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Protective role of intravenous macelignanagainst unilateral hind limb ischemia-reperfusion injury in a murine model

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Ischemia-reperfusion injury (I/RI) is a complex pathological process in which tissue damage caused by oxygen deprivation is further aggravated upon reoxygenation. This study aimed to investigate the protective effects and underlying mechanisms of macelignan in an experimental rat model of unilateral hind limb I/RI. Rats were divided into five experimental groups: control, sham, I/RI, and two treatment groups receiving intravenous macelignan either before ischemia (M1) or after ischemia (M2). Ischemia was induced by clamping the unilateral femoral artery for 3 hours, followed by a 3-hour reperfusion period. Macelignan was administered intravenously at a dose of 15 mg/kg: 60 minutes before ischemia in the M1 group and at the onset of reperfusion in the M2 group. Blood and tissue samples were collected for biochemical and histopathological analyses.Significant differences were observed among the groups in serum levels of alanine aminotransferase, creatinine, lactate dehydrogenase, and creatine phosphokinase-3 (P<0.05). Total antioxidant status (TAS) was significantly higher and total oxidant status (TOS) was significantly lower in the drug-treated groups compared to the I/RI group (P<0.05). Serum nitric oxide levels were significantly reduced in the M1 group (P=0.022). Tissue TOS and oxidative stress index (OSI) levels also differed significantly among the groups (p<0.001). Tissue caspase-3 levels were highest in the I/RI group and lowest in the drug-treated groups (P=0.006). Tumor necrosis factor-alpha (TNF-α) levels were significantly lower in the M2 group compared to the I/RI group (P=0.004). Our findings suggest that macelignan has a promising protective effect against I/RI induced musculoskeletal tissue damage in rats.

Keywords: Macelignan, Anwuligan, *Myristica fragrans*, nutmeg, acute hind limb ischemia-reperfusion injury.

Nutmeg (*Myristica fragrans*), a tropical plant from the Myristicaceae family, is widely used in both industrial and culinary applications[1]. Pharmacological studies have identified macelignan, a compound isolated from nutmeg seeds, as a potential natural therapeutic agent for various diseases[2,3]. Previous research suggests that macelignan may exert cytoprotective and antioxidative effects through mechanisms involving the regulation of lipid peroxidation[3]. It has also been shown to possess anti-inflammatory and antioxidant properties by modulating splenocyte proliferation and cytokine production in response to mitogenic stimuli[4]. Additionally, another experimental study reported that macelignan exerts anti-inflammatory effects via the downregulation of pro-inflammatory gene expression and activation of AMP-activated protein kinase (AMPK) [5].

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Based on the available literature, this study is the first to investigate the protective effects of intravenously administered macelignan against hind limb ischemia-reperfusion injury (I/RI) in a rat model.

Materials and Methods

Study design and study population

This study was conducted at the Kobay Lab Experimental Animals Laboratory and approved by the Kobay Lab Experimental Animals Ethics Committee (Date: 08/12/2023, No: 702) in Ankara, Türkiye. All animal procedures complied with the guidelines of the RIH Institutional Animal Care and Use Committee and adhered to the 8th Edition of the NIH Guide for the Care and Use of Laboratory Animals (NRC 2011).

A total of 30 adult male Sprague-Dawley rats (weighing 200–250 g, mean weight: 225 g; aged 7–9 weeks) were obtained from Kobay Lab Experimental Animals Inc. (Ankara, Türkiye). Rats were housed in metal cages under standard laboratory conditions with ad libitum access to standard chow and water.

The rats were randomly assigned to five groups (n=6 per group): Group 1 (Control): Received anesthesia only; Group 2 (Sham): Underwent surgery without ischemia induction; Group 3 (I/RI): Subjected to 3 hours of ischemia followed by 3 hours of reperfusion; Group 4 (M1): Received 15 mg/kg intravenous macelignan 60 minutes before ischemia, followed by 3 hours of ischemia and 3 hours of reperfusion; Group 5 (M2): Received 15 mg/kg intravenous macelignan at the onset of reperfusion after 3 hours of ischemia.

Experimental I/RI design

Anesthesia was induced via intramuscular injection of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg). To maintain anesthesia, one-fourth of the initial dose was administered every 20–30 minutes. A midline incision was made to expose the femoral artery, which was then clamped unilaterally for 3 hours to induce ischemia. This was followed by a 3-hour reperfusion period after clamp removal (see Fig. 1).

At the end of reperfusion, blood samples were collected from the abdominal aorta, centrifuged at 4,000 rpm for 10 minutes, and the serum was stored at -20°C for biochemical analysis. Rats were euthanized under anesthesia, and soleus muscle tissues were harvested. The tissues were washed with ice-cold saline and fixed in 10% neutral-buffered formalin for histopathological analysis. Portions of the tissue were also stored at -80°C for further biochemical evaluations.

Drug preparation

Macelignan was dissolved in a 0.5% dimethyl sulfoxide (DMSO) solution for intravenous use. The administered dose was 15 mg/kg, a concentration selected based on its previously demonstrated efficacy in achieving optimal tissue distribution[6].

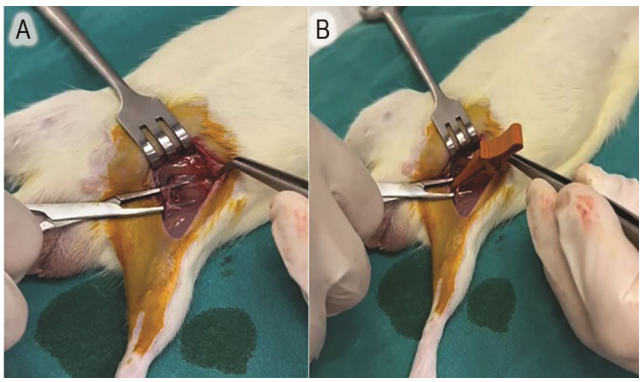

Fig. 1 —Unilateral femoral artery exposure and vascular clamping.

Reagents

Macelignan (CAS No: 107534-93-0) was sourced from Naturewill Biotechnology Co., Ltd. (Sichuan, China).Biochemical assay kits (ALT, AST, BUN, creatinine, LDH, and nitric oxide [NO]) were purchased from Otto Scientific™ (Ankara, Türkiye).ROS-related kits (TAS, TOS, paraoxonase-1 [PON-1]) were obtained from RelAssay Diagnostics® (Gaziantep, Türkiye); additional kits for superoxide dismutase (SOD) and malondialdehyde (MDA) were sourced from Otto Scientific™.Inflammatory and apoptotic marker kits (CK-MM, D-dimer, caspase-3 [CASP3], TNF-α, IL-6) were procured from Elabscience® (USA).

Measurement of serum biochemical parameters

Serum levels of urea, creatinine, AST, ALT, and LDH were measured to assess visceral organ damage. Samples were collected from the abdominal aorta and centrifuged at 4,000 rpm for 10 minutes at 4°C. These parameters were analyzed using a colorimetric method via an automated biochemical analyzer.

Measurement of serum ROS and ROS activity in skeletal muscle

Colorimetric assays were performed on both serum and tissue samples to measure TAS, TOS, OSI, SOD, MDA, PON-1, and NO levels. TAS, TOS, and OSI values were determined using previously established protocols[7–9]. Skeletal muscle tissues were homogenized in a 140 mmol/L KCl buffer at a 1:9 ratio (0.1 g tissue: 0.9 mL buffer), followed by centrifugation at 7,000 rpm for 5 minutes at 4°C.

Measurement of serum Caspase-3, D-dimer, TNF-α, and IL-6

Levels of serum CASP3, D-dimer, TNF-α, and IL-6 were measured using ELISA kits based on the competitive-ELISA principle. Optical densities (OD) were compared to standard curves to calculate the concentrations of each analyte.

Macroscopic scoring of the I/RI to the unilateral hind limb I/R infarct size determination and histopathology

Following fixation in 10% neutral-buffered formalin, muscle tissues were embedded in paraffin and sectioned at 5 µm thickness. Sections were deparaffinized, hydrated, and stained with hematoxylin-eosin (H&E). Histological evaluation was performed by a pathologist blinded to the study groups. Each sample was assessed under a light microscope at 400× magnification for muscle fiber degeneration/disorganization and inflammatory cell

infiltration. A semi-quantitative scoring system (0 = normal, 1 = mild, 2 = moderate, 3 = severe) was used[10,11].

Statistical analysis

Statistical analyses were conducted using Stata version 18.0 (StataCorp LLC, TX, USA). Data are presented as median (min–max) or as counts and percentages, as appropriate. The Shapiro–Wilk test was used to evaluate the normality of data distribution, and the Levene test assessed the homogeneity of variances. Group comparisons were performed using the Kruskal–Wallis test, and Dunn′s post hoc test was applied where significant differences were identified. A *P*-value of <0.05 was considered statistically significant.

Results

Serum and tissue sample results

No statistically significant differences were observed among the groups in serum urea, AST, or D-dimer levels (Table 1). However, serum ALT, creatinine, and LDH levels differed significantly among the groups ($P< 0.05$). Serum ALT levels were highest in the I/RI group and lowest in the sham, control, and M2 groups, which exhibited similar levels ($P= 0.017$). Serum creatinine levels were lowest in the control group and highest in the I/RI and M2 groups, while the sham and M1 groups showed comparable values ($P= 0.015$). Serum LDH levels were lowest in the M2 group and highest in the I/RI group ($P= 0.004$).

A statistically significant difference was also observed in serum CK-MM levels among the groups ($P= 0.002$), with the lowest levels in the M1 group and the highest in the I/RI group. In the macelignan-treated groups, serum ALT, creatinine, and LDH levels were all lower compared to the I/RI group (Fig. 2).

Significant differences were found among the groups in serum oxidative and antioxidative markers, including TAS, TOS, and OSI levels ($P< 0.05$) (Table 2). The highest TAS levels were detected in the control and sham groups, while the remaining groups showed similar, though slightly lower, levels. In both drug-treated groups, TAS levels were higher than those in the I/RI group ($P= 0.002$). Serum TOS levels were highest in the I/RI group and lowest in the sham group, while both drug-treated groups had lower TOS levels than the I/RI group ($P= 0.04$). OSI was lowest in the control and sham groups and highest in the I/RI group. In the drug-treated groups, OSI values were significantly lower than those in the I/RI group

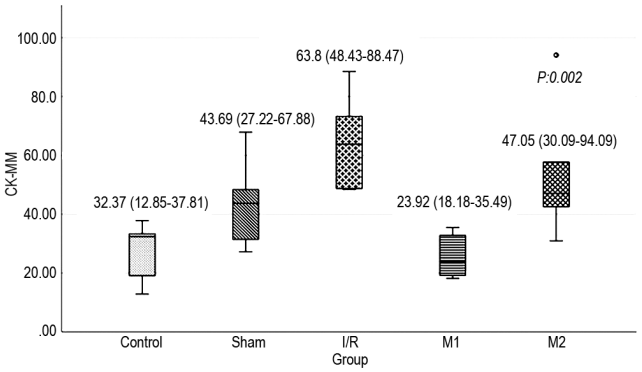

Fig. 2 —An example graph of serum CK-MM levels across groups. (Different letters within the graph indicate statistically significant differences between groups (P<0.05)).

Table 1 —Serum biochemical parameters

	Control (n=6) Median (min-max)	Sham (n=6) Median (min-max)	I/RI (n=6) Median (min-max)	M1 (n=6) Median (min-max)	M2 (n=6) Median (min-max)	P-value
AST	298.12 (226.03-336.53)	251.79 (165.44-312.09)	219.55 (90.99-321.74)	306.6 (181.72-352.84)	253.58 (159.03-336.63)	0.358
ALT	83.13 (58.31-109.32)^{b}	73.86 (50.05-135.34)^{b}	167.49 (87.97-234.99)^{a}	123.78 (84.66-192.53)^{ab}	87.07 (58.57-103.71)^{b}	0.017
Urea	123.62 (52.72-154.84)	119.77 (106.65-129.43)	138.94 (117.63-158.03)	157.77 (73.75-191.52)	135.22 (75.27-166.66)	0.171
Creatinine	0.63 (0.54-0.7)^{b}	0.66 (0.55-1.91)^{ab}	0.78 (0.69-0.84)^{a}	0.68 (0.64-0.91)^{ab}	0.75 (0.72-0.9)^{a}	0.015
LDH	782.06 (622.87-1174.18)^{bc}	1085.18 (885.39-1436.4)^{ab}	1670.83 (854.07-2565.29)^{a}	1081.19 (637.06-2129.24)^{abc}	630.97 (457.3-1067.95)^{c}	0.004
D-Dimer	564.43 (299.22-856.56)	651.88 (55.86-1431.26)	1194.8 (137.01-2667.8)	1091.55 (496.42-1377.64)	917.29 (272.77-2295.96)	0.31
CK-MM	32.37 (12.85-37.81)^{bc}	43.69 (27.22-67.88)^{abc}	63.8 (48.43-88.47)^{a}	23.92 (18.18-35.49)^{c}	47.05 (30.9-94.09)^{ab}	0.002

[^{a,b,c}For each variable separately, different letters in the same row indicate a statistically significant difference (P<0.05)]

and comparable between the M1 and M2 groups ($P = 0.012$).

No statistically significant differences were found among the groups in serum levels of SOD, MDA, or PON-1 ($P= 0.928$, $P= 0.240$, and $P= 0.079$, respectively). However, serum NO levels showed a statistically significant difference among the groups ($P= 0.022$), with the lowest level observed in the M1 group and the highest in the M2 group. The remaining groups had comparable NO levels.

Regarding oxidative and antioxidative markers measured in tissue, significant differences were found in TOS and OSI values ($P< 0.05$) (Table 3). Tissue TOS levels were highest in the I/RI and M1 groups and lowest in the control group ($P< 0.001$). OSI values were also lowest in the control group and highest in the M1 group ($P< 0.01$). When comparing the drug-treated groups to the I/RI group, tissue TOS levels were higher in the M1 group and lower in the M2 group. No significant differences were detected in tissue TAS, SOD, MDA, PON-1, or NO levels among the groups.

Tissue caspase-3 (CASP3) levels were significantly different across the groups ($P= 0.006$), with the highest levels in the I/RI group and the lowest in the drug-treated groups. In contrast, tissue CK-MM levels did not differ significantly among the groups ($P= 0.277$). Detailed tissue oxidative/antioxidative parameters and CASP3/CK-MM levels are shown in Table 3.

A statistically significant difference was also found in serum inflammatory markers among the groups ($P< 0.05$). TNF-α levels were highest in the I/RI and M1 groups, and lowest in the control and M2 groups ($P= 0.004$). Among the drug-treated groups, TNF-α levels were significantly lower in the M2 group compared to the I/RI group. Serum IL-6 levels were lowest in the control and sham groups and highest in the macelignan-treated groups ($P= 0.002$). Inflammatory parameter results are presented in Table 4.

Histopathological results

Histopathological analysis of soleus muscle samples revealed that muscle degeneration and inflammation were most prominent in the I/RI group. In contrast, muscle samples from rats in the macelignan-treated groups demonstrated markedly reduced histological signs of damage compared to the I/RI group (Fig. 3).

Table 2 — Serum oxidative and antioxidative parameters

	Control (n=6) Median (min-max)	Sham (n=6) Median (min-max)	I/RI (n=6) Median (min-max)	M1 (n=6) Median (min-max)	M2 (n=6) Median (min-max)	P-value
TAS	1.53 (1.3-1.79)a	1.52 (1.19-1.71) ab	1.05 (0.62-1.27) b	1.17 (0.96-1.24) b	1.13 (1.03-1.27) b	0.002
TOS	6.9 (2.66-8.12)b	5.37 (1.96-11.05)ab	11.74 (6.1-15.02)a	9.68 (6.78-12.98) ab	9.15 (4.78-11.32)ab	0.04
OSI	0.43 (0.17-0.59)b	0.33 (0.13-0.93) ab	1.21 (0.49-1.79) a	0.88 (0.55-1.18) a	0.78 (0.43-1.07) ab	0.012
SOD	445.64 (432.67-467.1)	443.44 (432.2-462.82)	447.71 (417.14-484.21)	447.79 (435.27-456.93)	449.71 (421.01-472.3)	0.928
MDA	21.91 (4.92-40.75)	19.11 (6.86-41.4)	44.41 (7.4-97.42)	31.21 (5.23-37.74)	31.67 (25.6-46.59)	0.24
pon-1	342.22 (262.97-458.01)	404.49 (365.09-566.7)	385.19 (287.85-481.91)	279.07 (225.03-408.93)	408.71 (227.25-496.8)	0.079
NO	34.72 (32.12-39.02)ab	34 (24.53-60.98)ab	39.46 (25.21-55.74)ab	26.91 (21.18-36.1) b	43.98 (36.2-72.13) a	0.022

[a,b,cFor each variable separately, different letters in the same row indicate a statistically significant difference (P<0.05)]

Table 3 — Tissue oxidative and antioxidative parameters

	Control (n=6) Median (min-max)	Sham (n=6) Median (min-max)	I/RI (n=6) Median (min-max)	M1 (n=6) Median (min-max)	M2 (n=6) Median (min-max)	P-value
TAS	0.57 (0.48-0.78)	0.41 (0.28-0.52)	0.49 (0.32-0.72)	0.39 (0.29-0.55)	0.52 (0.2-0.63)	0.069
TOS	1.21 (1.04-1.54)c	1.58 (0.94-1.9)bc	3 (2.37-3.45)a	3.31 (2.21-4.52)a	1.77 (1.54-2.09)b	<0.001
OSI	0.22 (0.14-0.31)d	0.34 (0.26-0.67)c	0.63 (0.43-0.85)ab	0.76 (0.58-1.56)a	0.34 (0.25-1.02)bc	<0.001
SOD	251.66 (247-254.66)	256.77 (247.39-264.59)	250.94 (242.07-263.02)	250.93 (236.09-262.74)	259.62 (238.36-266.3)	0.277
MDA	24.08 (19.45-35.23)	25.26 (18.32-48.46)	24.33 (22.42-36.5)	25.38 (22.07-31.36)	22.7 (19.37-28.48)	0.709
pon-1	25.18 (17.45-39.98)	17.87 (13.41-22.34)	20.29 (12.21-23.8)	15.66 (10.31-27.24)	20.57 (12.8-23.25)	0.147
NO	34 (28.46-42.03)	38.39 (33.39-51.56)	32.98 (23.87-57.0)	39.33 (24.28-51.82)	35.62 (22.47-58.75)	0.889
Caspase-3	3.58 (2.45-4.52)b	4.06 (2.86-6.41) ab	5.47 (4.69-8.69)a	2.78 (2.55-4.1)b	3.2 (2.61-5.31) b	0.006
CK-MM	22.4 (19.48-29.16)	23.14 (13.46-40.64)	22.84 (16.45-24.9)	18.75 (13.85-20.43)	16.82 (10.58-65.54)	0.277

[a,b,cFor each variable separately, different letters in the same row indicate a statistically significant difference (P<0.05)]

Table 4 — Serum inflammatory parameters

	Control (n=6) Median (min-max)	Sham (n=6) Median (min-max)	I/RI (n=6) Median (min-max)	M1 (n=6) Median (min-max)	M2 (n=6) Median (min-max)	P-value
TNF-α	38.89 (27.47-53.18)b	50.86 (3.51-72.13) ab	72.59 (60.52-77.36) a	73.6 (35.49-82.7) a	43.24 (25.18-66.36) b	0.004
IL-6	15.3 (12.28-19.36) c	16.59 (14.71-20.6) bc	22.29 (12.11-28.75) abc	26.03 (15.05-36.22) ab	32.72 (21.16-35.42) a	0.002

[a,b,cFor each variable separately, different letters in the same row indicate a statistically significant difference (P<0.05)]

Table 5 — Histopathological evaluation and injury scores of muscle tissue

	Control (n=6) Median (min-max)	Sham (n=6) Median (min-max)	I/RI (n=6) Median (min-max)	M1 (n=6) Median (min-max)	M2 (n=6) Median (min-max)	P-value
Degeneration of muscle cells	0.5 (0 - 2)	1 (1 - 1)	1 (1 - 2)	1 (0 - 1)	1 (0 - 2)	0.37
Inflammatory cell infiltration	0 (0 - 1)	1 (0 - 2)	1 (0 - 2)	0.5 (0 - 2)	0.5 (0 - 1)	0.22
Total Injury Score (Muscle)	0.5 (0 - 3)	2 (1 - 3)	2 (1 - 4)	1.5 (0 - 3)	1.5 (0 - 3)	0.27

[a,b,cFor each variable separately, different letters in the same row indicate a statistically significant difference (P<0.05)]

Muscle fiber disorganization/degeneration and inflammatory cell infiltration were evaluated separately for each rat across all groups using a semi-quantitative scoring system (0–3). The most severe muscle damage, characterised by extensive inflammation and fiber degeneration, was observed in the I/RI group. In the macelignan-treated groups, distinct patterns of damage were noted: inflammation was more pronounced in the M1 group, while muscle fiber degeneration was more evident in the M2 group.

The overall muscle damage scores were lower in both treatment groups compared to the I/RI group and were similar between M1 and M2. Although these differences did not reach statistical significance, the histological findings clearly suggest a protective effect of macelignan, with reduced muscle injury relative to the untreated I/RI group.A detailed summary of total muscle damage scores is provided in Table 5, and the graphical representation of the scoring results is shown in Fig. 4.

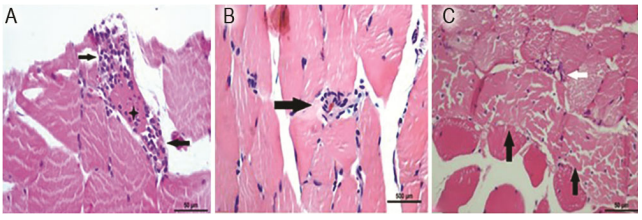

Fig. 3 — (A) Histopathological changes observed in the muscle tissue of rats in the I/RI group at 400× magnification, where degeneration and inflammation were predominant (asterisk: degeneration, black arrows: inflammation). (B) Histopathological changes observed in the muscle tissue of rats in the M1 group at 400× magnification, where inflammation was predominant (black arrow: inflammation). (C) Histopathological changes observed in the muscle tissue of rats in the M2 group at 400× magnification, where degeneration was predominant (white arrow: inflammation, black arrows: degeneration).

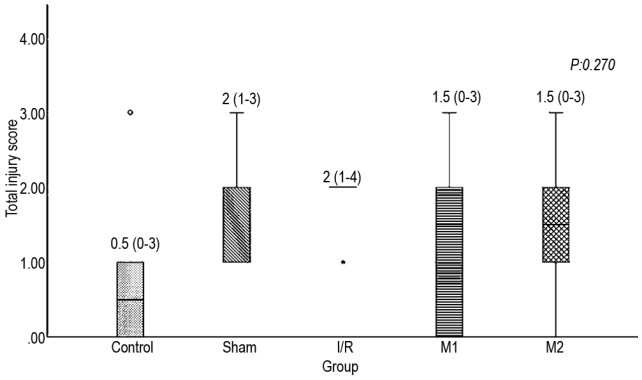

Fig. 4 —Total injury scores [Different letters in the graph indicate statistically significant differences between the groups (P<0.05)].

Discussion

In the present study, we investigated the protective effects of intravenously administered macelignan on hind limb ischemia-reperfusion injury (I/RI) using a rat model. To contribute to the limited existing literature, we evaluated tissue damage by assessing histopathological changes, oxidative/antioxidative parameters, and lipid peroxidation in skeletal muscle. Our findings demonstrated that macelignan administration significantly reduced oxidative stress and improved histopathological outcomes in a lower extremity I/RI model. To the best of our knowledge, this is the first study to explore the effects of macelignan on skeletal muscle injury in the context of peripheral I/RI.

In evaluating organ dysfunction, we observed significantly lower serum ALT and creatinine levels in the macelignan-treated groups compared to the I/RI

group. Lactate dehydrogenase (LDH), an established marker of generalized tissue damage[12], was also reduced in the treatment groups. Additionally, creatine kinase-MM (CK-MM), a muscle-specific injury marker[13], was lowest in the M1 group and reduced in the M2 group compared to the I/RI group.

We also assessed oxidative stress through multiple parameters, including TOS, TAS, OSI, SOD, MDA, NO, and PON-1. In the macelignan-treated groups, serum TAS levels were significantly higher and TOS levels lower than those in the I/RI group. OSI, calculated as the ratio of TOS to TAS, is considered a more comprehensive indicator of oxidative balance[14]. In line with this, OSI levels were significantly lower in the treatment groups than in the I/RI group, with comparable results between M1 and M2. Tissue TOS and OSI levels were also lower in the M2 group than in the I/RI group, supporting the biochemical data and aligning with the histopathological observations.

Histopathological examination confirmed these findings, with the I/RI group showing the most severe muscle fiber degeneration and inflammation. In contrast, the macelignan-treated groups exhibited substantially less muscle damage. These results suggest that macelignan may protect skeletal muscle cells from secondary oxidative injury by reducing the formation of reactive oxygen species (ROS) and lipid peroxidation.

In terms of inflammation and apoptosis, the drug-treated groups demonstrated divergent patterns in serum NO, IL-6, and TNF-α levels. Notably, the M2 group showed the lowest TNF-α expression, and caspase-3 (CASP3) levels in the tissue were also lowest in both treatment groups, indicating a possible anti-apoptotic effect. However, these results did not reach statistical significance in histopathological analysis. More comprehensive studies are necessary to fully validate these observations.

Previous studies have shown that macelignan exerts antioxidant and anti-inflammatory effects[15–18], primarily in models of neurological dysfunction[19–23]. Recent research has extended its therapeutic potential to conditions such as polycystic ovary syndrome[24] and colorectal cancer metastasis[25]. While Myristica fragrans essential oil has demonstrated cytoprotective, anticancer, and anti-inflammatory properties, data on its application in ischemia-reperfusion contexts remain scarce[1]. In one study, Benli *et al*. reported macelignan's protective effect against mesenteric I/RI via antioxidant pathways[26], and Long *et al*. demonstrated a reduction in renal I/RI damage through the downregulation of Bax and caspase-3[27]. However, no prior studies have evaluated macelignan′s effect on skeletal muscle injury. Our findings support and expand on these previous studies, confirming its protective role through enhanced antioxidant defense mechanisms.

Clinically, while surgical revascularization remains the primary treatment for acute limb ischemia, its inaccessibility in certain cases necessitates novel therapeutic strategies[28]. Despite advances in understanding I/RI, an effective pharmacological solution remains elusive. A recent murine study demonstrated that voluntary endurance exercise mitigated neuromuscular dysfunction following skeletal muscle I/RI[29]. This phenomenon may be linked to “hormesis” wherein repeated low-intensity stress enhances the tissue's resistance to subsequent insults[30–32].

In alignment with our findings, two recent experimental studies have demonstrated that plant-derived compounds, such as proanthocyanidins and Ginkgo biloba extract (EGb761), confer protective effects against I/RI by reducing oxidative stress and muscle damage[33,34].However, several limitations should be acknowledged. First, the sample size was restricted to the maximum number approved by the Ethics Committee in accordance with the 3Rs principle (replacement, reduction, and refinement). Second, limited serum volume prevented further analysis of additional oxidative stress parameters.

Conclusion

In conclusion, the findings of our study demonstrate the potential protective effects of intravenous macelignan against ischemia-reperfusion injury in rat musculoskeletal tissue. Macelignan significantly reduced oxidative stress and histopathological damage, suggesting a promising role in mitigating skeletal muscle injury. However, further comprehensive studies are needed to confirm its efficacy and safety in human models. We believe this research provides a foundational step toward the potential development of macelignan as a novel agent in natural therapeutic approaches for ischemia-related conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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