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Phytoconstituents and diuretic activity of *Cymbopogon citratus* leaf infusions in humans

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PEER REVIEW

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Comments

This is an interesting research contribution. The authors have established that *C. citratus* leaf infusions possessed diuretic potentials in humans. This reflected significant increase in diuretic indices of urine volume, urination frequency, natriuresis, saliuresis and renal fractional excretion of electrolytes following diuretic and biochemical evaluations.

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ABSTRACT

Objective: To assess the diuretic activity of infusions prepared from *Cymbopogon citratus* (*C. citratus*) leaves in healthy volunteers.

Methods: One hundred and five subjects (55 men and 50 women) aged 18 to 35 years were randomly assigned to groups set to orally receive infusions prepared from 2, 4, or 8 g of *C. citratus* leaf powder, once daily for 30 d. Urine volume, frequency of urination, urine specific gravity, and plasma and urinary levels of electrolytes were assessed 1 day before (baseline), and at 10 and 30 d after initiation of treatment. Computed diuretic indices were compared between experimental and baseline values.

Results: Subjects treated with infusions prepared from *C. citratus* leaf powder which tested positive for saponins, tannins, flavaniods, phenols, anthraquinones, alkaloids, and deoxy-sugar exhibited a significant increase in indices of diuresis including urine volume, urination frequency, diuretic action, natriuretic and saliuretic indices and renal fractional excretion of electrolytes and metabolic acidosis. A non-significant change in urine specific gravity was observed in all groups. eGFR showed a non-significant increase at Day 10, but decreased significantly ($P<0.05$) at Day 30. Thiazide and aldosterone secretion indices decreased at Day 10, whereas carbonic anhydrase index increased significantly ($P<0.05$) at both Days 10 and 30.

Conclusions: These results indicate a loop active diuretic action of *C. citratus* infusion.

KEYWORDS

Cymbopogon citratus, Phytochemistry, Diuretic activity, Human

1. Introduction

Diuretics are substances that act within the kidney and promote the loss of fluid and electrolytes from the body[1]. Over the years, the use of herbs and natural plant products as diuretics in management of cardiovascular disease and associated disorders in traditional medical practice has gained a global significance, not only due to poor access and cost of modern healthcare but also because of the growing awareness and interest in health benefits of herbs and natural plant products[2]. One such plant is *Cymbopogon citratus* (*C. citratus*) commonly known as lemongrass[3]. It is an aromatic perennial plant of the Poaceae family, widely grown around the world, and possesses thin grey-green leaves of about 90 cm in length and 1.5 cm in breadth[4]. The leaves contain various bioactive principles, including minerals (potassium, sodium, calcium, magnesium, copper, and phosphorus), phytochemicals (tannins, saponins,

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flavonoids, alkaloids, phenols, steroids, and anthraquinones), and macronutrients (carbohydrates, proteins, and fats)[5]. The leaves also contain essential oils in variable concentrations (about 0.2% to 1.4% and may be up to 3%), with citral as the major chemical constituent (30%-93.74%)[6]. Other monoterpenes present in the essential oil include myrcene, geranial, limonene, burneol, citronellol, neral, α-terpineol, elemicin, and geranyl acetate. Also, fumesol, furfurol, isopulegol, isovaleranic aldehyde, L-linanool, terpineone, and valeric ester have been isolated[7]. Previous studies have shown that, there could be variations in phytoconstituents of this herb due to factors such as species/genetic differences, method of extraction, geographical location and age/stage at harvest[6,7]. Such variation could affect its therapeutic efficacy and hence its usage.

C. citratus has a wide range of therapeutic, nutritional, and cosmetic uses. Traditionally, the plant is used as an antimicrobial, antioxidant, anti-inflammatory, hypoglycemic, insect repellant, cardioprotective, anticarcinogenic, and antipyretic[3].

Nutritionally, it is used in traditional cuisines, and in baked food and confections. Cosmetically, its essential oil is used in fragrances, soaps, detergents, and body creams. Recent evidence indicates that infusions prepared from dry or fresh leaves of *C. citratus* are extensively used in traditional medicine in many parts of the world, including Cuba, Brazil, India, and Indonesia, as a diuretic for treatment of hypertension and associated cardiovascular disorders, in bladder disorders, including inflammatory conditions of the urinary duct, and for treatment of renal stones and urine retention; it is also used in treatment of gout[8]. However, research evidence regarding its diuretic activity in human is scanty. Therefore, in this study, we examined the phytoconstituents and diuretic action of infusion prepared from *C. citratus* leaf powder in healthy volunteers.

2. Materials and methods

2.1. Plant materials

2.1.1. Collection, identification, and preparation of the C. citratus leaf infusion

Fresh *C. citratus* leaves were obtained from an agricultural farm in Uyo, Akwa Ibom state, Nigeria, in May 2012, a few days prior to utilization. The leaves were identified and authenticated by a taxonomist in the Department of Botany at the University of Uyo. Voucher specimen No. UUH3276/UYO was deposited at the herbarium in the Department of Botany at the University. The leaves were rinsed, sun-dried, and pulverized into powder using an electric blender to yield a weight of 200 g. The leaf powder was soaked in a container with 2 L of hot water and allowed to stand for approximately 8 h. Thereafter, the solution was filtered using No. 2 Whatman filter paper. The filtrate was evaporated by heating in a water bath at 40 °C to obtain the solid extract. The solid extract was weighed (ACS-ZE14, Surgifriend Medicals Ltd., England) to obtain a yield of 70 g (35% w/w), which was then stored in clean bottles at room temperature until required. Similar procedures were repeated using 2, 4, or 8 g of *C. citratus* powder, with extract yields of 410 mg, 810 mg, and 1 570 mg being obtained, respectively.

2.1.2. Phytochemical screening of C. citratus leaf extracts

The phytochemical analysis of extracts was carried out using standard procedures to determine the levels of saponins, phenolics, alkaloids, tannins, flavonoids, glycosides, steroids, deoxy sugars, trepans, and anthraquinones.

2.1.2.1. Frothing test for saponins

About 0.5 g of the extract was shaken vigorously with distilled water in a test tube. Frothy appearance on the upper interface of the solution which persisted after heating indicated the presence of saponins.

2.1.2.2. Ferric chloride test for tannins and phenolic compounds.

About 0.5 g of the extract was stirred with 10 mL of distilled water and filtered. The infusion was divided into 2 test tubes A and B. In the test A, ferric chloride was added while test tube B served as control. Appearance of bluish-black precipitate indicated the presence of tannins and phenolic compounds.

2.1.2.3. Shinoda test for flavaniods

Few pieces of magnesium metal were added to 5 mL of the *C. citratus* infusion previously treated with 1.5 mL of 50% methanol solution. Also, 5 drops of concentrated hydrochloric acid was added and red color was observed for flavaniods.

2.1.2.4. Dragendoff test for alkaloids

A total of 0.5 g of *C. citratus* extract stirred with 5 mL of 5% aqueous HCl, and heated on a steam bath when cold, few drops of Dragendroffs reagent (potassium bismuth iodide) was added. Appearance of the reddish brown precipitate indicated the presence of alkaloids.

2.1.2.5. Borntragers' test for anthraquinones.

About 0.5 g of the *C. citratus* extract was mixed with 10 mL

ether and then filtered. The filtrate was shaken with 5 mL of caustic soda and 10 mL of 10% ammonia solution was added. Appearance of red coloration indicated the presence of anthraquinones.

2.1.2.6. Keller- Killiani test for deoxy-sugar

To 0.5 mL of the *C. citratus* infusion, 1 mL of glacial acetic acid, ferric chloride acid solution and concentrated H_2SO_4 were added. Appearance of reddish-brown ring at the junction of the liquids signifies the presence of deoxy-sugars.

2.1.3. Determination of nutrient composition

Ash, crude fibre, fat, moisture, and protein content were determined by proximate analysis, using procedures provided by the Association of Official Analytical Chemists[9]. To determine the levels of mineral constituents, aliquots of extract were predigested with concentrated HNO_3, followed by digestion with a mixture (10:3) of concentrated HNO_3 and concentrated $HClO_4$. The acidified samples were heated in a covered 50 mL beaker until all traces of $HClO_4$ were eliminated, as reflected by the absence of white fumes. The resulting liquid in the beaker (approximately 2 mL) was diluted to a volume of 25 mL with deionized water, and assayed for copper, iron, magnesium, sodium, and calcium by atomic absorption spectrophotometry (Jarrel-Arh model 82-362).

2.1.4. Study design

This study used a pre-and post-experimental design to assess the effects of *C. citratus* leaf infusions on the BP of 105 normotensive participants (55 men and 50 women) who were selected by a simple random technique. Informed written consent was obtained from all participants. All participants underwent a thorough pre-trial medical screening performed by a medical officer to ensure medical fitness and to exclude those who did not meet the inclusion criteria. The exclusion criteria are as follows: inappropriate age, a history of kidney or liver disease, failure to satisfy the pre-trial clinical and biochemical assessment, pregnancy or lactation, allergy to any lemongrass constituents, and use of drugs known to affect or to be metabolized primarily in the kidney. Screening included determination of medical history, lifestyle assessment (such as smoking, drinking, physical activity, diet, and drug history), BP and heart rate (HR), weight, blood glucose level, full blood and platelet count, and urine and blood indices of renal and hepatic function. The participants were advised to avoid excessive physical activity and ingestion of drugs or alcohol and to remain on their regular diet throughout the study period. The study protocol was approved by the Institutional Research Ethics Committee and was conducted at the University of Uyo, Nigeria, according to the guidelines set forth in the Declaration of Helsinki governing the conduct of human research.

2.1.5. Safety evaluation/dose determination and administration of infusion

The participants were subdivided into 3 groups (n=35/group). Groups 1, 2, and 3 received infusions prepared from 2, 4, or 8 g of *C. citratus* leaf powder, respectively, in 150 mL of hot water, given once daily for 30 d. This infusion was prepared in this manner to correspond to the way in which lemongrass tea is usually prepared by the population[10]. The dose range employed was adapted from previous human studies in which participants exhibited no obvious clinical or biochemical evidence of toxicity[10]. To further confirm the safety of this dose range, we conducted a pilot study on 10 volunteers using infusions prepared from 2, 4, 8, and 10 g of *C. citratus* leaf powder in 150 mL of hot water. No evidence of adverse/toxic effects was observed, as judged by results of the tolerability evaluation.

Tolerability evaluation consisted of a range of clinical and laboratory tests, including tests for liver function (aminotransferase activity), renal function (serum creatinine and clearance rate), serum urea levels, and hematological indices. Physical examinations were also performed on each participant to check for the presence of jaundice or pallor (evidence of hepatotoxicity or hemolysis) and for abnormal skin reactions. Adverse effects reported by the participants or observed by the investigators during the clinical evaluation were recorded. In addition, participants were closely monitored during the study period. They were given a daily symptomatology and fluid intake assessment questionnaire containing 25 questions, which was designed by the authors. The presence of symptoms such as, lightness of the body, blurring of vision, insomnia, headache, dizziness, sweating, frequent micturition, belching, dyspepsia, diarrhea, constipation, vomiting, and conscious awareness of increased heart rate (palpitation) were to be reported.

We chose infusions prepared from 2 g of *C. citratus* leaf powder as the starting dose, which corresponds to the quantity usually employed by the Brazilian population to prepare lemon grass tea. Studies by others have shown that in Brazil, lemon grass tea or infusions, prepared by pouring 150 mL of boiling water over 2-3 g of fresh or dried lemon grass leaves, is one of the most popular traditional remedies for "nervous disturbances such as insomnia, irritability, and anxiety"[10]. In order to evaluate the dose-dependence of *C.*

citratus effects, the starting dose of 2 g of leaf powder was doubled and quadrupled, to give the final range of 2, 4, or 8 g used to prepare infusions in the present study.

2.1.6. Assessment of diuretic activity

Twelve-hour urine samples were collected from all participants between the hours of 6:00 pm and 6:00 am, at Days 0, 10, and 30 after initiation of the study. The volume of the urine was measured with a calibrated cylinder, while the 24 h urination frequency was reported in a chart designed by the authors. The Na^+ and K^+ concentrations in the urine were determined by Flame Photometry ("Jencon PEP 9", Jencons Scientific Limited, Bedfordshire, UK), Ca^{2+} was measured by atomic absorption spectrophotometry (Jarrel-Arh Model 82-36, UK), and Cl^- was measured using an ion selective meter (Orion 730", Orion Research Inc. Boston, USA). Urinary pH was measured using a digital pH meter (Model E9610, Equiptronics, England), while glucose and protein were measured using urine reagent test strips (Combi 9, Macherey-Negrel, Germany). On the days when urine specimens were collected (*i.e.*, Days 0, 10, and 30), fluid and food intake were restricted between the time of administration of the infusion and collection of the final urine specimen (6:00 pm to 6:00 am). Urinary electrolyte concentrations were measured at baseline, 10, and 30 d post-treatment. Data obtained were used to determine various saliuretic indices, including those for Na^+ (Na^+ test/Na^+ control), K^+ (K^+ test/K+control), Ca^{2+} test/Ca^{2+} control), and Cl^- (Cl^- test/Cl^- control).

Other indices calculated included aldosterone secretion index (Na^+/K^+), thiazide secretion index (Na^+/Cl^-), carbonic anhydrase inhibition index (Cl^-/Na^++K), diuretic action (urinary output of test group/urinary output of control group), saluretic index (Na^++Cl^-), and renal fractional excretion of electrolytes and other substances (urine volume X concentration of ion in test group/urine volume X concentration of ion in control group). Experimental values were compared with baseline values[11].

2.1.7. Biochemical estimation

Venous blood samples were obtained for biochemical analysis after a fasting period of about 8 h. All biochemical analysis were performed within 2 h of sample collection at the chemical pathology unit of the University of Uyo Teaching Hospital (UUTH). Parameters measured were serum creatinine, urea, and uric acid, liver enzymes (aspartate aminotransaminase, alanine transaminase, alkaline phosphatase, bilirubin, protein, glucose and serum lipid profile (high density lipoprotein, low density lipoprotein, VLDL-C, triglyceride and cholesterol).

Serum creatinine level was determined by Jaffe's method using 0.75 NaOH and 1% picric acid (Sigma chemicals, India) at a volume of 1 mL each to the serum specific specimen. A standard was similarly treated. A colour change that developed within 15 min at room temperature was measured spectrophotometrically (ESA Inc., Chelmsford, USA) at 520 nm. Serum total cholesterol (T-chol), triglyceride, low density lipoprotein, high density lipoprotein and glucose were measured using lipid profile and glucose automated measuring system (lipid proTM), Model ILM-0001 A, Infopia Co. Ltd., South Korea). Serum uric acid and urea was measured using multichannel automated analyser (SYNCHRON, Los Angeles, CA). Renal function [estimated glomerular filtration rate (eGFR)] was assessed primarily by using serum creatinine, estimation of glomerular filtration and by estimation of creatinine clearance calculation using modified Cockcroft and Gualt Formula[12]. All measurements were performed 1 day prior to, and at 10 and 30 d after the start of infusion administration.

2.1.8. Statistical analysis

Data (mean±SEM) were analyzed using One-way analysis of variance (ANOVA), followed by pair-wise comparison using the least significant difference test. Differences were considered statistically significant at $P<0.05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS 20.0).

3. Results

3.1. Analysis of the phytochemical constituents of C. citratus leaf hot water extract

Preliminary phytochemical screening of *C. citratus* leaf extract revealed the presence of a relatively high concentrations of saponins, moderate levels of tannins, flavonoids, and phenols, and relatively low concentrations of anthraquinones, alkaloids, and deoxy-sugars (Table 1).

Table 1
Phytochemical constituents of *C. citratus* leaf.

Phytochemical constituents	Comment
Saponins	+++
Tannins	++
Flavaniods	++
Phenols	++
Anthraquinones	+
Alkaloids	+
Deoxy-sugars	+
Steroids	-

-=absent; +=low; ++=moderate; +++=marked.

3.2. Analysis of nutritional composition of C. citratus leaf (per 100 g)

Analysis of the nutritional composition of *C. citratus* leaf extracts revealed the presence of moisture, ash, fibre, macronutrients (carbohydrates, fats and crude proteins), electrolytes (K^+, Na^+, Ca^{2+}, Mg^{2+}) and micronutrients (Cu, Zn, Pb, P, Mn, and Fe) (Table 2).

Table 2

Analysis of nutritional composition of *C. citratus* (per 100 g).

Nutritional constituents	Nutritional value (mg)
Moisture	2360.000
Total ash	4200.000
Fat	650.000
Crude proteins	2650.000
Crude fibre	3040.000
Carbohydrate	3602.000
K	475.000
Na	8.000
Ca	142.230
Fe	25.120
Cu	0.266
Zn	15.000
Pb	ND
Mg	122.250
Mn	48.380
P	12.300

ND=not detected.

3.3. Baseline demographic and clinical characteristics of study participants

Table 3 shows the baseline demographic and clinical characteristic of study participants.

Table 3

Baseline demographic and clinical characteristics of study participants.

Characteristics	Baseline values
Demographic characteristics	
Age (SD)	27.42±0.30
Age range (year)	18-35
Sex	
Male	55 (52.0)
Female	50 (48.0)
Race (% black)	105 (100)
Ethnic (% Ibibio)	105 (100)
Religion (% Christianity)	105 (100)
Clinical characteristics	
Weight (kg)	60.74±1.93
Height (m^2)	1.62±0.01
BMI (kg/m^2)	23.46±0.75
SBP (mmHg)	120.53±1.89
DBP (mmHg)	74.64±1.62
MAP (mmHg)	85.69±1.13
Heart rate (beats/min)	77.71±1.99
Pulse pressure (mmHg)	45.89±1.04
Respiratory rate (/min)	18.56±1.52
eGFR (mL/min)	99.88±1.52

Values=mean±SEM, values in parenthesis are percentages (%).

3.4. Effect on urine specific gravity

Urine specific gravity showed a non-significant change in all groups in both phases of the study.

3.5. Effects of C. citratus leaf infusions on renal fractional excretion of electrolytes and other substances

Table 4 shows that administration of *C. citratus* leaf infusions caused a significant increase in renal fractional excretion of some or all the electrolytes and some organic substances in both phases of the study.

Twelve-hour urine volume assessment revealed that urine

Table 4

Acute (10 d) and sub-chronic (30 d) effects of infusions prepared from 2, 4 or 8 g *C. citratus* leaf powder on renal fractional excretion of electrolytes and organic substances (12-h urine volume X urine ion concentration).

	12 h urine volume (L)	$U_{K+}V$ mmol/L	$U_{Na+}V$ mmol/L	$U_{Ca2+}V$ mmol/L	$U_{Cl-}V$ mmol/L	$U_{Urea}V$ mmol/L	$U_{Uricacid}V$ mmol/L	$U_{Cr}V$ mmol/L
Baseline (control)								
2 g	0.80±0.13	32.66±1.42	128.50±2.42	9.42±1.85	104.27±2.81	98.07±8.02	2.73±0.21	195.42±4.68
4 g	0.71±0.04	27.97±1.32	83.74±1.22	11.54±2.05	104.94±4.44	102.11±7.04	2.64±0.56	216.70±7.82
8 g	0.71±0.04	32.43±1.45	116.64±3.40	10.28±1.48	118.02±2.47	86.34±5.22	1.98±0.11	289.11±8.86
Acute								
2 g	0.90±0.04[a]	57.71±2.18[a]	153.30±1.22[a]	2.32±1.02	178.92±4.53[a]	115.24±8.02	3.15±0.21	421.11±.3.75[a]
4 g	1.10±0.02[ab]	58.16±4.03[a]	156.08±2.43[a]	22.42±1.03[a]	227.67±3.51[ab]	178.32±7.10[ab]	3.25±0.21	396.12±9.42[ab]
8 g	1.01±0.04[ab]	44.50±2.16[abc]	141.46±5.20[abc]	12.99±1.92[c]	183.33±6.22[ac]	113.82±5.24[abc]	2.42±0.11[ac]	477.09±8.60[abc]
Sub-chronic								
2 g	0.88±0.01	33.15±3.22	111.80±4.22[a]	111.80±4.22[a]	167.40±2.41[a]	112.68±8.63	2.32±0.15	326.48±8.06[a]
4 g	0.80±0.06	29.92±1.20	92.80±4.67[b]	16.34±1.89[a]	139.68±5.22[ab]	113.52±8.06	2.22±0.12	257.12±9.56[ab]
8 g	0.96±0.03[abc]	35.08±0.42[c]	121.07±4.82[bc]	16.37±1.25[a]	156.42±5.19[ac]	97.05±6.40	2.51±0.11[a]	302.75±9.05[c]

2g (410 mg yield); 4 g (810 mg yield); 8 g (1 570 mg yield; [a]=significantly different from baseline (P<0.05); [b]=significantly different from 2 g (P<0.05); [c]=significantly different from 4 g (P<00.05); Values reported as mean±SEM.

volumes significantly increased in all treatment groups by Day 10 (P<0.05). After 30 d, the urine output remained elevated in the participants receiving infusions prepared from either 4 or 8 g of *C. citratus* leaf powder.

3.6. Effect of C. citratus leaf infusions on eGFR

Table 5 shows that at Day 10, eGFR did not differ significantly from baseline values, except for a significant decrease observed in female subjects treated with an infusion prepared from 8 g of *C. citratus* leaf powder. At Day 30, eGFR significantly decreased in both male and female subjects in all the groups (P<0.05) respectively.

Table 5

Acute and sub-chronic effects of infusions prepared from 2, 4 or 8 g of *C. citratus* leaf powder on eGFR of study participants.

Dosage	Male			Female		
	Control	10 d	30 d	Control	10 d	30 d
2 g	99.27±5.28	100.70±5.47	84.08±4.73^{ab}	96.62±5.12	99.02±6.02	79.08±5.83^{ab}
4 g	101.85±6.03	99.83±5.47	86.17±4.08^{ab}	95.07±5.85	94.82±6.11	81.93±6.08^{ab}
8 g	103.17±5.17	101.83±5.23	85.18±4.97^{ab}	99.84±2.62	92.05±2.62^{a}	77.90±2.62^{ab}

^{a}=significantly different from baseline (P<0.05); ^{b}=significantly different from 2 g (P<0.05).

3.7. Effects of C. citratus leaf infusions on urine pH

Urine pH significantly decreased at both Days 10 and 30 in groups who received infusions prepared from 2 or 4 g of *C. citratus* leaf powder (P<0.5). Urine specific gravity showed a non-significant change in all groups in both phases of the study.

3.8. Effects of C. citratus leaf infusions on urine volume and urine frequency

Twenty-four hour urination frequency showed a significant increase in all groups; except participants treated with infusion prepared from 2 g of *C. citratus* leaf powder for 30 d (Figure 1).

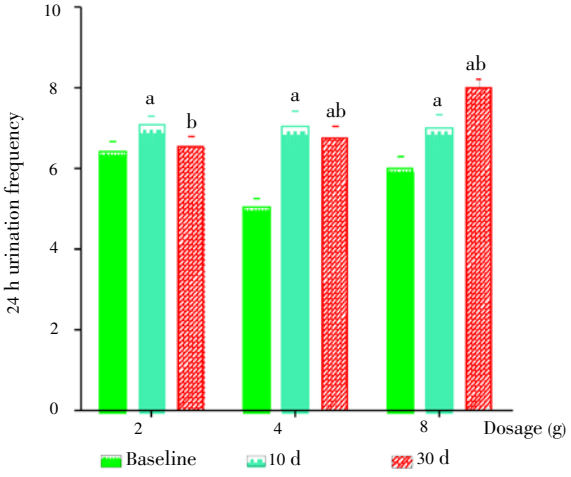

Figure 1. Comparison of 24 h urination frequency at baseline, 10 and 30 d after treatment with 2, 4 and 8 g of *C. citratus* leaf extract.

^{a}P<0.05 *v.s.* Baseline, ^{b}P< 0.05 *v.s.* 10 d. Values reported as means±SEM; ^{a}=significantly different from baseline; ^{ab}=significantly different from baseline and 10 d; ^{b}=significantly different from 10 d.

3.9. Effects of C. citratus leaf infusions on urinary Na^+, K^+, Cl^- and Ca^{2+} concentration

At Day 10, urinary Na^+ concentration increased significantly (P<0.05) in all groups except in those treated with infusion prepared from 8 g of the *C. citratus* leaf powder where there was a non-significant increase. At Day 30, urinary Na^+ returned to baseline in participants treated with infusion prepared from 4 g of *C. citratus* leaf powder, but was significantly lower in the group treated with infusions prepared from 2 or 8 g of *C. citratus* leaf powder (Figure 2).

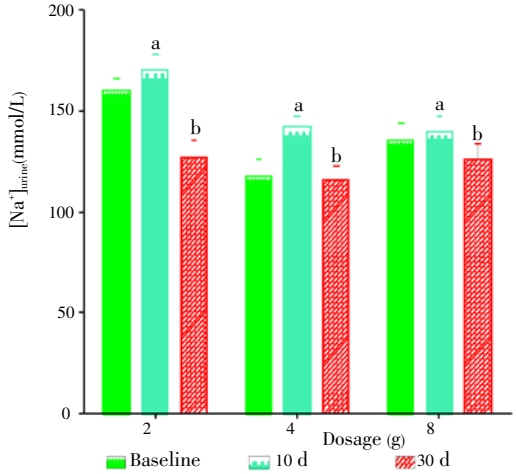

Figure 2. Comparison of urine Na^+ concentration at baseline, 10 and 30 d after treatment with 2, 4 and 8 g of *C. citratus* leaf extract.

^{a}=significantly different from baseline; ^{ab}=significantly different from baseline and 10 d; ^{b}=significantly different from 10 d.

Figure 3 shows that at Day 10, urinary K^+ concentration increased significantly in all groups (P<0.05) , but at Day 30, urinary K^+ concentration had decreased below baseline.

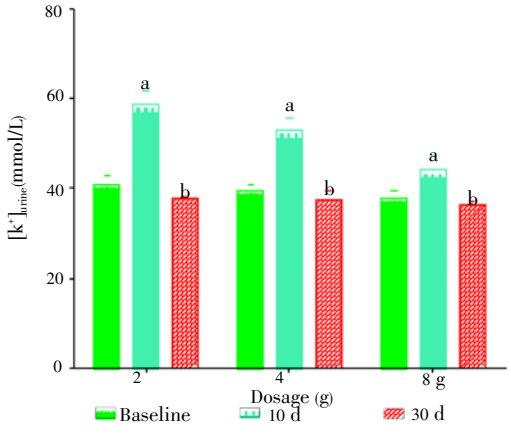

Figure 3. Comparison of urine K^+ concentration at baseline, 10 and 30 d after treatment with 2, 4 and 8 g of *C. citratus* leaf extract.

^{a}=significantly different from baseline; ^{ab}=significantly different from baseline and 10 d; ^{b}=significantly different from 10 d.

Urinary Cl^- concentrations increased significantly in both phases of the study ($P<0.05$) (Figure 4).

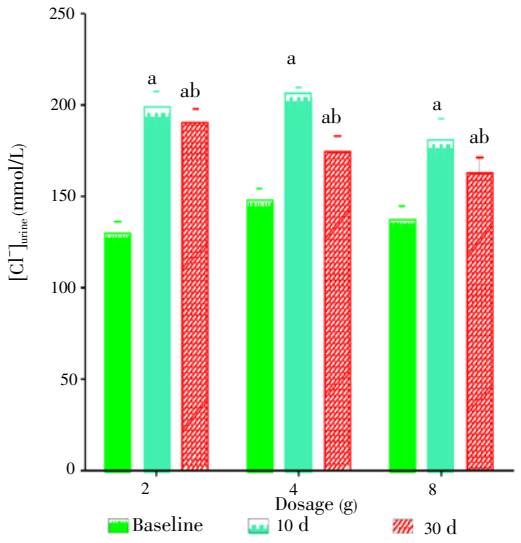

Figure 4. Comparison of urine chloride concentration at baseline, 10 and 30 d after treatment with 2, 4 and 8 g of *C. citratus* leaf extract.
[a]=significantly different from baseline; [ab]=significantly different from baseline and 10 d; [b]=significantly different from 10 d.

Urinary Ca^{2+} concentrations increased significantly ($P<0.05$) in all groups in both phases of the study except in those treated with infusion prepared from 8 g of *C. citratus* leaf powder for 10 d (Figure 5).

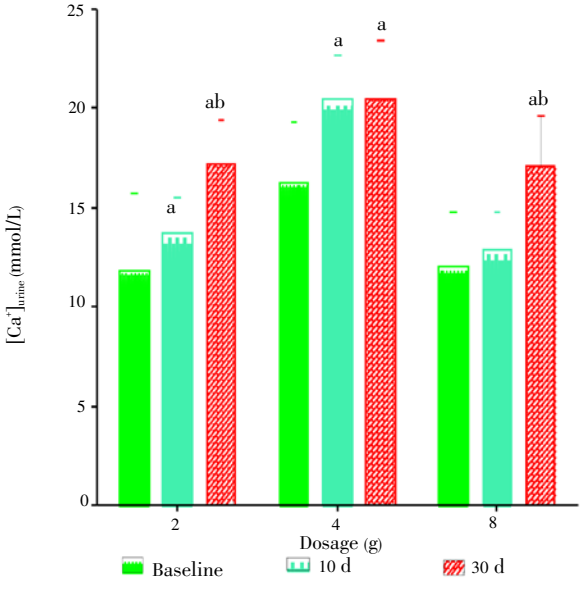

Figure 5. Comparison of urine calcium ions concentration at baseline, 10 and 30 d after treatment with 2, 4 and 8 g of *C. citratus* leaf extract.
[a]=significantly different from baseline; [ab]=significantly different from baseline and 10 d; [b]=significantly different from 10 d.

3.10. Effects of C. citratus leaf infusions on aldosterone, thiazide and carbonic anhydrase inhibition indices

Figure 6A shows a significant increase in carbonic anhydrase inhibition index in both phases of the study ($P<0.05$), whereas aldosterone and thiazide secretion indices decreased in both phases (Figure 6B and 6C), but significant in those who received infusion prepared from 4 g of the leaf powder in the acute phase (Figure 6B), and 2 or 8 g (Figure 6C).

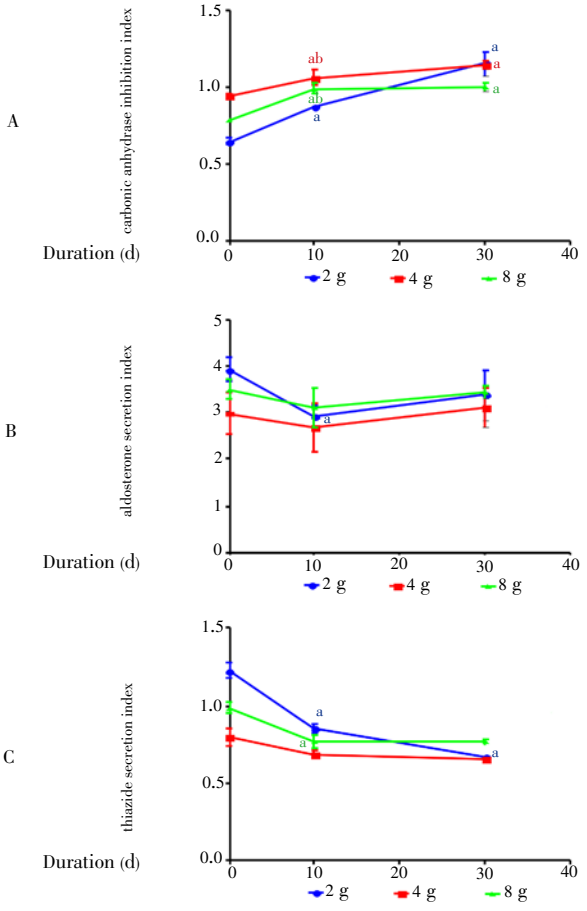

Figure 6. Comparison of carbonic anhydrase inhibition index (A) and aldosterone secretion index (B) and thiazide secretion index (C) at baseline (1 d before), and at 10 and 30 d after treatment with 2, 4, or 8 g of *C. citratus* leaf infusions.
[a]$P<0.05$ *v.s.* baseline [b]$P<0.05$ *v.s.* 10 d. Values reported as mean±SEM.

3.11. Effects of C. citratus leaf infusion on diuretic indices

The peak diuretic action and saliuretic activity (Figure 7A and B), were achieved during the acute phase (10 d of treatment) in participants who received infusions prepared from 2 or 4 g of the leaf powder. These effects were lower during the sub-chronic phase and in those treated with infusion prepared from 8 g of the leaf powder.

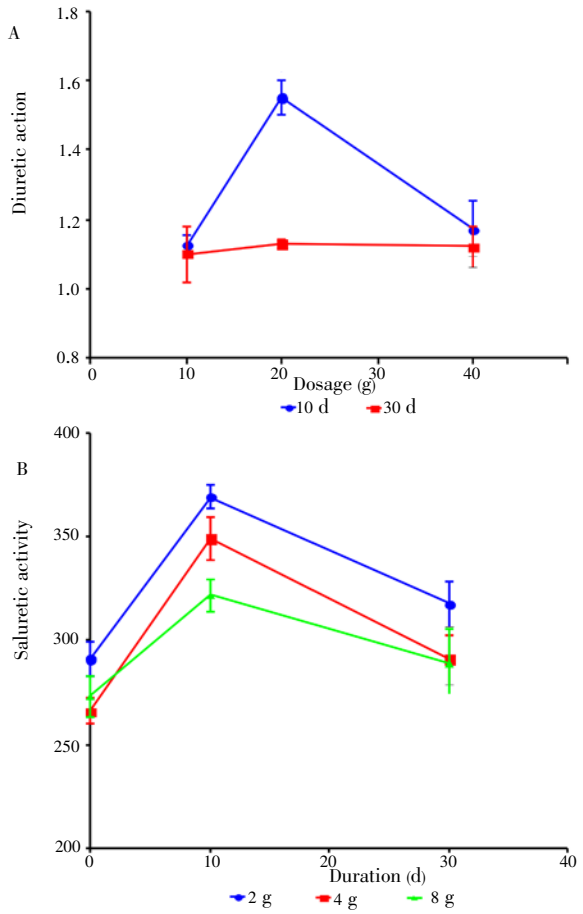

Figure 7. Comparison of the diuretic (A) and saliuretic (B) effects at baseline (1 d before), and at 10 and 30 d after treatment with 2, 4, or 8 g of *C. citratus* leaf infusions.

[a]$P<0.05$ *v.s.* baseline, [b]$P<0.05$ *v.s.* 10 d.

4. Discussion

The results of the present study indicate that oral intake of infusions prepared from *C. citratus* leaf powder significantly increased indices of diuresis including urine volume, urination frequency, saliuretic indices, diuretic action, and fractional excretion of electrolytes and other substances.

A non-significant change in eGFR was found at Day 10, in participants treated with infusions prepared from 2 or 4 g of the leaf powder. eGFR decreases significantly in both male and female subjects in all groups at Day 30. Urinary pH and uric acid levels decreased, whereas a non-significant change in urine specific gravity was observed. These findings are similar to those previously observed in individuals on standard loop-active (furosemide-like) diuretic therapy[13] and provide strong evidences in support of the diuretic property of *C. citratus* leaf infusions as reported by previous investigators[14] and also indicate its clinical effectiveness.

In support of this assertion, previous studies have shown that the phytochemicals (tannins, sapinins, flavonoids, alkaloids, phenolics, *etc.*) present in *C. citratus* extract induce diuresis and natriuresis[15] by individually or synergistically inhibiting the $Na^+/K^+/2Cl^-$ co-transporter activities, thereby interfering with the reabsorption of electrolytes (Na^+, K^+ and $2Cl^-$) and water through the walls of kidney tubules.

In a study by de Souza *et al*[16], the diuretic effect of saponins was found to be comparably higher than that of furosemide, a standard loop diuretic, which also acts by inhibiting the 3-ion co-transport system ($Na^+/K^+/2Cl^-$)[17], indicating similarity in their modes of action. Another study by Jouad *et al*[18], showed that flavonoids caused a decrease in blood pressure and a significant increase in urine electrolytes (Na^+, K^+ and $2Cl^-$) concentrations in a manner similar to that of furosemide but with twice the effect of placebo. Also, the non-significant changes in eGFR observed in the acute phase of the present study are similar in pattern to the eGFR changes observed in individuals on acute furosemide treatment, thus providing an additional evidence to support the similarity in their modes of action (inhibition of Na^+/K^+ ATPase ($Na^+/K^+/2Cl^-$ co-transporter))[19].

Interestingly, it was also observed that the peak diuretic, natriuretic and saluretic effects were achieved during the acute phase (10 d of treatment) in subjects who received infusions prepared from 2 or 4 g of the leaf powder, with a peak effect found among those who received infusion prepared from 4 g of the leaf powder. These effects were lower during the sub-chronic phase (30 d of treatment) and in those treated with the infusion prepared from 8 g of the leaf powder. In the absence of any contrary evidence, our findings suggest that the maximum effective dose for diuretic action is within the range of 2 to 4 g of the leaf powder, administered for a period of 10 d. These observations are consistent with the typical sigmoid diuretic dose-time response curve and the short duration of action observed with other loop diuretics[20].

According to Sica[21], the natriuretic response of a loop diuretic is optimal at a unique and clinically relevant dose/time point, beyond which no further gain in effects is observed. Our findings are consistent with this hypothesis, and provide additional evidence to support the use of 2-3 g of the leaf powder in 150 mL of hot water for preparing lemongrass tea (Abafado) in Brazil[9]. Moreover, this dose appeared to produce a beneficial effect in terms of a non-significant alteration in eGFR and other renal function indices in the present study. These findings are similar in pattern to the effects of acute administration of a loop active diuretic agent[21], and is attributed to the inhibition of the Na^+-K^+-ATPase (Na^+-K^+-$2Cl^-$ co-transporter) located in the apical membrane of the macula densa cell. This co-transporter is responsible for the transportation and delivery of adenosine across the macula densa cell membrane. Adenosine is the mediator of tubuloglomerular feedback vasoconstrictive system that acts to preserve volume homeostasis.

Accordingly, this action counteracted the postulated

vasoconstriction that would have followed the administration of a diuretic agent. This result in vasodilation, reduction in afferent arteriolar resistance and a non-significant alteration in the eGFR as observed in the present study. Therefore, the non-significant changes in eGFR observed in the acute phase of the present study provide additional evidence to support the loop-active diuretic (furosemide-like) mode of action of *C. citratus* leaf infusion.

The possible carbonic anhydrase inhibition, thiazide mode of action, osmotic diuretic effects and action of anti-diuretic hormone were excluded in the present study due to the absence of their biochemical indicators[22]. For instance, the observed increase in $Cl^-/(Na^+ + K^+)$ ratio (index of decreased carbonic anhydrase inhibitory activity)[23] ruled out carbonic anhydrase inhibition (acetazolamide-like) mode of action.

The absence of bicarbonaturetic and urine alkalinization effects further confirmed this assertion. In the same vein, increase calciuresis and decrease thiazide secreting index exclude thiazide (hydrochlorothiazide) mode of action[22,24]. Osmotic diuresis is unlikely to be operative, since the extract has a low Na^+ content, and ingestion of the infusion produced no significant effect on the urine specific gravity and osmolarity, though a high concentration of K^+ was detected in the extract[20]. The non-significant alteration in urine osmolarity excludes the possible diuretic effect associated with the impairment of the action of antidiuretic hormone.

Furthermore, inhibition of the aldosterone-sensitive Na^+ channels located on the cortical collecting tubules by saponins, causing a decrease in number of open Na^+ channels; a spironolactone-like diuretic mode of action has been suggested. In studies by Hiwatashi *et al.* and Chen *et al.*, saponin was found to inhibit the circulating and tissue rennin-angiotensin aldosterone system; a spironolactone effect. In a similar manner, quercetin, a flavonoid was found to inhibit the alpha epithelial Na^+ channel messenger RNA (αENaC-mRNA) expression in the kidney[25-27]. These may plausibly explain the metabolic acidosis, and decreases in kaliuretic index in the acute phase of the present study. The presence of a non-significant change in serum K^+ concentration and mild kaliuresis in the present study could partly be due to the balance between the furosemide-like (loop diuretic) and the spironolactone-like (K^+-sparing diuretic) mode of actions of *C. citratus* phytochemicals, causing little or no effect on serum K^+ level evidence of a good diuretic action[19].

Additionally, the diuretic effect of *C. citratus* leaf infusions may partly be due to the natriuretic and diuretic effects of its high K^+ content as observed in the present study, and as documented previously[1]. This could have partly contributed to the increase in serum K^+ concentrations in the acute phase of the present study.

The diuretic effect associated with increase serum K^+ concentration is explained on the basis of the behaviour of the Na^+/K^+ ATPase of intact human red cells. Empirical studies have shown that serum K^+ concentration act as a competitive inhibitor of the pump, and that the pump Na^+ efflux is an inverse function of the serum K^+ concentration[28]. The high K^+ content of *C. citratus* extract could produce diuresis similar to the action of saponin and other phytochemicals present in *C. citratus* and known to inhibit Na^+/K^+ ATPase.

In conclusion, the present study results show that *C. citratus* leaf infusion has a potent but dose-time dependent diuretic action/effect, mediated through multiple mechanisms: loop active-like activity and potassium-sparing effect. This additive or synergistic action of *C. citratus* phytoconstituents at a single or multiple target sites can be beneficial by eliminating the problematic side effects associated with the predominance of a single synthetic loop diuretic in the body. These characteristics present it as an effective diuretic agent.

Conflict of interest statement

There is no competing interest among the authors.

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Comments

Background

Infusions of leaves of *C. citratus* have been used extensively in traditional medicine worldwide especially as a diuretic in the treatment of various disease states in humans but little or no scientific evidence is available to corroborate this claim.

Research frontiers

This study evaluates the diuretic activity of infusions from the leaves of *C. citratus* using indices such as urine volume, urination frequency, natriuresis and saliuresis, renal electrolyte excretion and metabolic acidosis.

Related reports

The plant *C. citratus* contains bioactive ingredients capable of inducing diuresis and natriuresis.

Innovations and breakthroughs

C. citrtatus leaves are widely used in traditional herbology as diuretic for various conditions such as cardiovascular diseases and other related disaeses. The authors have

demonstrated that the plant has potent diuretic activity as depicted in the diuretic and biochemical evaluations in healthy humans.

Applications

The result of this study indicates that the leaves of *C. citratus* possess diuretic activity. This corroborates with the ethnobotanical use of this plant in humans in conditions requiring diuresis.

Peer review

This is an interesting research contribution. The authors have established that *C. citratus* leaf infusions possess diuretic potentials in humans. This reflected significant increase in diuretic indices of urine volume, urination frequency, natriuresis, saliuresis and renal fractional excretion of electrolytes following diuretic and biochemical evaluations.

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