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The effect of aloe vera on ischemia—Reperfusion injury of sciatic nerve in rats

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ABSTRACT

Purpose: Aloe vera is compound which has strong antioxidant and anti-inflammatory effects. We investigated the neuroprotective role of aloe vera treatment in rats with experimental sciatic nerve ischemia/reperfusion injury.
Methods: Twenty-eight male Wistar Albino rats were divided equally into 4 groups. Groups; Control group (no surgical procedure or medication), sciatic nerve ischemia/reperfusion group, sciatic nerve ischemia/reperfusion + aloe vera group and sciatic nerve ischemia/reperfusion + methylprednisolone group. Ischemia was performed by clamping the infrarenal abdominal aorta. 24 hours after ischemia, all animals were sacrificed. Sciatic nerve tissues were also examined histopathologically and biochemically.
Results: Ischemic fiber degeneration significantly decreased in the pre-treated with aloe vera and treated with methylprednisolone groups, especially in the pre-treated with aloe vera group, compared to the sciatic nerve ischemia/reperfusion group ($p < 0.05$). A significant decrease in MDA, an increase in NRF1 level and SOD activity were observed in the groups which obtained from the AV and MP groups when compared to the sciatic nerve ischemia/reperfusion group. When all results were analysed it was seen that the aloe vera group was not statistically different compared to the MP group ($p > 0.05$).
Conclusions: Aloe vera is effective neuroprotective against sciatic nerve ischemia/reperfusion injury via antioxidant and anti-inflammatory properties. Also aloe vera was found to be as effective as MP.

1. Introduction

Peripheral nerves are good vascularized structures that are perfused by independent external and internal microvascular systems. Disrupts this system; ischemia, plays significant roles in the development of pathological changes in many different peripheral neuropathies. In many tissues, the major mechanism of reperfusion injury is considered to be due to reduced oxygen species [1].

Peripheral nerve disorders are among the most common neurological problems that the clinicians face, yet few treatments and interventions are available to stopping or adverse the damage associated with them [2]. Therefore many pharmaceutical intervention, and/or physical rehabilitation, surgical repair are current therapeutic strategies for the treatment of the peripheral nerve injury and develop of regeneration [3–5]. Plants produce a various of antioxidants against molecular damage from reactive oxygen species (ROS), and phenolics compose the important class of plant-derived antioxidants. Aloe vera (AV) is a plant that has antioxidant properties [6,7], and made studies on the subject.

We know that AV is effective in protecting rats against cerebral ischemia/reperfusion injury induced damage [8]. In this study, we aimed to investigate the effect of AV in a rat model for sciatic nerve ischemia/reperfusion, and discuss the possible axon-protective and antioxidant mechanism of AV against ischemic damage and fiber degeneration.

2. Materials and methods

2.1. Animals

Twenty-eight male Wistar Albino rats each weighing 300 ± 50 g and 8–12 weeks old were used in the experiment. An automatic

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photoperiod with white fluorescent light was used to create an environment with 12 h light/12 h darkness. All rats were fed with standard pellet rat food (*Bil-Yem Ltd., Ankara, Turkey*) and water ad libitum. The temperature was set to 21 ± 2 °C. Ethical approval was obtained from Animal Experiments Ethics Committee. All methods used for animal experiments were organized in accordance with the protocols of the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Experimental groups

Rats were randomly divided into four equal groups.

Group 1: Control group (n = 7, no surgical procedure or medication),

Group 2*:* Ischemia group (n = 7, 24 h of reperfusion was created after 45 min sciatic nerve ischemia (SNI), and then the group was sacrificed),

Group 3: Aloe vera group (AV) (n = 7, 30 mg/kg/day aloe vera was applied via gastric gavage for 1 month. 24 h of reperfusion was created after 45 min sciatic nerve ischemia, and then the group was sacrificed),

Group 4: Methylprednisolone group (MP) (n = 7), Single dose 30 mg/kg MP (*Prednol, Mustafa Nevzat, Turkey)* was administered intraperitoneally immediately following SNI. 24 h of reperfusion was created after 45 min sciatic nerve ischemia, and then the group was sacrificed.

2.3. Drug treatment

Aloe vera gel (40% purity by extracted from *Aloe barbadensis*) was obtained from Herbalife International (Istanbul, Turkey). The dosage was determined as 30 mg/kg body weight based on preliminary studies with different doses (10, 20, 32, and 120 mg/kg/day) to find out the biological effects of AV [8–12]. Methylprednisolone was obtained from Mustafa Nevzat Drug Industry Inc. (Istanbul, Turkey). The drug was dispersed with isotonic NaCl 0.9%.

2.4. Surgical procedure

Premedication protocol [Intraperitoneal ketamine hydrochloride (Parke Davis, Istanbul, Turkey) (50 mg/kg) and xylazine (Bayer, Istanbul, Turkey) (5 mg/kg)] was applied to all rats. Anesthesia was continued with only ketamine injections at intervals. Surgical approach was supine position. Sterile laparotomy was performed with a standard midline incision. The retroperitoneum was opened and was reached to the abdominal aorta. Aort occlusion was induced by cross-clamping the aorta with mini aneurysm clip between just below the left renal artery and just proximal to the aortic bifurcation. It was confirmed by pulse palpation loss. Clamps removed after 45 min ischemia and was observed distal reperfusion. Surgical operation is finished, the abdominal wall was closed with 5/0 prolene sutures. Rats in the control group was surgical procedure similar to the other groups but the aorta was not clamped. All animals at 24th hour were anesthetized with penthobarbital (20 mg/kg) and sacrificed. Sciatic nerves were removed full length until bifurcation bilateraly in all animals. Half of the sample taken for histopathological investigation and it was fixed in%10 neutral formalin for 7 days. The other half was stored in a freezer at −80 ° C for biochemical evaluations.

2.5. Histopathological preparation and evaluation of rat sciatic nerves

The nerve samples were separated into two pieces, one for the transverse section and the other one for longitudinal section. Then, histologically processed and embedded in paraffin. 5 μm sections were taken from these blocks. Nerve samples were stained with hematoxylin&eosin (H&E) (Sigma-Aldrich, St. Louis, MO, USA) and also modified Gomori Trichrome (Bio-optica, Milano, Italy) (according to the manufacturer's protocol). Sciatic nerve samples were graded for ischemic fiber degeneration (IFD) using previously described method by Kihara et al. [13]. For IFD, the fibers were evaluated according to axonal changes. These changes were swollen or shrunken and dark axons. Changes in myelin were evaluated for breakdown, attenuation and collapse. The percentage of fibers undergoing ischemic fiber degeneration was graded histologically as; 0 = <5%; 1 = 5–25%; 2 = 26–50%; 3 = 51–75%; 4 = >76% (Table 1). All of the sections were evaluated under light microscope (Eclipse E-600 Nikon, Tokyo, Japan).

2.6. Immunohistochemistry protocol for NF-κB

Nuclear factor kappa B (NF-κB) is a redox-sensitive transcriptional factor activated by some stimuli such as hyperglycemia, oxidative stress, and pro-inflammatory cytokines. NF-κB has an important role in activation of inflammatory and immune responses which can cause cellular injury [14,15].

Tissue samples were first deparaffinized and rehydrated. Citrate buffer (pH = 6.0) was used for antigen retrieval with microwave heating. Endogenous peroxidase activity was blocked by%3 hydrogen peroxide in methanol. Then, NF-κB primary antibody (1/100 dilution) (anti-NF-κB p65 antibody, Santa Cruz Biotechnology, Texas, USA) was dropped and incubated overnight at 4 °C. After incubation, HRP secondary antibody kit was used as a secondary antibody. AEC kit was used for chromogen. At last, all the slides were counterstained with Mayers hematoxylin and mounted with water based mounting medium. All the chemicals were purchased from Labvision Corp. (Fremont, CA, USA). The immunoreactivity of NF-κB was investigated by immunohistochemical method.

A semiquantitative grading scale is used to evaluate antibody binding to NF-κB in axons and schwann cells. Fibers were scored using previously described method [16].

Grades 0–4 were defined as follows:

1 ≤2% of whole nerve showing staining; generally light staining
2 2–15% of whole nerve showing staining; moderate intensity of staining
3 16–25% of whole nerve showing staining; moderate to intense staining
4 26–35% of whole nerve showing staining; intense staining
5 ≥35% of whole nerve showing staining; intense staining

All of the sections were evaluated under light microscope (Eclipse E-600 Nikon, Tokyo, Japan). Image analysis was made with Image Analysis Software (NIS Elements Nikon, Tokyo, Japan) for assessing the samples.

2.7. Biochemical preparation and evaluation of rat sciatic nerves

The tissues were kept at −80 °C degree post-macroscopic examination. Nuclear respiratory factor-1 (NRF1),

Table 1
IFD (Ischemic fiber degeneration) and NF-kβ scores of sciatic nerves. All data were presented as mean ± S.D. In each line, (a–e) the difference between the means with the same letters are significant ($p < 0.05$, Mann Whitney *U* test, for each group n = 7).

Groups	IFD	NF-kβ immunreactivity
Control	$0.29 \pm 0.488^{a,b,c}$	$0.71 \pm 0.488^{a,b,c}$
SNI	$3.14 \pm 0.690^{a,d,e}$	$3.29 \pm 0.756^{a,d,e}$
AV	$2.00 \pm 0.577^{b,d}$	$1.71 \pm 0.488^{b,d}$
MP	$1.71 \pm 0.488^{c,e}$	$1.57 \pm 0.535^{c,e}$