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Evaluation of the Diuretic, Serum and Kidney Electrolyte Changes in Wistar Albino Rats after Aqueous Extract of *Cola nitida* Seeds Administration

Adeosun IO[1], Obajuluwa AO[2*], Chigozie FC[2,3]

[1]*Physiology Department, College of Health Sciences and Medicine, Afe Babalola University, Ado Ekiti, Nigeria*
[2]*Department of Biological Sciences, College of Sciences, Afe Babalola University, Ado Ekiti, Nigeria*
[3]*Human Biology Department, College of Sciences, Afe Babalola University, Ado Ekiti, Nigeria*

ABSTRACT

Alterations in water and electrolytes content of serum and tissues have been indicated in the management of hypertension amongst others. This study aims to evaluate the diuretic, saliuretic and natriuretic effect and acute toxicity of a crude Aqueous Extract of Cola nitida (AECONS) on urinary parameters over a 6 h (at 2 h interval) in Wistar Albino rats and their serum and kidneys after a three weeks' dose regimen. To evaluate the diuretic activity of the plant, Albino rats were divided into five groups. The control group received normal saline (10 ml/kg), the reference group received furosemide (20 mg/kg) and the test groups were administered different doses (i.e., 400, 600 and 800 mg/kg) of the crude extract by oral route (gavage) respectively. Student t-test and One-way ANOVA was used for the statistical analysis and p-values less than 0.05 were considered statistically significant. We observed significant diuretic, saliuretic and natriuretic effects in the treated groups in a dose dependent manner. However, urinary pH remained unchanged during the study except in the 800 mg/kg group. The diuretic index values showed good diuretic activity of the crude extract. The Lipschitz values demonstrated in the crude extract, at the dose of 600 mg/kg showed two-fold diuretic activity when compared with furosemide and control groups. Hence, this study confirms the significant diuretic, natriuretic and saliuretic activity of the AECONS. During the measurement period of the study (6 h) which provides empirical basis for its pharmacological action in the treatment of health conditions.

Keywords: *Cola nitida*, Natriuretic, Saliuretic, Lipschitz value, Diuretic index, Na^+/K^+ ratio

INTRODUCTION

Cola nitida is a very important economic crop that grows in the rainforests of tropical areas of West Africa and its evaluation among others have been suggested as an essential option that can be explored as a diuretic due to the natriuretic and kaliuretic potentials demonstrated by its extracts in animal studies [1,2]. Diuretics play important roles in the pharmacological treatments of diseases such as hypertension, heart failure, epilepsy among others and recent studies have demonstrated the diuretic potentials of a variety of herbs and crops from plant sources that are beneficial to improving health conditions managed by the administration of diuretics [3]. *C. nitida* has been used traditionally as a remedy for dysentery, coughs, diarrhoea, vomiting etc, [4]. Its phytochemical components include: Alkaloids, tannins, flavonoids, saponins, steroids, glycosides and reducing sugars [5,6]. Diuresis is the process of losing a great amount of water along with essential mineral salts through excessive urination [7].

Furosemide is a loop diuretic drug which is the most commonly used diuretic. It acts mainly by inhibiting water reabsorption in the nephron which is achieved through competitive inhibition at the chloride binding site on the co-transporter, thus preventing the transport of sodium from the lumen of the loop of henle into the basolateral interstitium of the nephron [8,9]. However, some adverse side effects have been documented such as dehydration and electrolytic depletion [10,11]. Also, some contra-indications of its pharmacological action include allergic reactions, kidney failure, irregular heartbeat etc., [12]. *C. nitida* is eaten casually and without prescription among the Nigerian population while little effort has been made to find out its diuretic function in urine excretion and electrolytic balance. Diuretic drug actions can be routinely examined using urine output/volume, vital urine composition where substances that are normally found in the urine are compared against abnormal urine constituents. Normal urine constituents include: water (About 95% of urine), urea, creatinine, uric acid, electrolytes [13], although it can be altered by a number of factors such as diet and nutritional status, condition of body metabolism, ability of kidney function, level of contamination with pathogenic microorganisms (Bacteria) or even non-pathogenic microflora [14].

MATERIALS AND METHODS

Experimental animals

Twenty five adult female albino rats weighing between 120 g and 150 g bred in the Animal House of Physiology Department, Afe Babalola University were used. Female rats were selected for this study as there are few reports of examination of Aqueous Extract of *Cola nitida* (AECONS) diuretic potential in female rats and we have earlier carried out a research examining the diuretic potential using male Wistar rats. They were housed under standard laboratory conditions in plastic cages with wire guaze covering under a 12 h daylight cycle and had free access to pelletized rat chow (Obtained from ABUAD Farms) and water. They were acclimatized to laboratory conditions for 2 weeks before the commencement of the experiments. The experimental procedures adopted in this research were in strict compliance with Experimental Animal Care and Use of Laboratory Animals in Biomedical Research Regulation of the College of Sciences, Afe Babalola University, Ado Ekiti.

Plant materials

Fresh seeds of *C. nitida* were locally sourced and authenticated in the taxonomy unit of the department of Agriculture, Afe Babalola University.

Preparation of the AECONS

Large quantities (245.00 g) of the fresh seeds of *C. nitida* were washed and cut into smaller bits and air-dried in a clean tray for three weeks, the dried specimens were pulverized using laboratory mortar and pestle. Weighed portion (232.50 g) of the pulverized specimens were macerated and extracted with distilled water (1:2 w/v) for 48 h at room temperature (26-28°C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The distilled water was later evaporated using rotary evaporator. Forty gram of the AECONS was dissolved in 100 ml of distilled water to give a concentration of 0.4 g/ml.

Experimental controls

A loop diuretic (Mark-Furosemide, Tianjin, Xinzheng, Henan, China), was used as positive control (Reference drug) and 0.9% sodium chloride (Merck, Germany) was used as control drug, respectively.

Experimental design

Twenty-five animals were randomly divided into five groups with each group consisting of five rats. Group I (Control) received 10 ml/kg of 0.9% NaCl (normal saline), Group II (standard) received 20 mg/kg of furosemide, Group III received 400 mg/kg AECONS, Group IV received 600 mg/kg AECONS and Group V received 800 mg/kg AECONS.

Urine collection and urinalysis

This was done at an interval of every 2 h (2, 4 and 6 h) after the first dose of AECONS and monitored using a constructed metabolic cage (One animal per cage) which was specially designed to separate urine and feces. The urine was collected in graduated vials and measured over a 6 h interval and expressed as ml/100 g of body weight. During this period, the rats were deprived of food or water. Diuretic index was calculated by Mean urine volume of the test group/Mean urine volume of the control group.

Blood and tissue collection

Animals were fasted for 12 hours before sacrifice by cervical dislocation after which blood was collected through cardiac puncture into heparin bottles for biochemical evaluation and plain bottles for serum electrolytes level. The blood samples were centrifuge for 5 min using a bench-top centrifuge and the supernatant plasma was then used for the determinations of the serum electrolytes. The kidneys were harvested and immediately processed for determination of electrolytes index as described in literature [15].

Saliuretic, natriuretic and carbonic anhydrase inhibition

The sum of Na^+ and Cl^- urinary excretion was calculated as a parameter of saliuretic activity in the serum. The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio $Cl/(Na^+K)$ was calculated to estimate carbonic anhydrase inhibition [16].

Statistical analysis

All values were expressed as mean values ± SEM (Standard error of mean) and data were analyzed by applying an Analysis of Variance (ANOVA) followed by Student's t-test. The results were considered statistically significant if $P < 0.05$.

RESULTS

Effect on urine output and diuretic activity

The effect of administration of AECONS on the urine volume after 2 h showed only groups IV (600 mg/kg AECONS) and V (800 mg/kg) of the values were statistically significant ($P < 0.05$). However, groups II-IV produced after 2 h. AECONS administration while group I (control). Group IV (600 mg/kg AECONS) produced the highest volume of urine after 2 post AECONS administration. The effect of administration of AECONS on the urine volume after 4 hours showed group II values were statistically significant ($P < 0.05$) which was highest value obtained at 4 h while group V (800 mg/kg AECONS) produced no urine. The effect of administration of AECONS on the urine volume after six hours showed statistically significant values in groups I, IV and V ($P < 0.05$). However, groups IV and V (600 mg/kg and 800 mg/kg AECONS respectively) produced more urine than the control group (group I) while group II (Furosemide) produced no urine after 6 h post administration. The diuretic activity of a drug is considered nil if it is less than 0.72, little if it is between 0.72 and 1.00, moderate if it is within 1.00-1.50 and good if it is above 1.50. In this respect, AECONS demonstrate good diuretic activity with group IV showing the highest diuretic activity which excreted more than two-fold the control and furosemide groups (Table 1).

Table 1: Urine output as a measure of diuretic activity of AECONS

Group	Urine vol (ml) after 2 h	Urine vol (ml) in 4 h (ml)	Urine vol (ml) in 6 h	Diuretic index after 6 h
I (Control-0.9% NaCl)	0.00	0.57 ± 0.31	1.02 ± 0.20*	1.00
II (Furosemide)	0.61 ± 0.27	1.17 ± 0.17*	0.00	1.19*
III (400 mg/kg AECONS)	0.59 ± 0.21	0.3 ± 0.3	0.91 ± 0.08*	0.75
IV (600 mg/kg AECONS)	2.03 ± 0.53*	0.67 ± 0.67	2.67 ± 0.58*	2.2*
V (800 mg/kg AECONS)	0.92 ± 0.60*	0.00	1.53 ± 0.73*	1.5*

Effects on electrolyte excretion and carbonic anhydrase inhibition

Treatment of rats with furosemide, 600 mg/kg and 800 mg/kg AECONS caused a significant ($P < 0.05$) change in the excretion of sodium and potassium electrolyte in experimental animals when compared with the control group over a 6 h period as shown Table 2 below. Groups II-V showed high and significant saliuretic index after 2 h post AECONS oral administration while group I didn't pass any urine. However, Group I-IV showed high saliuretic index at 4 h post AECONS administration however group V didn't pass any urine. Also, carbonic anhydrase inhibitory activity was observed in group II at 4 h post AECONS administration. The highest natriuretic and saliuretic effect was observed in Group III (400 mg/kg AECONS oral administration) did not show any statistical significant saliuretic effect when compared to the control group while group IV showed potent saliuretic activity as compared to all other groups. The natriuretic potential of AECONS administration was however found to be highest in group III followed by group V, IV and I 6 h post AECONS oral administration. However, group II (Furosemide group) didn't pass any urine (Tables 3 and 4).

Table 2: Saliuretic and natriuretic index of 2 h post AECONS oral administration

Group	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Na^+/K^+ ratio	SI	CAI ratio
I (Control-0.9% NaCl)	0.00	0.00	0.00	0.00	0.00	0.00
II (Furosemide)	474.00 ± 61.45	8.18 ± 1.23	32.24 ± 3.30	14.70	506.40*	0.07
III (400 mg/kg AECONS)	412.82 ± 219.41	4.15 ± 2.21	24.04 ± 21.78	99.47	436.86*	0.06
IV (600 mg/kg AECONS)	507.14 ± 58.36	6.59 ± 0.76	105.03 ± 22.80	76.95	612.17*	0.2
V (800 mg/kg AECONS)	414.00 ± 207.36		25.47 ± 23.05	90.78	439.47*	0.06

S.I-Saliuretic index

Table 3: Saliuretic and natriuretic index of 4 h post AECONS administration

Group	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Na^+/K^+ ratio	SI	CAI
I (Control-0.9% NaCl)	397.20 ± 200.53	5.91 ± 3.22	148.19 ± 74.63	67.20	545.39*	0.38
II (Furosemide)	394.34 ± 67.32	4.86 ± 3.20	276.66 ± 39.58	81.13	671.00*	0.69*
III (400 mg/kg AECONS)	190.63 ± 190.63	0.80 ± 0.80	60.43 ± 60.43	238.28*	251.06*	0.32
IV (600 mg/kg AECONS)	228.57 ± 228.57	0.86 ± 0.86	39.45 ± 39.45	265.77*	268.02*	0.17
V (800 mg/kg AECONS)	0.00	0.00	0.00	0.00	0.00	0.00

n=5; significance $p < 0.05^*$; S.I-Saliuretic index

Table 4: Saliuretic and natriuretic index of 6 h post AECONS administration

Group	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Na^+/K^+ ratio	SI	CAI
I (Control-0.9% NaCl)	165.13 ± 165.13	1.23 ± 1.23	61.41 ± 61.09	134.25*	226.54*	0.37
II (Furosemide)	0.00	0.00	0.00	0.00	0.00	0.00
III (400 mg/kg AECONS)	648.80 ± 82.95	5.70 ± 2.60	91.41 ± 16.12	113.82*	740.21*	0.14
IV (600 mg/kg AECONS)	388.67 ± 200.01	29.11 ± 23.96	103.28 ± 53.06	13.35	491.95*	0.25
V (800 mg/kg AECONS)	541.61 ± 20.38	8.28 ± 1.03	132.13 ± 43.54	65.41	673.74*	0.24

n=5; significance $p < 0.05^*$; S.I-Saliuretic index

Effects on kidney sodium, potassium and chloride levels after three weeks AECONS administration

The sodium, potassium and chloride ions assayed in the animals kidneys after a three week oral dose regimen showed significant increase ($P < 0.05$) in all the groups except in group III (400 mg/kg AECONS). However, there was significant increase in the AECONS groups in a dose dependent manner. Also, groups IV and V showed the highest saliuretic and natriuretic index while the lowest in all groups was Group III as shown in Table 5 (400 mg/kg AECONS). Also, no carbonic anhydrase inhibitory activity was observed in all the groups.

Table 5: Natriuretic and saliuretic effects of 3 weeks AECONS administration on rats

Group	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l	Na^+/K^+	S.I
I (Control-0.9% NaCl)	30.83 ± 12.07*	15.71 ± 2.34*	140.83 ± 24.74*	1.96*	171.66*
II (Furosemide)	39.16 ± 22.22*	14.52 ± 2.75*	92.51 ± 7.02	2.69*	131.67*
III (400 mg/kg AECONS)	13.64 ± 3.15	11.68 ± 1.10	100.30 ± 6.53*	1.17	113.94
IV (600 mg/kg AECONS)	46.70 ± 7.87*	12.25 ± 1.63	127.60 ± 23.52*	3.81*	174.30*
V (800 mg/kg AECONS)	61.54 ± 9.11*	12.71 ± 1.18	119.04 ± 9.60*	4.84*	180.58*

n=5; p<0.05; S.I-Saliuretic index

Effects on urine pH and conductivity

Urinary pH and specific gravity were measured over 6 h duration of post AECONS administration (Table 6). The urinary pH and specific gravity of control rats when compared over a 6 h post AECONS administration was statistically insignificant. However, all the groups showed higher urinary pH values after 3 weeks AECONS administration when compared with the furosemide and control group (Table 7). AECONS increased the urine pH thus making it more alkaline (Figures 1-3).

Table 6: Urine pH and specific gravity at 2, 4 and 6 h post AECONS administration

Group	pH	Specific gravity
I (2 h)	0	0
(4 h)	5.00 ± 2.09	1.00 ± 0.43
(6 h)	6.00 ± 2.38	1.00 ± 0.34
II (2 h)	6.00 ± 0.33	1.02 ± 0.03
(4 h)	7.00 ± 0.50	1.03 ± 0.30
(6 h)	0.00 ± 0.00	0.00 ± 0.00
III (2 h)	6.00 ± 0.28	1.02 ± 0.01
(4 h)	7.00 ± 2.17	1.00 ± 0.34
(6 h)	7.50 ± 2.42	1.00 ± 0.34
IV (2 h)	7.00 ± 0.00	1.02 ± 0.01
(4 h)	7.00 ± 3.00	1.00 ± 0.34
(6 h)	8.00 ± 2.56	1.00 ± 0.34
V (2 h)	7.00 ± 3.00	1.00 ± 0.34
(4 h)	0.00 ± 0.00	0.00 ± 0.00
(6 h)	7.00 ± 0.00	1.00 ± 0.00

Table 7: Urinalysis parameters after 3 weeks post AECONS administration

Group	pH	Specific gravity
I (Control-0.9% NaCl)	6.00 ± 0.76	1.03 ± 0.10
II (Furosemide)	7.00 ± 6.66	1.02 ± 0.02
III (400 mg/kg AECONS)	7.00 ± 1.00	1.02 ± 0.01
IV (600 mg/kg AECONS)	7.50 ± 0.86	1.02 ± 0.00
V (800 mg/kg AECONS)	9.00 ± 0.00	1.00 ± 0.12

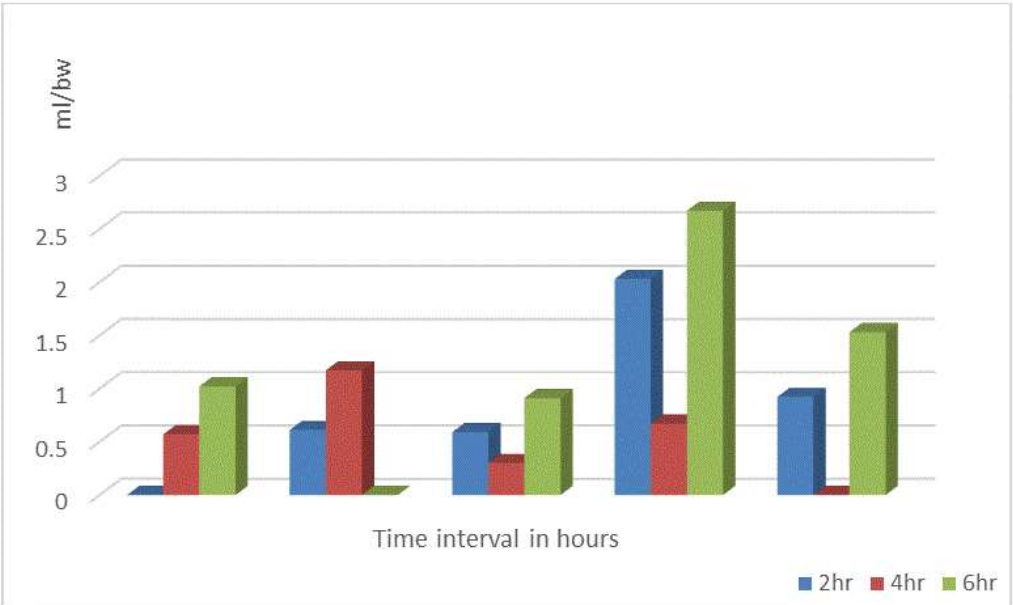

Figure 1: Urine excretion at 2, 4 and 6 h post AECONS administration

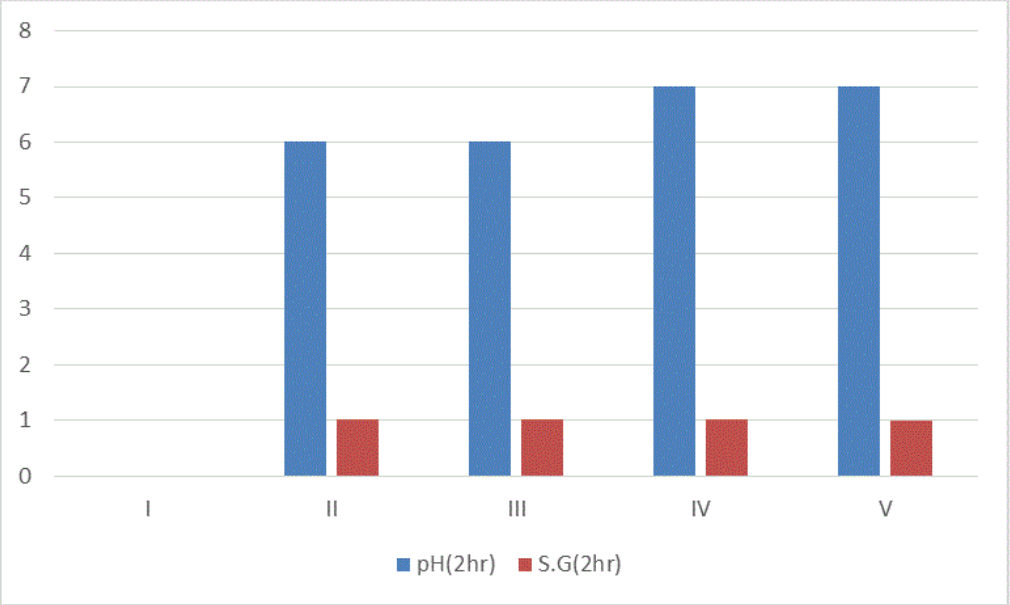

Figure 2: Two hour post AECONS administration effects in urine pH and specific gravity

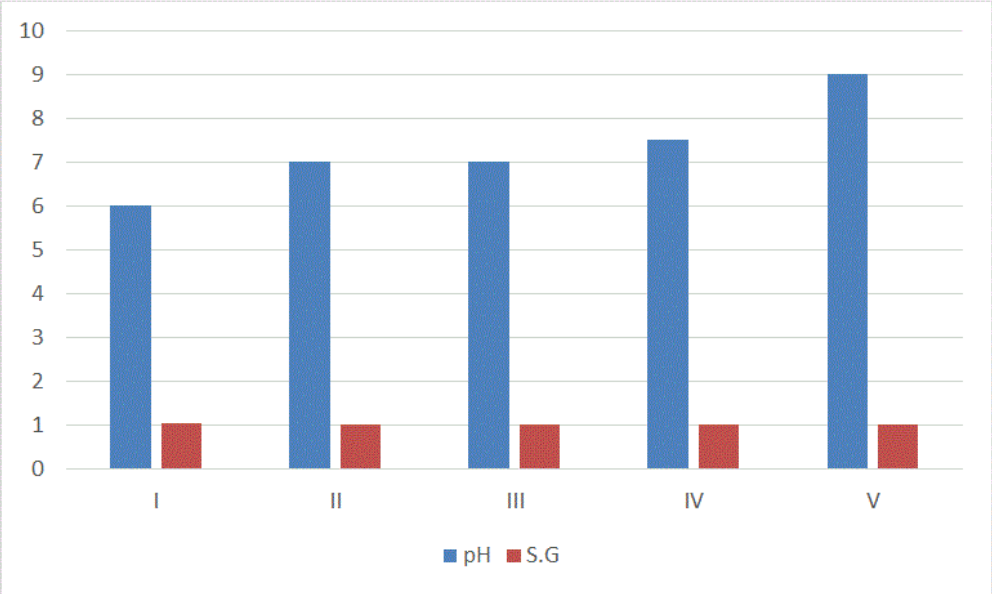

Figure 3: Three weeks post AECONS administration effects on urine pH and specific gravity

DISCUSSION

This study examined the diuretic, saliuretic, natriuretic, urine pH and specific gravity effects of three different doses of *AECONS* in female Wistar albino rats to examine diuretic index, urinalysis, serum and kidney electrolytes levels (Na^+, K^+, Cl^-). This was done to evaluate the diuretic, saliuretic and natriuretic potential of this plant extract in comparison with a standard diuretic agent (Furosemide). The results indicate that AECONS at all dose levels (400, 600 and 800 mg/kg) significantly increased urine output in a dose-dependent manner over a period of 2 h, 4 h and 6 h. Hence, good diuretic activity was observed among rats treated with furosemide and AECONS doses, except 400 mg/kg dose, where little diuretic activity was observed. AECONS also increased the urinary excretion of sodium, potassium and chloride ions significantly. Therefore, showing potency of saliuretic and natriuretic effects. Also, there was no observed carbonic anhydrase inhibition in all the groups studied. This effect may be due to the synergistic mechanism of the [$HCO3^-/Cl^-$], [$HCO3^+/H^+$] and the [Na^+/H^+] antiporter, leading to diuresis [17]. This can be explained in the light of most diuretics having weak carbonic anhydrase inhibiting activity thereby increasing urinary excretion of carbonic anhydrase (HCO_3) and phosphate [10]. Besides, the increased sodium and water excretion activity of AECONS also provides strong basis for presumed anti-hypertensive action [18] and the findings of this study is in consonance with the investigations of [19]. Consequently, a finding from this study reveals that AECONS had similar diuretic spectrum with Furosemide.

CONCLUSION

This study in reducing hypertension, body salt reduction acid-base balance essential in confirms the significant diuretic, natriuretic and saliuretic activity of the AECONS. During the measurement period of the study (6 h) which provides empirical basis for its pharmacological action clinical manifestations of some disorders. However, further studies are recommended for explaining the mechanism of diuretic activity and chronic toxicity as excessive use at high doses could lead to electrolytic depletion and water balance in the body.

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