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Diuretic Activity and Toxicological Assessment of the Aqueous Extract from the Aerial Part of *Commelina diffusa* (Commelinaceae) in Rats

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ABSTRACT

Background: *Commelina diffusa* (*C. diffusa*) is a plant, traditionally used in Africa and Asia to treat hypertension, pain and renal diseases. The aim of the present study was to evaluate the diuretic potential and toxicological profile of the aqueous extract from the aerial part of *Commelina diffusa* (AECD). **Materials and Methods:** The aqueous extract was prepared by decoction of the powder obtained from the aerial part of *C. diffusa* and administered orally at the doses 100 and 200 mg kg^{-1} b.wt. for the study of diuretic activity in male rats. For the acute toxicity study 1.5, 3, 6 and 12 g kg^{-1} b.wt. were used, while 150, 300 and 600 mg kg^{-1} b.wt. were used for the sub chronic toxicity study in mice for 4 weeks. Furosemide, the reference drug (20 mg kg^{-1}), was used as the positive control during the experiment. Blood sample collected was used to evaluate the concentrations of total proteins, creatinin and liver transaminases: Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), while the urine sample collected was used to evaluate Na^+, K^+ and creatinin concentrations. **Results:** After acute and 7 day subchronic diuretic study, AECD at all doses and furosemide produced important and significant increments in urinary excretion of water and sodium in rats with respect to control group. AECD also induced K^+ excretion increased but it was no significant and less than the one induced by furosemide. During the toxicity study, period, AECD may be devoided of acute toxicity and any other adverse effects, however, subchronic toxicity study revealed a significant increase in water consumption, ALT concentration and total proteins in serum. **Conclusion:** The strong diuretic, natriuretic properties and its K^+ sparing effect make the AECD to be regarded as a good diuretic confirming their ethno pharmacological use. Also, AECD was found to be practically nontoxic.

Key words: *Commelina diffusa*, diuretic activity, urine output, acute, chronic toxicity, furosemide

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INTRODUCTION

A diuretic is any substance which increase urine and solute excretion, thus, used to remove excess extra cellular water from edematous tissues (Sravani *et al*., 2010). They act either by increasing the glomerular filtration rate or by decreasing the rate of reabsorption of fluids from the tubules (Balasubramanian *et al*., 2009). They represent one of the classes of drugs most prescribed because of the strong correlation between fluid retention and diseases such as hypertension, heart failure, nephrotic syndrome, body fluid imbalance and pulmonary edema (Katedeshmukh *et al*., 2010). Loop diuretics such as furosemide are the most prescribed diuretics (Lahlou *et al*., 2007). They inhibit sodium and chloride transport in the renal tubule and lead to an increase in salt and water excretion (Danamma *et al*., 2011). However, these synthetic drugs are responsible of a number of adverse effects, including hypovolemia, hypokalemia, hyponatremia, electrolyte imbalance, metabolic alterations, development of new onset diabetes, activation of the renin-angiotensin-neuroendocrine systems and impairment of sexual function or deterioration of the reproductive system (Gupta and Neyses, 2005; Morganti, 2005; O'Brien *et al*., 2005). Hence, there is a need for new diuretics with less adverse effects.

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Medical plants play an important role in primary health care and as a reservoir of active principles, thus, they represent useful tools for the discovery of well tolerated new drugs. *Commelina diffusa* is a member of the family Commelinaceae, known to be a climbing dayflower or spreading dayflower, is an herbaceous plant of the dayflower family. It is used in the treatment of various ailments such as tuberculosis, veneral diseases, otitis, malaria, leprosy, dysentery and heart problems (Lans, 2007; Isaac and Brathwaite, 2007; Khan *et al*., 2011). *C. diffusa* is traditionally used in Africa and Asia to treat hypertension, pain and renal diseases (Hong *et al*., 2000; Faden, 2006). The empirical use of *C. diffusa* is to handle hypertension and heart problems which are conditions that require the prescription of diuretics, motivated this study which general objective is to evaluate the diuretic activity and the toxicological profile of the aqueous extract from the aerial part of *Commelina diffusa* (AECD).

MATERIALS AND METHODS

Experimental animals: Adult male Wistar rats, *Ratus norvegicus* weighing 180-250 g and adult mice, *Mus musculus* weighing between 20 and 25 g of both sexes were used in this study. Animals were raised in the animal house of the Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang, Cameroon, under environmental conditions. The animals had free access to tap water and standard laboratory rat food. Diuretic experiment was carried out in rats while mice were used to evaluate the toxicological profile.

Ethical consideration: Experimental protocols used in this study were approved by the Laboratory committee (Laboratory of Animal Physiology and Phytopharmacology, Department of Animal Biology, University of Dschang, Cameroon) according to the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive (EEC,1986).

Preparation of plant extract: The aerial part of *Commelina diffusa* was collected in Dschang, Menoua Division (west Region of Cameroon), in October 2011. The plant material was identified at the National Herbarium of Cameroon where a voucher specimen number SRFC/35189 was deposited. The aerial part of *C. diffusa* was dried at room temperature (24-25°C) away from sun light and ground into a coarse powder using an electric blender. Five hundred grams (500 g) of *C. diffusa* powder were boiled in 5 L of distilled water at 100°C for 20 min. The decoction obtained was filtered with a Whatman paper (pore size 3 μm). The filtrate was evaporated in a ventilated oven heated at 40°C. The 56 g of the dry extract was obtained and thus, correspond to an extraction yield of 11.2 %. The extract was then stored at room temperature for further studies. This extract was dissolved in distilled water upon administration.

Assessment of the diuretic activity in rats

Acute diuretic activity: Before the commencement of the experiment, each rat was placed in an individual metabolic cage for 48 h for adaptation purposes. The day before the experiment, animals were fasted overnight with free access to water only. Prior to the oral administration of the different test drugs, all animals received physiological (normal) saline (0.9% NaCl) at an oral dose of 10 mL kg^{-1} body weight (b.wt.), to impose a uniform water and salt load (Benjumea *et al*., 2005). Twenty four male rats were then assigned to four groups of six animals each. The first group received 10 mL kg^{-1} b.wt. of distilled water and serves as control. The second group (positive control) received 20 mg kg^{-1} b.wt. of furosemide (Lasilix, NOVARTIS) used as the reference drug. Groups 3 and 4 were administered 100 mg and 200 mg kg^{-1} b.wt. of AECD, respectively. All drugs were administered orally with a gavage needle.

During this experiment, 24 h urine was collected and measured after administration of the different substances. Sodium and potassium concentrations were determined in the daily urine sample of each rat (Patel et al., 2009; Jeunesse *et al*., 2007). The natriuretic activity was calculated using the ratio Na^+/K^+.

Subchronic diuretic activity: AECD (100 and 200 mg kg^{-1} b.wt.) and furosemide (20 mg kg^{-1} b.wt.) were administered to rats for 7 days. For each rat, 24 h urine was collected daily and its volume measured. Urinary sodium and potassium ions concentrations were measured by flame photometry in each urine specimen. Creatininemia and creatininuria levels were measured in rats on day 7 with an assay kit (Wako Pure Chemical Industries, Osaka, Japan) by the Jaffe method, with the use of a spectrophotometer and its clearance was calculated (Patel *et al*., 2009).

Biochemical analysis: At the end of day 7, blood was collected in test tubes containing ethylene-diamine tetraacetate by cardiac puncture under diazepam/ ketamine hydrochloride anesthesia at the dose of 0.2 and 0.1 mL^{-1} 100 g b.wt. for diazepam and ketamine hydrochloride, respectively. Plasma was obtained by centrifugation at 3000 rounds min^{-1} for 20 min and stored at -20°C until analyzed for biochemical parameters. Natriuria and kaliuria were quantified using flame photometry method. Total proteins, hepatic and serum transaminases (ALT and AST) were also quantified using standard assay kits (Roche Diagnostics

GmbH, USA). Plasma and urine creatinin concentrations were determined using Jaffe's method and creatinin clearance was calculated using the equation below:

$$\text{Creatinin clearance (mL min}^{-1}) = \frac{\text{Urine creatinin} \times \text{diuresis}}{\text{Toxicity study}}$$

Acute toxicity test in mice: In order to study any possible toxic effect or change in normal behavior, mice used in this experiment were divided into 5 groups of 6 animals each (3 males and 3 females) and were acclimatized for one week before the beginning of the experiment. They were fasted for 24 h but had free access to water. The plant extract (in mg) was diluted to the 10th with distilled water (in mL) and the volume administered was adjusted to 0.5 mL^{-1} 25 g b.wt. The animals were treated as follow: The first group received distilled water and serves as the control. Groups 2, 3, 4 and 5 received single doses of 1.5, 3, 6 and 12 g kg^{-1} b.wt. *per os* of the aqueous extract, respectively. Animals were observed for mortality and general behavioral changes, 6 h after treatment and the observation continued for a period of 7 days and monitored daily for changes in body weight, food and water consumption and for any sign of toxicity (Sireeratawong *et al*., 2013).

Subchronic toxicity: Animals were divided into four groups of six mice each. The first group, served as control group received distilled water while groups 2-4 received aqueous extract of *C. diffusa* at the dose of 150, 300 and 600 mg kg^{-1} of b.wt., respectively. Each animal received orally the plant extract once every 2 days for a period of 28 days. Animals were observed for signs of abnormalities during the treatment period. Besides, the body weight, food and water consumption were recorded at the end of each week during 4 weeks. On the last day of treatment, animals were fasted overnight but had free access to water. They were anesthetized with Diazepam/ketamine hydrochloride (0.2 mL^{-1} 100 g of b.wt. diazepam and 0.1 mL^{-1} 100 g of b.wt. of ketamine hydrochloride) and blood was collected without anticoagulant by cardiac puncture using capillary tubes for biochemical studies. After blood collection, organs such as heart, liver, lung, kidney and spleen were collected, blotted dry and the relative organ weight compared to that of control (Atsamo *et al*., 2011). Following this, only the liver was homogenized in phosphate buffer solution (0.1 M, pH 7.40). Fifteen gram of tissue was homogenized with 85 mL of buffer. The homogenates were centrifuged at 3000 rpm for 20 min and the supernatant was aliquoted and stored at -20°C for further biochemical analysis. Total proteins, Alanine Aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined in serum and liver tissue using standard assay kits (Roche Diagnostics GmbH, USA).

Statistical analysis: Results are expressed as the Mean±S.E.M. Data were analyzed by one way ANOVA (creatinin clearance) and two ways repeated measures (urine volume and electrolyte excretion) followed by Bonferroni post test. Probabilities less than 0.05 ($p<0.05$) represent signiflcant differences.

RESULTS

Acute diuretic activity

Effects of the AECD on the daily urine output and on electrolyte excretion: As shown in Table 1, furosemide increased urine volume by 63.35 mL^{-1} day (59%) compared to control. A significant increase in the production of urine was observed at the doses of 100 and 200 mg kg^{-1} of b.wt. of the aqueous extract by 56.84 mL^{-1} (54%) and 102.66 kg day^{-1} (74%), respectively, as compared to control (25.83 mL^{-1} kg day^{-1}).

Table 1 also shows the urinary electrolyte content following the administration of the extracts. AECD produced at the doses of 100 and 200 mg kg^{-1}, a highly significant ($p<0.001$) increase in Na^+ excretion, compared with the control group. Only Furosemide (20 mg kg^{-1}) increased significantly ($p<0.01$) the excretion of potassium.

Subchronic diuretic activity

Effect of the AECD on urine volume and urinary electrolyte excretion: In the chronic experiment, similar to furosemide, the AECD at all doses showed an increase in diuresis. Significant ($p<0.001$) increased in urine volume production was observed for the first 4 days with a peak of 65.87 mL^{-1} kg day^{-1} obtained on day one at the dose of 200 mg kg^{-1}, during 7 days of treatment compared to the control (26.52 mL^{-1} kg^{-1} day^{-1}) (Table 2).

Table 1: Diuretic effect of the single oral doses of AECD on the urine volume output and electrolyte concentration in rats at the 24th h

Groups	Urine volume (mL day^{-1})	Na^+ conc. in urine (mg L^{-1} kg^{-1})	K^+ conc. in urine (mg L^{-1} kg^{-1})	Natriuretic activity Na^+/K^+
Control 10 mL kg^{-1}	25.83±0.75	23.97±0.14	12.70±0.23	1.40±0.93
AECD 100 mg kg^{-1}	56.84±5.38[b]	166.28±1.90[c]	37.66±0.31	8.30±5.30
AECD 200 mg kg^{-1}	102.66±4.26[c]	123.72±1.90[c]	37.67±3.47	4.80±1.70
Furo 20 mg kg^{-1}	63.53±4.06[c]	131.58±3.75[c]	61.97±2.60[b]	4.10±1.90

Furo: Furosemide, Values are expressed as Mean±SEM (n = 6), [b]$p<0.01$, [c]$p<0.001$ compared with the control

Table 2: Subchronic diuretic activity of AECD and Furosemide on daily urine volume, sodium and potassium ions concentrations in rats during 7 days of treatment

	Time (days)						
Groups	1	2	3	4	5	6	7
Urinary volume							
Control 10 mL^{-1} kg^{-1}	26.52±0.52	28.18±0.17	26.44±0.42	21.90±0.01	24.05±0.02	23.36±1.1	20.16±0.38
AECD 100 mg kg^{-1}	46.74±2.84^{a}	49.60±1.09^{a}	42.55±8.75	34.74±5.36	38.90±3.92	36.50±2.93	34.36±2.18
AECD 200 mg kg^{-1}	65.85±1.92^{c}	59.60±2.74^{c}	53.40±4.47^{c}	44.25±4.37^{c}	38.88±4.75	34.97±4.05	35.28±4.96
Furosemide 20 mg kg^{-1}	55.16±1.89^{c}	58.68±2.69^{c}	46.30±4.49^{a}	42.98±8.57^{a}	33.91±9.03	31.05±6.48	40.67±3.79^{b}
Sodium							
Control 10 mL^{-1} kg^{-1}	62.20±0.53	81.32±0.12	79.08±0.42	62.20±0.17	44.60±0.92	53.64±1.11	34.80±0.38
AECD 100 mg kg^{-1}	166.30±2.84^{a}	121.32±1.09	399.10±8.25^{cz}	258.34±5.30^{cx}	164.34±3.27^{bz}	160.90±2.93^{ay}	144.12±2.90^{a}
AECD 200 mg kg^{-1}	133.72±1.92	182.70±2.74	228.00±4.07^{c}	221.13±4.34^{z}	218.60±4.25^{cz}	201.90±4.05^{c}	216.24±4.96^{c}
Furosemide 20 mg kg^{-1}	131.58±1.89	180.02±2.30	243.56±4.49^{c}	375.70±8.57^{c}	375.72±9.05^{c}	290.80±6.50^{c}	173.47±3.79^{b}
Potassium							
Control 10 mL^{-1} kg^{-1}	12.70±2.08	12.70±0.17	14.18±0.61	10.82±1.42	5.64±4.02	7.12±2.85	12.30±0.49
AECD 100 mg kg^{-1}	37.67±1.74	23.53±0.75^{z}	9.40±0.33^{z}	23.53±0.88	37.67±2.23	30.60±1.64	23.53±2.98
AECD 200 mg kg^{-1}	37.67±0.45	16.46±0.26^{z}	30.67±1.14	30.67±1.38	23.53±1.25	16.48±0.65^{y}	9.40±0.20
Furosemide 20 mg kg^{-1}	61.93±3.44^{b}	82.12±4.91^{c}	69.03±3.83^{c}	61.96±3.57^{b}	49.76±3.08^{a}	69.03±4.32^{c}	44.74±2.26^{a}

Values are expressed as Mean±SEM (n = 6), ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 compared to the control, ^{x}p<0.05, ^{y}p<0.01, ^{z}p<0.001 compared with Furosemide

Table 3: Variation of creatininemia and creatinin clearance on the 7th day of AECD and Furosemide treatment in rats

Parameters	Control 10 mL kg^{-1}	AECD 100 mg kg^{-1}	AECD 200 mg kg^{-1}	Furosemide 20 mg kg^{-1}
Creatininemia (mg kg^{-1})	54.60±2.42	75.12±5.22	84.00±5.07	106.00±4.56^{b}
Creatinin clearance (mL min^{-1})	2.53±0.98	1.94±0.36	1.60±0.78^{x}	5.76±1.24

Values are expressed as Mean±SEM (n = 6). ^{b}p<0.01 compared with the control, ^{x}p<0.05 compared with the furosemide group

For the electrolytes excretion (Table 2), the administration AECD provoked a highly significant (p<0.001) increase in the variation of sodium ions during the whole period of treatment. The dose 100 mg kg^{-1} b.wt., induced a prominent increase of 399.10 mg kg^{-1} b.wt. of Na^{+} obtained on day three compared to the control (79.08 mg kg^{-1} b.wt.) corresponding to a percentage increase of 80.18%. In contrast, the plant extract did not cause any significant variation in potassium ions compared to the control, whereas furosemide induced a significant (p<0.01) increase in potassium ions excretion compared to the control during the entire period of treatment.

Effect of the AECD on creatinin clearance: As shown in Table 3, creatinin clearance nor creatinemia measured on the last day of the AECD treatment did not show any variation compared to the control but compared to furosemide, we observed a significantly (p<0.05) decreased of creatinin clearance at the dose 200 mg kg^{-1} b.wt. of AECD. Similarly, apart from furosemide, no variation was observed on urine creatinin concentration after repeated administration of AECD compared with the control group (Fig. 1).

Acute toxicity of the aqueous extracts of *Commelina diffusa* (AECD): The results obtained within the 7 days of observation in acute toxicity showed no mortality, nor any sign of behavioural changes, at the doses of 0, 1.5, 3 and 6 g kg^{-1} b.wt. of AECD administered. However, at the dose of 12 g kg^{-1} b.wt., a decrease in locomotion, aggressiveness and sensitivity to noise were noticed and one dead was observed on the first day of treatment as shown in Table 4. All the observed signs appeared at least 1 h post treatment. The body weight, food and water consumption of mice treated with AECD for 7 days did not undergo significant variations compared with animals of the control group.

Table 4: Acute effect of AECD on behavioral parameters administered orally on mice

Dose (g kg^{-1})	D/T	Latency	Symptoms
0	0/6	-	None
1.5	0/6	-	None
3	0/6	-	None
6	0/6	-	None
12	1/6	1 h<signs<24 h	Locomotion↓, exploration↓ Aggressiveness↓, touch Sensibility↓, pain sensibility↓

D/T: Dead/treated mice, None: No toxic symptoms, Latency: Time at which appear toxic sign after treatment, ↓: Decrease

Subchronic toxicity of AECD

Effects of the AECD on body weight, water and food consumption: As shown in Table 5, a highly significant (p<0.001) increased in water consumption was observed independently of the dose administered, as from the 1st-4th week of treatment compared to the control. Whereas, no significant variation in the average food consumption and body weight were observed at the end of the experiment in all batches.

Table 5: Effects of the AECD on food consumption, water consumption and relative b.wt. during subchronic toxicity study

Parameters	Time (week)	Doses: Control 10 mL kg^{-1}	150 mg kg^{-1} b.wt.	300 mg kg^{-1} b.wt.	600 mg kg^{-1} b.wt.
Food consumption (g^{-1} rat week^{-1})	1	19 .00±0.94	20.00±0.60	19.00±0.10	20.00±0.90
	2	17.00±0.05	19.00±1.40	18.00±0.80	19.00±1.20
	3	16.00±0.02	20.00±2.50	18.00±1.50	18.00± 1.30
	4	17.00±0.95	20.00±1.90	18.00±0.70	17.00±0.50
Water consumption (mL^{-1} rat week^{-1})	1	8.30±0.40	12.00±3.60^{b}	13.00±4.50^{c}	13.00±4.20^{c}
	2	7.30±0.40	11.00±3.60^{b}	13.00±4.80^{c}	13.00±4.80^{c}
	3	7.30±0.40	11.00±3.60^{b}	12.00±3.90^{b}	12.00±4.20^{c}
	4	7.00±1.40	12.00±4.50^{c}	12.00±4.50^{c}	12.00±4.50^{c}
Relative body weight (g)	1	108.00±0.90	105.00±0.74	100.00±2.00	101.00±1.90
	2	101.00±0.03	102.00±0.37	105.00±1.20	107.00±1.60
	3	98.00±0.93	99.00±0.42	103.00±1.40	94.00±0.90
	4	104.00±0.67	101.00±0.85	100.00±1.20	104.00±0.05

Values are Mean±SEM (n = 6) $^{b}p<0.01$, $^{c}p<0.001$ significant difference with respect to the control group

Table 6: Variation of total proteins and hepatic enzymes (ALT an AST) in the serum and liver of mice treated with AECD in subchronic toxicity

Parameters	Control 10 mL kg^{-1}	AECD (mg kg^{-1}): 150	300	600
Serum				
ALT (IU L^{-1})	17.71±0.09	14.48±0.01	20.83±2.92	25.59±0.09
AST (IU L^{-1})	57.92±2.69	46.77±7.35	51.27±15.12	87.92±1.12
TP (g L^{-1})	70.89±5.98	86.92±5.11	128.9±19.62^{a}	119.9±17.76
Liver				
ALT (IU L^{-1})	26.22±6.53	39.40±4.01	38.73±4.01	46.53±6.01^{b}
AST (IU L^{-1})	37.47±0.24	28.30±0.69	17.15±0 .39	37.08±1.19
TP (g L^{-1})	11.95±0.10	11.31±0.1	10.92±0.12	11.83±0.1

ALT: Alanine amino transferase, AST: Aspartate amino transferase, TP: Total proteins. Values are Mean±SEM (n = 6), $^{a}p<0.05$, $^{b}p<0.01$ significantly different with respect to the control

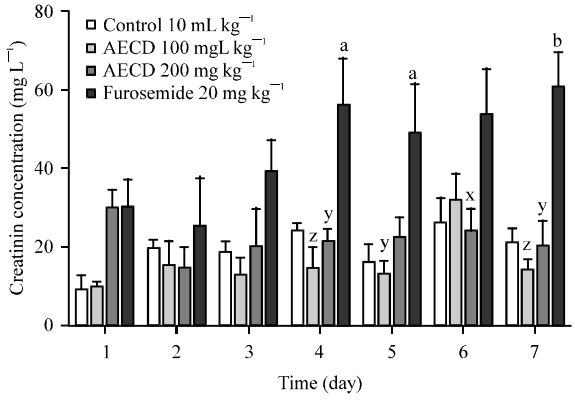

Fig. 1: Effect of the AECD and Furosemide on the variation of creatinin concentration after 7 days treatment. $^{a}p<0.05$; $^{b}p<0.01$ compared with the control group; $^{x}p<0.05$; $^{y}p<0.01$; $^{z}p<0.001$ compared with Furosemide

Effect of the AECD on some relative organs weight: The animals treated within 28 days with AECD presented at all doses no significant change in relative organ weight, as compared to control group. Macroscopic analysis of target organs of treated animals (liver, heart, lung, kidney and spleen) did not show signiflcant changes in color and texture when compared with the control group (Fig. 2).

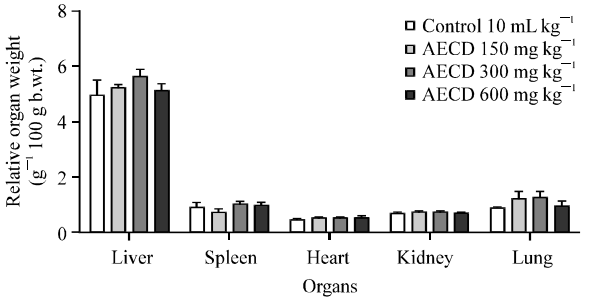

Fig. 2: Effect of AECD on the relative organ weights of mice after 4 weeks oral dosing. Each data column represents the Mean±S.E.M. (n = 6)

Effect of the aqueous extract on some biochemical parameters: A significant increase ($p<0.001$) in serum and liver ALT concentration was observed at the dose of 600 mg kg^{-1} b.wt. after the administration of AECD for 28 days treatment. More so, the serum total protein was significantly increased ($p<0.05$) at the dose of 300 mg kg^{-1}, as shown in Table 6.

DISCUSSION

C. diffusa is a plant used worldwide in traditional medicine for the treatment of various ailments. The present study was undertaken to evaluate the diuretic activity and to determine the toxicological profile of the aqueous extract from the aerial part of *C. diffusa*.

A diuretic is a substance which increases urine and solute excretion and to be therapeutically useful, it should increase the output of sodium and water, likewise their use in the treatment of diseases related to fluid retention (Camargo *et al*., 2004). Saluretic diuretic, furosemide (the reference drug), increases urine output and urinary excretion of sodium by inhibiting $Na^+/K^+/2Cl^-$ co-transporter at the level of the thick ascending limb of the loop of Henle (Meera *et al*., 2009). Also, Loop diuretics are renowned for their saluretic activity. Results from the acute diuretic study shows that maximum diuretic activity with the aqueous extract of *C. diffusa* was observed at the dose of 100 mg kg^{-1} b.wt. when administered orally. A similar pattern of diuresis is reported with some other plant diuretics: For instance, the ripe fruits of the *Carum carvi* and leaves of *Tanacetum vulgare* (Lahlou *et al*., 2008).

As from the third day of subchronic treatment, the AECD produced an effect on diuresis and urinary excretion of Na^+ similar to that of furosemide but at a lower potency which may be due to the crude nature of the extract. Similar to furosemide, it is likely that the active principle of the aqueous extract from the aerial part of *C. diffusa* has a furosemide-like action in terms of sodium ions excretion. However, the excretion of potassium ions was reduced as compared to the controlwhich is a very essential quality of a good diuretic agent with lesser hypokalemic side effect (Pawar *et al*., 2009). There are correspondences between the volume of urine and the concentration of Na^+, this aspect is logical because the excretion of this ion in urine increases its osmotic potential, thus responsible for the movement of water molecules alongside with it. Other explanation that can support this idea is the high ion concentrations in this medicinal plant (Cardenas *et al*., 2006). Despite these diuretic and natriuretic properties of the plant extract, there was no significant effect of repeated administration of the AECD on renal creatinin clearance, suggesting that, the plant extract has no renal toxicity at the studied dose. In addition, no apparent toxicity was observed in the rats during the 7 days of repeated dosing with the plant extracts. Nevertheless, additional studies have to be carried out to confirm the lack of renal toxicity and to rule out other organ toxicity, especially after chronic administration (Lahlou *et al*., 2007). These interesting diuretic properties of AECD have made us to evaluate its toxicological profile.

Acute toxicity showed no adverse effect up to the dose of 6 g kg^{-1} b.wt. However at the higher dose of 12 g kg^{-1} b.wt., a hypo activity characterized by a reduction in aggressiveness, locomotion and pain sensitivity was noticed. The reduction in pain sensitivity could be due to the effect of the AECD at the level of the free nerves ending of the skin, or by inhibiting the action of prostaglandin, synthesized from arachidonic acid, on its receptor. Hence, depressing the transmission of nociceptic impulses to the cerebral trunk (Nguessom *et al*., 2013). This could confirm the use of the decoction from the aerial part *of C. diffusa* in China to cure pain (Khan *et al*., 2011). Apart from one dead recorded with the higher doses (12 g kg^{-1}) 24 h after administration of AECD, all the other doses did not cause any d eath of the animals which survived beyond 14 days. The minimal lethal concentration (LD50) was found to be superior to 12 g kg^{-1} b.wt. Therefore, it can be suggested that AECD is devoid of acute oral toxicity.

As in acute toxicity studies some behavioral disturbances were recorded in subchronic toxicity. Indeed, treated animals showed an attitude of general body weakness at the end of the third week of treatment at higher dose with one death that occurred on day 21 at the dose of 600 mg kg^{-1} b.wt. This weakness may be due to dehydration of the treated animals (Ogata *et al*., 2004). No significant change in body weight of the animals has been reported after 4 weeks of treatment compared to the control. Also, no significant change in food consumption was observed at all doses of the aqueous extract. In contrast, water consumption has risen sharply during the period of treatment compared to the control. This result shows that the repeated administration of the aqueous extract of *C. diffusa* would lead to dehydration in mice due to its diuretic properties (Hong *et al*., 2000). Dehydration of the mice could lead to an increase in the osmolarity of extracellular fluids. This increase in osmolarity allowed osmoreceptors of the hypothalamus to stimulate the sensation of thirst (Meera *et al*., 2009), hence the increase in water consumption recorded throughout the treatment.

At the end of the 4th week of treatment with the aqueous extract of *C. diffusa*, during the subchronic toxicity study, macroscopic analysis of the relative organs weight such as the kidneys, heart, lungs, liver and spleen shows no significant difference between control and AECD treated groups. However, usual markers of liver toxicity (ALT and AST) reveal a significant increase level of ALT in the blood and liver of mice treated with higher doses (300-600 mg kg^{-1} b.wt.) of the aqueous extract of *C. diffusa*. This could be due to the damaging effect provoked by the AECD on hepatic cells in the treated groups (Ho *et al*., 2012). The significant increase in serum total protein could be due to the excessive loss of water from extracellular tissues, thus, increasing protein concentration in plasma.

CONCLUSION

The results obtained in this study provide a quantitative base to explain the traditional folkloric use of *C. diffusa* as a diuretic agentwhich might act by reducing the renal reabsorption of sodium ions in addition to potassium sparing effect and its relatively non toxic property. This good diuretic property justifies its use among the Cameroonian population and elsewhere.

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