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# Effects of *Justicia acuminatissima*, or Amazonian *Sara Tudo*, on ischemic acute kidney injury: an experimental study*

## Efeito da *Justicia acuminatissima*, Sara Tudo do Amazonas, na injúria renal aguda isquêmica: estudo experimental

## Efecto de la planta *Justicia acuminatissima*, "Sana Todo del Amazonas", en la injuria renal aguda isquémica: estudio experimental

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## ABSTRACT

**Objective:** To evaluate the effects of *Justicia acuminatissima*, or Amazonian *Sara Tudo*, on renal hemodynamics, oxidative profile, and renal histology in rats with ischemic acute kidney injury. **Method:** Preclinical assay with adult male Wistar rats, weighing from 250 g to 350 g, distributed into Sham, ischemia, and ischemia + *Sara Tudo* groups. Hemodynamic parameters, renal function, oxidative stress, and renal histology were evaluated. **Results:** Pretreatment with *Sara Tudo* reduced the functional injury, which was shown by the increase in creatinine clearance and thiols; reduction of oxidative markers, renal vascular resistance, and tubulointerstitial injury in the renal tissue; and the significant improvement in renal blood flow. **Conclusion:** The renoprotection provided by *Justicia acuminatissima*, or *Sara Tudo*, in cases of ischemic acute kidney injury was characterized by a marked improvement in renal function, reducing the oxidative injury, and impacting on renal histology positively.

## DESCRIPTORS

Acute Kidney Injury; Reperfusion; Phytotherapeutic Drugs; Complementary Therapies; Animal Experimentation.

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## INTRODUCTION

Acute kidney injury (AKI) is characterized clinically as a syndrome of sudden loss of renal function, defined by the Kidney Disease: Improving Global Outcomes guidelines as a decrease in urinary volume to less than 0.5 mL/kg/h for over six hours and/or an increase in serum creatinine levels to over 0.3 mg/dL in 48 hours or higher than 1.5 to 1.9 times the baseline creatinine, which is known or supposed to have occurred at least seven days before the manifestation of symptoms[1].

The incidence and prevalence of AKI vary according to the adopted criterion. In South America, the described incidence is 29.4%, and the highest prevalence of this syndrome in the hospital setting occurs in intensive care units, affecting up to 25% of patients and reaching a mortality rate of 50%. Late diagnosis and persistence of AKI triggering factors result in a longer hospital stay, the need for dialytic therapy, and a worse prognosis, which contribute to the evolution of the disease chronicity, cardiac dysfunctions, and even death. This scenario confirms the importance of early diagnosis together with the identification of the AKI primary cause, because these two actions provide better conditions for repair and regeneration of kidney cells[2-3].

Acute kidney injury is induced by a mechanism of renal ischemia/reperfusion (I/R) or the administration of nephrotoxic agents, resulting in acute tubular necrosis. Ischemic AKI involves physiopathological mechanisms, such as inflammatory response, hemodynamic alterations in the renal microvasculature as a consequence of the generation of reactive oxygen species, and endothelial injury, followed by tubular dysfunction and epithelial cell injury, which culminate in kidney injury and cell death[4-5].

Despite decades of research, there are few therapies that can influence the pathogenic progress of ischemic AKI. Consequently, feasible, low-cost, and accessible interventions that minimize the proinflammatory and oxidative effects of this syndrome remain being sought[6].

Some experimental studies with herbal medicines showed promising renoprotective effects. Among them, the antioxidant effect of *Uncaria tomentosa* and isoflavones, which were proven to provide renoprotection and associated with an increase in glomerular filtration rate and reduction of oxidative metabolites in the rat model of ischemic AKI, stands out[7-8].

In the Brazilian state of Amazonas, the difficulties to access healthcare services and drug therapies and the trust in homemade medications, with low costs and easy access, contribute for the local population to resort to herbal medicines, even if empirically[9-10]. In this context, *Justicia acuminatissima* (JA, Miq.) Bremek, Acanthaceae, a subshrub found in the North Region of Brazil, popularly known as *Sara Tudo* or *Sara Tudo de Quintal*, has gained prominence and is used in the preparation of teas taken to heal injuries, provide an anti-inflammatory action, and help treat urinary tract infections[11-12].

The alarming scenario of occurrence of hospital and community AKI indicates the most effective measure to control the disease is prevention. The use of herbal medicines has grown worldwide as a preventive and healing strategy, encouraging the investigation of ethnobotany to evaluate plant extracts with different mechanisms of action, including antibacterial, anti-inflammatory, antihemorrhagic, and anesthetic applications.

The routine use of *Justicia acuminatissima* by the population in the state of Amazonas with few or no consistent results on the real benefits of *Sara Tudo* and the lack of evidence confirming its effect on acute diseases motivated the present study. Its main goal was to evaluate the effects of *Justicia acuminatissima*, or Amazonian *Sara Tudo*, on renal function and hemodynamics, oxidative profile, and renal histology in rats with ischemic AKI.

## METHOD

### Study type

The present study was quantitative and experimental and applied an animal model with adult male Wistar rats, weighing from 250 g to 350 g, provided by the Institute of Biomedical Sciences of the University of São Paulo, kept with free access to water and rat food, in proper thermal conditions and submitted to alternating day and night cycles.

### Setting

The present study was developed at the Experimental Laboratory for Animal Models of the School of Nursing at the Universidade de São Paulo.

The intervention herbal medicine was *Justicia acuminatissima* (Amazonian *Sara Tudo*), used as a powder, at a dosage adjusted in a pilot study.

### Data collection

Animals: Experiments were carried out with 21 adult male Wistar rats, weighing from 250 g to 350 g. The animals were distributed into three groups: sham (n=7), consisting of rats submitted to laparotomy, with simulation of renal ischemia; ischemia (isc) (n=6), consisting of rats submitted to laparotomy to perform the bilateral clamping of the renal pedicles for 30 minutes followed by renal reperfusion; and ischemia + *Sara Tudo* (isc+st) (n=8), consisting of rats that received 1,000 mg/kg of body weight of *Justicia acuminatissima* by gavage 60 minutes before ischemia and renal reperfusion procedures.

The rats were anesthetized with Thiopentax® (thiopental sodium 40-50 mg/kg) administered intraperitoneally and submitted to laparotomy, according to the group distribution.

Collection of biological material: After a 24-hour rest for postsurgical recovery, the animals were put in metabolic cages for 24-hour urine collection, renal function studies, and evaluation of oxidative stress. Subsequently, the animals were anesthetized with Thiopentax® (thiopental sodium 40-50 mg/kg, intraperitoneal administration) and submitted to laparotomy for the following procedures to be executed: measurement of renal blood flow (RBF) in the left renal artery, which was isolated and involved by

an ultrasound probe (T402; Transonic Systems, Bethesda, Maryland, EUA); invasive evaluation of the average arterial pressure (AAP) after dissection and catheterization of the carotid artery for the insertion of a catheter (polyethylene tube – PE 60); collection of terminal blood by puncture of the abdominal aorta; and section of the kidneys for histopathological examination.

Renal function: Renal function was assessed by analyzing creatinine clearance. Serum and urine creatinine were measured using the Jaffe method. Creatinine clearance was calculated applying the following formula: creatinine clearance = urine creatinine × 24-hour urinary flow / serum creatinine[13].

Renal hemodynamics: This characteristic was measured using the RBF and renal vascular resistance (RVR), taking into account the AAP. The following formula was used to calculate RVR: RVR = AAP / RBF[14].

Oxidative metabolites: The assessment of oxidative metabolites was carried out with the following measurements: urinary peroxides (UP) using the FOX-2 method[15]; final products of lipid peroxidation detected by the TBARS method (substances that react with urinary thiobarbituric acids)[16]; nitric oxide (NO) dosing using the Griess test[17] and non-protein soluble thiols in the renal tissue, with correction of quantified total proteins using the Bradford assay[18].

Renal histology: The Shi score graded from 0 to 4 was applied to evaluate tubulointerstitial impairment[19]. The tubular injury index was obtained according to the examination of the area with necrosis, inflammatory cell infiltrate, dilation, or tubular atrophy. The images obtained using optical microscopy were captured by a light videocamera connected to an image analyzer and examined as 0.245-mm$^2$ fields from slides containing samples of the renal tissue of each animal.

### Data analysis and treatment

Results were shown as averages ± standard deviations. Data treatment was carried out by applying an analysis of variance, followed by the Newman-Keuls multiple post-test for comparisons among groups using the GraphPad Prism 6.01® software. Values of $p<0.05$ were considered significant.

### Ethical aspects

The present study was approved by the Animal Research Ethics Commission of the University of São Paulo Medical School as per protocol no. 126/15/CEUA – FMUSP. The procedures performed in the present study comply with the ethical procedures for animal experimentation adopted by the Brazilian School of Animal Experimentation.

## RESULTS

### Renal function and hemodynamics

According to Table 1, the isc group showed a significant creatinine clearance reduction in comparison with the sham group, and the preconditioning with *Justicia acuminatissima* improved the renal function of the animals submitted to ischemia in comparison with the isc group. The isc group had a decrease in RBF and an increase in the RVR in comparison with the sham group. The isc+st group showed an increase in RBF and a reduction in RVR when compared to the isc group.

**Table 1 –** Global renal function and renal hemodynamics of the examined groups – São Paulo, SP, Brazil, 2017.

| Groups | n | Weight (g) | Clcr/100 g (mL/min) | RBF (mL/min) | RVR (mmHg/mL min) |
|---|---|---|---|---|---|
| Sham | 6 | 296±18 | 0.97±0.06 | 11.0±2.0 | 10.9±1.8 |
| Isc | 5 | 292±11 | 0.12±0.01[a] | 4.0±0.6[c] | 26.3±4.1[c] |
| Isc+st | 8 | 291±31 | 0.42±0.17[ab] | 7.7±1.3[d] | 12.1±3.1[d] |

Isc: ischemia; Isc+st: ischemia + *Sara Tudo*; Clcr: creatinine clearance; RBF: renal blood flow; RVR: Renal Vascular Resistance.
The values are shown in the average ± standard deviation.
[a]p<0.001 versus Sham
[b]p<0.001 versus Isc
[c]p<0.05 versus Sham
[d]p<0.05 versus Isc

### Oxidative stress

The results of the variables that determine the oxidative profile (Table 2) demonstrate that normality reference values occurred in the sham group. Pretreatment with *Justicia acuminatissima* reduced oxidative markers (UP, TBARS, and NO) in the animals submitted to renal ischemia and increased thiols in the renal tissue in comparison with the levels found in the isc group.

**Table 2 –** Oxidative profile of the examined groups – São Paulo, SP, Brazil, 2017.

| Groups | n | UP (nmol/g of crU) | TBARS (nmol/g of Ucr) | NO (µmol/g of Ucr) | Thiols in the renal tissue (nmol/mg of protein) |
|---|---|---|---|---|---|
| Sham | 7 | 5.6±0.4 | 56.7±7.2 | 30.2±2.2 | 46.1±6.0 |
| Isc | 6 | 13.6±0.6[a] | 113.7±15.6[a] | 76.6±6.6[c] | 19.9±0.9[a] |
| Isc+st | 8 | 4.3±2.7[d] | 62.0±20.0[b] | 13.2±15.0[cd] | 31.0±5.9[ab] |

Isc: Ischemia; Isc+st: Ischemia + *Sara Tudo*; UP: urinary peroxides; TBARS: substances that react with thiobarbituric acid; NO: nitric oxide; Ucr: urinary creatinine.
The values are shown in the average ± standard deviation format.
[a] p<0.05 *versus* Sham
[b] p<0.05 *versus* Isc
[c] p<0.001 *versus* Sham
[d] p<0.001 *versus* Isc

### Renal histology

Analysis of the tubulointerstitial injury carried out using the Shi scale, with grades varying from 0 to 4, revealed that the renal tissue in the isc group showed injuries when

compared with the sham group (p<0.001). Treatment with *Sara Tudo* induced a statistically significant reduction in the injuries in comparison with the isc group.

The results related to the injuries revealed that the sham group (A – Figure 1) shows normality standards, that is, the absence of injuries in the renal tissue. The images from the isc group (B – Figure 1) revealed edema, diffuse interstitial inflammatory infiltrate, flattening of tubular cells with dilation of the tubular lumen, focal areas of denudation of the basement membrane, and tubular necrosis. Preconditioning with *Sara Tudo* reduced the deleterious effects induced by ischemia, as demonstrated in the images obtained from animals in the isc+st group (C – Figure 1).

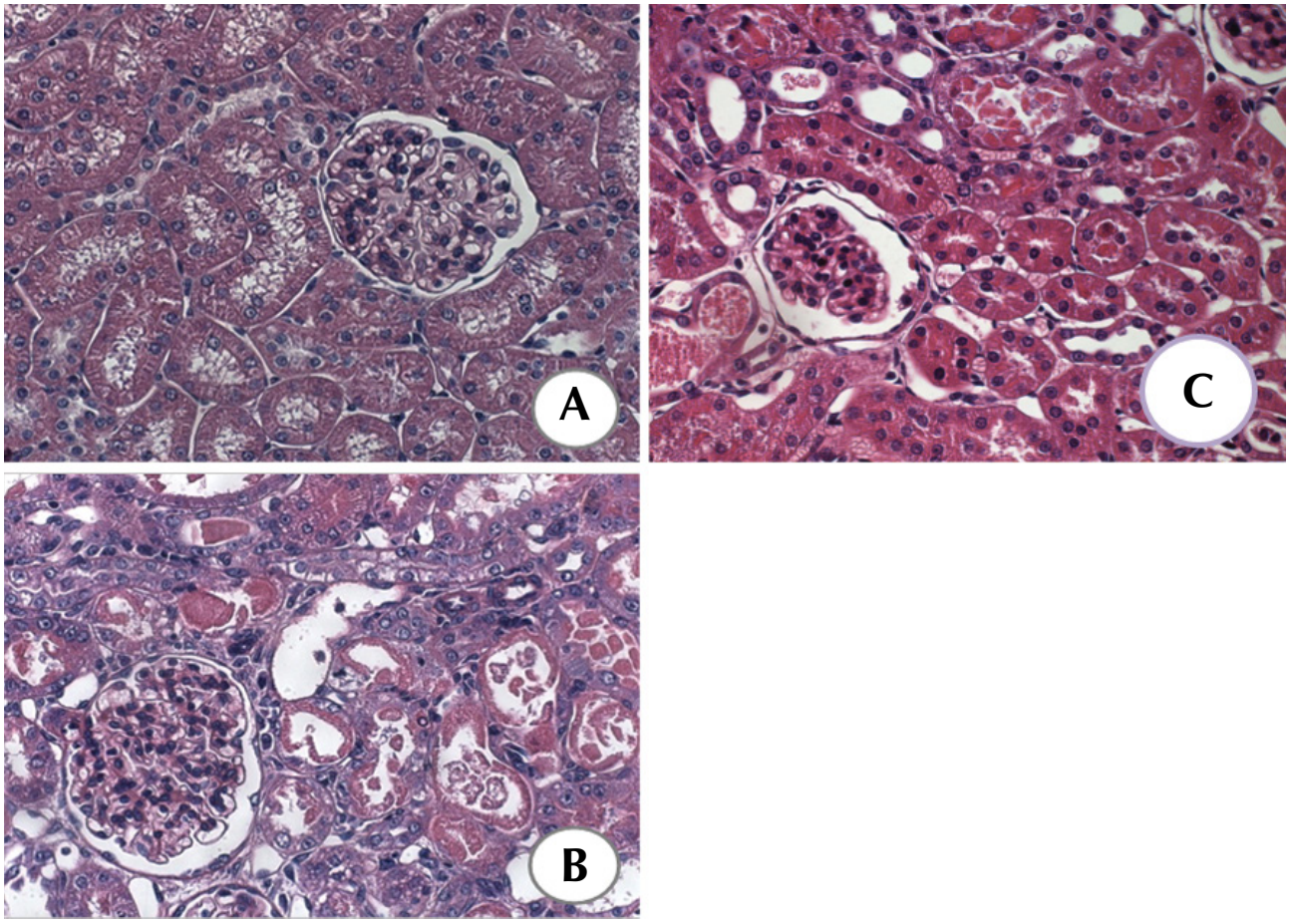


**Figure 1 –** Renal tissue histology. (A) sham, (B) isc, and (C) isc+st groups – São Paulo, SP, Brazil, 2017.

## DISCUSSION

The present study evaluated the effects of *Justicia acuminatissima*, or Amazonian *Sara Tudo*, on rats submitted to the model of I/R-induced AKI. The results confirmed the renoprotective effect of this herbal medicine on the animals used in the chosen experimental model.

I/R-induced AKI involves several complex physiopathological mechanisms, injury, and dysfunction in endothelial and tubular cells, inflammatory response, and formation of reactive oxygen species[20]. The experimental ischemic AKI model accurately reproduces what happens in the clinical post-transplant condition, a problem whose prevalence varies from 20% to 35% and is associated with a decrease in the glomerular filtration rate[20-21].

The isc group confirmed that the transitory 30-minute hypoperfusion resulting from clamping the renal pedicles triggers a response to renal ischemia demonstrated by the increase in the RVR and the consequent reduction of the RBF. The ischemic injury affects the endothelium barrier and reduces its integrity as a consequence of alterations in cell adhesion and the increase in the intratubular pressure. There is also an imbalance between vasodilator and vasoconstrictor agents, which leads to an increase in vasoconstriction, causing an increase in the total RVR and a decrease in the RBF, in addition to a reduction in the ultrafiltration coefficient[22-23].

The assessment of renal function in rats submitted to 30-minute renal ischemia demonstrated a creatinine clearance reduction, with the intensification of the redox mechanism, in addition to allowing the determination of renal parenchyma impairment. Authors reported a marked creatinine clearance reduction in animals submitted to 30-minute ischemia[8]. The results of oxidative profile analysis in the present study showed

an increase in UP, TBARS, and NO levels, as well as a reduction in thiols in the renal tissue in the isc group. It is well established that ischemia leads to several physiopathological events resulting from many metabolic and enzymatic processes, for instance oxidative stress. An animal experiment applying the 30- and 45-minute I/R model revealed similar results regarding oxidative injury, characterized by an increase in UP, TBARS, and NO levels and a reduction in thiols in the renal tissue. It is important to stress that the 45-minute ischemia was more aggressive and led to a more intense oxidative response[24].

The preconditioning with *Justicia acuminatissima* was associated with an improvement in hemodynamic parameters and confirms the increase in glomerular filtration rate and RBF and the reduction of RVR, which are interconnected, corroborated by the creatinine clearance increase. The improved renal function is probably related to the anti-inflammatory properties of *Justicia acuminatissima*, proven in other investigations. Glycosylated stigmasterol and glycosylated beta-sitosterol, two substances found in *Justicia acuminatissima*, act as anti-inflammatory agents, which was highlighted by the reduction of the injury-induced edema in animal paws[12,21].

A study that used *Dioclea violacea*, a herbal medicine with a composition similar to that of *Justicia acuminatissima*, in rats with the I/R model-induced AKI showed an improvement in hemodynamic parameters, with a decrease in RVR, demonstrating the similarity between the hemodynamic effects reported in that study and the present one[23].

Additionally, *Justicia acuminatissima* triggered an improvement in oxidation-related parameters, shown by a reduction in UP, TBARS, and NO, together with an increase in the levels of thiols in the renal tissue. The protective and antioxidant effect of the studied herbal medicine is similar to that provided by other plants, as illustrated by the use of isoflavones and *Uncaria tomentosa* in rats with I/R-induced AKI[7-8]. Investigations confirm that *Justicia acuminatissima* contains a set of substances denominated condensed tannins, which can reduce nitric oxide levels in rats, corroborating the antioxidant potential of the plant[20].

Renal histology analysis revealed that the isc group had significant interstitial injuries. However, *Sara Tudo* lessened the deleterious effects in the examined tissue. Observation of the kidneys of rats with AKI confirmed the tubulointerstitial alterations, with the presence of edema, diffuse interstitial inflammatory infiltrates, flattening of tubular cells with dilation of the tubular lumen, focal areas of denudation of the basement membrane, and I/R-induced necrosis. These findings are similar to those described for humans, including fading and loss of proximal tubule brush border, irregular loss of tubular cells, focal areas of proximal and distal tubular dilation, and areas of cell regeneration in conditions assumed as ischemic[24]. The previous conditioning with *Justicia acuminatissima* induced a decrease in alterations in the renal tissue submitted to I/R, confirmed by the reduction in diffuse inflammatory infiltrates, in edema, and in cell flattening in the renal tissue.

These findings suggest that *Sara Tudo* may be an adjuvant in preventing or treating renal injuries caused by ischemic events, such as transplants. In addition, these results can contribute to nurses' reasoning and provide scientific grounds for the routine use of this herbal medication by the general population and people who live in Amazonas in particular, strengthening a movement of clinical incorporation of therapeutic possibilities originated in the Brazilian flora.

## CONCLUSION

The previous treatment with *Justicia acuminatissima,* or Amazonian *Sara Tudo*, in rats submitted to the kidney ischemia model resulted in a reduction of renal injury, with an increase in creatinine clearance and in RBF and a reduction in RVR. The antioxidant activity of the herbal medicine was confirmed by the decrease in oxidative metabolites and in urinary peroxides, TBARS, and nitric oxide, combined with an increase in thiol levels, which reduced the tubulointerstitial injury. The results corroborate the therapeutic benefits of this herbal medicine commonly used by the population living in the Brazilian state of Amazonas.


## RESUMO

**Objetivo:** Avaliar o efeito da *Justicia acuminatissima*, Sara Tudo do Amazonas, na função renal, na hemodinâmica renal, no perfil oxidativo e na histologia renal em ratos com injúria renal aguda isquêmica. **Método:** Ensaio pré-clínico com ratos Wistar, adultos, machos (250-350 g), distribuídos nos grupos Sham, Isquemia e Isquemia + Sara Tudo. Foram avaliados os parâmetros hemodinâmicos, a função renal, o estresse oxidativo e a histologia renal. **Resultados:** O pré-tratamento com o Sara Tudo atenuou a lesão funcional, o que foi evidenciado pelo aumento no *clearance* de creatinina, redução dos marcadores oxidativos e elevação de tióis, pela melhora significativa do fluxo sanguíneo renal, diminuição da resistência vascular renal e redução da lesão tubulointersticial no tecido renal. **Conclusão:** A renoproteção da *Justicia acuminatissima*, Sara Tudo, na injúria renal aguda isquêmica, caracterizou-se por melhora significativa da função renal, reduzindo a lesão oxidativa, com impacto positivo na histologia renal.



## DESCRITORES

Lesão Renal Aguda; Reperfusão; Medicamentos Fitoterápicos; Terapias Complementares; Experimentação Animal.



## RESUMEN

**Objetivo:** Evaluar el efecto de la planta *Justicia acuminatissima*, "Sana Todo del Amazonas", en la función renal, la hemodinámica renal, el perfil oxidativo y la histología renal en ratones con injuria renal aguda isquémica. **Método:** Ensayo pre clínico con ratones Wistar, adultos, machos (250-350 g), distribuidos en los grupos Sham, Isquemia e Isquemia + Sana Todo. Fueron evaluados los parámetros hemodinámicos, la función renal, el estrés oxidativo y la histología renal. **Resultados:** El pre tratamiento con el Sana Todo atenuó la lesión funcional, lo que fue evidenciado por el aumento en el aclaramiento de creatinina, reducción de los marcadores oxidativos y elevación de tioles, por la mejora significativa del flujo sanguíneo renal, disminución de la resistencia vascular renal y reducción de la lesión tubulointersticial en el tejido renal. **Conclusión:** La renoprotección de la *Justicia acuminatissima*, "Sana Todo del Amazonas", en la injuria renal aguda isquémica se caracterizó por mejora significativa de la función renal, reduciendo la lesión oxidativa, con impacto positivo en la histología renal.

**DESCRIPTORES**

Lesión Renal Aguda; Repersusión; Medicamentos Fitoterápicos; Terapias Complementarias; Experimentación Animal.

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