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Hepatoprotective role of vitexin against cadmium-induced liver damage in male rats: A biochemical, inflammatory, apoptotic and histopathological investigation

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ABSTRACT

Cadmium (Cd) is one of the potent occupational and environmental toxicants, which induces oxidative stress to the multiple organs of the body, including liver. The present investigation was planned to evaluate the protective role of vitexin against Cd-prompted hepatotoxicity in rats. 24 male rats were divided into 4 groups viz. control, Cd-induced group (5 mg/kg), Cd + vitexin-treated group (2 mg/kg + 30 mg/kg), and vitexin-treated group (30 mg/kg). After 30 days of treatment, it was indicated that Cd escalated the level of liver function enzymes namely alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) as well as total bilirubin. Whereas the levels of albumin and total proteins were decreased in the rats. Additionally, it reduced the enzymatic activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSR) and glutathione-S-transferase (GST), in addition to glutathione (GSH) content, whereas levels of malondialdehyde (MDA) and reactive oxygen species (ROS) were escalated. Furthermore, level of nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) as well as the activity of cyclooxygenase-2 (COX-2) were increased. Besides, the level of Bax, caspase-9 and caspase-3 were elevated, while the Bcl-2 level was reduced following the Cd intoxication. Histopathological observation revealed significant hepatic tissue damage in Cd-administered rats. However, treatment of rats with vitexin significantly ($p < 0.05$) improved the Cd-induced disruptions in biochemical parameters as well as histological damages. Therefore, it is concluded that vitexin could be used as a therapeutic agent to counter the Cd-generated hepatic toxicity in rats owing to its anti-oxidant, anti-apoptotic and anti-inflammatory potential.

1. Introduction

Cadmium (Cd), a toxic heavy metal and environmental pollutant, is labelled as the seventh most harmful toxicant among the list of pollutants that causes serious health risks to humans and animals [1,2]. Cd mainly enters the body of living organisms through the respiratory and digestive tract as well as skin [3]. Occupational population is extensively exposed to Cd during the production of electronic devices and nickel-Cd batteries, combustion of metal ores, mining, waste incineration and electroplating processes [4]. On the other hand, food is the major source of Cd exposure for non-occupational population with cereals, potatoes and other vegetables being the source of more than the 80% of dietary intake of Cd. Long-term exposure of Cd impairs the functioning of various body organs due to its toxic nature as well as long half-life of

Abbreviations: Cd, cadmium; ALT, alanine transaminase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSR, glutathione reductase; GST, glutathione-S-transferase; GSH, glutathione; MDA, malondialdehyde; ROS, reactive oxygen species; NF-κB, nuclear factor-kappa B; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; COX-2, cyclooxygenase-2; MT, metallothioneins.

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approximately 30 years [5,6]. The detrimental effects of Cd exposure range from kidney damage, reproductive dysfunction, and hepatotoxicity to teratogenicity and carcinogenicity [7]. Half of the total absorbed Cd in body is reported to store in the kidney and liver [8].

Liver, the primary site of body for xenobiotics metabolism and detoxification, is the second major organ that faces the damages induced by Cd exposure [9]. Transportation of Cd to liver tissue occurs by attachment to the cellular membrane and through membrane transporters present in membrane of liver sinusoidal endothelial cells [10]. Upon translocation to the hepatic tissue, it inhibits the activity of hepatic antioxidant enzymes and generates ROS [11]. As a consequence, the increased level ROS promotes lipid peroxidation, damages the cellular macromolecules such as, DNA, proteins, and lipids, and ultimately causes systematic oxidative stress [12]. Begic et al. [13] reported that the Cd poses high affinity for various functional groups especially the sulfhydryl group, hence, it has the capability to affect the activity of multiple enzymes and consequently disrupt the metabolic homeostasis in liver [13]. A recent investigation has reported that Cd exposure results in the hepatotoxicity by causing vascular constriction as well as degeneration and vacuolization of hepatocytes [14].

Although the complete abstinence of Cd exposure to humans is inevitable; however, treatment interventions to alleviate the Cd-induced hepatotoxicity are gaining traction [15]. Flavonoids are an important class of polyphenols, which are proved to offer a wide range of benefits in pharmaceutical, nutraceutical and cosmetics industry [16]. Vitexin is a natural flavonoid which is found in several plants including bamboo, mung bean, common buckwheat and hawthorn. It is evinced to display antioxidant, anti-inflammatory, anti-carcinogenic and anti-bacterial effects [17]. Hence, present study was planned to determine the hepatoprotective role of vitexin against the Cd-generated liver damage by evaluating the liver function enzymes, antioxidant profile, oxidative stress markers, inflammatory and apoptotic markers as well as histopathological indices in the male Sprague-Dawley rats.

2. Material and methods

2.1. Chemicals

Cd and vitexin were bought from Sigma-Aldrich (St. Louis, MO). Remaining reagents were of analytical grade and taken from different commercial sources.

2.2. Animals

Experiment was performed on 24 male albino rats (180–220 g) in Animal facility of University of Agriculture, Faisalabad. Uniform temperature (24 ± 1 °C) and 12 h light/dark cycle were sustained. Additionally, free access to the water and standard food was given to rats. Rats were kept in laboratory condition for 1 week to acclimatize them with laboratory environment.

2.3. Experimental design

Rats were distributed into 4 groups (6 rats per group). The experiment was carried out for 30 days. In control group, tap water and animal feed were given to rats by oral gavage. Cd-treated group received the Cd at a dose of 5 mg/kg BW via oral gavage. Cd + vitexin group was provided the oral dose of 5 mg/kg of Cd. Additionally, 30 mg/kg of vitexin was orally given to rats of co-treated group. In vitexin (only) treated group, the dose of 30 mg/kg of vitexin was given by oral gavage. The Cd dose was selected as per the previous investigation by Renugadevi and Prabu [18], while the vitexin dose was chosen as per the study of Sun et al. [19]. After 30 days of treatment, rats were anesthetized by ketamine, cardiac blood was collected in heparinized syringes and the liver was taken out.

2.4. Sample processing

The blood was kept at room temperature for half hour, centrifuged at 3000 xg at 4 °C for almost 15 min and plasma was separated out. Plasma was stored at −80 °C, which was later used for assessment of hepatic function enzymes. Liver was washed with saline and cut into two halves. A part of liver was placed in ziploc pouch and stored at −20 °C following the decapitation. Later on, the homogenization was conducted in the 5 volumes (1:5; w/v) of the chilled Tris–HCl buffer (0.1 M; pH: 7.4) at 11,000 g for 20 min by the glass Teflon homogenizer. This homogenate would be used to assess inflammatory markers and antioxidant enzymes. Other part of liver was fixed in 10% of formaldehyde for histological observation.

2.5. Assessment of biochemical markers in serum

The levels of liver function enzymes, ALT (ab285264), AST (ab263883) and ALP (ab287823) were ascertained by ELISA kits (Abcam, MA, USA). The levels of total proteins were assessed by Bradford assay kit (BioRad, USA). Whereas, the albumin (ab108789, Abcam, MA, USA) and total bilirubin levels (MBS730053, MyBioSource, Inc., San Diego, USA) were evaluated using ELISA kits. The supplier's protocols were followed during all the assays.

2.6. Assessment of oxidative stress markers and anti-oxidative enzymes

Activity of CAT was determined as per the technique stated by Aebi [20]. SOD activity was assessed in accordance with the procedure of Sun et al. [21]. GSH content was evaluated by following the protocol of Sedlak and Lindsay [22]. GPx activity was evaluated by the technique of Lawrence and Burk [23]. GSR activity was evaluated as per the process stated by Factor et al. [24]. GST activity was assessed as per the protocol of Couri and Abdel-Rahman [25]. MDA level was analyzed in accordance with the procedure explained by Ohkawa et al. [26]. Level of ROS was determined by a method stated by Hayashi et al. [27].

2.7. Inflammatory markers analysis

Level of NF-κB (CSB-E13148r), TNF-α (CSB-E07379r), IL-1β (CSB-E08055r) and IL-6 (CSB-E04640r) as well as COX-2 (CSB-E13399r) were assessed using rat ELISA kits (Cusabio Technology Llc, Houston, TX, USA). Elisa Plate Reader (Bio-Tek, Winooski, VT, USA) was used and manufacturer's instructions were followed to perform the assays.

2.8. Apoptotic markers analysis

The levels of Bax (CSB-EL002573RA), Bcl-2 (CSB-E08854r), caspase-9 (CSB-E08863r) and caspase-3 (CSB-E08857r) were evaluated via ELISA kits (Cusabio Technology Llc, Houston, TX, USA) as per the manufacturer's instructions.

2.9. Histopathological examination

Hepatic tissues were fixed in 10% formaldehyde solution, dehydrated with ascending grades of alcohol, paraffin-embedded blocks were made, and sectioned into 4 μm thick slices with the help of rotary microtome. These slices were then stained by hematoxylin and eosin, and finally examined under light microscope. Photographs were captured using MoticTM 5.0 megapixels camera.

The hepatic injuries were scored by examining the several histological indices such as inflammatory cell infiltration as well as alterations in hepatic cells and the methodology was followed from [28]. The severity score of damages was ranging from 0 to 4, as displayed in the Table 1.

Table 1
Grading scheme for hepatic injuries.

Severity	Extent	Quantifiable finding	Score
Absent	None	0–1 foci	0
Minimal	Very small amount	2–3 foci	1
Slight	Small amount	4–6 foci	2
Moderate	Medium amount	7–12 foci	3
Many	Large amount	> 12 foci, coalescing	4

2.10. Statistical analysis

Data was expressed as mean ± SEM. One-way analysis of variance (ANOVA) technique was performed. After that, Tukey test was applied for comparing the various groups by GraphPad prism 5 software. $P < 0.05$ values were statistically significant.

3. Results

3.1. Effect of Cd and vitexin on biochemical parameters of serum

Cd significantly elevated ($P < 0.05$, $P < 0.01$) the level of hepatic function enzymes (ALT, AST and ALP) as well as total bilirubin, while decreased the level of total proteins and albumin in Cd-intoxicated group compared with control, which indicates the hepatotoxic effects of Cd (Table 2). Contrarily, treatment of rats with Cd and vitexin remarkably lowered ($P < 0.05$, $P < 0.01$) the level of hepatic function enzymes and total bilirubin, while escalating the level of total proteins and albumin in Cd + vitexin-treated group compared with Cd-intoxicated group, which precisely implies the hepatoprotective effects of vitexin. Additionally, no significant difference was noted among the rats of vitexin group and control.

3.2. Effect of Cd and vitexin on antioxidant profile

Cd exposure brought a prominent reduction ($P < 0.05$, $P < 0.01$) in activities of CAT, SOD, GPx, GSR and GST as well as GSH level in Cd-intoxicated group compared to control (Table 3). Nonetheless, vitexin treatment remarkably escalated ($P < 0.05$, $P < 0.01$) the activities of antioxidant enzymes and GSH level in Cd + vitexin-induced group compared to Cd group, which indicates the antioxidant potential of vitexin. Besides, no significant difference was noticed among rats of vitexin and control group.

3.3. Effect of Cd and vitexin on oxidative stress markers

Cd intoxication caused a pronounced elevation ($P < 0.01$) in level of MDA and ROS in Cd group compared with control, suggesting the role of Cd in increasing the oxidative stress (Table 4). However, co-treatment of rats with vitexin and Cd effectively brought down ($P < 0.05$, $P < 0.01$) the levels of these oxidative stress markers in Cd + vitexin group compared with Cd group. Nevertheless, no significant difference was seen among the vitexin group and control.

3.4. Effect of Cd and vitexin on inflammatory markers

Cd intoxication resulted in a profound increase in the level of NF-kB, TNF-α, IL-1β and IL-6 ($P < 0.01$, $P < 0.001$) as well as the COX-2 ($P < 0.05$) activity in liver of Cd group compared with control, which depicts the role of Cd in eliciting the inflammatory damages (Table 5). Conversely, vitexin treatment lowered the level of aforementioned inflammatory markers in co-treated (Cd + vitexin) group compared with Cd group, thereby displaying the anti-inflammatory nature of vitexin. Nevertheless, no significant difference was noticed in levels of inflammatory markers among vitexin and control group.

3.5. Effect of Cd and vitexin on apoptotic markers

Cd administration significantly ($P < 0.05$, $P < 0.01$) escalated the level of Bax, caspase-9 and caspase-3, while reduced the Bcl-2 level in Cd group compared with control, which indicated the potential of Cd in causing apoptotic damages (Table 6). Contrarily, vitexin treatment effectively brought down ($P < 0.05$, $P < 0.01$) the level of Bax, caspase-9 and caspase-3 as well as elevated the Bcl-2 level in co-treated (Cd + vitexin) group compared with Cd group, thereby revealing the anti-apoptotic effect of vitexin in Cd-intoxicated rats. However, no significant difference was spotted in levels of apoptotic markers among vitexin and control group.

3.6. Effect of Cd and vitexin on histopathological parameters

Provision of Cd culminated in the significant ($P < 0.05$) escalation in histological damages of hepatic tissues such as, degeneration of nucleus and lobules, inflammatory cell infiltration, degenerated hepatocytes, unclear hepatocyte boundaries, necrotic cells with nuclear dissolution, congested central veins, dilated sinusoid and fatty alterations in hepatic tissues of Cd-induced group compared with control (Fig. 1**A–D**). Histological scoring also displayed significant ($P < 0.05$) injuries in liver of rat (Fig. 1**E**). However, vitexin treatment significantly ($P < 0.05$) alleviated all the detrimental changes in liver morphology in Cd + vitexin-treated rats compared with Cd group, suggesting the histoprotective effect of vitexin. Additionally, no significant difference was noted among rats of vitexin group and control.

4. Discussion

Concentration of heavy metals particularly Cd is continuously increasing in the environment due to industrial and agricultural activities that is posing inevitable threat to human health [29]. Liver is the main organ for metabolism of xenobiotics and second most important target site that incurs toxic effects of Cd [30]. Previous investigations have revealed that antioxidants have capability to reduce the damaging effects of Cd exposure by mitigating the biochemical alterations in the body. Vitexin is a polyphenolic compound, which has seven hydroxyl groups in its chemical structure that probably contribute to its pharmacological and ROS scavenging activities [31]. Therefore, present investigation was designed to evaluate the hepatoprotective role of vitexin against the Cd-prompted hepatic damages by assessing the liver function enzymes, antioxidant profile, oxidative stress markers,

Table 2
Role of Cd and vitexin on biochemical parameters in serum.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total proteins (g/dL)	Albumin (g/dL)	Total bilirubin (mg/dL)
Control	47.76 ± 4.26	70.72 ± 4.12	109.56 ± 3.79	6.96 ± 0.17	4.41 ± 0.19	0.16 ± 0.02
Cd	90.56 ± 3.18 #	133.56 ± 6.12 ##	252.19 ± 4.69 ##	2.70 ± 0.23 ##	2.18 ± 0.12 ##	0.45 ± 0.06 ##
Cd + Vitexin	85.46 ± 2.89 *	81.12 ± 4.23 **	161.19 ± 4.19 **	5.37 ± 0.26 * *	3.38 ± 0.13 **	0.25 ± 0.01 **
Vitexin	46.12 ± 3.48 **	71.53 ± 4.13 **	112.85 ± 6.69 **	6.97 ± 0.25 * *	4.42 ± 0.23 **	0.15 ± 0.02 **

Values are Mean ± SEM (6 rats per group). Significant differences displayed as # $P < 0.05$, ## $P < 0.01$ compared to control; * $P < 0.05$, ** $P < 0.01$ compared to Cd-treated group.

Table 3
Role of Cd and vitexin on antioxidant profile of hepatic tissues.

Groups	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	GSH (μM/g tissue)	GSR (nM NADPHoxidized/min/mg tissue)	GST (nM/min/mg protein)
Control	7.95 ± 0.56	6.71 ± 0.19	16.49 ± 0.49	13.64 ± 0.75	2.35 ± 0.18	21.85 ± 0.91
Cd	3.17 ± 0.16 #	2.85 ± 0.13 ##	7.99 ± 0.48 ##	5.99 ± 0.45 ##	1.29 ± 0.21 ##	10.75 ± 0.45 ##
Cd + Vitexin	6.47 ± 0.39 *	4.90 ± 0.18 **	16.75 ± 0.55 **	12.13 ± 0.49 *	2.97 ± 0.12 **	19.21 ± 0.38 **
Vitexin	7.36 ± 0.28 *	5.09 ± 0.19 **	17.85 ± 0.29 **	13.85 ± 0.89 **	3.16 ± 0.29 **	22.75 ± 0.81 **

Values are Mean ± SEM (6 rats per group). Significant differences displayed as # $P < 0.05$, ## $P < 0.01$ compared to control; * $P < 0.05$, ** $P < 0.01$ compared to Cd-treated group.

Table 4
Role of Cd and vitexin on oxidative stress markers of hepatic tissues.

Groups	MDA (nmol/g)	ROS (μmol/g)
Control	0.49 ± 0.13	2.55 ± 0.14
Cd	1.75 ± 0.21 ##	5.13 ± 1.19 ##
Cd + Vitexin	2.09 ± 0.19 *	1.95 ± 0.21 **
Vitexin	1.34 ± 0.12 **	1.52 ± 0.09 **

Values are Mean ± SEM (6 rats per group). Significant differences displayed as # $P < 0.05$, ## $P < 0.01$ compared to control; * $P < 0.05$, ** $P < 0.01$ compared to Cd-treated group.

inflammatory and apoptotic markers as well as histopathological parameters in the liver of male Sprague-Dawley rats.

Cd intoxication led to the significant elevation in the levels of liver function enzymes (ALT, AST and ALP) and total bilirubin, while decreasing the level of total proteins and albumin. Hepatic function enzymes are generally regarded as reliable markers to assess the liver function [32]. Elevated levels of these enzymes indicate the disordered state of hepatic tissues and the resultant hepatic dysfunction [33]. The over-generation of ROS affects the performance of cell organelles (mitochondria and endoplasmic reticulum) and DNA, thereby affecting the protein formation. The aforementioned activities result in the necrotic damages in the liver tissues as well as the hepatic functions, which lead to the reduction in the level of albumin and total proteins [34,35]. Moreover, Cd exposure leads to the lipid peroxidation in cells, which causes the impairment of plasma membrane of hepatocytes [36], ultimately resulting in excessive release of ALT, AST and ALP into the blood [37]. However, treatment of rats with the vitexin significantly lowered the levels of the aforementioned hepatic markers . It was indicated that vitexin has the ability to restore the structural integrity of plasma membrane of hepatocytes by reducing the level of ROS and lipid peroxidation, which could ultimately reduce the release of ALT, AST and ALP into the blood. Furthermore, the level of total bilirubin was decreased, whereas the level of total proteins and albumin were increased due to the ROS-scavenging nature of vitexin.

In the present investigation, Cd exposure significantly lowered the activity of CAT, SOD, GPx, GSR and GST in addition to the GSH level indicating the presence of oxidative stress. Metallothioneins (MT) are metal-binding proteins in mammals, which have tendency to protect cytotoxicity generated by Cd and other toxic metals [38]. When the Cd level rises beyond the MT-binding capacity of liver, then non-MT-bound Cd ions produce toxic effects by generating ROS including $O_2^{\bullet-}$, HO• and H_2O_2 [39]. When the build-up of ROS exceeds the antioxidant capacity, entire antioxidant defense system gets distressed [40]. SOD, CAT and GPx are considered as first-line defense endogenous antioxidant enzymes, which have the strong capability to counter ROS [41]. CAT mediates the conversion of H_2O_2 to H_2O and O_2 by preventing the formation of the toxic ions, OH^- [42]. SOD dismutates superoxide ions formed as by-products during oxidative stress into H_2O_2 [43]. GPx takes part in reduction of H_2O_2 and lipid peroxides level to alleviate oxidative stress [44]. GST plays a pivotal role in detoxification process in the hepatic tissues by catalyzing the conjugation of GSH to xenobiotic substrates [45]. It was indicated that Cd exposure results in generation of ROS, that leads to peroxidation of lipids by inhibiting GSH content or by the depletion of antioxidant enzymes [46].

Apart from the depletion of antioxidant enzymes, Cd prompted the lipid peroxidation (MDA) and ROS in hepatic tissues of rats. It was conceived that the free radicals released due to Cd exposure directly reacted with the lipid part of cell membrane and resulted in its peroxidation as indicated by increased MDA level, a biological marker of lipid peroxidation. However, vitexin administration effectively increased the activities of CAT, SOD, GPx, GSR and GST, along with the elevation in GSH content. Whereas, levels of MDA and ROS were decreased. Previous reports stated that the apigenin isomer, vitexin has ability to revert the oxidative effects of a number of chemical species. It can scavenge free radicals, ameliorate oxidative stress, induce multiorgan damage and inhibit systemic oxidative stress [47–49]. It was indicated that the ROS scavenging potential of vitexin could be due to the adjacent dihydroxyl groups in its chemical structure [50]. Thus, the ability of vitexin in

Table 6
Role of Cd and vitexin on apoptotic protein levels.

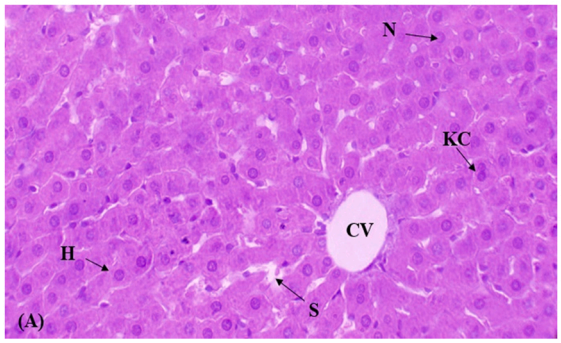
Groups	Bax (pg/mL)	Bcl-2 (ng/mL)	Caspase-9 (pg/mL)	Caspase-3 (pg/mL)
Control	1.97 ± 0.28	16.01 ± 0.75	3.25 ± 0.13	1.65 ± 0.11
Cd	8.02 ± 0.27 #	5.60 ± 0.44 #	16.28 ± 0.81 ##	12.56 ± 0.74 ##
Cd + Vitexin	2.84 ± 0.14 *	13.23 ± 0.40 *	4.64 ± 0.28 **	2.44 ± 0.28 **
Vitexin	1.93 ± 0.25 *	16.18 ± 0.76 *	3.23 ± 0.11 *	1.61 ± 0.12 **

Values are Mean ± SEM (6 rats per group). Significant differences displayed as # $P < 0.05$, ## $P < 0.01$ compared to control; * $P < 0.05$, ** $P < 0.01$ compared to Cd-treated group.

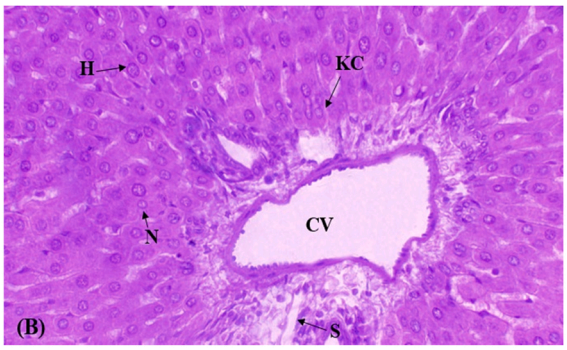
Table 5
Role of Cd and vitexin on inflammatory markers of hepatic tissues.

Groups	NF-κB (ng/g tissue)	TNF-α (ng/g tissue)	IL-1β (ng/g tissue)	IL-6 (ng/g tissue)	COX-2 (ng/g tissue)
Control	15.49 ± 0.39	6.49 ± 0.29	23.71 ± 0.49	4.82 ± 0.49	25.32 ± 0.79
Cd	59.75 ± 1.36 ##	15.59 ± 0.52 ###	82.32 ± 1.91 ###	22.12 ± 0.79 ###	67.49 ± 2.12 #
Cd + Vitexin	25.32 ± 2.14 *	10.48 ± 0.59 ***	34.27 ± 1.91 ***	10.25 ± 0.61 ***	36.76 ± 1.15 **
Vitexin	15.52 ± 1.41 *	6.99 ± 0.49 ***	22.49 ± 0.79 ***	4.99 ± 0.42 ***	24.95 ± 1.61 *

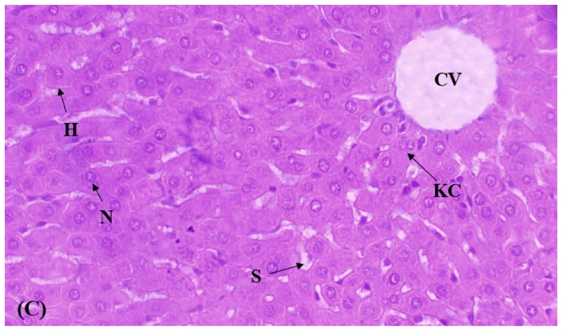
Values are Mean ± SEM (6 rats per group). Significant differences displayed as # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ compared to control; * $P < 0.05$, ** $P < 0.01$, ** $P < 0.001$ compared to Cd-treated group.



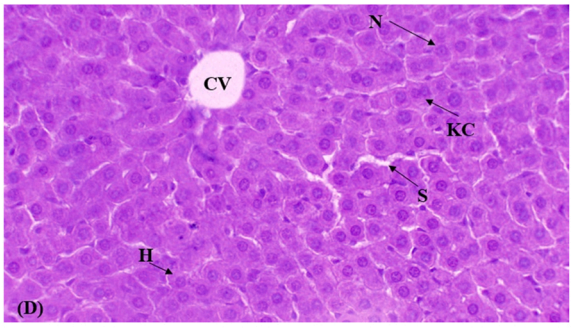
(A) Control



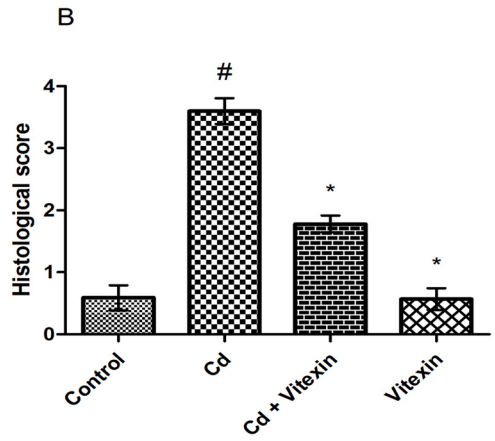
(B) Cd-treated group



(C) Cd + Vitexin-treated group



(D) Vitexin-treated group



(E) Quantitative histopathological scores

Fig. 1. Photomicrographs of Cd and vitexin-induced rat liver tissues (40x). A) Control group showed normal histology, and no damage in central veins and hepatocytes was observed. B) Cd exposure led to adverse changes in histology of liver tissues, including degenerated hepatocytes, unclear hepatocyte boundaries, necrotic cells with nuclear dissolution, congested central veins, as well as dilated sinusoid. C) Treatment of vitexin along with Cd showed improvement in morphology of liver tissues. D) The photomicrographs of control and vitexin group looked quite similar and no prominent changes were observed among them. Cd: Cadmium, S: sinusoids, CV: central vein, KC: kupffer cell, N: nucleus, H: hepatocytes. E) Histopathological scoring displaying infiltration of inflammatory cells and necrotic damages as well as nuclear and fatty degenerations. Significant differences were noted among treated and non-treated groups as $^{\#}$ $P < 0.05$ compared to control; * $P < 0.05$ compared to Cd-treated group.

mitigating Cd induced disruption in hepatic antioxidant defense system and lipid peroxidation is ascribed to its antioxidant as well as radical scavenging potential.

Cd exposure led to a profound increase in level of NF-kB, TNF-α, IL-1β and IL-6 as well as the COX-2 activity in rat hepatocytes. In the current study, Cd exposure resulted in oxidative stress, which subsequently activated the NF-κB. The NF-κB is a cytosolic protein complex whose activation triggered the release of inflammatory mediators, such as cytokines and chemokines that ultimately induced liver injury [51]. COX-2 is an inducible enzyme, which also contributes to the overall inflammation in the tissues [52]. In the present investigation, levels of the inflammatory biomarkers were increased, which indicated the inflammation in tissues of rat liver. However, vitexin supplementation significantly decreased the levels of aforementioned inflammatory markers. Our results are in harmony with the investigation of Jiang et al. [53], who stated that the vitexin could remarkably reduce the release of inflammatory biomarkers in cerebral stroke-induced inflammation in rats.

Cd intoxication as well as protective role of vitexin in testicular tissues was determined by assessing the levels of apoptotic markers. Cd

administration increased the level of Bax, caspase-9 and caspase-3, whereas reduced the Bcl-2 level. Apoptotic cell death is one of the factors which accounts for the hepatic dysfunction [54]. Bax and Bcl-2 are related to Bcl-2 protein family, which mediate the intrinsic (mitochondrial) apoptotic pathway [55]. Bcl-2 is considered as anti-apoptotic protein, whereas Bax, being a pro-apoptotic protein, performs opposing functions [56]. Escalation in Bax level and reduced Bcl-2 level trigger the eviction of cytochrome c from the mitochondrial membrane into cytoplasmic matrix, which culminates in the activation of caspase-9 [57]. Caspase-9 acts as the catalyst for further activation of caspase-3, which subsequently causes the apoptotic damages [58]. However, vitexin supplementation reduced the level of Bax, caspase-9 and caspase-3, whereas the level of Bcl-2 was elevated. This finding revealed the anti-apoptotic potential of vitexin against Cd-intoxication.

In the present study, histomorphological assessment of hepatic tissues displayed the several detrimental effects of Cd on liver histology such as, dilated sinusoids and disrupted central venules as well as adverse alterations in kupffer cells and nuclei of hepatocytes. Our findings coincide with the study of Al-Baqami and Hamza, in which it was revealed that the Cd brought histological alterations in hepatic tissues of rats [59]. Cd stimulated these damages through lipid peroxidation and generation of free radicals. The free radicals can potentially impair the macromolecules and result in oxidative damage, which eventually affect the cellular functions and lead to necrosis and degeneration of liver tissues [60]. However, vitexin administration effectively reduced the Cd-prompted histopathological damages in rat liver. As mentioned earlier, vitexin is a potent anti-oxidant and free radical scavenger as well as it possesses anti-inflammatory property. Therefore, it was deduced that vitexin could exert protective role in maintaining the structure of hepatic tissues owing to its anti-oxidant anti-apoptotic and anti-inflammatory effects.

5. Conclusion

Taken together, Cd exposure led to hepatotoxicity in rats by escalating the level of liver function enzymes, total bilirubin, oxidative stress markers (MDA and ROS) and inflammatory markers. Whereas, the levels of albumin and total proteins as well as the activities of antioxidant enzymes and GSH were reduced. Besides, histological damages were induced in rat liver following the Cd exposure. Furthermore, Cd escalated the level of Bax, caspase-9 and caspase-3, while reduced the Bcl-2 level. However, vitexin treatment effectively abrogated all the aforementioned Cd-prompted hepatic damages in rats owing to its antioxidant, anti-apoptotic, anti-inflammatory and hepatoprotective effects. In conclusion, it is stated that treatment with vitexin can be a promising therapeutic strategy to ameliorate Cd-induced hepatic damage.

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CRediT authorship contribution statement

Kiran Kousar Noor: Conceptualization, Methodology, Formal analysis, Investigation, Visualization. **Muhammad Umar Ijaz:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization. **Nazia Ehsan:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization. **Arfa Tahir:** Conceptualization, Data curation, Methodology, Formal Analysis, Investigation, Visualization, Supervision. **Derya Kertas Yeni:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization. **S. M. Neamul Kabir Zihad:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – review & editing. **Shaikh Jamal Uddin:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – review & editing. **Asma Ashraf:** Conceptualization, Data curation, Methodology, Formal analysis, Investigation, Visualization, Supervision. **Jesus Simal-Gandara:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Supervision, Writing – review & editing.

Author contributions

MUI, NE and KKN formulated the current study and executed the experiments. AT and AA help in data curation. DKY helped in statistical analysis. AT and MUI wrote the manuscript. SMNKZ, SJU, and JSG wrote and reviewed the manuscript. Final form of manuscript was approved by all the authors.

Animal ethics approval

All the experimental protocols for animal handling and treatment were reviewed and monitored by ethical committee of University of Agriculture, Faisalabad, in line with the European Union of animal care and experimentation approved (CEE Council 86/ 609) approved protocol.

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.

Data availability

Data will be made available on request.

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