ORIGINAL RESEARCH

Spinal Manipulation Therapy Improves Tactile Allodynia and Peripheral Nerve Functionality and Modulates Blood Oxidative Stress Markers in Rats Exposed to Knee-Joint Immobilization

Felipe C.K. Duarte, BS (Chiro), MSc, PhD,[a,b] Carolina Kolberg, BSc (Chiro), PhD,[b,c] Ana Paula K. Riffel, BBiolSc, Msc,[b] Jessica A. Souza, BBiomedSc,[b] Adriane Belló-Klein, PhD,[b] and Wania A. Partata, PhD[b]

ABSTRACT

Objective: The purpose of our study was to evaluate the effect of manually assisted lumbar spinal manipulation therapy on tactile allodynia, peripheral nerve functional recovery, and oxidative markers in rats exposed to knee immobilization-inducing hypersensitivity.

Methods: Tactile allodynia and sciatic, tibial, and peroneal functional indices were assessed before the knee joint immobilization, 24 hours after the knee cast removal, and 24 hours after 3 weeks of lumbar therapy with the Activator Adjusting Instrument, model 4 (AAI 4). Subsequently, the blood was collected from each rat, and oxidative markers such as lipid hydroperoxide levels; nitric oxide metabolites; and superoxide dismutase, catalase, and glutathione peroxidase activities were assessed.

Results: The AAI 4 improved the immobilization-induced allodynia and recovered the peripheral nerve functional indices impaired after knee immobilization. Immobilized rats treated with AAI 4 therapy presented a lack of significant changes in lipid hydroperoxides and nitric oxide metabolites in the plasma contrasting with rats that were kept freely in their cages, with no therapy applied, which presented elevated lipid hydroperoxides levels. Also, the antioxidant catalase enzymatic activity decreased in the blood of rats immobilized and treated with AAI 4.

Conclusion: These results suggest that manually assisted lumbar spinal manipulation therapy modulates systemic oxidative stress, which possibly contributes to the analgesia and recovery of peripheral nerve functionality.

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Key Indexing Terms: *Manipulation, Spinal; Immobilization; Oxidative Stress; Hyperalgesia; Central Nervous System Sensitization*

INTRODUCTION

Spinal manipulative therapy (SMT) is widely used to manage and treat painful musculoskeletal conditions.[1,2] Among nonpharmacologic treatments, SMT has shown good effectiveness to treat pain.[2-4] However, the biological mechanisms associated with SMT remain unclear, and further studies are necessary to better understand the analgesic effect of SMT.

Chiropractors and other manual therapists who apply SMT commonly use high-velocity and low-amplitude SMT in their clinical routine. However, techniques involving the use of instruments (manually assisted spinal manipulation

[a] Department of Life Sciences, Canadian Memorial Chiropractic College, Toronto, Ontario, Canada.
[b] Department of Physiology, Institute of Basic Health Science, Federal University of Rio Grande do Sul/UFRGS, Porto Alegre, RS, Brazil.
[c] America Latina College, Caxias do Sul, RS, Brazil.
Corresponding author: Felipe C. K. Duarte, BS(Chiro), MSc, PhD, Sarmento Leite, 500, Porto Alegre, Rio Grande do Sul, Brazil CEP 90050-170. Tel.: +55 51 3308 3305.
(e-mail: *felipe.quiropraxia@gmail.com*).
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therapy [MASMT]) are also employed, such as the Activator Adjusting Instrument (AAI).[3-5] This tool is a hand-operated and spring-loaded device designed for clinical practice that applies a high-speed thrust force in a specific line of drive.[3-5] Importantly, it has determined analgesic effects comparable to SMT[6,7] in patients with different painful etiologies during clinical practice and in experimentally pain-induced animal studies.[8-10] Although investigation using AAI has risen in the last decade,[8] the mechanisms behind its analgesic effects are still poorly understood, and further investigation in this field is demanded.

Previous studies have demonstrated that SMT modifies the activity of antioxidant enzymes such as catalase,[2] superoxide dismutase (SOD), and glutathione peroxidase (GPx) in human blood.[11] According to Poljsak et al,[12] oxidative stress may be defined as an excessive amount of reactive oxygen species (ROS), which is the net result of an imbalance between production and destruction of these species. The ROS include singlet oxygen, superoxide radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2). There is also reactive nitrogen (nitric oxide [NO]–derived molecules), iron, copper, and sulfur species, which could contribute to increased ROS formation and oxidative stress and impair the redox balance.[12] Diverse physiological enzymes such as SOD, catalase, and GPx are used to counterattack the ROS. The SOD converts the superoxide anion to H_2O_2, and catalase and GPx decompose H_2O_2 to water and oxygen.[12-14] The ROS and NO play an essential role in the pathogenesis of pain, and the scavenging of these species appears to present an opportunity to normalize the oxidative status altered by pain.[13-17]

Immobilization-inducing hypersensitivity has been used to study abnormal nociception and pain mediated by enhanced production of ROS and spinal sensitization.[18] In addition, spinal motor neuron degeneration and electrophysiological changes in the sciatic nerve have also been reported, suggesting that gait performance variability may occur after hind limb immobilization.[19-24] Although the antinociceptive effect of the Activator Adjusting Instrument, model 4 (AAI 4), has successfully been reported when it was applied on the immobilization site in rats with hind limb immobilization,[10] no studies have explored whether intervention with the AAI 4 on the lumbar spine leads to similar antinociceptive effects. Also, the mechanism of the AAI 4 leading to antinociceptive benefits in addition to its potential effect of improving walking gait performance have not been explored so far.

Thus, the primary purpose of this study was to investigate the causal relationship between the AAI 4 modulating mechanical nociception and free walking gait patterns in animals exposed to knee immobilization. To reveal the potential mechanism of the AAI 4, the secondary aim was to evaluate the effects of AAI 4 on pro-oxidative stress parameters such as lipid hydroperoxide levels and NO metabolites and on antioxidant enzyme SOD, catalase, and GPx activities.

We set out the hypothesis that the AAI 4 on segments L4-L5 induces antinociceptive effect and better performance on free walking pattern in rats exposed to 4 weeks of knee immobilization. We also tested the hypothesis that the AAI 4 on segments L4-L5 modulates the ROS and activity of antioxidant enzymes in the blood of rats exposed to 4 weeks of knee immobilization.

These findings will have important implications to enhancing our understanding of the underlying mechanisms responsible for the clinical manifestation of analgesia and benefits on motor functionality seen during chiropractic therapy.

Methods

Experimental Procedures

Animals. All animal procedures were approved by the ethics committee of the Universidade Federal do Rio Grande do Sul (#21462). Thirty adult male Wistar rats, weighing 200 to 300 g, were provided by the Center for Reproduction and Experimentation of Laboratory Animals of the Universidade Federal do Rio Grande do Sul, and were divided into experimental groups described later. All rats were housed in the room with a stable temperature of 23°C ± 1°C and fed a regular pellet diet ad libitum.

- Naive: Rats that did not undergo right knee joint immobilization for 4 weeks (n = 6).
- Naive-AAI 4: Rats that did not undergo right knee joint immobilization for 4 weeks and had the AAI 4 with adapter applied to the spinous process of L4-L5 for 3 weeks, 3 times/wk, used in force setting "1" (n = 6).
- Immobilization-AAI 4 (I-AAI 4): Rats that had their right knee joint immobilized for 4 weeks and had the AAI 4 with adapter applied to the spinous process of L4-L5 for 3 weeks, 3 times/wk, used in force setting "1" (n = 6).
- Immobilization-sham (I-sham): Rats that had their right knee joint immobilized for 4 weeks and had a lighter and gentler AAI 4 with adapter applied when compared with the I-AAI 4 group, to the spinous process of L4-L5 for 3 weeks, 3 times/wk (n = 6).
- Immobilization-remobilization free: Immobilization-remobilization free (I-RF) rats that had their right knee joint immobilized for 4 weeks and were kept freely in their cages for 3 weeks (n = 6) without application of the AAI 4.

For more details of the experimental groups, see Figure 1.

The AAI 4 with the adapter was used as described by Duarte et al.[25] Lumbar spinal segments were chosen as a therapeutic site to prevent the manipulation of the hind limb that had been immobilized. In addition, the lumbar spine is the location where most afferent fibers from neurosegmentally linked joints such as the hip, knee, and ankle enter.[22]

The AAI 4 was used in force setting "1," because the adapter practically determines the maximum peak force value at force setting 1 of the AAI 4.[25] The I-sham group

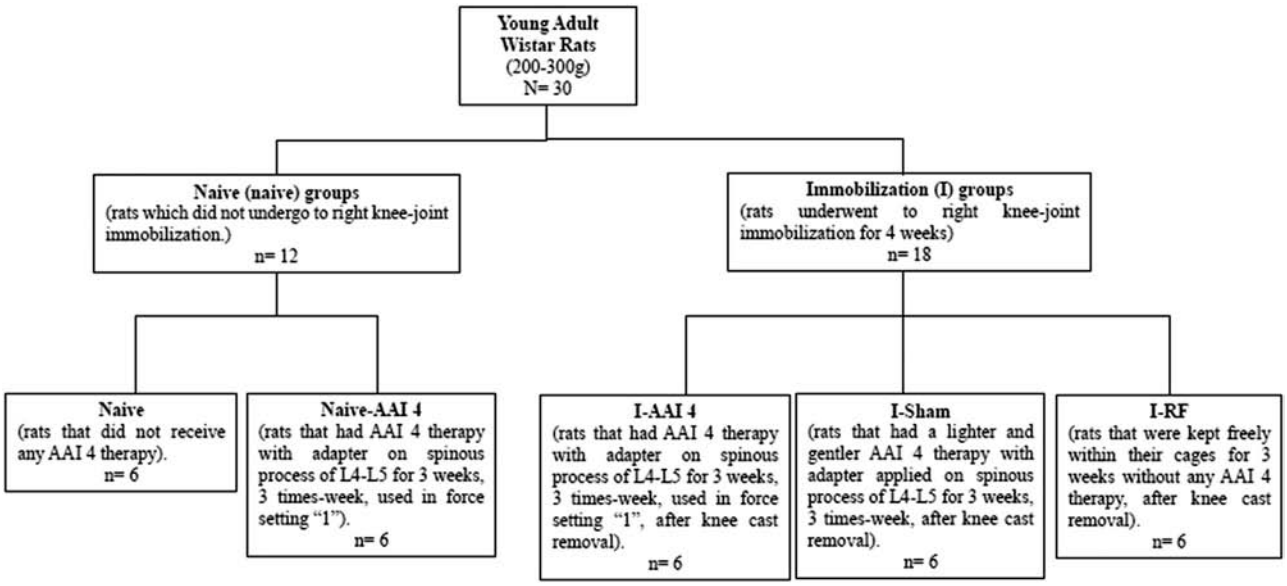

Fig 1. *A schematic diagram shows the experimental groups designated in this study. Thirty adult male Wistar rats, weighing 200 to 300 g, were divided into 2 main groups, the naive group containing 12 Wistar rats and the immobilization group containing 18 Wistar rats. The naive group originated 2 different subsets: naive, containing 6 Wistar rats, composed of rats that did not undergo the immobilization and had no AAI 4 therapy; naive-AAI 4, containing 6 Wistar rats, composed of rats that did not undergo immobilization but had AAI 4 therapy carried out on the L4-L5 spinous process for 3 weeks 3 times per week, used in the force setting "1." In addition, the immobilization group originated 3 different subsets: immobilization-AAI 4 (I-AAI 4), containing 6 Wistar rats, composed of rats that underwent right knee joint immobilization for 4 weeks and, after its removal, had the AAI 4 therapy carried out on the L4-L5 spinous process for 3 weeks, 3 times per week, used in the force setting "1"; immobilization-sham (I-sham), containing 6 Wistar rats, composed of rats that underwent right knee joint immobilization for 4 weeks and, after its removal, had a lighter and gentler AAI-4 therapy applied on the L4-L5 spinous process for 3 weeks 3 times per week; and immobilization-remobilization free (I-RF), containing 6 Wistar rats, composed of rats that underwent right knee joint immobilization for 4 weeks and after cast removal, were kept freely within their cages for 3 weeks.* AAI 4*, Activator Adjusting Instrument, model 4.*

aimed to discard any effects of the touch and audible "click" sound when the AAI 4 is triggered. Contrasting the naive-AAI4 and IIA4, the sham group had the AAI4 lightly touching the L4-L5 spinous processes. Since a preload is required to properly deliver the force of the AAI4 and consequently elicit its effects[4] we intentionally did not perform preadjustment compression onto the target prior to firing the instrument in the sham group which substantially decreased the force delivered by AAI4.[11]

Immobilization Procedure

The knee joint immobilization procedure was performed as described by He and Dishman.[24] After anesthesia (90 mg/kg of ketamine and 10 mg/kg of xylazine), the right knee joint was shaved, and 2 plastic glass splints (Fig 2A) were placed on the medial and lateral sides of the knee joint and then gently wrapped with adhesive plaster around the limb to restrain the movement of the joint. The right knee joint was immobilized in the extended position (150°). A plastic glass belt was attached surrounding the abdomen area to ensure rats would keep their hind paw apparatus (Fig 2B). After the knee immobilization, the foot skin color and movements of the foot, knee, and hip joints were checked daily by the same researcher. No immobilization casts were placed on the contralateral hind limb. After regaining consciousness, the rats returned to their cages and were maintained in the same conditions described earlier.

Mechanical Threshold

The mechanical threshold was assessed by electronic von Frey apparatus (Insight Equipamentos LTDA, Ribeirão Preto, SP, Brazil) to evaluate nociceptive changes after immobilization or after the therapy. A positive response was indicated by an abrupt withdrawal of the paw, and the intensity of the stimuli that provoked the withdrawal response was automatically recorded (in grams). Only the ipsilateral hind paw was subjected to the von Frey test. A single trial consisted of 5 applications of the plastic tip, once every 0 to 30 seconds. The mean of 5 readings was taken as the threshold for a particular timing trial to each rat. This mean indicated the value of mechanical allodynia in that point.[14]

The mechanical threshold was assessed before the knee joint immobilization procedure (pre-immobilization value). After immobilization, the mechanical threshold was evaluated 24 hours after knee-joint cast removal and 24 hours after 3 weeks with AAI 4 application. This test was conducted at the

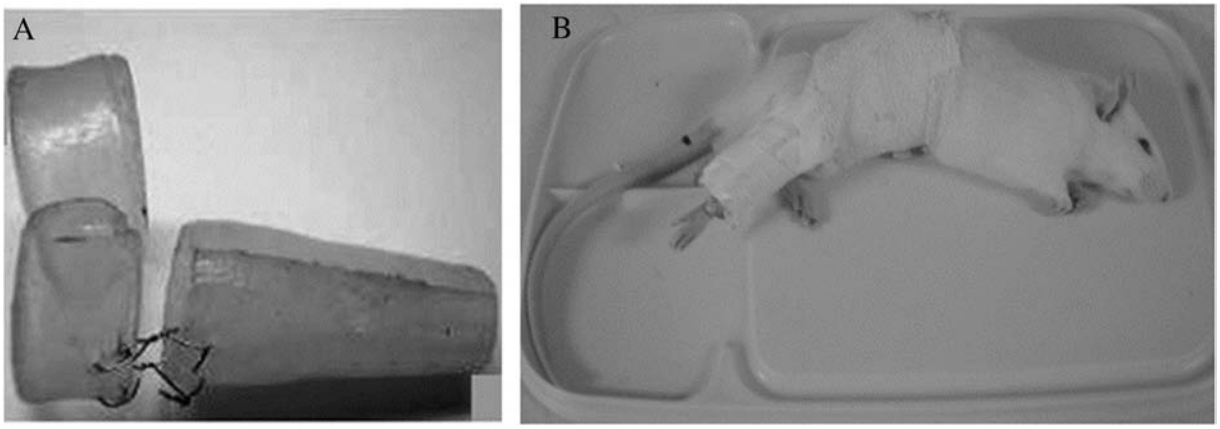

Fig 2. *(A) The plastic glass splints used for the knee immobilization, and (B) a rat with knee joint immobilized and wrapped with adhesive plaster around the right hind limb.*

same time of day (7:00 AM), by the same researcher, and in a room with a stable temperature of 23°C ± 1°C.

Sciatic, Peroneal, and Tibial Functional Indices

To estimate the functional sciatic, peroneal, and tibial nerve indices, a largely accepted method that determines nerve function abnormalities of innervated target organs post-nerve injury was employed.[26-28] Thus, the recovery of the right hind limb locomotor activity was monitored by analysis of the footprints after free walking gait analysis.[27,28] Rat footprints were used to determine the following measurements: (1) distance from the heel to the third toe, the print length (PL); (2) distance from the first to the fifth toe, the toe spread (TS); and (3) distance from the second to the fourth toe, the intermediate toe spread (ITS). The 3 measurements were obtained from the experimental (E) and normal (N) sides. Several prints of each foot have been achieved on each track, but only 3 prints of each foot were used to determine the mean measurements on the experimental and normal sides. These means were included in the functional indices formulas.

$$\text{Sciatic Functional Index} = -38.3\,(\text{EPL}-\text{NPL})/\text{NPL} + 109.5\,(\text{ETS}-\text{NTS})/\text{NTS} + 13.3\,(\text{EIT}-\text{NIT})/\text{NIT}-8.8$$

$$\text{Tibial Functional Index} = -37.2\,(\text{EPL}-\text{NPL})/\text{NPL} + 104.4\,(\text{ETS}-\text{NTS})/\text{NTS} + 45.6\,(\text{EIT}-\text{NIT})/\text{NIT}-8.8$$

$$\text{Peroneal Functional Index} = -174.9\,(\text{EPL}-\text{NPL})/\text{NPL} + 80.3\,(\text{ETS}-\text{NTS})/\text{NTS} + 13.4$$

The resulting functional index was considered to be the index of the functional condition of the nerve, where 0 (±11) represents the normal function and -100 represents the complete loss of functionality.[23,24,26] The functional indices were assessed before the knee joint immobilization procedure (pre-immobilization value), 24 hours after knee joint cast removal, and 24 hours after 3 weeks with AAI 4 application. The tests were conducted at the same time of the day (8:00 AM), by the same researcher, and in a room with a stable temperature of 23°C ± 1°C.

Blood Sample Preparation

All rats were *euthanized* by decapitation and blood was promptly collected. For oxidative stress parameters analysis, the blood samples were centrifuged at 1000 g for 20 minutes in a refrigerated centrifuge (Sorvall RC-5B, Rotor SM-24, DuPont Instruments, Wilmington, Delaware). Plasma samples were aliquoted for later analysis of lipid hydroperoxides and NO metabolites and stored at -70°C. For analysis of lipid hydroperoxides, the plasma was diluted 1:1 (v/v) in butylated hydroxytoluene (90% in methanol) before being stored.[29] Red blood cells (RBCs) were washed 3 times with cold 0.9-N phosphate-buffered saline. Aliquots of RBCs were stored at -70° C in 1 mM HCl and 4mM $MgSO_4$ buffer diluted 50:500 (v/v) for later analyses of enzymatic antioxidant activity.[29]

Determination of Lipid Hydroperoxides

The method is based on oxidation of Fe^{2+} to Fe^{3+} in the presence of lipid hydroperoxides and formation of complexes of Fe^{3+} with xylenol orange.[30] According to the technique adapted from Sodergren et al[31] for each sample, a blank reduced with 10 mmol/L of triphenylphosphine (TPP) in absolute methanol was used.

At the time of the test, a working reagent was prepared with 81% (vol/vol) of 90% methanol, 2 mmol/L of xylenol orange (o-Cresolsulfonphthalein-3′-3″-bis [methylimino-diacetic acid sodium salt]) to a final concentration of

100 μmol/L, 1 mol/L of sulfuric acid to a final concentration of 25 mmol/L, 40 mmol/L of 2,6-di-tert-butyl-4-methylphenol to a final concentration of 4 mmol/L, and 10 mmol/L of ferrous sulfate to a final concentration of 250 μmol. Samples were prediluted 1:10 in Milli-Q (Direct-Q3, Millipore SAS, Molsheim, France) water before the test. Then, they were divided into 2 tubes with 90 μL of the sample in each. The samples were incubated for 30 minutes with 10 μL of 90% methanol or 10 μL of 1 mmol/L TPP. After incubation, the samples were pipetted in duplicate in the microplate and incubated with working reagent (1:9) with stirring for 1 hour at room temperature. Absorbance at 560 nm was obtained with a Zenyth 200 spectrophotometer (Anthos, Salzburg, Austria), and the absorbance values of the duplicates obtained with TPP were subtracted from the values for the duplicates without TPP. Results are expressed in nanomoles of lipid hydroperoxides per milligram of protein.

Determination of NO Metabolites

Pure plasma samples without previous preparation or dilution were analyzed for NO metabolites.[29] Nitrites were determined using the Griess reagent, to measure NO metabolites, in which a chromophore with a strong absorbance at 540 nm is formed by reaction of nitrite with a mixture of 0.1% naphthyl-ethylenediamine and 1% sulfanilamide. Nitrates were determined as total nitrites (initial nitrite plus nitrite reduced from nitrate) after their reduction using nitrate reductase from *Aspergillus* species in the presence of nicotinamide adenine dinucleotide phosphate. A standard curve was established with a set of serial dilutions (10^{-8} – 10^{-3} mol/L) of sodium nitrite. Absorbance at 540 nm was obtained with a Zenyth 200 spectrophotometer (Anthos). Results were expressed as millimoles of NO metabolites per liter.[29]

Determination of Antioxidant Enzyme Activities

In this study, Cu-Zn SOD activity was determined. The activity of SOD expressed as units per milligram of protein (U/mg protein) was measured based on its action to neutralize the superoxide radicals to prevent oxidation of adrenaline to adrenochrome, a colorful byproduct that can be measured at 480 nm (SP22 spectrophotometer, Bioespectro, Curitiba, PR, Brazil).[32] The SOD activity was determined by measuring the velocity of inhibition of adrenaline oxidation. The reaction medium contained glycine buffer (50 mmol/L, pH 11.3) and adrenaline (60 mmol/L, pH 2.0).[33]

The GPx activity was measured by following nicotinamide adenine dinucleotide phosphate oxidation at 340 nm (T60U spectrophotometer, PG Instruments Ltd, Leicestershire, United Kingdom) as described by Flohé and Gunzler.[33] The GPx results were expressed as nanomoles of peroxide/hydroperoxide reduced per minute per milligram of protein.

Catalase activity was determined by following the decrease in absorption at 240 nm (T60U spectrophotometer, PG Instruments Ltd) in a reaction medium containing 50 mmol/L phosphate buffer (pH 7.2) and 10 mmol/L hydrogen peroxide (H_2O_2) and expressed as picomoles of H_2O_2 reduced per minute per milligram protein.[33-35]

Protein Measurement

Protein was measured by the method of Lowry using bovine serum albumin as standard.[36]

Statistical Analysis

The sample size for the present study was established based on previous studies using WINPEPI software version 9.1. A significant α level of .05 and 95% power resulted in a sample size of 6 rats per group. Data were analyzed by 2 independent researchers. All data are reported as means ± standard error of the values of 6 rats per group. Normal Gaussian distribution of the data was analyzed by the Shapiro-Wilk test. Levene's test analyzed constant variance. Biochemical results were analyzed using 1-way analysis of variance (factor: treatment) followed by the Tukey post hoc test. Mechanical threshold and functional indices were compared by repeated-measures 2-way analysis of variance (factors: treatment and time) followed by the Tukey post hoc test. Differences were considered statistically significant when P was <.05. All statistical analyses were carried out with Sigma State package version 3.1 (Systat Software Inc, Chicago, Illinois) for Windows.

Results

Mechanical Threshold

The mechanical threshold did not change significantly in naive and naive-AAI 4 groups ($P > .05$). Twenty-four hours after the end of the immobilization period, the mechanical threshold reduced in I-AAI 4 (mean difference [MD] = 30.45; 95% confidence interval [CI] (12.68-48.22); $P < .0001$), I-sham (MD = 36.9; 95% CI [19.13-54.67]; $P < .0001$), and I-RF rats (MD = 22.77; 95% CI [5.004-40.54]; $P = .0030$) (Fig 3). The reduction was around 38% in all groups when compared with pre-immobilization levels. A decrease of 38% in the mechanical threshold suggests the presence of hyperalgesia in these rats. In addition, 3 weeks of the AAI 4 therapy improved the mechanical threshold, returning the mechanical threshold to pre-immobilization levels. This was implied because no significant difference was found when compared with pre-immobilization values within group (MD = -1.465; 95% CI [-19.23 to 16.3]; $P > .9999$), compared with post-24 hours cast removal (MD = -31.92; 95% CI [-49.68 to -14.15]; $P < .001$), or when compared with naive and naive-AAI 4 (MD = [9.601], [0.1403]; 95% CI [-8.167 to 27.37], [-17.63 to 17.91];

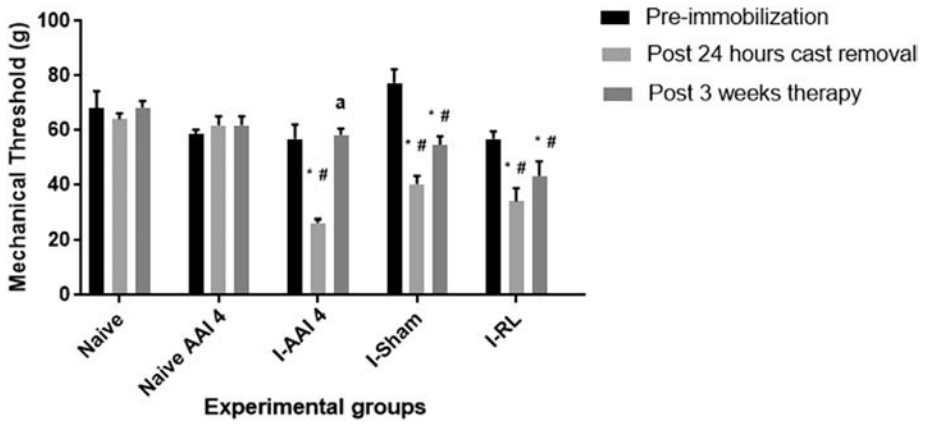

Fig 3. *Assessment of mechanical threshold in rats that underwent knee joint immobilization for 4 weeks and, after cast removal, had the AAI 4 therapy applied on the lumbar spinal segments L4-L5 for 3 weeks, 3 times per week. Data represent mean ± standard error of the mean. *Indicates significant difference compared to pre-immobilization values. #Indicates significant difference compared with naive groups. "a" represents significant difference compared to post-cast removal (repeated-measures 2-way analysis of variance followed by Tukey post hoc test,* P < *.05). Experimental groups are as described in Figure 1.* AAI 4*, Activator Adjusting Instrument, model 4;* I*, immobilization;* RL*, right leg.*

P > .999), indicating total recovery of the mechanical threshold. Interestingly, 3 weeks post-sham therapy did not show significant improvement of the mechanical threshold compared with post-24 hours cast removal (MD = -14.33; 95% CI [-32.1 to 3.435]; P = .2340). The mechanical threshold did not return to pre-immobilization levels within group (MD = 22.57; 95% CI [4.799-40.33]; P = .0034). After 3 weeks with sham therapy, the mechanical threshold was significantly decreased in these rats compared with naive (MD = 23.66; 95% CI [5.893-41.43]; P = .0018) and naive AAI 4 (MD = 18.54; 95% CI [0.7765-36.31]; P = .0335) groups. This result indicates that the value of force developed by the instrument appears to influence the recovery of mechanical threshold in immobilized rats. Similarly to I-sham, the mechanical threshold of the I-RF rats did not show significant recovery compared with post-24 hours cast removal (MD = -9.272; 95% CI [-27.04 to 8.496]; P = .8471), indicating that the recovery of mechanical threshold was small. In addition, the mechanical threshold was significantly different compared with the pre-immobilization threshold (MD =13.5; 95% CI [2.61-24.39]; P = .0117). After 3 weeks with free remobilization, the mechanical threshold was significantly decreased in these rats compared with naive (MD = 24.66; 95% CI [6.888-42.42] P = .0010) and naive AAI 4 (MD = 18.54; 95% CI [0.7765-36.31]; P = .0335) groups.

Sciatic, Peroneal, and Tibial Functional Indices

The functional indices did not differ significantly in naive and naive-AAI 4 groups (P > .05). The indices values were between -12 and -4 in these rats through all nerve indices assessed. The walking gait analysis values significantly decreased after 24 hours, ranging between -25 and -20 in the immobilized rats in the sciatic, peroneal, and tibial functional indices, respectively, within each group compared with pre-immobilization levels on the I-AAI 4 (MD = 12.92, 17, 14.12; 95% CI [10.71-15.13], [14.98-19.02], [12.11-16.14]; P < .0001), I-sham (MD = 8.541, 15, 16.07; 95% CI [6.333-10.75], [12.98-17.02], [14.06-18.09]; P < .0001), and I-RF (MD = 6.542, 14, 11.91; 95% CI [4.334-8.75], [11.98-16.02], [9.895-13.93]; P < .0001) as well as when I-AAI 4, I-sham, and I-RF were compared to naive (I-AAI 4: MD = 14.79, 19, 15.79; 95% CI [11.55-18.03], [16.04-21.96], [12.83-18.75]; P < .0001) (I-sham: MD = 9.674, 17, 17.51; 95% CI [6.435-12.91], [14.04-19.96], [14.55-20.46]; P < .0001) (I-RF: MD = 9.724, 17, 14.58; 95% CI [6.485-12.96], [14.04-19.96], [11.62-17.53]; P < .0001) and naive-AAI 4 (I-AAI 4: MD = 11.57, 16, 14.12; 95% CI [8.327-14.8], [13.04-18.96], [11.17-17.08]; P < .0001) (I-sham: MD = 6.453, 14, 15.84; 95% CI [3.214-9.692], [11.04-16.96], [12.88-18.8]; P < .0001) (I-RF: MD = 6.503, 14, 12.91; 95% CI [3.264-9.742], [11.04-16.96], [9.954-15.87]; P < .0001) (Figs 4A, 4B, and 4C). The indices of these nerves showed recovery in I-AAI 4 rats. At the end of 3 weeks of therapy, the sciatic functional index was similar to the pre-immobilization (MD = 1.1912; 95% CI [-0.2963 to 4.12]; P > .05) and to the naive-AAI 4 groups (MD = 0.557; 95% CI [-2.682 to 3.796]; P > .9999), which shows a full recovery of this nerve after AAI 4 therapy. We found an important improvement after 3 weeks of AAI 4 therapy in the sciatic (post-cast removal: -18.566; post-3 weeks AAI therapy: -7.557; MD = 3.726; 95% CI [0.4872-6.965]; P = .01), peroneal (post-cast removal: -23; post-3 weeks AAI 4: -10; MD = 6; 95% CI [3.039-8.961]; P < .0001), and tibial nerve indices (post-cast removal: -20; post-3 weeks AAI 4: -9; MD = 5.405; 95% CI [2.448-8.362]; P < .0001), reaching indices values close to naive

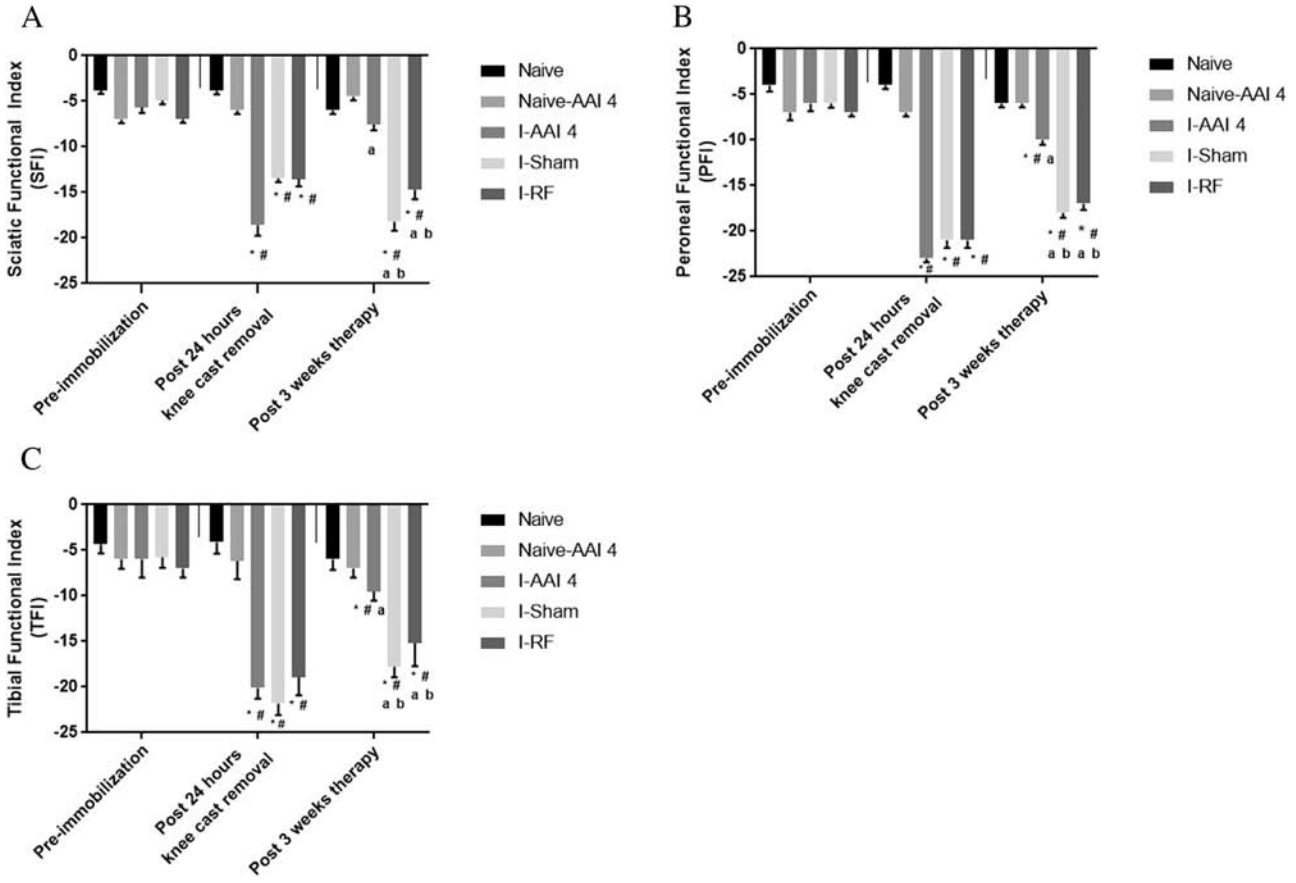

Fig 4. *Functional recovery assessed by the SFI, TFI, and PFI. Rats underwent knee joint immobilization for 4 weeks and, after joint cast removal, had the AAI 4 therapy applied on the lumbar spinal segments L4-L5 for 3 weeks, 3 times per week. Data represent mean ± standard error of the mean. *Indicates significant difference compared with pre-immobilization values. #Indicates significant difference compared to Naive groups. "a" represents significant difference compared with post-cast removal. "b" represents significant differences between 3 weeks post-sham or remobilization-free therapy compared with AAI 4 therapy (repeated-measures 2-way analysis of variance followed by Tukey post hoc test,* P < *.05). Experimental groups are as described in Figure 1.* AAI 4*, Activator Adjusting Instrument, model 4;* I*, immobilization;* PFI*, peroneal functional index;* RF*, remobilization free;* SFI*, sciatic functional index;* TFI*, tibial functional index.*

animals; however, neither the peroneal indices (MD = 4; 95% CI [1.981-6.019]; $P < .0001$) nor the tibial indices (MD = 3.515; 95% CI[1.499-5.531]; $P = .0002$) fully returned to pre-immobilization values. In addition, although a robust reduction post-3 weeks AAI 4 therapy was found, it still presented a significant difference compared with the peroneal naive and naive-AAI 4 indices (MD = 6, 3; 95% CI [3.039-8.961], [0.03926-5.961]; $P < .0001$, $P = .0439$) and tibial naive and naive AAI 4 indices (MD = 5.182, 3.515; 95% CI [2.225-8.139], [0.5582-6.472]; $P < .01$).

The sciatic, peroneal, and tibial indices of these 3 nerves did not show recovery 3 weeks post-I-sham therapy when compared with pre-immobilization values (MD = 13.28, 15, 12.02; 95% CI [10.04-16.52], [12.04-17.96], [9.064-14.98]; $P < .0001$). Despite this, we have found significant differences on I-sham post-cast removal compared to 3 weeks of post I-sham therapy on sciatic, peroneal, and tibial nerve indices (MD = 14.36, -3, -4.053; 95% CI [11.12-17.6], [-5.961 to -0.03926], [-7.01 to -1.096]; $P < .05$), which would suggest a successful rehabilitation; these indices values post-sham therapy were significantly lower (-18.18, -18, -17.78) and different than I-post-AAI 4 therapy (-7.55, -10, -9.51) (MD = 10.63, 8, 8.273; 95% CI [7.393-13.87], [5.039-10.96], [5.316-11.23]; $P < .0001$), implying that the AAI 4 therapy was more effective compared with I-sham. The sciatic, peroneal, and tibial post-3 weeks sham therapy were also different than naive (MD = 14.41, 14, 17.51; 95% CI [11.17-17.65], [11.04-16.96], [14.55-20.46]; $P < .0001$) and AAI 4 (MD = 11.19, 11, 15.84; 95% CI [7.95-14.43], [8.039-13.96], [12.88-18.8]; $P < .0001$).

Similar to I-sham, I-RF did not show sciatic, peroneal, and tibial indices recovery 3 weeks post-remobilization free compared with pre-immobilization indices (MD = 7.75, 10, 8.17; 95% CI [4.511-10.99], [7.039-12.96], [5.213-11.13]; $P < .0001$). The sciatic, peroneal, and tibial post-3 weeks remobilization-free therapy were also different than naive (MD = 10.93, 13, 10.84; 95% CI [7.693-14.17], [10.04-

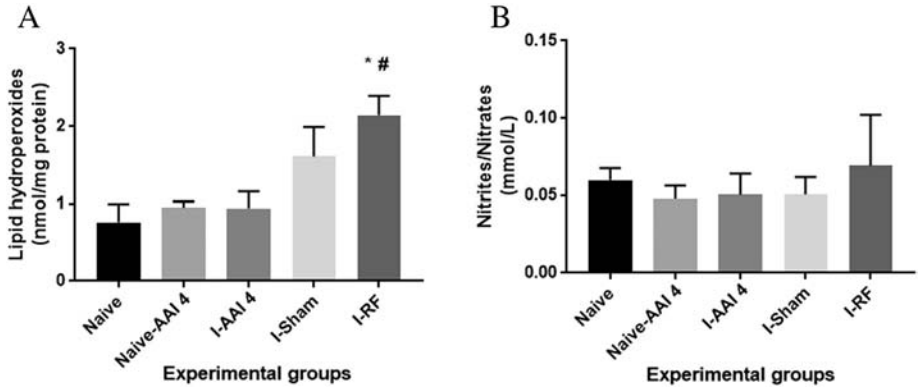

Fig 5. *Assessment of the blood pro-oxidative parameter activity lipid hydroperoxides (A) and nitric oxide metabolites (nitrites + nitrates) (B) in rats that underwent knee joint immobilization for 4 weeks and, after joint cast removal, had the AAI 4 therapy applied on the lumbar spinal segments L4-L5 for 3 weeks, 3 times per week. Data represent mean ± standard error of the mean. *Indicates significant difference compared with naive groups (1-way analysis of variance followed by Tukey post hoc test,* P < *.05). #Indicates significant difference compared with I-AAI 4 group (1-way analysis of variance followed by Tukey post hoc test,* P < *.05). Experimental groups are as described in Figure 1.* AAI 4*, Activator Adjusting Instrument, model 4;* I*, immobilization;* RF*, remobilization free.*

15.96], [7.88-13.79]; $P < .0001$) and naive-AAI 4 (MD = 7.711, 10, 12.91; 95% CI [4.472-10.95], [7.039-12.96], [9.954-15.87]; $P < .0001$). Although some improvement was found on these 3 nerve indices between post-3 weeks remobilization-free therapy compared to 24 hours knee cast removal (MD = 10.88, 13, -6.671; 95% CI [7.641-14.12], [10.04-15.96], [-9.628 to -3.714]; $P < .0001$), the sciatic, peroneal, and tibial indices were significantly lower in the post-3 weeks remobilization-free therapy group (-14.71, -17, -15.17) than in the post-3 weeks AAI 4 therapy group (-7.55, -10, -9.51) (MD = 7.154, 7, 5.655; 95% CI [3.915-10.39], [4.039-9.961], [2.698-8.612]; $P < .0001$). Interestingly, no difference between 3 weeks post-sham and remobilization-free therapy was found on the sciatic (MD = -2.93; 95% CI [-5.887 to 0.02676]; $P = .0546$), peroneal (MD = -1; 95% CI [-3.961 to 1.961]; $P = .9970$), and tibial (MD = -2.618; [-5.575 to 0.3388]; $P = .1401$) indices.

Oxidative Stress Parameters

The lipid hydroperoxide levels did not show a significant change in plasma of I-AAI 4 and I-sham rats compared with naive (MD = -0.182, -0.844; 95% CI [-1.34 to 0.97], [-1.96 to 0.27]; $P > .05$) and naive-AAI 4 (MD = 0.005, -0.656; 95% CI -1.10 to 1.11, -1.72 to 0.41; $P > .05$) groups, respectively (Fig 5A). However, the values of lipid hydroperoxides in plasma of I-sham rats were higher than those found in the plasma of naive, naive-AAI 4, and I-AAI 4 rats, although no significant differences were found among them. In I-RF rats, the lipid hydroperoxide levels significantly increased in plasma. The increase was 65% compared with both naive (MD = -1.382; 95% CI [-2.54 to -0.22]; $P = .0141$) and naive-AAI 4 (MD = -1.194; 95% CI [-2.30 to -0.08]; $P = .030$) and 56% compared to I-AAI 4 rats (MD = -1.2; 95% CI [-2.30 to -0.09]; $P = .029$). No significant difference was found in lipid hydroperoxides between I-RF and I-sham (MD = -0.537; 95% CI [-1.60 to 0.53]; $P = .584$).

The level of NO metabolites did not significantly change in the plasma of rats with immobilization that received AAI 4, sham therapy, or remobilization free and were similar to naive and naive-AAI 4 ($F [4, 18] = 1.037$, $P = .415$) (Fig 5B).

The SOD activity did not show any significant change in RBCs of I-AAI 4 rats nor I-sham, I-RF, naive, and naive-AAI 4 ($F [4, 25] = 0.870$, $P = .495$) (Fig 6A).

The GPx enzymatic activity did not show any significant change in RBCs of I-AAI 4, I-RF, nor both naïve groups. However, GPx activity was significantly higher in RBCs in I-sham rats (higher than 50%) compared with naive-AAI 4 and I-AAI 4 rats (MD = -10.4, -11.74; 95% CI [-20.61 to -0.1873], [-21.65 to -1.831]; $P < .05$) (Fig 6B). Catalase activity, in turn, decreased 53% in the RBCs of I-AAI 4 rats compared with naive (MD = 3.367, 95% CI [0.09-6.63]; $P = .041$), naive-AAI 4 (MD = 2.982, 95% CI [0.52-5.43]; $P = .011$), and I-sham (MD = -3.129, 95% CI [-5.66 to -0.596]; $P = .01$) groups. No significant change was found when compared wtih I-RF rats (Fig 6C).

Discussion

This study is the first to establish that AAI 4 therapy induces recovery of the sciatic, peroneal, and tibial nerve functional indices decreased by 4 weeks of immobilization. Also, this is the first study showing the effect of the AAI 4 on ROS. Although it is early to speculate on the role of MASMT on oxidative stress, our results demonstrated that this therapy might have a protective role, counteracting ROS formation. Its modulatory effect on systemic oxidative stress may be associated with the antinociceptive effect of MASMT, recovery of peripheral nerve functionality, and motor performance.

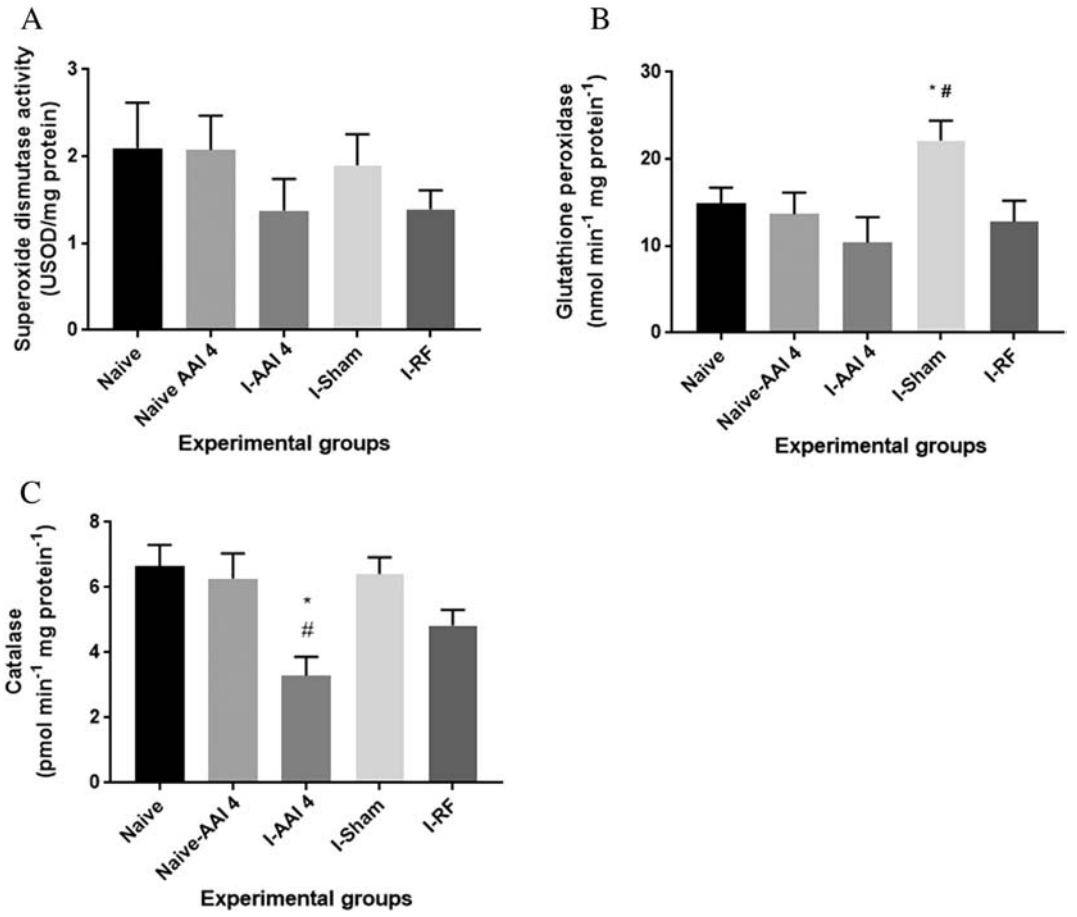

Fig 6. *Assessment of antioxidant enzyme activities such as superoxide dismutase (A), glutathione peroxidase (B), and catalase (C) in the blood of rats that underwent knee joint immobilization for 4 weeks and, after joint cast removal, had the AAI 4 therapy applied to the lumbar spinal segments L4-L5 for 3 weeks, 3 times per week. Data represent mean ± standard error of the mean. *Indicates significant difference compared with naive groups (1-way analysis of variance followed by Tukey post hoc test,* $P < .05$*). #Indicates significant difference compared with I-AAI 4 on (B) or to I-sham group on (C) (1-way analysis of variance followed by Tukey post hoc test,* $P < .01$*). Experimental groups are as described in Figure 1.* AAI 4*, Activator Adjusting Instrument, model 4;* I*, immobilization;* RF*, remobilization free.*

In this study, the immobilization condition increased mechanical nociception and promoted peripheral nerve function impairment and plasmatic changes in oxidative stress parameters. Otherwise, the MASMT induced antinociception; recovered the gait pattern assessed through the sciatic, peroneal, and tibial nerve functional indices; and modulated pro- and antioxidative stress parameters in the blood of these animals. Therefore, these findings support our hypothesis that the AAI 4 on lumbar spine segments ameliorates the mechanical hyperalgesia, recuperates the free walking pattern, and stabilizes the ROS activity induced by knee immobilization. Previous studies developed and validated the applicability of the AAI 4, replacing its original larger silicone rubber tip with a smaller adapter tip made in nylon, aiming to bridge the contact between the instrument and smaller joint such as those seen in rodents (spine segments, knee, etc).[10,25] Thus, the AAI 4 with the adapter becomes a useful tool to examine the effects of chiropractic treatment in animal studies.[10,25]

Similar to other studies, our results showed that 4 weeks of knee immobilization induces mechanical hypersensitivity in the ipsilateral hindpaw.[10,18] In addition, the hypersensitivity was persistent, not returning to pre-immobilization levels as verified when spine AAI 4 was applied. Despite the importance of the pain system as a warning and protective system to maintain tissue integrity, its mechanism of function differs when pain becomes persistent or chronic.[8,13,16] In such a situation, nociceptors no longer only respond to vigorous suprathreshold stimulus, but the threshold needed to evoke a response is lowered.[13,16,37-40] Persistent and chronic pain may be associated with peripheral or central sensitization (CS).[8,37,38,42] Previous studies reported that acute peripheral knee inflammation and knee immobilization sensitizes pain-related non-myelinated or low-myelinated fibers in the knee joint, increasing their activity during the rest (spontaneous activity) and when innocuous stimuli are applied.[11,13,18,27] One remarkable characteristic of CS is the augmented electrophysiological activity in nociceptive neurons within the spinal cord

elicited by normal inputs.[8,18,42] Central sensitization can be maintained by an ongoing peripheral noxious input such as occurs in neuropathic pain or during inflammation.[37,39,42] Interestingly, an increase of pro-oxidation peripherally has been suggested as one of the mechanisms contributing to peripheral inflammation after joint immobilization.[18] Also, Hamaue et al[18] showed an increase of spinal cord neuropeptides related to the onset of the CS (substance P and calcitonin gene-related peptide), after 4 and 8 weeks of joint immobilization. Interestingly, He and Dishman[24] showed that 4 weeks of immobilization enhances the oxidative activity, triggering neurotoxicity within the spinal cord. Although the cast apparatus itself it is not considered a painful stimulus, a knee cast apparatus for 4 weeks may lead to peripheral inflammation, which in turn sensitizes peripheral nociceptors, altering the expression of factors that are driven up to the central nervous system, such as those related to the onset of CS as substance P, which was previously demonstrated to be increased in rodents after inflammation.[42] Therefore, some of the benefits of the AAI 4 in this model might involve the modulation of factors linked to peripheral inflammation and central sensitization.

Only the AAI 4 therapy improved the abnormal mechanical threshold, returning to the normal baseline values such as given by naive and naive-AAI 4 groups. The antinociceptive effect of the AAI 4 therapy is in line with a previous study using alike animal model[10] and other studies with a similar therapy approach in animals and humans.[9,41,43-46] An earlier study demonstrated that the attenuation of dorsal root ganglia and spinal neuron hyperexcitability reduce mechanical allodynia in rats.[46] Although we found the critical antinociceptive effects of the AAI 4 focusing on lumbar spine therapy, Trierweiller et al[10] found analogous antinociceptive results by applying the AAI 4 therapy directly on the tibial tubercle of the immobilized knee. Thus, the antinociceptive effect of the AAI 4 and the possible modulation of CS likely appear to be independent of the application site, at least in this model of study.

Importantly, because an increase of NO was shown on motor neurons after knee immobilization,[24] this neurotoxic factor may be contributing to the findings of the walking track analysis performed through our study. We did show for the first time that I-AAI 4 rats improved sciatic, tibial, and peroneal functional indices decreased after 4 weeks of knee immobilization. In addition, the peripheral nerve functional indices did not recover at the same proportion in the I-sham and I-RF rats. The measures of the sciatic, peroneal, and tibial nerve functional indices from naive and naive-AAI 4 are in line with other studies,[24,26] thus reinforcing our measures made in all rats. Although any histological assessment was performed in our study, a mild nerve injury may have occurred since Alves et al[21] showed that hind limb immobilization for 14 days causes variation in the nerve excitability and fragmentation in the myelin sheath of the sciatic nerve of rats. Some of these changes were reversible after cast removal.[21] Also, He and Dishman[24] showed that 4 weeks of knee immobilization leads to motor neuronal degeneration and axonal demyelination. A normal and intact afferent input, which is conducted up to the central nervous system, is a key player for a normal and tuned locomotor activity.[47] Again, only animals exposed to AAI 4 therapy had their walking track patterns improved. Thus, we speculate that such an improvement may be due to modulatory effects on the inflammatory cascade and neuronal excitability, such those demonstrated by Song et al.[45]

On the other hand, abnormal motor activity can be caused by CS.[38] In this context, it is possible that 4 weeks of the knee immobilization was enough to fire a salvo of abnormal afferent inputs to the central nervous system, generating improper sensory and motor output. Our results comparing the effectiveness of 3 weeks of different therapies employed through this study suggest that the high-speed force delivery thrust application of the AAI 4 performed in the I-AAI 4 group is the primary factor inducing the physiological effects involved in the recovery of peripheral nerve functional indices and walking track.

Our study also focused on the role of AAI therapy on plasmatic oxidative stress markers owing to the emerging role of ROS in altered nociception and chronic pain[16,17,48,50] and the promisor effects of the SMT modulating oxidative stress parameters in patients with neck and back pain.[2,11] For the first time, we demonstrated that AAI 4 therapy on lumbar spine L4-L5 segments stables lipid hydroperoxide and decreases catalase activity, but does not change GPx and SOD activities in RBCs in the I-AAI 4 rats. These results differ partially from those found in patients with chronic back or neck pain who received high-velocity, low-amplitude manipulation. In these patients, plasmatic catalase, SOD, and GPx activity increased in the RBCs.[2,11] Therefore, these antioxidant enzymes likely acted to prevent the increase of plasmatic lipid peroxidation.[2,11] It is possible that AAI 4 activated other enzymatic or non-enzymatic pathways to counteract ROS and therefore prevent lipid peroxidation. In addition, the controversial findings between rats and humans may be related to the difference in the metabolic rate and the distinct chiropractic protocols used through these studies. Differences in the blood collection points after spinal manipulation therapy may also be another cause of such a difference. However, it is also necessary to consider that these results may be connected to the distinct painful conditions to which patients and rats were exposed. Regardless, MASMT modulated systemic ROS activity, and it may be linked with its antinociceptive effect found throughout the present study. Knee joint immobilization for 4 weeks changed the behavior of the plasmatic oxidative markers. The systemic blood cell activity may be changed by any modification in the immunological system, such as those influenced by inflammation or any biological stress.[49,51] Thus, we suggest that the systemic changes 4 weeks post-immobilization may be related to changes in the endothelium and skeletal muscle functions caused by a stressor factor (knee immobilization). Data from animals, and more recently from humans, indicate that cast immobilization increases xanthine

oxidase activity.[51] Xanthine oxidase seems present in the capillary endothelium and infiltrated leukocytes, and this enzyme catalyzes the oxidation of hypoxanthine and xanthine to produce xanthine and uric acid, respectively; these reactions are coupled with the reduction of dioxygen into superoxide anions. In addition, it also has been demonstrated that cast immobilization increases superoxide and H_2O_2 emissions in the skeletal muscle.[51] Taking these studies into account, we suggested that the immobilization protocol increased ROS in the blood of the immobilized rats by mechanisms that involve xanthine oxidase or emission of superoxide and H_2O_2 from skeletal muscle.

The enhanced ROS formation might be related to the plasmatic elevation of the lipid hydroperoxides found in the I-RF rats. Augmented production of ROS elevates lipid peroxidation.[54] Interestingly, we reported that elevated plasmatic lipid hydroperoxide levels persisted for at least 3 weeks after cast removal. Because the central nervous system and particularly the spinal cord is highly composed of polyunsaturated fatty acids,[48] lipid peroxidation may be occurring as consequence of enhanced formation of ROS. These species may contribute to maintenance of the nociceptive signaling, thus decreasing the mechanical threshold found in this group.

Importantly, the lack of significant changes in lipid hydroperoxide levels in the plasma of I-AAI 4 rats might be indicating a protective effect of the AAI 4 on ROS formation and subsequently in the oxidative damage that affects cellular membranes in this group. Interestingly, a decrease of oxidative stress and lipid peroxidation when an oxidative stress scavenger N-acetylcysteine is applied intraperitoneally has been associated with the reverse of the mechanical hyperalgesia and CS.[48] Thus, our results suggest that the protective effects of the AAI 4 on ROS formation and its overall inhibition of lipid peroxidation may possibly be linked with the improvement of the mechanical hyperalgesia in rats undergoing knee immobilization. Inflammatory molecules increase ROS formation.[45] Because AAI 4 reduces the manifestations of dorsal root ganglia inflammation,[44] the lack of significant changes in plasmatic lipid hydroperoxide levels of I-AAI 4 rats may be related to an anti-inflammatory effect of the therapy with AAI 4. Otherwise, the lipid hydroperoxide was elevated in the plasma of I-sham rats when compared with the I-AAI 4 rats. Although this is an exciting finding, it lacks statistical difference. A possible reason for the lack of significant difference would be the limited number of rats used in our study. It is likely that increasing the number of rats would result in a significant difference between the groups because the standard error of the mean was high in the I-sham group. Yet, the difference in lipid hydroperoxide levels in the plasma of I-sham and I-AAI 4 rats may also be related to the proper application of the AAI 4 and not merely casualty. Thus, we speculate that the smaller force developed by the instrument in the sham condition fired a minor modulatory effect on systemic ROS and consequently led to more elevated plasmatic lipid hydroperoxide values in the I-sham rats.

Elevated ROS formation in the blood of the I-sham rats is the latent reason for the increased GPx activity in these rats. Glutathione peroxidase decomposes H_2O_2 into water and oxygen,[12,17,55] and GPx activity is most useful for small amounts of H_2O_2 produced continuously by cellular metabolic activities.[58] Because ROS formation was likely reduced in the blood of the I-AAI 4 group, this reduction potentially contributed to the lack of significant changes in plasmatic GPx activity in these rats. In this context, the lack of significant changes in GPx activity in the blood of the I-RF rats is intriguing, mainly because they showed elevation in lipid hydroperoxide levels. This finding may likely be related to the highest use of the glutathione (GSH) defense system. Glutathione is the primary intracellular thiol-disulfide redox buffer that serves as a cofactor for many antioxidant enzymes, including GPx.[52] The oxidation of GSH leads to the production of glutathione disulfide (GSSG). In humans and animals, the GSSG concentration is low, but pro-oxidant overproduction is accompanied by activation of the GSH defense system, which enhanced GSSG levels to protect proteins and membrane lipids from oxidation.[56,57] Since in the I-RF a higher lipid hydroperoxide was observed without changes in SOD and catalase, the use of GSH defense system is likely limiting the effects of plasmatic ROS formation in this group.

On the other hand, the catalase activity was decreased in the RBCs of the I-AAI 4 rats, while no changes occurred in the I-sham rats. This result might be related to the effect of the therapy on ROS formation. Catalase has less affinity to H_2O_2 and is useful during peaks of H_2O_2 production.[53] The ROS formation appeared reduced in the RBCs of the I-AAI 4 rats, but more elevated in I-sham rats, as discussed earlier. Despite that ROS formation was lower in the RBCs of the I-AAI 4 rats, the activity of the catalase is decreased in the RBCs of these rats, without changing the GPx activity. Otherwise, because only the I-sham rats showed an increase in GPx activity, the activity of this enzyme was responsible for maintaining the low ROS in the RBCs of I-sham rats. Nevertheless, the catalase activity may be associated with the muscle injury. After muscle injury, the induction of the catalase is delayed, and its peak of activity occurs in the second day post-injury, followed by thioredoxin activity.[58,59] Thioredoxin is an antioxidant protein that limits the activity of ROS.[59] Thus, it is possible that the thioredoxin activity may have some role in the complex interaction of pro-oxidant and antioxidant parameters in RBCs in immobilized rats.

In our study, no significant change was found in NO metabolites in the plasma of the I-AAI 4, I-sham, and I-RF rats. These results suggest that no change occurred in NO production in the immobilized rats. Nitric oxide is the major player for the maintenance of vascular homeostasis, and reduction of its bioavailability and increased NO degradation by superoxide anion mark the onset of endothelial dysfunction.[59] Thus, the lack of changes in NO metabolites indicates that a normal bioavailability of NO to maintain the vascular homeostasis in the plasma of the I-AAI 4, I-sham, and I-RF rats is occurring. In

addition, the maintenance of NO production could be explained by the GSH system. Glutathione plays a major role in NO metabolism.[55] Glutathione reacts with peroxynitrite from S-nitrosothiols. Peroxynitrite is produced by the reaction of NO and superoxide anion.[53,56] Peroxynitrite by itself may react with lipids, proteins, and other cellular structures, causing structural and functional modifications.[56] The reaction of GSH with peroxynitrite may have prevented the systemic adverse effects of the peroxynitrite in the immobilized rats. The reaction of GSH with peroxynitrite also releases NO over a prolonged time to extend the half-life of NO,[55] thereby preventing the adverse effects of NO scavenging by superoxide anions. Thus, the GSH reaction with peroxynitrite possibly contributed to maintaining the NO metabolite levels in the blood of immobilized rats. Indeed, the reaction of GSH with peroxynitrite may be limiting the availability of superoxide anions to SOD. This enzyme converts the superoxide anions to H_2O_2.[12,17,52] Thus, the use of GSH may explain the lack of significant changes in NO metabolites and SOD activity in the plasma of immobilized rats.

Because ROS are involved in the nociceptive process, central sensitization, and chronic pain mechanisms,[17,22,50] the modulatory action of the AAI 4 therapy on oxidative stress parameters in the blood in the immobilized rats may be contributing to the AAI 4-induced antinociception. However, further studies are necessary to better understand the relation between AAI 4 and oxidative stress parameters in the blood of immobilized rats.

Limitations

This study did not assess other oxidative stress parameters such as the total content of thiols, which would allow for better understanding of the relation between GSH and our results. This study did not evaluate the concentration of oxidized and reduced glutathione (GSH-to-GSSG ratio), which forms the major redox couple in cells. We did not analyze the thioredoxin system and the pro-oxidant cytokine levels in the plasma and other tissues, such as a spinal cord and dorsal root ganglion of the I-AAI 4 rats. In future studies, investigating the effects of MASMT on ROS at treatment points will help to understand how AAI 4 modulates ROS activity over time. It is necessary to analyze the immobilization-induced muscle changes in future studies.

Future studies on this topic are needed to clarify the relationship between the AAI therapy and the oxidative stress parameters in the blood of immobilized rats and to determine the potential association between the oxidative markers and the antinociceptive effect of MASMT.

Conclusion

Our study showed that MASMT by application of the AAI 4 on lumbar segments L4-L5 induced an antinociceptive effect and a functional recovery of peripheral nerves in rats exposed to the knee immobilization. Also, our findings for the first time showed a modulatory effect of the AAI 4 on plasmatic ROS formation in rodents. Thereby, we speculated that the effects of MASMT on ROS formation may be contributing to the improvement of the sensorial and functional abnormalities post knee immobilization.

Funding Sources and Conflicts of Interest

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Contributorship Information

Concept development (provided idea for the research): F.C. K.D., C.K., W.A.P.
Design (planned the methods to generate the results): F.C. K.D., W.A.P.
Supervision (provided oversight, responsible for organization and implementation, writing of the manuscript): F.C.K. D., W.A.P.
Data collection/processing (responsible for experiments, patient management, organization, or reporting data): F.C. K.D., J.A.S., A.P.K.R., C.K., A.B.-K.
Analysis/interpretation (responsible for statistical analysis, evaluation, and presentation of the results): F.C.K.D., C.K., W.A.P.
Literature search (performed the literature search): F.C.K. D., W.A.P.
Writing (responsible for writing a substantive part of the manuscript): F.C.K.D., W.A.P.
Critical review (revised manuscript for intellectual content, this does not relate to spelling and grammar checking): F.C. K.D., C.K., W.A.P.

Practical Applications

- Manually assisted spinal manipulation therapy on lumbar segments improved hind paw mechanical allodynia, a clinical finding of central sensitization, caused by 4 weeks of knee immobilization.
- Manually assisted spinal manipulation therapy on lumbar segments recovered the peripheral nerve function and the locomotor activity impaired after 4 weeks of knee immobilization.
- Manually assisted spinal manipulation therapy on lumbar segments modulated systemic blood oxidative stress parameters, which might be contributing to the antinociceptive effects of SMT.

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