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Nephroprotective effect of *Rudgea viburnoides* (Cham.) Benth leaves on gentamicin-induced nephrotoxicity in rats

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ABSTRACT

Relevance: *Rudgea viburnoides*, popularly known as "congonha-de-bugre" or "erva de bugre", is used in folk medicine as hypotensive, blood depurative, anti-rheumatic, diuretic and in the treatment of kidney and bladder pain.

Aim: Based on the popularly acclaimed nephron-protective effect of *R. viburnoides*, we investigated, using rats, the protective effect of this plant extract on gentamicin-induced kidney injury.

Material and methods: Urinary volume, water and food intakes were assessed in adult male Wistar rats (naive or gentamicin-induced model of nephrotoxicity) treated with *R. viburnoides* extract. Also blood and kidney samples were collected for further laboratory and histological analyses.

Results: *R. viburnoides* leaves extract improved renal function. It also improved the renal function impairments caused by gentamicin-induced nephrotoxicity, as revealed by glomerular filtration rate, urine output and proteinuria.

Conclusion: *R. viburnoides* exert renoprotective effect, which may support its popular use for renal diseases treatment.

1. Introduction

Brazil displays a rich and diverse flora, from which substances with differential pharmacological activity have been identified. In addition, the discussion on the use of medicinal plants is getting greater through the last decades (Elisabetsky and Costa-Campos, 1996). Among the plants most popularly used for kidney dysfunctions in Goiânia (Goiás state capital), *Rudgea viburnoides* (Cham.) Benth, from the Rubiaceae family, is of interest in Brazilian folk medicine mainly due to its diuretic effects (Morais et al., 2005).

Popularly known as "congonha-de-bugre" or "erva de bugre", *R. viburnoides* is common in the Brazilian Cerrado region (Alves et al., 2004; Silva and Proença, 2008). The leaves of *R. viburnoides* have green-brownish color on abaxial face, slightly bitter taste and are odorless (Alves et al., 2004). Roots and bark are used in folk medicine as diuretic, hypotensive, blood depurative and anti-rheumatic (Balbach, 1980; Siqueira, 1981; Vieira and Martins, 2000); and the leaves are used in slimming diets (Alves et al., 2004) and to treat kidney and bladder pain (Nunes et al., 2003). Although some Rubiaceae species, as *Palicourea marcgravii*, exhibit high toxicity to cow herd (Gorniak et al., 1994; Kemmerling, 1996; Morita et al., 1989), toxic effects were not observed after acute treatment with *R. viburnoides* ethanolic extract in rats (Pucci et al., 2010). Phytochemical screening and the thin layer chromatography (TLC) profile determination reported the presence of tannins, flavonoids, triterpenes, sterols and saponins (Alves et al., 2004). Despite being common in Rubiaceae family, such as Psychotria ipecacuanha (Brot.) Strokes and Cinchona spp, Alkaloids were not detected in the leaves of *R. viburnoides*. Young et al. (1998) isolated and identified, the saponins arjunglucoside I and trachelosperosides B-1 and E-1, and the

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triterpenes trachelosperogenin B and arjungenin from *R. viburnoides* fruits (Young et al., 1998).

Within several experimental models used to provoke renal diseases, Gentamicin may be of choice, since it provokes tubular damages similar to those detected in clinical routine (Ali et al., 2011). Gentamicin is an antibiotic of the aminoglycosides group, an important antibacterial agent since its discovery in the 40 s (Oliveira et al., 2006). All aminoglycosides have the potential to induce renal toxicity; however, gentamicin, compared to other aminoglycoside, has the highest nephrotoxicity (Balakumar et al., 2010). Nephrotoxicity of gentamicin in the proximal convoluted tubules results from its internalization by lysosomes and causes release of hydrolase enzymes, thereby causing cell necrosis and proximal tubule obstruction (Hanslik et al., 1994). In this case, aminoglycosides endocytosis probably damages renal pathways processing amino acids and small peptides (Kaloyanides and Ramsammy, 1993; Rougier et al., 2003). The disturbances in the cell membranes structure induced by Gentamicin (Tavafi, 2012; Valipour et al., 2016) also recruits pathways of vasoconstrictor hormones release (Schor et al., 1981), besides involving release of platelet aggregating factors (Dos Santos et al., 1991), cellular debris deposition, which jointly, may block nephrons (Neugarten et al., 1983). As consequence, changes in glomerular permeability with decreased glomerular ultrafiltration coefficient may be found (Baylis et al., 1977; de-Barros-e-Silva et al., 1992).

In spite of being regularly used in Brazilian folk medicine, literature lacks in demonstrating the effects of the *R. viburnoides* leaves in renal function, as well as its renoprotective potential during renal diseases remains unknown. Therefore, the aim of this study was to evaluate the renal function in rats treated with *R. viburnoides* and its protective effect against gentamicin-induced kidney injury.

2. Material and methods

2.1. Animals

Adult male Wistar rats (180–250 g), provided by the Central Animal House of Federal University of Goiás (UFG) were used in this study. Animals were kept under constant room temperature of 23 ± 2 °C with free access to water and food, under a 12:12 h light/dark cycle (lights on at 7:00 h). Animals were acclimatized for one week before the beginning of the experiments. All experimental protocols were developed in accordance with the principles of local ethics committee and animal welfare and in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Experimental procedures approved by the institutional ethics committee (CEUA – UFG nº 088/10). The total number of animals used was 45 (please see Experimental protocol and Results sections for details).

2.2. Chemical

Gentamicin sulfate (Gentatec® - Chemitec, Brazil) and the crude extract were dissolved in a 0.9% NaCl solution w/v to oral treatment. Solvents with analytical grade were purchased from Vetec, Brazil.

2.3. Extractions

The *Rudgea viburnoides* (RV) leaves were collected from plants in natural habitat in FLONA IBAMA, city of Silvânia, Goiás, Brazil (950 m, 16°38′33.3″ S, 48°39′07″ W) by Prof. Dr. José Realino de Paula authenticated the samples, and a voucher specimen was deposited at the Herbarium of the UFG (catalog number UFG-24328).

The leaves were dried in an oven with forced circulating air at 40 °C and ground in a Wiley mill. The powder of *R. viburnoides* leaves were extracted with 95% ethanol solution, in a proportion of 1:5 of vegetal material/solvent, by maceration for 4 h. This process was repeated twice to ensure the exhaustion of extractable compounds by ethanol. The extract was filtrated and the solvent was removed using, rotary evaporator at 40 °C (Ferri, 1996). The yield in the extract production was about 10%, which is close to previously reported (Alves et al., 2004; Young et al., 1998).

2.4. Experimental protocol

All animals were submitted to 24 h of adaptation period in the metabolic cages. Following, rats were divided into six groups (n=7–8), which underwent different treatments. Oral treatments were performed by gavage twice per day, during 7 days, with vehicle (NaCl 0.9%) (SAL) as control or with two doses (50 or 200 mg/kg) of plant extract (RV). Acute renal injury (ARI) was started at the third day, subsequent to the beginning of the oral treatments. Subcutaneous (sc) injections of saline (SAL) or gentamicin (GT) (Gentatec®, 80 mg/kg/day) were given twice per day for ARI induction. Experimental groups were as follows: i) SAL+ SAL; ii) SAL + GT; iii) RV (50) + SAL); iv) RV (200) + SAL; v) RV (50) + GT; vi) RV (200) + GT.

Animals remained at metabolic cages throughout experiments. Urinary volume, water and chow intake were measured every 24 h. Blood and urine samples were collected at first and last days of experimental protocols. These samples were centrifuged and stored at −20 °C until analyses. At the end of the experiments, the rats were anesthetized with thiopental (40 mg/kg) to allow removal of the kidney for morphometry.

2.5. Laboratory investigation

The sodium and potassium concentrations in the blood and urine were determined by flame photometer (910 M, Analyser), calibrated with a standard solution containing 140 mEq / l Na^+ and 5 mEq / l K^+. The fractional excretion of sodium and potassium were calculated by standard methods.

The GFR (mL/min) was determined colorimetrically by the clearance of creatinine (CCr). The plasma and urine levels of creatinine were determined by spectrophotometry using Bioclin® kit, as instructed by the supplier.

Urine samples were added in a test tube with color reagent, homogenized and incubated in a water bath at 37 °C for 10 min. Protein concentrations in the urine samples were determined by spectrophotometer (BELphotonics 1105 with a wavelength of 600 nm) using the commercial kit MICROPROTE pyrogallol (Doles). The values were calculated based on the calibration factor, and the protein concentration was expressed in mg/24 h.

2.6. Morphological examination of the kidney

To determine the morphological alterations resulting from tubular necrosis, at the end of 5 days of treatment, the kidneys were immersed in a solution of 10% buffered formalin, and were submitted to the conventional staining techniques (hematoxylin-eosin). The team of pathologists coordinated by Prof. Dr. Ruy de Souza Lino Júnior made blind morphological analyses. Semi quantitative analyses of tubular necrosis, tubular vacuolization, interstitial changes and hyaline cylinders were performed.

2.7. Data analysis

The results were expressed as mean ± standard error of mean. Unpaired student's *t*-test or by one-way ANOVA followed by 'Newman–Keuls' were applied when necessary. All statistical analyses were carried out using GraphPad Prism 6 software. Statistical difference was set at $p < 0.05$.