PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.1586

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RESEARCH ARTICLE

Protective effect of *Vitex altissima* L.f. bark extract on cisplatin-induced renal injury in Wistar rats

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OPEN ACCESS

ARTICLE HISTORY

Received: 08 November 2021
Accepted: 15 April 2022

Available online
Version 1.0: 22 May 2022

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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CITE THIS ARTICLE

Vedula G S, Isukapatla T, Ketha A. Protective effect of *Vitex altissima* L.f. bark extract on cisplatin-induced renal injury in Wistar rats . Plant Science Today (Early Access). https://doi.org/10.14719/pst.1586

Abstract

Cisplatin (CP) is a commonly used chemotherapeutic drug. The major limiting factor in the use of CP is the side effects in normal tissues, including the kidney. Since ancient times, medicinal plants are rich sources of various bioactive constituents used to treat multiple ailments, including drug toxicities. The present work is a preliminary study to explore the renoprotective actions of methanolic extract of *Vitex altissima* L.f. bark (**Va**) against CP-induced renal damage in Wistar rats. **Va** was found to have potent radical scavenging activity than metal ion reducing power properties, compared with ascorbic acid. Further, **Va** was evaluated for nephroprotective activity in rats induced by CP (8 mg/kg; intraperitoneal) on the 7th day. The animals were grouped (n = 6) and treated with **Va** (100 and 200 mg/kg) orally for 14 days. The outcomes of the study found that CP significantly (P < 0.001) altered the oxidative stress markers (MDA, SOD and CAT), serum urea and creatinine levels. The administration of **Va** significantly halted the toxic condition and maintained it towards normal levels. The higher dose of **Va** significantly (P < 0.001) raised the SOD and CAT levels and halted the MDA levels than the low dose. Also, a higher dose of **Va** maintained the normal integrity of the histopathological studies of kidneys than a low dose. The present study demonstrates that *V. altissima* can attenuate the oxidative stress induced by CP by enhancing the endogenous antioxidant levels and depleting the lipid peroxidation levels.

Keywords

antioxidant activity, cisplatin, nephrotoxicity, oxidative stress, *Vitex altissima*

Introduction

The kidney is the principal organ that plays a vital role in the excretion of xenobiotics and their metabolites (1, 2). Nephrotoxicity is one of the major leading causes of death worldwide, of which 20% of deaths are accounted for drug-induced toxicity with various classes of life-saving drugs (2-4). The nephrotoxicity symptoms include the change in urine volume, increased kidney weight, and alteration of kidney biochemical parameters (serum urea nitrogen and creatinine levels) (5, 6).

Cisplatin (CP) is platinum derived first-line anticancer drug that shows the efficient suppression of malignancies. CP interacts with DNA *via* the formation of covalent adducts between certain DNA bases and the platinum compound (7, 8). The toxic effects of CP include nausea, vomiting, ototoxicity, neurotoxicity and bone marrow suppression, but its chief dose-limiting side effect is nephrotoxicity (8). But prolonged usage of CP exhibits

irreversible nephrotoxicity, ototoxicity and neurotoxicity (7-10). Even though less toxic anticancer drugs were available, CP remains the drug of choice due to its potential, availability and cost-effectiveness (11).

Although the exact mechanism underlying the renal toxicity induced by CP is unclear, the *in vitro* and *in vivo* studies suggested that it was attributed to the combination of the excess generation of reactive oxygen species (ROS), apoptosis and lipid peroxidation (15-17). The chronic usage of CP leads to oxidative stress that culminates in the generation of various classes of ROS, such as superoxide anion, nitric oxide and hydroxyl free radical (18). The platinum substances form complexes with the nitrogen bases of DNA and induce apoptosis (19). ROS plays a crucial role in cellular injury and necrosis *via* several mechanisms, namely increased lipid peroxidation, decreased endogenous antioxidants, DNA damage and protein denaturation by reacting with thiols (20, 21). CP-induced renal injuries increase the serum markers of kidney function such as blood urea nitrogen and creatinine (8). Moreover, CP administration reduces the renal level and/or activity of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione (8, 15).

There is a continuous search for agents which provide nephroprotection against the renal impairment induced by drugs like CP for which allopathy offers no remedial measures. It is thus imperative that we turn toward alternative systems of medicine for solutions. The *Vitex altissima* L.f. belongs to the family Verbenaceae. The phytochemical analysis of leaves of *V. altissima* consists of iridoid glucosides, flavonoids and triterpenoids (22, 23). The parts of *V. altissima* have been reported for antioxidant, anti-inflammatory and 5-lipoxygenase enzyme inhibitory activities (22, 23). Taken together, the present study made and attempted to evaluate the nephroprotective activity of methanol extract of barks of *V. altissima* in CP-induced renal oxidative stress in Wistar rats.

Materials and Methods

Plant material

The barks were collected from matured plants of *V. altissima* L.f. from Seshachalam hills, Tirupati, Andhra Pradesh, India, in February 2019 and a voucher specimen (No. 0732) was deposited in the Department of Botany herbarium, Sri Venkateswara University, India.

Extraction

The shade dried barks of *V. altissima* were ground into a coarse powder using an electrical blender. By hot continuous percolation technique (24), the powdered material (250 g) was extracted with methanol for 48 hr using a Soxhlet apparatus (25). The obtained solvent mixture was concentrated under reduced pressure using rotavapor (Buchi R-210 Rotavapor, Marshall Scientific, USA) yielded methanolic extract of barks of *V. altissima* (**Va**, 20 g, 2.0% w/w) as dark black solid and stored in amber-colored bottles at 4 °C for further use.

Total phenol and flavonoid contents

The **Va** total flavonoid and phenolic content were evaluated using aluminum chloride (26) and Folin- Ciocalteau reagent (27) respectively, in triplicate. The total flavonoid and phenolic content of **Va** were expressed as rutin and gallic acid equivalent respectively.

In vitro antioxidant activity

Evaluation of DPPH radical scavenging activity

By employing the 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma Aldrich Co., USA) assay (28) in triplicate, **Va** were evaluated for antioxidant activity. To the known concentrations of **Va** added 0.004% DPPH, and incubated for 30 min at 37 °C. Later, absorbance was measured at 517 nm against the blank. Ascorbic acid was used as a reference drug.

Evaluation of ferric ion reducing power assay

The ferric ion reducing power assay was determined in triplicate by the modified method (29). To 2.5 ml of potassium ferricyanide added various concentrations of **Va** and incubated at 50 °C for 20 min. To it, 0.5 ml of ferric chloride (0.1%) and 2.5 ml trichloroacetic acid (10%) were added, and the absorbance was noted at 700 nm. Ascorbic acid was used as a reference drug.

Test animals

Adult Wistar albino rats (weighing 190 ± 10 g, age 6-8 weeks) of either sex were used in this study. The animals were given food and water *ad libitum* and were housed in the Animal House of the Andhra University of Pharmacy under the standard condition with a temperature of 25 ± 2 °C, the relative humidity (50 ± 10%) and a 12-hr light/12-hr dark cycle. This study was approved by the Institutional Animal Ethics Committee of Andhra University, India (Code: 516/PO/c/01/IAEC).

Acute oral toxicity

The OECD main test 420 was utilized for acute toxicity studies. Rats were randomly divided into two groups (five males and five females) and dosed with 2000 mg/kg body weight (b.w) of **Va** suspended in 1% gum acacia and 0.1% tween-80. The test animals have undergone fasting overnight before administering the **Va** using oral gavage. The testing was ended until the last three animals survived the upper bound dose, and all of the test animals were observed up to 14 days (30).

Experimental design

Rats were randomly divided into 4 groups (six rats in each group). In group 1 (normal control), rats were administered orally with only 1% gum acacia for 14 days. In group 2 (toxic control), rats were dosed orally with a single dose of CP (8 mg/kg; intraperitoneal (i.p)) on the 7th day (31). Rats in groups 3 and 4 received 100 mg/kg b.w (as a low dose) and 200 mg/kg b.w (as a high dose) of **Va** orally for 14 days, respectively, and CP (8 mg/kg; i.p) on 7th day.

Serum sampling

Blood samples were obtained from the portal vein 0.5 ml of blood samples on the 14th day from retro-orbital plexus under mild anesthesia. They were transferred into labora-

tory tubes containing pre-autoclaved nutrient broth medium (Sigma-Aldrich, Germany) and put in an incubator at 37 °C. The remaining blood samples decanted gently into collection plastic tubes, centrifuged at 4000 rpm for 5 min. Then serum was obtained, aliquoted into microtubes and stored for 24 hr at -80 °C for biochemical analysis.

Tissue preparation and homogenization

At the end of the study, by cervical dislocation, all the rats were sacrificed. The kidneys were removed, weigh up, and washed thoroughly. Some portion of tissue was stored immediately in buffered formalin (10%) for histopathological studies, and the remaining tissue was processed. In 0.05 M of ice-cold phosphate buffer saline (pH 7) the tissue was minced into small pieces and homogenized with a Homogenizer (Remi Homogenizer, Mumbai, India) to obtain 10% whole homogenate. To the homogenate, an equal volume of trichloroacetic acid (10%) was mixed and centrifuged (Sigma-3-30 KS, USA) for 10 min. at 5000 rpm.

Assessment of oxidative stress parameters

The above-obtained supernatant was subjected to estimate the oxidative stress parameters, namely malondialdehyde (MDA) levels, superoxide dismutase (SOD) levels and catalase (CAT) activity using the established procedure (31, 32).

Assessment of the renal function

Measurement of blood urea nitrogen: Blood urea nitrogen was determined in serum using colorimetric assay kit according to manufacturer instructions (EXCEL kits, India) using semiautomatic biochemistry analyzer (Carex- EA112, India). The amount of urea nitrogen was determined by recording the absorbance per minute at 340 nm against the urea nitrogen standard.

Measurement of serum creatinine: Serum creatinine was determined by the method described in the kit leaflet (EXCEL kits, India) using a semiautomatic biochemistry analyzer (Carex- EA112, India). The concentration of creatinine was determined by recording the absorbance at 510 nm against the blank.

Histopathological studies

The thin sections of formalin-fixed kidney tissues were made using paraffin blocks and stained by 0.5% hematoxylin and eosin stain. These stained sections were inspected under a light microscope.

Statistical analysis

All the values were expressed as mean ± SD (n = 3), where $^{a}P < 0.05$; $^{b}P < 0.001$; $^{c}P < 0.0001$ was considered as statistically significant when compared with the normal control and $^{x}P < 0.05$, $^{y}P < 0.001$, $^{z}P < 0.0001$ as compared with toxic control using one-way ANOVA followed by Dunnett's multiple comparison test.

Results

Total flavonoid and phenol contents

The total flavonoid and total phenolic value of **Va** were equivalent to 10.11 ± 1.11 mg/g rutin and 112.2 ± 5.12 mg/g of gallic acid respectively.

In vitro antioxidant activity

The antioxidant activity of **Va** was evaluated by DPPH radical scavenging activity and ferric ion reducing power assay. The results showed that the **Va** exhibited a dose-dependent antioxidant activity (Fig. 1). **Va** was most active in DPPH radical scavenging activity with an IC_{50} value of 29.37 ± 2.11 µg/ml than ascorbic acid (32.53 ± 3.90 µg/ml). In reducing power assay, **Va** (IC_{50} value = 93.33 ± 3.14 µg/ml) was threefold less potent than the ascorbic acid (IC_{50} value = 35.47 ± 2.12 µg/ml) (Fig. 1). For antioxidant activity, it was proposed that **Va** showed antioxidant activity by its radical scavenging mechanism.

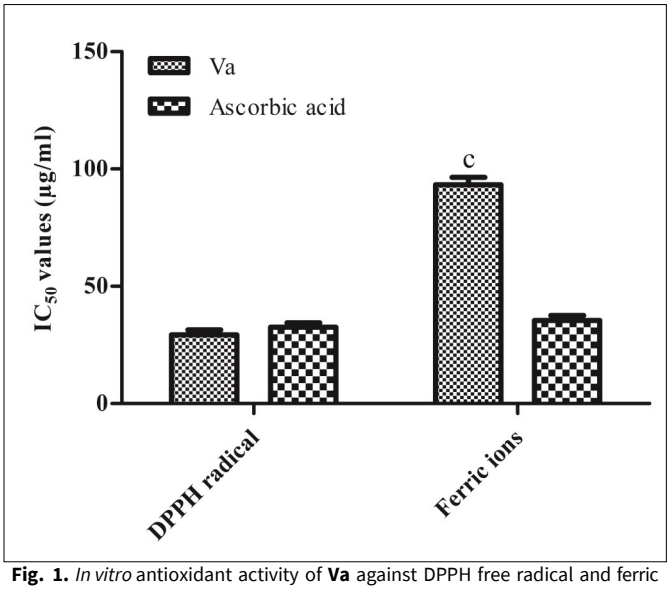
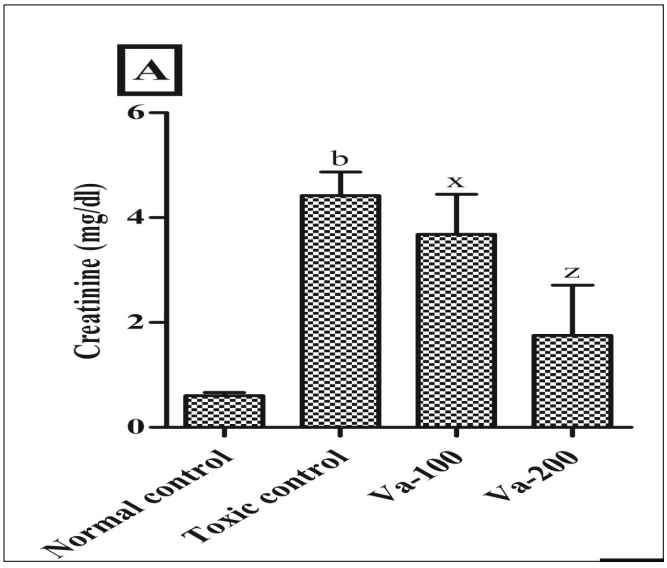
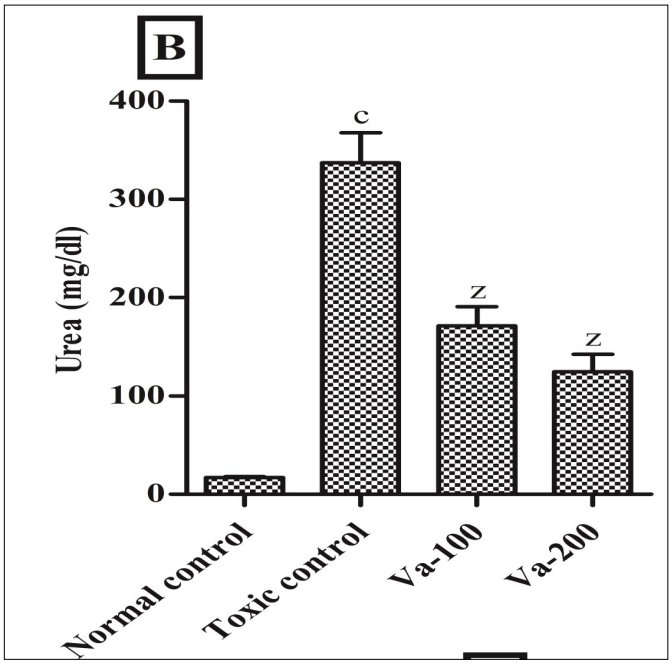


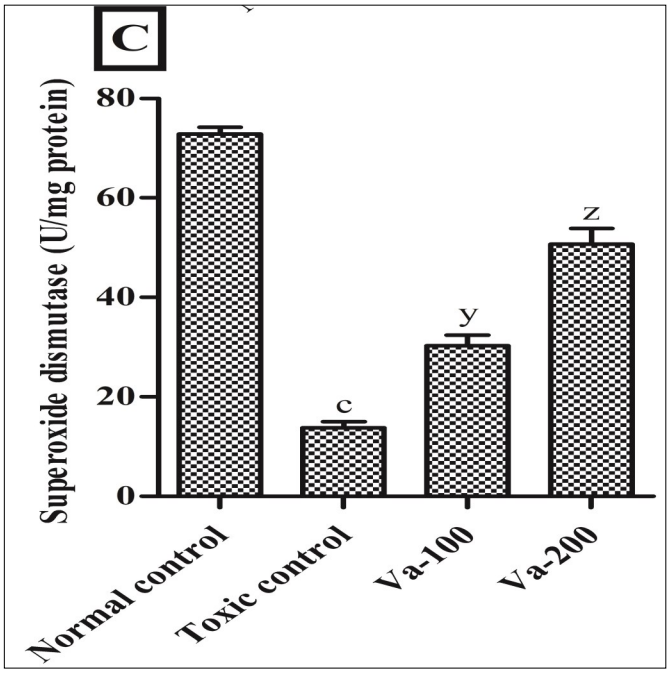
Fig. 1. *In vitro* antioxidant activity of **Va** against DPPH free radical and ferric ions. All the values were expressed as mean ± SD (n = 3). The $^{c}P < 0.0001$ was considered as statistically significant when compared with the ascorbic acid using one-way ANOVA followed by Dunnett's multiple comparison test.

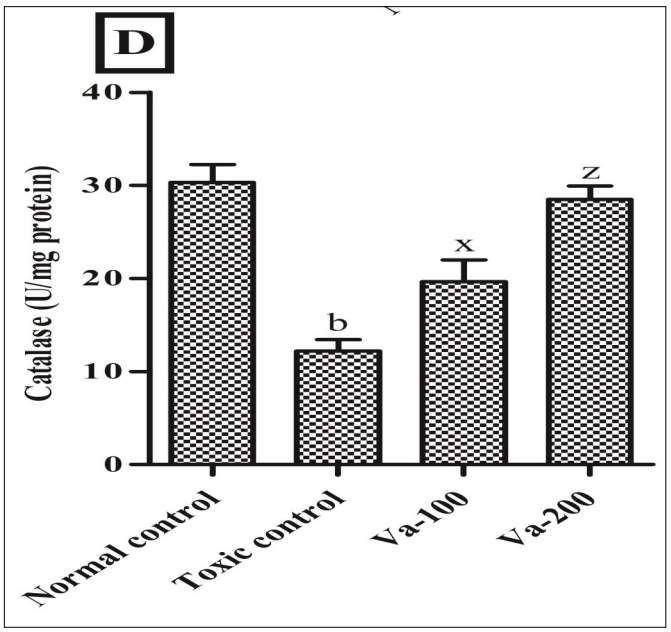
Renal function tests

The prior administration of **Va** (100 and 200 mg/kg) showed a significant (P < 0.05-0.0001) dose-dependent renoprotective activity when compared with the toxic control. The toxic control (P < 0.05-0.0001) significantly elevated the serum creatinine and urea levels to 4.42 ± 0.45 and 337.33 ± 30.42 mg/dl, respectively, compared with normal control (Fig. 2A-B). Besides, the higher dose of **Va** (P < 0.001-0.0001) exhibited higher renoprotection by reducing the elevated serum creatinine and urea levels to 1.75 ±









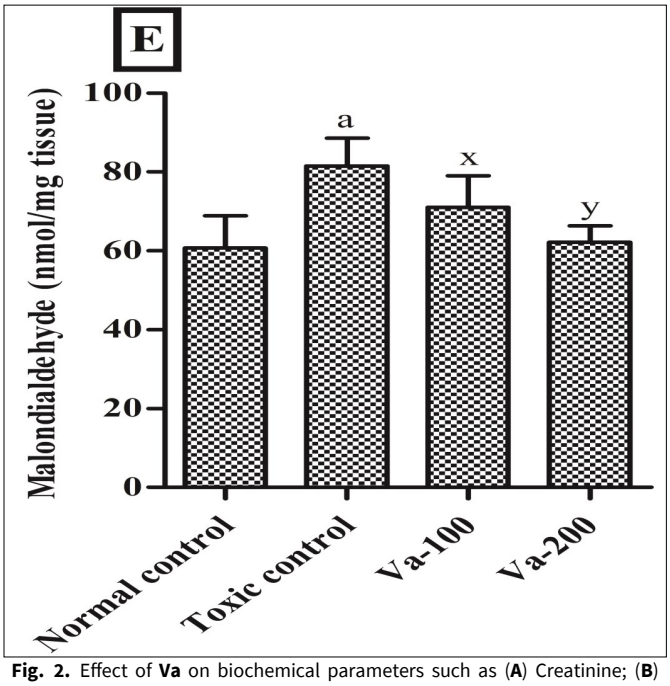


Fig. 2. Effect of **Va** on biochemical parameters such as (**A**) Creatinine; (**B**) Urea; (**C**) Superoxide dismutase; (**D**) Catalase; and (**E**) Malondialdehyde. All the values were expressed as mean ± SEM ($n = 6$). The $^{a}P < 0.05$; $^{b}P < 0.001$; $^{c}P < 0.0001$ was considered as statistically significant when compared with the normal control, and $^{x}P < 0.05$, $^{y}P < 0.001$, $^{z}P < 0.0001$ as compared with toxic control using one-way ANOVA followed by Dunnett's multiple comparison test. Where Va-100 is 100 mg/kg dose and Va-200 is 200 mg/kg dose.

0.96 and 124.5 ± 17.96 mg/dl, respectively, compared to its low dose (3.68 ± 0.77 and 171.33 ± 19. 66 mg/dl, respectively) (Fig. 2A-B).

Tissue oxidative stress markers

The oxidative stress induced by CP depleted the endogenous antioxidants (SOD and CAT) by increasing the MDA compared to the control. The pre-administration of **Va** ($P < 0.05$-0.0001) significantly ameliorated the CP's oxidative stress. At 200 mg/kg dose, **Va** ($P < 0.001$) showed a significant rise in SOD and CAT with 50.67 ± 3.24 and 28.50 ± 1.41 units/mg protein, respectively, and also significantly ($P < 0.01$) depleted the MDA levels with 71.00 ± 8.01 nmol/mg tissue, compared to toxic control (Fig. 2C-E).

Histopathological studies

The histopathological slides of the kidneys of the CP treated group altered the Bowman's capsules' structural integrity, signs of tubular necrosis, vacuolization and accumulation of inflammatory tissue (Fig. 3B). The pre-administration of **Va** (100 and 200 mg/kg) attenuated the histological alterations induced by CP (Fig. 3C-D).

Discussion

Since ancient times, medicinal plants are rich sources of various bioactive constituents used to treat multiple ailments. Instead, the major drawback of the currently available pharmaceutical aids is their toxic side effects, limiting their usage (33, 34). Nephrotoxicity is one such common side effect caused by numerous anticancer drugs. CP is the first-line anti-neoplastic drug used in the treatment of various cancers. CP produces platinum complexes that interact with DNA and leads to denaturation (35, 36). The exact

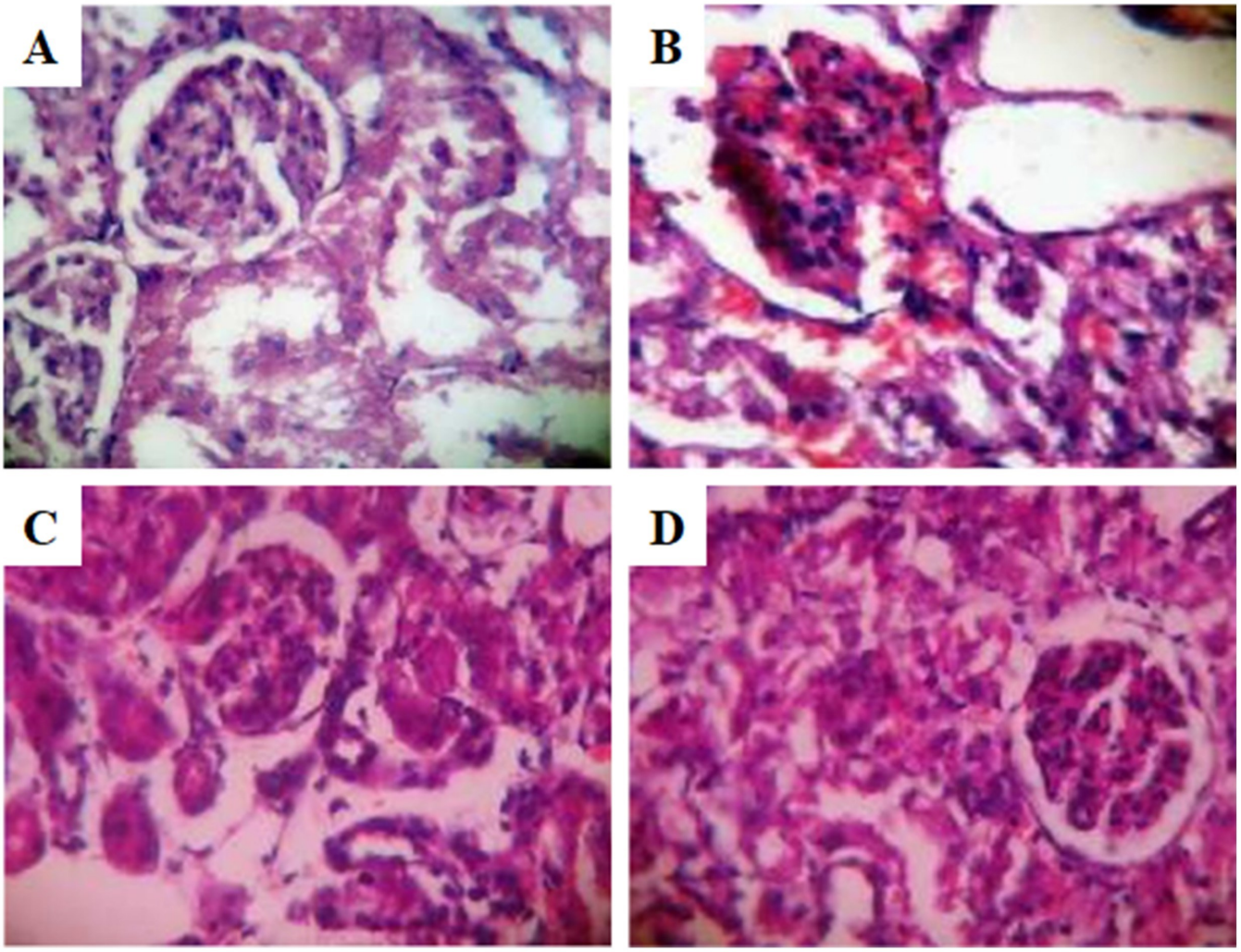


Fig. 3. Histopathological sections of the kidney at 40x magnification. (**A**) Normal control; (**B**) Toxic control; (**C**) **Va** (100 mg/kg, orally) for 14 days + cisplatin (8 mg/kg, i.p) on 7th day; (**D**) **Va** (200 mg/kg orally) for 14 days + cisplatin (8 mg/kg, i.p) on 7th day.

mechanism involved in CP-induced nephrotoxicity is unclear. Still, numerous research reports indicate that ROS, increased lipid peroxidation levels, apoptosis induced by the formation of DNA adducts, increased levels of TGF-1β, TNF and down-regulation of proliferative markers play a crucial role in the pathophysiology of nephrotoxicity (1, 7, 37-39). The ROS and lipid peroxidation together induces oxidative stress, leading to cellular damage of renal tubules (36, 39). Hence, investigation involving antioxidant strategies that lead to the diminution of oxidative injury was more concentrated.

In the present study, *V. altissima* is selected to investigate the renoprotective activity in the CP-induced rat model. Earlier, phytochemical screening of this plant has been acknowledged for iridoid-glucosides, flavonoids, and triterpenoids with good antioxidant properties (22, 23). In the present study, the **Va** also exhibited significant antioxidant activity (DPPH radical and ferric ion) attributed to the substantial quantity of phenol and flavonoid compounds (40) (Fig. 1). The current study results are concordant with the previous reports (22, 23). It was interesting to note that the **Va** was more active against DPPH free radicals than ferric ions (Fig. 1). For this observation, we proposed that **Va** showed antioxidant activity by its radical scavenging mechanism. Based on this *in vitro* preliminary analysis, further *in vivo* investigation of renoprotective activity of **Va** was performed in CP-induced rat model. The outcomes of these findings support the usage of *V. altissima* in protecting renal tissues.

The kidney is a vital organ that is involved in detoxification, concentration and excretion. The oxidative stress induced by CP damages the glomerulus' integrity, leading to tubular necrosis and accumulation of toxic substances resulting in increased serum urea and creatinine levels (40-42). The pretreatment of **Va** (P < 0.05-0.0001) markedly attenuated the levels of urea and creatinine (Fig. 2A-B). Both the doses (100 and 200 mg/kg) of **Va** (P < 0.05-0.0001) significantly depleted the urea and creatinine levels to the normal level (Fig. 2A-B). The oxidative stress induced by acute administration of CP damages the renal tubules due to the fivefold increased accumulation of CP in the S-segment of proximal tubule (43). The induced oxidative stress depletes the endogenous antioxidants and raises the lipid peroxidation levels (36, 44).

The endogenous antioxidant enzymes (SOD and CAT) play a vital role in catalyzing the H_2O_2 into the water that is the primary precursor of ROS. The depletion of SOD and CAT results in oxidative stress in surrounding tissues and leads to tissue and organ necrosis (45, 46). In the present study, the CP decreased the SOD and CAT and in-

creased elevated levels of MDA. The pretreatment of **Va** haltered the oxidative stress conditions by enhancing the SOD and CAT levels and depleting the MDA levels (Fig. 2C-E). The decreased levels of lipid peroxidation were attributed to the hypolipidemic activity of *V. altissima* (43-47).

The histopathological studies further support the CP-induced oxidative stress in renal tissue. The CP altered the renal architecture, increased tubular space, accumulated inflammatory cells, and decreased the urinary space (48-50). The **Va** recovered the histological alterations induced by CP. The high dose of **Va** showed marked histological changes compared with its low dose and toxic control (Fig. 3). The histological and biochemical studies suggest that **Va** possesses a potent renoprotective activity.

Conclusion

As per our knowledge, this is the first report of the nephroprotective activity of *V. altissima*. To conclude, the present study showed that *V. altissima* has a good amount of total flavonoid and phenolic contents with potent DPPH radical inhibitory activity. Also, *V. altissima* unexpectedly improved oxidative stress markers in rats induced by CP. Taken together, it could be proposed that *V. altissima* showed potent renoprotective activity by its antioxidant mechanisms. Hence, the present study can be used as a basic study to explore the other possible mechanisms that may betide in a renoprotective activity of *V. altissima* and also assist to screen other species from this genus for renoprotective properties.

Acknowledgements

None to acknowledge.

Authors contributions

VGS conceived of the study and participated in its design and coordination. VGS and AK participated in the design of the study and performed the statistical analysis. AK carried out the *in vivo* studies, and drafted the manuscript. TI participated in the biochemical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: This study was approved by the Institutional Animal Ethics Committee of AU College of Pharmaceutical Sciences, Visakhapatnam, India (Code: 516/PO/c/01/IAEC).

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