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Furosemide Reduces TNF Levels and Increases Antioxidant Activity in Animal Models of Nephrotic Syndrome

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Authors' contributions

This work was carried out in collaboration among all authors. Author WFP designed and coordinated the study. Authors BLSA, TPS, RMS, PFPA, ARG, VJL, KBC, DAF, BFM and, GEBAM collected the data. Authors BLSA, WFP and ARG analyzed the data and drafted the article. All authors read and approved the final manuscript.

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ABSTRACT

Nephrotic syndrome (NS) is a renal condition characterized by proteinuria, edema formation, hypoalbuminemia and dyslipidemia. Evidence indicates that the immune response plays a fundamental role in disease evolution and maintenance. Although diuretics are used in the NS treatment, it is not known whether they have any effect on immune and redox responses. Therefore, the aim of the present study was to evaluate the furosemide effects in the inflammatory and redox responses in a doxorubicin- induced NS model. Eighteen male adult Wistar rats were divided into 3 groups: Control (n = 6) - received intravenous injection of saline solution; DOXO (n = 6) - received intravenous injection of doxorubicin (7.5 mg/kg); DOXO-F (n = 6) - received intravenous injection of doxorubicin (7.5 mg/kg) and were later treated with furosemide by gavage (5.0 mg/kg). At the end of 36 days of treatment were evaluated: urine protein concentration, blood leukocyte count, kidney histology, cytokine levels (TNF-α, INF-γ and TGF-β), antioxidant levels (FRAP) and enzyme activity (CAT and SOD), besides markers of oxidative stress (TBARS and protein carbonyl) in renal tissue. Data were analyzed with ANOVA and Tukey test when necessary ($p < 0.05$). Furosemide, at the dosage used in this study, promoted increased in global blood leukocytes and reduced lymphocyte blood count. It was also observed that furosemide reduced TNF-α and increased TGF-β levels in renal tissue. In addition, furosemide increased the levels of oxidative stress markers (TBARS and protein carbonyls) and the activity of antioxidant enzymes (SOD and CAT). Thus, furosemide showed anti-inflammatory effects in rats with nephropathy, by reducing TNFα levels and increasing antioxidant activity in kidney tissue.

Keywords: Nephrotic syndrome; furosemide; animal model; cytokines; redox status.

1. INTRODUCTION

Nephrotic syndrome (NS) is one of the commonest renal conditions in the world [1–3]. The global incidence of NS can range from 1.2/100,000 to 4.71/100,000 among children aged 1 to 18 years [1,4,5], with a higher incidence in females [5]. In adults, a higher incidence has been reported in men, and increased with age to 11.77 per 100,000 person-years [6]. In a study carried out in Brazil, the average age for the manifestation of NS was 2.9 years, and in 13.8% of these cases it occurred in the first year of life [2].

The NS is caused by primary kidney diseases or induced by secondary causes, such as diabetes mellitus, lupus erythematosus, bacterial infections or medications [7]. Clinically, NS is characterized by intense proteinuria, edema formation, hypoalbuminemia and dyslipidemia [8]. These conditions develop from the increased permeability of the renal glomerular membrane, leading mainly to urinary elimination of massive amounts of proteins and others complications [8].

Although the NS mechanism is not fully understood, evidence indicates that the immune system plays a fundamental role in the disease evolution. For example, immune response may cause the disruption of the glomerular filtration barrier, which would be a major factor in the clinical manifestations of the primary or secondary NS [9]. Also, inflammatory infiltrates of macrophages and T lymphocytes were observed in the tubulointerstitial zone in NS early stages [10]. In addition, other studies show that cytokines and reactive oxygen species can alter the permeability of the glomerular capillary wall [11–13]. Aggravatingly, proteinuria provides the recruitment of more macrophages, prolonging the inflammatory response in the interstitium [14,15].

Regarding NS treatment, in patients with greater renal impairment, loop diuretics are used to contain the disease evolution [16,17]. The oral treatment with furosemide reduces edema formation and controls blood pressure through its natriuresis and diuresis mechanisms [16–19]. Additionally, furosemide also showed anti-inflammatory and antioxidant effects when using other experimental models [20–22], suggesting that this drug could also have an effect on NS inflammatory and oxidant responses.

In this context, it remains to be explored whether furosemide has any action on inflammatory and redox state markers in NS. Therefore, the aim of the present study was to evaluate the effects of furosemide on proteinuria, leukocyte response, production of the cytokines TNF, IFN and TGF, redox response, as well as on histological alterations, in an experimental model of NS.

2. MATERIALS AND METHODS

2.1 Sample Collection

All experimental procedures were approved by the Ethics Committee on the Use of Animals of Federal University of Jequitinhonha and Mucuri Valleys - UFVJM (protocol number 001/2019) and occurred according to the ethical principles of animal use [23]. Eighteen male Wistar rats were obtained from Experimental Physical Training Laboratory/UFVJM and keep under controlled environmental conditions, with free access to food (Nuvilab® CR-1) and potable water.

2.2 Experimental Design

On the first day of the experiment (D0), 18 male Wistar rats (six-week-old) were randomly placed in three groups: Control (n = 6) - received intravenous injection of saline solution (isotonic and pyrogen-free sodium chloride solution 0.9%); DOXO (n = 6) and DOXO-F (n = 6) - received intravenous injection of doxorubicin hydrochloride (7.5 mg/kg of body weight) [24].

The furosemide administration (5.0 mg/kg of body weight) occurred by gavage only for the DOXO-F group, from the seventh day (D7) onwards (D36). The Control and DOXO groups received potable water under the same conditions. The treatments were performed daily until the day of euthanasia (D36).

2.3 Protein Evaluation

Protein levels were measured using 24-hour urine samples, collected weekly in metabolic cages (Insight®, Ribeirão Preto, São Paulo, Brazil) on D0 (before doxorubicin injection), D7, D14, D21, D28 and D35 (after doxorubicin injection). First, the urine samples (24 hour) were diluted in the proportion of 100 microliters of the sample for each 9.9 mL of deionized water [25]. Proteinuria analyses was carried out by colorimetric Bradford assay [26].

2.4 Euthanasia and Sample Collection

On D36, the animals were anesthetized (xylazine 8 mg/kg; ketamine 60 mg/kg) and euthanized by xsanguinations process. The blood was collected to perform blood leukocyte count. The left kidney was removed and sectioned for histological analysis, in addition to cytokines and redox state evaluations.

2.5 Blood Leukocyte Count

For global leukocyte count, 10µL of the blood sample from each animal was diluted in 190µL of Turkey's solution (red cell lyser) and count in a Neubauer hemocytometric chamber (Neubauer Improved®, Marienfeld – Germany) under light microscopy (Olympus Optical Co., Ltd. Microscope – BX41 TF – Japan). Differential white blood cell counts were performed in blood smears using an optical microscope (Olympus-BX41 TF, Japan) after May-Grunwald-Giemsa staining [24].

2.6 Histological Analysis

The left kidney was segmented into coronal sections, fixed and stained with hematoxylin and eosin (HE) and Masson's Trichrome (MT) for the analysis of morphological changes and kidney fibrosis, according to Pereira et al., 2015a. Histological images were analyzed using light microscopy (Microscope Olympus Optical CO. Ltd – BX41 TF-Japan) and scanned by a camera (NIKON, Eclipse E220) attached to the microscope. The ImageJ® (ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA) software was used for the analysis.

2.7 Cytokines Analyses

For cytokines analyses, renal samples were homogenized in the extraction solution (0.4 M NaCl, 0.05% Tween 20, 0.5% bovine serum albumin (BSA), 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA, and 20 kIU aprotinin) prepared in PBS.

The homogenates were centrifuged at 10,000 × g for 10 min at 4 °C. Tumor necrosis factor-α (TNF-α), Interferon-γ (INF-γ) and Transforming growth factor-β (TGF-β) levels were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems®) and as previously described [27]. TGF-β/TNF-α ratio was calculated and classified as an anti-inflammatory marker [28].

2.8 Redox State

For tissue preparation, the kidney was homogenized in cold PBS (50mM, pH 7.0), and centrifuged at 750 g for 10 min at 4 °C, as previously described [29]

The total antioxidant capacity was evaluated using the ferric reducing antioxidant power (FRAP) method, monitored at 550nm, with FeSO4 used as standard [27,30]. For the activity of the antioxidant enzyme superoxide dismutase (SOD), the pyrogallol oxidation method was used. The oxidation process was measured at 420nm for 250s at intervals of 10s and 37ºC [29,31]. Catalase (CAT) activity was assessed by metabolizing hydrogen peroxide. The readings were performed in a microplate reader every 15 seconds for 1 minute at 25ºC [2 9 , 3 2] .

The lipid peroxidation evaluation was performed using the thiobarbituric acid reactive substances (TBARS) method with malondialdehyde (MDA; 1,1,3,3- tetramethoxypropane) as the standard, being monitored at 532nm using [29,33]. Protein carbonyls were evaluated in pellets from the homogenates and were determined using the 2,4-dinitrophenylhydrazine (DNPH) method, monitored at 370 nm [29,34].

Protein content was quantified using BSA (1 mg/mL) as the standard [26]. The results of the redox state were corrected for the amount of protein in the samples. All redox analyses were performed in triplicate, using a plate reader (UV/visible U-200 L Spectrophotometer).

2.9 Statistical Analyses

Analyses were performed using the GraphPad Prism 8.0 (GraphPad, La Jolla, California, USA). The results were expressed as mean and standard deviation (SD), with a significance level of 95% ($p < 0,05$). According to normality, the data were analyzed using the one-way or two-way analysis of variance (ANOVA), with Tukey post hoc test or Kruskal Wallis test. Area under the curve (AUC) were calculated from the baseline using the trapezoidal method in the GraphPad Prism 8.0.

3. RESULTS

Significant differences were observed in the protein levels of the DOXO group from D21 and in the DOXO-F group from D14 both compared to the Control group (Fig. 1A). AUC analysis showed that the DOXO and DOXO-F groups had higher proteinuria compared to the Control group (Fig. 1B).

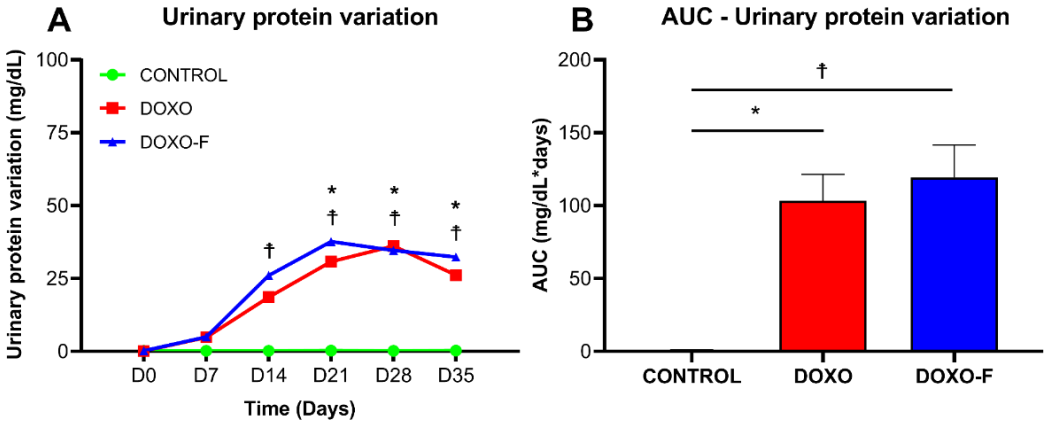

Fig. 1. Urinary protein variation (A) and its respective area under the curve (B) from rats with doxorubicin-induced NS

*Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment). DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Urinary protein variation was analyzed by two-way ANOVA and area under the curve (AUC) by one- way ANOVA, both with Tukey post-test. n = 6; *p < 0.05 DOXO vs. Control groups; ŧp < 0.05 DOXO-F vs Control groups*

Histological evaluation by HE staining showed a reduction in the number of glomeruli, increased hyalinization of the renal parenchyma and interstitial cellularity, indicative of an inflammatory infiltrate in DOXO group compared to Control. In the DOXO-F group, more glomeruli count and mild atrophy were observed compared to theDOXO group (Fig. 2).

MT staining of kidney sections revealed an accumulation of collagen fibers among glomerular capillaries in the Bowman's capsule of the glomeruli in the DOXO group. The DOXO-F group was more similar to the control group and showed a slight deposition of collagen fibers in the glomeruli (Fig. 3).

The DOXO-F group had higher values in the global leucocyte count when compared to the DOXO and CONTROL groups (Fig. 4). Regarding the percentage of lymphocytes, the DOXO and DOXO-F groups presented lower values compared to the CONTROL group (Fig. 4). It was also observed that the DOXO-F group had a lower percentage of lymphocytes than the DOXO group. Furthermore, the DOXO-F group presented a higher percentage of monocytes compared to the DOXO group (Fig. 4).

Regarding the neutrophils number, higher amounts of these cells were observed in the DOXO-F group (Fig. 4).

Significant increase in the renal concentration of TNF-α were showed in DOXO and DOXO-F groups when compared to the CONTROL group. In addition, the DOXO group had higher concentration of this cytokine compared to the DOXO-F group (Fig. 5).

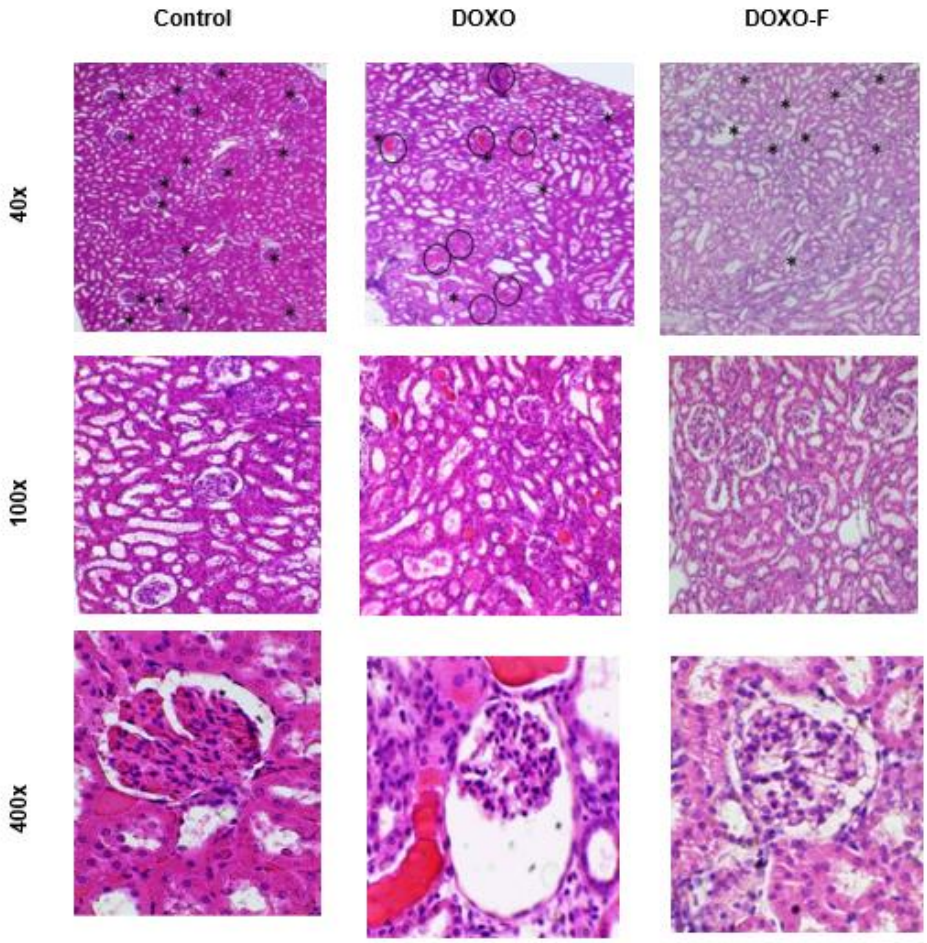

Fig. 2. Renal histology from rats with doxorubicin-induced NS. Control – Negative Control Group (no nephropathy and no treatment)

*DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). The circled areas represent hyaline formations, while the * shows the presence of glomeruli. The photomicrographs are presented in HE staining at 40x, 100x and 400x*

The renal concentration of TGF-β was significantly higher in the DOXO and DOXO-F groups compared to the CONTROL group. There was also increase in TGF-β in DOXO-F group compared to the DOXO group. Furthermore, DOXO-F group showed a higher TGF-β/TNF-α ratio compared to DOXO and CONTROL groups. No difference was showed for the INF-γ renal levels (Fig. 5).

Regarding FRAP levels, a higher antioxidant capacity was showed in the DOXO-F group compared to the DOXO and CONTROL groups. In addition, the activities of SOD and CAT enzymes were higher in DOXO-F and CONTROL groups compared to the DOXO group (Fig. 6).

DOXO-F group showed an increase in TBARS levels compared to the CONTROL and DOXO groups. Also, there was an increase in the expression of the protein carbonyl in the DOXO-F group compared to the CONTROL group (Fig. 6).

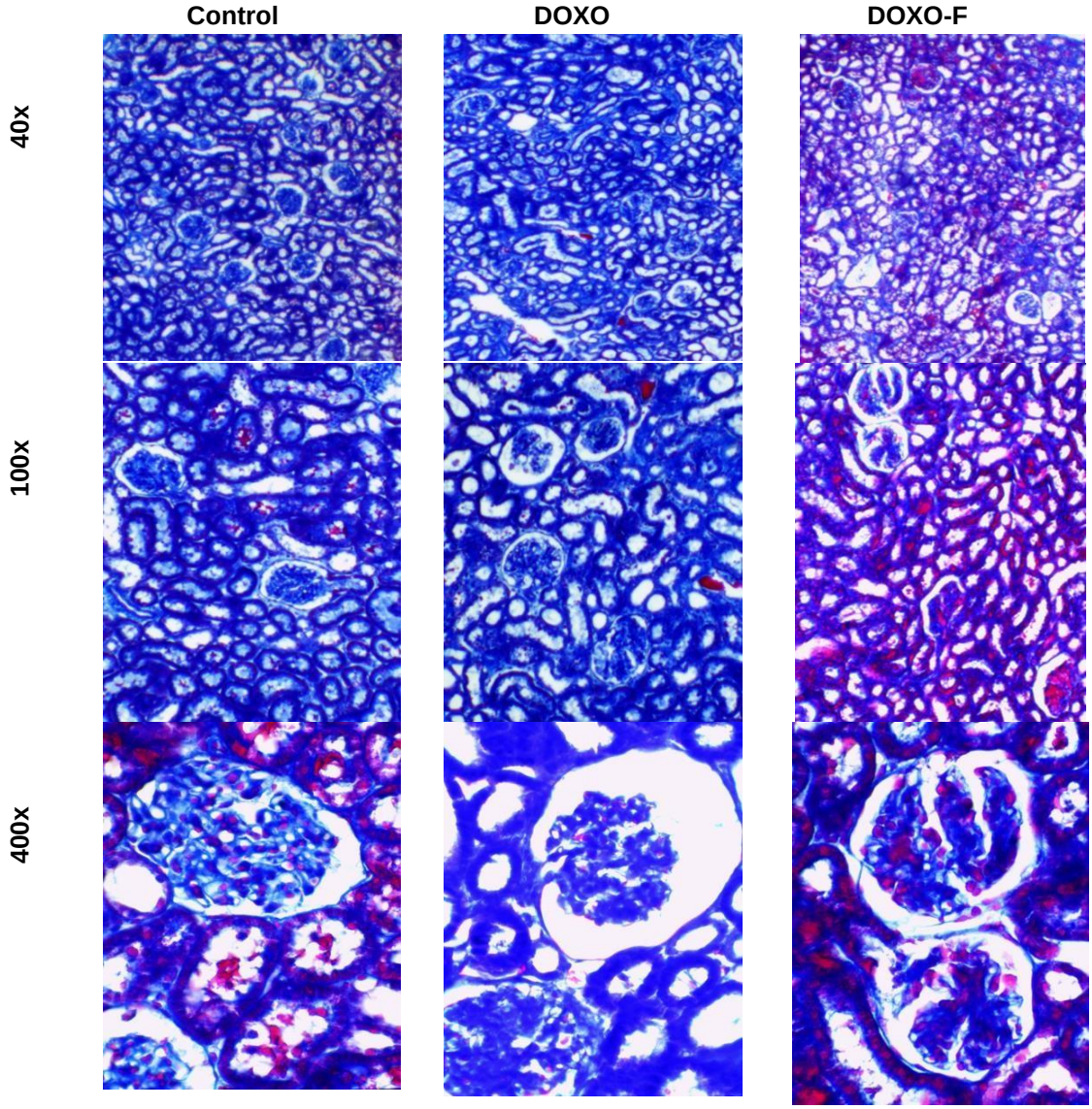

Fig. 3. Renal histology from rats with doxorubicin-induced NS. Control – Negative Control Group (no nephropathy and no treatment)

DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). The photomicrographs are presented in MT staining at 40x, 100x and 400x

4. DISCUSSION

The use of diuretics has been recognized as one of the main treatments for primary or secondary NS, mainly due to their benefits in reducing body edema [16,17]. Furthermore, some studies have demonstrated possible anti-inflammatory action of furosemide [35–37], however, none of these studies, regarding anti-inflammatory action of furosemide was carried out in an experimental model of Nephropathy.

Here, it was shown that the doxorubicin- induced NS model (DOXO and DOXO-F groups) led to proteinuria and high glomerular damage, both characteristic conditions in NS. Additionally, the DOXO group showed increased levels of inflammatory cytokines and low activity of antioxidant enzymes in renal tissue. In contrast, furosemide (DOXO-F group) reduced the damage caused by doxorubicin, promoted an anti-inflammatory response and increased activity of antioxidant enzymes in the kidney.

The presence of proteinuria is considered an expressive marker of kidney damage and is part of the NS diagnosis [38]. Proteinuria occurs due to increased protein filtration by glomeruli and a reduction in the rate of tubular reabsorption, causing podocyte and tubular lesions, interstitial changes, edema formation, fibrosis and inflammatory cell infiltrates [39,40]. Corroborating other studies, the groups that received the doxorubicin injection (DOXO and DOXO-F) showed an increase proteins concentration in the urine [41,42], confirming the effectiveness of the model in inducing NS.

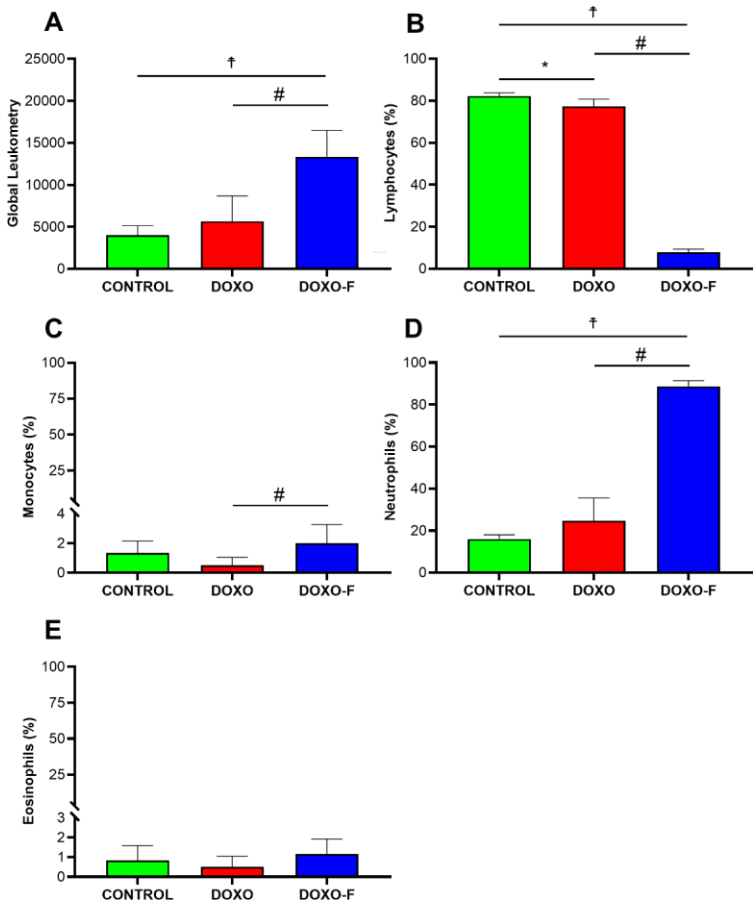

Fig. 4. Global leukocyte count (A); percentage of lymphocytes (B), monocytes (C), eosinophils (D) and neutrophils (E) in the blood of rats with doxorubicin-induced NS

Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment). DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Data were analyzed by one-way ANOVA with Tukey post-test. n = 6;

**p < 0.05 DOXO vs. Control groups; ŧp < 0.05 DOXO-F vs Control groups; # p < 0.05 DOXO vs DOXO-F groups*

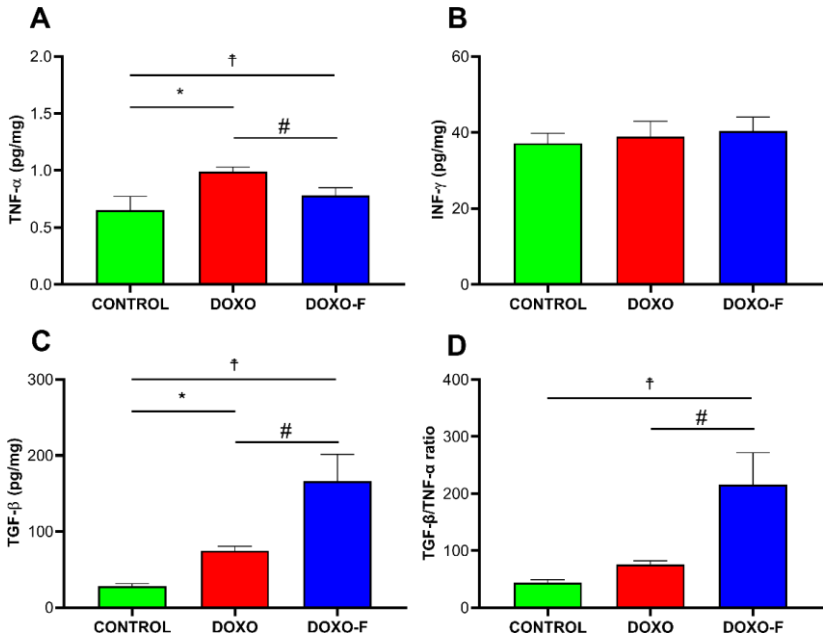

Fig. 5. Renal levels of TNF-α (A), INF-γ (B), TGF-β (C) and TGF-β/TNF-α ratio from rats with doxorubicin-induced NS. Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment)

*DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Data were analyzedby one-way ANOVA with Tukey post-test. n = 6; *p < 0.05 DOXO vs. Control groups; ŧp < 0.05 DOXO-F vs Control groups; # p < 0.05 DOXO vs DOXO-F groups*

The use of doxorubicin is also linked to glomerular atrophy and increased capillary permeability, factors that are associated with the proteinuria [43]. This condition can be observed in the histological analysis of the present study, in which the DOXO and DOXO-F groups showed a reduction in the glomeruli number in relation to the CONTROL group. However, it is important to highlight that the furosemide administration attenuated glomeruli loss in relation to the DOXO group, which may suggest an improvement in the NS pathophysiological evolution.

The increase in the leukocytes migration in animals with nephropathy may be related to an exacerbated immune response, also described using other experimental nephropathy models [44,45]. Therefore, the lower levels of blood lymphocytes presented by the DOXO-F group are suggestive of the migration of these cells to the renal tissue, since lymphocytes are one of the several cells responsible for the TGF-β production [46,47].

Several studies have identified the role of inflammation in the development and NS evolution [9,12,24]. In the present study, the DOXO and DOXO-F groups showed an increase in the levels of renal TNF-α. This is one of the main cytokines involved in the inflammatory processes, being released by activated mast cells and macrophages, also stimulating other cells of the immune system [48]. Hence, it is possible to assume that the elevation of TNF-α is linked to the doxorubicin injection and NS development, with consequent inflammatory response and tissue damage [21,49,50].

Interestingly, the DOXO-F group had lower levels of renal TNF-α compared to the DOXO group. Some studies had already shown anti-inflammatory action of furosemide in vitro using human peripheral blood mononuclear cells, reporting reduction of cytokines, such as IL-6, IL-8 and TNF-α [21,35]. In addition, the DOXO-F group had higher concentrations of renal TGF-β and a greater TGF-β/TNF-α ratio compared to the other groups. TGF-β has an important role in the regulation of processes such as tissue repair and apoptosis, besides acting in the control of immune system homeostasis and inflammation [51]. To the best of our knowledge, this is the first study to shown that the furosemide application can reduce renal levels of a pro-inflammatory cytokine (TNF-α) and increase the concentration of one involved in

tissue repair (TGF-β). These data suggest an anti-inflammatory role in renal tissue promoted by furosemide, with a consequent attempt to return to homeostasis.

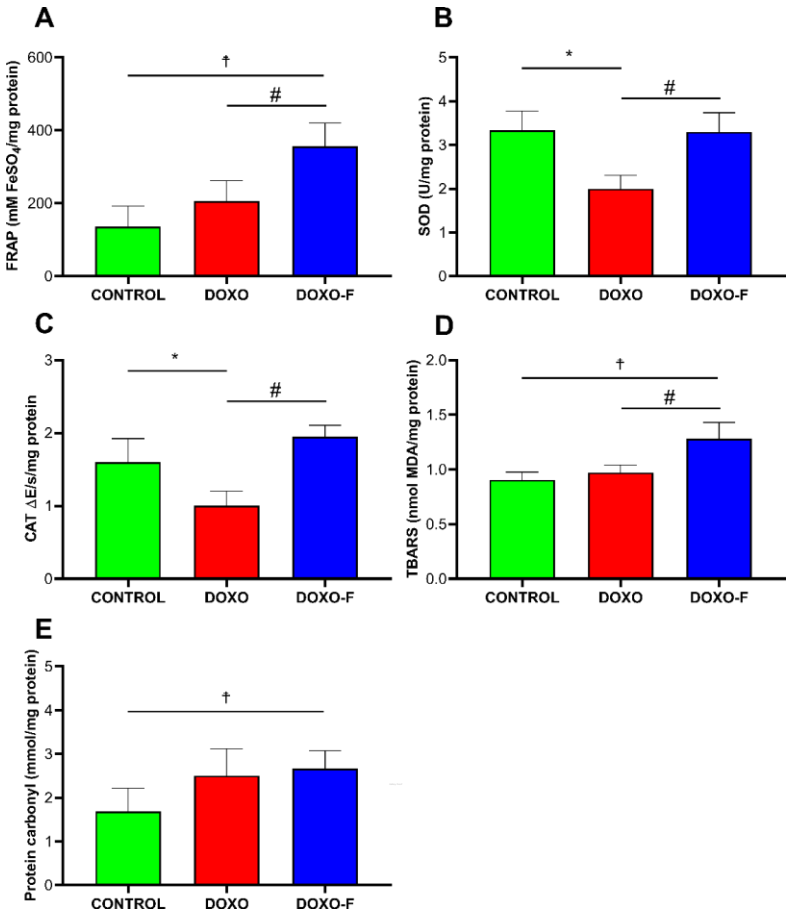

Fig. 6. Renal FRAP total antioxidant capacity (A); SOD (B) and CAT (C) activity; TBARS (D) and protein carbonyl (D) concentrations from rats with doxorubicin- induced NS.

*Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment). DOXO – Positive Control Group (with nephropathy andno treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Data were analyzed by one-way ANOVA with Tukey post-test. n = 6; *p < 0.05 DOXO vs. Control groups; ŧp < 0.05 DOXO-Fvs Control groups; # p < 0.05 DOXO vs DOXO-F groups*

Table 1. Urinary protein

Urinary protein (mg/dL)	Control	DOXO	DOXO-F
D0	0.14 ± 0.05	0.09 ± 0.04	0.16 ± 0.02
D7	0.17 ± 0.04	4.77 ± 1.02	4.93 ± 1.06
D14	0,18 ± 0.07	18.59 ± 8.92	26.02 ± 12.83†
D21	0.23 ± 0.02	30.73 ± 14.63*	43.78 ± 22.89†
D28	0.19 ± 0.03	36.15 ± 16.43*	34.60 ± 19.13†
D35	0.21 ± 0.05	26.12 ± 12.92*	32.36 ± 12.46†

*Urinary protein variation from rats with doxorubicin-induced NS. Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment). DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Urinary protein variation was analyzed by two-way ANOVA and area under the curve (AUC) by one- way ANOVA, both with Tukey post-test. n = 6; *p < 0.05 DOXO vs. Control groups; ŧp < 0.05 DOXO-F vs Control groups*

Table 2. Cytokines

Cytokines (pg/mg)	Control	DOXO	DOXO-F
TNF-α	0.65 ± 0.12	0.99 ± 0.04	0.78 ± 0.07†#
INF-y	37.17 ± 2.67	38.93 ± 4.02	40.35 ± 3.72
TGF-β	28.25 ± 3.32	74.76 ± 5.93*	166.16 ± 35.34†#
TGF-β/TNF-α ratio	43.99 ± 5.09	75.76 ± 14.63	215.71 ± 56.04†#

*Renal levels of TNF-α, INF-γ, TGF-β and TGF-β/TNF-α ratio from rats with doxorubicin-induced NS. Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment). DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Data were analyzedby one-way ANOVA with Tukey post-test. n = 6; *p < 0.05 DOXO vs. Control groups; †p < 0.05 DOXO-F vs Control groups; # p < 0.05 DOXO vs DOXO-F groups*

Table 3. Leucometry

Leucometry	CONTROL	DOXO	DOXO-F
Total Leukocytes (mm^3)	4000.00 ± 1143.68	5650.00 ± 3044.17	13330.00 ± 3156.29†#
Lymphocytes (%)	82.17 ± 1.60	77.40 ± 3.36*	7.83 ± 1.47†#
Monocytes (%)	1.33 ± 0.82	0.50 ± 0.55	2.00 ± 1.26#
Neutrophilis (%)	16.00 ± 2.00	24.67 ± 10.91	88.67 ± 2.73†#
Eosinophilis (%)	0.83 ± 0.75	0.50 ± 0.55	1.17 ± 0.75

*Global leukocyte count; percentage of lymphocytes, monocytes, eosinophils and neutrophils in the blood of rats with doxorubicin-induced NS. Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment). DOXO – Positive Control Group (with nephropathy and no treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Data were analyzed by one-way ANOVA with Tukey post-test. n = 6; *p < 0.05 DOXO vs. Control groups; †p < 0.05 DOXO-F vs Control groups; # p < 0.05 DOXO vs DOXO-F groups*

Table 4. Redox status

Redox status	CONTROL	DOXO	DOXO-F
FRAP (mg $FeSO_4$/mg protein)	136.17 ± 56.30	206.33 ± 55.46	356.67 ± 63.81†#
SOD (U/mg protein)	3.34 ± 0.44	2.00 ± 0.31*	3.30 ± 0.44#
CAT (ΔE/s/mg protein)	1.60 ± 0.32	1.01 ± 0.19*	1.95 ± 0.16#
TBARS (mmol MDA/mg protein)	0.91 ± 0.07	0.97 ± 0.07	1.28 ± 0.15†#
Protein carbonyl (mmol/mg protein)	1.69 ± 0.53	2.50 ± 0.62	2.66 ± 0.41†

*Renal FRAP total antioxidant capacity; SOD and CAT activity; TBARS and protein carbonyl concentrations from rats with doxorubicin- induced NS. Data are presented as mean ± SD. Control – Negative Control Group (no nephropathy and no treatment). DOXO – Positive Control Group (with nephropathy andno treatment). DOXO-F – Test Group (with nephropathy and treatment with oral Furosemide). Data presented as mean ± SD. Data were analyzed by one-way ANOVA with Tukey post-test. n = 6; *p < 0.05 DOXO vs. Control groups; †p < 0.05 DOXO-Fvs Control groups; # p < 0.05 DOXO vs DOXO-F groups*

The increase in reactive oxygen and nitrogen species can lead to oxidative stress, causing damage to proteins and lipids, raising the levels of TBARS and carbonyl derivatives in proteins. In contrast, to contain tissue oxidative stress advance, there is an increase in the production of antioxidant enzymes to maintain the redox state homeostasis [52]. In the present study, the DOXO-F group showed an increase in the expression of TBARS and carbonyl protein, but also an elevation in the activity of SOD and CAT enzymes, as well as in the FRAP levels. The renal oxidative stress has been shown in other studies that used doxorubicin or different models of kidney injury [53,54]. Furthermore, patients with NS have a strong correlation between oxidative stress and disease progression [55]. However, the increased activity of antioxidant enzymes in the DOXO-F group suggests that furosemide may triggered a response to contain oxidative damage [20,21]. These results reinforce those observed in cytokine levels, indicating an anti-inflammatory and antioxidant action of furosemide.

Some limitations were noted in this study. For a better understanding of the anti- inflammatory action of furosemide on renal tissue, other

inflammatory markers, as well as evaluation at different timepoints are necessary. In addition, the direct dosage of reactive species, evaluation of Nrf2 (nuclear erythroid factor 2-related factor 2) and the markers of its pathway expression, could be key elements to further understand the interaction of the redox state with the NS evolution. Finally, the need for future studies with other doses of furosemide and longer exposure time is evident.

The present study showed that the use of furosemide can attenuate the NS progression, possibly through an anti-inflammatory and antioxidant action. These findings are important to increase the discussion around NS therapy, since in some cases corticosteroids and immunosuppressants are used, which have serious long-term adverse effects [56,57]. Hence, the demonstration of the anti-inflammatory properties of furosemide may be useful for discussing new clinical approaches.

5. CONCLUSION

Furosemide reduced TNFα levels and increased antioxidant activity in the renal tissue of rats with doxorubicin-induced nephropathy, thus demonstrating a beneficial effect in attenuating the inflammatory response in an experimental model ofnephropathy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was previously approved by the Animal Ethics Committee of Federal University of Jequitinhonha and Mucury Valleys (UFVJM) regarding the Guiding Principles in the Care and Use of Animals, with an approved protocol number of 001/19.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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