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Diuretic activity and acute oral toxicity of *Palicourea coriacea* (Cham.) K Schum

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ABSTRACT

Aim of the study: Palicourea coriacea (Cham.) K Schum, is an endemic plant used in the Midwestern Region of Brazil, popularly known as "douradinha do campo" and "congonha do campo". This plant has been used in traditional medicine for several ailments, especially to treat kidney diseases. Since no formal studies on the biological activities and medicinal properties of the ethanolic extract of *Palicourea coriacea* (PCEE) have been carried out previously, the present study represents the first research into the efficacy of this plant as a diuretic agent employing laboratory rats as test animals.
Materials and methods: For diuretic activity evaluation we assayed three doses of PCEE (20, 40 and 80 mg/kg) and measurement of the urinary volume and electrolytes (Na^+, K^+) concentration were taken. The acute oral toxicity of PCEE was investigated according to OECD Guideline 423.
Results: The oral administration of a single dose of PCEE significantly increased the urinary volume in 24 h. Additionally, the treatment with PCEE increased, in a dose-dependent manner, the excretion of both, Na^+ and K^+. No sign of toxicity was observed in the animals.
Conclusions: The present study confirmed the ethnopharmacological use of *Palicourea coriacea* as a diuretic agent in the experimental condition tested here. Additionally, this work supports the importance of the preservation of local knowledge as well as the conservation of Brazilian biodiversity.

1. Introduction

Palicourea coriacea (Cham.) K Schum, is an endemic plant used in the Midwestern Region of Brazil, popularly known as 'douradinha do campo' and 'congonha do campo'. The genus *Palicourea* belongs to the tribe Psychotrieae of the Rubiaceae family and includes about 50 genera with 200 species, which occur in the tropics of the New World (Taylor, 1997; Silva et al., 2008). *Palicourea coriacea* has long been used in traditional medicine for several ailments, especially to treat kidney diseases (Nunes et al., 2003).

A number of alkaloids, saponins, tannins, cumarins, calycanthine, anthraquinone, allantoin, and triterpenes have been isolated from *Palicourea coriacea* in previous studies on the chemical composition of this plant (Laureano, 2001; Silva et al., 2008; Nascimento et al., 2008). Ursolic acid, a triterpenoid, was identified as the major phytoconstituent found in *Palicourea coriacea*. This compound has been associated with diuretic property (Somova et al., 2003a,b; Nascimento et al., 2006).

Even though the aqueous and ethanolic preparations of the aerial parts and the roots of *Palicourea coriacea* have long been empirically used in diuretic therapy by the population in the Midwestern Region of Brazil, there are no published experimental pharmacological data demonstrating the effectiveness of this plant species as a diuretic agent. Moreover, the oral acute toxicity of this plant had not been investigated. On the other hand, other species of the genus Palicourea, such as *Palicourea marcgravii*, have been considered the most toxic plants to animals in Brazil, presenting high lethality (Gorniak et al., 1992; Kemmerling, 1996).

Since no formal studies on the biological activities and medicinal properties of the ethanolic extract of *Palicourea coriacea* (PCEE) have been carried out previously, the present study represents the first research into the efficacy of this plant as a diuretic agent employing laboratory rats as test animals. We assayed three doses of PCEE and measured the urinary volume and electrolytes (Na^+, K^+) concentration. The oral acute toxicity of PCEE was also investigated.

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2. Materials and methods

2.1. Plant material and extract preparation

Palicourea coriacea aerial parts were obtained, in March 2003, in Goiânia, state of Goiás, Brazil. The identification and authentication of the plant samples were performed by Dr. José Realino de Paula, Pharmacy Faculty, Federal University of Goiás – Brazil. Specimen voucher is housed at the herbarium of this University under the register number 27153.

The botanical material (leaves and stalk) was air-dried at 40 °C and grounded to powder (248 g). The extraction was performed by maceration (three times of 24 h each; 1 part powder: 5 parts solvent) with ethanol 95% at room temperature. After filtration the solvent was eliminated by a rotator vacuum evaporator at 40 °C yielding 11 g of raw extract.

2.2. Drug

Furosemide (F), from Sigma Chemical Co., was used as reference diuretic drug.

2.3. Animals

The experiments were carried out on adult male normotensive Wistar rats (208 ± 8 g) obtained from Laboratório de Ciências da Saúde do Centro Universitário de Brasilia-DF (UNICEUB). All rats were kept under constant environmental conditions with 12:12 h light–dark cycle. All were fed standard granulated chow and given drinking water *ad libitum*. Animal experiments were approved and done in accordance with Institutional Protocols of Animal Care of this University (protocol number 118/07).

2.4. Diuretic activity

For the evaluation of diuretic activity, normotensive male Wistar rats (n = 6/per group) weighing 208 ± 8 g were placed in individual metabolic cages and had free access to water but no food. The control rats received saline only (1 mL/kg b/w orally administered), while treated animals received single dose of 20, 40 or 80 mg/kg of PCEE, by gavage. The positive control group received a single oral dose of furosemide (20 mg/kg). The urinary volume was measured 1, 2, 4, 6, 8, 12 and 24 h after the treatment. Natriuresis and kaliuresis were also evaluated after the treatment.

2.5. Analytical procedures

Urine Na^+ and K^+ concentrations were measured using a flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+. We determined pH and conductivity directly on fresh urine samples using a pH-meter and a conductivity meter, respectively (data not shown).

2.6. Acute toxicity

For the acute toxicity assay, two groups of three male Wistar rats (208 ± 8 g) were randomly allocated. From 12 h before until 3 h after the oral administration, the animals were kept without access to food and water. The assay was followed as OECD Guideline 423 (OECD, 2001). The control group received normal saline 1 mL/kg by gavage while the exposed group received 2000 mg/kg of PCEE. The safety of 2000 mg/kg dose was subsequently confirmed in another three animals as recommended in the OECD guideline. Immediately after dosing, the animals were observed continuously for symptoms of toxicity for 4 h in terms of autonomic and neurobehavioral alterations. They were then kept under observation up to 14 days in terms of weight loss and chow consumption. On day 15, the animals were euthanized and their vital organs were individually observed for overt pathology.

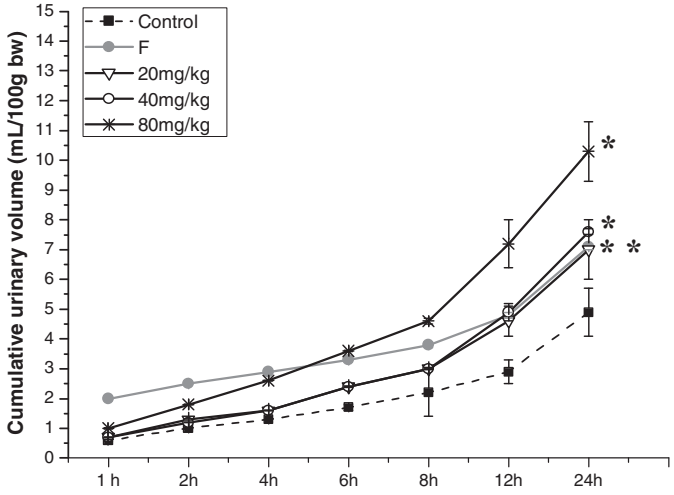

Fig. 1. Effects of *Palicourea coriacea* ethanolic extract (20, 40, and 80 mg/kg) on the cumulative urinary volume excretion (1–24 h). In all treatments schedule, rats were orally treated with a single dose of the ethanolic extract or with the positive control, furosemide (F, 20 mg/kg). The control group received saline only. Data are expressed as means ± SEM; n = 6 rats per group; $^*p < 0.05$.

2.7. Statistics

The results are expressed as mean ± SEM. Significance of differences between control and treated groups was determined using ANOVA followed by Tukey test $P < 0.05$.

3. Results

The effects of the administration of a single oral dose of PCEE (20, 40, and 80 mg/kg) on the cumulative urinary volume excretion volume excretion in 24 h are presented in Fig. 1. The oral administration of PCEE significantly increased the urinary flow in 24 h, compared with the control group ($p < 0.01$). This effect was observed, in a dose-dependent manner, between the third and the fourth hour after PCEE administration ($p < 0.001$). Similarly, furosemide (20 mg/kg) induced a comparable and cumulative effect; the urinary flow was also significantly elevated between the third ($p < 0.01$) and the fourth hour after treatment ($p < 0.001$). Fig. 2 shows that in parallel with the excretion of water, the administration of PCEE produced, in a dose-dependent manner, significant ($p < 0.001$) increases in the excretion of both ions studied, Na^+ and K^+. These effects were especially observed in relation to the excretion of K^+ when compared with furosemide. Animals treated with the highest dose of PCEE (80 mg/kg) showed more K^+ loss, approximately two fold, in comparison with furosemide. Higher electrolyte excretions were accompanied by higher urinary volumes.

Our results showed that 2.000 mg/kg of PCEE produced no toxic effects as evidenced by the absence of signs of toxicity or mortality in the animals during the study period (14 days of observation). Additionally, no weight loss, alteration of consumption of chow or macroscopic alterations in the viscera of treated animals were detected (data not shown).

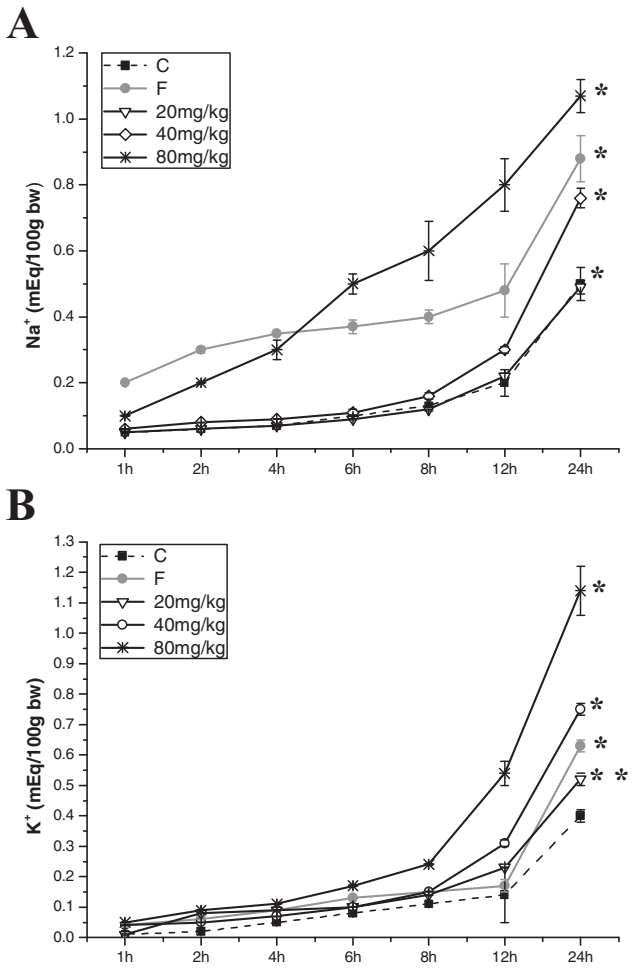

Fig. 2. Effects of *Palicourea coriacea* ethanolic extract (20, 40 and 80 mg/kg) on cumulative (1–24 h) Na^+ (A) and K^+ (B) excretion. In all treatments schedule, rats were orally treated with a single dose of the ethanolic extracts or with the positive control, furosemide (F, 20 mg/kg). The control group received saline only. Data are expressed as means ± SEM. $n = 6$ rats per group. $^*p < 0.05$.

4. Discussion

Usually, information about the potential of medicinal plants is handed down orally and, in some cases, valuable modern drugs have come into use through the study of traditional remedies. Moreover, chemists keep on researching plant-derived drugs as prototypes to develop more effective and, if possible, less toxic medicines. Although the vast arsenal of drugs used in the treatment of cardiovascular and nerve disorders, liver and kidneys diseases, medicinal plants are still a valuable source of new therapeutic agents. In this context, for the first time, a bioassay directed to study the effectiveness of *Palicourea coriacea* as a diuretic agent was performed. The administration of a single oral dose of PCEE (20, 40 and 80 mg/kg) significantly increased the urinary flow in 24 h. Additionally, the treatment with PCEE increased, in a dose-dependent manner, the excretion of both ions studied, Na^+ and K^+ especially in relation to the latter. Animals treated with the highest dose (80 mg/kg) showed marked kaliuresis. Considering the kaliuresis induced by PCEE, interactions between herbs and synthetic drugs, as well as the polypharmacy practice, the use of PCEE deserves attention especially in association with drugs used to the treatment of cardiovascular diseases, such as digoxin.

The diuretic effects presented by *Palicourea coriacea* could be explained by the presence of naturally bioactive compounds, in particular its major compound, ursolic acid, a known diuretic agent (Somova et al., 2003a,b). Somova et al. (2003a,b) demonstrated that African wild olive leaves contain a mixture of oleanolic and ursolic acids with potent antihypertensive, diuretic/natriuretic, antihyperlipidemic, and hypoglycemic. Moreover, plants of the Rubiaceae family, such as *Randia echinocarpa,* showed marked diuretic activity in different experimental models (Vargas and Perez, 2002; Santos-Cervantes et al., 2007).

Our results showed that PCEE produced no acute toxicity at the assayed doses, as evidenced by the absence of mortality of the animals during the study period and no macroscopic alterations were detected in the viscera of exposed animals. According to the Globally Harmonised Classification System for Chemical Substances and Mixtures (GSH) the PCEE was classified as class 5. Substances or mixtures classified under category 5 usually are of relatively low acute toxicity hazard but, under certain circumstances may present a danger to vulnerable populations (OECD, 2001). On the other hand, the specie of Palicourea, *Palicourea marcgravii*, has been demonstrated high toxicity due to the presence of fluoroacetate in the plant (Gorniak et al., 1992; Kemmerling, 1996).

The present study confirmed the ethnopharmacological use of *Palicourea coriacea* as a diuretic agent, but further studies are necessary to evaluate the mechanisms involved in its biological activity and safety following repeated exposure. Additionally, this work supports the importance of the preservation of local knowledge as well as the conservation of Brazilian biodiversity.

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References

Gorniak, S.L., Neto, J.P., Oliveira, G.H., Spinosa, H.S., 1992. *Palicourea marcgravii* intoxication in rats: effects of different fractions. Veterinarian Human Toxicology 34-3, 216–218.

Kemmerling, W., 1996. Toxicity of *Palicourea marcgravii*: combined effects of fluoroacetate, N-methyltyramine and 2-methyltetrahydro-beta-carboline. Zeitschrift für Naturforschung C, Journal of Biosciences 51, 59–64.

Laureano, L.C., 2001. Caracterização morfoanatômica, perfil fitoquímico e aspectos etnobotânicos das espécies medicinais do cerrado: *Palicourea coriacea* (Cham.) Schum.; *Rudgea virbuoides* (Cham.) Benth (Rubiaceae). In: Programa de Pós-graduação no Departamento de Biologia Geral, Universidade Federal de Goiás, Goiânia, p. 138.

Nascimento, C.A., Gomes, M.S., Lião, L.M., Oliveira, C.M.A., Kato, L., Silva, C.C., Tanaka, C.M.A., 2006. Alkaloids from *Palicourea coriacea* (Cham.) K. Schum. Zeitschrift für Naturforschung B, Journal of Biosciences 61, 1443–1446.

Nascimento, C.A., Liao, L.M., Kato, L., Silva, C.C., Tanaka, C.M.A., Schuquel, I.T.A., Oliveira, C.M.A., 2008. A tetrahydro beta-carboline trisaccharide from *Palicourea coriacea* (Cham.) K Schum. Carbohydrate Research 343–6, 1104–1107.

Nunes, G.P., Silva, M.F., Resende, U.M., Siqueira, J.M., 2003. Plantas medicinais comercializadas por raizeiros no Centro de Campo Grande, Mato Grosso do Sul. Revista Brasileira de Farmacognosia 13, 83–92.

OECD, 2001. Guidelines for Testing of Chemical, Guideline 423, Acute Oral Toxicity – Acute Toxic Class Method. OECD, Paris.

Santos-Cervantes, M.E., Ibarra-Zazueta, M.E., Loarca-Piña, G., Paredes-López, O., Delgado-Vargas, F., 2007. Antioxidant and antimutagenic activities of *Randia echinocarpa* fruit. Plants Foods for Human Nutrition 62, 71–77.

Silva, V.C., Carvalho, M.G., Alves, A.N., 2008. Chemical constituents from leaves of *Palicourea coriacea* (Rubiaceae). Journal of Natural Medicines 62, 356–357.

Somova, L.I., Shode, F.O., Ramnanan, P., Nadar, A., 2003a. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies africana leaves. Journal of Ethnopharmacology 84, 299–305.

Somova, L.O, Nadar, A., Rammanan, P., Shode, F.O., 2003b. Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. Phytomedicine 10, 115–121.

Taylor, C.M., 1997. Conspectus of the genus Palicourea (Rubiaceae: Psychotrieae) with the description of some new species from Ecuador and Colombia. Annals of the Missouri Botanical Garden 84, 224–262.

Vargas, S.R., Perez, G.R.M., 2002. Diuretic and urolithiatic activities of the aqueous extract of the fruit of *Randia echinocarpa* on rats. Journal of Ethnopharmacology 83, 145–147.