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Vitexin attenuates cisplatin-induced renal toxicity by reducing oxidative stress and inflammation

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ABSTRACT

Cisplatin (CP) is one of the most effective chemotherapeutic drugs used to treat different tumors. Vitexin (VIT) is a natural flavonoid having various pharmacological activities along with its curative effects. The present research was planned to evaluate the therapeutic potential of VIT on cisplatin-induced renal damage in male albino rats. Twenty-four male albino rats were divided into four equal groups. These groups were treated with CP (10 mg/kg injection on the first day of trial) administered group, cotreated (CP; 10 mg/kg + VIT; 10 mg/kg) and only VIT (10 mg/kg orally till the end of the trial) treated group and a control. CP administration significantly decreased the activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione S-transferase (GST), glutathione (GSH), and glutathione reductase (GSR). The levels of thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2) were increased. CP-treatment significantly increased the levels of urea, creatinine, kidney injury molecule-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL) while considerably reducing the creatinine clearance. The results demonstrated that CP significantly increased the inflammation markers, including tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) levels and cyclooxygenase-2 (COX-2) activities and histopathological damages. However, co-treatment with VIT efficiently minimized the CP-induced biochemical, inflammatory, and histopathological impairments in rat kidneys. The study's outcomes indicated the significant curative efficacy of VIT to overcome CP-induced nephrotoxicity in male albino rats.

1. Introduction

Continuous advancements in chemotherapy have increased life expectancy up to five years in about 82% of cancer patients (Gatta et al., 2014). Platinum-based anticancer medicines like cisplatin (CP) are being used to treat head, neck, lung, testicular, bladder, and ovarian cancer. CP has become the most extensively used anticancer drug due to its practical therapeutic effects against cancer (Ijaz et al., 2020a). However, residual effects and medicinal resistance are the main challenges due to CP and other metal-based anticancer drugs. Especially, CP-induced nephrotoxicity had become a significant problem (Dasari and Tchounwou, 2014).

Oxidative stress, inflammation, and apoptosis are the main pathways involved in cell damage. Multiple studies have shown that CP-induced nephrotoxicity is linked with reactive oxygen species (ROS) (Abdellatief et al., 2017). For several years, efforts have been made to produce derivatives of CP with decreased side effects which resulted in the formation of oxaliplatin and carboplatin with a narrow therapeutic spectrum (Kruger et al., 2015). The other

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method to reduce the nephrotoxic effects of CP is extensive hydration to wash out CP from the kidneys (Dasari and Tchounwou, 2014).

Flavonoids are significant natural plant chemicals; that commonly exist in fruits, vegetables, and certain beverages. Flavonoids have shown numerous pharmacological properties to cure several diseases, i.e., Alzheimer's, atherosclerosis, and cancer (Ovando et al., 2009). Flavonoids are linked with an extensive range of health-improving effects. They are an essential component of pharmacological, medicinal, nutritional, and beautifying applications due to their anti-mutagenic,anti-carcinogenic, antioxidant, and anti-inflammatory properties and their proficiency to control critical functions in cellular enzymes (Walker et al., 2000).

Trigonella foenum-graecum (Fenugreek) is an annual leguminous herb. Seeds of this plant have a higher content of polyphenolic flavonoids (Kaviarasan et al., 2004), which have shown a stimulating effect on the reproductive system (Jamalan et al., 2016). Vitexin, one of the essential bioactive flavonoids extracted from Fenugreek seeds (Khole et al., 2014), has anticancer, anti-inflammatory, antioxidant, and anti-diabetic effects (He et al., 2016). This experiment was planned to evaluate the protecting potential of vitexin against cisplatin-induced renal damage in male albino rats considering the above-stated facts.

2. Material and methods

2.1. Chemicals

CP and VIT were purchased from *Sigma-Aldrich* (Germany).

2.2. Animals

The experiment was carried out on 24 male albino rats, having 150–200 g weight. The rats were kept in standard laboratory conditions at room temperature (25-27℃), and suitable moisture with 12 h light/dark cycle was provided. They were provided with regular feed and tap water. Rats were handled in compliance with the European Union of animals care and experimentation (CEE Council 86/609) guidelines.

2.3. Experimental layout

Four experimental groups of 24 rats (having six rats in each group) were made. The first group was given with normal saline and considered as control. The second group was treated with an injection of CP intraperitoneally (10 mg/kg) on the first day of treatment. The third group was administered with both vitexin (10 mg/kg) orally for seven days and an injection of CP (10 mg/kg) on the first day of treatment. The fourth group was treated with vitexin alone (10 mg/kg) during the experiment (once a day). All the rats were anesthetized on the eighth day of the experiment with diethyl ether, decapitated, and sterile syringes were used to take trunk blood for biological estimation of serum profile. After dissection, both the kidneys were separated; one was packed in zipper bags and stored at −80 °C for biochemical analysis. The other was preserved in a 10% formalin buffer solution for histological examination.

2.4. Biochemical analysis

The activity of CAT was determined with the help of the Aebi (1984) procedure. Protocol of Kakkar et al. (1984) was followed for the estimation of SOD activity. Chance and Maehly (1955) procedure was applied for the assessment of POD activity. The protocol of Moron et al. (1979) was used for the evaluation of GSH activity. The protocol was followed to assess the GST activity as described by Younis et al. (2016). Protocol of Carlberg and Mannervik (1975) was followed for the determination of GSR activity.

2.5. Estimation of H_2O_2 and TBARS

The methodology of Pick and Keisari (1981) was used for the estimation of H_2O_2. To estimate the TBARS level was assessed as described by Iqbal et al. (1996).

2.6. Biological study of serum and urine

The standard diagnostic kits were used for the estimation of urea, creatinine, and creatinine. Urinary KIM-1 and Serum NGAL were determined according to the manufacturer's command using KIM-1 Quantikine ELISA Kit and NGAL Quantikine ELISA Kit (R and D Systems China Co. Ltd., Changning, China).

2.7. Inflammatory markers assessment

Commercially available kits were used to assess the inflammatory markers of the hepatic tissues. TNF-α, NF-κB, IL-6, IL-1β levels, and COX-2 activity were determined with a rat ELISA kit (Shanghai-YL-Biotech. Co. Ltd., China). Analyses were completed by following the manufacturer's instructions through ELISA Plate-Reader (BioTek, Winooski-VT, USA).

2.8. Histopathological study

The samples were fixed with a mixture of 20% formaldehyde, 10% glacial acetic acid, and 70% absolute alcohol. After fixation, samples were embedded in paraffin and fixed in blocks. Thin slices (3–4 μm) were cut down, stained with Hematoxylin/eosin, fixed on slides, and analyzed under the light microscope (Nikon Eclipse E100 LED, Tokyo, Japan) at 40X.

2.9. Statistical study

The data were presented as means ± SEM. One-way ANOVA followed by Tukey's test was applied by using Minitab software for comparison between groups. The level of significance was considered as $p < 0.05$.

3. Results

3.1. Protective effect of vitexin on antioxidant enzyme activity

The CP-administered group showed a significant ($p < 0.05$) reduction in antioxidant enzyme activities such as CAT, POD, SOD, GSR, GSH, and GST compared to the control group. Cotreatment of CP + VIT exhibited significantly ($p < 0.05$) amplified antioxidant activity as compared to CP-treated rats. The treatment of vitexin alone showed regular activity of antioxidant enzymes near to control (Table 1).

3.2. Protective effect of vitexin on the level of TBARS and H_2O_2

A remarkable elevation ($p < 0.05$) in TBARS and H_2O_2levels was found in the CP-treated rats when matched with the control group. Rats administered with CP + VIT showed reduced TBARS and H_2O_2-levels when compared to CP-treated rats. However, vitexin alone administration maintained the TBARS and H_2O_2 levels as in the control rats (Table 2).

Table 1
Effect of cisplatin and vitexin on the activities of CAT, SOD, POD, GSH, GSR, and GST.

Groups	CAT (U/mg protein)	SOD (nanomole)	POD (U/mg protein)	GST (mg/dl)	GSR (Nm NADPH oxidized/ min/mg tissues)	GSH (nM/min/mg protein)
Control	8.73 ± 0.34[a]	6.48 ± 0.11[a]	7.19 ± 0.09$_a$	24.84 ± 0.29[a]	4.54 ± 0.06[a]	17.33 ± 0.29[a]
CP (10 mg/kg)	4.29 ± 0.16[b]	2.99 ± 0.07[c]	3.44 ± 0.13[c]	14.14 ± 0.38[c]	2.11 ± 0.12[b]	8.28 ± 0.46[c]
CP (10 mg/kg) + VIT (10 mg/kg)	8.41 ± 0.23[a]	5.95 ± 0.10[b]	6.58 ± 0.06[ab]	21.66 ± 0.32[ab]	4.25 ± 0.07[c]	15.13 ± 0.30[ab]
VIT (10 mg/kg)	8.88 ± 0.11[a]	6.44 ± 0.13[ab]	7.13 ± 0.09[b]	24.73 ± 0.51[b]	4.56 ± 0.03[a]	17.45 ± 0.25[b]

Vales sharing different superscripts are significantly different from each other.

Table 2
Effect of cisplatin and vitexin on the TBARS and H_2O_2 levels.

Groups	TBARS (nM/min/mg protein)	H_2O_2 (μM/min/mg protein)
Control	13.80 ± 0.32[a]	1.49 ± 0.09[a]
CP (10 mg/kg)	26.66 ± 0.26[c]	3.84 ± 0.14[c]
CP (10 mg/kg) + VIT (10 mg/kg)	16.74 ± 0.28[b]	1.86 ± 0.07[b]
VIT (10 mg/kg)	14.08 ± 0.26[ab]	1.62 ± 0.19[ab]

Vales sharing different superscripts are significantly different from each other.

3.3. Protective effect of vitexin on renal function markers

CP-treatment significantly ($p < 0.05$) escalated the creatinine, urea, KIM-1, and NGAL, while a considerable decline ($p < 0.05$) was witnessed in creatinine clearance when compared to the control group. Co-treatment of CP + VIT showed a remarkably lowered creatinine level, urea, KIM-1, and NGAL, and a considerable ($p < 0.05$)rise in creatinine clearance. Group of rats treated with VIT alone exhibited average values of urinary markers as in the control group (Table 3).

3.4. Protective effect of vitexin on inflammatory markers

CP-treatment substantially ($p < 0.05$) elevated the inflammatory parameters; TNF-α, NF-κB, IL-1β, IL-6 levels, and COX-2 activities in contrast to the control group. While VIT administration considerably ($p < 0.05$) decreased the levels of these inflammatory parameters in the cotreated rats compared to the CP-treated group. No increase in inflammatory markers was noted in the VIT alone administered group (Table 4).

3.5. Protective effect of vitexin on histology of renal tissues

Microscopic analysis of kidneys showed that the treatment of rats with CP caused severe damage in renal parenchyma. CP made some tubular dilation in the cortex and focal epithelial cell damage throughout constrained areas, while in the outer medulla, it persuaded chronic and noticeable cytolysis of epithelial cells. Capillaries that interacted with tubules were dilated and dilated, and pyknotic nuclei were observed in the inner medulla. The co-treatment of CP + VIT showed more minor damage and significant recovery in the tubules of the renal parenchyma. (Fig. 1).

4. Discussion

In the current study, CP administration showed a substantial decline in antioxidant enzyme activities of CAT, POD, SOD, GSR, GSH, and GST. In contrast, a remarkable elevation in the level of TBARS and H_2O_2 was observed. CP induces an immune response and reactive oxygen species-mediated tissue damage (Abdellatief et al., 2017). Amplified generation of these reactive oxygen species reduces antioxidant enzymes (Ashraf et al., 2020; Ijaz et al.,2020b; Latif et al., 2020; Qamar et al., 2020) activity (CAT, SOD, POD, GSH) and increases lipid peroxidation in the kidneys. The evaluation of lipid peroxides and H_2O_2 in our samples helps validate renal irregularities. These results are inconsistent with the results shown in an earlier study where the level of oxidative stress markers was investigated four days, followed by CP treatment in rats (Darwish et al., 2017). In our study, improvement in antioxidant enzyme activities and decrease in TBARS and H_2O_2 levels due to the co-treatment of VIT with CP apprised the defensive potential of VIT in CP instigated renal injuries in rats. As in many other flavonoids, VIT inhibits ROS production and shows its antioxidant potential, which may be the main reason for increasing antioxidant enzyme activity and reducing the TBARS and H_2O_2 levels.

The toxicity induced by CP administration exhibited considerable escalation in urea and creatinine, while a significant decline was observed in creatinine clearance. Renal toxicity persuaded by CP can be described by considerably reduced kidney functions indicated by increased blood urea and serum creatinine (Farooqui et al., 2017). Creatinine is a metabolite that is excreted entirely in the urine through glomerular filtration, and a rise of its level in the blood is a sign of reduced renal function. Along with this, the augmented urea, creatinine, and decreased creatinine clearance are the markers for severe oxidative damage to the kidneys (Khan et al., 2010). This study revealed that VIT maintained the serum and urine profile towards normal conditions, which depicts the reno-protective role of VIT.

CP administration raised the KIM-1 and NGAL levels in treated rats. KIM-1 and NGAL are the prominent biomarkers of AKI (Lei et al., 2018). KIM-1 is nearly not expressed in the healthy renal tissue, but it is expressed during the early stages of nephrotoxic injury (Luo et al., 2016). NGAL is a cytosolic protein discharged in the urine, blood, and renal/proximal–distal tubule due to nephrotoxicity, kidney parenchymal damage, and renal ischemia (Mori et al., 2005). It is usually discharged into the blood to greater extents after damage and evacuated through the urine (Yim,

Table 3
Effect of cisplatin and vitexin on the level of urea, creatinine, creatinine clearance, KIM-1, and NGAL.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Creatinine clearance (ml/min)	KIM-1 (ng/day)	NGAL (mg/ml)
Control	17.90 ± 0.16[a]	1.54 ± 0.08[a]	1.4 ± 0.03[a]	0.37 ± 0.02[a]	0.52 ± 0.03[a]
CP (10 mg/kg)	39.28 ± 1.70[b]	4.58 ± 0.10[b]	0.54 ± 0.05[c]	1.51 ± 0.03[b]	1.90 ± 0.04[b]
CP (10 mg/kg) + VIT (10 mg/kg)	23.37 ± 1.20[c]	2.01 ± 0.07[c]	1.26 ± 0.03[ab]	0.85 ± 0.04[c]	0.81 ± 0.03[c]
VIT (10 mg/kg)	18.36 ± 0.58[d]	1.59 ± 0.12[a]	1.38 ± 0.05[b]	0.35 ± 0.05[a]	0.49 ± 0.02[a]

Vales sharing different superscripts are significantly different from each other.

Table 4
Effect of cisplatin and vitexin on inflammatory parameters (TNF-α, NF-κB, IL-1β, IL-6 levels, and COX-2 activities) in the renal tissues of rats.

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	17.7 ± 0.62 [a]	6.95 ± 0.31 [a]	23.8 ± 0.53 [a]	5.59 ± 0.28 [a]	21.0 ± 0.81 [a]
CP (10 mg/kg)	81.2 ± 2.54[b]	19.6 ± 1.19[b]	79.3 ± 1.95[b]	16.9 ± 0.38[b]	68.2 ± 1.79[b]
CP (10 mg/kg) + VIT (10 mg/kg)	28.9 ± 1.24[c]	9.18 ± 0.63[c]	30.5 ± 1.31[c]	11.4 ± 0.46[c]	29.8 ± 0.72[c]
VIT (10 mg/kg)	17.3 ± 0.63 [a]	6.58 ± 0.29 [a]	23.0 ± 0.73 [a]	5.46 ± 0.29 [a]	20.5 ± 0.98 [a]

Vales sharing different superscripts are significantly different from each other.

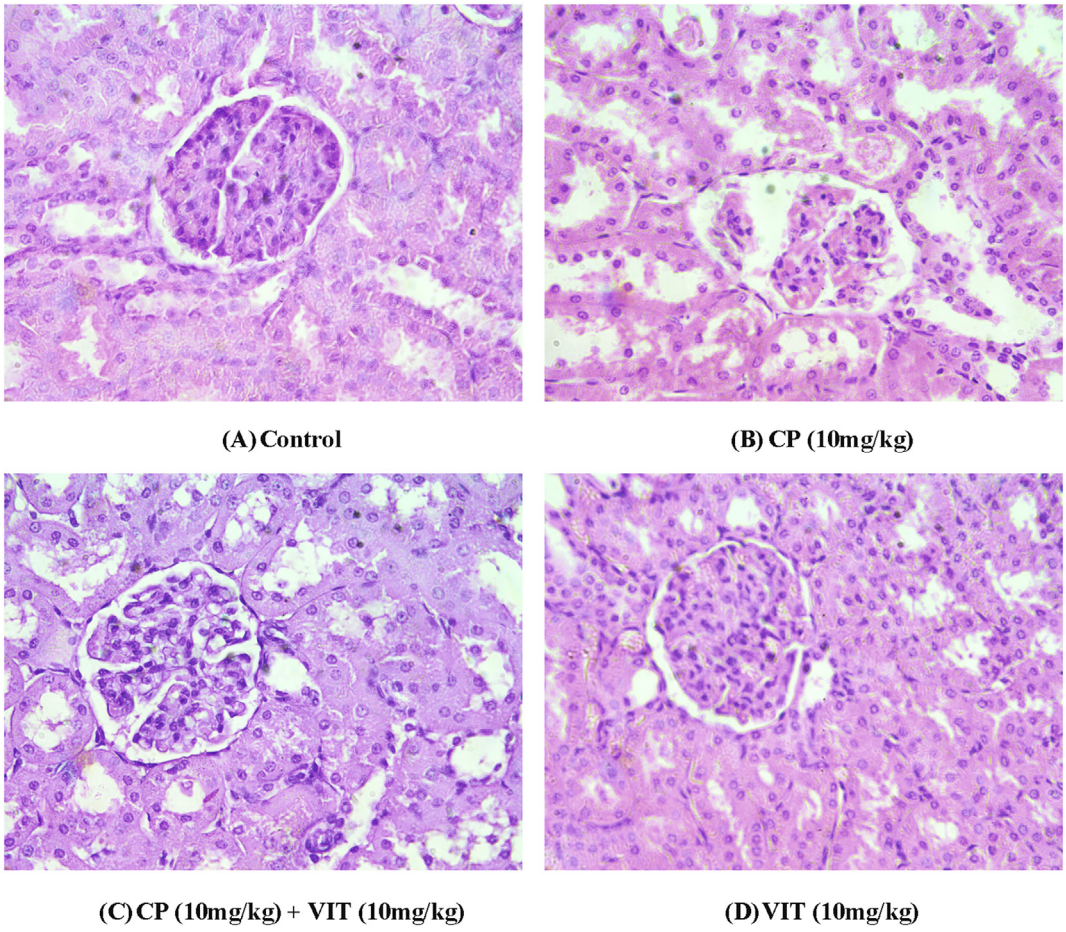

Fig. 1. Histopathological analysis of the various groups of kidney tissues. (A) a microphotograph of kidney section of control group rats, revealing normal histological structure of glomeruli and renal tubules. (B) a microphotograph of kidney section of CP (10 mg/kg) treated rats, demonstrating significant degenerative changes, granular deposits in their lumens and desquamation of the kidney epithelium. (C) a microphotograph of VIT (10 mg/kg) + CP (10 mg/kg) administered rats displaying degenerative alterations in renal epithelium and granular deposits in their lumens. (D) a microphotograph of section of kidney showing mild glomerulo-nephrosis associated with comparatively normal kidney lining epithelium in rats treated with VIT alone. (H&E, 400X).

2015). Abdelsalam et al. (2018) stated that substantial elevation was observed in renal KIM-1 and NGAL levels in platinum-based drugs induced nephrotoxicity. VIT administration substantially decreased the KIM-1 and NGAL expressions when cotreated with CP. Our studies are in line with the findings of Wang et al. (2019), who reported the curative nature of VIT against lipopolysaccharide-induced acute kidney injury in rats.

In the present study, CP administration raised inflammatory-markers NF-κB, TNF-α, IL-1β, IL-6, and COX-2 activities in treated rats. NF-κB activation is fundamental in the expression of proinflammatory cytokines like IL-1β, TNF-α, IL-6, and COX-2 that are concerned with acute inflammatory responses and other disorders linked with elevated ROS production (Rehman et al., 2014). COX-2 is an inductive form of COX and an additional critical inflammation marker, playing a critical biological role in inflammation (Subbaramaiah and Dannenberg, 2003). In this analysis, the level of inflammatory-markers TNF-α, NF-κB, IL-6, IL-1β, and the activity of COX-2 was escalated in kidney tissues of CP-treated groups. In line with the findings of Rehman et al. (2014), the present work confirmed that the CPadministration showed significant elevation of pro-inflammatory cytokines, including TNF-α, NF-κB, IL-6, IL-1β, and COX-2. Our results solidify the inflammatory role of CP on renal tissues. Cotreatment of VIT with CP substantially reduced the level of inflammatory markers TNF-α, NF-κB, IL-6, IL-1β, and COX-2 activities in treated rats. Our findings are in line with Raghu and Agrawal (2016), who reported the anti-inflammatory actions of vitexin. These findings indicate the anti-inflammatory role of VIT.

Histological study of kidneys showed that CP persuaded some tubular dilation and focal epithelial cell destruction throughout restricted areas in the cortex. In contrast, it induced chronic and marked cytolysis of epithelial cells and pyknotic nuclei in the outer

medulla. Capillaries that interacted with tubules were dilated, and dilation of tubules was also observed in the inner medulla. Animals treated with CP showed damage in renal tissues in the form of tubular dilation and lesions, which is in line with a previous study conducted by Saifi et al. (2018).VIT reduced tissue damages and inflammation in renal tissues due to the inhibitory effects of vitexin on inflammatory cytokine generation (Rosa et al., 2016), subsequently improved renal histology.

5. Conclusion

Our research showed the therapeutic capability of vitexin against cisplatin-induced renal injuries. Vitexin can restore the antioxidant enzyme activity, renal functional markers, regulated the inflammatory markers, and histological architecture. In conclusion, it is proposed that VIT has a therapeutic consequence over CP-induced nephrotoxicity due to its free radical scavenging ability.

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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