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# Clinical Correlates of Physiochemical Changes in Urinary Composition in Subjects treated with *Cymbopogon citratus* infusion

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**ABSTRACT**

Previous studies have shown that the physiochemical properties of urine could change after ingestion of medicinal plants, and may provide significant clinical and diagnostic information. This study examined the urinary profile of healthy subjects who consumed *Cymbopogon citratus* (*C. citratus*) infusion. Urine samples were obtained from 105 participants at days 0, 10 and 30 after treatment with infusions prepared from 2, 4 and 8g of *C. citratus* powder. Biochemical analyses of the urine to determine its chemical constituents were performed using standard procedures. Results obtained on days 10 and 30 were compared with baseline values. UV and UF increased in all groups. Urinary excretion of some electrolytes ($Na^+$ and $K^+$) and DA increased at day 10 only, whereas others ($Ca^{2+}$ and $Cl^-$) increased at both days 10 and 30. USG and colour remained unchanged. Urinary creatinine levels significantly increased ($p<0.05$) in all groups, whereas urinary urea significantly ($p<0.05$) increased in all groups except the group treated with 8g for 30 days. Urine uric acid and pH decreased in all groups. Urinary protein, glucose, bilirubin and bicarbonate were undetected. Ingestion of *C. citratus* infusion is associated with some physiochemical changes in urinary composition and could provide significant clinical information on the systemic effects of the plant in humans.



## Introduction

Human urine is one of the biological body fluids that has been most examined, and the first body fluid to be scientifically studied [1]. This is probably because urine is regarded as the inner window through which the metabolic activities of the body can be viewed [2].Furthermore, of all the body fluids, urine is the most freely accessible, noninvasive, and informative to clinicians. Its analysis, which is one of the most commonly ordered clinical tests, has been used as a reliable diagnostic tool in medicine for centuries [3], although recent advancements in medicine have decreased the diagnostic value of urinalysis in selected conditions, particularly those concerning the urinary system and metabolic activities of the body.

Therefore, changes in the physiochemical properties of urine (i.e., color, pH, osmolarity, red blood cells, sugar, protein, electrolytes, uric acid, urea, and creatinine levels) may indicate the presence of a primary kidney pathology (nephrotic syndrome, renal failure, acute glomerular nephritis, nephrolithiasis, and other forms of nephropathy), systemic diseases affecting the kidney (diabetes mellitus, hypertension, and acidic/base disturbances), or liver abnormalities. Furthermore, changes in the physiochemical properties of urine may be consequent to secondary effects of drugs or chemicals.

This is likely because besides urine production, the kidneys are also involved in the maintenance of homeostasis in the body by regulating urinary excretion to match excess intake, and eliminating metabolic end products. Thus, the kidney is uniquely susceptible to toxicity and is the target of many xenobiotics and environmental toxicants [4], including herbs and natural plant products.

Using changes in urine physiochemical parameters, Luycky *et al.* [5] reported metabolic acidosis and volume depletion in 80.8% and 62.8% of patients with renal failure after a recent use of traditional herbal medicine, respectively. Furthermore, changes in urine electrolyte levels ($Na^+$, $K^+$, $Ca^{2+}$, $HCO_3^-$, $Cl^-$, and $Po_4^{3-}$) have been associated with the effects of herbal preparation, thus altering the optimum pH required for normal homeostasis.

In patients with Chinese herbs nephropathy, changes in physiochemical properties of urine included mild proteinuria, glycosuria, or aseptic leukocyturia in about 40% of the patients and normal urine sediment in 46% of the patients [4]. These findings underscore the clinical significance of evaluating the physiochemical changes in urinary composition associated with some commonly used herbs and natural plant products such as *C. citratus*, which hitherto has not been investigated.

*C. citratus* is a widely cultivated herb from the Poaceae family of plants. It is a perennial grass with smooth green leaves. The leaves contain various bioactive substances including phytochemicals, macronutrients, minerals and vitamins.

It has a wide range of therapeutic applications including its antibacterial, antifungal, antiprotozoal, anticarcinogenic, analgesic, hypoglycemic, antioxidant, insect repellant, antihypertensive, cardio protective, and anti-dyslipidemic effects [6].

Literature evidence regarding its effects on physiochemical properties of urine is scanty, hence the present study.

## Materials and methods

### *Plant materials*

### *Collection, identification, and preparation of the infusion of C. citratus leaves*

Fresh *C. citratus* leaves were obtained from an agricultural farm in Uyo, AkwaIbom state, Nigeria few days prior to utilization, in the month of May 2012. The leaves were identified and authenticated by a taxonomist in the department

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of Botany at the University of Uyo. Voucher specimen NO. UUH3276/UYO was deposited at the herbarium in the department of Botany of the University. The leaves were rinsed, sun-dried, and pulverized into powder using electric blender to give a gram weight of 200g. This was soaked in a container with 2 liters of hot water and allowed to stand for about 8hours. Thereafter the solution was filtered using No. 2 Whatman filter paper.The filtrate was evaporated by heating in water bath at $40^0$C to obtain the solid extract. The solid extract was weighed with an electric weighing balance (ACS-ZE14, Surgifriend Medicals Ltd, England) to obtain a yield of 70grams (35% w/w), which was stored in clean bottles at room temperature until required for use. Similar procedures were repeated using 2, 4 or 8g of *C. citratus* powder and yields of 410mg, 810mg and 1570mg extract were obtained respectively.

***Phytochemical screening of C. citratus leaves extract***

The phytochemical analysis of the extract was carried out using standard procedures to determine the levels of saponins, phenolics, alkaloids, tannins, flavonoids, glycosides, steroids, deoxy sugars, trepans, and anthraquinones [7].

***Study design***

This study used a pre and post experimental design to assess the effect of *C. citratus* leaves infusions on physiochemical changes in the urinary composition of 105 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-survey 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, proteinuria, serum createnine(>1.15mg/dl),hematuria, failure to satisfy the pre-research 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 the study was conducted at the University of Uyo, Nigeria, according to the rules set forth in the Declaration of Helsinki governing the conduct of human research.

***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 8g of *C. citratus* leaves powder in 150ml of hot water respectively, given once daily for 30days. This infusion was prepared in this pattern to correspond to the pattern usually employed by the population to prepare lemongrass tea [8]. The dose range was adapted from previous human studies [8,9] in which participants exhibited no obvious clinical or biochemical evidence of toxicity. To further confirm the safety of this dose range, the investigators of the present study conducted a pilot survey on 10 human volunteers using 2, 4, 8 and 10g of *C. citratus* leaves powder. Participants who had received at least a dose of the infusion prepared from 2, 4, or 8g of *C. citratus* in equal volume of hot water (150ml) daily for 1 week showed no evidence of adverse/toxic effect, as judged by the results of the tolerability evaluation.

Tolerability evaluation consisted of determining clinical and laboratory test abnormalities (liver function test-aminotransferase activities) and renal function test, serum creatinine and clearance rate (Cr), serum urea levels and hematological indices.

The authors had chosen 2g of *C citratus* leaves powder as the starting dose to correspond to the quantity usually employed by the Brazilian population to prepare their lemon grass tea [8]. In order to evaluate dose effect, the 2g was doubled and quadrupled, to give 2, 4 or 8g of *C. citratus* leaves powder used in the present study.

***Biochemical estimation***

Venous blood and urine samples were obtained for biochemical analysis after a fasting period of about 8 hours. All biochemical analysis were performed within 2-hr of sample collection at the chemical pathology unit of the University of Uyo Teaching Hospital (UUTH). Parameters measured were serum (SCr) and urinary creatinine, urea, uric acid, liver enzymes (aspartate aminotransaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin, protein, glucose and serum lipid profile (HDL-C, LDL-C, VLDL-C, TG and Chol).

Serum creatinine level was determined by Jaffe's method using 0.75NaOH and1% Picric acid (Sigma chemicals, India) at a volume of 1ml each to the serum specific specimen. A standard was similarly treated. A colour change that developed within 15minutes at room temperature was measured spectrophotometrically (ESA Inc., Chelmsford, USA) at 520nm. Serum total cholesterol (T-chol), triglyceride (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and glucose were measured using lipid profile and glucose automated measuring system (lipid pro$^{TM}$), Model ILM-0001 A, Infopia Co. Ltd, South Korea). Serum uric acid and urea were 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 [10]. All measurements were performed 1 day prior to, and at 10 and 30 days after the start of infusion administration.

***Assessment of Diuretic Activity***

Twelve-hour urine sample was collected from all participants in their respective sample containers between the hours of 6pm and 6am, at days 0, 10 and 30 after the start of the infusion administration. The urine volume (UV) was estimated by using a calibrated cylinder, while the 24-h urination frequency (UF) was reported in a 24-h UF chart designed by the authors. The $Na^+$ and $K^+$ concentrations in the urine were determined with Flame Photometer ("Jencon PEP 9", Jencons Scientific Limited, Bedfordshire, UK), $Ca^{2+}$ was measured by an atomic absorption spectrophotometer technique (Jarrel-Arh Model 82-36, UK). Urine $Cl^-$ was measured by ion selective meter (Orion 730", Orion Research Inc. Boston, USA). The urine pH was measured with a digital pH meter (Model E9610, Equiptronics, England). Urine glucose and protein were measured using urine reagent test strips (Combi 9, Macherey-Negrel, Germany). On the days urine specimen was collected (days 0, 10 and 30), fluid and food intake were restricted within the hours of administration of infusion and collection of the final urine specimen (6pm to 6am). The urine electrolytes concentrations were measured at baseline, 10 and 30 days post

treatment. Data obtained were used for computing various saliuretic indices including saliuretic index 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 (DA)(urinary output of test group/ urinary output of control group), saluretic index ($Na^+ + Cl^-$) and other substances (urine volume *multiplied by* concentration of ions in test group/urine volume *multiplied by* conc. of ion in control group). Experimental values were compared with baseline values [11].

***Statistical Analysis***

Data (Mean ± SEM) were analysed using one-way analysis of variance (ANOVA) followed by pair-wise comparison using the least significant difference (LSD) 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).

**Results**

Preliminary phytochemical studies of *C. citratus* leaf extract showed the presence of saponins, tannins, alkaloids, anthraquinones, phenols, flavonoids, and terpenoids (Table 1).

The baseline demographic and clinical characteristics of the study participants are shown in table 2. Table 3 shows UV, UF, DA and urinary excretion of electrolytes( $K^+$, $Na^+$, $Ca^{2+}$ & $Cl^-$ ),pH and urine specific gravity (USG) at baseline, days 10 and 30 whereas table 4 shows the urinary excretion of urea, creatinine and uric acid within the same period.

Figures 1A and B show the effects of *C. citratus* leaves infusion on DA (**A**), and saluretic activity (**B**). The peak DA and saluretic activity were achieved at the day 10 (acute phase) in participants who received infusions prepared from 2 and 4g of the leaves powder. These effects were lower at day 30 (sub-chronic phase), and in those who received infusion prepared from 8g of *C. citratus* leaves powder.

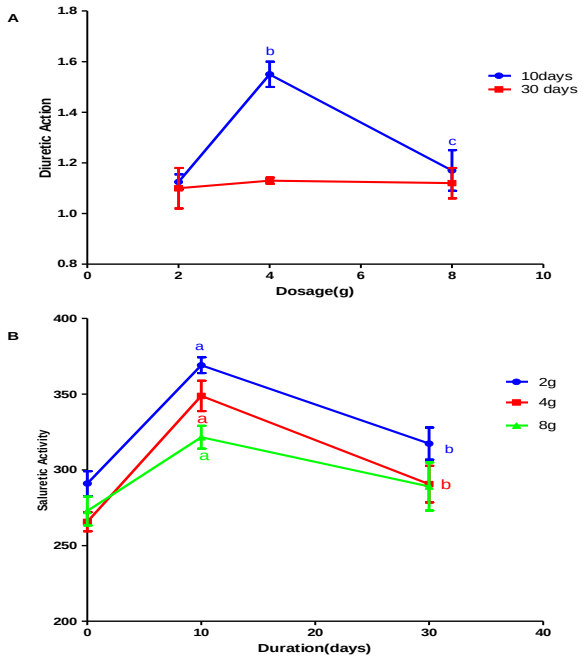


**Figure 1. Comparison of diuretic (A) and saliuretic (B) actions at baseline (1 day before), and at 10 and 30 days after treatment with 2, 4, or 8 g of *C. citratus* leaves infusions. [a]p< 0.05 vs. baseline, [b]p< 0.05 vs. 10 days. Values reported as Means ± SEM.**

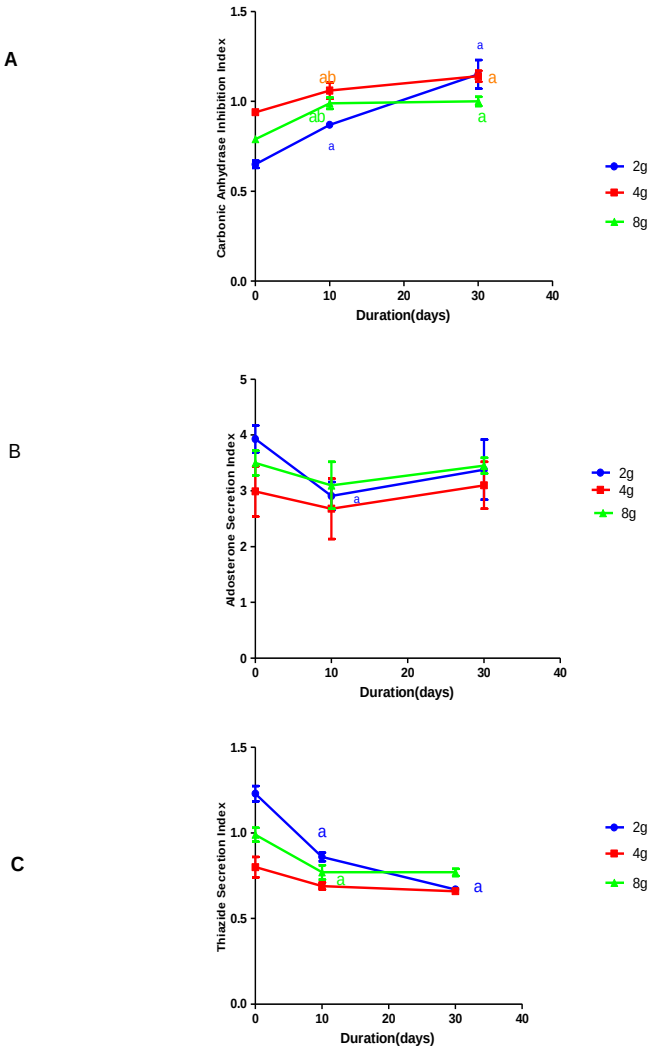


**Figure 2. Comparison of carbonic anhydrase inhibition (A) and aldosterone secretion (B) and thiazide secretion (C) indices at baseline (1 day before), and at 10 and 30 days after treatment with 2, 4, or 8 g of *C. citratus* leaves infusions. [a]p< 0.05 vs. baseline, [b]p< 0.05 vs. 10 days. Values reported as Means ± SEM**

Figures 2**A**, **B** and **C** show the effects of *C. citratus* infusion on carbonic anhydrase inhibition (**A**), aldosterone secretion (**B**) and thiazide secretion (**C**) indices. Carbonic anhydrase inhibition index increased significantly at days 10 and 30 (2**A**), whereas aldosterone and thiazide secretion indices decreased at day 10 and remained decreased at day 30 (2**B** and **C**).

**Discussion:**

The physiochemical changes in urinary composition in subjects treated with *C. citratus* infusion in the present study support the diuretic activities of the plant extract as reported by previous investigators [12] and are similar to those observed in individuals on a short (acute) or prolonged (chronic) treatment with a standard loop-active (furosemide-like) diuretics [11]. Evidently, these changes include acute increase in UV, UF, DA, urinary excretion of electrolytes ($K^+$, $Na^+$, $Ca^{2+}$ and $Cl^-$), renal fractional excretion of electrolytes and other substances. A non-significant effect on eGFR was observed at day 10, however, eGFR decreased significantly at day 30.

**Table 1. Phytochemical constituents of *Cymbopogon citratus* leaf extract**

| Phytochemical constituents | Concentration |
|---|---|
| Saponins | +++ |
| Tannins | ++ |
| Flavonoids | ++ |
| Phenols | ++ |
| Anthraquinones | + |
| Alkaloids | + |
| Deoxysugars | + |
| Steroids | - |
| Cyanate | - |
| Phlobatannins | - |

-absent
+present
++moderate
+++marked

**Table 2. Baseline demographic characteristics of study participants**

| Characteristics | Baseline values |
|---|---|
| Demographic characteristics | |
| Mean age(yr) | 27.42 ± 0.30 |
| Age range (yr) | 18 – 35 |
| Sex<br>Male<br>Female | <br>55 (52%)<br>50 (48%) |
| 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

**Table 3. Acute (10 days) and sub-chronic (30days) effects of infusions prepared from 2,4 or 8g *C. citratus* powder on some urine physiochemical parameters.**

| | Mean 12 hr urine volume (liter) | Mean 24 hr urination frequency | $K^+$ mmol/L | $Na^+$ mmol/L | $Ca^{2+}$ mmol/L | $Cl^-$ mmol/L | pH | SG | Urine output in test group/ urine output in control group |
|---|---|---|---|---|---|---|---|---|---|
| Baseline (control)<br>2g<br>4g<br>8g | 0.80±0.13<br>0.71±0.04<br>0.86±0.05 | 6.42±0.24<br>5.06±0.20<br>6.02±0.28 | 40.83±1.87<br>39.40±1.37<br>37.71±1.68 | 160.63±5.57<br>117.94±8.56<br>135.63±8.20 | 11.78±3.87<br>16.25±2.97<br>11.95±2.84 | 130.34±6.37<br>147.80±6.44<br>137.23±7.46 | 5.57±0.24<br>5.88±0.19<br>5.20±0.17 | 1.010±0.00001<br>1.010±0.00001<br>1.010±0.00001 | - |
| Acute<br>2g | 0.90±0.04[a] | 7.08±0.22[a] | 58.57±3.18[a] | 170.37±8.02[a*] | 13.69±1.83[a] | 198.80±8.47[a] | 4.59±0.19[a] | 1.010±0.00001 | 1.125±0.03 |
| 4g | 1.10±0.02[ab] | 7.04±0.37[a] | 52.87±2.87[a] | 141.89±5.53[ab] | 20.38±2.30[ab] | 206.97±3.35[a] | 5.00±0.23[a] | 1.011±0.00001 | 1.55±0.05[b] |
| 8g | 1.01±0.04[ab] | 7.02±0.34[a] | 44.06±3.06[abc] | 140.06±7.20[b] | 12.86±1.86[c] | 181.51±11.72[ac] | 4.99±0.11 | 1.014±0.00012 | 1.17±0.08[c] |
| Subchronic<br>2g | 0.88±0.01 | 6.56±0.25 | 37.67±0.39 | 127.14±8.21[a] | 17.13±2.26[a] | 190.23±7.43[a] | 4.02±0.14[a] | 1.013±0.00002 | 1.10±0.08 |
| 4g | 0.80±0.06 | 6.74±0.31[a] | 37.40±1.85 | 116.00±6.73 | 20.43±2.99[a] | 174.60±8.44[a] | 4.11±0.16[a] | 1.011±0.00001 | 1.13±0.12 |
| 8g | 0.96±0.03[abc] | 8.00±0.22[abc] | 36.54±0.65 | 126.11±7.70[a] | 17.05±2.54[a] | 162.94±8.16[ac] | 5.01±0.18[bc] | 1.014±0.00001 | 1.12±0.06 |

2g (410mg yield), 4g (810mg yield), 8g (1570mg yield) a = significantly different from baseline ($p<0.05$), b = significantly different from 2g ($p<0.05$), c = significantly different from 4g ($p<00.05$). Values reported as Mean±SEM

**Table 4. Comparison of urine creatinine, urea and uric acid levels at baseline, 10 and 30 days after treatment with infusion prepared from 2, 4 and 8g of *C citratus* powder**

| | | Creatinine | | | *Urea* | | | Uric Acid | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | baseline | 10 days | 30 days | baseline | 10 days | 30 days | baseline | 10 days | 30 days |
| male | 2g | 248.92 ± 15.14 | 482.95 ± 25.92$^{a**}$ | 379.53 ± 15.83$^{a** b**}$ | 3.48 ± 0.32 | 3.57 ± 0.28 | 2.70 ± 0.16$^{a** b**}$ | 123.47 ± 10.02 | 139.52 ± 9.22$^{a*}$ | 159.42 ± 10.62$^{a** b**}$ |
| | 4g | 310.54 ± 14.92 | 371.15 ± 30.14$^{a**}$ | 330.40 ± 16.17$^{a** b**}$ | 3.75 ± 0.82 | 2.97 ± 0.25$^{a*}$ | 2.81 ± 0.13$^{a*}$ | 141.93 ± 12.23 | 163.92 ± 11.15$^{a*}$ | 145.13 ± 10.09$^{a* b*}$ |
| | 8g | 339.19 ± 17.95 | 479.95 ± 20.22$^{a**}$ | 369.58 ± 25.10$^{a** b**}$ | 2.39 ± 0.16 | 2.46 ± 0.18 | 2.71 ± 0.15 | 102.45 ± 6.23 | 114.33 ± 8.45$^{a*}$ | 102.65 ± 11.90 $^{b*}$ |
| female | 2g | 240.12 ± 14.12 | 452.02 ± 25.63$^{a**}$ | 363.05 ± 15.78$^{a** b**}$ | 3.34 ± 0.45 | 3.48 ± 0.22 | 2.58 ± 0.32$^{a** b**}$ | 121.71 ± 9.07 | 136.56 ± 8.92$^{a*}$ | 155.56 ± 12.23$^{a** b**}$ |
| | 4g | 300.42 ± 15.93 | 349.24 ± 28.92$^{a**}$ | 312.92 ± 30.40$^{a** b**}$ | 3.69 ± 0.25 | 2.93 ± 0.21$^{a*}$ | 2.81 ± 0.41$^{a*}$ | 145.69 ± 10.02 | 160.3 ± 9.62$^{a*}$ | 158.67 ± 11.04$^{a* b*}$ |
| | 8g | 332.57 ± 19.82 | 465.02 ± 20.15$^{a*}$ | 351.08 ± 19.02$^{a**b**}$ | 2.21 ± 0.10 | 2.34 ± 0.12 | 2.51 ± 0.36 | 98.35 ± 8.45 | 111.05 ± 7.22$^{a*}$ | 99.53 ± 6.24 $^{b*}$ |

a* = significant different from baseline at 5 %( p<0.05); b* = significantly different from 10 days at 5% (p<0.05); a*b* = significantly different from baseline and 10 days at 5 %( p<0.05);a**b** = significantly different from baseline and 10 days at 1 %( p<0.01). Values reported as Mean ± SEM.

In terms of diuretic mode of action, carbonic anhydrase inhibition (acetazolamide-like)diuretic action was unlikely due to the observed increase in urinary $Cl^-/Na^+ + K^+$ ratio (index of decreased carbonic anhydrase inhibitory action) [13]. The absence of bicarbonaturetic and urine alkanization effects further confirms this assertion. In a similar manner, increased calciuresis and decreased urinary thiazide secretion index exclude thiazide (hydrochlorothiazide) diuretic mode of action [14]. Osmotic diuresis was unlikely to be operative, since the extract has low $Na^+$ content, and ingestion of the infusion produced no significant effect on the USG. The presence of kaliuresis and decreased urinary aldosterone secretion index ruled out the possible $K^+$-sparing diuretic mode of action. These changes in urinary physiochemical parameters provide additional evidence in support of the loop-active (furosemide-like) diuretic action of *C. citratus*, as previously documented. It is an established fact that, the phytochemicals in *C. citratus* can induce diuresis and natriuresis and associated hemodynamic changes by individually or synergistically inhibiting the $Na^+/K^+/2Cl^-$ co-transporter activities thereby interfering with the re-absorption of electrolytes ($K^+$, $Na^+$, and $2Cl^-$) and water through the walls of the renal tubules. In the study by Jouad et al [15], the diuretic effect of flavonoids was similar to that of furosemide. Also, in de Souza et al's [16] study, saponins were found to induce diuresis similar in action to furosemide (that is inhibition of $Na^+/K^+/2Cl$-co-transporter) but higher in effects.

These results may have a significant clinical relevance in terms of the associated therapeutic uses due to its diuretic effects, and adverse health outcomes associated with its loop active diuretic actions. For instance, increase in diuretic activities may plausible explain for its hypotensive effects observed in the present study, and hence its use in lowering BP, treating edematous conditions caused by disorders of extracellular fluid volume regulation (congestive cardiac failure, nephritic syndrome, pulmonary edema and liver cirrhosis).

Also, the natriuretic, calciuretic, kaliuretic and chloriuretic effects of C. citratus infusion may provide therapeutic benefits in conditions associated with hypernatremia, hyperkalemia, hypercalcemia, hypermagnesemia, and metabolic acidosis and in other conditions associated with anion overdose.

In a similar manner, prolonged use may be associated with significant serum electrolytes depletion leading to hypokalemia, hyponatriemia, hypocholoremia, hypomagnesemia, hypocalcemia and metabolic alkalosis. Additionally, extracellular volume depletion, dehydration, plasma contraction, reduction in renal blood flow, cardiac output, BP and changes in neural, hormonal and local mechanisms may ensue.

Physiologically, the hemodynamic changes following these effects could lead to enhance baroreceptor activity and renal sympathetic tone, increase activity of the renin-angiostenin system [17] and other vasoconstrictor systems, with resultant efferent arteriolar constriction and a decrease in glomerular filtration rate(GFR) and other renal function indices such as altered serum urea and creatinine clearance as observed in the present study. Other changes in participants' urine physiochemical parameters included: significant increase in urea and creatinine levels in some or all groups. Urinary pH and uric acid concentrations decreased significantly in some or all groups, whereas USG showed a non-significant change. $HCO_3$, protein, glucose and bilirubin were below detectable limits.

The increased urinary urea and creatinine levels may be due to the high plasma levels of these substances. This assertion is supported by the findings of high protein content in C. citratus extract in the present and previous studies [18]. In their study, Arhoghro et al [18] observed that compared to the control group, rats treated with C. citratus extract had higher plasma protein levels, with a subsequent increase in liver ureagenesis [19] and hence increase in urinary urea levels, as observed in the present study. The observed decrease in urine pH and the absence of urinary $HCO_3$ typify pH changes in individuals on acid-producing diet, as it may also be true of *C. citratus* extract with the pH of 5.4 obtained in the present study. It also indicates the kidneys' role in acidification of urine to rid the body of excess acid load. This response includes a reduction, if not complete elimination of bicarbonate in the urine, and increase in titratable acids (phosphoric acid, uric acid, creatinine,) and ammonium excretion. This provides a plausible explanation for the increase in urinary excretion of creatinine, acids and urea and absence of $HCO_3^-$ in the urine of the present study participants. The decreased urinary uric acid may partly reflect a decreased production and hence plasma levels, probably due to the effect of xanthine oxidase inhibitor found in *C. citratus* extract [20]. This further supports the anti-gout activity of *C. citratus*.

**Conclusion:**

Ingestion of C. citratus infusion is associated with increase in urination frequency, urine output, diuretic action, renal fractional excretion of electrolytes, urea and creatinine, decreased urinary pH and uric acid.

These findings may provide significant clinical information on the systemic effects of the plant, specifically the renal system, and also evidence for its indication or contraindication in certain medical conditions.

**Conflict of interest:**

None declared

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