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Effects of N–acetyl cysteine on serum podocalyxin and pentraxin levels in an experimental lower extremity ischemia–reperfusion injury model

Efectos de la N–acetilcisteína en los niveles séricos de podocalixina y pentraxina en un modelo experimental de lesión por ischemia–reperfusión en extremidades inferiores

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ABSTRACT

Ischemia–reperfusion injury causes oxidative stress and inflammation, leading to skeletal muscle damage. This study investigates the role of N–acetylcysteine in modulating oxidative stress and inflammatory biomarkers specifically Podocalyxin and Pentraxin 3 in a rat model of lower extremity Ischemia–reperfusion injury. An experimental, controlled animal study conducted at the Experimental Research Center of Firat University. Twenty–four female Sprague–Dawley rats were allocated into four groups: control, sham, Ischemia–reperfusion, and Ischemia–reperfusion treated with N–acetylcysteine. Ischemia was induced by clamping the infrarenal abdominal aorta for 120 min, followed by 120 min of reperfusion. A single dose of N–acetylcysteine (150 mg·kg^{-1}, i.p.) was administered at the onset of reperfusion in the treatment group. Levels of serum Total Oxidative Status and Total Antioxidant Status, as well as the expression of Podocalyxin and Pentraxin 3 in tissue, were evaluated. A significant increase in Oxidative Status levels and a significant decrease in Antioxidant Status levels were observed in the Ischemia–reperfusion group compared to the control group. After administering N–acetylcysteine, there was a significant decrease in Oxidative Status levels and a significant increase in Antioxidant Status levels when compared to the Ischemia–reperfusion group. Histological evaluation showed that N–acetylcysteine reduced edema, hemorrhage, and overall tissue injury scores. Immunohistochemical analyses revealed increased Podocalyxin and Pentraxin 3 expression in Ischemia–reperfusion group tissues, which was notably diminished in the N–acetylcysteine – treated group. N–acetylcysteine demonstrated protective effects against Ischemia–reperfusion–induced oxidative and inflammatory damage in skeletal muscle by reducing serum and tissue levels of Podocalyxin and Pentraxin 3. These findings suggest its therapeutic potential in mitigating Ischemia–reperfusion injury and highlight Podocalyxin and Pentraxin 3 as promising biomarkers for tissue damage and treatment monitoring.

Key words: Ischemia–Reperfusion Injury; N–Acetylcysteine; Podocalyxin; Pentraxin 3; Oxidative Stress; Skeletal Muscle

RESUMEN

La lesión por isquemia–reperfusión provoca estrés oxidativo e inflamación, lo que conduce a daños en el músculo esquelético. Este estudio investiga el papel de la N–acetilcisteína en la modulación de biomarcadores de estrés oxidativo e inflamación, específicamente la podocalixina y la pentraxina 3, en un modelo de rata con lesión por isquemia–reperfusión en extremidades inferiores. Se realizó un estudio controlado en animales en el Centro de Investigación Experimental de la Universidad Firat. Veinticuatro ratas Sprague–Dawley hembras se distribuyeron en cuatro grupos: control, sham, isquemia–reperfusión y isquemia–reperfusión tratado con N–acetilcisteína. La ischemia se indujo mediante clampeo de la aorta abdominal infrarenal durante 120 min , seguido de 120 min de reperfusión. En el grupo de tratamiento, se administró una dosis única de N–acetilcisteína (150 mg·kg^{-1}, intraperitoneal) al inicio de la reperfusión. Se evaluaron los niveles séricos de estado oxidativo total y estado antioxidante total, así como la expresión de podocalixina y pentraxina 3 en el tejido. En el grupo de isquemia–reperfusión, se observó un aumento significativo de los niveles de estado oxidativo total y una disminución significativa de los niveles de estado antioxidante total en comparación con el grupo control. Tras la administración de N–acetilcisteína, se observó una disminución significativa de los niveles de estado oxidativo total y un aumento significativo de los niveles de estado antioxidante total en comparación con el grupo isquemia–reperfusión. La evaluación histológica mostró que la N–acetilcisteína disminuyó el edema, la hemorragia y las puntuaciones generales de daño tisular. Los análisis inmunohistoquímicos revelaron un aumento de la expresión de podocalixina y pentraxina 3 en el grupo isquemia–reperfusión, la cual se redujo notablemente en el grupo tratado con N–acetilcisteína. La N–acetilcisteína demostró efectos protectores contra el daño oxidativo e inflamatorio inducido por isquemia–reperfusión en el músculo esquelético, reduciendo los niveles séricos y tisulares de podocalixina y pentraxina 3. Estos hallazgos sugieren su potencial terapéutico para mitigar la lesión por isquemia–reperfusión y destacan a podocalixina y pentraxina 3 como biomarcadores prometedores para el daño tisular y la monitorización del tratamiento.

Palabras clave: Lesión por Ischemia–Reperfusión; N–Acetilcisteína; Podocalixina; Pentraxina 3; Estrés Oxidativo; Músculo Esquelético

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INTRODUCTION

Skeletal muscles work together with bones and joints to enable movement in living beings. For individuals to carry out daily physical activities efficiently, these muscles must function effectively and contract properly. The continuity of this functionality largely hinges on an adequate blood supply to the muscle tissue [1].

Ischemia occurs when tissues fail to receive essential oxygen and nutrients due to restricted blood flow. Although restoring circulation to the affected area after ischemia can mitigate tissue damage to some extent, paradoxically, it can also exacerbate the injury. Common clinical scenarios that can lead to ischemia–reperfusion (IR) injury in skeletal muscle include bone and soft tissue trauma, thrombotic or embolic events, limb reattachment surgeries, acute physical injuries, and the prolonged use of tourniquets during operations [2].

When oxygen levels drop, cells must transition to anaerobic metabolism. This switch results in a marked decrease in ATP levels within the cells and an increase in intracellular calcium (Ca^{2+}) levels. Consequently, this leads to the buildup of reactive oxygen species (ROS), which triggers acidosis. Acidosis, in turn, initiates both apoptotic (programmed) and necrotic cell death at the tissue level [3].

Podocalycin (Podxl) is a sialomucin that belongs to the CD34 family and is primarily found in renal podocytes and vascular endothelial cells. It plays a crucial role in maintaining the structure of endothelial cells, ensuring cell adhesion to the extracellular matrix, and preserving the integrity of the glomerular filtration barrier [4]. In addition to these localization areas, Podxl is also found in mesothelial cells and hematopoietic stem cells, where it is believed to help protect the endothelial barrier by regulating cell adhesion, vascular permeability, and the inflammatory response. [5].

When podocytes are damaged, Podxl is released through microvilli and vesicle–like structures. Since it is excreted in the urine, the levels of Podxl can serve as a biomarker for damage to podocytes and vascular endothelial cells in diabetic nephropathy and other glomerular disorders. [6].

Pentraxin proteins (PTXs) are part of a family of multimeric soluble proteins that play a crucial role in the acute phase response during inflammation. These proteins are synthesized in hepatocytes in response to interleukin-6 (IL-6) and include short structures such as serum amyloid P and C–reactive protein (CRP).These proteins bind to pathogens, necrotic cells, and foreign particles, facilitating their clearance via the complement system and phagocytic cells. Through these mechanisms, short pentraxins enhance immune defense [7].

Unlike short pentraxins, long pentraxins such as PTX3 are synthesized in peripheral cells and act as components of the innate immune response by activating the complement cascade [8]. PTX3 is uniquely secreted at the site of inflammation by various immune–related cells including dendritic cells, fibroblasts, and mononuclear phagocytes. Neutrophils store a pre–synthesized form of PTX3 in their granules, allowing for rapid secretion upon microbial stimulation. Endothelial cells also upregulate PTX3 expression under the influence of cytokines such as TNF–α and IL-1β during inflammation, triggering pro–inflammatory and pro–coagulant responses and modulating microvascular activity. Moreover, PTX3 has been shown to inhibit granuloma formation by suppressing macrophage activation through complement regulation. For these reasons, PTX3 levels are considered a valuable indicator of inflammatory activity [9].

Given the significant involvement of oxidative stress and cellular apoptosis in tissue injury, antioxidant treatments have gained attention. N–acetylcysteine (NAC), an acetylated derivative of L–cysteine, functions as a potent antioxidant due to its thiol group and role as a glutathione precursor. NAC can neutralize ROS directly and thereby attenuate oxidative stress. It also enhances cellular defense mechanisms and suppresses apoptosis. Research indicates that NAC may protect against cellular damage by reducing oxidative stress, inhibiting programmed cell death, and modulating heat shock responses [10, 11].

Two objectives were defined for this experimental lower limb ischemic reperfusion study. First, to evaluate the possible effect on the level of podocalyxin and pentraxin in smooth muscle tissue after ischemic reperfusion and the possible preventive effect of NAC on the morphological changes of smooth muscle.

MATERIALS AND METHODS

This study received ethical approval from the Firat University Animal Experiments Ethics Committee, as indicated in the decision dated January 31, 2024, with the approval number 2024/03-04. The research was conducted at the Experimental Research Center of Firat University.

Animals and groups

The rats (*Rattus norvegicus)* used in the experiment were maintained under controlled environmental conditions, with the ambient temperature set between 22–25°C and a 12–hour light/dark cycle (lights on from 07:00 to 19:00, and off from 19:00 to 07:00). Each rat was housed in specially prepared cages, which were cleaned daily. Standard laboratory chow was provided in stainless steel containers, and clean tap water was supplied ad libitum through glass water bottles.

A total of 24 female Sprague–Dawley rats, aged between 8 and 10 weeks and confirmed to be in the same estrous phase via vaginal smear examination, were included in the study. These animals were randomly divided into four groups, each containing six rats:

- **Group I – Control (n = 6):** No treatment or surgical intervention was applied to this group.
- **Group II – Sham (n = 6):** Rats underwent a laparotomy and abdominal aorta dissection, mimicking the surgical duration and stress of other groups, but without inducing ischemia.
- **Group III – Ischemia–Reperfusion (IR) (n = 6):** In a controlled experimental setting, a midline laparotomy was conducted with the rats positioned supine. To induce ischemia, the infrarenal abdominal aorta (IAA) was temporarily occluded using a non–traumatic microvascular clamp for a duration of 120 min. After the ischemic period, the clamp was removed, and reperfusion was permitted for an additional 120 min, concluding the

procedure. This methodology allows researchers to study the effects of ischemia and subsequent reperfusion on the abdominal organs.

- **Group IV – Ischemia–Reperfusion + N–Acetylcysteine (IR + NAC) (n = 6):** The same surgical protocol as Group III was followed. However, immediately after clamp removal, a single dose of N–acetylcysteine (150 mg·kg^{-1}, intraperitoneally) was administered [Asist, 300 mg/3 mL (10%), Hüsnü Arsan Farma, Türkiye]. Reperfusion was then carried out for 120 min.

All rats were anesthetized through intramuscular injections of 75 mg·kg^{-1} Ketamine hydrochloride (Ketalar, Pfizer, Groton, CT, USA) and 10 mg·kg^{-1} Xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany). If needed, additional doses of one–third of the initial amounts were given to maintain the desired depth of anesthesia.

To prevent hypothermia, the animals were placed under a heat lamp in a supine position. The abdominal area was then disinfected, followed by a midline laparotomy. In order to maintain fluid balance, 10 mL of warmed physiological saline was gently introduced into the peritoneal cavity. The intestines were carefully shifted to the left using moistened gauze to facilitate access to the abdominal aorta. Two min. before aortic clamping, anticoagulation was judiciously induced through an intravenous injection of Heparin at a dose of 150 U·kg^{-1} via the tail vein (Nevparin, Mustafa Nevzat Pharmaceuticals, Türkiye).

Lower limb ischemia was induced by applying a non–traumatic microvascular clamp to the infrarenal abdominal aorta for a duration of 120 min (FIG. 1).

To prevent heat and fluid loss during the occlusion phase, the abdominal incision was maintained in a closed position. Following the ischemic period, the abdomen was reopened to remove the clamp, and reperfusion was subsequently initiated, lasting for an additional 120 min.

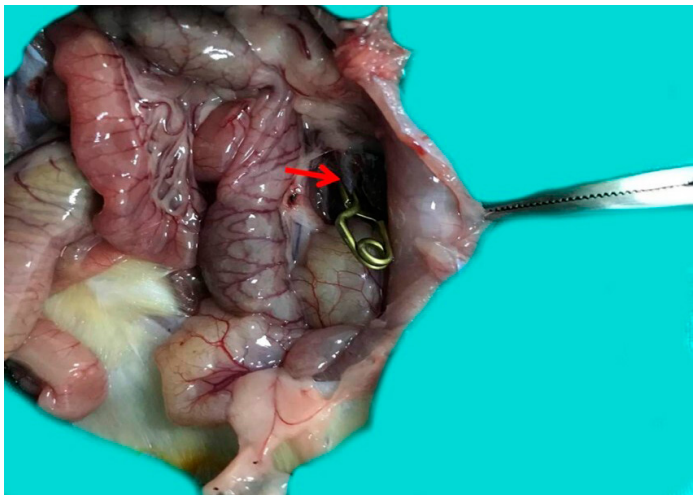

FIGURE 1. Lower limb ischemia was induced by applying a non–traumatic microvascular clamp to the infrarenal abdominal aorta. A non–traumatic microvascular clamp is shown with red arrow

Sampling and tissue processing

At the conclusion of the experimental procedures, blood samples were collected from all rats via intracardiac puncture under anesthesia. Immediately afterward, skeletal muscle tissues from the lower limbs were excised promptly. Following collection, blood samples were processed via centrifugation (NF1200R, Nuve, Ankara, Türkiye) at 4000 G for 5 min. The isolated serum was stored (NUAIRE, NU-9483E, Mexican) at -80°C pending biochemical analyses.

Muscle tissues were preserved in a 10% formaldehyde solution to facilitate immunohistochemical analysis. Once fixation was complete, the samples were rinsed with tap water and underwent a standard histological preparation protocol. Following dehydration and clearing, the tissues were embedded in paraffin blocks. The sections were carefully sliced to a thickness of 4-6 μm and placed on glass coated with poly–L–lysine.

Biochemical evaluation

Rat–specific enzyme–linked immunosorbent assay (ELISA) kits were utilized to assess serum Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) levels. For standard evaluation of these biomarkers, kits were used (SunRed, China; TOS: 201-11-1669, TAS: 201-11-2672).

Immunohistochemical assessment

Sections carefully removed from paraffin blocks were placed on poly–L–lysine coated slides and were thoroughly cleaned by passing through a graded series of alcohol after deparaffinization. To enhance antigen accessibility, the slides were then heated in citrate buffer (pH 6.0) using a 750 W microwave oven (Beko, MD 1610, Türkiye). In this two–stage process, the first cycle lasted for 7 min, followed by a second cycle that lasted for 5 min. After heating, the slides were allowed to cool to room temperature for approximately 20 min. They were then washed three times with phosphate–buffered saline (PBS), with each wash lasting 5 min, to ensure optimal conditions for analysis. To inhibit the activity of endogenous peroxidase, slides were treated with Hydrogen Peroxide Block solution (TA-125-HP, Lab Vision, USA) for 5 min. Following this treatment, Ultra V Block (TA-125-UB, Lab Vision, USA) was applied for an additional 5 min to minimize nonspecific background staining.The sections were allowed to incubate at room temperature for 60 min within a humidified chamber. During this time, primary antibodies were applied, diluted to a concentration of 1:200.

- PTX3 antibody (PA5-36156, Thermo Fisher Scientific, Invitrogen, USA)

- Podxl antibody (39-3800, Thermo Fisher Scientific, Invitrogen, USA)

Following the incubation period, slides were washed extensively with PBS in three 5 min cycles. Following this, they were incubated for thirty min with a biotinylated secondary antibody, specifically the Biotinylated Goat Anti–Polyvalent IgG (TP-125-BN, Lab Vision, USA). The next step involved applying Streptavidin Peroxidase (TS-125-HR, Lab Vision, USA) for an additional thirty min to facilitate further visualization.

The process of color development involved a mixture of a substrate and chromogen, specifically utilizing 3-amino-9-ethylcarbazole (AEC) (TA-015 and TA-002-HAC, Lab Vision, USA). Following this, the samples underwent rinsing with PBS to ensure proper cleanup. Counterstaining was then carried out using Mayer's hematoxylin to enhance contrast. After the counterstaining step, the slides were briefly immersed in distilled water, and finally, they were sealed with Vision Mount (TA-125-UG, Lab Vision, USA) to preserve the specimens.

Prepared slides were analyzed using a Leica microscope (DM500, Leica Microsystems, Switzerland), with digital images captured through a Leica camera system (DFC295, Leica Microsystems, Switzerland). The evaluation of immunoreactivity was conducted using a semi–quantitative scoring system, allowing for a systematic assessment of the results.

- **Extent of staining:**
  - 0.1: < 25%
  - 0.4: 26–50%
  - 0.6: 51–75%
  - 0.9: 76–100%
- **Staining intensity:**
  - 0: None
  - +0.5: Very weak
  - +1: Weak
  - +2: Moderate
  - +3: Strong

A histological score, commonly referred to as the H–score, is obtained by multiplying two key parameters: the extent and the intensity values. H–score = Extent × Intensity

Statistical analysis

Statistical evaluations were performed using IBM SPSS Statistics version 22.0 (Armonk, NY, USA). Quantitative data were described using median values along with their respective minimum and maximum ranges. For comparisons involving more than two independent groups, the Kruskal–Wallis test was employed. In cases of significant differences, pairwise group comparisons were conducted using Dunn's post–hoc method. Statistical significance was considered at a *P*–value threshold below 0.05.

RESULTS AND DISCUSSION

Biochemical Findings

Serum Total Oxidant Status

Biochemical analysis of serum Serum Total Oxidant Status (TOS) levels revealed no significant statistical difference between the Control and Sham groups (*P*=0.919). However, the rats in the IR group demonstrated a significantly higher TOS level compared to the Control group (*P*=0.029). Furthermore, the administration of NAC in the IR + NAC group resulted in a marked reduction in TOS levels compared to the IR group alone. (*P*=0.006) (TABLE I).

Serum Total Antioxidant Status (TAS)

Total Antioxidant Status levels did not differ significantly between the Control and Sham groups *P*=0.870), whereas the IR group demonstrated a statistically significant decline relative to the Control group (*P*=0.037). Interestingly, TAS levels in the IR+NAC group were significantly higher than those in the IR group. (*P*=0.003) (TABLE I).

TABLE I
Serum Total Oxidant Status and Total Antioxidant Status of the groups

	TOS (μmol·L^{-1}) Median (min–max)	TAS (U·mL^{-1}) Median (min–max)
CONTROL	2.98 (1.45–4.12)	19.91 (14.36–32.12)
SHAM	2.87 (2.31–3.95)	18.48 (13.45–33.41)
IR	17.28 (12.35–18.42)[a]	1.74 (1.12–3.27)[a]
IR+NAC	2.02 (1.23–4.85)[b]	23.68 (18.25–37.62)[b]
*P**	0.004	0.003

Values are presented as median and minimum–maximum. *Kruskal–Wallis test: [a]compared to the control group, [b]compared to the IR group (*P*<0.05). IR: Ischemia–Reperfusion; IR + NAC: Ischemia–Reperfusion plus N–Acetylcysteine group. TOS: Serum Total Oxidant Status; TAS: Serum Total Antioxidant Status

Histological findings

Microscopic evaluation of H&E–stained skeletal muscle sections revealed varying degrees of edema and hemorrhage among groups. A semi–quantitative scoring system was applied to assess the severity of these findings (TABLE II).

TABLE II
Statistical analysis results of the groups following the histopathological evaluation score

	CONTROL				SHAM				IR				IR+NAC			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Edema	5	1			4	2					1	5	5	1		
Hemorrhage	5	1			4	2				1	1	4	5	1		

(0): absent, (1): mild, (2): moderate, (3): severe. IR: Ischemia–Reperfusion, IR + NAC: Ischemia–Reperfusion plus N–Acetylcysteine group

Histopathological Score Analysis

A statistical analysis of the histopathological scores revealed no significant difference between the Control and Sham groups (*P*=0.695). However, muscle tissue from the IR group exhibited significantly higher damage scores compared to the Control group (*P*=0.006). Notably, the administration of NAC significantly reduced tissue injury in the IR + NAC group when compared to the IR group *P*=0.006) (TABLE III).

TABLE III
Histopathological evaluation scores of the groups

	Histopathological evaluation score Median (min–max)
CONTROL	0.00 (0.00-1.00)
SHAM	0.50 (0.00-1.00)
IR	6.00 (4.00-6.00)[a]
IR+NAC	0.00 (0.00-1.00)[b]
*P**	0.002

Values are presented as median and minimum–maximum. *Kruskal–Wallis test: [a]compared to the control group, [b]compared to the IR group (*P*<0.05). IR: Ischemia–Reperfusion, IR + NAC: Ischemia–Reperfusion plus N–Acetylcysteine group

Immunohistochemical Findings

Podocalyxin Immunoreactivity

Immunostaining showed marked Podocalyxin expression in myocytes (indicated by black arrows) (FIG. 2).

There was no significant difference observed between the sham group and the control group (*P*=0.221).However, IR group rats demonstrated significantly elevated Podocalyxin immunoreactivity compared to the Control (*P*=0.008). This increase was significantly reversed in the IR + NAC group (*P*=0.023)

Pentraxin 3 Immunoreactivity

Pentraxin 3 was notably found to be expressed in muscle cells, especially within IR group. No significant differences were observed between the Control and Sham groups (*P*=0.541). However, expression levels were significantly increased in the IR group (*P*=0.014) and were significantly decreased following NAC treatment (*P*=0.024) (TABLE IV) and FIG. 2).

Oxidative stress occurs when there is an imbalance between the excessive generation of ROS and the tissue's capacity to neutralize these harmful compounds following IR injury. This imbalance can impair the ability of the tissue to detoxify intermediates and repair any resultant damage effectively.

In skeletal muscle, oxidative stress disrupts basal energy homeostasis, leading to a range of structural and metabolic alterations. After these changes, there may be a loss of strength

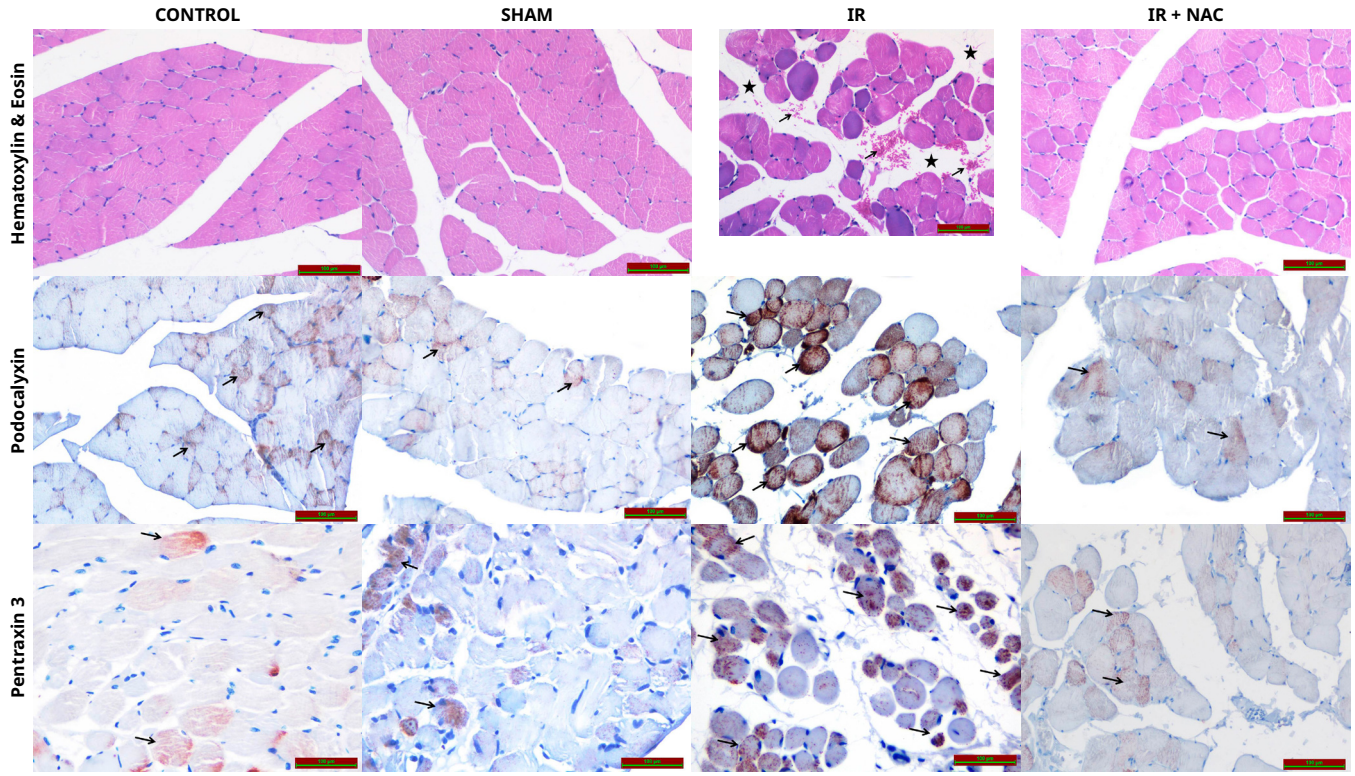

FIGURE 2. immunohistochemical images of the groups in the study. IR: Ischemia–Reperfusion, IR+NAC: Ischemia–Reperfusion plus N–Acetylcysteine group. In Hematoxylin Eosin staining, edema and hemorrhage in the IR group are shown with star and Black Arrow, respectively

TABLE IV
Histoscore of Podocalyxin and Pentraxin 3 immunoreactivity of the groups

	Podocalyxin Median (min–max)	Pentraxin 3 Median (min–max)
CONTROL	0.20 (0.10–0.30)	0.25 (0.20–0.40)
SHAM	0.30 (0.20–0.40)	0.30 (0.20–0.45)
IR	1.20 (0.90–1.80)[a]	1.20 (1.20–1.80)[a]
IR+NAC	0.25 (0.10–0.30)[b]	0.30 (0.10–0.40)[b]
*P**	0.002	0.003

Values are presented as median and minimum–maximum. *Kruskal–Wallis: [a]Compared to the control group, [b]Compared to the IR group ($P<0.05$). IR: Ischemia–Reperfusion, IR+NAC: Ischemia–Reperfusion plus N–Acetylcysteine group

and function in the lower extremity muscles, a decrease in regeneration capacity, and, as a result of atrophy, an increase in connective tissue.

This study is the first to evaluate levels of Podxl and PTX3 after lower extremity IR injury. These findings indicate that NAC a precursor to glutathione that has been used for many years in the treatment of pulmonary and cardiac diseases, contributes to histopathological recovery by reducing oxidative stress. Additionally, NAC significantly lowers tissue levels of Podxl and PTX3. These results emphasize the effectiveness of NAC in preventing IR–induced injury in the lower extremities.

Podocalycin is expressed in various cell types, including vascular endothelial cells, lung tissue, platelets, and neurons. It plays a crucial role in maintaining the integrity of the vascular endothelial layer by modulating inflammation. Studies have shown that the levels of podocalycin increase in conditions of tissue damage, such as peripheral artery disease and myocardial infarction, due to its release from podocytes or endothelial cells. This increase has been associated with vascular permeability and endothelial dysfunction, suggesting that podocalycin could serve as a definitive biomarker, particularly in cases of vascular inflammation and tissue ischemia. [6, 12].

A study found elevated serum Podxl levels in patients with preeclampsia and those experiencing recurrent pregnancy loss, suggesting that these conditions are linked to maternal endothelial dysfunction [13, 14]. Consistent with previously summarized literature, this study also observed significant increases in serum Podxl levels in the IR group. The rise in Podxl levels corresponds with increased TOS and decreased TAS, indicating the presence of tissue–level oxidative stress and inflammation.

Furthermore, the notable decrease in Podxl levels following NAC administration may be due to the antioxidant and anti–inflammatory properties of NAC. Pentraxin proteins interact with the complement system at various levels and can display both pro–inflammatory and anti–inflammatory properties [15]. Pentraxin 3 is an acute–phase protein produced by local inflammatory cells that plays a crucial role in the immune response.

Numerous studies have demonstrated that PTX3 levels increase dramatically following IR injury. [16, 17]. As an acute–phase reactant, PTX3 increases systemically after ischemic events and interacts with P–selectin to limit neutrophil migration from activated endothelial cells. This interaction reduces leukocyte infiltration and ROS generation, thereby dampening excessive inflammatory responses and aiding in the resolution of sterile inflammation [18, 19].

Interestingly, some studies have reported that PTX3 deficiency can mitigate tissue damage by attenuating local and systemic inflammatory responses, as seen in intestinal IR models [17]. Similarly, increased plasma PTX3 levels have been noted in ovarian torsion/detorsion models, with anti–inflammatory treatment using losartan shown to reduce PTX3 levels and offer tissue protection [20].

In this study, consistent with existing literature, serum PTX3 levels significantly increased in the IR group. This biochemical finding was further supported by elevated TOS and decreased TAS values. The observed increase in PTX3 immunoreactivity suggests that complement–mediated inflammatory mechanisms become activated during IR injury. The marked decrease in PTX3 levels after NAC treatment serves as molecular evidence of its protective effect against IR damage.

The results of this study indicate that plasma PTX3 levels may serve as a valuable marker for detecting lower extremity injury, monitoring the clinical course, and evaluating treatment follow–up.

CONCLUSION

In a model of experimentally induced IR injury in the lower extremities, treatment with NAC led to favorable changes in oxidative stress parameters, such as TAS and TOS. Additionally, NAC treatment positively affected histopathological and immunohistochemical markers at the tissue level. This treatment significantly mitigated damage to the lower extremity muscles, which was indicated by a reduction in the levels of Podxl and PTX3. NAC, well–known for its antioxidant properties, should also be considered for its potential therapeutic benefits, particularly during lower extremity revascularization procedures or in cases of ischemic attacks. Additionally, This study demonstrates the potential of Podxl and PTX3 as biomarkers for diagnosing IR injury, monitoring disease progression, and assessing treatment effectiveness.

Conflicts of interest

The authors declare no conflict of interest.

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