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Original Article

Diuretic Activity of *Chandraprabha vati* (an Ayurvedic Herbo-mineral formulation) in Rats

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ABSTRACT

Chandraprabha vati (CV) is a herbo-mineral formulation consisting of 37 ingredients (28 herbs, 3 mineral salts, 2 alkali, 2 metal ashes, sucrose and Aspelt mineral pitch) which is used as a diuretic in the Ayurvedic system of medicine. However, the diuretic potential and its mechanisms of action are not scientifically investigated. This study examined these two aspects using rat conscious hydrated model. Three different doses of CV in 1 ml (1000, 2000, 4000mg/kg) or furosemide (positive control) (13 mg/kg) or 1 ml of distilled water (negative control) was orally administered to different groups of rats (N=6/group) which were previously starved (for 18 h) and subsequently hydrated (15 ml of isotonic saline). The rats were individually placed in metabolic cages and their urine output was monitored hourly for 6h. The result revealed that, CV markedly increased the urinary output at1st hour itself in an inversely dose – related manner (r^2 = -1). The onset of the diuretic action of CV was very rapid (within 1h) and so was the peak diuresis (within 1h) but the effect was short lived (2h) as furosemide. Interestingly, the diuretic potential of CV was superior (by 2 fold) to furosemide. Further, CV increased the specific gravity, conductivity, urinary Na^+ level, urinary Na^+/ K^+ ratio, urinary Na^+/ K^+ ratio, urinary Na^+/Cl^- ratio, urinary Na^+/ H^+ ratio and creatinine clearance. The CV did not induced toxicity (general, renal, hepatic and neuro toxicity). These results significantly show that CV can function as a diuretic as claimed in Ayurvedic medicine and acts via multiple mechanisms. (osmotic, thiazide, potassium sparing, loop diuretic and promoting glomerular filtration rate). It is concluded that, CV can as function as potent, safe diuretic as claimed in Ayurvedic medicine.

Keywords: *Chandraprabha vati*, Diuretic, Ayurveda, Herbo-mineral formulations

INTRODUCTION

Currently, there is a big demand for diuretics, the drugs that increase urinary output and its sodium iron level [1]. Diuretics are generally used in the treatment of hypertension, congestive heart failure, ascites, pulmonary oedema, nephritic syndrome, renal failure, cirrhosis of lever and pregnancy toxemia [2]. However, most diuretics produce undesirable side effects such as fatigue, weakness, impotence, electrolyte imbalance, development of diabetes, or activation of the renal-angiotensis-neuroendocrine system [3]. Further, some are not available or expensive, especially, in underdeveloped countries and often they require special storage facilities used as refrigeration and have drug interactions [4]. Hence, there is a need for development of safe, orally active inexpensive and effective diuretics, preferably from herbal sources as they are more user friendly. In this regard, in this study, we scientifically investigated the diuretic potential of CV, an Ayurvedic herbo-

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mineral formulation consisting of 28 botanicals which is recommended as a diuretic. Further, it is used in several clinical conditions such as obesity, diabetes mellitus, skin infections, gastro intestinal disorders, impotency, premature aging, urinary tract diseases [5].

MATERIALS AND METHODS

Experimental animals: Healthy adult cross bred male albino rats (200-225g) from our own colony were used. They were kept under standard environment condition (temperature: 28-31^{0}C, photoperiod: approximately 12h natural light per day, relative humidity: 50-55%). The animals were fed with pelleted food (Ceylon Grain Elevators, Colombo, Sri Lanka) and clear drinking water *ad labium*. All the experiments were conducted in accordance with the international accepted laboratory animal used and care and guidelines and rules of the Faculty of Science, University of Colombo, Sri Lanka. Further, ethical clearance (Registration No; ERC 12/07) was obtained from the Ethical Committee of the Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Preparation of Chandraprabha vati: Rhizomes of *Acorus calamus, Zingiber officinale* and *Curcuma longa*, tubers of *Cyperus rotandus*, whole plant of *Berberis aristata, Andrographis paniculata* and *Tinospora cordifolia*, heartwood of *Cedrus deodara*, roots of *Ipomea turpethum, Aconite heterophyllium, Plumbago zeylanica, Baliosperum montanum*, fruits and dried spikes of *Piper lomgum*, fruits of *Coriandrum sativum, Terminalia belarica, Terminalia chebula, Emblica officinale, Emblica ribes, Scindasus officinalis, Piper nigrum, Piper cheba, Elettaria cardomomum*, bark of *Cinnamomum zeylanicum* , sugar and leaves of *Cinnamomum tamala* were purchased from registered Ayurvedic drug sales outlets in Colombo Sri Lanka. These were authenticated by Dr. S.M.K.Harapathdeniya, Institute of Indigenous Medicine, Sri Lanka and shade dried for 3 days. Leaves of *Cinnamomum tamala*, Camphor, 3 salts (Rock salt, Black salt, Ammonium chloride), 2 alkali (Sodium chloride and Potassium carbonate) and 2 metal ashes (Ferrum and Copper), Aspelt mineral pitch (Shilajatu), The manne of bamboo (Vanshalocana) and purified resinous gum of *Balsamodendron mukul* (Gugul) were purchased from the Indian Medical practitioners co-operative pharmacy and stores, Chennai, India. Using appropriate each of these ingredients of CV was made according to details description given in the Ayurvedic Pharmacy text [6].

Evaluation of the diuretic activity: Thirty rats were deprived of water but not food for 18h. Their urinary bladder was emptied by gentle compression of the pelvic area and by pull of their tails. Then these each rats was orally administered with 15ml of isotonic saline (NaCl 0.9%w/v) to impose a uniform water load. Forty five minutes later, these rats were randomly divided into 5 groups (N=6/per group) and treated orally in the following manner: Group 1:1ml of distilled water, group 2:1000 mg/kg of test drug, group 3: 2000 mg/kg of test drug, group 4: 4000mg/kg of test drug and group 5: 13mg/kg of furosemide (State Pharmaceutical Corporation, Colombo, Sri Lanka), the reference drug [7,8]. Each of these rats was individually placed in metabolic cages and their cumulative urine output determined at hourly intervals for 6 hours. The colour of urine was also noted. For the evaluation of the broad mechanism of diuretic action, the urine collected from group 1 (control) and group 4 (2000mg/kg of test drug) were subjected to the following investigations: pH (by pH meter, Toa Electronics Ltd., Tokyo, Japan), Na^+ , K^+ and Cl^- levels by flame photometry (compact atomic absorption spectrometer, GFS Sciencetific Equipment Pvt. Ltd., Sydney, Australia), conductivity (by conductivity meter, Type TW2, Advanced Instrument Inc., Massachusetts, USA), specific gravity, glucose and proteins (using Combistrix®, reagent strips, Bayer Diagnostics Manufacturing Ltd., Bridgent, UKC). Na^+/K^+ (aldosterone secretion index), Na^+/Cl^- (thiozide secretion index), Na^+/H^+ (urine alkaline index), $Cl^-/Na^+{+}K^+$ (carbonic anhydrase index), diuretic action (urinary output of treated group/ urinary output of control group) and diuretic activity or potency (urinary output of treated group/urinary output of furosemide treated group) ratios were computed. Saliuretic index for Na^+ (Na^+ in treated group/ Na^+ in control group), K^+ (K^+ in treated group/ K^+ in control group) and Cl^- (Cl^- in treated group/ Cl^- in control group) were also calculated [9,10,11,12,13].

Estimation of creatinine clearance: Twelve rate were randomly divided into two groups (N=6/group). They were kept fasting for 18 h and hydrated as described previously. One group was orally administered 1ml water and other group was treated with 2000 mg/kg of test drug and these rats were separately placed in metabolic cages. Then their cumulative urine output was measured after 1 and 24 hours and their blood was collected from tails under mild ether anesthesia using aseptic precautions and serum was separated. Urine and serum creatinine levels were determined using Randox kits (Randox Laboratories Ltd., Antrim, UK) as per instruction given by the manufacturer, creatinine clearance was then determined using

these data. Creatinine clearance was then taken as an estimation of the glomerular filtration rate [14].

Evaluation of acute and sub chronic toxicity: Twelve rats were randomly divided into 2 groups (N=6/group) One group was orally administered with the highest dose (4000mg/kg) daily for 7 consecutive days and the other group was treated with 1ml of water. During this period each rat was observed for the overt signs of toxicity such as salivation, lacrymation, breathing distress, ptosis, stupor, squint, teeth exposure, writhing, convulsions, tremors, yellowing of fur and loss of fur and also stress (erection of fur and exophthalmia), behavioral abnormalities (impairment of spontaneous movements, climbing, cleaning of face, ataxia, rolling and other postural changes) and aversive behaviours (biting and scratching, licking of tail, paw and penis, intense grooming and vocalization) and diarrhoea. On day 1 post treatment, these rats were anaesthetized with ether (BDH Chemical Co., Poole, UK). Blood was collected from tails using aseptic precautions, serum separated and, urea and creatinine (to examine renal toxicity), and SGOT and SGPT (to examine liver toxicity) levels determined using respective assay kits (Randox Laboratory Ltd., UK).

Evaluation of neurotoxicity: 12 male rats used in the evaluation of toxicity of the CV were individually subjected to the bar holding test [15] Bridge test [15] and righting reflex test [16] and their respective latencies (in seconds) were recorded.

Statistical analysis: Data are expressed as Mean ± SEM. Statistical comparisons were made by Mann-Whitney, U-test as appropriate using Minitab 13.0 version statistical package. Significance was set at $P \leq 0.05$.

RESULTS

Evaluation of diuretic activity: As shown table 1, CV significantly ($P<0.05$) and markedly increased (low, mid and high dose respectively by 146 %, 137 % and 46 %) urinary output at the 1st h. At 1h, furosemide, reference drug, increased the urinary output only 32 % which was approximately 2 fold lower than that induced by the lowest dose of CV. The increase of urinary output by CV at 1 h was inversely dose-dependent ($r^2 = -1$). As shown in Table 2, among the urinary parameters determined an indices computed, the mid dose of CV significantly ($P<0.05$) increased the specific gravity (by 64%), conductivity (by 87%), urinary Na^+ iron level (by 76%), urinary Na^+/K^+ ratio (by 63%), urinary Na^+/Cl^-ratio (by 83%), urinary Na^+/H^+(by 81%). The rest of the parameters were not significantly ($P<0.05$) altered. cumulative urine output at 1st hour. High dose of(4000mg/kg) CV and furosemide significantly (*$P<0.05$) and profoundly increased the cumulative urine output at 1st hour. Further, the increase in urine output induced by the CV and furosemide were evident from the first hour and lasted until the termination of the experiment.

***Evaluation of creatinine clearance*:** The highest dose of CV induced a marked and significant ($P<0.05$) increase by 358% in glomerular filtration rate at 1h as examined by creatinine clearance.

Evaluation of acute and chronic toxicity: The highest dose of CV did not provoke any overt signs of toxicity, stress or aversive behaviour. Interestingly, the highest dose of CV did not significantly ($P<0.05$) changed any of the serum parameters investigated: SGOT (control vs treatment, 30.5±3.73vs 26.5±2.99mg/dl), SGPT (21.5±2.66 vs 27.5±2.58mg/dl), serum urea (32.0±2.7 vs 34.7±4.0mg/dl) and serum creatinine (1.7±0.3 vs 1.1±0.2mg/dl) levels. There was also no sign of diarrhoea and none of the treated rats died.

***Evaluation of neurotoxicity*:** The highest dose of CV failed to alter the latency to fall significantly ($P>0.05$) in the Bar holding test (control vs treatment: 60.0±0.0 vs 60.0±0.0) and the latency to the slide off in the Bridge test (control vs treatment: 45.9±9.6 vs 53.8±6.3 sec) and in writing reflex test (control vs treatment: 2.0 ±0.3 vs 1.7 ±0.4).

DISCUSSION

This study examined the oral diuretic potential of CV, an Ayurvedic herbo-mineral formulation using conscious hydrated rat model. This model is a validated, sensitive and a reliable technique which is widely used in the investigation of potential diuretics and anti diuretic agents [17]. The results showed, for the first time, that CV possesses potent (in terms of urinary output, diuretic action) and true (in terms of urinary Na^+ level and Sodium saliuretic index) diuretic activity and not aquatic diuretic activity. The diuretic activity was short lived (1h) and inversely dose dependent. This is indicative of genuine, intrinsic, cousel and specific receptor modulated diuretic action of CV. However, it is worth noting that even the receptors for clinically used diuretics are yet unknown.

Interestingly, the diuretic activity of CV was superior (by two fold) to the frequently clinically used synthetic loop diuretic, furosemide. The onset

of the diuretic activity of CV was extremely quick (within 1h) reaching its peak in the 1st hour itself as furosemide. This indicates prompt absorption of CV and/ or its active ingredients from the gastrointestinal tract and that the action is unlikely to be mediated via a secondary metabolite/s. The diuretic activity was, however, short lived suggesting rapid hepatic distruction (metabolism) and/ or rapid renal clearance. Another important observation was that CV increased GFR (interms of criatinine clearance) and produce marked natiuresis (increase urinary Na^+ level) indicating that it acts both on the glomerulus and tubular parts of nephrones.

Another important feature observed in this study was that CV was well tolerated with no un desirable side effects even with high daily subchronic administration: as judged by absence of overt signs of toxicity, hepatotoxicity (in terms of serum SGPT and SGOT), nephrotoxicity (in terms of serum urea and creatinine levels) and neurotoxicity (in terms of latencies of bar, Bridge and righting tests).

Some herbal medications induce diuresis by stimulating thirst centre in the hypothalamus of brain [18]. However, such a mechanism of action is unlikely to be operative there since the rats had no access to water intake during the study period. CV provoked marked increase in GFR as indicated by enhanced creatinine clearance which is an obvious mode of action for the observed diuretic action. Several diuretics are known to potentiate GFR [19] in mediation their diuretic action mainly via increasing the renal blood flow. Such a mode of action is possible with CV.

CV induced an increase in urinary specific gravity and conductivity. There is a direct correlation between conductivity and ionic content [20]. Therefore, it is possible that osmotic mechanism plays a important role in CV induced diuresis. It is well recognized that ADH plays a vital role regulation of water balance of the body [19]. So, an impairment of ADH release from posterior pituitary and/ or an inhibition of water reabsorption at the distal convoluted tubule of nephron and collecting ducts may increase urine conductivity anproduce osmotic type of diuresis in this study. Alternatively, CV, may possesses ADH antagonistic activity as reported with drug like tolvotan and vlixiveptal [21]which could trigger a diuretic action in this study. Further studies are warranted to pinpoint the exact osmotic mechanism of CV induced diuresis. In complete contrast, diuresis could have also resulted from high salt and sugar content of CV which obviously increase osmolality of urine. However, it is worth noting that none of the currently available osmotic diuretics are orally active and should be given intravenously [4].

CV simultaneously elevated the urinary aldosterone index (urinary Na^+/K^- ratio) and urinary alkali index (Na^+/H^- ratio) significantly. This suggests a mild potassium sparing diuretic action [22]. Potassium sparing diuretics act by blocking of aldosterone action in the distal convoluted tubule of nephron leading to a suppression of release of K^+ from plasma causing an increase in the urinary Na^+/K^+ ratio [7].

In this study, CV treated rats were markedly hypernatremic (in terms of urinary Na+ level and sodium saliuretic index). Morever, it elevated (by 75%) the thiozide diuretic index (Na^+/Cl^- ratio). Collectively, this indicates thaiozide like mode of diuretic action. Thaiozide diuretics act by inhibiting the Na^+/Cl^- symporter (co-transpotar system) in the distal convoluted tubule of the nephron by competing for Cl^- binding sites and inhibiting Na^+ reabsorption resulting in an increased urinary Na^+ excretion [7].

CV also significantly and profoundly decreased the urinary $Cl^-/ Na^+ + K^+$ ratio (carbonic anhydrase index) indicating carbonic anhydrase inhibition in inducing diuresis [23]. Generally carbonic anhydrase inhibiting diuretics increase urinary HCO_3^-/ H^+ ratio (extra and intracellular pH regulatory index). But, unfortunately we could not calculate this ratio as we did not determine the urinary HCO_3^- level.

The diuretic profile exhibited by CV was very much similar to that of reference drug furosemide: rapid onset of diuresis and quick atteinment of peak diuresis, rapid loss of diuretic activity and marked increase in urinary Na^+ level and mild increase in urinary K^+ level. Furosemide is a loop diuretic [7] and therefore it is possible that CV also have a loop diuretic mode of action. Loop diuretics act by inhibiting the $Na^+/K^+/Cl^-$ co-transpoter in the thick region of the ascending limb of loop of Henle of the nephrone [7]. However, loop diuretics usually increase urinary Cl^- level, but in this study, there was no chlorouresis. Nevertheless, such a mode of loop diuretic action has been reported previously with *Ruta graveolens* leaves[24] and and tea brew of Sri Lankan low grown Orange Pekoe grade black tea [25]. Thus, it is clear that CV excerts its diuretic action by multiple mechanisms. In this regard, it is noteworthy that the present trend in developing novel therapeutics is to seek collective activity of (synergestic action) multiple components, preferably from several plants, rather than on a single active principle from a single plant

[26]. Further, use of polyherbal preparations would not only enhance efficacy but also impair toxicity by having detoxicant effects as well known in Ayurvedic and traditional medicinal systems [26]. Interestingly, it was evident that when patients are treated with CV their urine becomes non turbid and colourless possibly indicating a detoxicant effect (unpublished data).

CONCLUSION

The result of this study scientifically validates the Ayurvedic claim that CV is a potent and safe oral diuretic. Further, it shows a multiple mechanism of action in executing the diuretic activity.

Table1: Cumulative urine output in rats at 1 h period following oral administration of *Chandraprabha vati* (Mean ± SEM) N=6

Treatment	Urine output at 1st hour (ml/100g/b.wt./h)
Control	2.56±0.13
1000mg/ kg	6.30 ±0.72**
2000mg/ kg	6.08±0.56**
4000mg/ kg	3.75±0.49*
13mg/ kg of furosemide	3.38±0.44*

*P<0.05 and **<0.01 as compared with control (Mann-Whitney U- test)

Table 2: Effect of orally administered *Chandraprabha vati* on some urine parameters (up to 6 hour) of rats (Mean ± SEM) N=6

Parameter	Control group	Human equivalent dose (Mid dose)
pH	6.75±0.11	6.16±0.40
H^+ (ppm)	0.8289±0.005	0.7897±0.007
Specific gravity	1.0175±0.0011	1.0241±0.0008*
Conductivity (ms)	6.76±0.168	12.65±0.69**
Na^+ (ppm)	4494±222	7888±209**
K^+ (ppm)	2326±210	2368±168
Cl^- (ppm)	7549±214.11	8058±682.54
Na^+/K^+	2.100 ±0.37	3.426±0.314**
Na^+/Cl^-	0.5986±0.037	1.0927±0.080**
Na^+/H^+	5463.26±272.58	9896.45± 288.81**
$Cl^-/Na^+ + K^+$	0.03135 ±0.0014	0.02215 ±0.0018
Sodium saliuretic index	1.0	1.766
Potassium saliuretic index	1.0	1.018
Chloride saliuretic index	1.0	1.067
Diuretic index	1.0	1.39
Diuretic activity (potency)	-	1.525

*P<0.05 and **<0.01 as compared with control (Mann-Whitney U- test)

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