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NEPHROPROTECTIVE POTENTIAL OF *CURCUMA CAESIA* ROXB. IN ANIMAL MODEL

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Abstract: A perennial medicinal herb, *Curcuma caesia* Roxb. (Zingiberaceae) known as black turmeric in Ayurveda, used to treat human ailments such as inflammation, wounds healing and kidney disorders. The aim of present study was to evaluate the protective effect of methanolic extract of *Curcuma caesia* (CCE) against gentamicin (GM) induced nephrotoxicity. Nephrotoxicity was induced in rats with GM (100 mg/kg b.w.; i.p. for 8 days) and were treated with a graded dose of CCE (250 mg/kg and 500 mg/kg b.w.; p.o. for 8 days) or 0.9% sodium chloride (vehicle). Biomarkers like; plasma (urea, creatinine) and urine (urea, creatinine); renal enzymatic and non-enzymatic antioxidants along with blood profile and histopathology were evaluated in all experimental groups. GM induced significant elevation ($p<0.01$ and $p<0.001$) in physiological, haematological parameters, plasma and urine (both urea, creatinine) along with a significant reduction ($p<0.01$ and $p<0.001$) in renal enzymatic and non-enzymatic antioxidants. The graded dose of CCE treatment over GM treated rats (GM+CCE-250 and GM+CCE-500) recorded significant improvements ($p<0.001$ and $p<0.001$) in physiological, haematological parameters, plasma and urine (both urea, creatinine) along with a dose dependent significant increment ($p<0.01$ and $p<0.001$) in renal enzymatic and non-enzymatic antioxidants. The methanolic extract of *Curcuma caesia* Roxb. ameliorates GM induced nephrotoxicity and ability to maintain the functional baseline of the kidneys; thus validates its therapeutic use.

Key Words: *Curcuma caesia* Roxb. (CCE), Gentamicin (GM), Nephrotoxicity, Reduced glutathione (GSH), Oxidised glutathione (GSSG), Malonaldehyde (MDA), Catalase (CAT).

INTRODUCTION

The Zingiberaceae family comprises a variety of medicinal plants used in the treatment of various human ailments. A perennial medicinal herb, *Curcuma caesia* Roxb. (Family-Zingiberaceae) known as black turmeric in Ayurveda is widely cultivated and found in Southeast Asian countries like India, Indonesia, Thailand and Malaysia for traditional purposes[1]. It is a wonder herb in the midst of a chemical substance Curcumin, which have many therapeutic properties. It is conceivably one of the marginal medicinal plants that are used in black magic as well as herbal remedies. However, scientific studies suggest that the rhizome possesses the characteristic properties like anti-oxidative, anti-inflammatory, anti-diarrheal, diuretic, anti-emetic, wound healing, hypoglycaemia, anticoagulant and antimicrobial activities[2].

Recent research showed that the volatile oils from rhizomes of *Curcuma caesia* contains 30 major components such as; camphor, ar-turmerone, (Z)-ocimene, ar-curcumene, 1, 8-cineole, borneol bornyl acetate etc., which is responsible for major pharmacological activities[3].

The present study was designed to evaluate the protective effect of methanolic extract of *Curcuma caesia* against gentamicin induced nephrotoxicity. However the antioxidant and free radical scavenging effect of phenolic compounds, flavonoids and volatile oils remarkably showed nephroprotective action[4]. Thus, the biological screening of this plant was carried out to find nephroprotective potential, which is remarkably a new herbal treatment of kidney disorders. The extent of protective effect was determined by studying blood plasma and urine toxicity markers and biochemical estimation of antioxidant enzymes followed by histopathology of kidney tissues.

MATERIALS AND METHODS

Plant material

The rhizomes of *Curcuma caesia* Roxb. was composed in the month of September from the foothills of Himalayan region, Pithoragarh (Uttarakhand, India). The plant was identified by Mr. Gaurav Upadhyay, Assistant Professor, Siddhartha Institute of Pharmacy, Dehradun, India.

Preparation of extracts

About 450 g fine powder of dried rhizomes of *Curcuma Caesia* was subjected to straight extraction in a Soxhlet apparatus with 750 ml methanol at a temperature of 55 ± 5°C for a period of 16 hrs. The obtained crude extract was filtered and evaporated to dryness at vacuum in a Buchi evaporator. The residue was weighed (30 g) and stored at a temperature 4 °C until use[5].

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Preliminary phytochemical screening

A preliminary phytochemical screening was carried out to assess presence/absence of various groups of phytochemicals[6].

Experimental animals

Healthy adult male wistar rats (180-250 g) were obtained from Siddhartha Institute of Pharmacy, Dehradun, India. They were housed in separate groups under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 h light/dark cycle. Animals had free access to standard pellet diet and water ad libitum. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and investigation procedure was approved by Institutional Animal Ethical Committee Siddhartha Institute of Pharmacy, Dehradun, India (Approval No. 1891/PO/2A/105/CPCSEA).

Gentamicin induced nephrotoxicity in rats

Animals were divided into four groups consist of 6 animals in each group. Group I (CON) received 10ml/kg/day oral dose of 0.9% of NaCl, Group II (GM) received 100 mg/kg/day i.p. of gentamicin and Groups III (GM + CCE-250) & IV (GM + CCE-500) were received CCE at a dose of 250 mg/kg/day and 500 mg/kg/day, respectively with 100 mg/kg/day i.p. of gentamicin for eight days. The body weights of the animals were also recorded daily. After 8 days, animals were anaesthetized with ether for collection of blood serum (using retro orbital plexus) for estimation of various haematological and biochemical analysis. The rats were then sacrificed by cervical dislocation and kidneys were isolated for estimation of tissue parameters and histopathological study.

Plasma and urine markers of renal damage

Rats were individually housed in metabolic cages as per divided in separate groups for 24 h and urine was collected on the 8th day of the treatment. Blood samples were collected from these overnight fasted animals through retro-orbital sinus puncture in ethylene diamine tetra acetic acid (EDTA) coated vials and plasma was separated by cold centrifugation of vial (REMI Centrifuge R23) at 3000rpm for 10 min. Urea and creatinine were assayed in plasma and urine using commercially available kits (DIATEK Healthcare Pvt. Ltd. Kolkata, India) and the hematological variables viz. RBC, WBC and Hb were recorded by using hematological auto-Analyzer (Lal pathology lab., Dehradun, India).

Preparation of homogenate of renal tissue

A small portion of the kidney tissue (300-600 mg) were excised and homogenized in 8 mL of 0.02 M EDTA and then 4 mL of cold distil water was added in it. After that, 2 mL of suspension medium was taken from the supernatants of the 10% (w/v) tissue homogenate in 1.15% KCl and centrifuged at 10,000 RPM for 20 min in a high speed cooling centrifuge; supernatant and sediment were used for further biochemical estimations.

Measurement of renal lipid peroxidation

Measurement of malonaldehyde (MDA) as an index of lipid peroxidation was done using thiobarbituric acid assay as per Buege and Aust method[7].

Measurement of renal antioxidants

Superoxide dismutase (SOD) was assayed in the tissue supernatant by the method of Kakkar *et al.* 1984,[8] based on the inhibition of the formation of nicotinamide adenine dinucleotide-phenazine methosulfate-Nitro blue tetrazolium formazan. Catalase (CAT) activity in tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of hydrogen peroxide, the substrate of the enzyme[9]. Reduced glutathione (GSH) content in tissue supernatant was measured spectrophotometrically by using Ellman's reagent (Dinitrothiobenzoic acid) as a colouring reagent, following the method described by Beutler 1963.[10] Whereas, oxidized glutathione (GSSG) was measured according to the method described by Aseni 1999,[11] based on the principle of glutathione reductase enzyme reducing GSSG to GSH with the concomitant oxidation of NADPH to $NADP^+$.

Acute oral toxicity study

The toxicity of extract was studied as per organization for economic co-operation and development (OECD) guideline number 425. Male wistar rats (180-250 g) were used in the sub-acute toxicity study. Six rats were successively administered a dose of 2000 mg/kg body weight of CCE prepared with water as recommended in the guideline. After the administration of drugs, each animal was observed every other hour for signs of toxicity and abnormality in behavior up to the 48th hour. After this, daily observations for toxicity and mortality were concluded up to the 14th day. The body weights of the animals were recorded every third day. At the end of study duration, all the mice were sacrificed and processed for gross necropsy (OECD, Adopted in December 2001)[12].

Preparation of extracts dose

Graded dose of 250 mg/kg and 500 mg/kg (based on toxicity study) of methanolic extract *Curcuma caesia* (CCE) were suspended in distilled water using 1% tween 80 and administered orally to experimental animals. Suspension of extract was prepared freshly. The extracts were administered at a constant volume of 10 ml/kg for each animal[13].

Histopathological study

Kidney tissues of rats were removed and washed with normal saline. Formaldehyde (4% buffered) or 10% buffered formalin was employed as universal fixative particularly for routine paraffin embedded sections. The cleared tissue was fixed in 10% natural buffered formalin solution (pH 7.0-7.2). All the sections of kidney tissues were examined under a microscope for the analysing of altered structural design of kidney tissue due to gentamicin challenge and improved kidney architecture due to pre-treatment with drug extracts. These were examined under the microscope for histopathological changes such as glomerular congestion, degeneration of epithelial cells of tubular cells.

Statistical analysis

Numerical results are expressed as Mean ± SEM. The data was analysed using one way analysis of variance (ANOVA) test. Calculations were performed using commercial software (GraphPad Software, San Diego, CA). ANOVA followed by Bonferroni post hoc test. The *p* values <0.05, <0.01 and <0.001 were considered as statistically significant. *p<0.05; **p<0.01 and ***p<0.001 significant when compared with the GM group as applicable.

RESULTS

Preliminary phytochemical screening of methanolic extract of *Curcuma caesia* showed that the plant contains phenolic compounds, flavonoids, carbohydrates, proteins, tannins, alkaloids, glycosides, saponins and volatile oils which may possessing antioxidant and free radical scavenging activity. There were no any abnormal behavior and physiological changes recorded in CCE fed rats up to a dose of 2000 mg/kg body weight.

Measurement of Curcuma caesia Roxb extract (CCE) on body weight, kidney weight and kidney volume in gentamycin (GM) induced nephrotoxicity

The graded dose (250 mg/kg and 500 mg/kg) of methanolic extract of CCE was given as pre-treatment dose by the oral route for both the treatment group with gentamicin (100 mg/kg i.p.) induced nephrotoxicity in rats (n=6). The measured parameters are mentioned in Table 1 which summarised that, the GM treated group has been scored significant (p<0.001) decrease in body weight, while significant (p<0.05 and p<0.01) increase in kidney weight and kidney volume as compared to CON group. Whereas, these sets of changes were remains same in graded dose of CCE treatment groups as compared to GM treated animals.

Table 1: Effect of *Curcuma caesia* Roxb extract on physiological activity like bodyweight, kidney weight & kidney volume in gentamicin induced nephrotoxicity

Experimental Groups[a]	Bodyweight (g)		Kidney Weight (g)	Kidney Volume (ml)
	Initial	Final		
CON	182.5±3.80	183.0±3.96	0.685±0.025	4.82±0.05
GM	200.6±4.33$^{\#\#}$	190.3±4.05$^{\#\#}$	0.947±0.032$^{\#\#\#}$	6.04±0.12$^{\#\#}$
GM+CCE-250	208.8±6.75*	206.6±5.55*	0.812±0.020*	5.78±0.08*
GM+CCE-500	201.3±5.68**	200.8±4.78**	0.700±0.03**	5.02±0.11**

Values are expressed as mean ± SEM, n=6. $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001 compared with CON; *p<0.05 and **p<0.01 compared with GM.
[a]Where, CON: normal saline (0.9% NaCl), GM: Gentamicin (100 mg/kg b.w.) treated rats, GM+CCE-250: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (250 mg/kg b.w.) treated rats, GM+CCE-500: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (500 mg/kg b.w.) treated rats.

Effect of CCE on RBC, WBC count and Haemoglobin in GM induced nephrotoxicity

As per above mentioned treatment sequence, the hematological parameters are expressed in Table 2. In which, the GM treated group showed significant (p<0.01) increase in WBC and RBC count as compared to CON group, while Hb showed significant (p<0.01) decrease in parameter when compared to the same group. The pre-treated group of CCE at graded dose of 250 mg/kg and 500 mg/kg showed significant (p<0.01 and p<0.001) improvements in WBC, WBC count and Hb parameter as compared to GM treated group.

Table 2: Effect of *Curcuma caesia* Roxb extract on haematological activity like RBC, WBC & Haemoglobin in gentamicin induced nephrotoxicity

Experimental Groups[a]	RBCs Count (million/cmm)	WBCs Count (million/cmm)	Haemoglobin (Hb) (g/dL)
CON	16.5±0.96	5.0±0.36	13.6±0.33
GM	19.8±1.03$^{\#\#}$	6.3±0.55$^{\#\#}$	10.8±0.54$^{\#\#}$
GM+CCE-250	17.2±0.75**	4.9±0.46**	11.9±0.38**
GM+CCE-500	16.0±0.861***	4.6±0.254***	14.2±0.43**

Values are expressed as mean ± SEM, n=6. $^{\#\#}$p<0.01; compared to CON; *p<0.05, **p<0.01 & ***p<0.001 compared to GM.
[a]Where, CON: normal saline (0.9% NaCl), GM: Gentamicin (100 mg/kg b.w.) treated rats, GM+CCE-250: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (250 mg/kg b.w.) treated rats, GM+CCE-500: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (500 mg/kg b.w.) treated rats.

Effect of CCE on plasma (urea & creatinine) and urine (urea & creatinine) level in GM induced nephrotoxicity

Table 3 shows that, the GM treated animals registered significant (P<0.01 and P<0.001) increase in urea and creatinine levels in plasma and urine compared to CON rats whereas, these set of changes were assumed base line gradually in CCE treated group of animals.

Table 3: Effect of *Curcuma caesia* Roxb extract on plasma (urea & creatinine) and urine (urea & creatinine) level in gentamicin induced nephrotoxicity

Experimental Groups[a]	Estimation in Plasma		Estimation in Urine	
	Urea (mg/dl)	Urea (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
CON	23.54±2.17	0.87±0.13	68.37±3.18	10.86±2.08
GM	68.38±3.93$^{\#\#}$	2.03±0.20$^{\#\#}$	106.13±4.25$^{\#\#}$	35.79±1.89$^{\#\#}$
GM+CCE-250	30.29±2.15*	1.20±0.08**	81.65±3.36**	21.08±1.50**
GM+CCE-500	24.83±3.02**	0.96±0.092***	70.09±2.323***	12.16±1.443***

Values are expressed as mean ± SEM, n=6. $^{\#\#}$p<0.01; compared to CON; *p<0.05, **p<0.01 & ***p<0.001 compared to GM.

[a]Where, CON: normal saline (0.9% NaCl), GM: Gentamicin (100 mg/kg b.w.) treated rats, GM+CCE-250: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (250 mg/kg b.w.) treated rats, GM+CCE-500: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (500 mg/kg b.w.) treated rats.

Effect of CCE on renal activity levels of superoxide dismutase (SOD), catalase (CAT), Malonaldehyde (MDA), reduced glutathione (GSH) and oxidized glutathione (GSSH) level in GM induced nephrotoxicity

As summarized in Table 4, GM treated animals registered significant ($p < 0.01$) decrement in the enzymatic and non-enzymatic antioxidants along with significant increment ($p < 0.05$) in renal MDA as compared to CON rats whereas, GM+CCE (250 mg/kg and 500 mg/kg) groups significantly (P<0.05, <0.01 & <0.001) prevented these set of changes and achieved base line of GM+CCE-500 treatment group.

Table 4: Effect of CCE on renal activity levels of superoxide dismutase (SOD), catalase (CAT), Malonaldehyde (MDA), reduced glutathione (GSH) and oxidized glutathione (GSSH) level in GM induced nephrotoxicity

Experimental Groups [a]	SOD (µg/g tissue)	CAT (µg/g tissue)	MDA (µg/g tissue)	GSH (µg/g tissue)	GSSG (µg/g tissue)
CON	5.81±1.26	4.07±0.85	48.62±5.92	4.28±1.03	0.174±0.09
GM	3.47±2.00$^{\#\#}$	0.98±0.71$^{\#\#}$	70.69±4.30$^{\#\#}$	3.22±0.90$^{\#\#}$	0.095±0.11$^{\#\#}$
GM+CCE-250	4.31±1.53*	1.62±0.83*	61.93±3.53**	3.89±0.65**	0.108±0.20**
GM+CCE-500	5.20±1.32**	3.16±0.98**	50.68±4.21**	4.06±0.88**	0.168±0.08**

Values are expressed as mean ± SEM, n=6. $^{\#\#}$p<0.01; compared to CON; *p<0.05, **p<0.01 compared to GM.

[a]Where, CON: normal saline (0.9% NaCl), GM: Gentamicin (100 mg/kg b.w.) treated rats, GM+CCE-250: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (250 mg/kg b.w.) treated rats, GM+CCE-500: (100 mg/kg b.w.) and *Curcuma caesia* Roxb extract (500 mg/kg b.w.) treated rats.

Histopathology

GM lead to major histopathological changes including glomerular congestion, degeneration of the tubular epithelial cells and necrosis of cells in compared to control group. The kidney tissue of rats treated with CCE at a graded dose of 250 mg/kg and 500 mg/kg bodyweight/day for a period of 8 days, showed the excellent recovery with little epithelial cell de-generation and few blood vessel congestion (Figure 1).

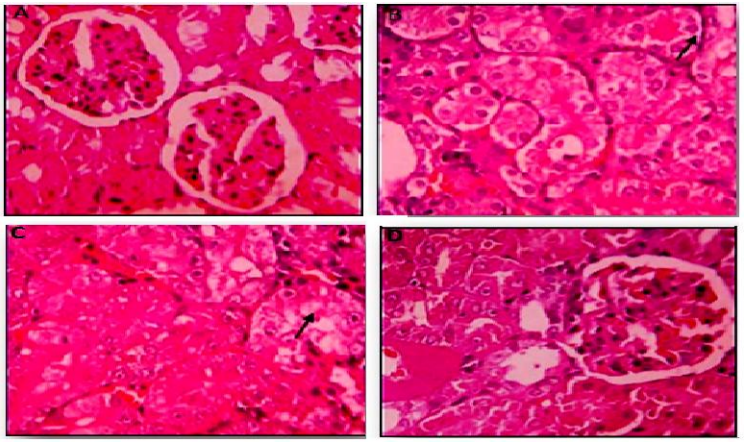

Figure 1: Histological appearance of kidney samples. (A) Glomeruli and tubules have a normal appearance in samples from the control group. (B) Marked tubular necrosis is observed in samples from the GM treated group. The tubular epithelial cells have large nuclei (arrows) and notice the swelling cytoplasm, which is a result of edema. (C) Sample from GM+CCE-250 group showing a marked reduction in tubular damage. Distal tubules appear normal while some of the proximal tubules show moderate intracellular edema (arrows). (D) Sample from GM+CCE-500 treated group showing basement membrane appears orderly and continuous narrowing of the Bowman's space. Hematoxylin and eosin stain. Bar, 100 mm.

DISCUSSION

The present study investigated that, the nephroprotective activity of CCE on GM induced nephrotoxicity in rats, consisting various biochemical parameters such as plasma and urine (urea, creatinine) levels[14]. Further it was supported by analysing various enzymatic parameters such as superoxide dismutase, catalase, malonaldehyde, oxidized glutathione and reduced glutathione in kidney. These parameters were analysed after per-treatment with extract in graded doses (250 & 500 mg/kg, orally) with gentamicin (100 mg/kg, i.p.).

On the experimental basis it was observed that, there was a significant ($p<0.05$) reduction in urea and creatinine levels against gentamicin treated group, followed by significant decrease in kidney weight after treatment with *Curcuma caesia* extract, offering an excellent nephroprotective potential. Furthermore a significant ($p<0.05$) decrease in renal blood urea, serum creatinine and lipid peroxides, whereas significant ($p<0.05$) increase in glutathione level was observed when compared with Gentamicin treated group, confirming the nephroprotective potential of *Curcuma caesia*. Based on the above observations revealing, improvement in levels of serum marker and antioxidant potential, it is conferred that *Curcuma caesia* possess tremendous nephroprotective activity and subsequently supports the traditional application of the same under the light of modern science[15].

Thus *Curcuma caesia* has revealed the ability to maintain the normal functioning of kidney, offering an excellent nephroprotective potential, which was further facilitated by analysing the following parameters:

Physical Parameters

The present study revealed that there was non- significant decrease in organ weight as well as body weight in comparison to control group[16]. But certainly, in the case of *Curcuma caesia* treated group, it was observed that a minor change in organ weight as well as body weight in compared to toxicant control group (table 1).

Biochemical Parameters

An increase in serum creatinine and blood urea levels in case of gentamicin treated groups mimic the conditions of the kidney diseases as in clinical practice offering the diagnostic importance for confirmation of this disease[17]. In the present study utilizing *Curcuma caesia* extract, it observed that there was a significant ($p<0.05$) reduction in the above mentioned serum creatinine and blood urea levels as compared to gentamicin treated group, facilitating nephroprotective potential of *Curcuma caesia* (table 3).

Antioxidant Parameter

The increased tissue MDA and decreased SOD, CAT, GSH & GSSG levels in case of gentamicin treated group imitate the conditions of kidney diseases in clinical practice and hence are having diagnostic importance in the assessment of kidney function[18]. In the present study, pre-treatment with *Curcuma caesia* there was a significant ($p<0.05$) improvement in the above mentioned antioxidant tissue parameters, conferring the nephroprotective potential of the plant (table 4).

Histopathological study

Histopathological disturbance of the kidneys in case of toxicant treated animals depict the conditions of humans, suffering from major kidney disorders. In the present study, animals treated with extract were observed with minimal glomerular congestion and the few degenerated epithelial cells, when compared to control group indicating an outstanding nephroprotective potential (fig. 1).

CONCLUSION

Based on improvement in above mentioned physical parameters, serum markers, antioxidant levels and histopathological study, it was concluded that the *Curcuma caesia* extract possess an excellent nephroprotective potential, supporting the traditional utility of the same under the light of modern science.

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