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Diuretic activity of aqueous extract and smoothie preparation of *Verbesina crocata* in rat

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Abstract

The aim of this study was to evaluate the diuretic activity of aqueous extract of *Verbesina crocata* at different doses in rat. The aqueous extract (100 and 400 mg/kg for decoction; 200 and 400 mg/kg for smoothie form), furosemide (4 mg/kg and vehicle were administered *per os* to rat. After 6 hours in metabolic cages, the urinary flow, glomerular filtration rate and electrolyte balance (Na+ and K+) were assessed. All doses produced an increment of urinary flow and Na+/K+ excretion. Glomerular filtration rate was incremented only on smoothie form at 400 mg/kg. These findings provide a basis explaining the traditional folk medicine use of this plant as a diuretic agent.

Introduction

Diuretic is prescribed to increase the sodium and water excretion in the treatment of disorders such as edematous states (congestive heart failure, hepatic cirrhosis and nephritic syndrome) and hypertension. Conventional widely used diuretic like furosemide or thiazides, has been associated with several adverse effects, such as electrolytic imbalance, glomerular impairment, stimulation of renin-angiotensin system, etc. (Gupta and Neyses, 2005; Morganti, 2005).

Medicinal plants such as *Smilax canariensis, Retama reatam, Artemisia thuscula, Urtica dioica, Erica multiora, Cynodon dactylon, Eysenhardtia polystcahya* constitute an alternative to diuretic with efficacy and low adverse effects in the ethnomedicine (Sadki et al., 2010). It has been estimated that 80% of people in developing countries are almost completely dependents on traditional medicine practices and utilize plants as drugs for their primary health care needs (Abdala et al., 2012).

Verbesina crocata (Cav.) Less. is an endemic plant traditionally used in Tequesquitengo, State of Morelos, Mexico, commonly known as "*capitaneja*". It has long been used in traditional medicine like decoction or smoothie form mainly to treat kidney diseases, as diuretic agent, and other ailments such as diabetes (Pérez et al., 1984). Now-a-days, there is not enough information about pharmacological effect of this plant, on renal diseases. Thus, this study was aimed to assess the possible diuretic effect of the aqueous extracts of *V. crocata* in order to verify its ethnomedicinal use in an experimental model of diuresis.

Materials and Methods

Collection of plant material

V. crocata was collected in Tequesquitengo (18° 37′ 0″ N 99° 16′ 0″ O), State of Morelos, Mexico in January 2012. The botanical identification and authentication of the plant samples were performed by the Biologist Laura

Doval Ugalde at the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. The plant samples were compared with a voucher specimen deposited in the ENCB herbarium under number 2405. The aerial part of plant was divided in two portions, the first one was dried at room temperature and other part was used fresh.

Ethnobotanical survey

A survey was carried out around Tequesquitengo, State of Morelos using a questionnaire to interview 100 individuals with knowledge on the subject matter as herb seller, traditional medical healers and consumers about the part of plant and the usefulness of *V. crocata* in ethnomedicine. A specimen of the plant was shown to people at the time of the interview to avoid confusion (Pablo-Pérez et al., 2016).

Preparation of the aqueous extract and smoothie of the plant

Two types of extracts from the aerial part of *V. crocata* were prepared, one from the dry plant and the other from the fresh plant. For decoction, the plant was air-dried at room temperature in the shade, and then was cut into small pieces. The extract was freshly prepared just before administration as follows: 2 g of the plant were placed in water, the mixture was boiled at 100°C for 5 min and subsequently filtered. On the other hand, the smoothie was performed with 1.2 g of fresh plant and blended with water until the mixture was homogeneous. All doses of extract used were calculated respect to the weight of starting dry or wet material.

Phytochemical screening

The secondary metabolites of the aqueous extract of *V. crocata* were detected by qualitative chemical reactions. Briefly, Dragendorff, Mayer, Sonnenschain and Wagner reactions were used to identify alkaloids, Fehling and Benedict reactions for reductor sugar, Erlich reaction and observation under UV light of alkalinized extracts for coumarins, Kedde, Legal´s and Baljet´s reactions for cardiac glycosides, a frothing test for saponins. The tannins were identified by $FeCl_3$ test and jelly reagent. Liebermann-Buchard reaction for triterpenoids and steroids. Bornträger reaction for quinones and Shinoda reaction for flavonoids were used among others (Saleem et al., 2015; Pablo-Pérez et al., 2018).

Reagents and drug

Reagent-grade reagents were used. Furosemide, a high-ceiling loop diuretic, was used as reference diuretic drug (Lasix®, vial 20 mg/mL). It was dissolved in water prior to administration.

Animals

Adult female Wistar rats (180-220 g body weight) and female NIH mice (20-30 g) were used. They were housed and maintained in the animal house at room temperature (22–24°C) and 50–55% relative humidity, with day/night cycles of 12 × 12 hours. They were fed with standard rodent diet and water *ad libitum*.

Diuretic activity of the aqueous extract of V. crocata

For the evaluation of diuretic activity, the animals were carried out in un-anesthetized and randomly allocated to 6 groups: Control group received vehicle (water, 1 mL/kg), furosemide-treated group (4 mg/kg) and the other four groups were treated with the aqueous extract from *V. crocata* at the doses of 100 and 400 mg/kg for decoction and 200 and 400 mg/kg for smoothie form. In all groups, the administration was *per os* and there were at least six animals per group.

At the beginning of the experiment, the urinary bladder was emptied by gentle compression of the abdomen, as previously described (Meléndez et al., 2004). Once the bladder was emptied, either the vehicle, furosemide or aqueous extract was administered. The animals were kept during 6 hours in individual metabolic cages in a quiet and warm environment (21–23°C) under fluid and food deprivation. At the end of this period, the sample of urine was obtained; also by abdomen compression to ensure complete emptiness of the bladder and urine volume was measured. The animals were decapitated and blood samples were obtained.

Effect of aqueous extract on glomerular filtration rate

Glomerular filtration rate was estimated by the clearance of endogenous creatinine. To avoid the error due to tubular secretion of creatinine, only female animals were used. Plasma and urinary creatinine were determined by the Jaffe alkaline picrate modified method (Meléndez et al., 2004). Creatinine clearance and fractional excretion of sodium and potassium were calculated using the conventional equations (1 and 2):

$$Cx = (Ux * V/Px) \quad \text{Equation 1}$$

$$FEx = (Cx/Ccreat) * 100 \quad \text{Equation 2}$$

Where, Cx and Ccreat are the clearance (mL/min) of substance x and creatinine, respectively. Ux and Px are the concentrations (µEq/L) of substance x in urine and plasma, respectively, and V is the urinary flow (mL/min). FEx is the fractional excretion (%) of the substance x

Effect of aqueous extract on electrolyte balance

To study the effect of aqueous extract from *V. crocata*, sodium and potassium concentrations were measured in the urine and plasma samples by Flame photometer (Corning 400, Corning Medical and Scientific, England). The instrument was calibrated with standard solutions of Na^+ and K^+. Urinary excretions and clearances of sodium and potassium were calculated (Estévez-Carmona et al., 2013).

Sodium and potassium concentration in plant extracts

The content of Na^+ and K^+ were determined in both

extracts for each 100 mg of plant material following the previous procedure.

Acute toxicity

Among the five groups of mice, one as a control and the rest four groups treated with different doses of aqueous extract (2, 4, 8 and 16 g/kg of body weight) with at least 4 animals per group. The mice had access to food and water *ad libitum* and they were observed for number of deaths, clinical signs and behaviour changes during the first 3 hours following *per os* administration. Lethality was assessed using death within 14 days as an index of toxicity (Hilaly et al., 2004; Mukinda and Eagles, 2010; Pablo-Pérez et al., 2018).

Statistical analysis

All data are expressed as mean ± SEM (standard error of mean). To perform statistical analyses, SigmaPlot® 11.0 software was used. Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple range median test. Significant differences were set at p values less than 0.05.

Results

Ethnobotanical survey

According to interviewed people, *V. crocata* is used specially to treat kidney diseases as pain and kidney infection (46%), urolithiasis (14%), anuria and oliguria (38%). The majority of the remedies in the study area were prepared from dried form (50%) followed by fresh parts (33%), especially as smoothie of this plant and (17%) prepared as infusion.

Phytochemical screening

Phytochemical screening of decoction and smoothie preparations of *V. crocata* showed the presence of alkaloids, coumarins, quinones, saponins and tannins. Saponins, quinones and tannins types detected in the extract were: Steroidal saponins (Liebemann Burchard reaction), anthraquinones (sulfuric acid reaction) and phenolic compounds (reaction of ferric chloride 1% + potassium ferrocyanide 1%) respectively.

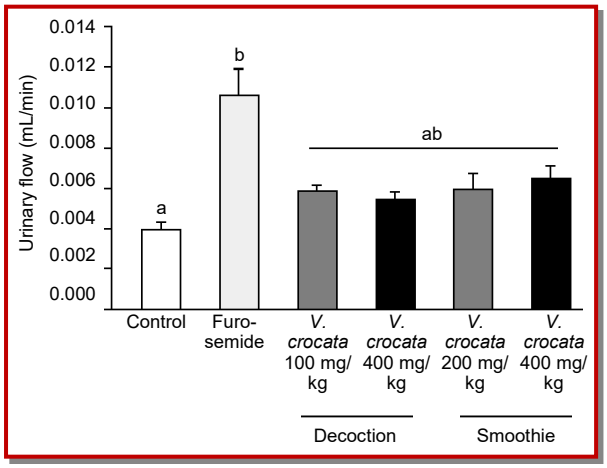

Figure 1: Urinary flow. Data are mean ± SEM., n = 6, [a]p<0.05 compared to control group, [b]p<0.05 compared to furosemide group

Diuretic activity of aqueous extract of V. crocata

The doses of aqueous extract produced a slight increase ($p<0.05$) in the urine flow compared with the control group remaining, but this variable did not reach the value produced by the reference diuretic drug. The increment on urine flow does not depend on the dose administered (Figure 1). Glomerular filtration rate was not affected by decoction treatment, however at dose of 400 mg/kg of smoothie of plant there was an increment of this variable (Table I).

Sodium clearance increased only in the group administered with 100 mg/kg of decoction of plant (Figure 2A). Two doses of smoothie and the dose of 100 mg/kg of decoction increased potassium clearance similarly to the effect of furosemide group (Figure 2B).

Also, we observed an increment on the urinary excretion of sodium at decoction dose of 100 mg/kg. On the other hand the urinary excretion of potassium only was augmented in animals treated with smoothie doses as it is observed in Table I.

Sodium concentration of decoction of plant was 6.2

Table I

Effect of *V. crocata* on glomerular filtration and sodium and potassium excretion

	Control	Furosemide	Decoction		Smoothie	
			100 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg
Glomerular filtration (mL/min)	1.3 ± 0.2	0.9 ± 0.1	1.2 ± 0.2	1.4 ± 0.2	1.7 ± 0.2	2.9 ± 0.5[a]
Urinary excretion Na+ (mEq/min)	0.6 ± 0.1	1.3 ± 0.2[a]	1.2 ± 0.3[a]	0.5 ± 0.1	0.6 ± 0.2	0.4 ± 0.2
Urinary excretion K+ (mEq/min)	0.7 ± 0.1	1.4 ± 0.1[a]	1.1 ± 0.2	0.6 ± 0.1	1.2 ± 0.2[a]	1.6 ± 0.2[a]

Data are mean ± SE. of six animals in all groups. [a]p<0.05 compared with control group, [b]p<0.05 compared with furosemide treated group

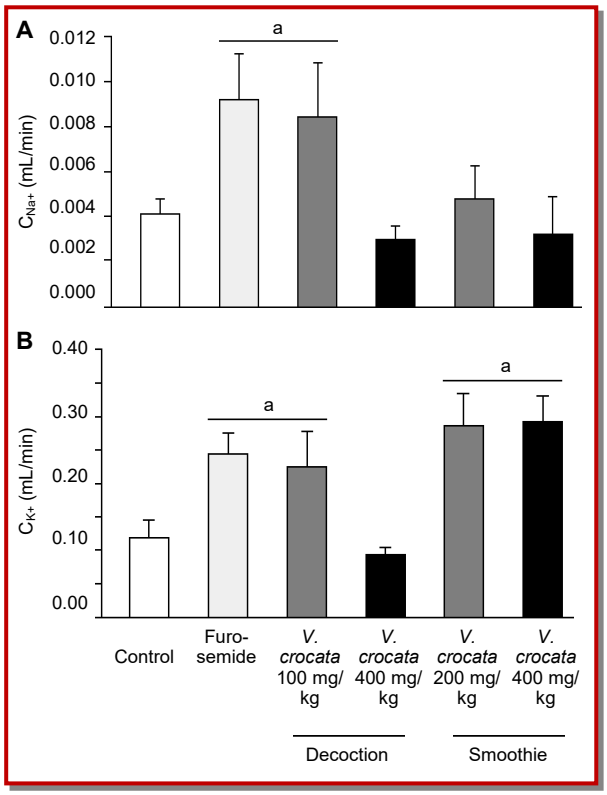

Figure 2: A) Sodium clearance (C_{Na+}), B) Potassium clearance (C_{K+}). Data are mean ± SEM., n = 6, $^{a}p<0.05$ compared to control group

mEq/L, while for the smoothie the sodium content was 1.9 mEq/L. On the other hand, the potassium concentration in decoction was 3.2 mEq/L and for smoothie was 1.2 mEq/L.

Acute toxicity

None of the doses of aqueous extract produced mortality or any behavioural disorders in animals. Because no deaths were observed at the dose levels used, the LD_{50} was assumed greater than 16 g/kg.

Discussion

Decoction and smoothie forms of *V. crocata* induced diuretic effect in rat, which is reflected in the increase in urinary flow and electrolytic excretion similar to furosemide group.

Since no conclusion could be drawn from the present work about the mechanism of action of the extract, and although evidence of diuretic efficacy of the plant tested have been obtained, further investigations are necessary to determine the precise mechanism by which the extract affect diuresis and electrolyte urinary excretion. Although the mechanism of action of the diuretic effect of aqueous extracts is unknown, it is possible that preparations of *V. crocata* exerted its pharmacological activity by inhibiting tubular reabsorption of water and accompanying anions, as such action has been hypothesized for some tested plants in previous reports (Jouad et al., 2001; Adam et al., 2009).

Increased urinary excretion of sodium and potassium could not be due to electrolytes present in *V. crocata* because of the low concentration of eletrolytes present in this plant. The pharmacodynamics studies on medicinal plants stressed that there is often no correlation between the diuretic effect observed and the content of K^+ in the extract (Galati et al., 2002). The potassium loss that occurs with many diuretics may lead to hypokalaemia. For this reason, generally potassium-sparing diuretics are recommended (Meera et al., 2009). In this work, only the groups treated with smoothie had an important excretion of potassium, although, the loss of K^+ is not beneficial during medical therapies, it should be compensated with potassium supplements or a diet rich in this mineral.

The fact that there is not any report on the chemical composition of the studied specie it was required phytochemical screening that allows identifying the main chemical groups present in the plant. The active principles responsible for the diuretic effects of the extracts of this plant have not yet been elucidated but preliminary phytochemical screening of the extracts revealed the presence of alkaloids, saponins, quinones and tannins.

Previous reports have reported that the diuretic activity is related to plants rich in alkaloids and saponins (Khan et al., 2010; Lahlou et al., 2007), in the case of saponins, depending on their chemical structures, they are known to have various physiological activities such as hemolytic properties, alteration of membrane permeability and the modulation of renal sodium excretion (Amuthan et al., 2012). It is probable that constituents of the aqueous extract of *V. crocata*, could be acting individually or in synergism with other molecules to produce the diuretic effect reported in this work.

The observed effect does not exclude the possibility that the changes in urine output may occur as a result of the presence of the chemicals identified in the phytochemical screening. Also, do not rule out the idea that the extract may exert a possible effect against diseases whose etiology is direct to the structural components of the kidney.

In the toxicological evaluation, this plant did not show any toxic effects at the doses evaluated in this study. With the non-toxic data and the encouraging results obtained in diuretic study, it is possible to consider that the *V. crocata* aqueous extract could be a safe drug in used doses.

Conclusion

This study supports the ethnomedical use of *V. crocata* as a diuretic agent and the plant extracts do not seem to have toxicity *per os* at the doses studied.

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Ethical Issue

Care and handling of animals followed international accepted procedures according to the Institute for Laboratory Animal Research´s Guide for Care and Use of Laboratory Animals.

Conflict of Interest

Authors declare no conflict of interest

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