

Modulatory Effect of Chandraprabha Vati on Antimicrobial Peptides and Inflammatory Markers in Kidneys of Mice With Urinary Tract Infection

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Keywords. plant extracts,
urinary tract infections, Tamm-
Horsfall protein, inflammation,
animals

Introduction. *Chandraprabha Vati* (CV) is an Indian polyherbal Siddha drug, traditionally used as an anti-inflammatory agent for arthritis and urinary ailments. This study explores its effect on mice with urinary tract infection.

Materials and Methods. The in-organic constituents of CV were determined by atomic absorption spectroscopy and phytochemical analysis was carried out. The supplementing dose of CV to infected experimental mice was determined by in vitro antimicrobial assay. Transurethrally infected animals were supplemented with CV extract for 20 days after confirmation of UTI. The animals were euthanized as per the guidelines and the tissues were harvested from the control and infected mice for histopathological examination the antimicrobial peptide Tamm-Horsfall protein (THP) and inflammatory markers (tumor necrosis factor- α and nuclear factor kappa-light-chain-enhancer of activated B cells) to ascertain the modulatory effects of CV. Indicators for oxidative stress and protein levels were also quantified to validate the efficacy of CV.

Results. Terpenoids and flavanoids were majorly found to constitute CV along with zinc and iron as in-organic content. Histological and immunohistochemical studies confirmed the pronounced infection in the kidney of the uropathogenic *Escherichia coli*-infected animals. Supplementation of CV significantly restored the increased levels of the antimicrobial proteins, THP, and inflammatory markers.

Conclusions. This study explored the efficacy of the aqueous extract of CV as an alternative medication for the synthetic analogues administered for UTI. This study also provides information on the possible role of THP as an antimicrobial protein in the kidney in preventing infection due to uropathogenic *E coli*.

IJKD 2013;7:390-8
www.ijkd.org

INTRODUCTION

The coordinated actions of the antimicrobial proteins, cytokines, chemokines, and the phagocytes maintain the sterile niche in the urinary system (ie, the kidneys, ureters, and bladder).¹ However, in the case of urinary tract infection (UTI), the physiological alterations, decrease in the

glycoproteins lining the urinary system,² increase in the inflammatory markers,³ and bacterial invasion call for an antibiotic treatment. The alarming and exponential use of nonspecific antibiotics and the high cost of prescribed medications for the management of the infective microorganisms⁴ raise the need for alternate and safe medication.⁵

Although, phyto-based traditional medicine systems such as Siddha have been practised for centuries in India to treat various ailments, the lack of valid documentations relating to usage, toxicity regime, and pharmacovigilance limits their wide-spread use.⁶ *Chandraprabha Vati* (CV) is a polyherbal preparation consisting of *Cyperus rotundus*, *Phyllanthus emblica*, *Piper longum*, *Zingiber officinale*, *Coriandrum sativum*, *Curcuma longa*, and essential minerals. This study aims to explore its effect against UTI in a mouse model and its modulatory role in the levels of inflammatory markers and antimicrobial protein expression.

MATERIALS AND METHODS

Chemicals and Reagents

The organic chemical compound 2,2-diphenyl-1-picrylhydrazyl and pH indicator strips were procured from Sigma Aldrich (Bangalore, India). Chemicals, solvents, and reagents used were purchased from Sisco Research Laboratories (Mumbai, India) and the microbiological media from Himedia Pvt Ltd (Mumbai, India). The antibodies against tumor necrosis factor (TNF)- α raised in goats, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) raised in rabbits were procured from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA) and the polyclonal antibody against normal Tamm-Horsfall protein (THP) was raised in-house in New Zealand white rabbits.

Chandraprabha Vati

Chandraprabha Vati was procured from the Indian Medical Practitioners Co-operative Pharmacy and Stores (IMCOPS) Ltd, Chennai, India. The infusion, obtained after mixing 10 g of the drug in 100 mL of water at 60°C,⁷ was used for further analysis. The drug was analyzed for heavy metals,⁸ using atomic absorption spectroscopy (VARIAN, AA240).

The CV was subjected to preliminary phytochemical analysis and the various groups were separated on a silica column of mesh sized 300 to 400, using distilled water, methanol, ethanol, ethyl acetate, butanol, acetone, chloroform, and dichloromethane in the increasing order of their polarity; the fractions containing terpenoids and flavanoids were identified. The high-performance liquid chromatography method was performed with a Waters high-performance liquid chromatography model fitted with a 20- μ L rheodyne injector, on a RP C-18

column (5 mm, 4.6 \times 150 mm), waters 1525 binary high-performance liquid chromatography pump, and a dual λ absorbance detector. The terpenoids were separated using methanol:water:phosphoric acid (85:15:1 v/v/v) in isocratic conditions with a flow rate of 2 mL/min; while the flavanoids using the mobile-phase solvents water:acetic acid (99:1, v/v) and MeCN under gradient conditions (18% of the latter to 32% in 15 minutes and finally to 50% in 40 minutes) with a flow rate of 1 mL/min.

Microbial Strains

Escherichia coli 25922 strain (MTCC No 443, IMTECH Chandigarh, India), a wild nonpathogenic strain was utilized as the standard strain. The clinical isolate *E coli* RRL-02 (ECRRL02; Genbank accession number, JQ398845.1; Supplementary method 1) served as the pathogenic strain for in vitro and in vivo antimicrobial studies.

A single colony of ECRRL02 was inoculated into 10 mL of Luria Bertani broth and statically incubated overnight at 37°C to ensure piliation. The bacterial culture was spun down at 5000 g for 5 minutes at 4°C and the pellet was resuspended in sterile phosphate buffered saline (pH 7.4). The 10-fold diluted bacterial suspension was used for further analysis after determining the colony-forming units per milliliter.⁹

In Vitro Antimicrobial Growth Kinetics Study

The antibacterial activity of the CV was assessed by tube dilution method using varying concentrations of the drug (zero to 1000 mg/mL), which were added to Luria Bertani broth inoculated with 100 μ L of McFarland matched 4-hour culture of standard strain or ECRRL02. The tubes were incubated at 37°C for 24 hours, and the minimum inhibitory concentration and minimum bactericidal concentration of the drug were determined by growth curve analysis. Amoxicillin, which acts by inhibiting the bacterial cell wall synthesis, was chosen as the standard drug for the study.¹⁰

Initiation of Infection in Mice

Female Swiss Albino mice (8- to 10-week old), weighing 25 \pm 2 g, were housed in polypropylene cages (6 animals per cage) lined with paddy-husk bedding. An ambient temperature of 24 \pm 1°C, with a 12-hour light-dark light cycle and 65 \pm 2% relative humidity was maintained. The animals were fed

with food and water ad libitum and the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines for laboratory animal facility (New Delhi, India) were followed. The Institutional Animal Ethical Committee, VIT University, approved the study design and protocols (Approval number, VIT/SBST/IAEC/III/2011/19).

Each anesthetized mouse ($n = 12$) was infected with 2.5×10^8 colony-forming units per milliliter of ECRRL02 and 30 μL of sterile phosphate-buffered saline, transurethrally using lubricated catheters delivering about 10^{-1} μL of inoculum into the bladder. After inoculation, the catheter was removed and the animal was monitored regularly for any discomfort, injury or inflammation due to the procedure.⁹ The bladders and kidneys were harvested from the control animals prior to the infection and the infected animals were sacrificed after 6 hours, 7 days, 10 days, and 20 days of treatment with CV. The tissues were processed for biochemical studies and a portion was stored in 10% neutral buffered formalin for histopathological analysis.

Groups and Treatments

Upon confirmation of pyelonephritis, the mice were separated into 2 groups of 6 each and scheduled for treatment protocols for 20 days. Animals in group 1 were orally administered with 100 mg/kg bodyweight of amoxicillin (standard drug) and those in group 2 received CV extract (500 mg/kg body weight derived after the growth kinetics studies using the drug on ECRRL02). The body weights of the animals were determined before sacrifice.

Biochemical Parameters

Protein, nitrite, and pH were determined by indicator strips,^{11,12} and the leukocytes enumeration on a hemocytometer (Neubauer, Luetzellinden, HBG, Germany) were carried out to determine their levels in the urine of the control, infected, and treated groups. The tissue homogenate was analyzed for markers of oxidative stress such as superoxide dismutase, catalase, and total protein content.^{11,13,14}

Histopathology

The 10% neutral buffered formalin-fixed tissues were processed for paraffin embedment and 4- μm tissue sections were stained with hematoxylin and

eosin. The histological structures of the kidney and bladder were studied for evidence of UTI such as inflammation; inflammatory cell infiltrates; destruction of renal epithelium and urothelium; changes in the architecture of the glomeruli, tubules, interstitium and vessels; resolved and persistent bacteriuria; and the degree of nephritis using a light microscope at $\times 400$ magnification.

Immunohistostaining for Inflammatory Markers and Tamm-Horsfall Protein

Paraffin sections (4- μm thick) were mounted on silanized slides, dewaxed in xylene and rehydrated. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 15 minutes. After washing with phosphate buffered saline, containing 0.1% polysorbate 20, the slides were incubated overnight with the primary antibody for TNF- α , NF- κB , and THP (1:200) at 4°C. Immunodetection was performed by incubating with horseradish peroxidase-conjugated goat-antirabbit immunoglobulin G antibody for 30 minutes at room temperature and 3,3'-diaminobenzidine (Dako) served as the chromogen. The slides were counterstained with hematoxylin, rinsed in tap water, dehydrated, placed in xylene, mounted, and read at $\times 400$ magnification.

Statistical Analyses

Data were represented as mean \pm standard error of mean. The mean differences between the groups were assessed using the 1-way analysis of variance. A *P* value less than .05 was considered significant. Statistical analysis was performed using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA).

RESULTS

The preliminary phytochemical analysis of CV revealed the presence of various phytochemicals such as terpenoids, flavanoids, and anthroquinone (Table 1). Metal analysis revealed the presence of 6.1 ± 0.3 mg/L of zinc and 0.2 ± 0.1 mg/L of iron in the water extract of CV. The methanolic fraction of CV collected after silica separation was found to be concentrated with 9 different flavonoids, while the acetone fraction with 5 terpenoids.

The antioxidant profile of CV was carried out up to 0.5-mg/mL concentration. Chandraprabha Vati scavenged 60% of the free radicals produced

Table 1. Phytochemical Analysis of the Water Extract of Chandrababha Vati (CV)

Phytocompound	Presence in CV
Tannins	Yes
Saponins	No
Flavanopids	Yes
Steroidal ring	Yes
Glycosides	Yes
Terpenoids	Yes
Alkaloids	Yes
Coumarins	No
Steroids	Yes
Anthraquinone	Yes
Reducing sugars	Yes
Phenols	Yes
Pholabotamines	Yes
Pratas xanthopota	No
Total flavanoid assay	Yes
Lipids and fats	Yes

at 0.1 mg/mL concentration, 100% of the generated superoxide radicals at 0.15 mg/mL, 50% hydroxyl radical scavenging activity at 0.1 mg/mL, an

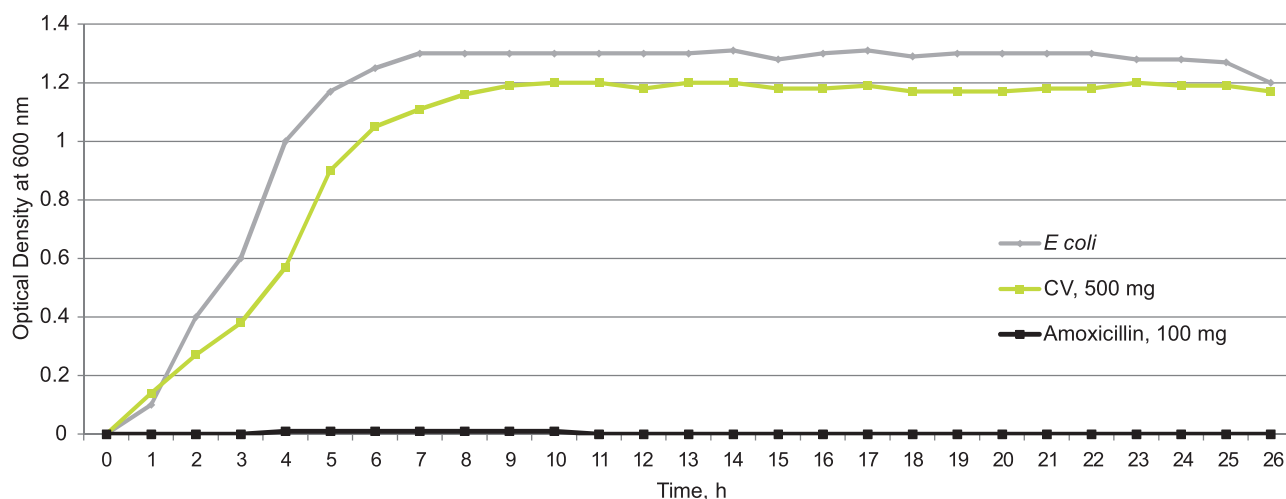
increasing total antioxidant activity and reducing power activity (Table 2).

The 24-hour supernatant from each of the macrodilution tubes was subcultured on Luria Bertani agar plates at 37°C in the absence of drug. Amoxicillin showed complete inhibition at 100 mg, whereas a reduction in the number of colony-forming units was observed after 500 mg of CV. There was no complete inhibition in the subsequent concentrations of the drug, indicating its bacteristatic nature, and 500 mg of CV was set as the concentration for further elucidation of its in vitro and in vivo properties. The growth kinetics of ECRRL02 in the presence of Amoxicillin (100 mg) and CPV (500 mg) are shown in Figure 1.

Protein quantification on infected and control kidney homogenates confirmed a 50-fold decrease in protein content in infected mice relative to controls. Upon treatment for 20 days with the specified dosage of CV, the protein concentration significantly increased compared to the infected mice. The entry of the uropathogen into the luminal

Table 2. Antioxidant Profile of the Water Extract of Chandrababha Vati

Assay	Chandrababha Vati Dose, µg/mL					
	0	100	200	300	400	500
Diphenylpicrylhydrazyl free radical, % inhibition	0 ± 0.20	34.44 ± 0.30	66.63 ± 0.70	52.25 ± 0.25	51.15 ± 0.71	46.72 ± 0.50
Reducing power, butylated hydroxytoluene equivalents	0 ± 0.01	16.50 ± 0.01	19.50 ± 0.03	22.60 ± 0.02	26.90 ± 0.01	30.10 ± 0.01
Hydroxyl radical, % inhibition	0	31.59 ± 0.10	0 ± 0.01	0 ± 0.01	0 ± 0.50	0 ± 0.03
Total Antioxidant, % inhibition	0	16.50 ± 0.01	19.50 ± 0.03	22.60 ± 0.02	26.90 ± 0.01	30.10 ± 0.02
Lipid peroxidation, % inhibition	0	0 ± 0.03	17.41 ± 0.20	21.64 ± 0.06	29.02 ± 0.03	15.43 ± 0.01
Superoxide scavenging, % inhibition	0	6.81 ± 0.01	11.99 ± 0.03	14.73 ± 0.10	23.43 ± 0.02	83.36 ± 0.02

**Figure 1.** Growth curve kinetics of Chandrababha Vati (CV) and amoxicillin used for treatment of the uropathogenic *Escherichia coli*.

cells and its re-emergence caused the disruption of the phospholipid membranes and elevation of the kidney antioxidant enzymes such as catalase, nitrite, and superoxide dismutase (Table 3).

Control (day 0) and the infected mouse kidney sections obtained on the 7th day revealed that acute pyelonephritis was established in the model with neutrophilic infiltration in the ureters and urethropelvic junction with the sparing of the renal parenchyma. Dense infiltration by the lymphocytes and plasma cells was also noticed, with disruption of the urothelium. Upon treatment with CV for 20 days at 500 mg/kg body weight, no evidence of pyelonephritis was noticed. The glomerular,

interstitial, and vascular compartments were within the normal limits as seen in Figure 2.

On the 7th day, the levels of the THP were found to be drastically decreased in the proximal and distal tubules with dense lymphocytic infiltration due to the establishment of acute pyelonephritis. The expression of this protein was restored to normal levels after 20 days of treatment with CV. The markers of inflammation were expressed in elevated levels in the glomerular capillaries and peritubular capillaries in the case of TNF- α , while NF- κ B was expressed in the peritubular capillaries (Figure 3). Dense lymphocyte infiltration of the interstitium was a common feature noticed in both

Table 3. Biochemical Parameters in the Kidneys of Mice Infected With *Escherichia Coli* Treated With Chandraprabha Vati (CV) and Amoxicillin

Kidney	Kidney Tissue			
	Normal	Infected	Treated With CV	Treated With Amoxicillin
Protein, mg/g tissue	2.33 \pm 0.20	1.47 \pm 0.10	2.17 \pm 0.10*	2.25 \pm 0.30†
Catalase, U/ mg protein	0.68 \pm 0.01	2.09 \pm 0.01	0.87 \pm 0.01‡	0.74 \pm 0.10
Nitrite, μ g/ μ L	0.06 \pm 0.01	0.09 \pm 0.00	0.06 \pm 0.01‡	0.04 \pm 0.01‡
Superoxide dismutase, μ mol/min/mg protein	1.65 \pm 0.10	1.98 \pm 0.20	1.71 \pm 0.02†	1.66 \pm 0.40*

* $P < .001$ as compared with the control group

† $P < .05$ as compared with the control group

‡ $P < .01$ as compared with the control group

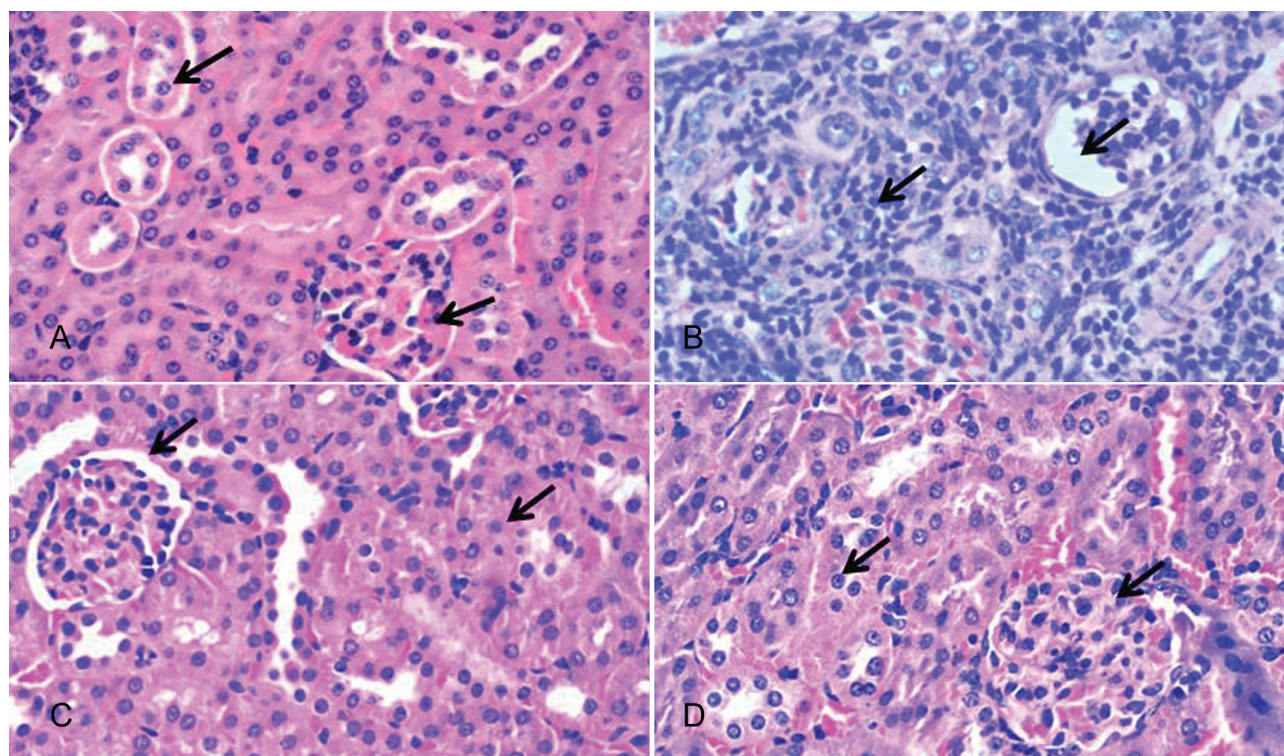


Figure 2. Sections of the kidney tissues obtained from the mice. **A**, control (day zero); **B**, infected kidney on day 7; **C**, kidney treated with Chandraprabha Vati (day 2); and **D**, kidney treated with amoxicillin (day 20). The 7-day postinfection specimen shows the establishment of acute pyelonephritis and also in the urothelium of the bladder (hematoxylin-eosin, $\times 400$).

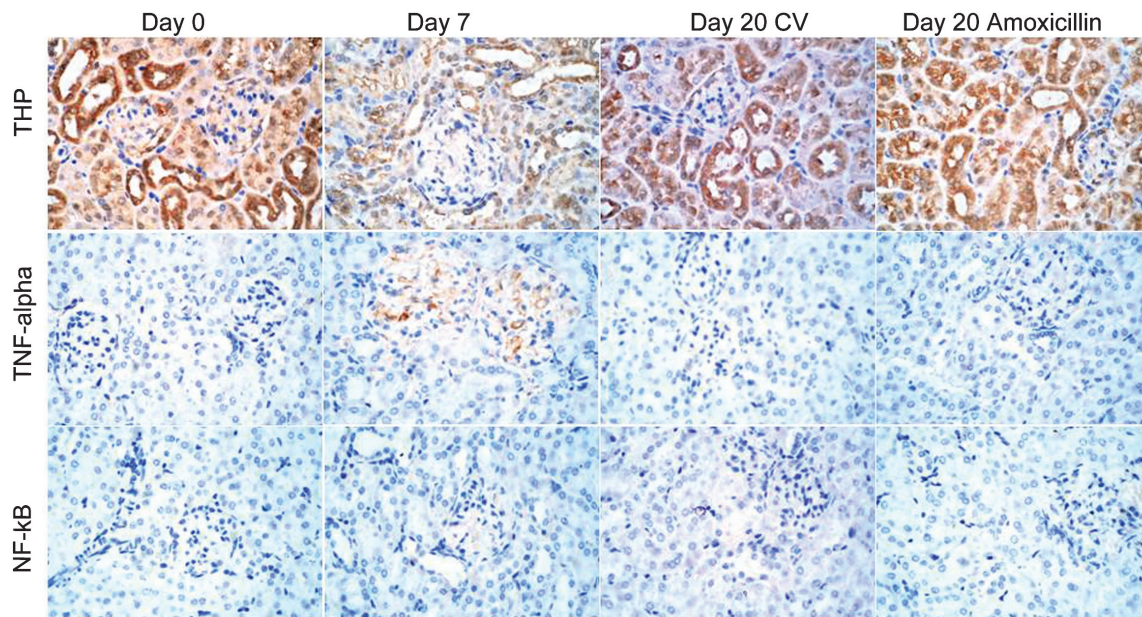


Figure 3. Immunohistochemistry panel of the sections obtained on day zero (control), day 7 postinfection, and day 20 after treatment with Chandraprabha Vati (CV) and amoxicillin with the antibodies against the Tamm-Horsfall protein (THP), and the inflammatory markers tumor necrosis factor (TNF)- α and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B).

the sections. The elevated levels of TNF- α were completely restored to normal in the glomerular capillaries, while the levels remained elevated in the peritubular region, indicating that drug was effective in treating the induced the pyelonephritis. The levels of NF- κ B were reduced and the sections appeared normal after 20 days of treatment.

DISCUSSION

The misuse of various antibiotics such as amoxicillin, cefotaxime, fluoroquinolone, fosfomycin, trimethoprim-sulphamethoxazole, nitrofurantoin, and ciprofloxacin have increased by 40% in the span of 4 years from 2005 to 2009.^{15,16} This has led to the emergence of broad-spectrum resistance disease pathogens.¹⁷ To prevent generation of antibiotic resistant microorganisms, the World Health Organization recommended the search for safer and alternative medication for infectious diseases in humans.¹⁵

Siddha, an ancient medicine system followed in India, is based on the preparation of drugs and formulations from the plant parts which offer an array of chemicals, with synergistic interactions for treating ailments.¹⁸ This has led to revitalization of plant products as sources of new drugs.¹⁹ In vitro and in vivo evidence support synergism between the constituents in herbal extracts. However, there

exists the lack of clinical evidence and uncertainty in the extent of the active principle raise concerns.²⁰ Chandraprabha Vati is one such polyherbal drug, which has been prescribed for treatment of various urinary problems, “strengthening the kidney,” and for general malaise for the past 40 years in India.¹⁴ The exact mechanism and principles responsible for its activity still remain a mystery.

The metal analysis of the drug revealed the presence of zinc and iron in CV. Zinc is required in optimum concentration (0.16 mM per 1.8×10^6 colony-forming units per milliliter) for the growth of microorganisms, but at higher concentrations, it is cytotoxic to the bacteria.²¹ During the establishment of UTI in vivo, the bacteria scavenge the zinc available in the host, thus causing depletion in the environment rapidly, due to which the progression of the infection was not inhibited.²² The external supplementation of zinc, as in CV, not only restores the depleted zinc pools in the body fluids, but also acts as a cytotoxic agent to the bacteria.²³ The supplementation of iron in CV could be utilized by the bacteria for their growth and thus prevents iron scavenging from the chelated proteins,²⁴ maintain its levels in the serum, and prevent anemia in the UTI patients during pyelonephritis.^{25, 26} These nutrient limitations of zinc and iron are known to have effects on the composition of the bacterial

envelope,²⁷ changes in the antibiotic sensitivity pattern,²⁸ host defence mechanisms,²⁹ and the bacterial virulence factor production.³⁰

The majority of the ascending UTIs are due to the uropathogenic *E coli*.³¹ Fimbriation of uropathogenic *E coli* aids in the adhesion of bacterium to the bladder epithelium, and colonization of the sterile urinary tract.³² The bacteria internalize by penetrating the lipid rafts of the urothelium and reside before they produce an infection,³³ and thereby silently evade the innate immunity of the host.³⁴ At this stage the pathogens are resistant to the antibiotic regimen thus paving way for recurrent UTI in the bladder and the kidneys.³⁵ The entry and re-emergence of the uropathogenic *E coli* from the urothelium, leads to the increased levels of reactive oxygen species and reactive nitrogen species in the body; it also results in inflammation due to activation of NF- κ B.³⁶ This results in the damage of the surrounding macromolecules,³⁷ and the sloughing of the infected bladder and renal epithelial cells resulting in the loss of the various glycoproteins lining the epithelial surface such as caveolin-1,³⁸ THP, transferrin, lipocalin, etc.

The innate immune response impedes the invasion of the uropathogenic *E coli* in the urinary tract within minutes, by recruiting neutrophils and macrophages of the site of action. This increased neutrophil presence was noticed in the pelvi-uretic section of the mice. Invasion of the uropathogen increases cytokine levels, due to which inflammatory receptors such as TNF- α and NF- κ B are expressed at the cell surface and lead to urothelial inflammation.^{3,36} This increase in the reactive oxygen species and reactive nitrogen species has to be prevented through the administration of antioxidants.

The terpenoids present in CV are capable of combating the infection and thereby bring down inflammation. This was demonstrated by the immunostaining of the histological sections with TNF- α and NF- κ B, obtained on 10th and 20th day of treatment with the CV. The terpenoids from plant sources have been reported in earlier cases, to have the capacity to act against the *E coli* responsible for oral infections.³⁹ This would be the first time, where the terpenoids from varied plant sources synergistically have been responsible for the reduction of infection and inflammation due to uropathogenic *E coli*, as the number of bacteria have been shown to be reduced considerably in

the sections obtained after 20 days of treatment with the CV.

The flavanoids present in CV act by quenching the ROS and RNS levels, which were increased in the urinary system upon infection and thereby reducing their damage to the lipid bi-layer and the various bio-molecules, present on the luminal wall of the tubules in the kidney. This potential antioxidant nature of the drug is evident from the increase in the THP expression, an abundant protein in the normal urine, produced and secreted by the proximal convoluted tubules. This protein has a reduced expression during the infection as indicated by the immunostaining of the kidney sections on the 7th day. Upon treatment, the levels of this protein were increased, indicating that this could be due to the antioxidant activity of the drug.

Studies in THP knockout mice for the establishment of UTI have revealed the presence of numerous bacteria in the urine, larger and swollen bladders, frequent discoloration of the kidneys with abscess formation necessitate THP for the prevention of UTI. Also bacteria have been shown to bind to renal cells through the extraneous THP, thus preventing the adherence of the type 1 piliated uropathogenic *E coli*. This study also indicated that the binding of THP does not reduce all competitive uropathogenic adhesion, and therefore aid in bacterial colonization.⁴⁰ A third study showed bound THP with *E coli* in the case of renal calculi.⁴¹

These insights suggest the probable role of THP as an antimicrobial protein in preventing the uropathogenic *E coli* from adhering to the kidney and bladder epithelia.² This can be brought about when fimbriated UPEC binds with THP (in abundance) as they gain entry into the kidney and are removed before they can establish pyelonephritis. In our study, the infected mice were allowed to develop pyelonephritis and the sections were stained with anti-THP antibodies. THP levels were remarkably reduced in the kidney on the 7th day post infection, in corroboration with Raffi and colleagues' study.⁴² Also, the presence of mannose in the media effectively reduced the colonization of bacteria in the in vitro systems.⁴³ This implies that THP, prevents the progression of UTI by uropathogenic *E coli* and thereby serves as a host defence molecule, due to which its levels have decreased in the renal tubular sections as shown in Figure 3.² This is supported by the earlier

study involving the role of THP in UTI due to *Proteus mirabilis*, where this protein was found to be bound to the pathogen in the immunostained sections and in the urine of the infected animals. The levels of THP were increased upon treatment for 20 days with CV and amoxicillin (Figure 3) and the levels were subsequently close to normal in the immunostained sections. This also can be correlated to the protein levels present in the kidney and in the excreted urine. On the 7th day post infection, about 50% reduction in the protein levels in the infected kidney were noticed which corresponds to the increased protein levels in the urine collected from the animals. This increased protein levels returned to normal levels upon treatment, indicating that the decreased protein concentration in the kidney could be due to the increased excretion rate of THP, which calls in for an entirely new study which are out of the aims of the current study being discussed here. Also, it has to be noted here that THP cannot only be the sole protein that could be responsible for the elevation of the protein content in the urine. The other candidate proteins which are excreted out in an elevated condition have to be identified to portray the exact mechanism by which the antimicrobial proteins/ present in the kidney ward-off an uropathogenic *E coli* mediated infection. Also, more studies are warranted to reveal the exact mechanism of how THP interacts with the bacteria and thus prevents the progression of infection.

In addition, the use of this drug as an alternative to the existing chemical analogues is recommended based on the fact that this drug is capable of increasing, the in vivo antioxidant enzyme levels such as superoxide dismutase and catalase levels, in order to reduce the inflammation caused due to the presence of microorganisms. Also the generated nitrite radicals and decreased protein content of the kidney, due to infection were brought to normal upon treatment with the drug.

CONCLUSIONS

Our study explored the possible role of THP as an antimicrobial protein and the use of CV as a potential source of antioxidants and antimicrobial agent that can be effectively used to remove the invading and persisting bacterial population in the bladder and kidney of the experimental animal models. The reduction in the inflammatory

markers TNF- α and NF- κ B shows that CV is a potential anti-inflammatory drug. In addition, the restoration of the THP to its normal level concludes that the drug is capable of reducing the pathological changes caused by UTI. From the findings of the present study, we can thus conclude that CV could effectively serve as an alternative treatment to combat UTI when compared to the costly synthetic analogues. The exact mechanism of action for the drug against UTI and the role of THP during UTI have to be elucidated.

ACKNOWLEDGEMENTS

Authors acknowledge Dr Girish Kumar CP, Scientist, Indian Council of Medical Research, NIE, Ayapakkam, Chennai- 600077, for his comments on results and discussion.

CONFLICT OF INTEREST

None declared.

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Received October 2012

Revised March 2013

Accepted April 2013