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Protective effects of vitexin on cadmium-induced renal toxicity in rats

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ABSTRACT

Cadmium (Cd) is an industrial contaminant that poses severe threats to human and animal health. Vitexin (VIT) is a polyphenolic flavonoid of characteristic pharmacological properties. We explored the curative role of vitexin on Cd-induced mitochondrial-dysfunction in rat renal tissues. Twenty-four rats were equally divided into four groups and designated as control, Cd, Cd + vitexin and vitexin treated groups. The results showed that Cd exposure increased urea and creatinine levels while decreased creatinine clearance. Cd reduced the activities of antioxidant enzymes, i.e., catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione content in the Cd exposed group. Cd exposure significantly ($p < 0.05$) elevated the reactive oxygen species (ROS) and Thiobarbituric acid reactive substances (TBARS) levels in rat kidney. Cd also caused a significant ($p < 0.05$) reduction in the mitochondrial TCA-cycle enzymes, including isocitrate dehydrogenase, succinate dehydrogenase, alpha-ketoglutarate dehydrogenase, and malate-dehydrogenase activities. Besides, mitochondrial respiratory chain enzymes, including NADH-dehydrogenase, coenzyme Q-cytochrome reductase, succinic-coenzyme Q, and cytochrome c-oxidase activities were also decreased under Cd exposure. Cd exposure also damaged the mitochondrial membrane potential (MMP). However, VIT treatment potentially reduced the detrimental effects of Cd in the kidney of rats. In conclusion, our study indicated that the VIT could attenuate the Cd-induced renal toxicity in rats.

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1. Introduction

Cadmium (Cd) is among the critically harmful environmental pollutants that pose several threats to animal and human health (Zhu et al., 2019). It is scientifically established as a highly toxic metal with no known essential role in the biological systems (Dai

et al., 2016). It exists universally in nature and damages humans and animals (Liu et al., 2019). Cd is one of the most harmful metals throughout the world, and its exposure is a threat to approximately 10% of the world population with a higher mortality rate (Moulis and Thévenod, 2010). Cd exposure permanent sources are industrial applications such as a corrosive reagent, used in Ni-Cd batteries, color pigments and phosphate fertilizer, and polyvinyl chloride (PVC) products (Genchi et al., 2020). An essential route of Cd exposure in humans is rice consumption (Shi et al., 2020). Therefore, Cd-contaminated food has become a severe and constant threat to human health and food safety (Wang et al., 2019). Once it enters the body, it causes adverse health issues such as nephrotoxicity, hepatotoxicity, carcinogenesis, and ototoxicity (Seif et al., 2019).

The kidney is one of the main targets of cadmium toxicity. Cd ions impersonate as the essential metal ions like Zn, Mn, Fe, Cu,

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and Ca and cross the kidney's cell membrane barriers using their transport-pathways (Thévenod, 2018). In eukaryotic cells, mitochondria are the most critical organelles that mediate many biological functions and provide a vital energy source to cells (Davila et al., 2018). Cd induces oxidative stress by excessive mitochondrial reactive oxygen species (ROS) production (Belyaeva et al., 2006) and can directly affect renal mitochondria (Thévenod, 2009). Cd damages the mitochondrial structure via initiating a shortage of cristae by reducing cristae numbers (Lee and Thévenod, 2020).

Polyphenolic flavonoids have great concern nowadays due to their distinctive pharmacological properties (Bakar et al., 2019; Ijaz et al., 2020). Vitexin (apigenin-8-C-D-glucopyranoside) is a bioactive flavonoid present in various plants such as bamboo (Wang et al., 2012), fenugreek (Khole et al., 2014), and mung beans (Hou et al., 2019). It possesses potential pharmacological properties such as anti-cancer, anti-inflammatory, neuroprotective, antioxidant, and anti-hyperalgesic. However, vitexin's possible effect on Cd-induced mitochondrial toxicity has not been studied to date and hold greater significance. Therefore, we planned this research to elucidate the curative effect of vitexin on Cd-induced toxicity in rats' renal tissues.

2. Materials and methods

2.1. Animals

The current trial was conducted on adult male Sprague Dawley rats (180–200 mg/kg). Rats were kept in the bioterium of the University of Agriculture, Faisalabad. We maintained the standard conditions (photoperiod 12 h light/dark; humidity 40–60%; temperature $25 \pm 1^\circ\text{C}$) and provided the tap water and standard food chaw. Animals were treated in strict compliance with international instructions for the use of experimental animals.

2.2. Experimental design

Twenty-four male rats were equally distributed into four groups having six rats in each and treatment continued for 30 days. Group A: Control group, provided with normal food and tap water. Group B received 2 mg/kg BW dose of Cd injection i.p. daily. Group C: Received Cd (2 mg/kg BW i.p.) and vitexin (30 mg/kg BW) orally. Group D received vitexin at a dose of 30 mg/kg BW orally. The dose of Cd was chosen according to the study conducted by El-Maraghy et al. (2001) while dose of vitexin was selected according to Sun et al. (2016). Animals were treated in compliance with the European Union of Animal Care and Experimentation (CEE Council 86/609) approved protocol. Retro-orbital venous plexus was used to collect blood; serum was isolated from blood and stored at 4°C . Rats were given anesthesia by diethylether before slaughtering, and kidneys were removed, rinsed in normal saline and 10% w/v homogenate was formed in PBS at neutral pH. Centrifugation was carried out at $12,000 \times g$ for 60 min at 4°C . The Supernatant was separated and stored at -20°C until used in further analysis.

2.3. Isolation of kidney mitochondria

The process of Mingatto et al. (1996) was applied for the mitochondrial isolation from kidneys. The kidney tissues were blended in the Medium-I (250 mM mannitol, 70 mM sucrose, 1 mM EDTA, 50 mM Tris-HCl, 10 mM HEPES, 120 mM KCl and pH 7.4). We centrifuged the homogenate for 5 min at $755 \times g$. The resultant homogenate was centrifuged again for 15 min at $13300 \times g$. The medium-II (250 mM mannitol, 50 mM Tris-HCl, 10 mM HEPES, 70 mM sucrose, pH 7.4) was used in suspension of the resulting

pellets cleaned two times using the same buffer by centrifuge for 15 min at $13,300 \times g$. The final mitochondrial pellets were suspended again in the same media and then immediately used for further analysis.

2.4. Assessment of kidney function marker

Concentrations of serum urea, creatinine and creatinine clearance were assessed using laboratory procedures provided with the Randox standard laboratory kits (Crumlin, Co. Antrim, UK).

2.5. Assessment of antioxidant enzymes activity, ROS and TBARS levels

The activity of CAT was assessed according to the procedure of Aebi, 1984. The GPx and SOD activities were evaluated according to Das et al. (2010) and Manna et al. (2009), respectively. GSH content was assessed by following the procedure of Ellman, 1959. The reactive oxygen species (ROS) level was assessed by employing ELISA kits (Shanghai Enzyme-Linked Biotechnology Company, Ltd., Shanghai, China) following the guidelines provided with kit. Thiobarbituric acid reactive substances (TBARS) level was assessed according to Ohkawa et al. (1979) methodology.

2.6. Assessment of TCA-cycle enzymes

We applied the Bernt and Bergmeyer (1974) procedure to determine the activities of isocitrate dehydrogenase (ICDH). The process of Reed and Mukherjee (1969) was followed to evaluate the α -KGDH activity. Succinate-dehydrogenase (SDH) activity was assessed via following the technique of Slater and Borner (1952). Mehler et al. (1948) method was followed to determine Malate dehydrogenase (MDH).

2.7. Analysis of respiratory chain complex activity in renal mitochondria

Mitochondrial respiratory chain complexes activities were assessed by using the standard kits manufactured by Suzhou Comin Biotechnology LTD., China.

2.8. Assessment of mitochondrial membrane potential

The MMP was assessed by mitochondrial staining with a cationic-fluorescent dye (Rhodamine 123). To incubate suspension of mitochondria ($0.5 \text{ mg protein ml}^{-1}$), the tubes were slightly shaken for 10 min at 37°C with Rh 123 ($1.5 \mu\text{M}$). At emission (490 nm) and excitation (535 nm) wavelength, the Elmer LS-50B Luminescence fluorescence spectrophotometer was applied for the estimation of fluorescence (Baracca et al. 2003).

2.9. Statistical analysis

The obtained values are shown as Mean \pm SEM. To analyze the mean variations in the experimental treatments, we used the one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test. All the statistical analyses were performed in Minitab software and the significance level was set at $p < 0.05$.

3. Results

3.1. Effect of vitexin on serum markers of kidney in Cd-exposed rats

Cd administration caused a significant ($p < 0.05$) increase in serum urea and creatinine levels, however, a significant

($p < 0.05$) reduction in creatinine clearance was observed in Cd administered rats (Table 1). Co-administration of vitexin with Cd showed a remarkable reduction in urea and creatinine levels while increasing the creatinine clearance compared to Cd-exposed rats. The VIT-treated group displayed the average level of these renal function markers in contrast to the control group. This showed good efficacy of the vitexin in controlling the urea and creatinine levels in kidney.

3.2. Effect of vitexin on oxidative stress and antioxidative capacity

Cd exposure significantly ($p < 0.05$) reduced the suite of antioxidant enzymes activities i.e., CAT, SOD, GPx and GSH content along with increased ROS and TBARS levels in the renal tissues (Table 2). Co-administration of VIT with Cd elevated the CAT, SOD, GPx activities and GSH content while remarkably ($p < 0.05$) reduced the ROS and TBARS levels compared to Cd exposed rats. The VIT alone treatment showed normal antioxidants activities, ROS and TBARS levels as in the control group.

3.3. Effect of vitexin on the activities of renal mitochondrial respiratory chain complexes

As shown in table 3, we observed a significant ($p < 0.05$) decline in the activities of mitochondrial complexes (I-IV) upon exposure to Cd when compared with control group. Co-treatment of VIT with Cd significantly ($p < 0.05$) restored the mitochondrial respiratory chain complexes activities compared to Cd administered rats. VIT alone treated group showed normal activities of mitochondrial respiratory-chain complexes.

3.4. Effects of vitexin on activities of renal mitochondrial TCA cycle enzymes

Table 4 presents that the Cd exposure significantly ($p < 0.05$) reduced the TCA-cycle enzymes (α -KGDH, ICDH, SDH and MDH) activities when compared with the control group. Co-administration of VIT with Cd significantly ($p < 0.05$) reestablished the TCA enzymes activities compared to Cd-treated rats. However, the VIT alone treatment group did not exhibit any significant variations in the TCA enzyme activities in contrast to the control group.

3.5. Effect of the vitexin on mitochondrial membrane potential

Results displayed that rats Cd-administered rats showed a substantial ($p < 0.05$) depolarization of mitochondrial membrane potential ($\Delta\Psi_m$) when matched with the rats in control group (Table 4). However, co-administration of VIT with Cd restored the loss of $\Delta\Psi_m$, in comparison to Cd exposed rats. VIT alone treated group showed an average mitochondrial membrane potential as in the control group.

4. Discussion

Mitochondria are the hub of bioenergetic metabolism and the largest ATP production generator (Lee et al., 2020). However, the

production of oxidative stress conditions and perturbation of the mitochondrial respiratory chain is considered the primary factor of mitochondrial damage (Khan et al., 2016). The ETC impairment immensely damages the tissues and causes oxidative stress conditions (Nita and Grzybowski, 2016). Such damages in the mitochondrial processes directly disturb ATP production (Zanellati et al., 2015). Previous studies have shown that Cd disrupts the mitochondrial enzyme activities (Wang et al., 2004), induces mitochondrial swelling, MMP collapse and inhibits respiration (Lee et al., 2020). This experimental study is the first of its kind to explore vitexin's effect, which is usually used as a curative agent, on the mitochondrial dysfunction in Cd exposed rat's kidneys.

The present research outcomes demonstrated that the serum urea and creatinine levels were escalated; while the creatinine clearance declined after Cd exposure. The urea and creatinine levels are used as serum biochemical markers in kidney function (Sahu et al., 2020). Urea is the waste product of protein metabolism. Creatinine is a nitrogenous compound formed by creatine and phosphocreatine during muscular metabolism and primarily eliminated through glomerular filtration (Sepulveda, 2019). Kidney damage lowers the renal glomerular filtration rate (GFR), resulting in elevated serum urea and creatinine. The reduced GFR causes the accumulation of xenobiotics, endogenous waste, and various toxicants (Orr and Bridges, 2017). However, the Cd has been reported to reduce the GFR, which is also witnessed by elevated serum urea and creatinine (Poosa and Vanapatla, 2020). However, the treatment with VIT showed a reduction in urea and creatinine levels, with increased creatinine clearance, that could prevent Cd's nephrotoxic effect by increasing the GFR.

Our results stated that Cd treatment reduced the antioxidant enzymes (CAT, SOD, GPx) activity and the GSH content; however, it increased ROS and TBARS levels in the renal tissues. Antioxidants are a central hub against an excessive amount of ROS (Latif et al., 2020). SOD combines two oxygen radicals and converts them into hydrogen peroxide. CAT is an important antioxidant enzyme that helps GSH and GPx converts the H_2O_2 into oxygen and water (Ighodaro and Akinloye, 2018). One of the critical mechanisms of Cd-induced toxicity is oxidative stress (OS). Cd induces OS by disturbing the production and elimination balance of ROS in cells and tissues, which impair the protein and membranes (Akinyemi et al., 2017). ROS generation and increased TBARS level are linked to Cd-induced toxicity in the kidney, ultimately responsible for the change in the defensive mechanism. Cd-exposure also stimulates the maximum ROS level in rats by suppressing antioxidants such as CAT, SOD, GSH, and GPx (Seif et al., 2019). The previous investigations have shown a substantial increase in lipid peroxidation after Cd exposure in rat kidney (Poosa and Vanapatla, 2020). However, the vitexin administration substantially decreased the ROS and TBARS levels by restoring the antioxidant enzyme activities.

Our study indicated that Cd significantly reduced the TCA cycle enzymes (α -KGDH, ICDH, MDH and SDH) activity. The mitochondria are among the most critical subcellular organelles that produce energy and are susceptible to OS. Mitochondrial enzymes (ICDH, α KGDH, SDH and MDH) trigger various substrates oxidation by the TCA cycle that yielded reducing equivalents. The electron transport chain (ETC) channels these reducing equivalents for ATP production through oxidative phosphorylation (Chandramohan et al., 2015). The investigation by Hu et al. (2019) reported that Cd increased TCA cycle enzyme oxidation, causing lower ATP production in rat lungs. However, the co-treatment with VIT restored TCA cycle enzyme activities, potentially by reducing the oxidative stress.

The present study indicated that Cd exposure reduced mitochondrial complexes (I-IV) activity of the ETC. Mitochondria is the primary source and target of ROS (Li et al., 2012). The ETC consists of various multimeric complexes (I-IV), located in the inner

Table 1
Values having different superscripts in the same column are significantly different.

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Creatinine Clearance (mg/dL)
Control	17.51 ± 0.61 ^a	2.60 ± 0.15 ^a	1.78 ± 0.12 ^a
Cd	45.88 ± 1.57 ^b	5.67 ± 0.31 ^b	0.38 ± 0.03 ^b
Cd + vitexin	24.97 ± 0.8 ^c	3.37 ± 0.20 ^a	1.06 ± 0.08 ^c
Vitexin	17.46 ± 0.9 ^a	2.48 ± 0.19 ^a	1.82 ± 0.10 ^a

Table 2

Values having different superscripts in the same column are significantly different.

Groups	CAT (U/mg protein)	SOD (U/mg protein)	GSH (μ M/g tissue)	Gpx (U/mg protein)	TBARS (nm TBARS/min/mg tissue)	ROS (U/g tissue)
Control	4.81 \pm 0.22 ^a	6.62 \pm 0.19 ^a	24.13 \pm 0.84 ^a	16.46 \pm 0.63 ^a	10.48 \pm 0.41 ^a	0.84 \pm 0.15 ^a
Cd	1.81 \pm 0.37 ^b	3.20 \pm 0.14 ^b	11.73 \pm 0.51 ^b	7.973 \pm 0.25 ^b	23.91 \pm 0.78 ^b	7.93 \pm 0.66 ^b
Cd + vitexin	4.22 \pm 0.23 ^a	4.93 \pm 0.15 ^c	19.66 \pm 0.57 ^c	13.17 \pm 0.28 ^c	14.5 \pm 0.70 ^c	1.55 \pm 0.19 ^c
Vitexin	4.83 \pm 0.21 ^a	6.56 \pm 0.29 ^a	24.74 \pm 0.70 ^a	16.77 \pm 0.64 ^a	10.20 \pm 0.50 ^a	0.81 \pm 0.21 ^a

Table 3

Values having different superscripts in the same column are significantly different.

Groups	ICDH (units/min/mg of protein)	α -KGDH (units/min/mg of protein)	SDH (units/min/mg of protein)	MDH (units/min/mg of protein)
Control	811.1 \pm 13.5 ^a	165.0 \pm 3.10 ^a	65.4 \pm 2.73 ^a	552.0 \pm 15.6 ^a
Cd	300.9 \pm 12.7 ^b	35.22 \pm 1.51 ^b	19.82 \pm 1.20 ^b	207.0 \pm 9.51 ^b
Cd + vitexin	661.3 \pm 16.8 ^c	93.81 \pm 2.91 ^c	51.79 \pm 1.63 ^c	430.5 \pm 16.0 ^c
Vitexin	820.2 \pm 13.3 ^a	172.7 \pm 2.60 ^a	71.96 \pm 2.07 ^a	561.7 \pm 9.41 ^a

Table 4

Values having different superscripts in the same column are significantly different.

Groups	Complex-I (NADH dehydrogenase)	Complex-II (Succinate-dehydrogenase)	Complex-III (Succinic-coenzyme Q)	Complex-IV (Cytochrome c oxidase)	MMP%
Control	34.13 \pm 1.94 ^a	88.2 \pm 2.42 ^a	0.92 \pm 0.03 ^a	259.1 \pm 9.9 ^a	89.4 \pm 1.38 ^a
Cd	18.47 \pm 0.68 ^b	29.3 \pm 1.76 ^b	0.19 \pm 0.01 ^b	106.3 \pm 8.2 ^b	38.1 \pm 1.90 ^b
Cd + vitexin	28.93 \pm 1.13 ^a	57.6 \pm 1.84 ^c	0.58 \pm 0.02 ^c	195.2 \pm 4.94 ^c	75.2 \pm 1.57 ^c
Vitexin	34.37 \pm 2.42 ^a	89.5 \pm 2.59 ^a	0.9 \pm 0.02 ^a	256.8 \pm 15.2 ^a	85.6 \pm 1.91 ^a

mitochondrial membrane from where electrons are transferred from one complex to the next complex, which helps produce energy (Letts and Sazanov, 2017). However, Cd may block various mitochondrial proteins by inhibiting respiratory chain enzymes (Wang et al., 2004). Cd damage the mitochondria by increasing mitochondrial ROS (Belyaeva et al., 2006).

Furthermore, the Cd ions act like a xenobiotic that inhibits complexes II and III activities in the ETC more than complexes I and IV. The primary site of ROS induction appears to be complex III. Accumulation of ROS disturbs the mitochondrial membrane potential and may cause apoptosis (Chatterjee et al., 2008). However, our investigation has demonstrated that the administration of VIT substantially reversed the activities of ETC complexes, which might be linked to the declining production of ROS.

Our findings presented that Cd exposure significantly reduced the mitochondrial membrane potential ($\Delta\Psi_m$). The excessive production of ROS may cause mitochondrial dysfunction, which results in lower ATP formation (Zorov et al., 2014). Oxidative damage has a deleterious effect on mitochondria. It stands up the mitochondrial membrane permeability, causing protons and ions asymmetrical distribution on both sides of the membrane, leading to mitochondrial membrane potential ($\Delta\Psi_m$) reduction. The previous data by Lee et al. (2005) has shown that Cd exposure leads to mitochondrial membrane potential ($\Delta\Psi_m$) breakdown in isolated renal mitochondria. However, our findings display that the VIT can reverse the Cd-induced mitochondrial membrane potential ($\Delta\Psi_m$) loss.

5. Conclusion

In conclusion, our experimental findings have demonstrated that Cd exposure is one of the critical factors behind renal damage. Our results also indicated that morin administration exhibited protective effects against CP-induced adverse effects on urea, creatinine, creatinine clearance, antioxidant enzymes, ETC complexes, and mitochondrial membrane potential. Therefore, the VIT could maintain the standard renal functions by reducing ROS and TBARS levels and protecting TCA cycle enzymes and ETC complexes. This

ameliorative role may be attributed to the antioxidant potential of the vitexin.

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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