Research Article

Investigations on *Canna indica* rhizomes for nephroprotective activity with antioxidant effects

Preeti Singh, Himanshu Bhusan Sahoo*, Alok Pal Jain

RKDF College of Pharmacy, SRK University, Hoshangabad road, Misrod, Bhopal - 462026 (MP) India

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Abstract

Objective: Present study was aimed for screening of *Canna indica* rhizomes for phytochemical investigation and nephroprotective activity on experimentally induced kidney toxicity. **Material and methods**: Nephroprotective activity of *Canna indica* rhizomes was investigated on cisplatin, and gentamicin induced kidney toxicity in rats. Animals were divided in 8 groups (n=6) and initial body weight was recorded. The group-I was administered vehicle for 15 days (p.o.). In II group, gentamicin (40 mg/kg b.w., s.c.) was administered for 15 days. The group III and IV were administered with ethanol extract of *Canna indica* rhizomes (EECA) (p.o.) at dose of 200 and 400 mg/kg, p.o, respectively. On 16th day, weight of animals was taken and blood samples were withdrawn through retro-orbital plexus. Changes occurring in body weight of animals along with other morphological changes were studied. Biochemical parameters during *in vivo* study were blood urea, serum creatinine and urine albumin studied. In addition, estimation of extent of lipid peroxidation and reduced glutathione (GSH) was also carried out to find out for observation of antioxidant effect of the extract. **Results:** In present study, significant increase in blood urea and creatinine has revealed toxicant effect of gentamicin on kidney/nephrons in negative control group. Gentamicin (40 mg/kg, s.c.) intoxicated group has shown significant increase in blood urea 83.61±3.62 mg/dL and creatinine levels 4.62±0.62mg/dL as compared to control group 31.82±1.27 mg/dL and 1.53±0.17 mg/dL, respectively. The ethanolic extract of *Canna indica* rhizomes at dose levels of 200 and 400 mg/kg have significantly protective effect in gentamicin induce nephrotoxicity. Results were also supported by histopathological studies in both preventive and curative groups. The significant improvement in GSH level was observed after treatment with EECI (200 mg/kg and 400 mg/kg. Results were confirmed that DDPH scavenging effects were observed in dose dependent manner. GSH and MDA levels were supporting to antioxidant activity. **Conclusion**: In conclusion, ethanolic extract of *Canna indica* rhizomes have significant nephroprotective activities also supported with antioxidant activity. It is further concluded that this antioxidant activity is attributed to phenolic compounds, and flavonoids present in the extracts.

***Keywords*:** *Canna indica,* nephroprotective, flavonoids, cisplatin, gentamicin, antioxidant

Introduction

Nephrotoxicity is a poisonous effect of both toxic substances and medication on the kidney. Alternatively, nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals. Some chemicals target one discrete anatomical region of the kidney and may affect only one cell type (Bennett, 1993). The nephrotoxic effect of most drugs is more profound in patients who already have renal impairment. Nephrotoxicity occurs in kidney cortex by generation of free radical, which may be super oxide anion, hydroxyl radical, hydrogen peroxide etc. Nephrotoxicity can be evaluated by measuring concentration of urea and creatinine in serum, reduced glutathione concentration and reduced super oxide activity in renal cortex. Amino glycosides antibiotics are the most common antibiotics, which produce severe nephrotoxicity. Gentamicin induced nephrotoxicity is manifested functionally by urine concentration, tubular proteinuria, lysozyme enzyme, and decreased glomerular filtration rate.

Canna indica commonly known as Keli belongs to the

Address for Corresponding Author:
Dr. Himanshu Bhusan Sahoo
RKDF College of Pharmacy, SRK University, Hoshangabad road, Misrod, Bhopal - 462026 (MP) India
Email: rkdfcp95@gmail.com

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family Cannaceae is widely cultivated throughout India, tropical and sub-tropical regions of Southern United State and South to Northern Argentina & Philippines in settled areas (Chopra et al., 1999). Root contains the chemical constituents as cannagenins. Rootstock contains enzymes, triacontanal and mixture of stigmasterol, β-sitosterol, campesterol and β lectin and traces of alkpiels (Ghani, 2003). Decoction of fresh rhizome is used as jaunditic symptoms fevers, dropsy and dyspepsia. Phytochemical screening yielded phenols, sterols, flavonoids and saponins. Composition of the unsaponifiable matter from *Canna indica* rhizome are 5, 8 Henicosdiene, 7- Henicosyne, 3, 15- Dihydroxy-2-octadecene, 6-Hydroxy eicosane, Tricosane, and Tetracosane.

The objective of the present study was to perform phytochemical screening of *Canna indica,* for different chemical constituents and screening of extract for nephroprotective activity. However, the literature review revealed that *Canna indica* has been used traditionally for inflammatory disorders and free radical scavenging effects. In connection of these reports, the present study aimed for screening of *Canna indica* rhizomes for phytochemical investigation and nephroprotective activity on experimentally induced kidney toxicity.

Material and methods

Identification and collection of plant material

The rhizomes of *Canna indica* was collected in the month of August from campus of SRK University, Bhopal. Plant was identify and authenticated at the Department of Botany, SRK University, Bhopal (MP). The rhizomes were clean and cut in small pieces and then sun dried and was powdered moderately.

Qualitative analysis of extracts

The plant extracts were subjected for different qualitative chemical tests to detect the plant constituents of the plant extracts (Kokate, 2003; Khandelwal, 2006).

Animal selection

Wistar albino rat of either sex weighing between 150 and 160 gm. were selected for acute toxicity studies and nephroprotective activity. The animals were acclimatized to standard laboratory conditions of temperature (22±3^{0}C) and maintained on 12:12 h light: dark cycle. The animal care and experimental protocols were in accordance with CPCSEA/ IAEC. The animal are randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute toxicity study

The acute oral toxicity study of ethanol extract of *Canna indica* rhizomes was carried out as per the guidelines set by Organization for Economic Co–operation and Development (OECD) guideline 423. The acute toxicity study was conducted in animals of either sex were divided into 4 groups (10 rats per group, 5 male and 5 female) for all extracts after a single oral dose. Ethanol extract (0.5, 1, 2, and 4 g/kg) was mixed in 0.5% Tween-80 solution and administered orally. All experimental animals were fed with standard diet and clean water *ad libitum* and kept under observation. The mortality or behavioral changes, including hyperactivity, tremors, ataxia, convulsions, salivation, diarrhea, sleep, and coma, were regularly observed for 14 days.

Nephroprotective activity

The *in vivo* method is a convenient method for evaluation because screening can be performed in a normal laboratory without requiring additional infrastructures or chemicals. By administrating the toxins, corresponding symptoms of the disease can be observed easily.

Changes in blood and urine profiles are observed in addition to morphological and histological parameters in nephrotoxicity, *in vivo*. Following various parameters were monitored to assess the activities *in vivo.*

Changes occurring in body weight of animals along with other morphological changes were studied. The necrosis induced, the type and the extent of degeneration in the kidney tissues occurred were studied by histological studies.

The most reliable biochemical parameters in the *in vivo* study are blood urea, serum creatinine and urine albumin was estimated. In addition, estimation of extent of lipid peroxidation and reduced glutathione (GSH) was also carried out to find out for observation of antioxidant effect of the extract.

Preparation of toxin solution

Injection of gentamicin (Genticyn, 10 mg/ml, 2 ml, Nicholas Piramal, India) and cisplatin (Cytoplatin-50, 1 mg/ml, 50 mg, Cipla Laboratories, India) were purchased from the local market. Solution were freshly prepared in 0.1 M citrate buffer (Sekar et al., 1990) and administered to animals in calculated dose according to body weight.

Gentamicin induced nephrotoxicity

Animals were divided in 8 groups (n=6) and initial body weight was recorded. The group-I was administered vehicle for 15 days (p.o.). In II group, gentamicin (40 mg/kg b.w., s.c.) was administered for 15 days. The group III and IV were administered with ethanol extract of *Canna indica* rhizomes (EECA) (p.o.) at dose of 200 and 400 mg/kg, p.o, respectively.

On 16th day, weight of animals was taken and blood samples were withdrawn through retro-orbital plexus. To get serum from the blood sample, freshly drawn blood was centrifuged at 2500 rpm for 30 minutes and used for estimation of various biochemical parameters. Animals were sacrificed by cervical dislocation and kidney were dissected and kept in formalin solution (10%) for biochemical estimation and histopathological studies.

Cisplatin induced nephrotoxicity

Animals were divided in 8 groups (n=6) and weighed. The group I was administered vehicle (distilled water) for 15 days (p.o.). The group II was administered cisplatin (5 mg/kg b.w., i.p.) on first day. In the III and IV groups, EECI was administered at dose of 200 and 400 mg/kg, p.o. respectively. Cisplatin was administered on 11th day in III and IV groups.

On 16th day, weight of animals was taken and blood samples were withdrawn through retro – orbital plexus. To get serum from the blood sample, freshly drawn blood was centrifuged at 2500 rpm for 30 minute and used for estimation of various biochemical parameters. Animals were sacrificed by cervical dislocation and kidney were dissected and kept in formalin solution (10%) for biochemical estimation and histopathological studies.

Biochemical assessment of renal function

Alkaline picrate method was used for the estimation of serum creatinine (Bonses & Taussky, 1945).

Creatinine in basic picric acid solution forms an orange red complex. The absorbance at predetermined times during conversion is proportional to the concentration of creatinine in the sample.

Estimation of urea

Method of urease enzyme was used for the estimation of urea in test samples (Coulambe and Favrean, 1965).

Urea reacts with hot acidic diacetylmonooxime in presence of thiosemicarbazide and produces a rose purple red complex, which is measured at 525 nm.

Estimations of reduced glutathione (GSH) and Malondialdehyde (MDA)

Kidney samples were dissected out from the tested groups and washed immediately with ice cold saline to remove as much blood as possible. Kidney homogenates (5% w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000 rpm for 10 min using a Remi centrifuge. The supernatant was used for the estimation of reduced glutathione GSH (Ellaman, 1959), malondialdehyde (MDA) (Yagi and Rastogi, 1979).

Histopathological Study

The changes in the normal architecture of any tissue, due to untoward effect of drugs and chemicals can be observed by histopathological studies. The different techniques used are light microscopy, electron microscopy, fluorescent microscopy, autoradiography and immunocytology (Mcmanus, and Mowry, 1965).

Statistical analysis

The data were expressed as mean standard deviation (SD). The statistical significance of the difference in each parameter among the groups was evaluated using one-way analysis of variance (ANOVA) followed by the followed by the multiple comparison test of Tukey–Kramer tests. Criterion for statistically significant difference was chosen to be at $P<0.01$.

Results

The plant material was identified and powdered material was subjected to successive extraction with petroleum ether and ethanol. The percentage yield obtained from non polar (petroleum ether) and polar solvents i.e. ethanol 3.8 and 6.1%w/w respectively. The Preliminary phytochemical investigations revealed the presence of various phytoconstittuents in ethanol extract of *Canna indica* were as Steroid, Tannins, and flavonoid compounds.

Acute toxicity study

Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases no one death was observed within first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and motor activity and behavioral pattern. Attention was also given to observation of tremors and convulsions. We have selected one tenth dose of highest toxic dose level.

Nephroprotective Activity

The most common form of nephrotoxic injury is antibiotic nephropathy, which usually occurs when antibiotics are given to patients with already weakened kidneys. Analgesic nephropathy is another common form of nephrotoxic injury and occurs as a result of long-term abuse of analgesics, usually NSAIDs (e.g., ibuprofen). Present study amongst various neprotoxins, we selected gentamicin and antineoplastic drug cisplatin as nephrotoxins to evaluate the efficacy of *Canna indica* rhizomes extract for nephroprotective activity.

In present study shade dried rhizomatous part of *canna*

indica belonging to family canaceae having medicinally important bioactive constituents is reviewed with special emphasis on the biological activities.

Gentamicin induced nephrotoxicity

Gentamicin, an aminoglycoside antibiotic is used as an effective agent against gram negative infections. Its chemical stability, rapid bactericidal action has made it a first line drug in a variety of clinical situations. However, nephrotoxicity is the major side effect of aminoglycosides accounting for 10–15% of all cases of acute renal failure (Shifow et al., 2000). Studies have shown that 30% of the patients treated with gentamicin for more than 7 days show signs of nephrotoxicity (Mattew, 1992). It has been also shown that the specificity of gentamicin renal toxicity is related to its preferential accumulation in the renal convoluted tubules and lysosomes (Nagai and Takano, 2004). Major signs of nephrotoxicity are increased concentrations of blood urea and serum creatinine.

In present study, significant increase in blood urea and creatinine has revealed toxicant effect of gentamicin on kidney/nephrons in negative control group. Gentamicin (40 mg/kg, s.c.) intoxicated group has shown significant increase in blood urea 83.61±3.62 mg/dL and creatinine levels 4.62±0.62mg/dL as compared to control group 31.82±1.27 mg/dL and 1.53±0.17 mg/dL, respectively (Table 1). The ethanolic extract of *Canna indica* rhizomes at dose levels of 200 and 400 mg/kg have significantly protective effect in gentamicin induce nephrotoxicity. Results were also supported by histopathological studies in both preventive and curative groups.

Numerous studies have shown that gentamicin activates phospholipases and alters the lysosomal membrane in addition to oxidative stress (Laurent et al., 1990). Although the mechanism of gentamicin induced nephrotoxicity is not completely known. However, studies have implicated reactive oxygen species particularly superoxide anion radical in the pathophysiology of gentamicin nephropathy (Cuzzocrea et al., 2002). It has been demonstrated that gentamicin administration increases renal cortical lipid peroxidation, renal nitric oxide generation and mitochondria H_2O_2 generation (Parlakpinar et al., 2005; Yanget et al., 1995). Ethanolic extract of *Canna indica* have shown dose dependent radical scavenging activity has shown in the form of GSH and MDA level. After intoxication, level of GSH was decreased as 25.70±1.60 from 51.63±2.48 mg/100g tissue. The significant improvement in GSH level was observed after treatment with EECI (200 mg/kg and 400 mg/kg. Results were

Table 1. Effects of ethanolic extract of *Canna indica* rhizomes on blood urea and serum creatinine in Gentamicin induced nephrotoxicity

Treatment groups	Blood urea (mg/dL)	Serum creatinine (mg/dL)
Control (vehicle only)	31.82±1.27	1.53±0.17
Gentamicin intoxicated (40 mg/kg b.w., s.c.)	83.61±3.62	4.62±0.62
EECI (200 mg/kg)+Gentamicin	54.18±2.43*	2.17±0.42*
EECI (400 mg/kg)+Gentamicin	35.92±2.06**	1.85±0.15**

All the values are expressed as Mean± SEM. *P<0.05, **P<0.01, shows level of significance as compared to intoxicated group, and control group. EECI: ethanolic extract of *Canna indica*

Table 2. Effects of ethanolic extract of *Canna indica* rhizomes on GSH and MDA level of kidney in Gentamicin induced nephrotoxicity

Treatment groups	GSH (mg/100g tissue)	MDA (nmol/mg protein)
Control (vehicle only)	51.63±2.48	174.82±5.37
Gentamicin intoxicated (40 mg/kg b.w., s.c.)	25.70±1.60	442.87±11.72
EECI (200 mg/kg)+Gentamicin	42.18±1.82*	245.11±6.37*
EECI (400 mg/kg)+Gentamicin	48.75±2.16**	185.64±5.94**

All the values are expressed as Mean± SEM. *P<0.05, **P<0.01, shows level of significance as compared to intoxicated group, and control group. EECI: ethanolic extract of *Canna indica*

confirmed that DDPH scavenging effects were observed in dose dependent manner (Table 2). In addition, MDA level also found significantly decreases in both doses of the extracts (200 mg/kg and 400 mg/kg) have shown as 245.11±6.37 and 185.64±5.94, respectively (Table 2) was compared with normal control group of animals. GSH and MDA levels were supporting to antioxidant activity.

Natural and synthetic antioxidants and free radical scavengers are claimed to provide nephroprotection in gentamicin renal injury. Vitamin C, Vitamin E, selenium, etc. are among the natural free radical scavengers. Natural antioxidants ascorbic acid and α-tocopherol have also been found as nephroprotective in animal model (Ajith et al., 2007). Flavonoids and phenolic compounds are well known potent antioxidant and free radical scavengers. Renoprotective effects have been reported for polyphenols such as quercetin (Ishikawa & Kitamura, 2000). Phytochemical study revealed that flavonoids and phenolic compounds are present in ethanol extract of *Canna indica* rhizomes. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups. Hence, the probable mechanism of nephroprotective activity by EECI may be attributed to antioxidant and free radical scavenging property, due to presence of flavonoids and phenolic compounds.

Cisplatin induced nephrotoxicity

The changes in renal function observed in the rat system correlate well with the nephrotoxic effects of cisplatin in man (Daugaard et al, 1988). Alterations in values of blood urea and serum creatinine levels were taken as indications of an abnormal kidney function. Our findings confirmed that a single dose of cisplatin induced a significant increase in blood urea and serum creatinine in rats. Cisplatin administration (5 mg/kg, i.p.) caused intoxication in animals revealed by elevated level of blood urea (42.08±1.42 mg/dL) and serum creatinine (3.48±0.62 mg/dL) as compared to control group (18.67±0.84 and 1.15±0.07 mg/dL, respectively) (Table 3). The results showed that pretreatment with the EECI (200 and 400 mg/kg) has efficacy to prevent the elevation in these levels of blood urea and serum creatinine. EECI pretreatment has significantly prevented elevation in blood urea (28.34±1.38 and 20.22±1.11 mg/dL) and serum creatinine (2.16±0.75 and 1.52±0.18) at tested doses, respectively as compared to cisplatin intoxicated groups and normal control group.

Although the exact mechanism of cisplatin induced nephrotoxicity is not well understood, several studies have

Table 3. Effects of ethanolic extract of *Canna indica* rhizomes on blood urea and serum creatinine in Cisplatin induced nephrotoxicity

Treatment groups	Blood urea (mg/dL)	Serum creatinine (mg/dL)
Control (vehicle only)	18.67±0.84	1.15±0.07
Cisplatin intoxicated (5 mg/kg b.w., i.p.)	42.08±1.42	3.48±0.62
EECI (200 mg/kg)+Cisplatin	28.34±1.38*	2.16±0.75*
EECI (400 mg/kg)+Cisplatin	20.22±1.11**	1.52±0.18**

All the values are expressed as Mean± SEM. *P<0.05, **P<0.01 show level of significance as compared to intoxicated group, and control group. EECI: ethanolic extract of *Canna indica*

Table 4. Effects of ethanolic extract of *Canna indica* rhizomes on GSH and MDA level of kidney in Cisplatin induced nephrotoxicity

Treatment groups	GSH (mg/100g tissue)	MDA (nmol/mg protein)
Control (vehicle only)	28.66±1.49	142.31±4.82
Cisplatin intoxicated (5 mg/kg b.w., i.p.)	14.21±1.10	391.75±7.61
EECI (200 mg/kg)+Cisplatin	23.46±1.30**	243.44±5.62*
EECI (400 mg/kg)+Cisplatin	26.71±1.42**	151.76±4.55**

All the values are expressed as Mean± SEM. *P<0.05, **P<0.01, show level of significance as compared to intoxicated group, and control group. EECI: ethanolic extract of *Canna indica*

suggested the involvement of lipid peroxidation and free radicals. Cisplatin generates active oxygen species such as superoxide anion and hydroxyl radicals, and stimulates renal lipid peroxidation (Matsushima et al., 1998). The role of lipid peroxidation and its position in the chain of events that leads to cisplatin nephrotoxicity still remains controversial. It has been (Kruidering, et al., 1997) suggested that although free radical generation is not the direct cause of cisplatin induced renal injury, it may be important for cisplatin nephrotoxicity, since the *in vivo* administration of antioxidants in animals can attenuate or inhibit renal damage. In present study, the administration of a single dose of cisplatin resulted in renal lipid peroxidation revealed by significant decrease in GSH level (14.21±1.10 mg/100g tissue) and increase in MDA (391.75±7.61 nmol/mg protein) as compared to control group i.e. 28.66±1.49 mg/100g tissue and 142.31±4.82 nmol/mg protein. Many antioxidants have shown protection against antitumoral agent induced peroxidative damage in the renal tissue, such as isoeugenol (Rao et al., 1999), dimethylthiourea (Matsushima et al., 1998), Vitamin C (Antunes et al., 2000), and the iron chelating deferoxamine that markedly reduced the peroxidative damage induced in the renal tissues in rats treated with doxorubicin (Saad et al., 2001). EECI has attenuated decrease in GSH content 23.46±1.30 and 26.71±1.42 mg/100g tissue at both doses, respectively. It has also been found able to prevent lipid peroxidation and reduced MDA content to 243.44±5.62 and 151.76±4.55 nmol/mg protein at 200 and 400 mg/kg, respectively (Table 4).

Several studies suggested a critical role for glutathione in mechanisms of tumor cell resistance to alkylating agents, such as cisplatin (Silva et al., 2001). The depletion in the renal glutathione level has been observed in rats in response to oxidative stress caused by cisplatin treatment (Kroning et al., 2000). Various phenolic compounds are having important role for synthesis of the endogenous antioxidant glutathione. These phenolic compounds have protective effect in renal injury due to its antioxidant activity (Mora and Antunes et al., 2003).

Histopathological study

Histopathological observations of kidney tissue of animals from various groups were shown in figure 1 and 2. It shows structure of normal control group which has shown normal kidney structure with normal glomeruli and Bowmen's capsule. No tubular damage was seen. Gentamicin intoxicated group (40 mg/kg, b.w., s.c.) for 15 days has shown severe damage in kidney. The renal tubules are damaged with breaking up tubular lining. Glomerular capsule shows degenerative changes. Tubular cast was found due to breakdown of RBC of glomeruli (Figure 1). Administration of

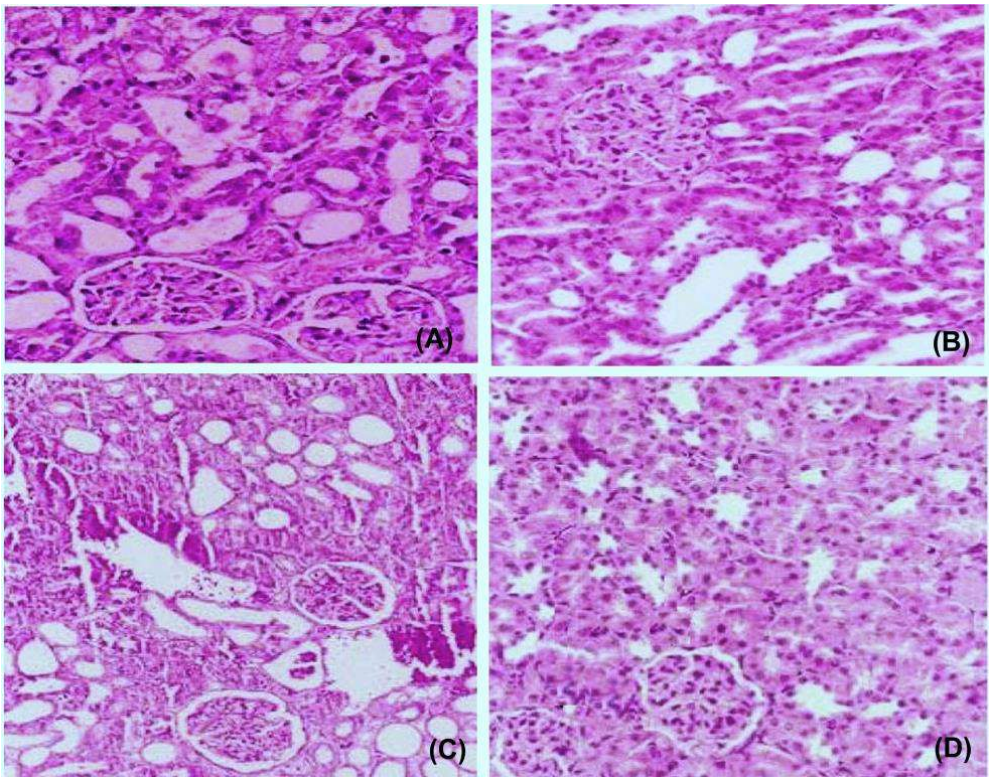

Figure 1. Photomicrograph of kidney in Gentamicin induced nephrotoxicity showed effects of different treatments: (a) Control (vehicle only); (b) Gentamicin intoxicated (40 mg/kg b.w., s.c.); (c) EECI (200 mg/kg)+Gentamicin; (b) EECI (400 mg/kg)+Gentamicin

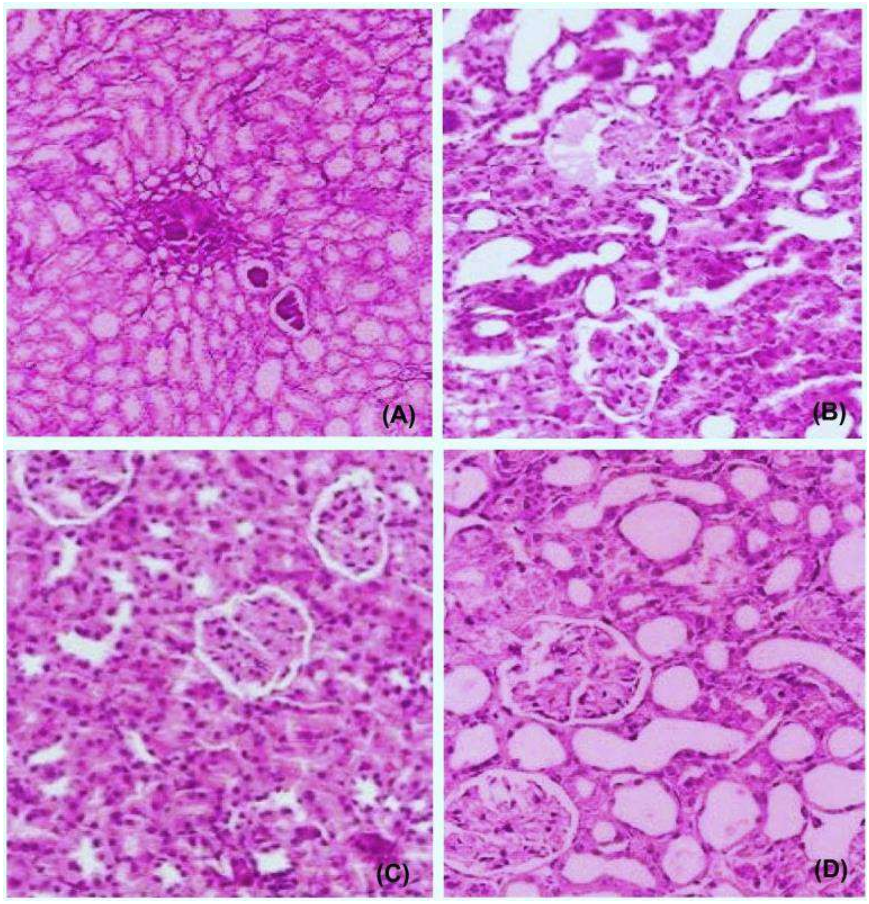

Figure 2. Photomicrograph of kidney in Cisplatin induced nephrotoxicity showed effects of different treatments: (a) Control (vehicle only); (b) Cisplatin intoxicated (5 mg/kg b.w., i.p.); (c) EECI (200 mg/kg)+Cisplatin; (b) EECI (400 mg/kg)+Cisplatin

EECI 30 minutes before gentamicin intoxication, EECI at 200 and 400 mg/kg has prevented generation of cell necrosis and renal tubules damage to great extent. In addition, no glomeruli congestion was found although slight edema and inflammation was there. Extract treated group has shown inhibition in cell necrosis to certain extent and still cell necrosis was found, renal tubules were not damaged and there was no glomeruli congestion. Inflammatory cells were found to be absent.

In case of Cisplatin induced nephrotoxicity, histopathological structure of kidney tissue has been shown in figure 5.6. In control group normal kidney structure has been found with normal glomeruli and Bowmen's capsule. No tubular damage was seen. Extensive tubular epithelial cellular necrosis, desquamation, vacuolization and swelling were observed in the cisplatin intoxicated animals. Group treated with EECI (200 and 400 mg/kg) for 1- 10 days followed by single dose of cisplatin (5 mg/kg b.w., i.p) resulted prevention of damage in histological appearance and tubular cell damage (Figure 2).

In conclusion, ethanolic extract of *Canna indica* rhizomes have significant nephroprotective activities also supported histological observations. The probable mechanism responsible is antioxidant activity. It is further concluded that this antioxidant activity is attributed to phenolic compounds, and flavonoids present in the extracts.

Conflict of interest

None

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