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Honey protects against cisplatin-induced hepatic and renal toxicity through inhibition of NF-κB-mediated COX-2 expression and the oxidative stress dependent BAX/Bcl-2/caspase-3 apoptotic pathway

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The protective effects of both manuka and talh honeys were assessed using a rat model of cisplatin (CISP)-induced hepatotoxicity and nephrotoxicity. The results revealed that both honeys exerted a protective effect against CISP-induced hepatotoxicity and nephrotoxicity as demonstrated by decreasing liver and kidney function. Manuka honey also prevented CISP-induced histopathological changes observed in the liver and decreased the changes seen in the kidneys. Talh honey decreased CISP-induced liver histopathological changes but had no effect on CISP-induced kidney histopathological changes. Both honeys reduced the oxidative stress in the liver. Conversely, they have no effect on kidney oxidative stress, except that manuka honey increased CAT activity. GC-MS analysis showed the presence of the antioxidant octadecanoic acid in talh honey while heneicosane and hydrocinnamic acid were present at a higher content in manuka honey. The molecular mechanism was to limit the expression of inflammatory signals, including COX-2 and NF-κB, and the expression of the apoptotic signal, BAX and caspase-3 while inducing Bcl-2 expression.

1. Introduction

Cisplatin (CISP) is a widely used chemotherapeutic anti-cancer drug. It is an important component of many chemotherapy regimens for cancers such as breast, metastatic ovarian, testicular, colorectal, lung, and neuroblastoma.[1] Several mechanisms explain the anticancer effect of CISP. The platinum component of CISP interacts with certain DNA bases to form covalent adducts, causing apoptosis in cancerous cells and other rapidly dividing cells.[2] CISP also triggers a cascade of proinflammatory interleukins, produces oxidative and nitrosative stress and activates the apoptotic cell death inducer protein BAX.[3–7] In spite of the tremendous advantages of CISP in cancer treatment, its clinical use is compromised by multiple systemic toxicities including gastrotoxicity, ototoxicity, testicular toxicity, myelosuppression, hepatotoxicity and nephrotoxicity.[8–12]

Honey is a complex natural solution, consisting mainly of fructose and glucose. Manuka (MAN) honey, native to New Zealand, is produced by bees that pollinate the manuka shrub. It is a unifloral honey with many biological effects, including antibacterial, antioxidant, and antiulcer activities. The major antibacterial constituents of MAN honey are hydrogen peroxide and methylglyoxal. MAN honey also contains a considerable number of flavonoid compounds, the main one being chrysin, which possesses potent free radical-scavenging activity.[13,14]

Talh (TALH) honey originates from the acacia plant of Saudi Arabia. Similar to MAN honey, it is a unifloral honey with antibacterial activity against select pathogenic bacteria. TALH honey is a dark honey that contains a significant number of phenolic compounds compared to the other types of Saudi honey, which tend to be light in color. It is suggested that

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TALH honey may have antioxidant properties based on its phenolic contents.[15,16]

Until now, there has been no effective, conclusive therapy that can prevent CISP-induced kidney and liver toxicity. In addition, no studies have examined the possible protective effects of MAN honey and TALH honey against CISP-induced nephrotoxicity and hepatotoxicity.

This study aimed to test the hypothesis that both honeys could reduce CISP-induced oxidative stress and hence protect against its liver and kidney toxicities compared to silymarin (SYL) as a control drug. The study also assessed the mechanism(s) of protection offered by MAN honey and TALH honey with special emphasis on free-radical scavenging and anti-inflammatory and anti-apoptotic activity.

2. Materials and methods

2.1. Chemicals

CISP (1 mg mL^{-1}; Mylan Institutional LLC, Rockford, IL, USA), SYL (Sigma, USA), and Royal Bee 20+ Active MAN Honey 100% (Royal Bee, New Zealand) were used in this study. TALH honey was purchased at the local market in Jeddah, Saudi Arabia. All the other chemicals used were of analytical grade, and all solvents were of HPLC grade.

2.2. Characterization of volatile compounds in TALH honey and MAN honey using solid phase extraction-gas chromatography/mass spectrometry analysis (SPE-GC/MS)

The method adopted was previously described in detail by El-Shitany *et al.*[17] A PerkinElmer Clarus 500 system (PerkinElmer, Shelton, CT, USA) was utilized throughout the experiments. The software controller/integrator was a TurboMass version 5.4.2.1617. An Elite-1 GC capillary column, Crossbond, 100% dimethyl polysiloxane (30 m × 0.25 mm id × 0.25_mdf, PerkinElmer) was used. The carrier gas was helium (purity 99.9999%), and the flow rate was 0.9 ml min^{-1}. The source (EI+) temperature was 250 °C. The GC line temperature was 200 °C. The electron energy was 70 eV, and the trap emission was 100 V. The oven was programmed as follows: the initial temperature was 80 °C (held 5 min) rising to 250 °C (rate 15 °C min^{-1}, held 5.0 min), followed by an increase to 280 °C (rate 20 °C min^{-1}, held 2 min). The injector temperature was 260 °C. The MS scan was from 45 to 350 *m/z* (500 scan per s). The injection volume was 1.0 μL, and the split ratio was 50 : 1. Samples were acquired by applying the positive total ion chromatogram (TIC). The NIST 2008 program was used for matching characterized compounds.

2.3. Animals

The male Sprague–Dawley rats used in the study weighed 150–180 g and were purchased from King Fahad Medical Research Center, KAU. The rats were maintained at room temperature with a 12-hour light cycle and had free access to a standard rodent diet and water *ad libitum*.

2.4. Experimental protocol

The rats were randomly divided into 5 groups (n = 6 rats per group). In Group I (the control group, CONT) the rats received intraperitoneal (i.p.) injections of normal saline. In Group II (the cisplatin-treated group, CISP), the rats received a single dose of CISP (7.5 mg kg^{-1}, i.p.).[7] In Group III (the silymarin-treated group, SYL), the rats received silymarin (100 mg kg^{-1}, i.p.)[18] for 10 continuous days and then a single dose of CISP (7.5 mg kg^{-1}, i.p.) on day 10. In Groups IV (the talh honey-treated group, TALH), the rats received TALH honey orally (2.5 g kg^{-1})[19] for 10 continuous days and then a single dose of CISP (7.5 mg kg^{-1}, i.p.) on day 10. In Group V (the manuka honey group, MAN), the rats received MAN honey orally (2.5 g kg^{-1})[19] for 10 continuous days and then a single dose of CISP (7.5 mg kg^{-1}, i.p.) on day 10. The experimental protocol was reviewed and approved by the Faculty of Pharmacy Research Ethics Committee at King Abdulaziz University, Saudi Arabia (reference no. 1438-109).

2.5. Sample collection

Twenty-four hours after the CISP injection, the animals in all the groups were anesthetized by ether inhalation. Blood samples were collected, through a direct intracardiac puncture, into sterile, labeled heparinized test tubes, allowed to stand for half an hour and then centrifuged at 500 g for 15 min at 4 °C to separate the plasma; they were then stored at −80 °C for the measurement of liver and kidney function. All the animals were then sacrificed by fast decapitation, and the liver and kidneys were dissected out. Parts of the liver and kidney tissue were fixed immediately in 10% buffered formaldehyde for histopathological and immunohistochemical studies. The other parts were weighed and homogenized immediately to obtain 50% (w/v) homogenate in ice-cold phosphate buffer (pH 7.4) +2% Triton X-100, then centrifuged at 500 g for 10 min at 4 °C. The supernatant was stored at −80 °C for the measurement of oxidative stress measures and antioxidant enzymes.

2.6. Measurements of liver and kidney function

The levels of plasma aspartate aminotransferase (AST),[20] alanine aminotransferase (ALT),[20] alkaline phosphatase (ALP),[21] creatinine[22] and urea[23] were assessed using detection kits from Human (Germany) for liver enzymes and Crescent Diagnostics (Saudi Arabia) for kidney function markers.

2.7. Measurement of oxidative stress markers and antioxidant enzymes

The levels of reduced glutathione (GSH),[24] malondialdehyde (MDA),[25] nitric oxide (NO),[26] catalase (CAT),[27] and glutathione peroxidase (GPx)[28] were assessed in the liver and kidney homogenates using commercially available kits (Biodiagnostic, Egypt) based on the manufacturer's instructions.

2.8. Histopathological examination

Formalin-fixed liver and kidney tissues were paraffin-embedded and sectioned into 4 μm slices. The sections were fixed onto slides and further stained with hematoxylin and eosin (H&E). The stained slides were examined and photographed using light microscopy.

2.9. Immunohistochemical staining

An immunoperoxidase (PAP, peroxidase/antiperoxidase) technique was used to stain the liver and kidney sections. BAX (catalog no. MS711B0), Bcl-2 (catalog no. MS123R7), nuclear factor-kappa B (NF-κB)/p65 (catalog no. RB-9034-R7), cyclooxygenase-2 (COX-2) (catalog no. RM9121R7) and caspase-3 (catalog no. RB1197R7) antibodies were purchased from Lab Vision (Fremont, CA). Images of the sections were acquired using light microscopy (Nikon Eclipse TE2000-U, NIKON, Japan) and analyzed with ImageJ analysis software (ImageJ, 1.46a, NIH, USA).

2.10. Semiquantitative reverse-transcriptase-polymerase chain reaction (RT-PCR)

Liver and kidney total RNA was extracted using an RNA extraction kit (Bio Basic Inc., Markham, ON, Canada) following the manufacturer's instructions. RNA was then transcribed using a Revert Aid(TM) first strand cDNA synthesis kit (Ferments Life Science, Fort Collins, CO, USA). Beta-actin (β-actin) was used as an internal control. Primers used were as follows: NF-κB sense primer: 5′ CATGAAGAGAAGACACTGACCATGGAAA3′, and the corresponding antisense primer: 5′TGGATAGAGGCTAAG TGTAGACACG 3′ (primer size 329 bp); BAX sense primer: 5′ GTTGCCCTCTTCTACTTTG 3′, and the corresponding antisense primer: Reverse 5′ AGCCACCCTGGTCTTG 3′ (primer size 194 bp).

2.10. Statistical analysis

The data are presented as the mean ± SEM. The values were compared by a one-way analysis of variance (ANOVA) followed by a Tukey–Kramer *post hoc* test (package Minitab 18, Minitab. Inc., State College, PA, USA). A P value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Characterization of volatile compounds in TALH honey and MAN honey (SPE-GC/MS)

MAN honey and TALH honey contain diverse volatile compounds. The most important are kojic acid, hydrocinnamic acid, eicosane, heneicosane, pentacosane, hexadecanoic acid, octadecanoic acid and oleic acid (Tables 1 and 2, Fig. 1A and B).

Octadecanoic acid constitutes 10.25% of the total volatile compounds of TALH honey, while heneicosane and hydrocinnamic acid constitute 7.03% and 6.9%, respectively, of the total volatile compounds of MAN honey (Fig. 1C).

3.2. Liver and kidney function

Liver enzymes (AST, ALT and ALP) were measured in the CONT and the CISP, SYL, TALH honey and MAN honey-treated animals in order to assess the influence of the treatment on liver function (Table 3). All three enzymes were found at markedly higher levels in the CISP-treated rats than in the CONT rats, which displayed normal levels for all. Pre-treatment with SYL, TALH honey and MAN honey resulted in reduced AST, ALT and ALP levels compared to those of the CISP-treated group.

The creatinine and urea levels were used to assess kidney function in the CONT and treated animals (Table 3). CISP-treated rats had significantly higher creatinine and urea levels compared to CONT rats. These levels were not restored by SYL pre-treatment. However, pretreatment with TALH honey and MAN honey resulted in a significantly lower creatinine and urea levels than in the CISP-treated group.

3.3. Oxidative stress markers

Malondialdehyde (MDA) levels were measured in the kidneys and liver and found to be significantly higher in CISP-treated rats than in CONT rats. The treatment of the rats with SYL, TALH honey and MAN honey significantly lowered the liver MDA levels compared to those in the CISP-treated rats. However, the kidney levels of MDA were similar in CISP, SYL, TALH honey and MAN honey-treated animals (Table 4).

Similarly, nitric oxide (NO) levels were measured in both the renal and hepatic tissues and were significantly higher in the CISP-treated animals than in the CONT group. SYL, TALH honey and MAN honey treatment resulted in statistically significant reductions of both liver and kidney NO concentrations when compared to CISP-treated animals (Table 4).

The levels of reduced glutathione (GSH) were measured in the liver and kidneys of the CONT group and the CISP, SYL, TALH honey and MAN honey treated animals (Table 4). The liver GSH levels were significantly lower in CISP-treated rats than in CONT rats. In contrast, the SYL, TALH honey and MAN honey-treated groups showed significantly higher hepatic GSH levels than the CISP-treated animals. In the kidneys, the CISP-treated animals displayed a significantly lower GSH concentration than the CONT animals. The levels of kidney GSH were similar in the CISP, SYL, TALH honey and MAN honey-treated animals.

3.4. Antioxidant enzymes

The levels of catalase (CAT) and glutathione peroxidase (GPx) were measured in the hepatic and renal tissues of all the groups of animals (Table 5). There was a significant reduction in CAT and GPx activity in the liver of CISP-treated rats. However, pretreatment with SYL, TALH honey and MAN honey resulted in a significant increase in the activity of liver CAT and GPx compared to the CISP group. In the kidneys, there was a significant drop in the activity of CAT and GPx enzymes in CISP-treated animals compared to CONT animals (Table 5). Pre-treatment with SYL resulted in a significantly higher

Table 1 Volatile constituents of TALH honey detected by SPE-GC/MS

Serial number	Retention (min)	Compound name	Relative area (%)
1	8.45	3-Methyl-2-buten-1-ol, trimethylsilyl ether	0.754
2	8.68	Propanoic acid, 3-(trimethylsilyl)-	1.702
3	9.05	Ethanol, 2-(trimethylsilyl)-, acetate	0.144
4	10.46	3,3-Dimethyl-2-butanol, trimethylsilyl ether	7.85
5	14.87	Butane, 2,3-bis(trimethylsiloxy)-	4.308
6	15.47	D-(−)-Lactic acid, trimethylsilyl ether, trimethylsilyl ester	7.675
7	16.07	Acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester	0.176
8	18.09	Trimethylsilyloxycyclobutane	0.141
9	19.13	Propanedioic acid, dimethyl-, bis(trimethylsilyl) ester	0.527
10	19.22	Propanoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.357
11	22.97	Silanamine, 1,1,1-trimethyl-*N*-(trimethylsilyl)-*N*-[2-[(trimethylsilyl)oxy]ethyl]-	2.410
12	23.66	Silane, trimethyl[1-phenyl-2-[2-(trimethylsilyl)-1-cyclopropen-1-yl]ethoxy]-	0.046
13	24.93	Trimethylsilyl ether of glycerol	1.386
14	25.08	Pentenoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (levulinic acid enol di-TMS)	1.231
15	27.63	2,3-Dimethyl-3-hydroxyglutaric acid, tris(trimethylsilyl)	0.066
16	29.63	2-Hydroxycyclohexane-1-carboxylic acid, di-TMS	0.052
17	30.37	2-(2,2,2-Trifluoroacetamido)acetic acid; *N*-trifluoroacetylglycine	0.259
18	31.27	Pentonic acid, 2-deoxy-3,5-bis-*O*-(trimethylsilyl)-, γ-lactone	0.123
19	33.44	Hydrocinnamic acid, α-(trimethylsiloxy)-, trimethylsilyl ester (benzenepropanoic acid, α-[(trimethylsilyl)oxy]-, trimethylsilyl ester)	0.329
20	35.87	4H-Pyran-4-one, 5-[(trimethylsilyl)oxy]-2-[[(trimethylsilyl)oxy]methyl]-(kojic acid, bis(trimethylsilyl) ether)	0.013
21	36.25	2-Propenoic acid, oxybis(methyl-2,1-ethanediyl) ester	0.324
22	38.67	Silane, dimethyl(2,6-dimethoxyphenoxy)butoxy-	0.025
23	39.39	Benzeneacetic acid, α-methoxy-, trimethylsilyl ester (mandelic acid)	0.964
24	40.20	Glucofuranoside, methyl 2,3,5,6-tetrakis-*O*-(trimethylsilyl)-, α-D-	0.010
25	40.72	D-Fructose, 1,3,4,5,6-pentakis-*O*-(trimethylsilyl)-	5.775
26	40.90	D-Fructose, 1,3,4,5,6-pentakis-*O*-(trimethylsilyl)-	3.878
27	41.35	Glucofuranoside, methyl 2,3,5,6-tetrakis-*O*-(trimethylsilyl)-, α-D-	0.547
28	41.79	D-Fructose, 1,3,4,5,6-pentakis-*O*-(trimethylsilyl)-	2.292
29	42.63	Xylulose, tetrakis(trimethylsilyl)-	3.862
30	43.51	Acrylic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.292
31	44.01	3,4-Dimethoxymandelic acid, di-TMS	0.176
32	45.02	Mannose, 2,3,4,5,6-pentakis-*O*-(trimethylsilyl)-, D-	1.539
33	45.70	2,3,4-Trimethoxymandelic acid, di-TMS	0.162
34	48.92	Oleic acid, trimethylsilyl ester	0.167
35	49.90	Octadecanoic acid, trimethylsilyl ester (stearic acid, trimethylsilyl ester)	1.105
36	56.57	Eicosane	0.661
37	57.57	Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	1.454
38	58.34	Heneicosane	0.136
39	58.48	Tetracosanoic acid, trimethylsilyl ester (lignoceric acid)	0.009
40	59.07	Bis(trimethylsilyl)monostearin (octadecanoic acid, 2,3-bis-(OTMS) propyl ester)	4.819
41	60.11	Pentacosane	0.009

activity of the two enzymes than in the CISP-treated group. TALH honey had no significant effect on the activity of CAT and GPx in the kidneys. However, in comparison with the CISP-treated group, pretreatment with MAN honey significantly augmented renal CAT activity without influencing GPx.

3.5. Histopathology

A histopathological examination of the rat liver was carried out in order to detect the protective effects of pre-treatment with SYL, TALH and MAN honey compared with CISP (Fig. 2A). CONT animals had a normal hepatocyte appearance. In contrast, CISP-treated animals showed centrilobular hepatocyte swelling and hydropic degeneration. Pre-treatment with SYL resulted in a normal hepatocyte architecture with few degenerated nuclei. Similarly, pre-treatment with TALH honey resulted in significant protection, with only a few cells displaying karyomegaly. Furthermore, MAN honey pre-treatment resulted in a normal hepatocyte architecture.

An examination of the kidney tubules was also carried out (Fig. 2B). Although CONT rats showed a normal intact epithelial lining with narrow lumina, CISP-treated animals showed tubular cells and dilated lumina with the presence of a few casts. SYL pre-treatment resulted in a normal appearance of the proximal tubules with an intact epithelium and distal tubules with dilated lumina. TALH honey pre-treated rats showed a marked dilation of the distal tubules and the presence of casts, while the MAN honey group showed protected tubules with few casts present.

3.6. Immunohistochemistry

The immunohistochemical expression of BAX, Bcl-2, NF-κB, COX-2 and caspase-3 was determined in the livers and kidneys of all the animal groups (Fig. 3A and B).

BAX expression was significantly higher in the livers and kidneys of the CISP-treated animals than in the CONT animals (Fig. 3C). Pre-treatment with SYL, TALH honey and MAN honey

Table 2 Volatile constituents of MAN honey detected by SPE-GC/MS

Serial number	Retention (min)	Compound name	Relative area (%)
1	8.34	3-Methyl-2-buten-1-ol, trimethylsilyl ether	1.105
2	8.5	Silane, trimethyl(pentyloxy)-	0.055
3	8.73	Propanoic acid, 3-(trimethylsilyl)-	0.30
4	8.93	Ethanol, 2-(trimethylsilyl)-, acetate	1.261
5	10.33	3,3-Dimethyl-2-butanol, trimethylsilyl ether	2.512
6	14.76	Butane, 2,3-bis(trimethylsiloxy)-	0.053
7	15.26	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.021
8	16.1	Acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester	0.082
9	16.66	Propanoic acid, 2-oxo-3-(trimethylsilyl)-, trimethylsilyl ester	0.051
10	17.65	Pentanoic acid, 4-oxo-, trimethylsilyl ester	0.017
11	18.13	Trimethylsilyloxycyclobutane	0.165
12	19.15	Propanedioic acid, dimethyl-, bis(trimethylsilyl) ester	0.047
13	19.24	Propanoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.083
14	22.08	3,7-Dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl-	0.103
15	22.2	3,7-Dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl-	2.575
16	22.98	Silanamine, 1,1,1-trimethyl-*N*-(trimethylsilyl)-*N*-[2-[(trimethylsilyl)oxy]ethyl]-	0.79
17	23.66	Silane, trimethyl[1-phenyl-2-[2-(trimethylsilyl)-1-cyclopropen-1-yl]ethoxy]-	0.086
18	24.95	Trimethylsilyl ether of glycerol	0.588
19	25.11	Pentenoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (levulinic acid enol di-TMS)	0.594
20	26.24	3,8-Dioxa-2,9-disiladec-5-ene, 2,2,9,9-tetramethyl-, (*E*)-	2.436
21	27.52	2,3-Dimethyl-3-hydroxyglutaric acid, tris(trimethylsilyl)	0.071
22	29.63	2-Hydroxycyclohexane-1-carboxylic acid, di-TMS	0.082
23	30.39	2-(2,2,2-Trifluoroacetamido)acetic acid; *N*-trifluoroacetylglycine	0.142
24	31.25	Pentonic acid, 2-deoxy-3,5-bis-*O*-(trimethylsilyl)-, γ-lactone	0.364
25	33.61	Hydrocinnamic acid, α-(trimethylsiloxy)-, trimethylsilyl ester (benzenepropanoic acid, α-[(trimethylsilyl)oxy]-, trimethylsilyl ester)	3.908
26	35.8	4H-Pyran-4-one, 5-[(trimethylsilyl)oxy]-2-[[(trimethylsilyl)oxy]methyl]-(kojic acid, bis(trimethylsilyl) ether)	0.193
27	36.24	2-Propenoic acid, oxybis(methyl-2,1-ethanediyl) ester	0.121
28	38.77	Silane, dimethyl(2,6-dimethoxyphenoxy)butoxy-	0.827
29	39.26	Benzeneacetic acid, α-methoxy-, trimethylsilyl ester (mandelic acid)	1.411
30	40.13	Glucofuranoside, methyl 2,3,5,6-tetrakis-*O*-(trimethylsilyl)-, α-D-	0.353
31	40.73	D-Fructose, 1,3,4,5,6-pentakis-*O*-(trimethylsilyl)-	9.807
32	40.91	D-Fructose, 1,3,4,5,6-pentakis-*O*-(trimethylsilyl)-	6.964
33	41.34	Glucofuranoside, methyl 2,3,5,6-tetrakis-*O*-(trimethylsilyl)-, α-D-	0.45
34	41.77	D-Fructose, 1,3,4,5,6-pentakis-*O*-(trimethylsilyl)-	1.821
35	42.63	Xylulose tetrakis(trimethylsilyl)-	4.839
36	43.51	Acrylic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.326
37	43.91	3,4-Dimethoxymandelic acid, di-TMS	0.188
38	45.02	Mannose, 2,3,4,5,6-pentakis-*O*-(trimethylsilyl)-, D-	1.964
39	45.72	2,3,4-Trimethoxymandelic acid, di-TMS	0.286
40	48.94	Oleic acid, trimethylsilyl ester	0.207
41	49.90	Octadecanoic acid, trimethylsilyl ester (stearic acid, trimethylsilyl ester)	0.204
42	56.57	Eicosane	1.288
43	57.57	Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	0.422
44	58.34	Heneicosane	3.959
45	58.42	Tetracosanoic acid, trimethylsilyl ester (lignoceric acid)	1.622
46	60.04	Pentacosane	1.546

resulted in a significant reduction of BAX expression in both liver and kidneys compared to the CISP-treated animals (Fig. 3C).

Conversely, the Bcl-2 levels in the hepatic and renal tissues were significantly lower in the CISP-treated animals than in the CONT animals (Fig. 3D). Pre-treatment with SYL, TALH honey and MAN honey resulted in the restoration of Bcl-2 levels in both the liver and kidneys compared to the CISP-treated group (Fig. 3D).

Furthermore, NF-κB expression in the liver and kidneys was augmented after CISP treatment and showed significantly higher expression levels than in the CONT animals (Fig. 3E). This increase was reversed by pre-treatment with SYL, TALH honey and MAN honey (Fig. 3E).

Similarly, COX-2 expression was found to be much higher in the renal and hepatic tissues of CISP-treated rats than in CONT rats (Fig. 3F). The expression of COX-2 decreased significantly after pre-treatment with SYL, TALH honey and MAN honey compared to the CISP group (Fig. 3F).

Caspase-3 expression in the liver and kidneys was augmented after CISP treatment and showed significantly higher expression levels than in the CONT animals (Fig. 3G). This increase was reversed by pre-treatment with SYL, TALH honey and MAN honey (Fig. 3G).

3.7. Semiquantitative RT-PCR

Both NF-κB mRNA and BAX mRNA expressions in the liver and kidneys were augmented after CISP treatment and showed sig-

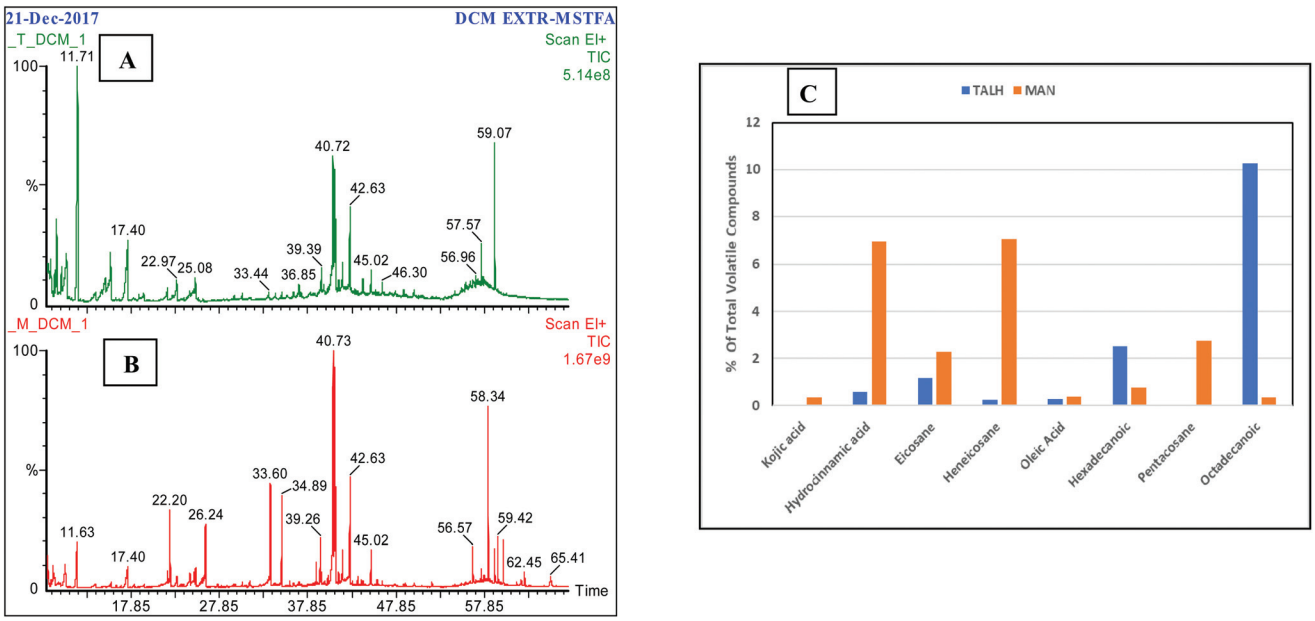

Fig. 1 (A) Chromatogram obtained from SPE-GC/MS of TALH honey; (B) chromatogram obtained from SPE-GC/MS of MAN honey; (C) antioxidant bioactive constituents present in both TALH and MAN honey.

Table 3 Measurements of liver and kidney function in control (CONT), cisplatin (CISP), silymarin (SYL), talh honey (TALH) and manuka honey (MAN) groups

	Liver function		Kidney function		
Group	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})
CONT	94.7 ± 12.7	18.2 ± 2.2	128.1 ± 9.5	0.53 ± 0.04	27.56 ± 1.87
CISP	179.5 ± 6.4^a	43.5 ± 5.2^a	197.9 ± 23.9^a	2.32 ± 0.08^a	191.82 ± 8.05^a
SYL	105.5 ± 6.1^b	13.8 ± 2.4^b	120.9 ± 11.3^b	2.38 ± 0.45	192.29 ± 8.19
TALH	131.8 ± 8.9^b	18.83 ± 2.0^b	134.0 ± 14.3^b	0.72 ± 0.07^b	147.82 ± 12.87^b
MAN	131.8 ± 5.0^b	30.5 ± 1.3^b	126.7 ± 8.9^b	0.67 ± 0.02^b	163.71 ± 3.56^b

Results are presented as mean ± SEM (n = 6). ^{a} $P \leq 0.05$ compared to CONT. ^{b} $P \leq 0.05$ compared to CISP.

Table 4 Measurement of liver and kidney malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH) in control (CONT), cisplatin (CISP), silymarin (SYL), talh honey (TALH) and manuka honey (MAN) groups

	Liver			Kidney		
Group	MDA (μM g^{-1} tissue)	NO (μM g^{-1} tissue)	GSH (mg g^{-1} tissue)	MDA (μM g^{-1} tissue)	NO (μM g^{-1} tissue)	GSH (mg g^{-1} tissue)
CONT	103 ± 6	43 ± 2.8	5.6 ± 0.4	60 ± 9	58 ± 12.3	5.2 ± 0.2
CISP	176 ± 14^a	130 ± 6.3^a	4.1 ± 0.1^a	102 ± 8^a	163 ± 16.3^a	4.4 ± 0.2^a
SYL	113 ± 24^b	94 ± 7.4^b	5.8 ± 0.4^b	117 ± 18	46 ± 8.6^b	4.8 ± 0.5
TALH	129 ± 14^b	98 ± 7.9^b	4.9 ± 0.2^b	90 ± 8	92 ± 7.5^b	5.3 ± 0.3
MAN	106 ± 10^b	69 ± 10.8^b	4.9 ± 0.1^b	89 ± 10	73 ± 8.7^b	4.9 ± 0.4

Results are presented as mean ± SEM (n = 6). ^{a} $P \leq 0.05$ compared to CONT. ^{b} $P \leq 0.05$ compared to CISP.

nificantly higher expression levels than in the CONT animals. Treatment of the rats with SYL, TALH honey and MAN honey significantly lowered the liver and kidney NF-κB mRNA and BAX mRNA expression compared to CISP-treated rats (Fig. 4A and B).

4. Discussion

In the present study, the protective effects of two types of honey, New Zealand MAN honey and Saudi TALH honey, were assessed using a rat model of CISP-induced hepatotoxicity and

Table 5 Measurement of liver and kidney catalase (CAT) and glutathione peroxidase (GPx) in control (CONT), cisplatin (CISP), silymarin (SYL), talh honey (TALH) and manuka honey (MAN) groups

	Liver		Kidney	
Group	CAT (U g^{-1} tissue)	GPx (U g^{-1} tissue)	CAT (U g^{-1} tissue)	GPx (U g^{-1} tissue)
CONT	21.1 ± 3.7	128.1 ± 9.5	19.6 ± 3.9	334.4 ± 11.4
CISP	8.2 ± 2.5[a]	92.4 ± 3.7[a]	9.2 ± 2.1[a]	90.0 ± 6.7[a]
SYL	31.6 ± 3.2[b]	167.5 ± 18.6[b]	19.0 ± 3.0[b]	190.6 ± 22.8[b]
TALH	40.5 ± 5.3[b]	174.7 ± 12.6[b]	9.4 ± 1.0	115.3 ± 15.8
MAN	35.6 ± 1.8[b]	151.5 ± 15.3[b]	26.9 ± 6.1[b]	75.4 ± 10.8

Results are presented as mean ± SEM (n = 6). [a] $P \leq 0.05$ compared to CONT. [b] $P \leq 0.05$ compared to CISP.

nephrotoxicity. The results revealed that both MAN honey and TALH honey exerted a protective effect against CISP-induced hepatotoxicity and nephrotoxicity, as demonstrated by analyses of liver and kidney function. In addition, MAN honey prevented CISP-induced histopathological changes observed in the rat liver and decreased the changes seen in the kidneys. In contrast, TALH honey decreased CISP-induced liver histopathological changes but had no significant effect on CISP-induced kidney histopathological changes. Because the elimination of urea and creatine from the body occurs mainly through the kidneys, the serum levels of these compounds are reliable as signs of the renal toxicity of CISP or as indicators of prevention.[29]

In agreement with our data, Karadeniz *et al.*[30] found a protective effect of royal jelly against CISP-induced hepatotoxicity and nephrotoxicity in rats. Recently, Osama *et al.*[31] reported a protective effect of bee honey and royal jelly against CISP-induced kidney toxicity in cancer patients treated with CISP. Similarly, royal jelly and bee honey protected against CISP-induced nephrotoxicity in rats.[32,33]

We suggest that the nephrotoxicity induced by CISP is unlikely to be fully cured by honey consumption because CISP accumulates significantly in the kidneys, more so than in other organs such as the liver. This effect on the kidneys occurs through a special organic cation receptor-2 (Oct-2).[34,35] In addition, the kidneys are considered the primary route by which CISP is eliminated from the body. The CISP concentration in the S3 segment of the proximal tubule is about five times the concentration in the serum.[36,37]

This study showed that both TALH honey and MAN honey reduced CISP-induced oxidative stress in the liver. Conversely, neither TALH honey nor MAN honey showed protection against CISP-induced oxidative stress in the kidneys, except that MAN honey protects against a CISP-induced reduction in CAT antioxidant enzyme activity in the kidneys. The inability of TALH honey and MAN honey to decrease CISP-induced oxidative stress in the kidneys is because both honey types have insufficient capability to completely prevent CISP-induced nephrotoxicity. CISP binds to the sulfhydryl groups of all molecules with low and high molecular weights. This could explain the reduction in GSH levels measured in the liver and kidneys, as well as the altered cellular redox state and, hence, CISP-induced toxicity.[38]

Previous studies have shown that honey contains numerous phenolic and non-phenolic antioxidant ingredients.[39] The phenolic constituents participate significantly in the antioxidant activity of honey but are not the sole contributing compounds. Gheldof *et al.*[40] reported that the antioxidant power of honey was a consequence of the integrated activity of a vast range of constituents including phenolics, peptides, organic acids,

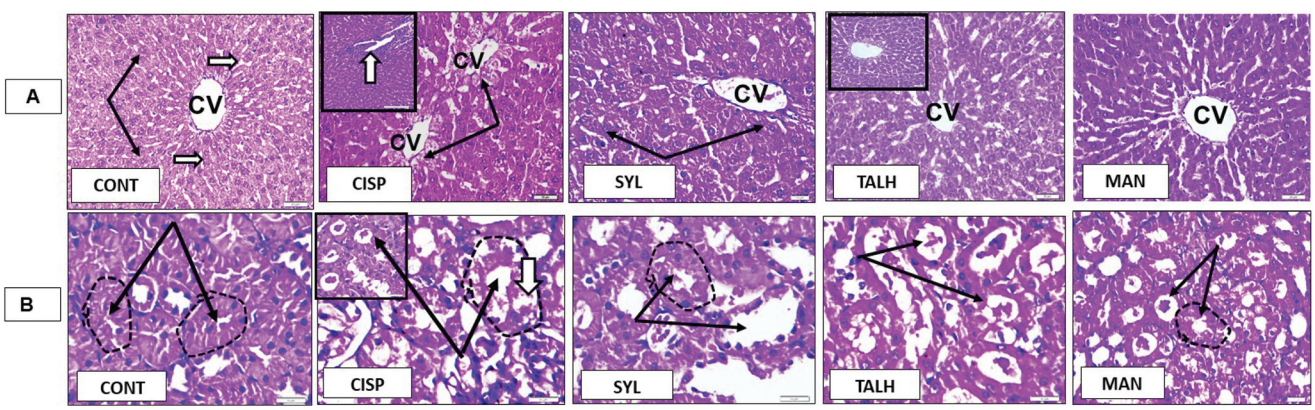

Fig. 2 (A) Rat liver at the central vein region (CV), CONT: control showing normal hepatocyte cell cords (arrows) radiating from the central vein (CV) and blood sinusoids among the cells of normal appearance (white arrows); CISP: cisplatin treatment shows centrilobular hepatocyte swelling and hydropic degeneration (black arrows), and bile duct proliferation (inset with white arrow); SYL: silymarin pre-treatment shows normal hepatocyte architecture with few degenerated nuclei; TALH: talh honey pre-treatment shows marked protection with only few cells showing karyomegaly (arrows); MAN: manuka honey pre-treatment shows normal hepatocyte architecture (arrows). (B) Rat kidney tubules, CONT: control shows normal, intact epithelial lining (dotted circles) and narrow lumina (arrows); CISP: cisplatin treatment shows tubular cells, unstained regions (white arrow) and dilated lumina (black arrows); some contain casts (insert); SYL: silymarin pre-treatment shows normal proximal tubules with intact epithelium (dotted circles), distal tubules with dilated lumina (arrows); TALH: talh honey pre-treatment shows marked dilation of distal tubules and presence of casts (arrows); MAN: manuka honey pre-treatment shows potential protection of tubules (dotted circle) with a few containing casts (arrows). (H&E stain ×400).

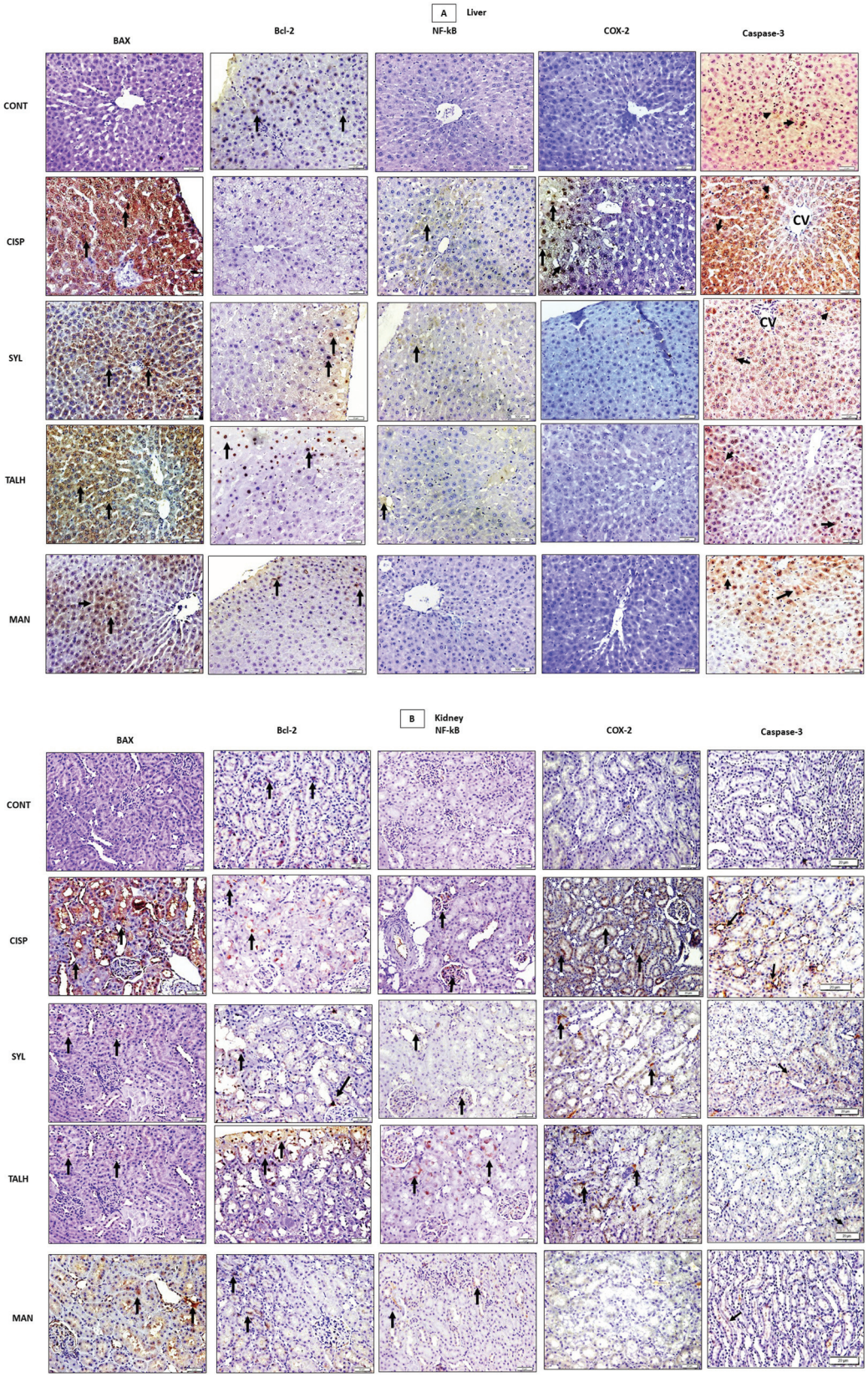

Fig. 3 Photomicrographs show (A) liver and (B) kidney immune expression of BAX, Bcl2, NF-κB, COX-2 and caspase-3 in control (CONT), cisplatin (CISP), silymarin (SYL), talh honey (TALH) and manuka honey (MAN). The bar charts C, D, E, F, and G show liver and kidney BAX, Bcl2, NF-κB, COX-2 and caspase-3 OD values, respectively, in the different experimental groups; results are presented as mean ± SEM (n = 6). *$P \leq 0.05$ compared to CONT; $^{\#}P \leq 0.05$ compared to CISP.

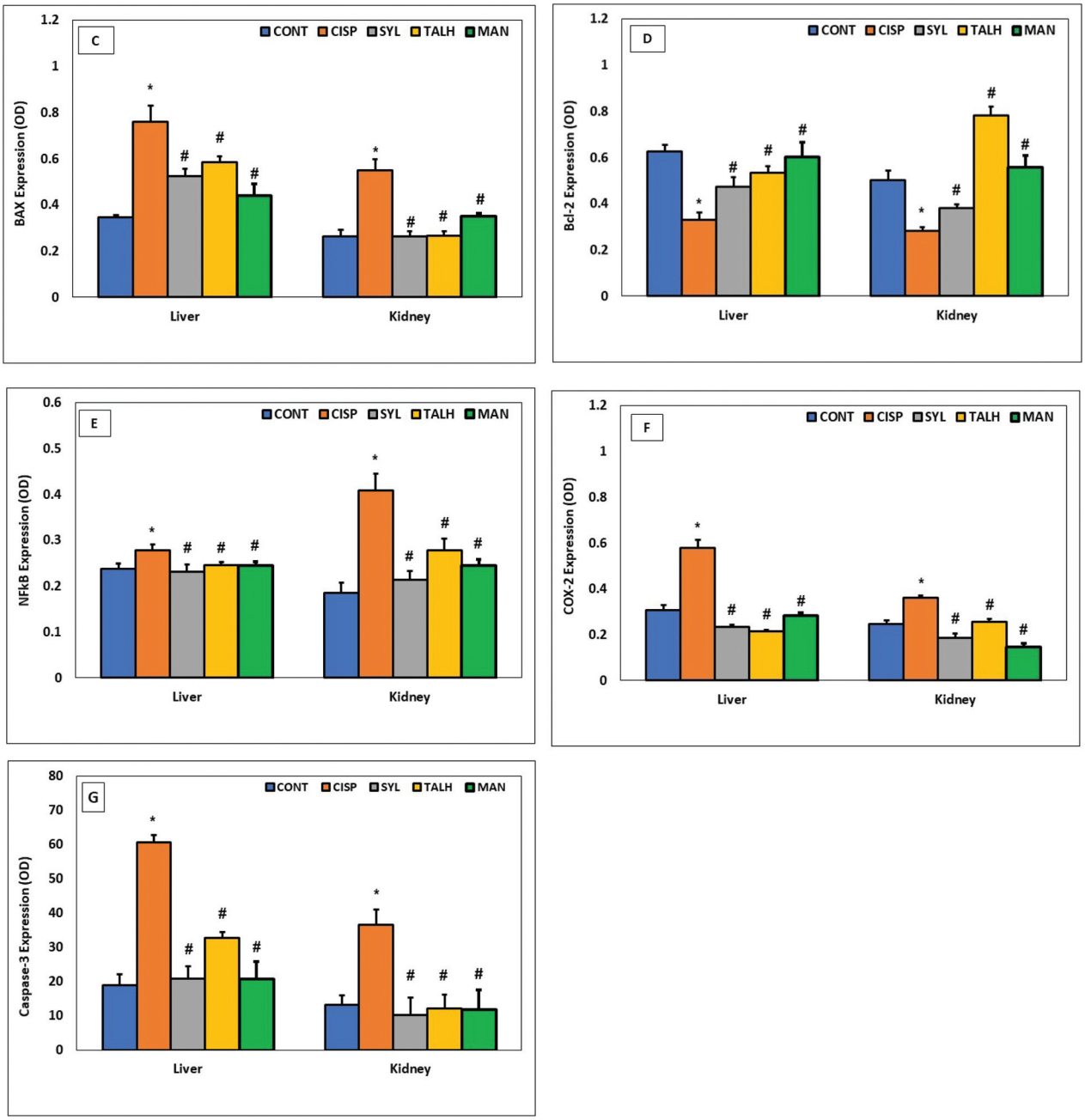

Fig. 3 *(Contd).*

enzymes and likely other minor ingredients. In this study, a GC-MS analysis showed the presence of kojic acid,[41] hydrocinnamic acid,[42] eicosane, heneicosane,[43] pentacosane,[44] hexadecanoic acid,[45] octadecanoic acid[46,47] and oleic acid.[46] These compounds, together with the detected α-hydroxy fatty acids, benzoic acids and their esters and, cinnamic acids and their esters, could be responsible for the antioxidant activity of both TALH honey and MAN honey.[45,48]

The molecular mechanism worked by limiting the prompt increase of CISP in the expression of inflammatory signals, including COX-2 and NF-κB, and the expression of an apoptotic signal, BAX. Furthermore, both honeys induced a CISP-mediated reduction in Bcl-2 expression. CISP-induced inflammatory reaction in the liver and kidneys are mostly attributed to NF-κB and COX-2 activation.[49–51]

NF-κB is an upstream regulator for proinflammatory mediators, including COX-2; hence, NF-κB-mediated COX-2 expression can be considered an important mechanism in the process of inflammation.[52] This study showed a significant CISP-induced increase in NF-κB and COX-2 expression in the liver and kidneys. This result agrees with the findings of Kang *et al.*[53] and Ma *et al.*[52] regarding NF-κB kidney expression. The

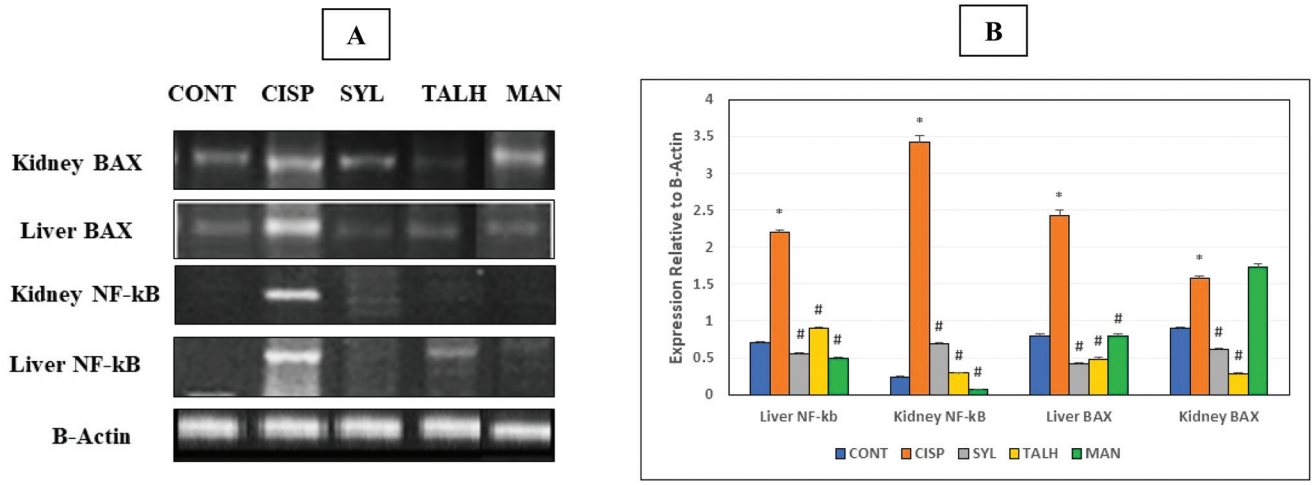

Fig. 4 Electrophoretic analysis of RT-PCR products. 'A' shows liver and kidney BAX and NF-κB gene expression in control (CONT), cisplatin (CISP), silymarin (SYL), talh honey (TALH) and manuka honey (MAN). The bar chart B shows liver and kidney BAX and NF-κB OD values to the corresponding internal control (β-actin) in the different experimental groups; results are presented as mean ± SEM (n = 6). *$P \leq 0.05$ compared to CONT; #$P \leq 0.05$ compared to CISP.

results of this study suggest that MAN honey and TALH honey reduced inflammation *via* the suppression of NF-κB and COX-2 pathway activation. Our results showed that a CISP-induced increase in the oxidative stress markers contributes to the activation of the NF-κB pathway, which, in turn, causes inflammation and apoptosis.[54,55]

Both the pro-apoptotic molecule BAX and the anti-apoptotic molecule Bcl-2 are involved in the regulation of CISP-induced apoptosis.[56] In the present study, CISP increased BAX and caspase-3 expression and decreased Bcl2 expression in both liver and kidneys. The kidney results agree with those of Hassan *et al.*;[6] the liver results confirmed our previously published data.[7] Furthermore, the consumption of both MAN and TALH honey decreased BAX and caspase-3 expression and increased Bcl-2 expression in the liver and kidneys. The binding of BAX to the mitochondrial membrane resulted in the liberation of cytochrome C, an activator of caspase Smac and Omi into the cytosol. Both activate the initiator procaspase-9, which activates caspase-9, and further caspase-3, which can cleave various protein substrates, leading to apoptosis.[57] Among the factors influencing apoptotic pathways, Bcl-2 is a protein encoded by the Bcl-2 proto-oncogene and is one of the molecular members of the cell survival factors in the Bcl-2 family.[58] Bcl-2 prevents the liberation of cytochrome C from the mitochondria, resulting in the prevention of apoptosis.[6,57]

5. Conclusion

The results of this study confirmed a protective role of both TALH honey and MAN honey against acute CISP-induced liver and kidney toxicity. The protective effect of both honeys is more pronounced in the liver than in the kidneys. The underlying mechanism involves the protection against oxidative stress, inflammation and apoptosis.

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	One-way analysis of variance
AST	Aspartate aminotransferase
CAT	Catalase
CISP	Cisplatin
CONT	Control
COX-2	Cyclooxygenase-2
GPx	Glutathione peroxidase
GSH	Reduced glutathione
H&E	Hematoxylin and eosin
MAN	Manuka
MDA	Malondialdehyde
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
SOD	Superoxide dismutase
SPE-GC/MS	Solid phase extraction-gas chromatography/mass spectrometry analysis
SYL	Silymarin
TALH	Talh

Conflicts of interest

There are no conflicts of interest to declare.

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