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Article - Human and Animal Health

Essential Oil of *Lippia alba* Protects Against Ischemic-Reperfusion Acute Kidney Injury

Mariana Maciel Cavalcanti[1]
https://orcid.org/0000-0002-8313-9264

Marilia Lopes Monteiro[1]
https://orcid.org/0000-0003-2559-8793

Tiago Lima Sampaio[1]
https://orcid.org/0000-0002-3962-6508

Janaina Serra Azul Monteiro Evangelista[2]
https://orcid.org/0000-0001-5416-2273

Dânya Bandeira Lima[1]
https://orcid.org/0000-0002-6395-8561

Mary Anne Medeiros Bandeira[3]
https://orcid.org/0000-0003-0550-8308

Marcus Felipe Bezerra da Costa[1]
https://orcid.org/0000-0002-6121-1202

Alice Maria Costa Martins[1]*
https://orcid.org/0000-0001-8160-2027

Isabella Evelyn Prado de Azevedo[1]
https://orcid.org/0000-0002-7047-9091

[1] Universidade Federal do Ceará, Escola de Farmácia, Departamento de Análises Clinicas e Toxicológicas, Fortaleza, Ceará, Brasil; [2] Universidade Estadual do Ceará, Escola de Medicina Veterinária, Ceará, Brasil; [3] Universidade Federal do Ceará, Escola de Farmácia, Departamento de Farmácia, Fortaleza, Ceará, Brasil.

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*Correspondence: martinsalice@gmail.com; Tel.: +55-85-33668263 (A.M.C.M.)

HIGHLIGHTS

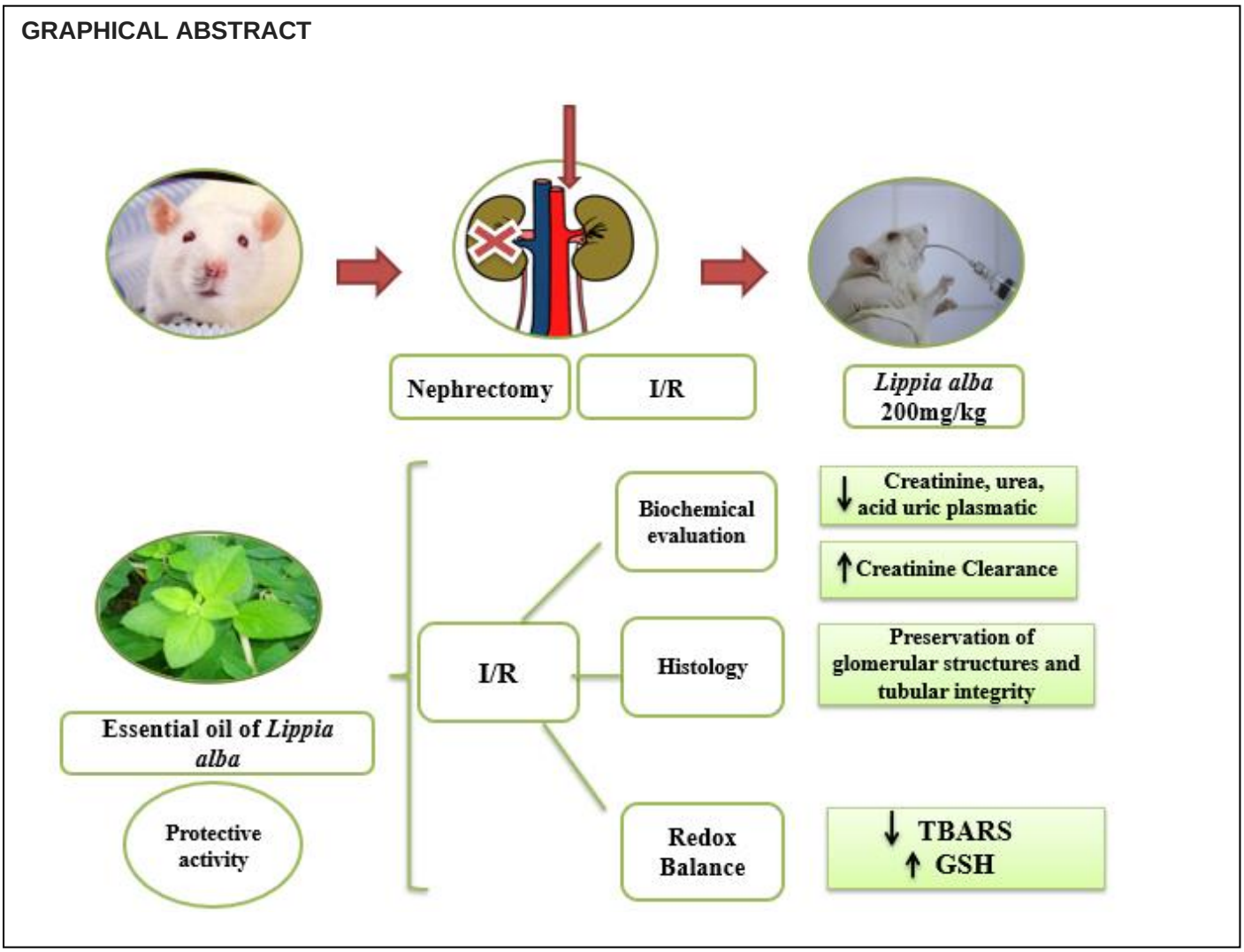
- Essential Oil of *Lippia alba* treatment reverted ischemia-induced biochemical alterations.
- Essential Oil of *Lippia alba* treatment ameliorated renal morphological alterations.
- Essential Oil of *Lippia alba* decreased oxidative stress in renal tissue.
- Essential Oil of *Lippia alba* reversed the ischemic damage on tubular cells.

Abstract: Ischemia/reperfusion (I/R) -induced Acute kidney injury (AKI) is characterized by hypoxia and production of reactive oxygen species (ROS), which could be prevented with antioxidant agents. The essential oil of *Lippia alba* (EOLA) chemotype citral-limonene is rich in components with antioxidant activity. So, this study aims to evaluate the nephroprotective effect of the EOLA on in vivo and in vitro models of renal I/R. Male Wistar rats were submitted to right nephrectomy, followed by ischemia by clamping the renal artery in the left kidney and a reperfusion. Animals received EOLA (200 mg/kg) or vehicle three days prior to I/R surgery. Blood and urine samples were obtained to evaluate creatinine, urea, uric acid, and creatinine clearance. The left kidney was collected for histological evaluations and analysis of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH). HK-2 cells were used to evaluate the effect of EOLA on I/R in vitro, using the MTT assay. Moreover, scanning electron microscopy (SEM) was performed to evaluate cell morphological alterations. The I/R resulted in biochemical parameter alterations, with EOLA

reverting all these alterations. Histological evaluation showed that EOLA protected the morphological changes caused by I/R. Also, EOLA was able to reduce TBARS and increase GSH levels in renal tissue. In the tubular cells, the EOLA partially reversed the damage caused by I/R, which was observed through the SEM. The EOLA showed effect against I/R-induced AKI through pre-treatment, protecting biochemical and oxidative parameters in vivo and reversing tubular cell damage in vitro.

Keywords: Ischemia; Reperfusion; Acute Kidney Injury; Citral-Limonene.

GRAPHICAL ABSTRACT



INTRODUCTION

Acute Kidney Injury (AKI) is characterized by metabolic changes such a rapid loss of kidney excretory function, accumulation of nitrogen metabolites, glomerular filtration barrier dysfunction and alteration on the tubular secretion and reabsorption. AKI also is associated with high morbidity and mortality, especially in hospitalized patients [1,2]. Ischemia/Reperfusion Syndrome is one of the predisposing factors for the installation of the AKI. The process of ischemia occurs with a decrease or interruption of blood flow, with a reduction or lack of oxygen supply, leading to hypoxia. When the oxygen supply is restored, the reperfusion results in the production of reactive oxygen species (ROS), increasing the tissue damage [3-5].

Antioxidant agents are extensively searching to treat this condition, especially those derived from natural sources. Citral and limonene, two antioxidant substances, are major components of the essential oil of one chemotype of *Lippia alba* [6-8]. *Lippia alba* is a plant known in Brazil as “erva-cidreira” used in folk medicine, as a sedative, analgesic, anti-inflammatory, antipyretic and antihypertensive. In the Brazilian Northeast exists chemotypes citral-myrcene, citral-limonene and limonene-carvone, based on major components. Each essential oil of different chemotypes has specific biological activity, which the citral-limonene chemotype has anxiolytic, sedative, and antispasmodic activities [9-10].

Isolated components such as citral and limonene have high production costs and low stability, which can be optimized using essential oils. Therefore, the present study aims to investigate the protective effect of the essential oil of *Lippia alba* chemotype citral-limonene on Ischemia/Reperfusion (I/R)-induced AKI.

MATERIAL AND METHODS

Collection of botanical material, extraction, and analysis of essential oil

Leaves of *Lippia alba* were collected in June, 180 days after planting, at the Medicinal Plants Garden Prof. Francisco José Abreu Matos of the Federal University of Ceará. The identification of the plant was carried out through exsiccate registered (n° 38.174) at the Prisco Bezerra Herbarium. The essential oil of *Lippia alba* (EOLA) was obtained from the Laboratory of Phytochemistry of Medicinal Plants located at the Pici Campus of the UFC by steam distillation with Clevenger's device, in 2-L steam distillation units at a maximum temperature of 100 °C until boiling, then reducing to 75 °C, for a period of approximately 2 h. Fresh samples (500 g) consisted of leaves. Prior to distillation, samples were chopped into 2.5 cm long pieces. To determine the yield of essential oil extracted from each 100 g of plant species, empty polypropylene microtubes ware weighed on an analytical balance and then the microtubes with the essential oils extracted from each plant were weighed. The percentage yield found was 2.69%, expressed as a function of the mass of oil obtained per 100 g of leaves. Essential oil sample was kept in a freezer at -20°C. All procedures were performed according to a previously established protocol [11].

The phytochemical characterization of the essential oil was made by mass spectrometry coupled gas chromatography (GC/MS) (Shimadzu GCMS QP2010s). The identification of the constituents was performed by interpreting the respective mass spectra by computerized comparison of the fragmentation records (m/z) with the spectra available in the NIST (National Institute of Standards and Technology) database.

Animals and surgical procedure

The experimental protocol was approved by the Ethical Committee on Animal Research of the Federal University of Ceará (UFC) (No. 110/2017) in accordance with the ethical guidelines. In order to avoid age-related impairment of renal function, male Wistar rats, aging between 7-8 weeks, weighting between 200-250 g, were maintained under controlled conditions (25 ± 2 °C ambient temperature, 12 h light-dark cycle). Food and water were offered ad libitum.

Animals were divided into 4 experimental groups (n = 6), and treated by oral gavage, once daily for 3 days before the surgical procedure. The 3-day time for treatment with EOLA was based in a previously established model [20]. SHAM and I/R groups received only water and SHAM+EOLA and I/R+EOLA received the essential oil of *Lippia alba* (EOLA). To calculate the treatment dose, the essential oil was initially weighed and diluted in pure dimethylsulfoxide (DMSO) in order to obtain a stock solution. Then, this solution was diluted in order to obtain 2% of DMSO v/v, a concentration considered innocuous for the animals. Next, the volume to be administered was calculated so that each animal would receive 200 mg/kg, as previously established [13].

For the surgery, animals were previously anesthetized using Ketamine 10% (100 mg/kg i.p.) and Xylazine 2% (10 mg/kg i.p.) and a laparotomy was performed through an incision in the midline, where the right nephrectomy was performed. The ischemia process was induced in the left kidney by clamping, which caused unilateral occlusion of the renal artery for 60 minutes, followed by reestablishment of renal blood flow and 48 hours of reperfusion [12].

Animals were kept warm by using a thermal plate at a constant temperature of approximately 36 ° C during surgeries. After the procedure, temperature, heart rate and respiratory frequency were checked every half hour. The surgery is not started until the body temperature is stabilized at the set-point, and the rat is under deep anesthesia and, thus, does not respond to pain stimulus induced by toe pinching.

The surgeries were performed equally in groups called "SHAM" (false surgery), excluding the right nephrectomy and renal artery clamping. During the last 24 hours of reperfusion, urine samples were obtained with the use of a metabolic cage. At the end of the 48-hour interval, blood samples were collected for biochemical tests and animals were anesthetized to collect the left kidney for further evaluation. The animals were submitted to euthanasia through the intravenous administration of potassium chloride, according to the ethical guidelines.

Evaluation of biochemical parameters

Blood samples collected from the animals were centrifuged in tubes containing lithic heparin (4500 rpm for 10 minutes) to obtain plasma. Biochemical parameters were measured using an automated analyzer (Roche Diagnostics Limited, Rotkreuz Switzerland). Urinary creatinine (uCr) and plasma levels of urea, uric acid, and creatinine (sCr) were determined. It should be noted that creatinine clearance (CrCl) was calculated as follows: CrCl = uCr x 24-hour urinary volume / sCr x Time (min) [14].

Histological analysis

After renal tissue removal, it was placed in a 10% buffered formaldehyde solution and, 24 hours later, transferred to a 70% ethanol solution. Then, tissue processing was performed and shortly thereafter, 5 µm median-sagittal paraffin sections were stained with hematoxylin-eosin for histological evaluation.

Determination of TBARS levels

The left kidney homogenate was prepared for oxidative stress analyses. The determination of malondialdehyde (MDA) in renal tissue was used as a lipid peroxidation indicator, as previously described, using the thiobarbituric acid reactive substance (TBARS) method [15]. Fragments of the left kidney were homogenized with 1.15% KCl in an ice bath, soon after the addition of 1% phosphoric acid and 0.6% thiobarbituric acid. The mixture was incubated at 96 °C for 20 min. Absorbance was measured by spectrophotometry at 532 nm (Asys UV 340, Biochrom, Cambridge, UK) and MDA was used for building a calibration curve. Results were expressed in µg/g tissue.

Determination of GSH levels

The concentration of reduced glutathione (GSH-γ-glutamyl-cysteinylglycine) was determined using renal tissue homogenate, in which a homogenate was prepared using 0.02 M EDTA in an ice bath. The samples were then mixed with 50% trichloroacetic acid, centrifuged (30.0 rpm, 15 min), and 400 µL of the supernatant were added to 800 µL of Tris-HCl buffer (0.4 M, pH 8.9) and 20 µL of the chromogenic DTNB (5,5-dithiobis-(2-nitrobenzoic acid)). Finally, a spectrophotometer (Asys UV 340, Biochrom, Cambridge, UK) was used to read absorbance at 412 nm expressed in µg/g tissue [16].

Culture of tubular kidney cells

HK-2 human renal tubular epithelial cells were donated by the Department of Biochemistry of the University of São Paulo and cultured in Dulbecco's Modified Eagle's medium (DMEM, Invitrogen, USA), plus 10% fetal bovine serum (FBS) and antibiotics and maintained in a CO2 oven at 37 ° C and 5% CO2 until confluence was reached [17].

The in vitro ischemia/reperfusion method was performed using the anaerobic chamber, as previously described [18]. Cells were plated at a concentration of 1 x 105 cells/mL in 96-well plates and incubated for 24 hours to allow cell adhesion and proliferation. For the induction of ischemia, the normal DMEM culture medium was changed to DMEM deprived of glucose, pyruvate and FBS. After that, the plates were incubated in the anaerobic chamber for 24 hours. Reperfusion was performed after the period in the chamber by adding a complete culture medium and returning the cells to a 5% CO2 atmosphere for 3 hours. Then the cells were treated with *Lippia alba* essential oil (EOLA) at concentrations of 1,000; 500; 250; 125; 62.5; 31.25; 15.62; and 7.81 µg/ml diluted in DMSO. Two-fold dilutions were made to avoid DMSO concentrations higher than 0.5%. After 24 hours, the percent cell viability was measured in the control, treated and I/R groups using the MTT assay [19].

Scanning electron microscopy (SEM)

Cells were cultured in 24-well plates and incubated for 24 hours as previously described. The samples were then fixed for 2 hours with 2.5% glutaraldehyde. Before SEM, these samples were dehydrated in increasing series of ethanol (30-100%), placed in glass coverslips, fixed at 37ºC with 5% CO2, covered with gold and observed in a FEG Quanta scanning electron microscope 450 (FEI, Oregon, USA).

Statistical analysis

Variable’s normality was analyzed statistically using a Shapiro–Wilk test. Parametric variables were expressed as mean ± standard error of the mean (SEM). For statistical comparison between the experimental groups, one-way ANOVA followed by the Bonferroni and Tukey’s post-test was used. The linear association

between continuous variables was analyzed using Pearson's correlation. Significance was set at $p<0.05$. Statistical analyses were performed using the GraphPad Prism 5.0 software (USA).

RESULTS

In order to investigate the composition of the essential oil, the GC-MS analysis was performed and allowed the identification of twenty constituents, among which the major were Geranial (trans-citral) (39.6%), Neral (cis-citral) (28.79%) and Limonene (10.05%). These components are shown in Table 1, with their respective retention times (RT) and percentage (%).

Table 1. Composition of the essential oil of leaves of *Lippia alba* citral-limonene chemotype analyzed by gas-chromatography coupled to mass-spectrometer (GC-MS) with their respective retention times (RT) and percentage (%).

Nº	COMPONENTS	RT (min)	%
1	Sabinene	14.556	0.66
2	6-metil-5-hepten-2-one	15.166	0.73
3	p-cimene	17.517	2.85
4	**Limonene**	17.835	10.05
5	γ-Terpinene	19.555	2.19
6	Linalool	21.908	0.90
7	β-copaene	25.831	1.46
8	Linalool oxide	26.923	1.99
9	α-Copaene	29.724	0.88
10	**Neral (cis-citral)**	30.751	28.79
11	Naftalene	30.949	2.48
12	**Geranial (trans-citral)**	32.511	39.60
13	Nerol	33.426	0.54
14	Germacrene	44.902	2.18
15	γ-murolene	46.391	0.49
16	Elemol	47.187	1.31
17	Nerolidol	47.362	0.79
18	Cariofilene oxide	47.929	0.56
19	Guaiol	48.067	0.56
20	β-eudesmol	48.847	0.98

Biochemical alterations were observed in the animal of I/R group compared to Sham. It was observed a decrease in creatinine clearance, increase in plasma levels of creatinine, urea, and uric acid. These alterations indicate the onset of AKI in the proposed model. However, no changes were observed in the diuresis of the animals of the different groups.

Therefore, the pre-treatment with EOLA showed a 6.2-fold increase in creatinine clearance compared to the I/R group, a decrease in 54.85% of creatinine, 36.56% of urea and 72.77% of uric acid plasma concentrations also compared to I/R group (Figure 1).

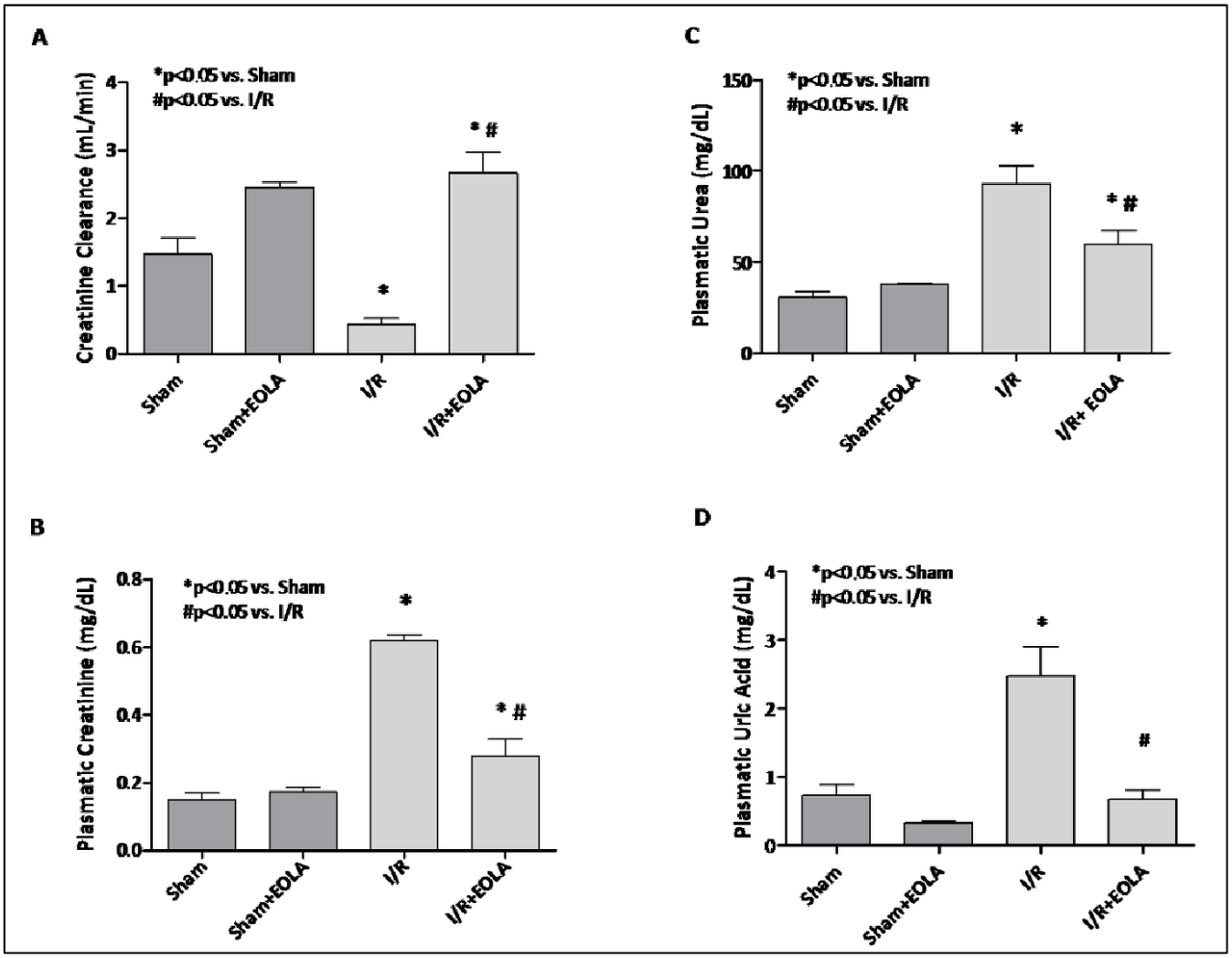


Figure 1. Effects of essential oil of *Lippia alba* on biochemical parameters after Ischemia/Reperfusion (I/R) process. (A) creatinine clearance; (B) plasmatic creatinine; (C) plasmatic urea; (D) plasmatic uric acid; Results are shown as mean ± SEM. *p < 0.05 vs. sham group and # p < 0.05 vs. I/R group.

Aiming to evaluate structural alterations on the renal architecture, histological analysis was performed (Figure 2). Sham group showed cell integrity, demonstrating the absence of injury (Figure 2A-C). I/R caused glomerular atrophy and intense protein deposition in the cortical region (Figure 2D); at the medullar level, vascular congestion process and the presence of intratubular proteins were observed (Figure 2E-F). Pre-treatment with EOLA showed the preservation of glomerular structures and tubular integrity, as well as lower vascular congestion foci than I/R group (Figure 2G-I).

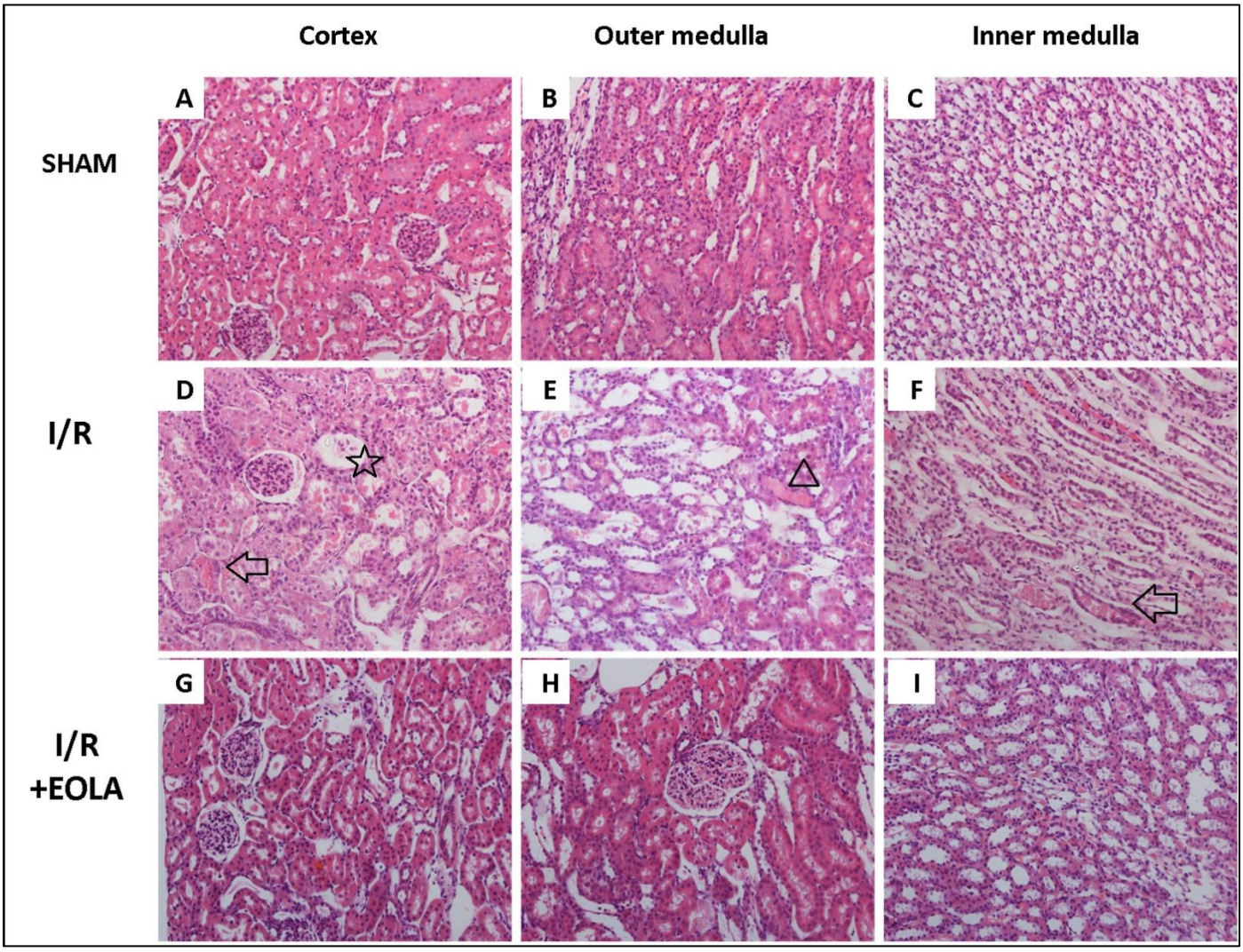


Figure 2. Photomicrography of kidneys stained with hematoxylin and eosin. Sham group is showed in A (cortex), B (outer medulla) and C (inner medulla). Ischemia/reperfusion (I/R) group is showed in D (cortex), E (outer medulla) and F (inner medulla). Treated group is showed in G (cortex), H (outer medulla) and I (inner medulla). Magnification of 200 ×; Microscope Nikon Eclipse Nis, Software Nis 4.0. Legend: glomerular atrophy (star); vascular congestion (arrow); intratubular proteins deposition (triangle).

For the oxidative stress analysis, the levels of TBARS and GSH were measured in renal tissue. I/R group showed an increase in TBARS formation and a decrease in GSH levels when compared to Sham group, indicating the formation of ROS after the injury. Pre-treatment with EOLA led to a reduction of TBARS by 30.48% and an increase in GSH formation by 68.91%, when compared to the I/R group, suggesting antioxidant effect (Figure 3A-B).

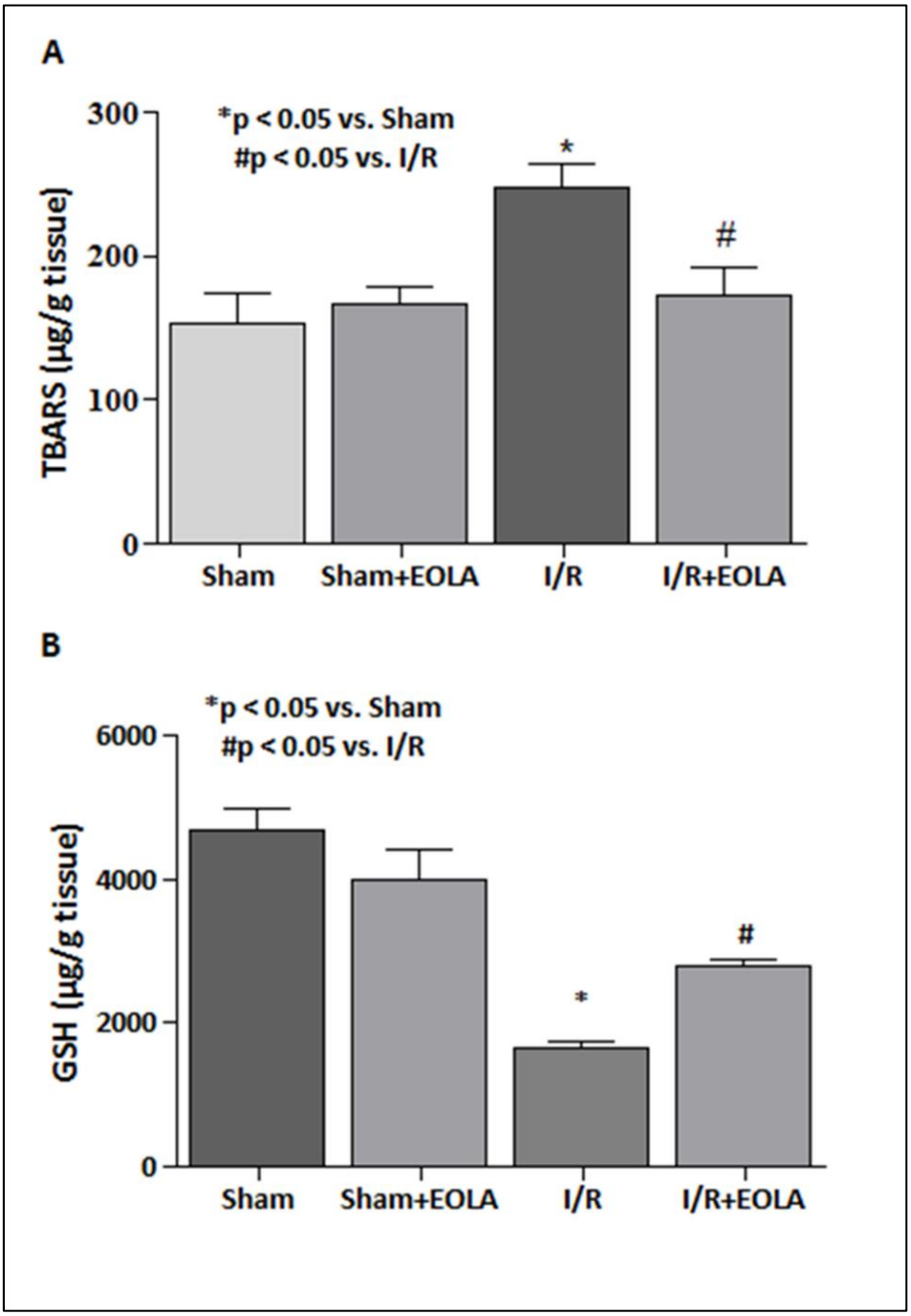


Figure 3. Oxidative stress evaluation on kidneys after the after Ischemia/Reperfusion (I/R) and the pre-treatment with essential oil of *Lippia alba* (EOLA). Thiobarbituric Acid Reactive Substances (TBARS) (A). Reduced glutathione (GSH) (B). Results are shown as mean ± SEM. *p < 0.05 vs. sham group and # p < 0.05 vs. I/R group.

Focusing on the antioxidant activity of EOLA for renoprotective effects, a correlation analysis was performed to evaluate the linear association between serum creatinine or creatinine clearance (used to estimate glomerular filtration rate - GFR) and oxidative parameters (GSH and TBARS), as shown in Figure 4. A strong (when 0.8 ≤ Pearson's r < 1) positive correlation ($p < 0.0001$) was observed between serum creatinine and tissue TBARS; in the same way that a negative correlation was observed between serum creatinine and tissue GSH ($p < 0.0001$; $r = -0.8148$). Thus, as serum creatine increases, lipid peroxidation increases and antioxidant defenses decrease in the same proportion. When the GFR estimate was considered, it was noticed that there was a negative correlation ($p = 0.0005$) of moderate intensity (when -0.5 ≤ r < -0.8) between TBARS and creatinine clearance; however, there was no significance in the analysis of the correlation between creatinine clearance and GSH.

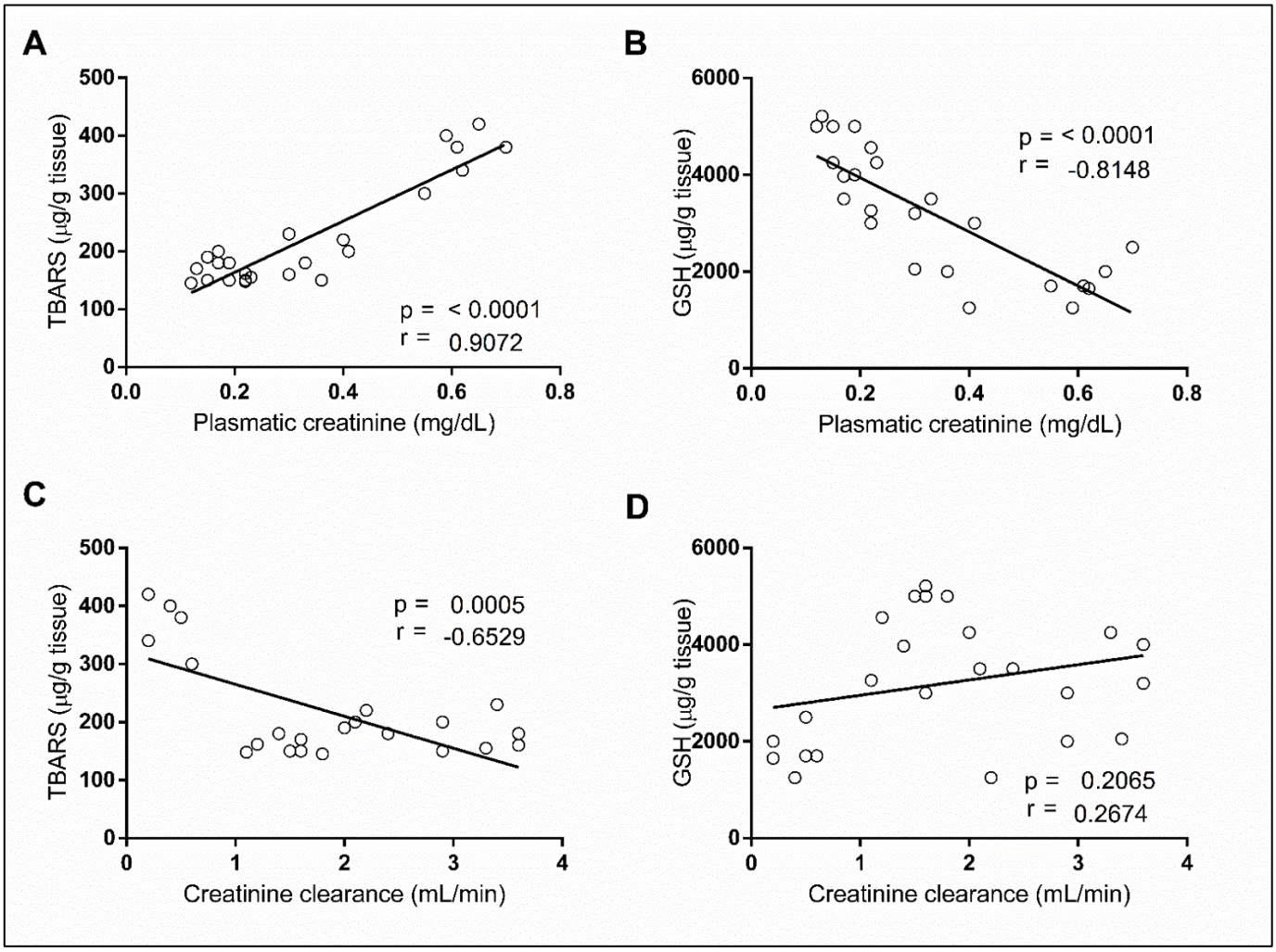


Figure 4. Correlation analyzes between renal function markers and oxidative parameters. (A) Correlation between plasmatic creatinine and thiobarbituric acid reactive substances (TBARS); (B) Correlation between plasmatic creatinine and reduced glutathione (GSH); (C) Correlation between creatinine clearance and TBARS; (D) Correlation between creatinine clearance and GSH. Pearson's correlation ($p < 0.05$), r = correlation's coefficient.

Aiming to evaluate the influence of EOLA in tubular cells viability, the MTT assay was performed. In the cell culture assay, a reduction in cell viability was observed in I/R group (54.8%±1.9), when compared to the control group (100%±2.1). Cells treated with EOLA showed an increase in cell viability at concentrations of 62.5 µg/mL (99.3% ± 4.0) and 125 µg/mL (67.3% ± 2.9) (Fig 5). Thus, the group treated with the 62.5 µg/mL concentration was chosen to undergo Scanning Electron Microscopy (SEM) analysis, since it promoted higher percentage of viability in HK-2 cells.

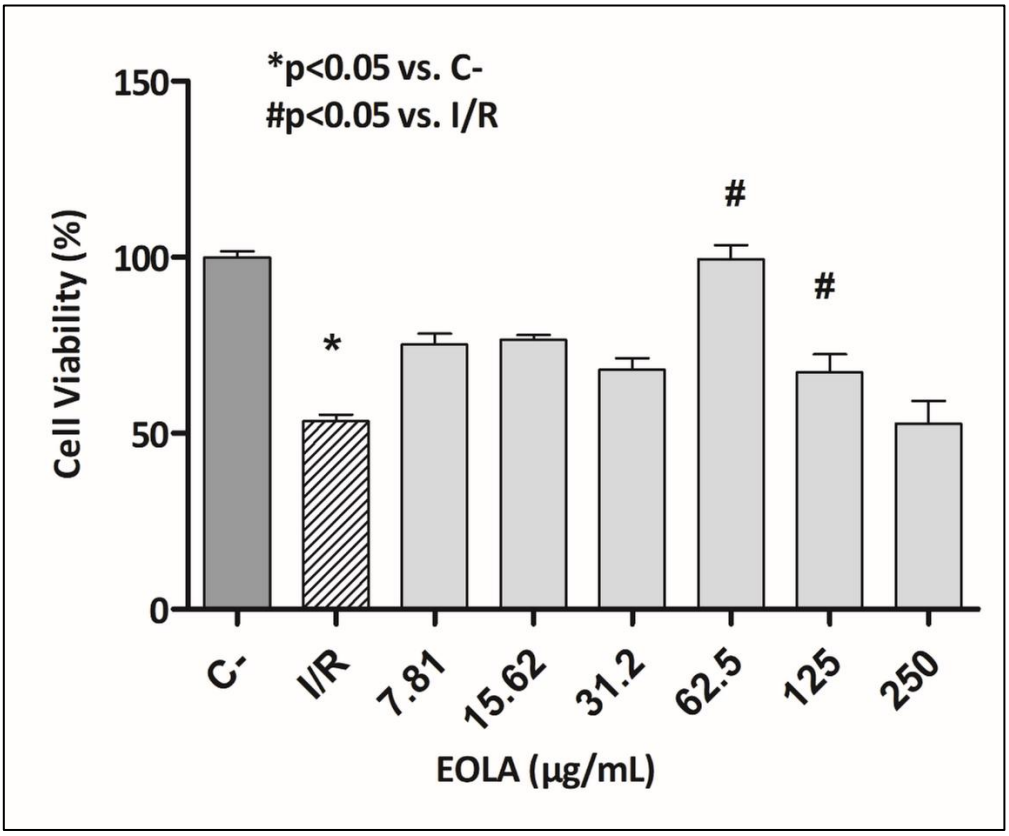


Figure 5. MTT assay showing the effect of the anaerobic chamber method on HK-2 tubular cells viability and the protective effect of essential oil of *Lippia alba* at different concentrations. Results are shown as mean ± SEM. *$p < 0.05$ compared to the control groups.

With the aim of visualize ultra-structural alterations on the cell surface, scanning electron microscopy was performed. Control group, composed of HK-2 cells submitted to aerobiotic conditions, presented preserved morphology (Figure 6A). In addition, cells submitted to hypoxia and reoxygenation showed changes in their morphology, such as cellular volume retraction, reduction of adhesion to the underlying cells and formation of blebbing, characteristic of apoptosis (Figure 6B). Finally, the treatment with the EOLA, at the concentration of 62.5 µg/mL, was able to promote a partial recovery of the morphological structure of the tubular cells submitted to I/R (Figure 6C).

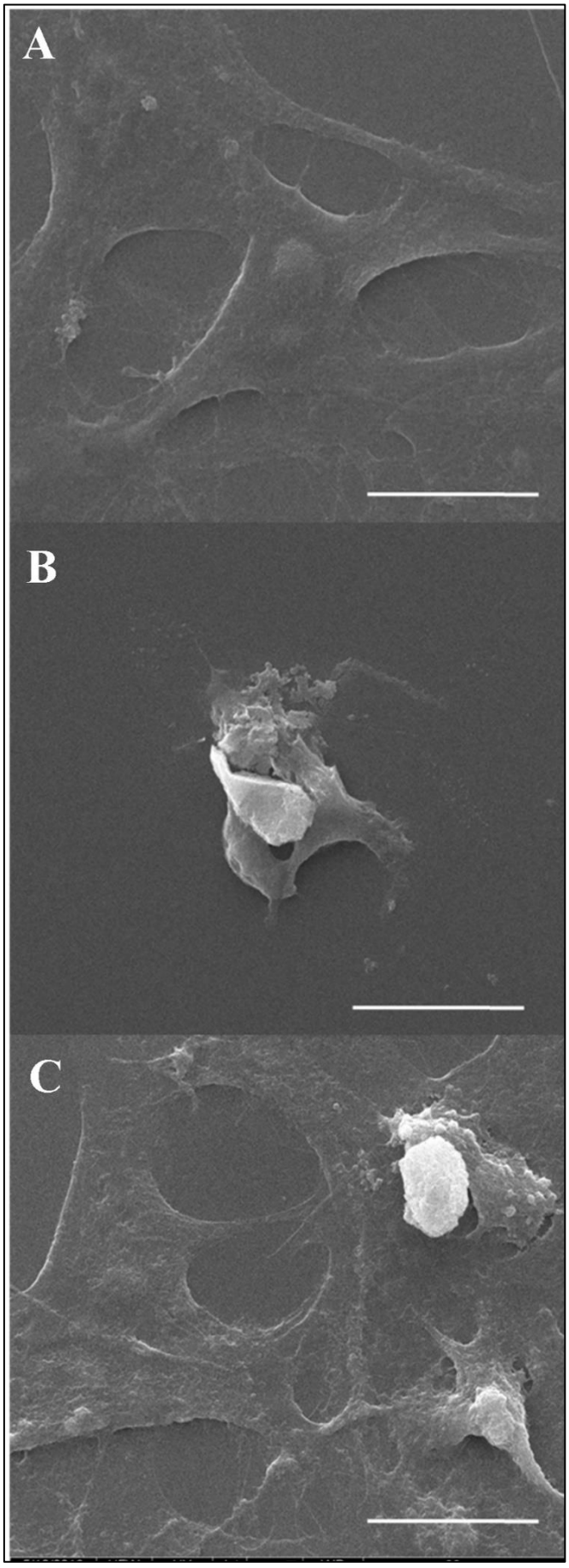


Figure 6. Scanning electron microscopy. (A) HK-2 cells under aerobic conditions; (B) HK-2 cells submitted to the process of ischemia/reperfusion without treatment and (C) HK-2 cells submitted to the ischemia/reperfusion process treated with essential oil of *Lippia alba* (62.5 µL/mL).

DISCUSSION

Natural products are sources of antioxidant substances of low-cost. They can protect, reverse, or improve I/R-induced AKI [12]. So, we decided to study the nephroprotective effect of EOLA chemotype citral-limonene on in vivo and in vitro models of AKI, because of antioxidant properties of their major components.

The in vivo model performed in this work was used in previous studies, involving natural nephroprotective substances. This model was able to show AKI through biochemical and histological parameters in I/R group, such as the beneficial effects of substances in the treated group. It was also able to induce changes in renal tubules and glomeruli [20].

The components of the EOLA could vary according to environmental factors, but the two major components of this chemotype have showed similar proportions in different regions (north and northeast of Brazil), with citral in 58-75% and limonene in 7-9.85% [20-22]. In this study, the concentrations obtained by CG-CM were also similar, showing citral (cis-citral and trans-citral) as 61% and limonene as 10%.

After the characterization of EOLA, we performed the in vivo assays in I/R-induced AKI model. AKI is characterized by a decrease in glomerular filtration rate (GFR), with consequent renal function deterioration [23]. It also is known that renal function reduction is manifested through the accumulation of nitrogen metabolites, such as urea and creatinine, indicating possible renal alteration [24]. In this study, I/R group showed increased plasmatic concentrations of creatinine, urea, and uric acid, suggesting renal damage. The pre-treatment with EOLA partially prevented the alterations caused by the injury process showed by reduced levels in nitrogen metabolites. In addition, the pre-treatment with EOLA was able to prevent the decreasing of glomerular filtration rate represented by creatinine clearance and was able to prevent partially histological alterations, such as vascular congestion, glomerular atrophy, and protein deposition, which were observed in this study in the I/R group.

In fact, data from the literature have shown that the inhibition of the inflammatory process, especially involving oxidative stress, is an important pharmacological tool in protecting against damage caused by I/R [25]. For example, recent work showed that I/R increased tissue levels of TBARS. Interestingly, these findings were associated with biochemical markers of renal function, such as an increase in plasma concentrations of creatinine and nitrogenous compounds, such as urea, which was associated with a decrease in GFR. The authors associate these phenomena, in part, with the synthesis of nitric oxide (NO), due to an increased tubular expression of inducible nitric oxide synthase (iNOS), potentiating nitrosative stress, leading to tubular damage due to the accumulation of peroxynitrite ($ONOO^-$). This could cause direct cellular damage, such as membrane lipid peroxidation and consequent apoptosis; vascular, due to inhibition of endothelial NOS (eNOS) by NO; and inflammatory, represented by increased mRNA expression for Tumor Necrosis Factor-alpha (TNF-α) and intercellular adhesion molecule-1 (ICAM-1), which would generate an inflammatory infiltrate, which was confirmed by histopathological observation. In addition, piperine, an alkaloid extracted from the seeds of *Piper nigrum* (black pepper), known for its antioxidant and anti-inflammatory potential, reversed biochemical and morphological changes, especially in GFR [26]. These data are important, as the present study found strong correlations between renal function markers and oxidative parameters, showing that an improvement in the oxidative state may be associated with an increase in GFR.

Previous studies have shown the of TBARS and GSH measurements for redox balance evaluation after treatment with natural products. The I/R process generates reactive oxygen species (ROS) responsible for exacerbating the AKI [12]. Citral is able to improve some oxidative stress markers, such as ROS-scavenging activity, increase the activities of antioxidant enzymes, like Glutathione-S transferase (GST), superoxide dismutase (SOD) and lactoperoxidase (LPO). The other major component, limonene, can up-regulate and increase the activity of antioxidant enzymes (SOD, catalase and peroxidases), reduce levels of thiobarbituric acid reactive substances (TBARS), inhibit of the phosphorylation of p38 MAPK, which mediates oxygen peroxide-induced apoptosis and increase ROS-scavenging activity [7-8]. The present study showed in I/R group a significant increase in the formation of TBARS and glutathione oxidation. The pre-treatment with EOLA showed lower levels of TBARS and higher levels of GSH than I/R group, demonstrating the antioxidant effect of the essential oil.

Some conditions, as hemi nephrectomy, vascular surgeries, hemorrhagic stroke, and dehydration are predisposing to I/R-induced AKI. Once these clinical outcomes are characterized by hypoxia, reduction on the blood flow and impairment on the energetic substrate offering. Thus, several strategies are used in order to prevent the renal function loss, such as the use of hemodynamic tools (fluid reposition and dialysis) or antioxidants tools (N-acetyl-cysteine, taurine and ascorbic acid) [27-28]. In this context, the pre-treatment with essential oil of *Lippia alba* has potential as a tool for prevent this injury.

In vitro studies also have been evaluating the I/R damage in tubular renal cells [12]. Hypoxic damage results in a series of physiological alterations, causing changes in tubular cells, impairing cellular ATP

production, leading to cell death by apoptosis or necrosis [29]. In the present study, the treatment with EOLA was able to reverse the damage caused by hypoxia renal tubular cells, which has a high regenerative potential.

Studies in the literature suggest that the use of antioxidant substances can prevent or reverse the occurrence of morphological changes in models of oxidative imbalance [30]. Considering that plant extracts rich in citral and limonene have previously been shown to have antioxidant potential [31], the results obtained in this work suggest a cytoprotective potential of *Lippia alba* based on its antioxidant effects. Oxidative stress leads to the ROS accumulation, which is responsible to modify the cellular architecture through oxidation of membrane phospholipids, dysfunction of structural proteins, enzymes, and nucleic acids. These phenomena are responsible to the modifications of cells glycocalyx, adhesion of epithelial and maintaining of glomerular negativity barriers, corroborating biochemical and microscopical findings [32]. In the present study, SEM was used to assess the morphological alterations of HK-2 cells after I/R. It was observed that the cells submitted to the process showed cell damage, such as loss of extracellular matrix and contact with neighboring cells and size and volume reduction, suggesting apoptosis blebbing. These alterations were partially reverted by the treatment with EOLA.

Lippia alba presents seasonality characteristics, being a plant that needs specific conditions of temperature, soil, and climate; therefore, it must be collected under specific conditions to essential oil extraction. Although these limitations, the production of essential oil have low costs and it could help poor countries to prevent AKI induced by I/R and the morbidity, mortality and high costs of health care related to AKI. In conclusion, essential oil of *Lippia alba* (lemon balm) chemotype citral-limonene showed itself as a potential pharmacological and biotechnological tool against I/R-induced AKI through pre-treatment, protecting biochemical and oxidative parameters in vivo.

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