue malondialdehyde (MDA) concentration (nmol/g) in CCl_4-treated rats. All values are expressed as the mean ± SEM of triplicate measurements ($^{**}p < 0.01$). [a] Compared with the control group. [b] Compared with the CCl_4-only group.

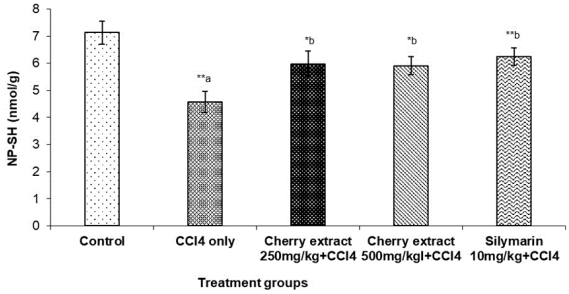

Fig. 3 Effect of LCE on kidney tissue nonprotein sulfhydryl (NP-SH) concentration (nmol/g) in CCl_4-treated rats. All values are expressed as the mean ± SEM of triplicate measurements ($^{*}p < 0.05$, $^{**}p < 0.01$). [a] Compared with the control group. [b] Compared with the CCl_4-only group.

Table 2 Effect of LCE on CCl_4-induced changes in kidney function parameters in rat serum.

Treatment group	Urea (nmol/l)	Uric acid (mg/dl)	Creatinine (mg/dl)
I. Control	38.53 ± 2.00	1.90 ± 0.05	1.03 ± 0.04
II. CCl_4 (1.25 ml/kg b.w)	$183.33 \pm 6.84^{***a}$	$5.08 \pm 0.11^{***a}$	$6.97 \pm 0.19^{***a}$
III. CCl_4+LCE (250 mg/ kg b.w.)	$160.33 \pm 5.42^{**b}$	$4.55 \pm 0.17^{*b}$	6.57 ± 0.13
IV. CCl_4+LCE (500 mg/kg b.w)	$125.50 \pm 5.95^{***b}$	$3.13 \pm 0.10^{***b}$	$4.68 \pm 0.17^{***b}$
V. CCl_4+Silymarin (10 mg/kg b.w)	$155.33 \pm 5.14^{*b}$	4.51 ± 0.28	$5.14 \pm 0.29^{***b}$

All values are expressed as the mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. [a] Compared with the normal control. [b] Compared with CCl_4-only group. LCE, lyophilized sweet cherry extract.

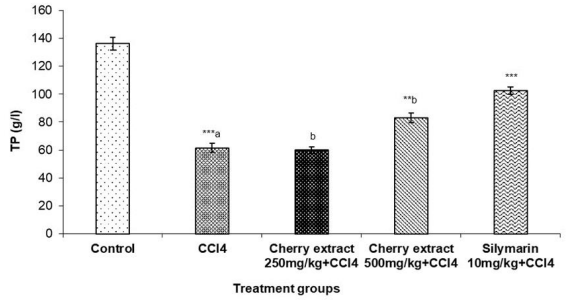

Fig. 4 Effect of LCE on kidney tissue total protein (TP) concentration (g/l) in CCl_4-treated rats. All values are expressed as the mean ± SEM of triplicate measurements ($^{**}p < 0.01$, $^{***}p < 0.001$). [a] Compared with the control group. [b] Compared with the CCl_4-only group.

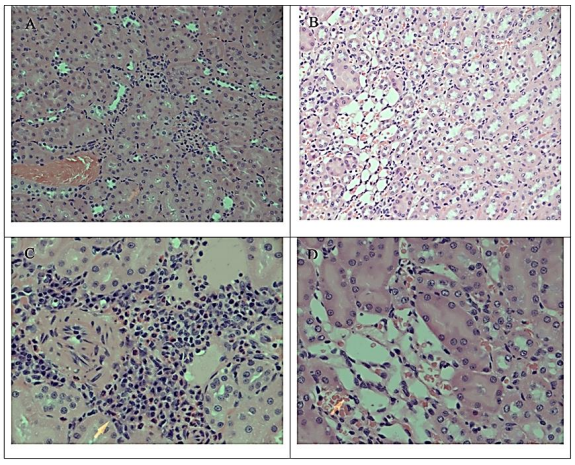

Fig. 5 Effect of LCE on histopathological changes in kidney tissues of CCl_4-treated rats. (A) Normal (control) tissues showing normal tubules and interstitium. (B) CCl_4-treated tissues with minimal interstitial inflammation and focal tubular atrophy. (C) LCE (250 mg/kg)-treated tissues with moderate interstitial nephritis, including some eosinophils. (D) LCE (500 mg/kg)-treated tissues with mild focal tubular atrophy. Photomicrograph (×400).

in living cells [32]. A quantitative analysis of caspase-3/7 production in cultured cells is a reliable tool for detecting and estimating cell death. Therefore, we also performed a caspase-3/7 activation assay to further establish the anti-apoptotic effect of LCE against DCF-induced cell death in both HEK and HUVEC cells.

Administration of toxins such as CCl_4 triggers oxidative damage to liver cells via mitochondrial membrane depolarization [33], which is paralleled by the upregulation of inflammatory cytokines and cyclooxygenases (COX-1 and COX-2) [34], and the downregulation of hepatic cytochrome enzymes (CYP450) [35]. Remarkably, in a previous report, bioactive constituents in cherry extracts were shown to inhibit

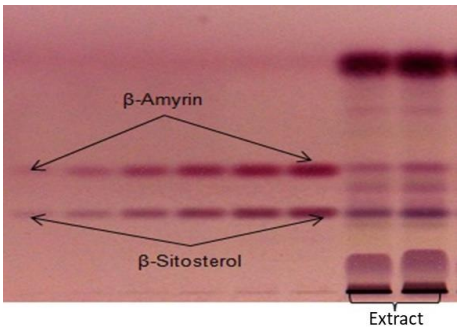

Fig. 6 HPTLC analysis (mobile phase: hexane:ethyl acetate; 7.5:2.5, v/v) of standard biomarkers, β-sitosterol and β-amyrin. Standard stock (100 µg/ml, each) further reconstituted and applied (left to right) at different concentrations (lanes 1–6: 10, 20, 40, 80, 100, and 120 ng/ml, respectively), followed by their detection in sweet cherry extract (lanes 7 and 8; in duplicate). The plate was derivatized with *p*-anisaldehyde in daylight.

CYP450 [14] as well as COX-1 and COX-2 [36]. In addition, in a recent study, lyophilized sweet cherry was demonstrated to decrease MDA content and activate antioxidant enzymes, viz., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), during the experimentally induced oxidative stress [37]. In our *in vivo* study, LCE treatment significantly abated CCl_4-elicited changes in serum and tissue biomarkers of renal injury. In addition, LCE improved CCl_4-triggered histological lesions by curtailing necrosis and inflammation in the kidney architecture. Taken together, sweet cherry significantly and potently protected against chemically induced renal oxidative injury in cultured kidney cells as well as in a rat model.

Mechanistically, the known biological or pharmacological effects of sweet cherry have been attributed to its bioactive phytoconstituents. Natural anthocyanins, known for their robust antioxidant properties, have been reported to attenuate oxidative stress and nephrotic damage in rats [38]. Because anthocyanins comprise the major portion of bioactive phytoconstituents in sweet cherry extracts, their nephroprotective effect is very likely mediated through the mitigation of cellular redox imbalances. In addition, bioactive triterpenes or triterpenoids, such as ursolic and oleanolic acids, have been previously reported in sweet cherry [7]. The biologically proven efficacy of plant extracts further needs to be validated to confirm the active constituents responsible for the bioactivity through analytical methods, such as spectroscopic and chromatographic techniques. In our quantitative HPTLC validation, considerable amounts of β-sitosterol and β-amyrin were found in sweet cherry extract. Notably, the triterpenes β-amyrin and β-sitosterol are known for their potent antimicrobial, anticancer, hepatoprotective, anti-inflammatory, and antioxidative properties

[39, 40]. Thus, consumption of sweet cherry fruits or fresh juice may have beneficial effects in counteracting oxidative damage to the kidney in humans. Taken together, we confirmed our results using two different toxins in two different experimental systems for renal protection, with validation by a chromatographic method.

CONCLUSION

In this study, the sweet cherry extract exhibited potent *in vitro* antioxidant and anti-apoptotic activities, efficiently restored kidney cell viability against dichlorofluorescein-induced oxidative and apoptotic cell death, and curtailed carbon tetrachloride-induced imbalances in serum biomarkers of renal injury, as supported by histological improvements in rat kidney. The observed bioactivity was further validated by the quantitative detection of two potent antioxidant phytoconstituents, β-sitosterol and β-amyrin, in the extract. Our data, therefore, warrant promising nephroprotective effects of sweet cherry against chemically induced oxidative kidney damage, mediated by its potent antioxidant constituents.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2026.077.

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Appendix A. Supplementary data

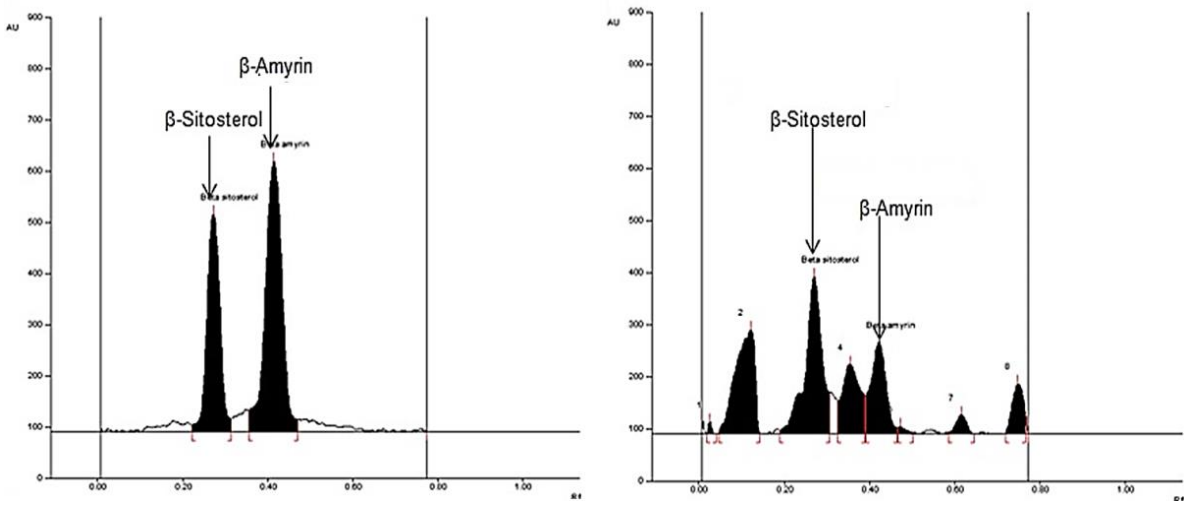

Fig. S1 Quantitative HPTLC analysis (mobile phase: hexane:ethyl acetate; 7.5:2.5, v/v) of standards: β-sitosterol ($R_f = 0.27$) and β-amyrin ($R_f = 0.42$) at 540 nm (left panel) and of extract: β-sitosterol (spot 3, $R_f = 0.27$) and β-amyrin (spot 5, $R_f = 0.42$) at 540 nm (right panel).