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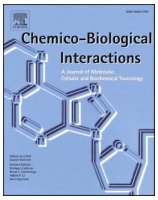

Research paper

Chemopreventive effects of hesperidin against paclitaxel-induced hepatotoxicity and nephrotoxicity via amendment of Nrf2/HO-1 and caspase-3/Bax/Bcl-2 signaling pathways

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ABSTRACT

Paclitaxel (PTX) is a widely used chemotherapeutic drug particularly effective against lung, breast, and ovarian cancer, though its usefulness is limited due to its multi-organ toxicity. The mechanisms underlying PTX toxicity are currently not yet known and there are no approved treatments for its control or prevention. This study aimed to investigate whether hesperidin (HSP) had a protective effect on paclitaxel-induced hepatotoxicity and nephrotoxicity from biochemical, and molecular perspectives. The rats were administered PTX 2 mg/kg, b.w. intraperitoneally for the first 5 consecutive days, then 100 or 200 mg/kg b.w. HSP orally for 10 consecutive days. Our results demonstrated that HSP decreased the PTX induced lipid peroxidation, improved the serum hepatic and renal functions (by decreasing the levels of AST, ALT, ALP, urea, and creatinine), and restored the liver and kidney antioxidant armory (SOD, CAT, GPx, and GSH). HSP also significantly reduced mRNA expression levels of NF-κB, TNF-α, IL-1β, IL-6, MAPK 14, Caspase-3, Bax, LC3A, LC3B, MMP2, and MMP9 whereas caused an increase in levels of Nrf2, HO-1, and Bcl-2 in the kidney and liver of PTX-induced rats. In addition, caspase-3, Bax, and Bcl-2 protein levels were examined by Western blot analysis, and it was determined that HSP decreased caspase-3 and Bax protein levels, but increased Bcl-2 protein levels. The findings of the study suggest that HSP has chemopreventive potential against PTX-induced hepatorenal toxicity plausibly through the attenuation of oxidative stress, inflammation, apoptosis, and autophagy.

1. Introduction

Although some patients successfully recover from cancer, which many people suffer from in the world, many others are defeated. One of the most important reasons for this is that the treatment cannot be completed due to the toxic effects of the chemotherapeutic agents given to the patient [1]. Peripheral neuropathy is among the most common side effects caused by chemotherapeutic agents. However, bone marrow suppression and liver, kidney, testis and heart toxicities are among the common problems [2–6]. It is thought that mitigating these side effects will both pave the way for more effective use of chemotherapeutics and increase the quality of life of patients.

Taxanes obtained from the bark extract of the Pacific yew are mitotic inhibitors with significant anti-cancer activity against various types of cancer [7]. Paclitaxel (PTX), which belongs to the taxane class, stimulates the polymerization of tubulin, thus leading to cell death by disrupting the normal microtubule dynamics required for interphase and mitotic processes [8]. PTX possesses activities towards a comprehensive range of tumour types that include head, breast, lung, neck and ovarian cancers [9]. In addition to these, PTX has activities in other malignant diseases which are insensitive to conventional chemotherapeutic drugs containing formerly treated small cell lung cancers. PTX is also active towards HIV-negative Kaposi's sarcoma [10]. PTX strongly binds to the N-terminal section of the β-subunit of tubulin and hence stabilises its polymerization and precludes active tumour cell division and results in cell cycle arrest in the G2/M phase [11].

In the treatment of patients with ovarian cancer, PTX is administered intravenously at 135 or 175 mg/m^2 every 3 weeks for a 3-h infusion period. In breast cancer, a 3-h intravenous infusion of 175 mg/m^2 every 3 weeks is recommended [12]. On the other hand, the amount of PTX

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and the program it will be administered vary depending on many factors such as height and weight, general health status, and the cancer or condition being treated. Side effects of PTX include alopecia, nausea and vomiting, mucositis, hypersensitivity reactions, arthralgia, myalgia, and fatigue [13]. Also, PTX-related toxicities comprise dosage-dependent severe neurotoxicity and haematological toxicities [14,15]. Although there is not enough information in the literature about liver and kidney toxicities of PTX, dose reduction is recommended for these drugs in patients with liver dysfunction [7]. For that reason, different approaches should be utilised in order to preclude, moderate and overwhelmed the unwanted side effects. Today, various treatment strategies have been applied practically to reduce side effects [2,16–18]. Antioxidants may be beneficial to limit the damage caused by cytotoxic drugs in non-tumour tissues, particularly to reduce the toxic effects of paclitaxel [19,20].

Flavonoids are a class of phenolic substances commonly found in plants. Presently, a great number of flavonoids are assessed in the form of freestate and glycoside and possess some biological activities containing anti-oxidant, anti-cancer, and anti-inflammatory properties [21–23]. Hesperidin (HSP) classified under flavanones class of flavonoids and retains wide range of applicability to preclude terrible diseases that include cardiovascular disease, neurodegeneration, and cancer [24–26]. HSP interacts with several well-known cellular targets and prevents proliferation of cancer cells through induction of apoptosis and cell cycle arrest [27].

There is no certain knowledge in on whether HSP has an ameliorative effect against liver and kidney damages caused by PTX. Therefore, the current study was carried out to investigate the anti-oxidant, anti-inflammatory, anti-apoptotic, and anti-autophagic effects of HSP against PTX-induced hepato- and nephro-toxicities in rats.

2. Material and method

2.1. Reagents and chemicals

Paclitaxel (Taksen 300 mg/50 ml) was obtained from Koçak Farma (Istanbul, Turkey). Analytical-grade reagents were used for the study. HSP and all other compounds were purchased from Sigma–Aldrich chemicals (St. Louis, MO, USA). Antibodies for RT-PCR and Western blot analysis were obtained from Santa Cruz Biotechnology (USA).

2.2. Experimental animals

Thirty five male Sprague Dawley rats, weighing 280–300 g, were used in this interventional study. They were housed in plastic cages under standard laboratory conditions (24 ± 2 °C, 45 ± 5% humidity, and a 12-h light/dark cycle). A commercial balanced diet and tap water ad libitum were provided throughout the experimental period. The experimental protocol was duly approved by Ataturk University Animal Experiments Local Ethics Committee (Permit number: 2021–3/102).

2.3. Experimental design

In the current study, 35 male rats were included, which were randomly divided into 5 groups with each group containing 7 rats. All groups were treated for 15 consecutive days. The doses of HSP and PTX were chosen based on the previous studies [3,28].

Group I (control): For the first 5 days, 0.2 ml of isotonic 0.9% NaCl solution was administered intraperitoneal (i.p.), and then again orally 0.5 ml of isotonic 0.9% NaCl solution was administered for last 10 days.

Group II (HSP): 0.2 ml isotonic 0.9% NaCl solution was given i.p. for the first 5 days, followed by HSP (200 mg/kg b.w. in 0.5 ml isotonic 0.9% NaCl solution) orally for last 10 days.

Group III (PTX): PTX (2 mg/kg b.w. in 0.2 ml isotonic 0.9% NaCl solution) was given i.p. for the first 5 days, followed by 0.5 ml of isotonic 0.9% NaCl solution orally for last 10 days.

Group IV (PTX + HSP 100): PTX (2 mg/kg b.w. in 0.2 ml isotonic 0.9% NaCl solution) was given i.p. for the first 5 days, followed by HSP (100 mg/kg b.w. in 0.5 ml isotonic 0.9% NaCl solution) orally for last 10 days.

Group V (PTX + HSP 200): PTX (2 mg/kg b.w. in 0.2 ml isotonic 0.9% NaCl solution) was given i.p. for the first 5 days, followed by HSP (200 mg/kg b.w. in 0.5 ml isotonic 0.9% NaCl solution) orally for last 10 days.

The end of the study period i.e., 16th day, the rats were decapitated under mild sevoflurane anesthesia (Sevorane liquid 100%, Abbott Laboratory, Istanbul, Turkey). The liver and kidney tissues taken from rats were stored at −80 °C to be evaluated in biochemical and molecular analyzes. Blood samples were collected and centrifuged at 3000 rpm for 10 min to separate the serum. Afterwards, serum was kept at −80 °C until biochemical parameters such as AST, ALT, ALP, urea and creatinine were evaluated.

2.4. Analysis of liver and kidney function markers

Serum levels of ALT, AST and ALP activities were determined using a ELISA Plate Reader (Bio-Tek, Winooski, VT, USA) according to the diagnostic kit obtained from the TML, Diagnostic Medical Products, (Ankara, Turkey). Also, serum levels of urea and creatinine were measured by colorimetric kits (Diasis Diagnostic Systems, İstanbul, Turkey). ALT, AST and ALP activities were expressed as U/L and urea and creatinine were expressed as mg/dL.

2.5. Analysis of oxidative stress markers

Tissues were ground with liquid nitrogen to obtain homogenate from liver and kidney tissues. Then, these ground tissues were diluted 1:10 with 1.15% KCl and homogenized in a homogenizer (Tissue Lyser II, Qiagen, Netherlands). The supernatants obtained after centrifugation were used for oxidative stress biomarkers and lipid peroxidation analyses. Superoxide dismutase (SOD) activity was estimated according to the method of Sun, Oberley and Li [29], and the results were given in U/g protein. Catalase (CAT) activity was determined according to Aebi [30] method and the results were expressed as catal/g protein. Glutathione peroxidase (GPx) activity was determined according to the method of Lawrence and Burk [31], and it was also expressed as U/g of protein. Assay of glutathione (GSH) concentration was done according to the method of Sedlak and Lindsay [32]. Malondialdehyde (MDA) levels (indicator of lipid peroxidation) were assayed colorimetrically according to the methods of Placer, Cushman and Johnson [33]. The protein content of the liver and kidney tissue homogenates were determined using the Lowry, Rosebrough, Farr and Randall [34].

2.6. Real-time PCR analysis

For Real-Time PCR analysis, liver and kidney tissues of the control and experimental groups were powdered in a homogenizer (Tissue Lyser II, Qiagen, Netherlands) after being treated with liquid nitrogen. Then, total RNA was isolated from each group with QIAzol Lysis Reagent (Qiagen, Cat: 79306, Germany). Total RNA concentrations were measured in NanoDrop (Epoch Microplate Spectrophotometer, USA). After the total RNA concentrations of the samples were equalized, cDNA synthesis was performed with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™ Cat: 4368814, USA) according to the manufacturer's instructions. The obtained cDNAs were used to determine the mRNA transcript levels of the genes given in Table 1. The mRNA transcript levels of the relevant genes were analyzed on the ROTOR-GENE Q (Qiagen, Germany) instrument using QuantiTect SYBR Green PCR Master Mix (Qiagen, Cat: 204143, Germany). Finally, fold change calculations were made by the method developed by Livak and Schmittgen [35]) using the CT values of actin, which is the internal control, and other genes.

Table 1
Primer sequences.

Gene	Sequences (5′-3′)	Length (bp)	Accession No
NF-κB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	106	NM_001276711.1
IL-1β	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	197	NM_031512.2
TNF-α	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	139	NM_012675.3
IL-6	F: AGCGATGATGCACTGTCAGA R: GGAACTCCAGAAGACCAGAGC	127	NM_012589.2
MAPK14	F: GTGGCAGTGAAGAAGCTGTC R: GTCACCAGGTACACATCGTT	170	NM_031020.2
Nrf2	F: TTTGTAGATGACCATGAGTCGC R: TCCTGCCAAACTTGCTCCAT	161	NM_031789.2
HO-1	F: ATGTCCCAGGATTTGTCCGA R: ATGGTACAAGGAGGCCATCA	144	NM_012580.2
Bcl-2	F: GACTTTGCAGAGATGTCCAG R: TCAGGTACTCAGTCATCCAC	214	NM_016993.2
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	154	NM_017059.2
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
MMP2	F: CTCTAGGAGAAGGACAAGTG R: CTCAAAGTTGTACGTGGTGG	158	NM_031054.2
MMP9	F: AGCTGGCAGAGGATTACCTG R: ATGATGGTGCCACTTGAGGT	230	NM_031055.2
LC3A	F: GACCATGTTAACATGAGCGA R: CCTGTTCATAGATGTCAGCG	139	NM_199500.2
LC3B	F: GAGCTTCGAACAAAGAGTGG R: CGCTCATATTCACGTGATCA	152	NM_022867.2
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

2.7. Western blotting analysis

Western blot analysis was performed similar to our previous study [36]. Briefly, the liver and kidney tissues were removed and homogenized in RIPA lysis buffer containing protease inhibitor cocktail and phenylmethylsulfonyl fluoride (PMSF) at 4 °C. The homogenates were centrifuged at 12000 g for 20 min and the supernatant was used for Western blot analysis. Protein concentration was measured by Pierce™ BCA Protein Assay Kit (Rockford, IL, USA) using bovine serum albumin (BSA) as standard. Homogenates dissolved in Laemmli sample buffer were loaded with 30 μg protein in each well and separated in sodium dodecyl sulphate polyacrylamide gel electrophoresis. Proteins transferred to polyvinylidene fluoride (PVDF) membranes were blocked for 1.5 h in 5% BSA in tris-buffered saline with 0.1% Tween 20 (TBS-T). After blocking, membranes were washed 5 times in TBS-T for 5 min and each of which, mouse monoclonal primary antibodies β-actin, Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2) and caspase-3 was incubated at 4 °C for overnight. Then the membranes were washed 5 times with TBS-T and left for 1.5 h in the presence of goat anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (1:2000 dilution, sc-2005). Protein bands were detected using enhanced chemiluminescence reagent Clarity™ Western ECL Substrate (Bio-Rad, Hercules, USA) and detected by a Western blotting system.

2.8. Statistical analysis

GraphPad Prism 5 was used for the analysis of data. Significance levels and statistical differences were determined by using the one-way ANOVA, and Tukey's post hoc test was used to determine differences among the groups. Data are expressed as mean ± standard deviations (SD). Differences between the groups were considered statistically significant at $p < 0.05$.

3. Results

3.1. Effect of HSP on PTX-induced hepatotoxicity and nephrotoxicity

As presented in Table 2, PTX-treated rats revealed a significant ($p < 0.001$) increase in serum levels of ALT, AST and ALP, compared with control group. Post-treatment with HSP at two doses 100 and 200 mg/kg significantly ($p < 0.001$) reduced the elevated levels of serum liver enzymes (AST, ALT and ALP).

Intraperitoneal injection of PTX caused a significant ($p < 0.001$) increase in serum creatinine and urea levels when compared to the control values which was shown in Table 3. However, post-treatment with HSP significantly ($p < 0.001$) reduced the increased levels of serum urea and creatinine in PTX group in both tissues. HSP-alone treatment did not exhibit a significant effect on levels of these parameters compared to the control group.

3.2. Effect of HSP on PTX-induced oxidative stress

The activities of the antioxidant enzymes, SOD, CAT and GPx, and levels of GSH in the both tissues of the rats have been shown in Tables 2 and 3 The SOD, CAT and GPx activities and GSH levels were significantly reduced in the PTX-treated group compared to the control group ($p < 0.001$). However, HSP administration produced a significant ($p < 0.001$) improvement in these parameters as compared to the PTX-treated group. Also, it was observed that MDA level was increased significantly in liver and kidney tissues comparison with the control group in the PTX-induced group ($p < 0.001$) and did not change in the HSP group. However, treatment of HSP decreased the level of MDA compared to the PTX group ($p < 0.001$).

3.3. HSP increases PTX-downregulated Nrf2 and HO-1 expressions in liver and kidney tissues

According to the results given in Fig. 1A–D, it was determined that

Table 2
Effect of HSP on hepatic serum markers and oxidative stress biomarkers in PTX-induced hepatotoxicity.

Parameters	Control	HSP	PTX	PTX + HSP 100	PTX + HSP 200
ALP (U/L)	43.30 ± 2.71	45.63 ± 1.97$^{###}$	93.43 ± 4.60***	75.50 ± 4.38***/###/✣✣✣	56.87 ± 3.49***/###
ALT(U/L)	40.41 ± 2.78	40.12 ± 3.72$^{###}$	76.01 ± 2.96***	61.86 ± 2.65***/###/✣✣✣	53.71 ± 4.77***/###
AST (U/L)	53.68 ± 2.99	55.59 ± 3.09$^{###}$	121.03 ± 4.61***	93.29 ± 2.45***/###/✣✣	76.71 ± 5.41***/###
MDA(nmol/g tissue)	40.52 ± 2.83	40.17 ± 2.39$^{###}$	61.98 ± 3.29***	51.95 ± 1.84***/###/✣✣✣	45.33 ± 1.74**/###
GSH (nmol/g tissue)	6.18 ± 0.20	6.27 ± 0.31$^{###}$	3.32 ± 0.15***	3.84 ± 0.10***/###/✣✣✣	5.18 ± 0.18***/###
CAT(katal/g protein)	50.19 ± 2.88	51.73 ± 1.72$^{###}$	26.78 ± 1.80***	32.42 ± 1.73***/###/✣✣✣	40.62 ± 1.46***/###
SOD (U/g tissue)	20.19 ± 1.98	21.10 ± 1.15$^{###}$	10.51 ± 0.88***	13.99 ± 0.81***/###/✣✣✣	17.60 ± 0.70**/###
GPx (U/g tissue)	25.34 ± 1.44	25.35 ± 2.17$^{###}$	14.81 ± 0.82***	18.29 ± 1.25***/##/✣	20.82 ± 1.69***/###

Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✣ P < 0.05, ✣✣ p < 0.01, ✣✣✣ p < 0.001) was analyzed using One Way ANOVA.

Table 3
Effect of HSP on renal serum markers and oxidative stress biomarkers in PTX-induced nephrotoxicity.

Parameters	Control	HSP	PTX	PTX + HSP 100	PTX + HSP 200
Urea (mg/dL)	3.09 ± 0.07	3.08 ± 0.11$^{###}$	8.14 ± 0.29***	6.42 ± 0.17$^{***/###/✣✣✣}$	5.29 ± 0.19$^{***/###}$
Creatinine (mg/dL)	0.40 ± 0.02	0.39 ± 0.03$^{###}$	1.73 ± 0.07***	1.34 ± 0.06$^{***/###/✣✣✣}$	0.76 ± 0.07$^{***/###}$
MDA(nmol/g tissue)	50.45 ± 2.09	51.09 ± 3.12$^{###}$	76.69 ± 2.47***	67.61 ± 2.04$^{***/###/✣✣✣}$	61.05 ± 1.93$^{***/###}$
GSH (nmol/g tissue)	4.09 ± 0.15	4.15 ± 0.09$^{###}$	2.18 ± 0.22***	2.76 ± 0.13$^{***/###/✣✣✣}$	3.47 ± 0.15$^{***/###}$
CAT(katal/g protein)	29.79 ± 1.52	30.09 ± 1.46$^{###}$	13.75 ± 1.19***	19.84 ± 1.70$^{***/###/✣✣✣}$	25.24 ± 0.91$^{***/###}$
SOD(U/g tissue)	17.55 ± 1.43	18.14 ± 1.59$^{###}$	6.67 ± 0.81***	11.74 ± 0.96$^{***/###/✣✣✣}$	15.38 ± 0.81$^{*/###}$
GPx(U/g tissue)	21.73 ± 1.69	22.79 ± 0.98$^{###}$	13.84 ± 0.85***	16.66 ± 0.94$^{***/##/✣✣✣}$	19.75 ± 1.25$^{***/###}$

Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✣ P < 0.05, ✣✣ p < 0.01, ✣✣✣ p < 0.001) was analyzed using One Way ANOVA.

the expression of Nrf2 and HO-1 in the liver and kidney tissues of the rats treated with PTX was significantly suppressed when compared to the control group ($p < 0.001$). It was observed that HSP administration increased both Nrf2 and HO-1 mRNA transcript levels compared to the PTX group ($p < 0.001$). In addition, it is among the findings that Nrf2 and HO-1 expressions in both tissues increase more in high-dose of HSP administration ($p < 0.001$).

3.4. HSP reduces the expressions of PTX-induced inflammatory markers in liver and kidney tissues

The mRNA transcript levels of inflammatory markers (NF-κB, IL-1β, TNF-α, IL-6 and MAPK14) analyzed by RT-PCR in liver and kidney tissues of rats treated with PTX and HSP are given in Fig. 2A–J. The data obtained show that PTX-treated rats triggered inflammation by up-regulating NF-κB, IL-1β, TNF-α, IL-6 and MAPK14 expression in their livers and kidneys compared to the control group ($p < 0.001$). However, it was observed that HSP treatment decreased the mRNA transcript levels of these inflammatory cytokines in both tissues, yet there was a difference between doses in some parameters. For example, it was determined that high dose administration was more effective on MAPK14 ($p < 0.001$), NF-κB ($p < 0.001$), IL-6 ($p < 0.01$), IL-1β ($p < 0.001$) and TNF-α ($p < 0.001$) expressions in liver tissue; nevertheless, it was found to be more effective only on MAPK14 ($p < 0.001$), NF-κB ($p < 0.01$) and TNF-α ($p < 0.05$) expressions in kidney tissue. It was determined that the expressions of IL-1β and IL-6 did not differ between doses of 100 mg/kg and 200 mg/kg in kidney tissue.

3.5. HSP attenuates PTX-induced apoptosis by acting on Bax, Bcl-2 and Caspase-3 in liver and kidney tissues

After PTX and HSP administrations, Bax, Bcl-2 and caspase-3 mRNA transcript levels were analyzed by RT-PCR to determine the apoptotic status in liver and kidney tissues, and the results are presented in Fig. 3A–F. Accordingly, it was observed that Bax and caspase-3 expressions in liver and kidney tissues of rats given PTX were up-regulated and triggered apoptosis compared to the control group ($p < 0.001$). It was found that HSP treatment dose-dependently suppressed caspase-3 ($p < 0.001$) expression and increased Bcl-2 ($p < 0.05$) expression in liver tissue. However, it was determined that Bax expression did not differ between the doses of HSP. When the effects of HSP were examined in kidney tissue, it was observed that the mRNA transcript level of Bax decreased in a dose-dependent manner ($p < 0.05$), while there was no

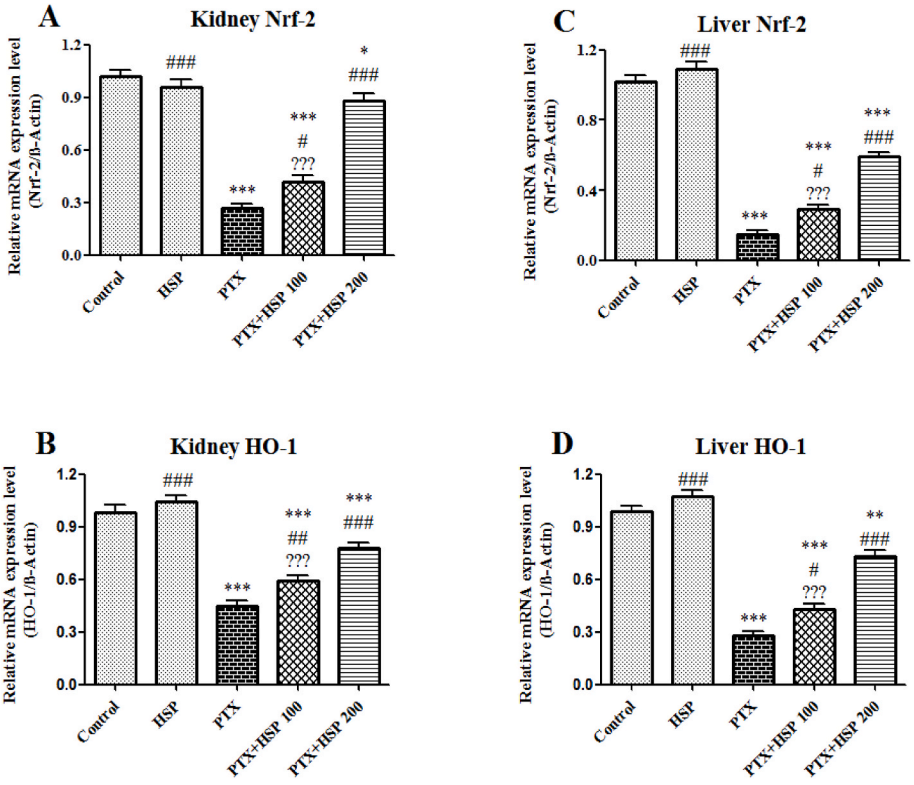

Fig. 1. (A–D) The mRNA transcript level of Nrf2 and HO-1 in the kidney and liver of rats. (A and C) Represent the relative mRNA expression levels of Nrf-2 in the kidney and liver tissues. (B and D) Represent the relative mRNA expression levels of HO-1 in the kidney and liver tissues. All data were expressed as mean ± SD. Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✣ P < 0.05, ✣✣ p < 0.01, ✣✣✣ p < 0.001) was analyzed using One Way ANOVA.

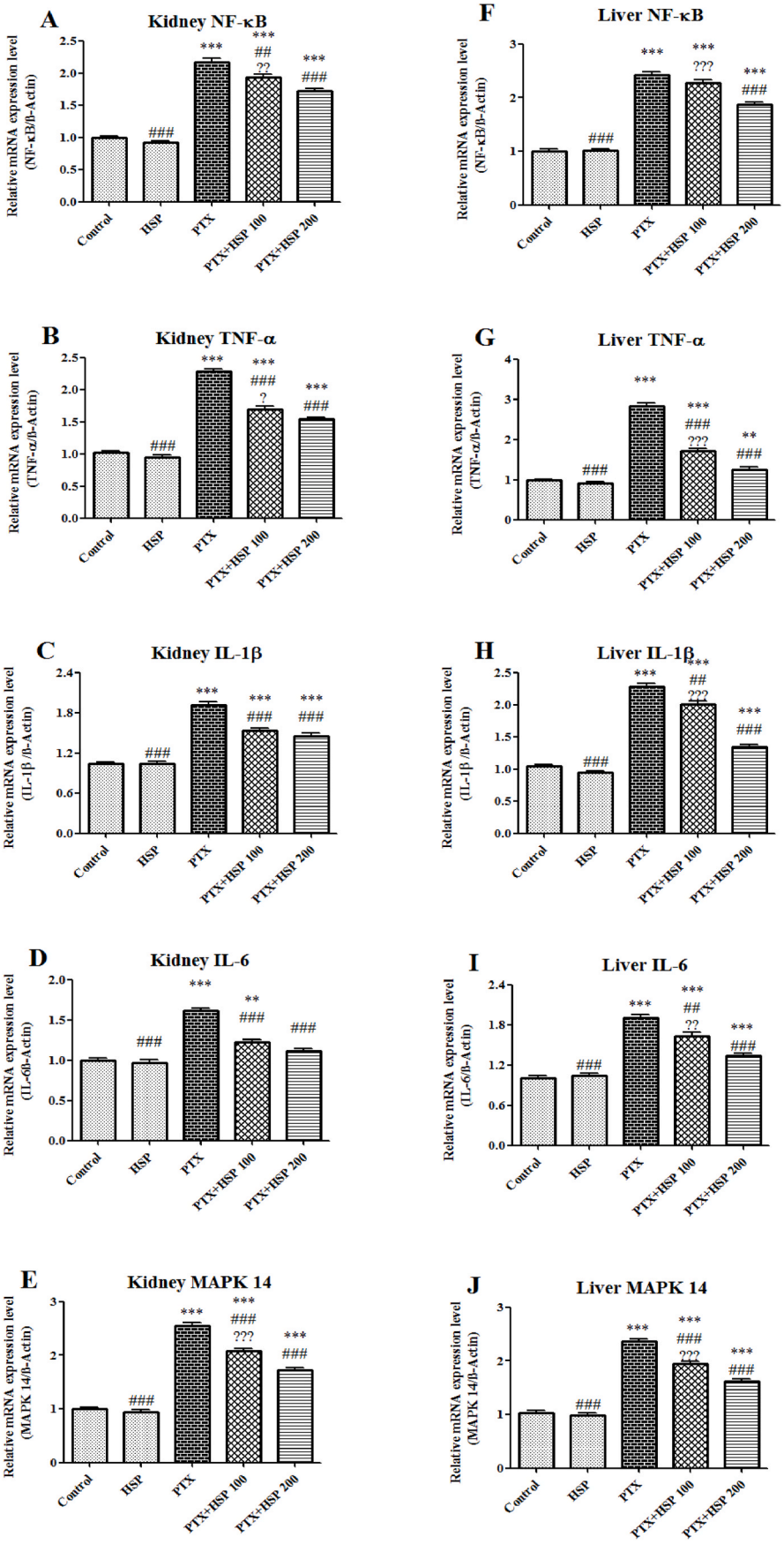

Fig. 2. (A–J) The mRNA transcript level of NF-κB, TNF-α, IL-1β, IL-6 and MAPK 14 in the kidney and liver of rats. (A and F) Represent the relative mRNA expression levels of NF-κB in the kidney and liver tissues. (B and G) Represent the relative mRNA expression levels of TNF-α in the kidney and liver tissues. (C and H) Represent the relative mRNA expression levels of IL-1β in the kidney and liver tissues. (D and I) Represent the relative mRNA expression levels of IL-6 in the kidney and liver tissues. (E and J) Represent the relative mRNA expression levels of MAPK 14 in the kidney and liver tissues. All data were expressed as mean ± SD. Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✢ P < 0.05, ✢✢ p < 0.01, ✢✢✢ p < 0.001) was analyzed using One Way ANOVA.

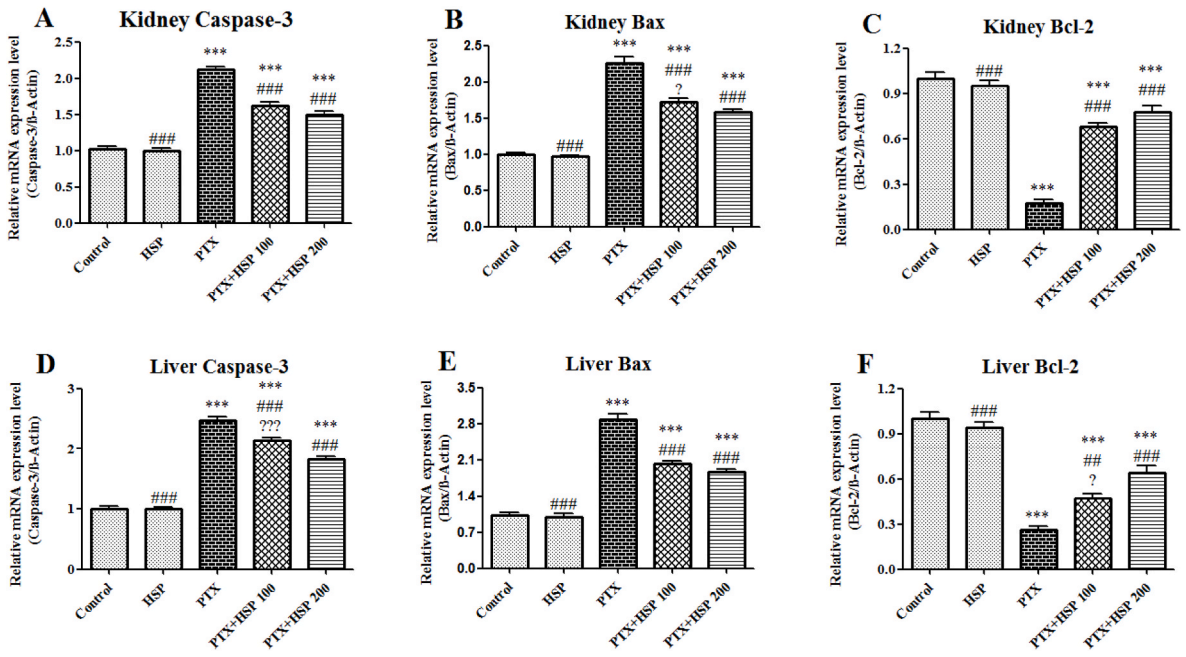

Fig. 3. (A–F) The mRNA transcript level of Caspase-3, Bax and Bcl-2 in the kidney and liver of rats. (A and D) Represent the relative mRNA expression levels of Caspase-3 in the kidney and liver tissues. (B and E) Represent the relative mRNA expression levels of Bax in the kidney and liver tissues. (C and F) Represent the relative mRNA expression levels of Bcl-2 in the kidney and liver tissues. All data were expressed as mean ± SD. Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✣ P < 0.05, ✣✣ p < 0.01, ✣✣✣ p < 0.001) was analyzed using One Way ANOVA.

statistical difference between HSP doses on caspase-3 and Bcl-2 expressions. On the other hand, it was determined from the data obtained that HSP treatment attenuated PTX-induced apoptosis in both liver and kidney.

Consistent with the results obtained by the RT-PCR method, the western blotting results revealed that the levels of Bax, Bcl-2 and caspase-3 were markedly increased in the liver and kidney of the PTX-induced rats. However, treatment with HSP decreased the levels of these apoptotic markers (Fig. 4A–F).

3.6. HSP attenuates PTX-induced autophagy by acting on LC3A and LC3B genes in liver and kidney tissues

In order to evaluate the effects of HSP against PTX-induced liver and kidney damage, LC3A and LC3B mRNA transcript levels, which are autophagic markers in tissues, were evaluated. According to the results (see Fig. 5A–D), it was determined that LC3A and LC3B mRNA transcript levels increased in liver and kidney tissues of rats administered PTX compared to the control group ($p < 0.001$). However, it was observed that HSP treatment had an anti-autophagic effect and down-regulated LC3A and LC3B expressions compared to the PTX group. It is also among our results that high dose is more effective on autophagy.

3.7. Effects of HSP and PTX administrations on MMP-2 and MMP-9 expressions in liver and kidney tissues

MMP-2 and MMP-9 mRNA transcript levels in liver and kidney tissues are given in Fig. 6A–D. The results showed that PTX administration increased MMP-2 and MMP-9 mRNA transcript levels in both liver and kidney compared to control group ($p < 0.001$). However, with HSP administration, it was determined that the MMP-2 and MMP-9 mRNA transcript levels were dose-dependently decreased in liver tissue compared to the PTX group. In kidney tissue, although HSP suppressed the MMP-2 and MMP-9 expressions compared to the PTX group, there was no significant difference between the doses.

4. Discussion

Paclitaxel, a drug used in cancer chemotherapy, has also been shown to use in combination with several chemotherapeutic drugs. Nonetheless, the augmented risk of drug-induced toxicities following chemotherapy has resulted in searches for alternate approaches to the current chemotherapeutic agents. Neurotoxicity is one of the well-known side effects of PTX however, its toxicity on liver and kidney tissues has yet to be studied. Therefore, the main aim of this study was to investigate the ameliorative effects of HSP on PTX induced toxicities in liver and kidney tissues of rats.

Measurement of serum ALT, AST, ALP, urea and creatinine levels is widely used to determine the degree of liver and kidney toxicity. ALT, AST and ALP enzymes are cellular enzymes and increases in serum indicate cellular destruction of the liver. In addition, elevation of urea and creatinine levels in serum indicates a decrease in glomerular filtration rate [37]. The results of this study pointed out the liver and kidney damages triggered by PTX intoxication as obvious by a rise in serum urea and creatinine levels for kidney, and increase in ALP, ALT and AST represents a hepato-cellular damage. The increase in serum ALT, AST and ALP activities are possibly owing to injury of hepatocytes and leakage of the corresponding enzymes to the blood. On the other hand, the increases in serum creatinine and urea levels after drug toxicity has been attributed to the possible DNA breakdown and by increased serum levels of 8-OHdG [38]. The data of our study indicated that HSP ameliorated the serum activities of liver enzymes and levels of serum urea and creatinine in kidney. HSP exhibited hepato and renal protective effects in several prior experiments. In a study carried out by Kücükler et al., administration of chlorpyrifos resulted in severe renal damage with rised serum urea and creatinine levels while HSP treatment considerably alleviated chlorpyrifos-induced renal dysfunction [39]. The results of another study showed that HSP remarkably alleviated methotrexate-induced rise in the activities of liver enzymes [40]. In a different study, liver and renal damages stimulated by sodium fluoride were certified by the change in levels of serum urea and creatinine in kidney tissue, levels of ALT, ALP and AST in liver whereas

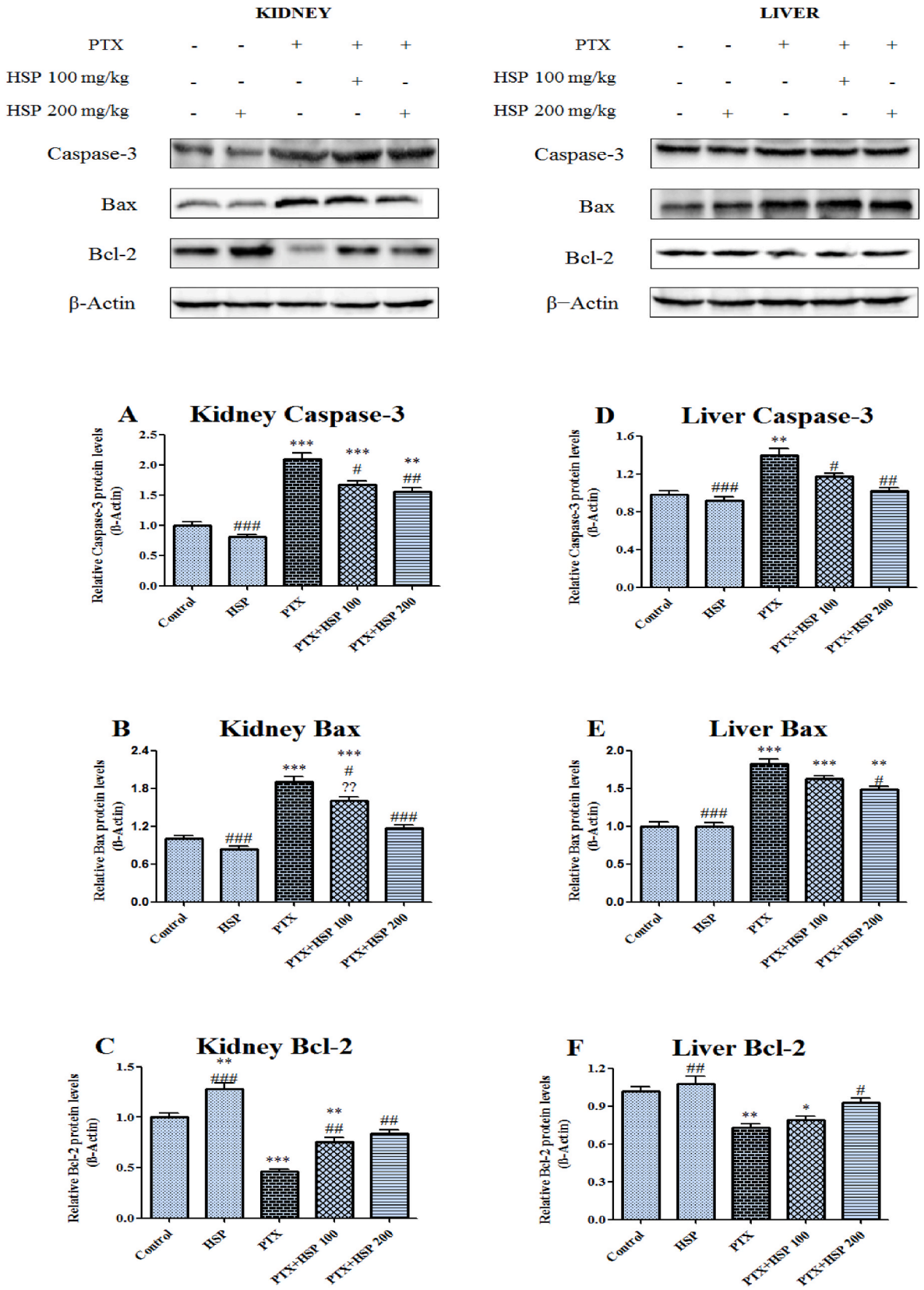

Fig. 4. (A–F) Effects of HSP on PTX-induced apoptosis in kidney and liver tissues. Protein levels caspase-3, Bax and Bcl-2 were measured by Western blotting analysis. β-Actin was used as housekeeping protein. All data were expressed as mean ± SD. Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✣ P < 0.05, ✣✣ p < 0.01, ✣✣✣ p < 0.001) was analyzed using One Way ANOVA.

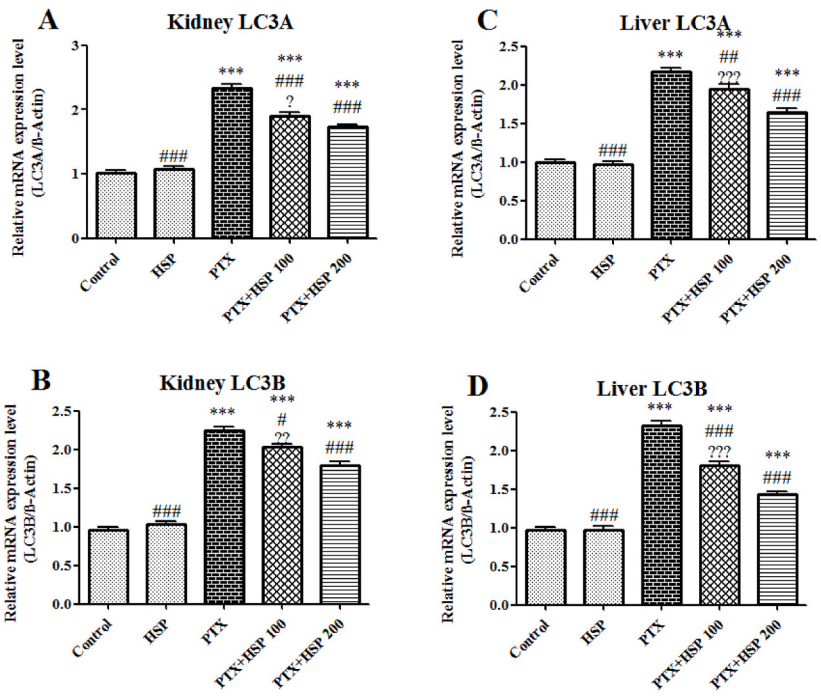

Fig. 5. (A–D) The mRNA transcript level of LC3A and LC3B in the kidney and liver of rats. (A and C) Represent the relative mRNA expression levels of LC3A in the kidney and liver tissues. (B and D) Represent the relative mRNA expression levels of LC3B in the kidney and liver tissues. All data were expressed as mean ± SD. Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✣ P < 0.05, ✣✣ p < 0.01, ✣✣✣ p < 0.001) was analyzed using One Way ANOVA.

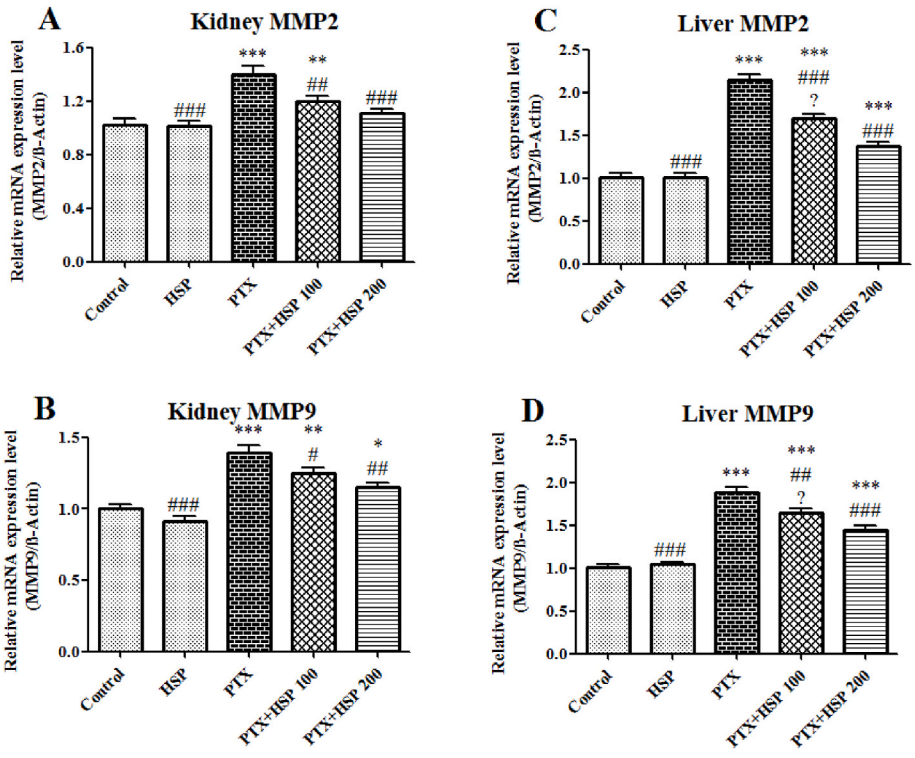

Fig. 6. (A–D) The mRNA transcript level of MMP2 and MMP9 in the kidney and liver of rats. (A and C) Represent the relative mRNA expression levels of MMP2 in the kidney and liver tissues. (B and D) Represent the relative mRNA expression levels of MMP9 in the kidney and liver tissues. All data were expressed as mean ± SD. Statistical significance (Control vs others: *P < 0.05, **p < 0.01, ***p < 0.001, PTX vs others: #P < 0.05, ##p < 0.01, ###p < 0.001, PTX + HSP 100 vs PTX + HSP 200: ✣ P < 0.05, ✣✣ p < 0.01, ✣✣✣ p < 0.001) was analyzed using One Way ANOVA.

administration of HSP and sodium fluoride administration remarkably alleviated the deviation in the these parameters [28]. These findings were consistent with our study indication protective role of HSP in PTX induced toxicities in hepatorenal tissues.

One of the most important causes of cellular damage is oxidative stress due to excessive production of reactive oxygen species (ROS) and suppression of the antioxidant system [41–43]. ROS are key mediators in various biological and pathological processes. Excess amount of ROS generation has been associated with oxidative damage to several important biomolecules containing lipids, proteins and DNA. The damages in these macromolecules are related to pathogenic conditions in kidney and liver [44,45]. In the current study, PTX caused marked oxidative stress in liver and kidney tissues as evident by an increase in MDA level, decrease in antioxidant enzyme activities (SOD, CAT and GPx) and GSH levels. The post-treatment with HSP alleviated these effects. There is limited amount of study on toxicity of PTX in liver and kidney. One of the study revealed that PTX has direct impacts on mitochondria to prompt ROS production in liver cells [46] and this may

possibly be reason for the substantial reduction in the levels of antioxidant enzymes. PTX-induced ROS generation was approximately 3 folds higher in the lipids than in the aqueous phase, signifying that the ROS generated in response to PTX treatment was predominantly localised in the membranes of mitochondria. The mitochondrial effects point to a new biomolecular mechanism for PTX that is probable to contribute to the toxic impacts of the chemotherapeutic agent. To clarify the significance of ROS in the development of PTX-induced neuropathy, ROS level and antioxidant enzyme activities were measured. The data revealed that neuronally-derived ROS and tiny antioxidant enzyme response, are responsible factors in PTX-induced neuropathy [47]. In a different study, HSP was reported to decrease MDA levels, but augmented the levels of SOD, CAT, GPx and GSH in liver ischemia/reperfusion (I/R) injury [48]. HSP was also shown to remarkably reduce levels of MDA, increase total antioxidant capacity in kidneys of mice supplemented with cyclophosphamide [49].

One of the commonly used markers in the evaluation of oxidative stress is the Nrf2/HO-1 signaling pathway [50,51]. Nrf2 is a transcription factor involves in regulating the balance of cellular redox and ameliorative phase II detoxification reactions and antioxidant levels in mammals [52,53]. The Keap1-Nrf2 pathway controls the expression levels of several cyto-protective genes such as the genes that encode the enzymes that involve in the both regeneration and synthesis of GSH and detoxifying molecules. Heme oxygenase-1 (HO-1) is one of the genes regulated through Nrf2 [3,54]. The activity of its products has been shown to regulate key biological processes such as inflammation, cell proliferation, apoptosis, angiogenesis and fibrosis [55]. In parallel to data obtained in other studies, the results of our study displayed that mRNA levels of Nrf2 and HO-1 in the PTX-induced liver and kidney tissues were lower than those of control groups, while after HSP administration Nrf2 and HO-1 were up-regulated. Consistent with results of our study, preceding researches informed that HSP offers cyto-protective mechanism through activation of the Nrf2 pathway [56,57].

Oxidative damage has also been shown to trigger inflammatory responses through activating some of the transcription factors [58]. In addition to this, inflammatory cells create some soluble mediators some of which include the metabolites of arachidonic acid, cytokines, and chemokines, all of which perform by further engaging the inflammatory cells to the site of damages and generating more ROS. These mediators could activate signal transduction cascades as well as stimulating some changes in transcription factors, such as NF-κB aberrant expression of inflammatory cytokines (TNF-α, IL-1, IL-6) and chemokines (IL-8) have been reported to play a role in oxidative stress-induced inflammation [59]. Studies further demonstrate that excessive ROS generation involves in activation of the MAPK14 signaling pathway, a potent activator of NF-κB [60,61]. Unfortunately, the research examining the impacts of PTX on these mechanisms are inadequate. In the current research, we determined that PTX upregulated the mRNA expressions of MAPK14 and NF-κB, probably because of excess ROS generation, and consequently, it might cause to up-regulation of IL-1β, TNF-α, and IL-6. Our study also revealed that HSP hampered the activation of pro-inflammatory genes through clearing the PTX derived ROS and alleviated toxicities in hepatorenal tissues against inflammation.

PTX was reported to stabilise tubulin dimers, leading to the cell arrest [62], and apoptosis. PTX has also been demonstrated to activate Raf-1 and lead to phosphorylation of Bcl-2 [63,64]. This process has been thought to cause inactivation of Bcl-2 and preventing it of making heterodimers with the pro-apoptotic Bax [65], consequently causing to augmented free intra-cellular Bax level, and eventually leading to the activation of the caspases via the Bax-triggered cytochrome *c* release from mitochondria. The studies documented that, Bcl-2 and Bcl-xL decrease apoptotic effects of PTX [66], whereas the proteins stimulating the opening of permeability transition pore enable the PTX-induced programmed cell deaths in tumour cell lines [67]. In our study, PTX increased levels of caspase-3 and Bax and reduced levels of Bcl-2 in liver and kidney tissues in parallel to literature while HSP alleviated these effects revealing protective effects of HSP in PTX induced toxicities in hepatorenal tissues. Moreover, HSP ameliorated the renal damage caused by acetaminophen through reducing expression level of caspase-3 and increasing level of Bcl-2 [68]. HSP also remarkably decreased mRNA levels Bax and caspase-3 in isoniazid, rifampicin and pyrazinamide induced toxicities [69].

Autophagy is one of the events mediated by oxidative stress and when it occurs at high levels, it triggers cell death and causes loss of function in tissues [70,71]. LC3A and LC3B are key proteins of autophagosomal membranes, extensively used as the biological markers of the autophagic processes [72]. In our study, PTX treatment increased the mRNA levels of LC3A and LC3B confirming PTX's function in induction of autophagy. The levels of authophagy related genes LC3A, LC3B, and Beclin-1 were shown to be repressed in the PTX-induced spinal cord injury [3]. In a different study, it was determined that the HSP supplementation down-regulated the expressions of LC3A and LC3B in toxicity resulted by abamectin in testicular tissues [73].

One of the crucial biomolecules devoting to renal miscarriage is signified by metalloproteinases (MMPs) [74,75]. MMPs have been sub-divided into collagenases, gelatinases and several other types of MMPs. They have been shown to contribute to the proliferation of cell, angiogenesis and apoptosis [76]. Majority of the studies have focused on plasma concentrations of gelatinase B (MMP-9) and gelatinase A (MMP-2) [77]. In liver fibrosis, MMP-2 was shown to be highly up-regulated in myofibroblasts and thought to possess a pro-fibrogenic role while MMP-9 is a multi-directional metalloproteinase with an important role in damaging liver regeneration [78,79]. The data of the current study revealed that expression levels of MMP-2 and MMP-9 in the PTX-induced liver and kidney were up-regulated while HSP treatment down-regulated levels of MMP-2 and MMP-9. Furthermore, up-regulation of these genes was reported to be suppressed in n-nitro L-arginine methyl ester rats treated with HSP [80].

5. Conclusion

The results of the present study suggest that suppression of oxidative stress, inflammatory response, apoptotic and autophagic cell death by HSP may be an effective strategy for the treatment of PTX-induced liver and kidney damage.

CRediT authorship contribution statement

The Author contribution as follows was accepted by all authors:

Cihan Gür: methodology, investigation, data curation, software, formal analysis. **Fatih Mehmet Kandemir:** investigation, data curation, software, formal analysis, supervision. **Cuneyt Caglayan:** conceptualization, investigation, visualization, writing - review & editing, supervision. **Emine Satıcı:** investigation, methodology, validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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