Renoprotective effect of the *Echinodorus macrophyllus* in induced renal injury

Efeito renoprotetor do *Echinodorus macrophyllus* na lesão renal induzida

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Keywords
Nursing research; Practice nursing; Acute kidney injury; Antioxidants; *Alismataceae*

Descritores
Pesquisa em enfermagem; Enfermagem prática; Lesão renal aguda; Antioxidantes; *Alismataceae*

Submitted
January 20, 2014

Accepted
February 26, 2014

Abstract

Objective: Evaluating the renoprotective effect of *Echinodorus macrophyllus* in acute kidney injury induced by cyclophosphamide in rats.

Methods: Experimental research with *Wistar* rats, male adults, distributed into groups, namely: Control – administration of 1.5 ml sodium chloride 0.9% intraperitoneally; Echinodorous – administration of 2g/kg of *Echinodorus macrophyllus* by gavage for five days; Cyclophosphamide – administration of cyclophosphamide 150mg/kg intraperitoneally; and Cyclosphosphamide + Echinodorus – administration of *Echinodorus macrophyllus* and cyclophosphamide. Renal function (creatinine clearance) and the oxidative metabolites (peroxides and urinary substances reactive to thiobarbituric acid, thiols in kidney tissue) were evaluated.

Results: Preconditioning with *Echinodorus macrophyllus* elevated the creatinine clearance and reduced the levels of oxidative metabolites.

Conclusion: The antioxidant action of *Echinodorus macrophyllus* has demonstrated renoprotective effects evidenced by the reduction of oxidative stress in acute renal injury induced by cyclophosphamide in rats.

Resumo

Objetivo: Avaliar o efeito renoprotetor do *Echinodorus macrophyllus* na lesão renal aguda induzida pela ciclofosfamida em ratos.

Métodos: Pesquisa experimental com ratos *Wistar*, machos e adultos que foram distribuídos nos grupos: Controle - administração de 1,5 ml de cloreto de sódio a 0,9,% por via intraperitoneal, *Echinodorous* – administração de 2g/kg de *Echinodorus macrophyllus* por gavagem durante cinco dias, Ciclofosfamida – administração de ciclofosfamida 150mg/kg por via intraperitoneal, *Ciclosfofamida* + *Echinodorus* – administração de *Echinodorus macrophyllus* e ciclofosfamida. Foram avaliados a função renal (*clearance* de creatinina) e os metabólitos oxidativos (peróxidos e substâncias reativas ao ácido tiobarbitúrico urinários, tióis no tecido renal).

Resultados: O pré-condicionamento com *Echinodorus macrophyllus* elevou o *clearance* de creatinina e reduziu os níveis dos metabólitos oxidativos.

Conclusão: A ação antioxidante do *Echinodorus macrophyllus* demonstrou efeito renoprotetor evidenciado pela redução do estresse oxidativo na lesão renal aguda induzida pela ciclofosfamida em ratos.

DOI: http://dx.doi.org/10.1590/1982-0194201400004

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Conflicts of interest: no conflicts of interest to declare.

Introduction

In recent years, the use of medicinal plants is notorious due to its potentially beneficial effects on human health. However, there is still a lack of information about its mutagenic effects in most of the active ingredients.[1]

In the tropics in particular, there is a diversity of species of medicinal plants that are used by the population to treat their illnesses. Medicinal plants are used for various purposes and based on historical or personal evidence that were incorporated by habits, traditions and customs throughout time, but studies confirming its safety and efficacy are still rare.[2]

The lack of scientific research to Brazilian native plants undermines the promotion of such products and causes difficulties for their use as raw material for the herbal medicines industry. In this context, we can mention the native Brazilian species of *Echinodorus macrophyllus* of the *Alismataceae* family, commonly known by chapéu-de-couro, chá-mineiro, erva-de-pântano, erva-de-bugre, congonha-do-campo ou erva-do-brejo (Portuguese names for the herb, does not translate), widely used in folk medicine in the Southeast and Midwest regions. The leaves are used for the treatment of various diseases such as rheumatism and syphilis.[3] The *Echinodorus macrophyllus* is known for its diuretic action, and has anti-inflammatory and antioxidant properties.[3]

Currently, medicinal plants are recognized as potent antioxidants and used primarily in experimental research in the prevention or treatment of cellular injury induced by the imbalance between oxidants and antioxidant enzymes.[2] In clinical practice, the use of drugs often results in renal cell injury that characterizes acute kidney injury (AKI) with rapid decline of the renal function. It is clinically defined by an absolute increase in serum creatinine of at least 0.3 mg/dl or 1.5 times the increase from baseline, or a reduction in urine flow (documented as oliguria), or lower than 0.5 mL/kg per hour for more than six hours.[4]

Acute kidney injury is a complication in 5% of hospitalizations and up to 30% of admissions to the intensive care unit.[5] The drugs with nephrotoxic potential are responsible for approximately 20% of cases of acute kidney injury in critically ill patients.[6] Examples include antibiotics, chemotherapeutic agents, analgesics and immunosuppressants.[7]

The cyclophosphamide stands out among the chemotherapeutic drugs. It is a cytostatic agent used in the treatment of neoplastic diseases of solid tumors, lymphomas and other non-neoplastic diseases, such as rheumatoid arthritis and systemic lupus erythematosus. This drug and its metabolites can cause nephrotoxicity with glomerular and tubular dysfunction.[8] The study models *in vivo* have demonstrated that during the administration of cyclophosphamide there was decrease of the antioxidants reserve in the plasma and the renal tissue of animals.[8,9]

The anatomical and functional characteristics of the kidney favor the incidence of acute kidney injury of nephrotoxic origin because 25% of cardiac output are directed to the organ and the high renal blood flow favors the high concentration of toxic substances and its metabolites in tissue. In addition, the cells of the proximal tubule and the loop of Henle are more sensitive to damage induced by nephrotoxic substances due to their location in a hypoxic environment of the outer portion of the renal medulla, and these cells require high concentrations of energy for the active transport of solutes.[7] The pathophysiology of acute kidney injury is composed of several mechanisms such as impairment of renal microvasculature, which reduces glomerular filtration rate, the direct tubular toxicity that triggers an intense inflammatory response and generates reactive oxygen species.[10]

Considering the nephrotoxic effect of cyclophosphamide and its indication for the treatment of various cancers and other diseases, this study aims to determine the protective effect of the *Echinodorus macrophyllus* in acute kidney injury induced by cyclophosphamide in rats.

Methods

Experimental study on animal model with the use of *Wistar* rats, male adults, weighing 250-300 g, that were divided into the following groups:

- Control – animals that received sodium chloride 0.9% intraperitoneally (ip), single dose;
- Echinodorus – animals that received a solution of *Echinodorus macrophyllus* (2g/kg) by gavage once a day for five days;
- Cyclophosphamide – animals that received cyclophosphamide (150mg/kg) intraperitoneally, single dose;
- Cyclophosphamide + Echinodorus - animals that received *Echinodorus macrophyllus* (2g/kg) by gavage once a day for five days and cyclophosphamide (150mg/kg) intraperitoneally, single dose in the fifth day of the experiment.

At the end of the protocol, the animals were placed in individual metabolic cages for 24h-urine collection, for evaluation of renal function and oxidative metabolites. After this period, the animals were anesthetized for terminal blood collection by puncture of the abdominal aorta and subsequent assessment of renal function.

The renal function was assessed by creatinine clearance. The colorimetric Jaffe method was used to determine the values of serum and urinary creatinine. Creatinine clearance was calculated by the formula: creatinine clearance = urine creatinine x urine flow of 24 hours / serum creatinine.[11,12]

The oxidative metabolites were assessed by measuring the dosage of urinary peroxides, of thiobarbituric acid reactive substances (TBARS) and thiols in kidney tissue. The evaluation of urinary peroxides was performed with the FOX-2 method using xylenol orange iron, which oxidizes the Fe^{2+} ion producing a purplish blue staining ($\alpha = 4.3 \times 10^4\ M^{-1}\ cm^{-1}$).[11,12] The assessment of urinary TBARS allows identifying the final products of the lipid peroxidation cascade that react in the presence of thiobarbituric acid in body fluids ($\alpha = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$).[11,12] The level of thiols in renal tissue was evaluated by reaction with DTNB ($\alpha = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$).[11]

The results were presented as mean ± standard deviation. Statistical analysis of results was performed by analysis of variance (ANOVA) followed by Tukey test for comparisons between groups. The level of significance was set at $p < 0.05$.

The development of the study followed national and international standards of ethics in research involving the use of animals.

Results

As shown in table 1, the groups of Control and Echinodorus showed no variability in relation to creatinine clearance. The administration of a single dose of cyclophosphamide resulted in significant reduction in creatinine clearance that characterized the model of nephrotoxic AKI ($p<0.001$). It was observed that preconditioning with *Echinodorus macrophyllus* in animals treated with cyclophosphamide showed significant elevation of creatinine clearance when compared with the Cyclophosphamide group ($p<0.05$).

Table 1. Results of renal function of various group

Groups	n	Weight (grams)	Urinary flow (ml/min)	Creatinine clearance/100g (ml/min)
Control	5	280±28	0.013±0.003	0.81±0.05
Echinodorus	6	290±11	0.011±0.002	0.77±0.14
Cyclphosphamide	11	286±14	0.016±0.005	0.20±0.05[ab]
Cyclophosphamide+Echinodorus	11	293±21	0.011±0.004	0.31±0.11[abc]

[a]p<0.001versus Control; [b]p<0.001versus Echinodorus; [c]p<0.05 versus Cyclophosphamide

The results of the oxidative metabolites (Table 2) showed that the values of urinary peroxides of the Echinodorus and Control groups were considered as normal reference. The Cyclophosphamide group showed a significant increase in the values of urinary peroxides when compared with Control groups ($p<0.001$). The group that received treatment with *Echinodorus macrophyllus* showed decreased excretion of urinary peroxides compared to the Cyclophosphamide group ($p<0.05$).

Table 2. Results of oxidative metabolites of various groups

Groups	n	Urinary peroxides (nmol/g creatinine)	Urinary TBARS (nmol/g urinary creatinine)	Thiols in renal tissue (nmol/mg protein)
Control	5	25.6±6.5	0.3±0.1	–
Echinodorus	6	27.0±7.3	0.3±0.1	2.1±0.2
Cyclophosphamide	11	73.9±24.0[ab]	4.6±1.9[ab]	1.2±0.4[a]
Cyclophosphamide+Echinodorus	11	47.7±20.2[abc]	1.3±1.0[abc]	2.4±0.4[c]

TBARS – thiobarbituric acid reactive substances. [a]p<0.001 versus Control; [b]p<0.001 versus Echinodorus; [c]p<0.05 versus Cyclophosphamide

The quantification of urinary TBARS followed the pattern described for urinary peroxides. The Cyclophosphamide group showed significant elevation of TBARS when compared with the Control and Echinodorus groups ($p<0.001$). The Cyclophosphamide + Echinodorus group showed a statistically significant reduction of TBARS compared to the Cyclophosphamide group ($p<0.05$).

The evaluation of thiols in renal tissue showed that the Cyclophosphamide group presented reduced levels of thiols in renal tissue compared to the Echinodorus group ($p<0.001$) and preconditioning with *Echinodorus macrophyllus* significantly increased thiol levels in kidney tissue in comparison with the Cyclophosphamide group ($p<0.05$).

Discussion

This study showed that preconditioning with *Echinodorus macrophyllus* attenuated the acute kidney injury induced by cyclophosphamide in rats, which was evidenced by the increase in creatinine clearance, reduction of oxidative metabolites in the urine, and increase of the reserve of antioxidant enzymes in renal tissue. However, the study has limitations regarding the extraction of *Echinodorus macrophyllus*, since it was not performed a specific quantification of the functional components of the plant. Thus, the results of this study allow the identification of the toxicity mechanism via generation of reactive oxygen species in the acute kidney injury model induced by cyclophosphamide.

These data allow nurses to identify cyclophosphamide as a drug with potential for nephrotoxicity and stratify patients at risk to implement preventive measures such as hydration and administration of antioxidant drugs. This practice can be demonstrated by care protocols with n-acetylcysteine for prophylaxis of nephrotoxicity induced by iodinated radiocontrast.[13]

Clinically, the toxicity of cyclophosphamide has mild side effects, such as irritative urinary symptoms and transient, mild hematuria, and more severe ones such as acute kidney injury.[14] The main objective of therapies for the prevention of adverse effects is to maintain the efficacy of drug treatment and the use of protectives that demonstrate inhibition or interference in pathological mechanisms of cell injury.[14] Strategies for preventing adverse effects of the cyclophosphamide are welcome, and antioxidant agents such as the *Echinodorus macrophyllus* flavonoid stand out.

Cyclophosphamide and its structural analog, ifosfamide, are widely used in therapeutic protocols for patients with cancer, and also for non-neoplastic conditions. The toxicity of cyclophosphamide is mainly related to the release of toxic metabolites by the liver, the cytochrome P-450, which converts cyclophosphamide in acrolein and chloroacetaldehyde. In the kidneys, acrolein and chloroacetaldehyde cause death of tubular epithelial cells. The primary toxic mechanism involves direct action of acrolein in the proximal tubule, and the secondary action of aldophosphamide - toxic nitrogenous and alkylating compounds - are responsible for the generation of reactive oxygen species.[15,16] This metabolism of cyclophosphamide induces the release of inflammatory cytokines and reactive oxygen species resulting in lipid peroxidation of the cell membrane and consequently, renal injury.[15,16]

Thus, the administration of cyclophosphamide chemotherapy induced AKI in rats, which was characterized by reduced glomerular filtration rate, evidenced by a decrease in creatinine clearance. The release of oxidative metabolites confirms the mechanism of tubular damage, such as the radical superoxide and hydroxyl, and of intermediate such as peroxides that result in the consumption of antioxidants reserve. Studies with different nephrotoxic drugs such as gentamicin, radiocontrasts and other chemotherapeutics reinforce the significant role of oxidative injury in nephrotoxic acute kidney injury.[12,16-18]

Antioxidants are compounds that attenuate or inhibit the oxidation of cellular protein, lipid peroxidation of the cell membrane and injury of nucleic acids.[7] In this scenario are the medicinal plants. Containing flavonoids, they have been used for thousands of years in Eastern medicine, have antioxidant activity and protective function in the treatment of diseases mediated by reactive oxygen species.[19,20] The *Echinodorus macrophyllus*, described

by its antioxidant and anti-inflammatory action, stands out. It is rich in flavonoids, confirmed by the phytochemical analysis of the leaves.[19]

In this context, it was observed that the antioxidant action of the *Echinodorus macrophyllus* phytomedicine attenuated the decrease in renal function and the reduction of levels of peroxides and aldehydes from the lipid peroxidation. Similar results of antioxidant protection of the *Echinodorus macrophyllus* were found in studies carried out with the model of acute kidney injury induced by gentamicin.[17] The administration of flavonoids has shown a protective effect on nephrotoxicity induced by cisplatin in rats by elevating creatinine clearance, reducing lipid peroxidation and inflammatory mediators.[20] A similar result was shown in a study with the *Uncaria tomentosa* phytomedicine in a model of ischemic acute renal injury.[21]

Studies with phytomedicines have evolved in recent decades in an attempt to contribute to the treatment of acute and chronic diseases. Although there is still a need for research on the adverse effects of *Echinodorus macrophyllus* for its final introduction into clinical practice, the antioxidant protective mechanisms of that phytomedication make it a possible alternative of treatment for the prevention of nephrotoxic AKI.

This study will make it possible that the nursing correlates the clinical and basic research, and allow professionals to have an improved understanding of the physiological and pathological mechanisms affecting the nephrotoxic acute kidney injury by cyclophosphamide.

Conclusion

The antioxidant effect of *Echinodorus macrophyllus* promoted functional renoprotection evidenced by the increase in creatinine clearance and reduction of oxidative metabolites in the model of acute kidney injury induced by cyclophosphamide in rats.

Acknowledgements

Research carried out with the financial support from the Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP. Project FAPESP: 2010/02155-3

Collaborations

Nascimento EL; Watanabe M; Fonseca CD; Schlottfeldt FS and Vattimo MFF declare that contributed to the project design, analysis and interpretation of data, drafting the article, critical revision of the important intellectual content and final approval of the version to be published.

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