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Red propolis ameliorates ischemic–reperfusion acute kidney injury

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ABSTRACT

Introduction: Acute kidney injury (AKI) remains a great problem in clinical practice. Renal ischemia/reperfusion (I/R) injury is a complex pathophysiological process. Propolis is a natural polyphenol-rich resinous substance collected by honeybees from a variety of plant sources that has anti-inflammatory and anti-oxidative properties. Red propolis (RP) protection in renal I/R injury was investigated.
Methods: Male Wistar rats underwent unilateral nephrectomy and contralateral renal I/R (60 min). Rats were divided into four groups: (1) sham group, (2) RP group (sham-operated rats treated with RP), (3) IR group (rats submitted to ischemia) and (4) IR–RP (rats treated with RP before ischemia). At 48 h after reperfusion, renal function was assessed and kidneys were removed for analysis.
Results: I/R increased plasma levels of creatinine and reduced creatinine clearance (CrCl), and RP provided protection against this renal injury. Red propolis significantly improves oxidative stress parameters when compared with the IR group. Semiquantitative assessment of the histological lesions showed marked structural damage in I/R rats compared with the IR–RP rats. RP attenuates I/R-induced endothelial nitric oxide-synthase down regulation and increased heme-oxygenase expression in renal tissue.
Conclusion: Red propolis protects kidney against acute ischemic renal failure and this protection is associated with reduced oxidative stress and eNOS and heme-oxygenase up regulation.

Introduction

Acute kidney injury (AKI) remains a great problem in clinical practice. It affects approximately 20% of hospitalized patients and half of critically-ill patients admitted to intensive care unit (Poukkanen et al. 2013; Uchino et al. 2005; Zeng et al. 2013). Despite improved strategies for supporting vital organs during AKI recovery and in renal replacement therapy (dialysis), AKI mortality rates remain quite high (Leite et al. 2013). Also, renal I/R injury is a common cause of early allograft dysfunction in renal transplanted patients and represents an additional risk factor for late renal allograft failure (Ditonno et al. 2013). The prevention of kidney lesions and their progression continue to represent a great challenge. Although renal injuries are multifactorial in many patients, in the clinical scenario, animal models of renal ischemia/reperfusion (I/R) remain important to understand the pathophysiology and potential treatment options for AKI.

Renal I/R injury is a complex pathophysiological process involving oxidative and inflammatory damage, endothelium-mediated injury and apoptosis. Nitric oxide (NO) is involved in the pathophysiology of ischemic AKI. Increased expression of proinflammatory inducible nitric oxide synthase (iNOS) is considered a pivotal step in renal damage, whereas the reduced activity of endothelial nitric oxide synthase (eNOS) contributes to renal impairment resulting from endothelial dysfunction (Heemskerk et al. 2009).

Many molecules have intrinsic cytoprotective properties that include anti-apoptotic, anti-inflammatory and antioxidant actions. Heme-oxygenase (HO) 1 and 2 are the rate-limiting enzymes in the catabolism of heme, a reaction that yields equimolar amounts of biliverdin, Fe^{2+} and carbon monoxide. Expression of HO-1 is readily increased upon organ I/R injury, becoming the rate-limiting factor in the generation of biliverdin, Fe^{+} and CO. Heme-oxygenase-1 pro-

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vides protection against renal I/R injury through its antioxidant, anti-inflammatory and cytoprotective activities (Agarwal and Nick 2000; Nath et al. 1992).

Propolis is a natural polyphenol-rich resinous substance collected by honeybees from a variety of plant sources. In recent years, propolis has gained popularity as a health drink, has been extensively used in food and beverages, and is thought to improve human health and prevent disease (Daleprane and Abdalla 2013). Beneficial health effects are largely attributed to its polyphenolic composition. Red propolis has been classified as a separate type based on its unique chemical composition, particularly rich in isoflavonoids (Righi et al. 2013). Anti-inflammatory and antioxidant properties have been attributed to red propolis (Bueno-Silva et al. 2013; Enis Yonar et al. 2012). In the present study, we aimed to evaluate the effects of red propolis extract on renal I/R injury.

Methods

Animals and red propolis

The experimental protocol was approved by the Ethical Committee on Animal Research of Federal University of Ceará (no. 39/13). Wistar rats, weighing 250–300 g, were obtained from the Pharmacology Department of Federal University of Ceara and maintained under controlled temperature (21 ± 2 °C) and humidity conditions (60 ± 5%) with a 12:12-h light: dark cycle. A standard commercial pellet diet and water were offered *ad libitum*.

Chemical characterization of red propolis

Red propolis was collected in the mangrove region in Marechal Deodoro (a city in the vicinity of Maceio, capital of Alagoas State, in the northeastern Brazil (SL 09.40 and WL 35.41). The botanic origin was *Dalbergia ecastaphyllum*. An ethanol extract of red propolis was used at a concentration of 0.25 g/ml. The chromatographic analysis by high-performance liquid chromatography (HPLC) was performed. The assay was performed on Alliance – Waters 2695 (Milford, MA) chromatograph with a binary pump, auto-sampler, and photodiode-array detector (Waters-2996 PDA) at 268 nm. The separations were performed with an analytical reverse-phase column C18 (Waters, 250 mm × 4.6 mm, 5 μm) at 40 °C in a thermostatic oven. The mobile phase was made from water/acetic acid 0.1% (solvent A) and methanol (solvent B) in a gradient elution for 65 min (total run time), starting with 30% B (0–15 min), increasing to 90% B (15–60 min), held at 90% B and decreasing to 30% B (60–65 min) with a solvent flow rate of 1 ml/min. The solvents were previously degassed under vacuum by sonication during 5 min and filtered through phenomenex nylon membrane (0.45 μm). The samples were dissolved in the initial mobile phase and filtered through a 0.45 μm filter unit (Millipore, USA) before injection (20 ml). The data was processed by Empower software (Waters, USA).

The identification of formononetin and biochanin A in RP by HPLC experiments was based on the retention time (rt) of external standards. The contents of the three flavonoids were calculated using calibration curves. The ranges of calibration curves were 0.04–0.12 mg/ml for formononetin and 0.005–0.013 mg/ml for biochanin A. The linear relationship was obtained correlating the concentration of flavonoids to the correspondent peak area.

For peak purity analysis, spectra in the range of 210–400 nm were recorded at a frequency of 1 Hz. Threshold was calculated employing noise and solvent angles. Reference spectra of formononetin and biochanin A standards were recorded in the Empower 2 software library for identification purposes.

The spectra search improves the identification of compounds in complex matrices since different substance can have identical retention times. Formononetin and Biochanin A were identified in propolis extract chromatogram through the comparison of peak apex spectrum against the results of reference standards solutions recorded previously in the software library. The peak height of biochanin A in propolis extract chromatogram is lower than formononetin (Fig. 1).

The peak purity analysis provided by diode array detectors is essential to ensure reliability and accuracy of the chromatographic measurements of analytes in complex matrices. In the present work, the formononetin and biochanin A peaks were found pure since the purity angles were lower than the threshold angles and the threshold curves do not intersect the purity curves.

The chromatographic method shows linearity over the range evaluated and the correlation coefficients for and formononetin and biochanin A were 0.9915 and 0.9996, respectively. The concentrations (mean ± standard deviation for $n = 12$) of formononetin and biochanin A in the propolis extracted were 10.25 ± 0.21 and 0.50 ± 0.02 μg/mg, respectively. The amount of formononetin in the propolis extract is greater than 1% and was approximately fifteen times larger than biochanin A.

Surgical procedure

Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A midline laparotomy incision was performed, the right kidney was removed and left ischemic renal failure was induced by clamping the renal artery (with a nontraumatic clamp) for 60 min, followed by reperfusion. After 48 h, animals were sacrificed to obtain blood samples for biochemical tests. Additionally, the left kidneys were collected for histological and immunohistochemistry evaluation.

Experimental groups

Rats were divided into the following groups ($n = 8$ in each group):

−*Sham* + vehicle group (SHAM):

Rats were submitted to identical surgical procedures, except for the nephrectomy and unilateral renal occlusion shock and were kept under anesthesia for the duration of the experiment.

−*Sham* + red propolis (RP):

Identical to SHAM group, receiving red propolis (150 mg/kg/day) was administered by gastric gavage 3 days before the procedure and 1 h prior to surgical procedure. To administration, the ethanol extract was filtered and then evaporated by using a vacuum evaporator. The propolis samples were maintained in a dark environment, inside a deep freezer (kept at – 20 °C). The dried form was suspended in water just before oral administration according to required dosage.

−I/R + vehicle group (IR):

Rats were submitted to nephrectomy and unilateral renal occlusion (60 min) followed by reperfusion.

−I/R + red propolis group (IR–RP):

Rats were submitted to the above mentioned surgical procedures and red propolis (150 mg/kg/day) was administered by gastric gavage 3 days before the procedure and 1 h prior to ischemia.

Measurement of biochemical parameters

Forty-eight hours after ischemia, rats were reanesthetized, and blood samples (1 ml) were collected via venipuncture. The samples were centrifuged (6000 rpm for 3 min) to separate plasma. Plasma and urine concentrations of urea (BUN) and creatinine (Cr) were measured as indicators of impaired glomerular function. Plasma and urine concentrations of sodium (Na^+) and potassium (K^+) were used