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Hesperetin attenuates cadmium-induced hepatotoxicity in rats through anti-inflammatory and antiapoptotic mechanisms: An *in vivo* and *in silico* study

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Cadmium (Cd) is a common pollutant in the industry and the environment, which causes serious health effects, especially deleterious liver injury. Hesperetin (Hp) is a bioflavonoid with various pharmacological properties, including antioxidant, anti-inflammatory, antiallergic, vasoprotective, anticarcinogenic, and hypolipidemic activities. The present study aimed to investigate the potential role of Hp on Cd-induced hepatic injury in male Wistar rats. The liver tissue was subjected to the comet assay, immunohistochemistry, transmission electron microscopy (TEM), and western blotting. Results of the study showed that rats treated with Cd alone displayed significantly altered markers of oxidative stress (comet assay), inflammation, apoptosis, and hepatic tissue histology compared with controls. Oral co-treatment with Cd to Hp-exposed rats for 3 weeks significantly ameliorated the changes. It was remarkably synthesized that Hp could bind to critical apoptotic and inflammatory proteins. It was bridged to Bcl-2 by three hydrogen bonds, with binding energies of –7.2 and –8.3 kcal/mol. It also established hydrogens bonds with Bax, IL-6, and TNF-α were –6.3, –6.6, and -6.2 kcal/mol, respectively. It suggested that Hp might be hepatoprotective and modulate oxidative stress, inflammation, and apoptosis *in vivo* and *in silico*.

Keywords: Apoptosis, Cadmium, Hepatocytes, Hesperetin, Inflammation, Liver damage, Oxidative stress

Cadmium (Cd) is a toxic heavy metal widely distributed in the environment from both natural and anthropogenic sources. It's an industrial byproduct of many processes, including smelting, mining, and battery manufacture. It is also present in certain fertilizers, sewage sludge, and tobacco smoke[1]. The persistence of Cd leads to its accumulation in soils, the aquatic environment, and the food chain, posing significant risks to human health. Long-term, Low-level exposure leads to chronic poisoning, whereas short-term, high-level exposure can lead to severe toxicity and even fatal conditions[2].

Several studies have highlighted Cd's tendency to accumulate in the body, leading to pathological changes in organ systems. For instance, it affects the kidney, liver, testis, and spleen. Among these organs, the liver is highly susceptible to the toxic effects of Cd, both in acute and chronic exposures[3-6]. Experimental and Epidemiological studies have provided evidence that Cd exposure can result in fibrosis, inflammation, and oxidative stress, leading to impairments in liver function[7-9].

Hesperetin (Hp) is a flavonoid commonly found in vegetables and fruits, particularly citrus fruits such as oranges, lemons, and grapefruits[10]. Its chemical structure consists of a flavanone backbone with an OH (hydroxyl group) at orientation 5 and an OCH_3 (methoxy group) at orientation 7 on the ring[11]. HP is known for its potential health benefits and has attracted attention for its antioxidant, anticarcinogenic, anti-atherogenic, anti-inflammatory, and antihypertensive effects[12,13]. Our previous biochemical and histological studies suggest that Hp protects the Cd-induced testicular, renal, and hepatic damage in Wister rats by improving its antioxidant status and reducing lipid peroxidation[14-16]. In this study, we explored DNA damage and the molecular

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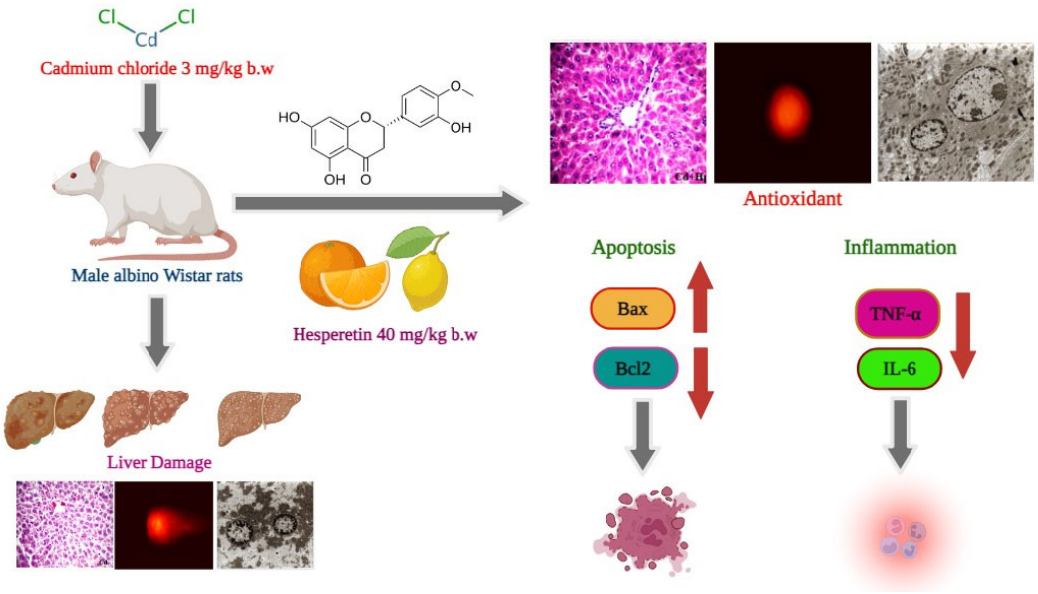

Graphical abstract

mechanisms underlying Cd-induced hepatic damage in Wistar rats by examining apoptotic and inflammatory markers.

Materials and Methods

Chemicals

Hesperetin and Cadmium chloride were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals used in the present study were analytical grade.

Animal model

Male albino Wistar rats weighing 200-220 g were used in this study. The rats were bred at the Central Animal House of Rajah Muthiah Medical College, Annamalai University. They were provided in polypropylene cages with six rats per cage, in adherence to the directives of the National Institute of Nutrition, located in Hyderabad, India, as specified by the Indian Council of Medical Research. The study protocol received approval from the Institutional Ethical Committee of Annamalai University. (Approval No. 643, 2009). The rats were provided with a standard pellet diet from Lipton India Ltd., Mumbai, India, and had access to water ad libitum.

Preparation of Cd and Hp

Rats were subcutaneously injected with 3 mg/kg body weight of Cd dissolved in isotonic saline. Additionally, they received Hp orally, suspended in 0.1% carboxymethyl cellulose, for 21 days.

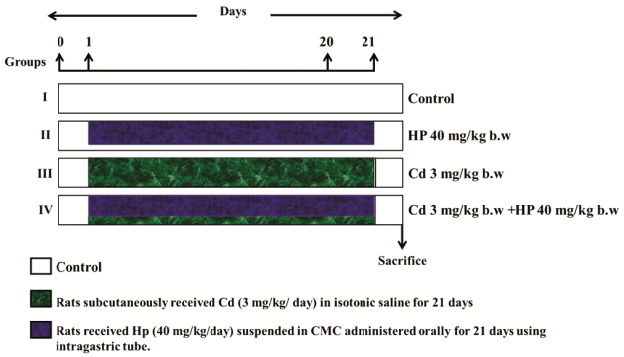

Fig. 1 — Experimental design

Experimental design

24 male albino Wistar rats, distributed randomly into four groups, with six rats allocated to each group. The experimental procedure followed in this study is illustrated in (Fig. 1).

After the experimental period of 21 days, the rats were fasted overnight and subsequently anesthetized for euthanasia *via* cervical decapitation. Immediately, liver tissues were removed and washed with ice-chilled physiological saline. For immunohistochemical and molecular analysis, the liver tissues were preserved in 10% buffered formalin and stored at –80°C.

Histopathological studies

Control and experimental animals' liver tissues were examined histopathologically. Tissues were regularly treated and embedded in paraffin after being fixed in 10 percent buffered formalin; 2-3 mm slices of the tissues were then cut using a rotary microtome,

placed on glass slides, and stained with H&E (hematoxylin and eosin).

Comet assay

Liver tissue from both the control and experimental groups underwent hepatocyte extraction, which was subsequently analyzed using the alkaline comet assay (Singh, 2000)[17]. The slides were placed in lysis buffer at 4°C for 1 h/20 min, then treated with an alkaline solution for 20 min. Subsequently, electrophoresis was performed at 25 V and 300 mA. Following electrophoresis, the slides were neutralized and stained with 50 µl of ethidium bromide (diluted 1:10000). The fluorescence microscope was utilized to capture the images. For each slide, twenty-five images were analysed using Komet v.50 (Kinetics Imaging Ltd., Liverpool, UK) image analyser to measure tail length and olive tail moment.

TEM (Transmission Electron Microscopy)

The fresh liver tissues were quickly immersed in a 3% glutaraldehyde solution at room temperature for 2 h. Then rinsed thrice for 5 min each with 0.1 M PBS. Subsequently, the tissues underwent overnight post-fixation with 1% Osmium Tetroxide (OsO_4) at room temperature. Afterward, they were embedded in epon resin and sliced into ultrathin sections (50 nm thickness) using an ultramicrotome. These samples were positioned on copper grids, stained with uranyl acetate and lead citrate, and examined with a TEM microscope (Philips 420).

Immunohistochemistry analysis

Tissue sections 5 µm thickness embedded in paraffin, 5 µm thick, were prepared, deparaffinized, and exposed to 3% H_2O_2 for 30 min—incubation overnight at 4°C with primary antibodies of Bax and Bcl2. After washing, sections were incubated with an HRP-tagged secondary antibody for 30 min. PBS was used for the subsequent washes, then the sections were exposed to diaminobenzidine (DAB) for 10 min. The sections were counterstained with hematoxylin. The expression levels of Bax and Bcl2 were determined by reading six fields at ×400 magnification using a light microscope with a full-HD imaging system. The Leica Application module was used for data analysis.

Immunoblotting

Liver proteins were extracted and separated by SDS-PAGE using 5% stacking gel and 15% separation Gel. The protein was loaded onto a 12% SDS-polyacrylamide gel at a concentration of 30 µg per well, and electrophoresis was conducted at 80 V for 2 h. After electrophoresis, the proteins were transferred onto a PVDF membrane at a constant current of 100 mA for 2 h, and nonspecific binding was blocked with nonfat milk powder in TBST for 2 h. After which, the primary antibodies (anti-IL-6, anti-TNF-α, and anti-β-actin as an internal control) were left to react overnight at 4°C. Subsequently, the membrane was washed with TBST and incubated with HRP-conjugated goat anti-mouse/rabbit Abs at normal temperature for 45 min. Further washing steps were performed as described before. The protein bands were visualized with DAB using a western blot detection reagent system (Genei) and images recorded with a Gel Documentation system (Bio-Rad). The bands were quantified using the Quantity One analysis software.

Molecular docking

Molecular docking studies were carried out using the automated protein-ligand docking tool CB-Dock, which can reliably detect potential binding sites. 3D structures of Hp (CID: 72281) were submitted to CB-Dock, together with the crystal structures of target proteins Bcl-2 (6QGG), Bax (6EB6), IL-6 (1ALU), and TNF-α (4TVS). The binding cavities of the protein were automatically located by CB-Dock, which analyzed structural and physicochemical features of the protein surface. The method generated a docking grid focused on the predicted binding site, enabling blind docking of compounds. Flexible ligand docking was performed, and the binding conformations were ordered by calculated binding affinity (kcal/mol).

The best binding poses, based on the lowest binding energies (kcal/mol), were selected, and interaction analysis was performed in Discovery Studio Visualizer to observe hydrogen bonding, hydrophobic interactions, and other significant interactions, and to estimate ligand efficiency and docking scores to evaluate the strength of binding and the stability of the complex.

Statistical analysis

The mean values were presented with their corresponding standard deviations (SD). Statistical analyzes were performed using SPSS. To compare the groups, a one-way ANOVA was conducted, followed by Duncan's multiple-range test (DMRT). Statistical significance was determined at a threshold of $P \leq 0.05$.

Results

Histological changes

Figure 2 shows the histopathological changes of control and experimental rats. No histopathological changes were observed in the liver of control (2a) and Hp-treated rats (2b). The light microscopic examination of the hepatic tissue of Cd-treated rats (2c) showed severe hepatocyte necrosis, along with inflammatory cell infiltration, portal inflammation, and derangement of hepatic cords. Rats treated with Cd and concurrently provided Hp supplementation (2d) showed a significant alleviation in the severity of necrosis observed in liver tissues, inflammation, and other histopathological changes. Administration of Hp to rats after Cd exposure significantly reduced the population of lymphocytes and degenerated cells in liver tissue, indicating the ameliorative effects of Hp on the lethal effects of Cd in the liver of rats.

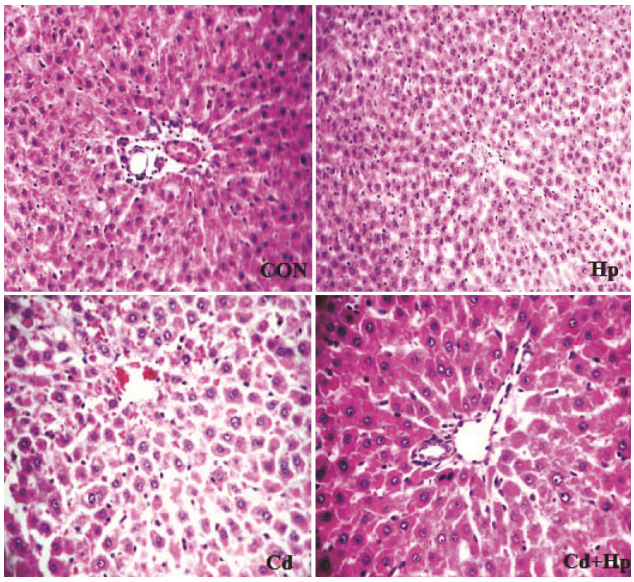

Fig. 2 — Effects of Hp on the liver histology of control and experimental groups (H&E, 20x). Control group with regular hepatic parenchymal structure; Hp-only administered rats show a normal hepatic architecture; Cd-only administered rats show necrosis, inflammatory cell infiltration, and degenerated hepatocytes; Cd+Hp-administered rats supplemented with Hesperitin show almost normal appearance of hepatic sinusoids and veins

Comet assay

The comet assays of hepatic tissue-derived cells showed distinct characteristics across groups (Fig. 3). In the control group (3a), cells exhibited a normal morphology, with a rounded shape and an undamaged nucleus, lacking any tail-like features. Conversely, the HP-treated groups (3b) exhibited a higher percentage of complete cells with undamaged DNA. In contrast, the Cd group (3c) showed greater damage and injury, with comet-shaped formations containing two apoptotic cells, each with a diminutive head and an elongated tail. In Cd-induced HP-treated rats, hepatic tissues exhibited enhancements, demonstrated by reduced tail lengths and a diminished percentage of damaged DNA relative to prior data, as illustrated in (Fig. 3d). The figure presents detailed data on the percentages related to tail length and DNA damage.

TEM examination of the hepatic tissues

Figure 4 presents the TEM images of control and experimental rats. The nuclei in the hepatic tissues displayed a typical structure, accompanied by mitochondria of regular size (4a). In the group receiving

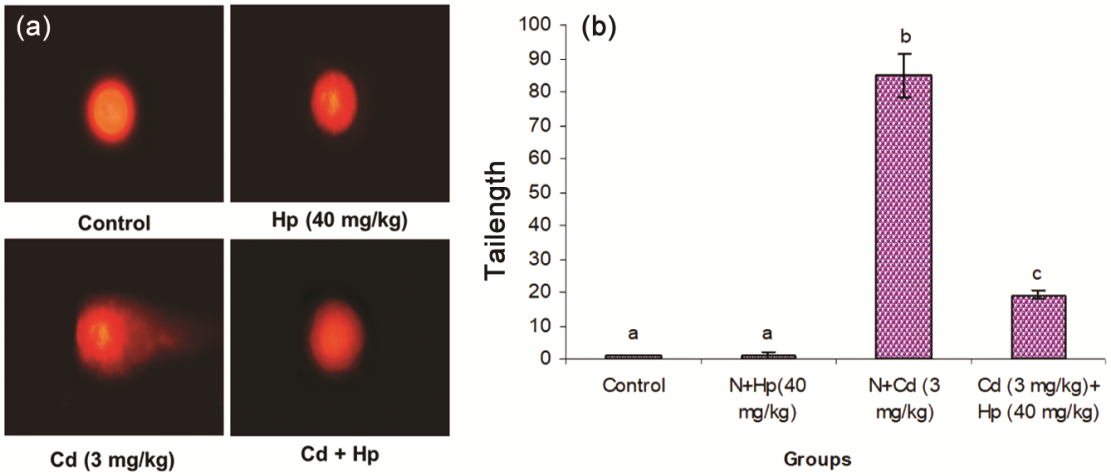

Fig. 3 — depicts representative images of comets stained with ethidium bromide at ×200 magnification, illustrating the DNA migration pattern in hepatocytes. Control rats display no DNA migration. Rats administered only Hp show no DNA migration. Cd-treated rats exhibit significant DNA migration. Hp-administered rats subjected to Cd intoxication display minimal DNA migration

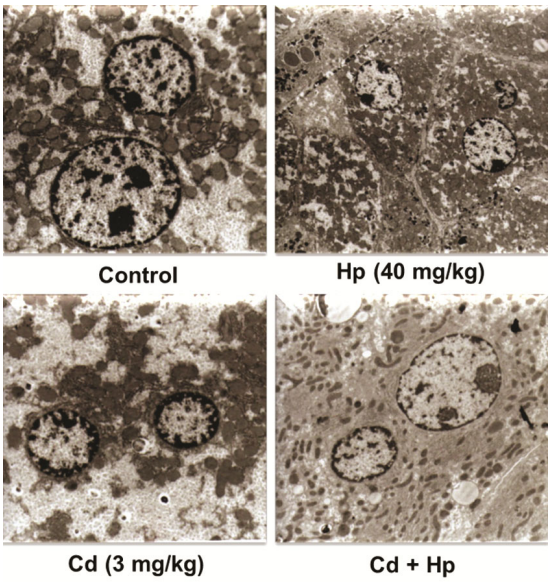

Fig. 4 — TEM images depicting the hepatic tissue of both control and experimental rats were utilized as representatives

HP treatment, there was a notable enhancement, showcasing the normal hepatic structures (4b). Conversely, the Cd-induced group exhibited acute hepatic toxicity, leading to the degeneration of the majority of nuclear components and hepatic structures, as well as the development of fatty changes (4c). However, in the group receiving both Cd and HP treatment, some hepatic tissues showed almost complete recovery of hepatic structure reclamation (4d).

The effect of HP on the levels of Bax and Bcl-2

Figures 5 and 6 illustrate the impact of HP on Bax and Bcl-2 levels in liver tissues, respectively. Hp alone-treated rats did not exhibit significant differences in Bax and Bcl-2 expression compared to the control group. However, in rats exposed to Cd, there was a distinct decrease in Bax and Bcl-2 levels in the liver, in contrast to the control. Notably, the administration of HP in the Cd-induced group effectively reversed these alterations, restoring levels closer to normal than in the Cd-treated group.

The effect of HP on the levels of IL-6 and TNF-α

As illustrated in Figure 7, the liver tissues of Cd-treated rats showed robust staining, indicating a marked increase in IL-6 and TNF-α immunoreactivity compared to the control group. Conversely, this effect was counteracted in the Cd-induced condition with Hp supplementation, as substantial reductions in Cd-induced expression of IL-6 and TNF-α were observed in liver tissue compared with rats treated with Cd

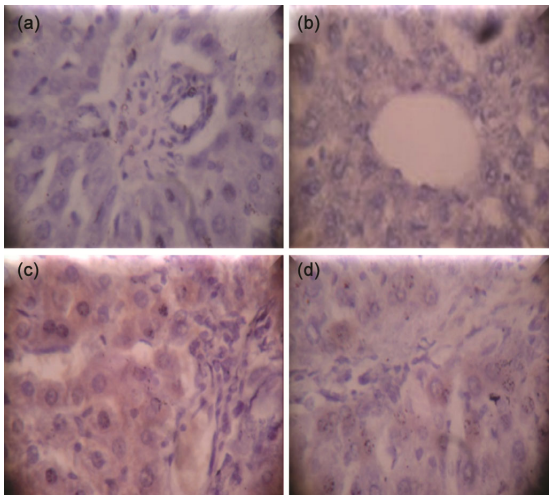

Fig. 5 — Immunohistochemical assessment of Bcl-2 expression in liver tissues of both control and experimental rats is depicted. (a) The photomicrographic image displays the immunohistochemical analysis of Bcl-2 in control rats (group I); (b) Rats treated solely with HP (group II); (c) Rats induced with Cd (group III); and (d) Cd-induced rats treated with HP (group IV). The magnification is 40×

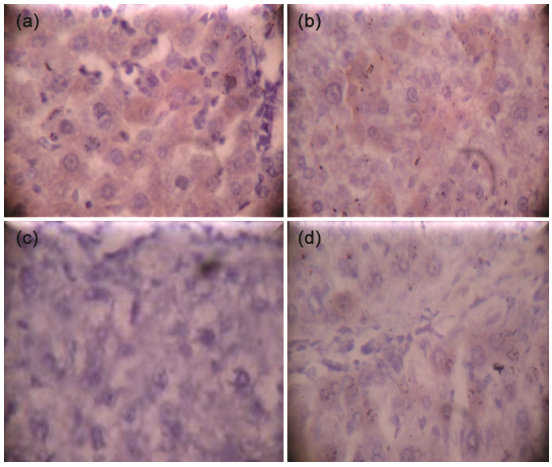

Fig. 6 — Immunohistochemical assessment of Bax expression in the liver tissues of both control and experimental rats. (a) Photomicrographic representation displaying the immunohistochemical analysis of Bax in control rats (group I); (b) Rats treated solely with Hp (group II); (c) Cd-induced rats (group III); and (d) Cd-induced rats subjected to Hp treatment (group IV). Magnification: 40×

alone. No significant differences were detected in IL-6 and TNF-α expression levels when comparing the Hp-treated group alone to the control group.

Molecular docking

Molecular docking studies demonstrated binding interactions between Hp and critical apoptotic and inflammatory proteins, suggesting its potential therapeutic significance (Table 1, Figs. 8 and 9). Hp had

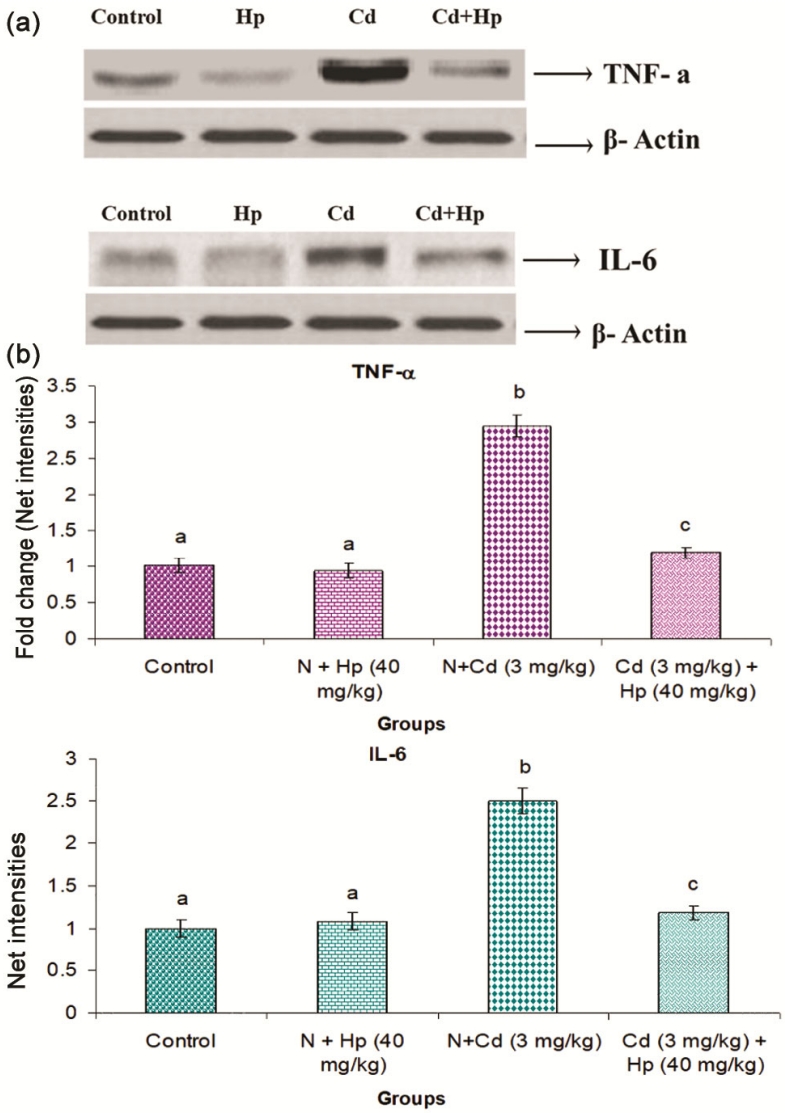

Fig. 7 — Western blot analysis was conducted to assess TNF-α and IL-6 in liver tissues of control and experimental rats. (I) Band intensities were quantified using densitometry and normalized to the respective β-actin loading control. Lane 1: Control rats (group I); Lane 2: Rats treated with Hp alone (group II); Lane 3: Cd-induced rats (group III); Lane 4: Cd-induced rats treated with Hp (group IV). (II) The accompanying graph represents the fold changes in relative protein expression observed in the western blot analysis. Mean values ± SD for groups of ten rats each are presented. Values without a common superscript differ significantly at the 0.05 level (DMRT)

Table 1 — Binding energy and hydrogen bond amino acid interactions of the Hp with apoptotic and inflammatory proteins

Sl. No	Ligand	Protein	Hydrogen bond Involved	Number of Hydrogen bond	Binding energy (kcal/mol)
1	Hesperetin (CID: 72281)	Bcl-2 (PDB:6QGG)	AGR146, ASN143 and ALA100	3	-7.2
2		Bax (PDB: 6EB6)	THR56, THR22, TRP158 and ASP159	4	-6.3
3		IL-6 (PDB:1ALU)	ASN63 and THR137	2	-6.6
4		TNF-α (PDB:4TSV)	TRP114, LYS65, THR72 and CYS101	3	-6.2

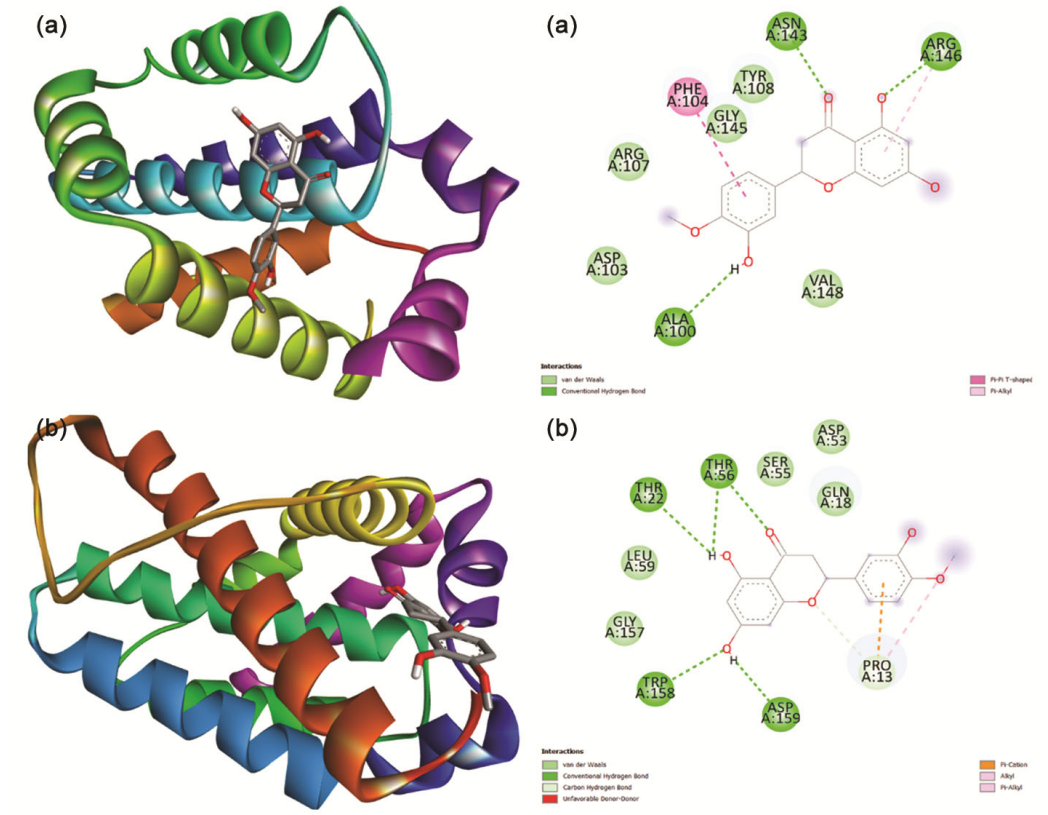

Fig. 8 — 3D and 2D interaction of Hp with apoptotic proteins (a) Bcl-2 (6QGG); and (b) Bax (6EB6)

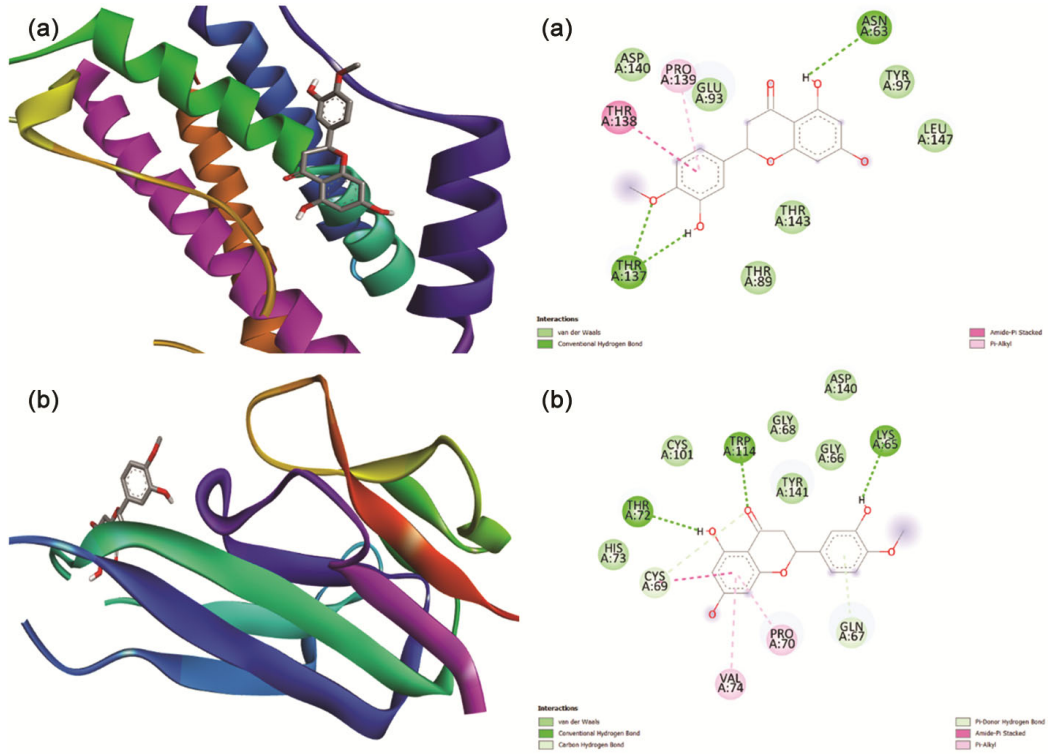

Fig. 9 — 3D and 2D interaction of Hp with inflammatory proteins (a) IL-6 (1ALU); and (b) TNF-α (4TSV)

a significant binding affinity for the anti-apoptotic protein Bcl-2 (PDB: 6QGG), with a binding energy of –7.2 kcal/mol, establishing three hydrogen bonds with AGR146, ASN143, and ALA100. The pro-apoptotic protein Bax (PDB: 6EB6) exhibited four hydrogen bonds with Hp, specifically with THR56, THR22, TRP158, and ASP159, resulting in a binding energy of –6.3 kcal/mol. HP demonstrated a binding energy of –6.6 kcal/mol with the inflammatory cytokine IL-6 (PDB: 1ALU), engaging in two hydrogen bonds with ASN63 and THR137. Furthermore, in combination with TNF-α (PDB: 4TSV), Hp exhibited a binding energy of –6.2 kcal/mol, establishing three hydrogen bonds with TRP114, LYS65, THR72, and CYS101. These connections indicate Hp may effectively regulate both apoptotic and inflammatory processes by forming stable associations with their corresponding regulatory proteins.

Discussion

Cd, an industrial and environmental contaminant, is widely disseminated in our environment and poses a significant ecological issue [18]. Humans and animals are exposed to Cd through multiple sources, including food, industrial waste, tobacco smoke, and shellfish [19]. Cd can induce significant toxicity in hepatocytes, which play a crucial role in eliminating Cd from the bloodstream[5]. The accumulation of Cd in the liver triggers multiple toxic responses in hepatocytes, leading to oxidative damage and the production of reactive oxygen species (ROS)[20]. With a half-life of 15–30 years, Cd persists in the body for a prolonged period[21]. The increased half-life in rats enables sustained release of Cd within the organism over a prolonged period.

Cd risk-mediated induction of ROS production causes oxidative stress within hepatic cells[22]. ROS can directly damage DNA through the introduction of strand breaks, base oxidation, and promote the formation of DNA adducts[23]. Additionally, Cd might indirectly promote DNA damage by interfering with DNA repair processes and cell cycle progression[24]. Accumulation of DNA damage in hepatocytes may lead to dysfunction and contribute to the progression of liver disease, such as hepatocellular carcinoma[25]. The comet assay has been useful for analyzing Cd-induced DNA damage in liver toxicity studies. It has clarified the genotoxic effects of Cd exposure and highlighted the importance of DNA damage in Cd-induced liver disease[26]. Miltonprabu *et al*. (2016)[27] also reported that Cd causes DNA damage in the liver cells. HP's free radical scavenging can protect against liver cell DNA damage[28]. SDS-PAGE has verified the protective effect of HP against Cd-induced genotoxicity in rat liver tissues. Cd induced higher cellular DNA fragmentation; the reversion was observed following pre-treatment with hydrogen peroxide, highlighting its protective role.

The TEM image is crucial for us to understand the changes in liver structure induced by Cd stress. Yang *et al*. (2021) observed severe damage in the liver, as evidenced by TEM images of liver tissue exposed to Cd. Liver tissue appeared to have a distortion in hepatocyte structure; the nuclei were pyknotic with irregular folding and dense chromatin border clustering. The nuclear membrane appeared irregular and constricted, with the rough endoplasmic reticulum lost, which also suggested damage to the liver of Cd. The reported changes reveal the core molecular and physiological function for liver injury[29]. The damaged mitochondria shown in the TEM image are consistent with a previous study on Cd-induced oxidative damage[30]. Disturbed mitochondrial function disrupts cellular energy metabolism and can initiate apoptotic pathways leading to hepatocyte death[31]. These nuclear abnormalities observed in TEM micrographs suggest that Cd-mediated DNA damage may lead to genomic instability and abnormal gene expression profiles. These alterations can interfere with key DNA repair pathways, normal cellular function, and the regulation of the cell cycle, leading ultimately to the potential for disruption of cellular integrity and malignant transformation[32]. The accumulation of lipid droplets in hepatocytes is a hallmark of Cd-induced steatosis. Excessive lipid accumulation can impair liver function, promote inflammation, and contribute to the progression of liver diseases such as non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), and promote inflammation[33]. The pharmacological activities of Hp have reduced the Cd-induced cellular changes in liver tissue. Our results suggest that Hp protects against nuclear and mitochondrial damage and lipid accumulation in Cd-induced rat liver cells.

Cd-induced liver toxicity involves dysregulation of apoptosis, a programmed cell death process[34]. This process relies on a balance between pro-apoptotic and

anti-apoptotic factors. B-cell lymphoma 2 (Bcl2) and Bcl-2-associated X protein (Bax) are pivotal in this mechanism: Bcl2, an anti-apoptotic protein, inhibits cell death, whereas Bax, a pro-apoptotic protein, promotes cell death[35]. Cd exposure in rats has been shown to disrupt the balance between Bcl2 and Bax, favouring the pro-apoptotic pathway. Previous studies have demonstrated that Cd upregulates Bax expression and downregulates Bcl2 expression in liver tissues[36]. This study yielded comparable findings. This dysregulation promotes apoptotic cell death in hepatocytes, leading to liver damage. Whereas Hp-supplemented rats show downregulation of Bax and upregulation of Bcl2 expression, indicating an anti-apoptotic effect of Hp.

Cd exposure over a long time alters the regulation of hepatic pro-inflammatory cytokine synthesis, in addition to altering the expression of apoptosis-related proteins. TNF-α and IL-6 are two important mediators in the inflammatory process[37]. Rat liver tissue exposed to Cd often shows higher levels of TNF-α and IL-6[38,39]. We also found similar results in our study. IL-6 is involved in the regulation of inflammation and immunological response. Cd exposure induces IL-6 secretion from the liver, triggering an inflammatory cascade. Alternatively, TNF-α is a potent pro-inflammatory cytokine and apoptotic factor[40]. Furthermore, in rats fed with Hp, the expression of TNF-α and IL-6 is downregulated. Hp was a methoxyl at the 4'-position of the B ring[41]. This modification appears to prevent the flavanone from inducing cytokine production. These structural modifications may influence their ability to block TNF-α and IL-6 expression[42].

The upregulation of Bcl2, Bax, IL-6, and TNF-α in Cd-induced hepatic injury indicates a complex relationship between apoptosis and inflammation. Bax-induced upregulation and Bcl2-downregulation led to cell death via apoptosis, accompanied by elevated IL-6 and TNF-α, exacerbating liver inflammation and degradation. All these molecular changes cooperate to make rats unwell when Cd is applied. In addition to treating with Hp as a drug can alter Bcl2, Bax, TNF-α, and IL-6 production, which reduces the formation of liver toxicity in Cd-exposed animals.

Conclusion

The present study demonstrates that Hp provides significant protection against Cd-induced hepatotoxicity in rats. Histological observations revealed that Hp greatly attenuated Cd-induced liver necrosis, inflammatory cell infiltration, and architectural alterations. Comet assay demonstrated that Hp is capable of reducing oxidative damage and DNA fragmentation. Transmission electron microscopy also proved that Hp played a protective role in hepatic ultrastructure during AIA. Furthermore, immunohistochemical and western blot studies revealed that Hp modulated the expression of apoptosis-related factors (Bcl-2/Bax) and pro-inflammatory cytokines (IL-6/TNF-α), bringing them close to normal levels. Molecular docking experiments also confirmed the strong, stable binding of Hp to key apoptotic and inflammatory proteins, supporting its *in vivo* actions. These results suggest a hepatoprotective role of Hp against heavy metal-induced liver damage through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms, and that Hp could be considered an effective agent in the treatment of heavy metal intoxication.

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Conflict of interest

All authors declare no conflicts of interest.

References

1 Jadaa W & Mohammed H, Heavy metals-Definition, natural and anthropogenic sources of releasing into ecosystems, toxicity, and removal methods-An overview study. *J Ecol Eng*, 24 (2023) 6.

2 Jaishankar M, Tseten T, Anbalagan N, Mathew BB & Beeregowda KN, Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol*, 7 (2014) 60.

3 Mishra S, Bharagava RN, More N, Yadav A, Zainith S, Mani S & Chowdhary P, Heavy metal contamination: An alarming threat to environment and human health. *Environ Biotechnol Sustain Future*, (2019) 103.

4 Rikans LE & Yamano T, Mechanisms of cadmium-mediated acute hepatotoxicity. *J Biochem Mol Toxicol*, 14 (2000) 110.

5 Yamano T, DeCicco LA & Rikans LE, Attenuation of cadmium-induced liver injury in senescent male Fischer 344 rats: Role of Kupffer cells and inflammatory cytokines. *Toxicol Appl Pharmacol,* 162 (2000) 68.

6 Xu S, Pi H, Chen Y, Zhang N, Guo P, Lu Y, He M, Xie J, Zhong M, Zhang Y & Yu Z, Cadmium induced Drp1-

dependent mitochondrial fragmentation by disturbing calcium homeostasis in its hepatotoxicity. *Cell Death Dis*, 4 (2013) e540.

7 Liu J, Qu W & Kadiiska MB, Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol Appl Pharmacol*, 238 (2009) 209.

8 Fan R, Hu PC, Wang Y, Lin HY, Su K, Feng XS, Wei L & Yang F, Betulinic acid protects mice from cadmium chloride-induced toxicity by inhibiting cadmium-induced apoptosis in kidney and liver. *Toxicol Lett*, 299 (2018) 56.

9 Zhang J, Wang Y, Fu L, Wang B, Ji YL, Wang H & Xu DX, Chronic cadmium exposure induced hepatic cellular stress and inflammation in aged female mice. *J Appl Toxicol*, 39 (2019) 498.

10 Ortiz AD, Fideles SO, Reis CH, Bellini MZ, Pereira ED, Pilon JP, de Marchi MÂ, Detregiachi CR, Flato UA, Trazzi BF & Pagani BT, Therapeutic effects of citrus flavonoids neohesperidin, hesperidin and its aglycone, hesperetin on bone health. *Biomolecules*, 12 (2022) 626.

11 Kim JY, Jung KJ, Choi JS & Chung HY, Hesperetin: a potent antioxidant against peroxynitrite. *Free Radic Res*, 38 (2004) 761.

12 Galati EM, Monforte MT, Kirjavainen S, Forestieri AM, Trovato A & Tripodo MM, Biological effects of hesperidin, a citrus flavonoid. (Note I): antiinflammatory and analgesic activity. *Farmaco*, 40 (1994) 709.

13 Garg A, Garg S, Zaneveld LJ & Singla AK, Chemistry and pharmacology of the citrus bioflavonoid hesperidin. *Phytother Res*, 15 (2001) 655.

14 Shagirtha K & Pari L, Hesperetin, a citrus flavanone, protects potentially cadmium-induced oxidative testicular dysfunction in rats. *Ecotoxicol Environ Saf*, 74 (2011) 2105.

15 Shagirtha K & Pari L, Hesperetin, a citrus flavanone, attenuates cadmium-induced nephrotoxicity in rat. *Biomed Prev Nutr*, 1 (2011) 139.

16 Pari L & Shagirtha KH, Hesperetin protects against oxidative stress related hepatic dysfunction by cadmium in rats. *Exp Toxicol Pathol*, 64 (2012) 513.

17 Singh NP, Microgels for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. *Mutat Res Fundam Mol Mech Mutagen*, 455 (2000) 111.

18 Bernard A, Cadmium and its adverse effects on human health. *Indian J Med Res*, 128 (2008) 557.

19 Järup L, Hazards of heavy metal contamination. *Br Med Bull*, 68 (2003) 167.

20 Wang Y, Fang J, Leonard SS & Rao KM, Cadmium inhibits the electron transfer chain and induces reactive oxygen species. *Free Radic Biol Med*, 36 (2004) 1434.

21 Genchi G, Sinicropi MS, Lauria G, Carocci A & Catalano A, The effects of cadmium toxicity. *Int J Environ Res Public Health*, 17 (2020) 3782.

22 Ikediobi CO, Badisa VL, Ayuk-Takem LT, Latinwo LM & West J, Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells. *Int J Mol Med*, 14 (2004) 87.

23 Ziech D, Franco R, Georgakilas AG, Georgakila S, Malamou-Mitsi V, Schoneveld O, Pappa A & Panayiotidis MI, The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. *Chem Biol Interact*, 188 (2010) 334.

24 Bertin G & Averbeck D, Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). *Biochimie*, 88 (2006) 1549.

25 Karakousis ND, Papatheodoridi A, Chatzigeorgiou A & Papatheodoridis G, Cellular senescence and hepatitis B-related hepatocellular carcinoma: an intriguing link. *Liver Int*, 40 (2020) 2917.

26 Knani L, Bartolini D, Kechiche S, Tortoioli C, Murdolo G & Moretti M, Messaoudi I, Reiter RJ & Galli F, Melatonin prevents cadmium-induced bone damage: first evidence on an improved osteogenic/adipogenic differentiation balance of mesenchymal stem cells as underlying mechanism. *J Pineal Res*, 67 (2019) e12597.

27 Miltonprabu S & Manoharan V, Hepatoprotective effect of grape seed proanthocyanidins on cadmium-induced hepatic injury in rats: possible involvement of mitochondrial dysfunction, inflammation and apoptosis. *Toxicol Rep*, 3 (2016) 63.

28 Famurewa AC, Renu K, Eladl MA, Chakraborty R, Myakala H, El-Sherbiny M, Elsherbini DM, Vellingiri B, Madhyastha H, Wanjari UR & Mukherjee AG, Hesperidin and hesperetin against heavy metal toxicity: insight on the molecular mechanism of mitigation. *Biomed Pharmacother*, 149 (2022) 112914.

29 Yang Z, He Y, Wang H & Zhang Q, Protective effect of melatonin against chronic cadmium-induced hepatotoxicity by suppressing oxidative stress, inflammation, and apoptosis in mice. *Ecotoxicol Environ Saf*, 228 (2021) 112947.

30 Sun J, Bian Y, Ma Y, Ali W, Wang T, Yuan Y, Gu J, Bian J, Liu Z & Zou H, Melatonin alleviates cadmium-induced nonalcoholic fatty liver disease in ducks by alleviating autophagic flow arrest via PPAR-α and reducing oxidative stress. *Poult Sci*, (2023) 102835.

31 Shi S, Wang L, Van der Laan LJ, Pan Q & Verstegen MM, Mitochondrial dysfunction and oxidative stress in liver transplantation and underlying diseases: new insights and therapeutics. *Transplantation*, 105 (2021) 2362.

32 Sun Y, Lv Y, Li Y, Li J, Liu J, Luo L, Zhang C & Zhang W, Maternal genetic effect on apoptosis of ovarian granulosa cells induced by cadmium. *Food Chem Toxicol*, 165 (2022) 113079.

33 Nam Y, Cadmium-induced nonalcoholic fatty liver disease: Implications of the heme oxygenase 1/biliverdin reductase enzymatic pathway. *PhD Thesis*, University of Alabama (2022).

34 Zhou XL, Wan XM, Fu XX & Xie CG, Puerarin prevents cadmium-induced hepatic cell damage by suppressing apoptosis and restoring autophagic flux. *Biomed Pharmacother*, 115 (2019) 108929.

35 Siddiqui WA, Ahad A & Ahsan H, The mystery of BCL2 family: Bcl-2 proteins and apoptosis – an update. *Arch Toxicol*, 89 (2015) 289.

36 Arab HH, Ashour AM, Eid AH, Arafa ES, Al Khabbaz HJ & Abd El-Aal SA, Targeting oxidative stress, apoptosis, and autophagy by galangin mitigates cadmium-induced renal damage: Role of SIRT1/Nrf2 and AMPK/mTOR pathways. *Life Sci*, 291 (2022) 120300.

37 Elsadek AE, Al-Shokary AH, Abdelghani WE, Kamal NM, Ibrahim AO, El-Shorbagy HH, Suliman HA, Barseem NF, Maksoud YH, Azab SM & El Din DM, Serum levels of interleukin-6 and tumor necrosis factor alpha in children with attention-deficit hyperactivity disorder. *J Pediatr Neurosci*, 15 (2020) 402.

38 El-Boshy ME, Risha EF, Abdelhamid FM, Mubarak MS & Hadda TB, Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. *J Trace Elem Med Biol*, 29 (2015) 104.

39 Noor KK, Ijaz MU, Ehsan N, Tahir A, Yeni DK, Zihad SN, Uddin SJ, Ashraf A & Simal-Gandara J, Hepatoprotective role of vitexin against cadmium-induced liver damage in male rats: A biochemical, inflammatory, apoptotic and histopathological investigation. *Biomed Pharmacother*, 150 (2022) 112934.

40 Frances D, Ingaramo PI, Ronco MT & Carnovale CE, Diabetes, an inflammatory process: oxidative stress and TNF-α involved in hepatic complication. *J Biomed Sci Eng*, 6 (2013) 645.

41 Ma YT & Cheung PC, Spectrophotometric determination of phenolic compounds by enzymatic and chemical methods: A comparison of structure−activity relationship. *J Agric Food Chem*, 55 (2007) 4222.

42 Vafeiadou K, Vauzour D, Lee HY, Rodriguez-Mateos A, Williams RJ & Spencer JP, The citrus flavanone naringenin inhibits inflammatory signalling in glial cells and protects against neuroinflammatory injury. *Arch Biochem Biophys*, 484 (2009) 100.