Saudi Journal of Biological Sciences 27 (2020) 3307–3312

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Nephroprotective effect of pigmented violacein isolated from *Chromobacterium violaceum* in wistar rats

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ARTICLE INFO

Article history:
Received 14 August 2020
Revised 3 October 2020
Accepted 5 October 2020
Available online 15 October 2020

Keywords:
Chromobacterium violaceum
Violacein
Creatinine
Gentamicin
Nephroprotective property

ABSTRACT

The present study aimed to analyze the nephroprotective property of violacein obtained from the bacterium, *Chromobacterium violaceum*. The nephrotoxicity in the animal model was induced by gentamicin, potassium dichromate, mercuric chloride, and cadmium chloride-induced nephrotoxicity in the Wistar rats was analyzed by measuring the serum creatinine, uric acid, and urea level. The present investigation revealed the nephroprotective property on convoluted proximal tubule (S1 and S2 segments) and the straight proximal tubule (S3 segment). Also, violacein significantly improved the renal function by the renal protective property on S2 segment of proximal tubule from the nephrotoxicity stimulated by mercuric chloride, potassium dichromate, cadmium chloride and gentamicin in animal models. Animal model studies revealed that violacein at 20 and 40 mg/kg p.o improved the renal function and significantly reduced the increased amount of uric acid, creatinine, and blood urea compared to the control.

1. Introduction

Kidney is an important organ affected by heavy metal toxicity. The toxic property of heavy metals on kidney has been illustrated previously. Therapeutic exposure or occupational hazard affected kidney function. In the environment, heavy metals are generally distributed various forms. Animals and human beings receive heavy metals through various routes, including consumption of highly contaminated water, heavy metal contaminated food than normal limit, used battery, mining and plastics. Kidney is an important organ involved in excretion process and is highly susceptible for heavy metal toxicity. The heavy metals such as, arsenic, mercury, cadmium and lead were highly toxic to kidney. Also, based on the dosage, these heavy metals affected blood parameters, brain and lung cells. In human beings, cadmium toxicity induced chronic rhinitis, anosmia, eosinophilia, non-hypertrophic emphysema, and osteoporosis. Heavy metals affect the secretion of insulin, and also associated with kidney failure. Exposure of cadmium induced kidney damage and studied *in vivo* and *in vitro* (Chen et al., 2006). Cadmium has direct cytotoxic effect and is categorized as type I carcinogen (Arroyo et al., 2012). The absorbed cadmium is generally deposited in the kidney and liver and affects these cells. The toxicity of cadmium was studied previously because of its high half-life. Many experimental studies were performed to characterize the toxic effect of cadmium. These findings revealed the cadmium cause toxicity to central nervous system, heart, testes, ovary, brain, lungs, liver and heart. Also, cadmium induced chronic rhinitis, eosinophilia, non-hypertrophic emphysema, anaemia, and osteoporosis (Valko et al., 2005). Cadmium affected various systems, including, nervous system, gastrointestinal system, cardiovascular system, urinary and respiratory system, by directly or indirectly affecting the function of the cells and cellular mechanism (Rani et al., 2014).

Heavy metal accumulation in kidneys leads to various functional and morphological defects (Conner and Fowler, 1993). The antibiotics such as, tetracyclines, cephalosporins, penicillins, as

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.10.004
1319-562X/

well as aminoglycosides, sulfonamides are effective nephrotoxins. Aminoglycoside affected kidney function and decreased urine concentrating capacity, mild glucosuria, lysosomal enzymuria, and tubular proteinuria, Also, decreased level of glomerular filtration rate and lowered ammonia excretion (Kaloyanides and Pastoriza-Munoz, 1980). *Chromobacterium violaceum* was reported various countries. It is a Gram-negative bacterium cause infection in animals and induced mortality in humans. This bacterium produced a violet coloured pigment called violacein (Chattopadhyay et al., 2002**).**

The whole genome of *C. violaceum* was sequenced and showed information about opportunistic pathogenic property and applications in pharmaceutical and biotechnological industry (Vasconcelos et al., 2003). The antichagasic and antagonistic property of *C. violaceum* was reported (Duran and Menck, 2001; Antonisamy and Ignacimuthu, 2010). This organism also showed potential antileishmanial, antioxidant (Leon et al., 2001), and antitumoral (Melo et al., 2000; Durán et al., 2012), antifungal activities. The culture medium was optimized to enhance the production of violacein in *Chromobacterium violaceum* (Ahmad et al., 2012). The purified violacein showed potential antimicrobial property against multi-drug resistant *Mycobacterium tuberculosis* (De Souza et al., 1999), *Trypanosoma cruzi* (Duran et al., 1989, 1994; Caldas et al., 1978) and *Leishmania* sp. (Leon et al., 2001). This organism showed apoptotic induction abilities in leukemia cells (Melo et al., 2000; Ferreira et al., 2004) and colon cancer cells (Carvalho et al., 2006; Kodach et al., 2006).

The purified violacein with 10% deoxyviolacein showed lot of potentials against polioviruses and herpes virus (May et al., 1991). Earlier, a patent has been filed for the formulation of violacein against viral, bacterial infection and also showed potent anticancer activity (Duran and Menck, 2001; Melo et al., 2000). At higher concentrations, violacein showed less inhibition property against Simian rotavirus SA11, Poliovirus type 2 and HSV-1 virus (Andrighetti-Frohner et al., 2003). Violacein also has ulcer-protective and anti-diarrhoeal properties (Antonisamy et al., 2009). Violacein was decolorized and biotransformed by various bacteria and basidiomycetes (Bromberg and Duran, 2001). The isolated violacein from *Chromobacterium violaceum* showed activity against various microbial pathogens (Pauer et al., 2018). Recently, Batista and da Silva Neto (2017) sequenced the whole genome of *Chromobacterium violaceum* and reported pathogenicity. In this study, a nephrotoxic effect was induced in experimental animal using heavy metals and antibiotics and nephroprotective property of violacein was analyzed. The renal function was monitored by studying serum creatinine, uric acid and urea level.

2. Materials and methods

2.1. Animals

Wistar albino rat was used as the model for *in vivo* analysis. Adult experimental animal with 225–250 g of male and female were subjected for experimental trials. These animals were carefully maintained at 25 ± 1 °C with 60–70% relative humidity and maintained on 12 h dark/light cycle. Water and food were provided to all animals. These animals were acclimatized for 15 days before to start the treatment. Animals were grouped into three experimental groups and six experimental animals were used for each group. Animals were maintained according to the recommendations of ethical committee approved by the guidelines of Institutional Animal Ethics Committee guidelines.

2.2. Chemicals

The chemicals such as, gentamicin, mercuric chloride, potassium dichromate and cadmium chloride were purchased from Sigma-Aldrich, USA. Diagnostic kits were purchased from Merck, Bangalore, India and Aspen Laboratories, India. Carboxymethyl cellulose was collected from Merck, Bangalore, India.

2.3. Isolation of C. violaceum

About 1.0 g soil sample was obtained from Kolli Hills, Tamilnadu, India (11°10′ to 11°30′N and 78° 15′ to 78°30′E). It was transported to the laboratory in ice and applied for the isolation of bacteria. Sample was serially diluted up to 10^{-7} and place on nutrient agar (Himedia, Mumabi, India) plates. It was incubated for 24 h at 37 °C and the development of violet pigmented colonies indicated the growth of *C. violaceum* (Antonisamy et al., 2009). The morphological and biochemical characters were analyzed as suggested by Cappuccino et al., 2004. It was characterized by 16S rDNA gene sequencing (Hao et al., 2007).

2.4. Batch culture of C. violaceum for the production of violacein

C. violaceum was cultured in 100 ml LB broth (Himedia, Mumbai, India) and incubated at 37 ± 2 °C for 18 h. About 2 ml of this inoculum was introduced into the production medium (250 ml) containing 0.5% peptone, 0.2% yeast extract, and 0.5% D-glucose in an Erlenmeyer flask (1000 ml). A sponge (4 × 4 × 3 cm) was also placed into the Erlenmeyer flask. It was incubated for 48 h at 37 ± 2 °C in an orbital shaker at 175 rpm. After 48 h incubation, bright violet colour was observed due to the production of violacein. It was trapped in the sponge and attained bright violet colour (Rettori and Duran, 1998).

2.5. Extraction of violacein

The violacein accumulated sponge was removed from the Erlenmeyer flask and the excess culture medium was pressed out and washed with double distilled water (two times). Then, 500 ml ethanol was added with the sponge and evaporated. This process was repeated several times.

2.6. Purification of violacein

The crude violacein (5 g) was used for extraction using a soxhlet apparatus. The sample was initially packed and washed with chloroform for 2 h and diethyl ether for another 2 h. This was repeated three times. Then ethanol was evaporated and violacein was crystallized using methanol and water at 1:1 ratio. The crystals of violacein were obtained after centrifugation at 10,000 rpm for 10 min and dried (Rettori and Duran, 1998). The crystallized sample was purified using a thin layer chromatography (TLC) and characterized by Mass and UV spectra analysis , IR spectra analysis, and ^{1}H NMR analysis (AL-300 MHz JEOL). The structure of violacein was analyzed and compared with previous reports.

2.7. Influence of violacein in normal rat kidney

The experimental animal was divided into four different groups. Six experimental animals were randomly selected for each group. Group I animals received 1% CMC in double distilled water at the dosage of 10 ml/kg body weight was served as the control group. To the other groups (II, III and IV), violacein was administered with 1% CMC at 10, 20 and 40 mg/kg body weight. These were administered daily using stomach tube at 10 ml/kg for 7 days. Then blood samples were collected using a capillary tube and serum sample

was obtained. It was centrifuged at 5000 rpm for 10 min and the pale yellow serum was obtained. It was used for the determination of creatinine, uric acid and urea.

2.8. Impact of violacein in $CdCl_2$-administered kidney damage

The experimental animals were categorized into 4 groups (n = 6). To the first group, 1% CMC was administered (10 ml/kg/d, p.o.). To the experimental groups, violacein was administered at three different concentrations (10, 20 and 40 mg/kg) and the experiment was conducted for eight days. The animals were also administered with cadmium chloride for four days (day 4–8) (Panda et al., 1997). Cadmium chloride was administered at 3 mg/kg/d through subcutaneous injection. After 24 h of injection, blood samples were collected and creatinine, uric acid and urea content were estimated.

2.9. Influence of violacein in potassium dichromate-induced nephrotoxicity in rats

The experimental animals were categorized into 4 groups (n = 6). To the first group of animal, 1% CMC was administered. To the second, third and fourth groups, 10, 20 and 40 mg violacein/kg/days. Further, potassium dichromate solution was injected through subcutaneous route at 20 mg/kg (single dose) (Jonker et al., 1993). After six days, creatinine, uric acid and urea content were estimated.

2.10. Influence of violacein in mercuric chloride-induced nephrotoxicity analysis

In this experiment, mercuric chloride was administered through subcutaneous injection in the neck region at the dose of 1.5 mg/kg/d. After six days, creatinine, uric acid and urea content were estimated.

2.11. Influence of violacein in gentamicin-induced kidney damage

The experimental animals were divided into four different groups (n = 6). Group I animals received 1% CMC, whereas Group II, III and IV received violacein at three different concentrations (10, 20 and 40 mg/kg) for eight days. To all experimental groups, gentamicin was administered subcutaneously at 100 mg/kg (Niazi, 1994). After six days, creatinine, uric acid and urea content were estimated.

2.12. Determination of creatinine, uric acid and urea

The amount of creatinine in the serum sample was testing as suggested by Jaffe's (1886) and Tietz (1987). Uric acid content of the serum sample was analyzed by enzymatic method (Caraway, 1955). Urea content of the serum sample was tested as suggested by Fawcett & Scott (1960).

2.13. Statistical analysis

One-way analysis of variance (ANOVA) and Student's *t*-test was performed to analyze the significance of data (Tallarida and Murria, 1987).

3. Results

3.1. Characterization of violacein from C. violaceum

The crude violacein was extracted using a soxhlet apparatus and washed with various solvent. The crystallized violacein showed a single prominent spot on TLC. The UV-spectrum and Mass spectrum were analyzed with standard. Also, proton NMR was used to characterize the molecule and compared with previous sources. The predicted structure of violacein was described in Fig. 1.

3.2. Effect of violacein in the kidney of control animal

The impact of violacein on kidney function in normal animals was tested after one week of treatment. No significant variation was observed in control animals (Fig. 2).

3.3. Effect of violacein in $CdCl_2$-induced kidney damage in rats

In this study, cadmium chloride induced nephrotoxicity and elevated level of serum urea (41.27 mg/dl), uric acid (2.47 mg/dl) and creatinine (2.13 mg/dl) were determined than normal animals. Co-administration of violacein with cadmium chloride effectively managed the kidney function and control the rise in blood urea (28.42 and 24.16 mg/dl), uric acid (1.68 and 1.56 mg/dl) and creatinine (1.46 and 1.21 mg/dl) (Fig. 3).

3.4. Effect of violacein in potassium dichromate-induced rats

The impact of violacein on potassium dichromate mediated nephrotoxicity was analyzed in experimental animals. The present finding revealed that potassium dichromate at 20 mg/kg affected kidney function. The serum uric acid (3.14 mg/dl), creatinine (2.68 mg/dl), and urea (44.82 mg/dl) level were increased. However, animals co-administered with violacein (20 and 40 mg/kg) controlled kidney function and reduced the levels of urea (30.75 and 26.33 mg/dl), uric acid (2.68 and 1.36 mg/dl) and creatinine (1.30 and 1.13 mg/dl) compared to control groups (Fig. 4).

3.5. Effect of violacein in mercuric chloride-induced kidney function in rats

The influence of violacein on mercuric chloride induced kidney function was evaluated in experimental animals. Mercuric chloride affected kindly function and showed elevated level of serum creatinine (2.12 mg/dl), uric acid (2.51 mg/dl) and urea (42.82 mg/dl). In the case of animals received violacein (20 and 40 mg/kg) controlled the elevated level of creatinine (1.40 and 1.21 mg/dl), uric acid (1.73 and 1.52 mg/dl) and serum urea (26.19 and 23.10 mg/dl) than control animals (Table 1).

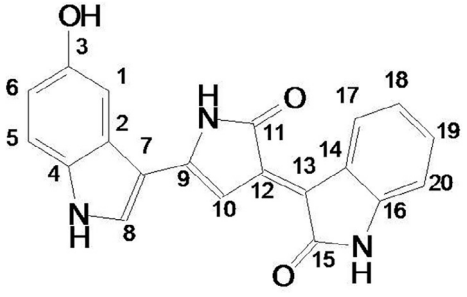

Fig. 1. Structure of violacein.

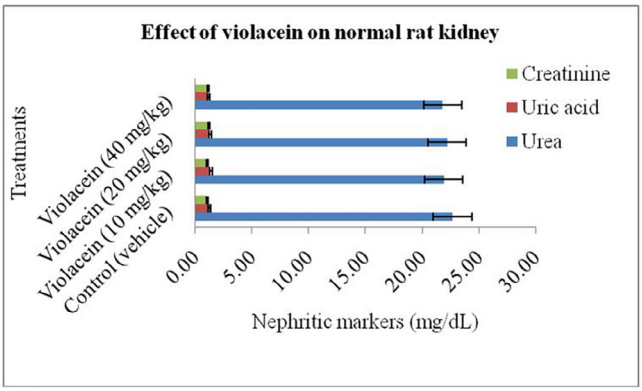

Fig. 2. Data represent mean ± S.D. (standard deviation) (n = 6). *p < 0.05 significant from the control.

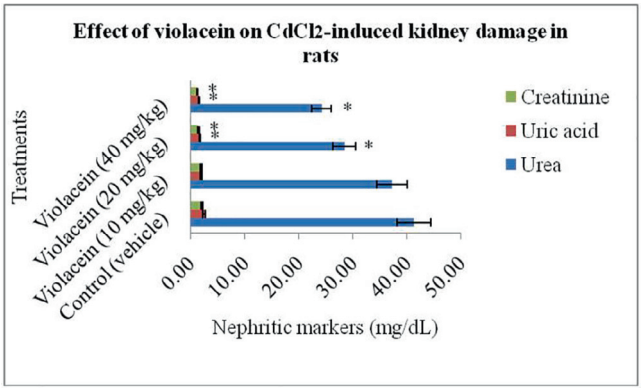

Fig. 3. Data represent mean ± S.D. (standard deviation) (n = 6). *p < 0.05 significant from the control.

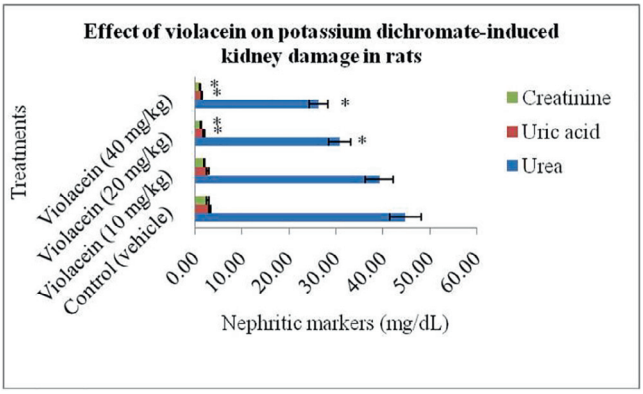

Fig. 4. Data represent mean ± S.D. (standard deviation) (n = 6). *p < 0.05 significant from the control.

3.6. Effect of violacein in gentamicin-induced kidney function

Gentamicin induced elevated level of serum creatinine (2.20 mg/dl), uric acid (3.26 mg/dl) and urea (45.17 mg/dl) in experimental animal than normal rat. Experimental animal co-administered with violacein (20 and 40 mg/kg) controlled the rise in serum urea (30.63 and 25.91 mg/dl), uric acid (1.91 and 1.34 mg/dl) and creatinine (1.33 and 1.18 mg/dl) compared to control animals (Table 2).

4. Discussion

C. violaceum was widely used for the batch production of violacein and the yield was analyzed in various culture conditions. The yield of violacein varied based on the type strains. In a study a plant associated *Duganella* spp. has been used for the production of violacein. A total of seven strains from *Duganella* spp. showed violacein production (Choi et al., 2015). The antibacterial activity of violacein has been reported. It showed activity against Gram-positive bacteria. The strains of *C. violaceum* was cultured in various media, including, molasses, vegetable waste, food waste, fruit waste and other nutrient sources (Ahmad et al., 2012). The pathogenic property of *C. violaceum* has been reported previously by Duran et al. (1989). In this study, this organism was isolated from the Hill region. In a study, Brito et al. (2004) isolated this bacterial strain from Amazon forest, Brazil. It was also isolated from various part of India and it is one of the opportunistic pathogen (Rai et al., 2011). Infection was severe among immunocompromised invididuals and symptoms like sepsis, urinary tract infection, wound infection and abscesses in brain, lymph nodes, lungs, skin and livers were reported (Orsetti et al., 2013).

Heavy metal such as cadmium, mercury and cobalt affect kidney function and these are occupational hazards. These heavy metals have length half life. In the case of cadmium, it's half-life was about 30 years. Cadmium primarily affected renal function, chronic exposure of cadmium leads to calciurea, tubular proteinuria and glucosuria. The tubular segments, S1 and S2 were mainly affected by cadmium (Conner and Fowler, 1993). The present results revealed the protective role of violacein on cadmium induced renal failure. The nephrotoxic property of cadmium has been described previously in humans (Conner and Fowler, 1993). $Cr^{+}6$ form of chromium critically damaged the S1 and S2 tubular segments (Franchini et al., 1993), however in rats the prime target is S1 tubular segments. The present findings clearly showed the protective property of violacein in rats due to potassium dichromate toxicity. The present finding revealed the protective role of violacein on proximal tubule segments (S1 and S2) of experimental rats.

The inorganic and organic forms of mercury affected the renal cells and have been described previously. Each form of mercury showed different specificity on tubules. Mercury (Hg^{2+}) at 2–3 mg/kg dosages induced necrosis in the proximal tubule, specifically S3 segment (Ganote et al., 1975). It leads to the damage in cell

Table 1
Effect of violacein on mercuric chloride-induced kidney damage in rats.

Treatment	Dose (mg/kg)	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)
Vehicle (1% CMC)	–	1.98 ± 0.15	2.33 ± 0.25	42.51 ± 5.28
Violacein	10	2.11 ± 0.35	2.00 ± 0.19	38.62 ± 2.94
	20	1.13 ± 0.05*	1.50 ± 0.11*	30.11 ± 3.10*
	40	1.00 ± 0.08*	1.34 ± 0.16*	27.21 ± 2.07*

Values are mean ± SD (n = 6).
* P < 0.05 vs. vehicle.

Table 2
Effect of violacein on gentamicin-induced kidney damage in rats.

Treatment	Dose (mg/kg)	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)
Vehicle (1% CMC)	–	2.01 ± 0.21	2.25 ± 0.17	42.81 ± 4.66
Violacein	10	2.18 ± 0.17	2.00 ± 0.30	42.68 ± 3.24
	20	1.54 ± 0.25*	1.70 ± 0.13*	37.13 ± 25.11*
	40	1.20 ± 0.09*	1.42 ± 0.24*	25.11 ± 1.91*

Values are mean ± SD (n = 6).
* $P < 0.05$ vs. vehicle.

membrane, loss of control because of variations in Ca^{2+} level (Kempson et al., 1977) and mitochondrial function (Trump et al., 1984). In a study, Sparrow et al. (1988) found the formation of necrosis in the S2 and S3 region of experimental animals treated with 0.5 mg/kg mercury. In the present investigation nephrotoxicity was initiated due to mercury toxicity and the impact of mercury toxicity was reduced by the application of violacein. The selected metals showed toxic effect and the administration of violacein showed protection to S1, S2 and S3 segments of proximal tubule.

Violacein was highly effective against *E. coli* and *Staphylococcus aureus* and has been recommended to combat bovine mastitis. This molecule also showed synergistic activity with various commercial antibiotics including, penicillin (Cazoto et al., 2011). Also, this compound showed antibacterial activity against, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Staphylococcus aureus, Bacillus megaterium, Bacillus subtilis, and Bacillus licheniformis (Nakamura et al., 2003). Gentamicin is one of the aminoglycoside antibiotics widely used to treat various bacterial pathogens from Gram-negative group. However, prolonged application of gentamicin showed nephrotoxicity. Gentamicin is accumulated by the renal proximal tubular cells and interactions between intracellular processes and drugs were reported (Humes et al., 1982). Most of the heavy metals induced oxidative stress and this stress enhance tissue injury in experimental animals (Walker et al., 1984). In a study, the protective role of superoxide dismutase on gentamicin mediated renal failure has been described (Nakajima et al., 1994). Also, co-administration of selenium, vitamin E and antioxidant showed protective role in gentamicin-induced nephrotoxicity in experimental animal (Ademuyiwa et al., 1990). The synergistic effect of violacein with various antibacterial agents has been reported earlier (Subramaniam et al., 2014). Microbial violacein with gentamicin and cefadroxil showed enhanced antibacterial activity against *Staphyloccus aureus*. Also, the combination of violacein–aminoglycoside and violacein–macrolide showed potent activity (Subramaniam et al., 2014). To conclude, administration of violacein showed nephroprotective activity against heavy metals and gentamicin through antioxidant property.

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.

Acknowledgments

Project of Qiqihar Science and Technology Bureau of Heilongjiang Province Subject No. SFZD-2016110. This work was supported by the Soonchunhyang University Research Fund. The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. RG-1440-107.

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