



Diuretic, Kaliuretic and Anti-Natriuretic Properties of Aqueous Extract of *Celosia trigyna* L. (*Amaranthaceae*) on Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author TAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors YO and LB managed the analyses of the study. Authors FLD and BB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this study was to determine the diuretic and electrolyte excretion properties of the aqueous extract of *Celosia trigyna* L. (*Amaranthaceae*) on female Wistar rats.

Methodology: The extraction of active principles was done by macerating aerial parts of the plant. The administration of the extract and other products was done by single-dose gavage. Measurements of urinary flow rate (UFR), natriuria, kaliuria and chloruria were taken on urine collected for twenty-four hours after each product was administered. Diuretic activity (DA) and

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diuretic index (DI), natriuretic and saluretic effects, and carbonic anhydrase inhibition were calculated. A NaCl solution (0.9%) was used as a negative control; furosemide and aldactone were respectively used as hypokalemic and hyperkalemic positive controls.

Results: We observed a significant increase in UFR, confirmed by the values of DA and DI, obtained after the administration of extract. For electrolyte excretion, we observed an increase of the kaliuria ($p < 0.001$) and a decrease of natriuria ($p < 0.001$) after the extract was administered; chloruria did not significantly changed. We also found a drastic anti-natriuretic dose-dependent effect while saluretic activity and carbonic anhydrase inhibition were not clearly observable.

Conclusion: These results confirm the ethnobotanical data about diuretic effect of *Celosia trigyna* L. extract. This diuretic effect would be supported by a specific increase in K^+ excretion suggesting that the extract is possibly hypokalemic. The anti-natriuretic effect suggests that extract possess an aldosterone-like properties.

Keywords: *Celosia trigyna*; furosemide; aldactone; diuretic; natriuretic; kaliuretic.

1. INTRODUCTION

The species of the genus *Celosia* (*Amaranthaceae*) are edible and ornamental plants. They have various pharmacological and phytotherapeutic properties. Several studies have shown that species of the genus *Celosia* had many interesting pharmacological properties: antidiabetic, anti-inflammatory, antioxidant, antibiotic anti-diarrheal, hepatoprotective and immunostimulant, etc. But only a few species, among them, *Celosia argentea*, *Celosia cristata* and *Celosia isertii* have been well studied (phytochemistry and pharmacological activities) [1].

Celosia trigyna Linn is found in tropical and subtropical regions of Central America, Australia, Africa, and Saudi Arabia [2-4]. According to ethnobotanical data, the whole plant or its different parts are used in traditional recipes as diuretic, and against urethral disorders, heart complaints, stomach-ache, diarrhea, etc. [5-7].

Diuretic substances are of great medical interest. They are among the most prescribed on the front line against hypertension [8]. They are also used in the treatment of congestive heart failure, liver cirrhosis and various kidney disorders [3,9]. There are two types, depending on whether they decrease or increase kalemia [10]: loop diuretics, such as furosemide, induce a hypokalemia, by reducing sodium reabsorption, via an inhibition of $[Na^+/K^+/2Cl^-]$ -cotransporter [11]; the potassium-sparing diuretics, such as spironolactone (aldactone) act mainly on renal distal convoluted and collector tubules and are anti-aldosteronics [12,13].

As for the diuretic, natriuretic and saluretic properties, they have been studied by many

authors on plant extract including *Amaranthaceae* family plants [1,14]. Phytochemical constituents, nutritional and medicinal properties, and other pharmacological effects of aqueous or alcoholic extract of *Celosia trigyna*, were also studied [3,4,15-17].

But experimental studies targeting diuretic and electrolyte excretion properties of *Celosia trigyna* extract remains extremely rare. In this study, we evaluated these effects on female Wistar rats, using aqueous extract of the aerial parts of the plant.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Animal material

Female Wistar rats, 8 to 12 weeks old, provided by the pet store of Joseph KI-ZERBO University (Ouagadougou, Burkina Faso) were used. Thirty-six rats were selected (see exclusion criteria below) and then subjected to an average temperature of $22 \pm 3^\circ C$, a humidity rate of $50 \pm 10\%$, and 12 hours of lighting (06:30 a.m. to 6:30 p.m.). They had access to water and food (29% protein granules) *ad libitum*; food was provided by the Ouagadougou-based Livestock Feed Manufacturing Society.

2.1.2 Plant material

The plant material consisted of the fresh aerial parts of *C. trigyna* collected in the village of Loumbila ($12^\circ 29' 32'' N$ and $12^\circ 24' 04'' W$). The samples collected were washed with water and dried in the shade under ventilation. Once dried, the samples were powdered using an electric grinder, and the powder was macerated in

distilled water for the extraction of the active ingredients.

The plant *Celosia trigyna* L. (*Amaranthaceae*) was authenticated at the Laboratory of Plant Biology and Ecology and a specimen was deposited there under the identification number of 17965 and the specie number of 6962.

2.2 Methods

2.2.1 Preparing the aqueous extract

A 250 g sample of *C. trigyna* powder was placed in a 5000 mL stainless steel beaker containing 1500 mL of distilled water. The mixture was homogenized with a glass rod and then macerated at laboratory temperature (approximately 30°C) for 24 hours under mechanical agitation. The macerated was filtered and the filtrate was centrifuged at 2000 rpm for 10 min; the supernatant was collected, distributed in laboratory crystallizers and frozen at -18°C for 24 hours, before being freeze-dried. The obtained lyophilizate was weighed and stored in the refrigerator for the various experiments.

2.2.2 Experiments and measures

A 3-day adjustment period in an individual metabolic cage was observed during which the animals had access to water and food *ad libitum*. During this period, product administrations were simulated. All products administrations were done by single dose gavage. Animals that did not adapt or those that lost weight at the end of the three days were excluded. In addition, basic diuresis was evaluated after administration of 25 mL/kg of body weight (bw) of NaCl solution (0.9%). Animals that excreted less than 2 mL of urine within following two hours were also excluded. After that, thirty-six (36) rats were selected and divided into 6 groups of 6 animals:

- Group 1, negative control: it was administered to animals the solution of NaCl at 0.9%;
- Groups 2, 3 and 4: test batches where aqueous extract solutions of *Celosia trigyna* (AECT) were administered, at doses of 50, 100 and 250 mg/kg bw, respectively (AECT50, AECT100 and AECT250);
- Group 5: hypokalemic positive control lot where animals received a furosemide solution at 10 mg/kg bw dose;

- Group 6: hyperkalemic positive control lot where animals received an aldactone solution at 25 mg/kg bw dose.

All animals were deprived of food and water 24 hours before the first treatments (product administrations). The volumes administered have been adjusted to 50 mL/kg bw. AECT, furosemide and aldactone were dissolved in NaCl (0.9%). After treatment, each rat was placed back in its cage. Urine from each batch was collected for 24 hours after administration: urinary excretion volume was considered as urinary flow rate (UFR) measured in mL per 100 g body weight; diuretic activity (DA) was determined according to the following formula [18]:

$$DA = \frac{\text{Excreted volume}}{\text{Administrated volume}} \times 100$$

The diuretic index (DI) was calculated as a quotient, UFRt/UFRc, where UFRt is the urinary flow rate of test batch and UFRc, the urinary flow rate for control batch.

The concentration of the Na⁺, K⁺ and Cl⁻ in urine were determined using the "HumaLyte Plus³ ISE System" automaton. The [Na⁺]/[K⁺]-ratio was considered as natriuretic activity, the total urine content in Na⁺ and Cl⁻, ([Na⁺]+[Cl⁻]), as saluretic activity and the ionic quotient, [Cl⁻]/([Na⁺]+[K⁺]) ratio, as carbonic anhydrase inhibition [8,19].

2.3 Statistical Analysis

The data were entered in Excel 2016 software for calculation of mean and standard deviation. The graphics were made by the Graph Pad Prism 5.03 software. The one-way analysis of variance (ANOVA) with the post-hoc test of Tukey-Kramer using GraphPad in SAT software was used to compare the data. The difference was considered significant when p < 0.05, very significant when p < 0.01, and highly significant when p < 0.001.

3. RESULTS AND DISCUSSION

3.1 Effect of Aqueous Extract of *Celosia trigyna* on Urinary Excretion

The urinary flow rate (UFR), the volume per 100 g bw, increased significantly at all utilized doses of AECT: its increase was highly significative (p < 0.001) after the administration of AECT at 50 mg/kg bw (AECT50), significant (p < 0.05) after

that of AECT100 and very significant ($p < 0.01$) with AECT250 (Fig. 1). There was not a dose-response relationship in these results. Furosemide and aldactone administration led also to an increase in UFR ($p < 0.001$).

For diuretic activity (DA) and diuretic index (DI), their values interpretation was reported by several authors [18,20]. According to these interpretations, our results revealed moderate to important activities after administration of AECT (Table 1).

These results showed a non-dose dependent effects on urinary flow rate (UFR): AECT50 had an important diuretic activity while AECT100 and AECT250 had a moderate one. Furosemide and aldactone both resulted in an important diuretic activity.

These non-dose dependent diuretic responses were qualitatively similar of those of many other studies using different plant extracts: aqueous extract of *Amaranthus spinosus* [14]; aqueous extract of *Moringa stenopetala* [21]; essential oil of *Cymbopogon densiflorus* [8], etc. All of this could be explained by actives principles interactions and/or by their own pharmacological properties. Indeed, AECT, such as other plants extract, is a complex mixture whose constituents have different and, sometimes, contradictory properties. However, a dose-dependent increase in diuresis was also found with ethanolic extract of *Sphaeranthus indicus* [22], with aqueous

extract of trunk bark of *Lannea microcarpa* [23], etc.

A wide range of phytoconstituents such as alkaloids, glycosides, tannins, coumarinic phenolics, triterpenoids, etc. are responsible for plant extract diuretic activity [21,24,25]. And in a previous paper [26], we detected alkaloids, saponosides, glycosides, and triterpene steroids in the AECT. The diuretic properties that we found could be due to alkaloids, glycosides and triterpenoids content in our extract.

3.2 Effect of AECT on Na^+ , K^+ and Cl^- Excretion

3.2.1 The excretion of Na^+

The AECT50, AECT100, furosemide and aldactone, all showed a highly significant decrease in Na^+ concentration in urines (Fig. 2). There was no dose-response relationship in the inhibition of Na excretion by AECT.

3.2.2 The excretion of K^+

For K^+ excretion, it was observed its dose-dependent stimulation after administration of AECT. AECT50 did not induce a significant effect while AECT100 and AECT250 triggered a highly significant effect ($p < 0.001$). Furosemide also induced a highly significant increase on K^+ excretion, while aldactone had no significant effect (Fig. 3). AECT effect on kaliuria was furosemide-like one.

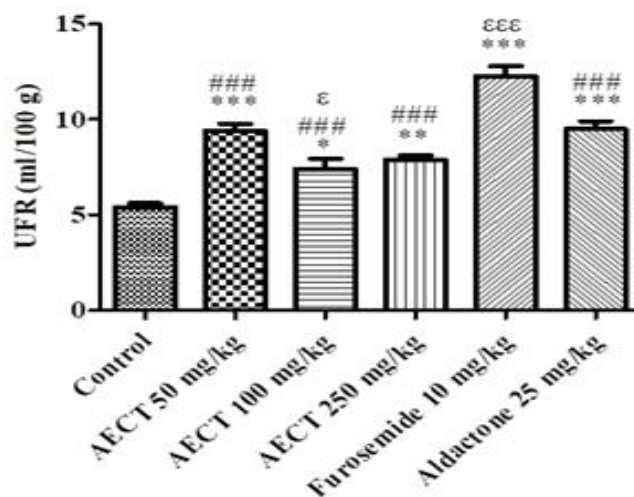


Fig. 1. Effect of AECT on urinary flow rate after 24 h (n = 6)

Compared to control: $p < 0.05$ (*), significant; $p < 0.01$ (**), very significant; $p < 0.001$ (***), highly significant.

Compared to furosemide: $p < 0.001$ (###), highly significant

Compared to aldactone: $p < 0.05$ (ε), significant; $p < 0.001$ (εεε), highly significant

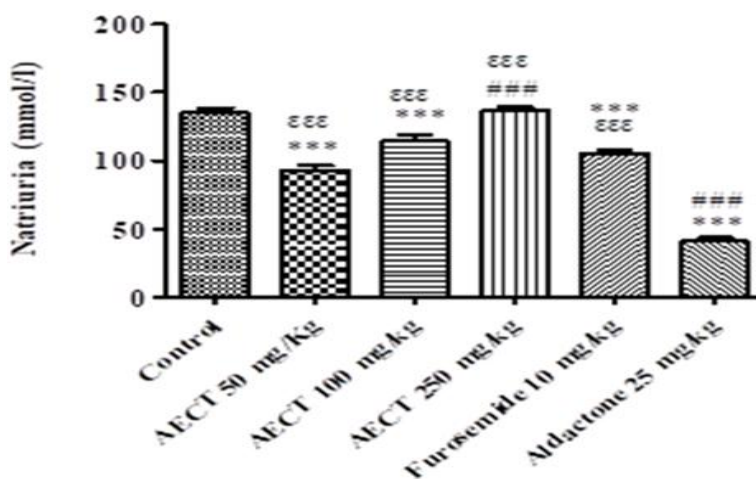


Fig. 2. Effect of AECT on natriuria (n = 6)
 Compared to control: $p < 0.001$ (***) , highly significant
 Compared to furosemide: $p < 0.001$ (###) , highly significant
 Compared to aldactone: $p < 0.001$ (εεε) , highly significant

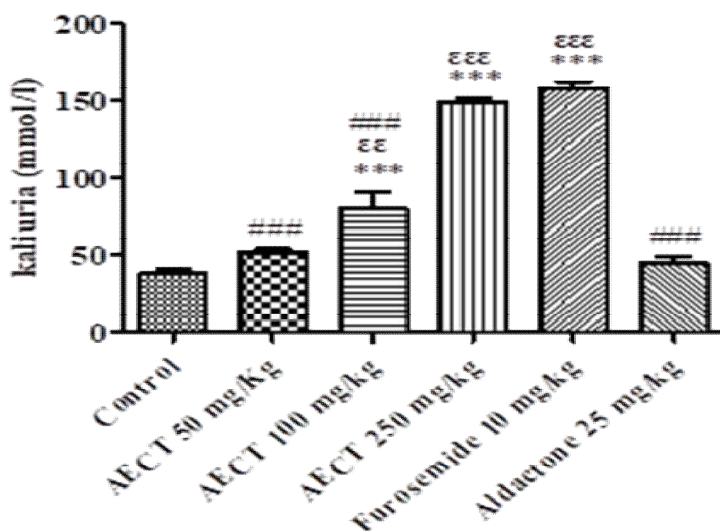


Fig. 3. Effect of AECT on kaliuria (n = 6)
 Compared to control: $p < 0.001$ (***) , highly significant
 Compared to furosemide: $p < 0.001$ (###) , highly significant
 Compared to aldactone: $p < 0.01$ (εε) , very significant; $p < 0.001$ (εεε) , highly significant

Table 1. Diuretic activity, diuretic index and interpretation

Treatment	DA (%)	DI	Interpretation
Control (NaCl 0.9%)	105.1	1,0	No activity
AECT (50 mg/kg)	187.8	1.5	Important activity
AECT (100 mg/kg)	145.6	1.4	Moderate activity
AECT (250 mg/kg)	148.5	1.2	Moderate activity
Furosemide (10 mg/kg)	244.6	2.3	Important activity
Aldactone (25 mg/kg)	188.9	1.8	Important activity

DA = diuretic activity; DI = diuretic index

3.2.3 The excretion of Cl^-

For Cl^- excretion, there was no significant effect when AECT was administered. Only animals treated with furosemide had a very significant increase in Cl^- concentration in the urine collected (Fig. 4).

Many authors found, using plant extracts, a stimulation of the excretion of all major electrolytes (Na^+ , K^+ and Cl^-) [14] with aqueous extract of *Amaranthus spinosus*, or both Na^+ and K^+ excretion with crude extract of *Trianthema portulacastrum* [27], with ethanolic extract of *Nigella sativa* and *N. damascena* [28], with extracts of *Mimosa bimucronata* [29], with methanol extracts of the root of *Euclea divinorum* [30], etc. The specific increase in kaliuria and the decrease, at the same time, of the natriuria, was unhabitual in diuresis and its related parameters study. This is an aldosteronic response [31] typically implicated in arterial pressure issues. Aldosterone is crucial in hydrosodium and potassium balance. It regulates the excretion of electrolytes and intravascular volume. It works mainly at renal distal and collector tubes levels to stimulate the reabsorption of Na^+ and the excretion of K^+ by the principal cells [32]. In this hypothesis, AECT would interfere with arterial pressure regulation via renin-angiotensin-aldosterone system. Additionally, this result suggests that the diuretic

effect of AECT in our experiments was underpinned mainly by the K^+ excretion [33].

3.3 AECT Effects on Natriuretic and Saluretic Activities and on Carbonic Anhydrase Inhibition

3.3.1 Natriuretic activity

The above results correspond to a decrease of the natriuretic activity ($[\text{Na}^+]/[\text{K}^+]$) after AECT administrations (all doses) compared to negative control ($p < 0.001$). Indeed, inhibition of natriuria and stimulation of kaliuria, both determined a drastically decrease in natriuretic activity ($[\text{Na}^+]/[\text{K}^+]$). This effect was dose dependent (Fig. 5) just like kaliuria increasing effect. Natriuretic inhibition effect was therefore determined by the increase of the K^+ excretion (fraction denominator) and not by the decrease of Na^+ excretion (fraction numerator).

Positive controls, furosemide and aldactone, both induced a highly significant decrease ($p < 0.001$) in $[\text{Na}^+]/[\text{K}^+]$ ratio.

3.3.2 Saluretic activity

For saluretic activity ($[\text{Na}^+]+[\text{Cl}^-]$), only the AECT50 (and aldactone) induced its significant decrease ($p < 0.001$). See Fig. 6.

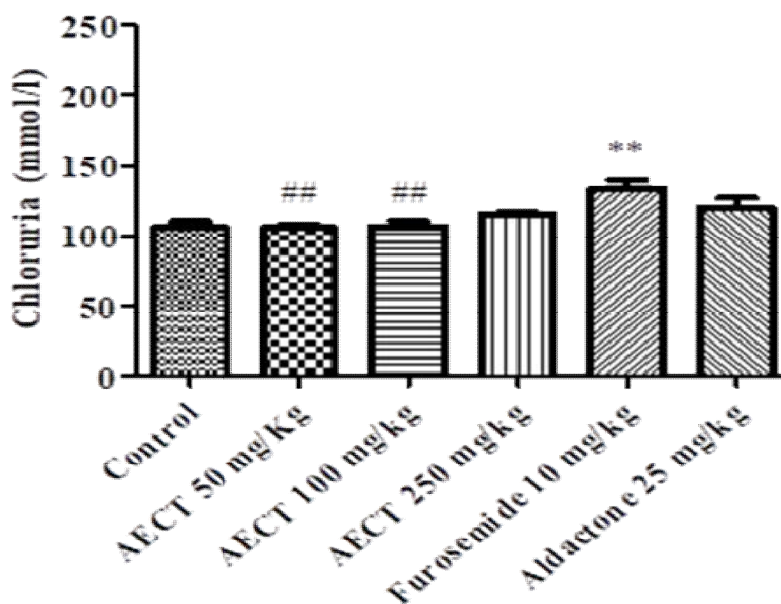


Fig. 4. Effect of AECT on chloruria (n = 6)
 Compared to control: $p < 0.01$ (**), very significant
 Compared to furosemide: $p < 0.01$ (##), very significant

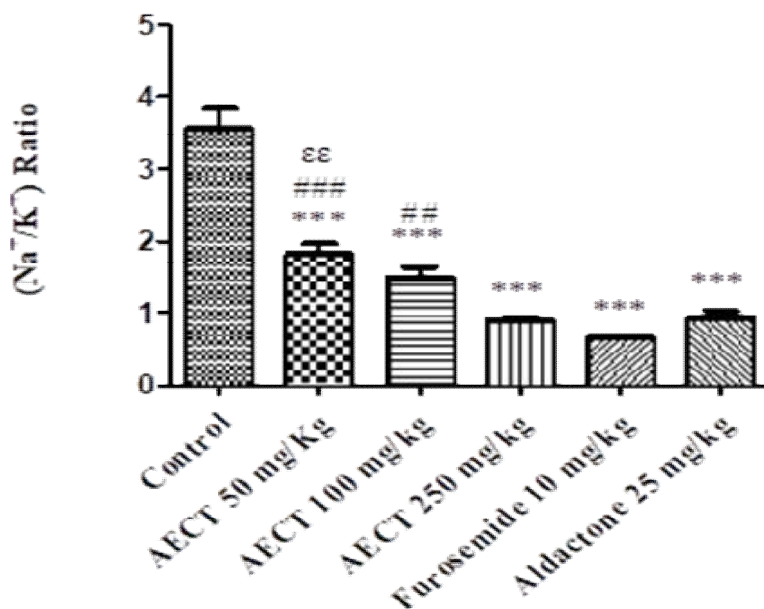


Fig. 5. Effect of AECT on natriuretic activity (n = 6)

Compared to control: $p < 0.001$ (***), highly significant
 Compared to furosemide: $p < 0.01$ (##), very significant
 Compared to aldactone: $p < 0.01$ (εε), very significant

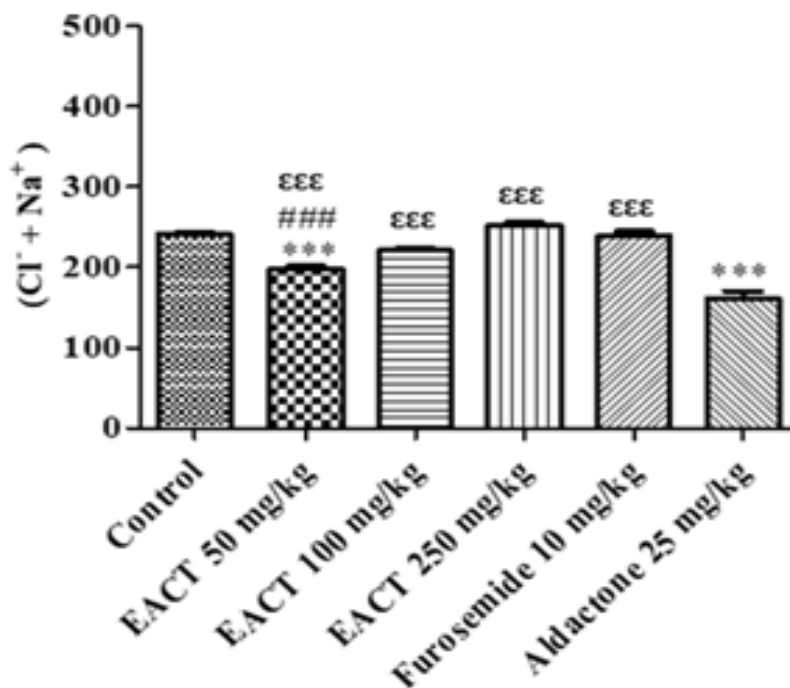


Fig. 6. Effect of AECT on saluretic activity (n = 6)

Compared to control: $p < 0.001$ (***), highly significant
 Compared to furosemide: $p < 0.001$ (###), highly significant.
 Compared to aldactone: $p < 0.001$ (εεε), highly significant

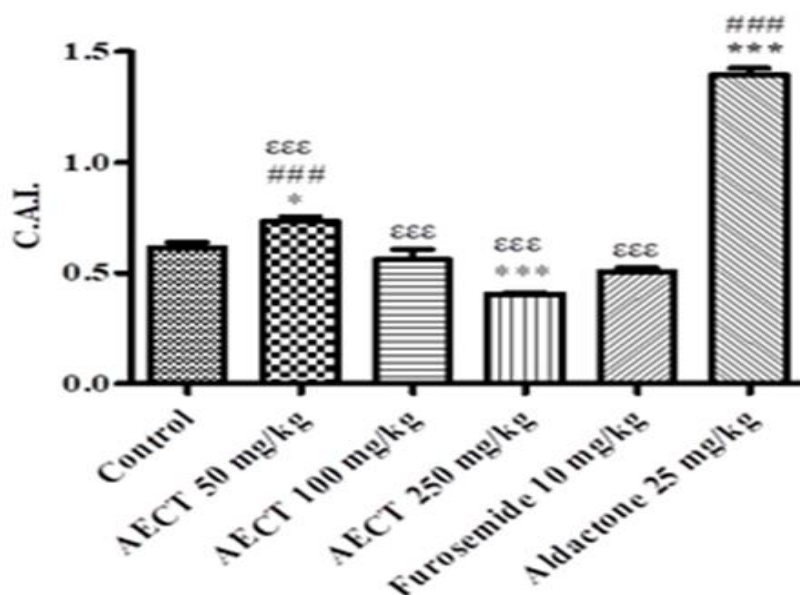


Fig. 7. Effect of AECT on carbonic anhydrase inhibition (CAI)
 Compared to control: $p < 0.05$ (*), significant; $p < 0.001$ (***), highly significant
 Compared to furosemide: $p < 0.001$ (###), highly significant
 Compared to aldactone: $p < 0.001$ (εεε), highly significant

Saluretic activity of our extract might be determined by natriuria; it had significantly decreased with the AECT50 but not at higher doses.

3.2.3 Carbonic anhydrase inhibition

As to carbonic anhydrase inhibition, $[Cl^-]/([Na^+]+[K^+])$, the effect of AECT administration was contrasted, compared to negative control: it was observed a increasing effect with AECT50 ($p < 0.05$) and a decreasing one with AECT250 ($p < 0.001$).

Furosemide had no significant effect on the CAI while aldactone induced its highly significant increase (Fig.7).

Similar results have already been reported: an increase of CAI with extract of *Amaranthus spinosus* [14], with aqueous extract of *Moringa stenopetala* [21]. In these cases, it was without dose-response relationship. In our results, diuretic effect could not be underpinned by CAI [34,35].

4. CONCLUSION

Our results about diuresis confirm ethnobotanical data and corroborate the traditional medicinal

use of this plant as a diuretic, and against related diseases. The stimulation of kaluria is furosemide-like effect and, on this basis, AECT could be considered as a hypokalemic loop diuretic. The stimulation of K^+ excretion and the inhibition of natriuria, at the same time, are aldosterone-like effect. The main sites of action of AECT would be the Henle loop and principal cells of distal and collecting renal tubules. However, these results must be deepened by other studies in several directions: AECT effect on blood ionogram, utilization of extract fractions, study of interactions with related references substances, etc.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animals' procedures were strictly within national laws and guidelines. The approval number for the research protocol is CE-UJKZ/2020-05.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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