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ORIGINAL ARTICLE

Neuromuscular degenerative effects of Ankaferd Blood Stopper® in mouse sciatic nerve model

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ABSTRACT

Purpose: Ankaferd Blood Stopper® (ABS), a licenced medicinal herbal extract, is commonly used as an effective topical haemostatic agent. This study is designed to investigate whether topical ABS application may cause peripheral nerve degeneration and neuromuscular dysfunction in a mouse sciatic nerve model.

Methods: Twenty mice were randomly divided into two groups; an ABS treated experimental group and a saline-treated control group. Left sciatic nerves were treated with 0.3 ml of ABS in the experimental group and 0.3 ml of sterile saline in the control group for 5 min. Peripheral nerve degeneration and neuromuscular dysfunction were evaluated by behavioural tests, electrophysiological analysis and weight ratio comparison of target muscles.

Results: The motor function, assessed by the sciatic function index, was significantly impaired in ABS-treated animals as compared to the animals treated with saline. Motor coordination, evaluated with the rotarod test, was significantly decreased (–42%) in ABS-treated animals compared to the saline-treated animals. The degree of pain, assessed by the reaction latency to thermal stimuli (hot-plate test), was significantly prolonged (313%) in ABS-treated mice when compared to the saline-treated mice. ABS-treated mice showed a significant reduction in motor nerve conduction velocity (MNCV) (–52%) and the compound muscle action potential (CMAP) (–47%); however, it significantly prolonged onset latency (23%). The gastrocnemius muscles weight ratio of the ABS group was considerably lower than that of the control group.

Conclusions: These findings demonstrate that ABS triggers peripheral nerve degeneration and functional impairment and, thus promotes a deterioration of sciatic nerves.

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KEYWORDS
Anakaferd blood stopper; functional impairment; peripheral nerve degeneration

Introduction

ABS (Immun Drug Cosmetic Co., Istanbul, Turkey) is a topical haemostatic agent composed of five distinct herbal extracts (Turk et al. 2017). In the 100 ml ABS solution, there are namely 9 mg of Glycyrrhiza glabra (dried leaf extract), 8 mg of Vitis vinifera (dried leaf extract), 7 mg Alpinia officinarum (dried leaf extract), 6 mg of Urtica dioica (dried root extract) and 5 mg of Thymus vulgaris (dried grass extract) (Garber and Jang 2016). It has been used for centuries in Turkish traditional medicine as a haemostatic agent (Dincol et al. 2016) and has been approved by the Turkish Ministry of Health as a topical haemostatic agent for the management of dermal, external, internal, post-operative and dental bleeding (Huri et al. 2010a; Ugur et al. 2016). This agent is efficacious in both bleeding individuals with standard haemostatic parameters and in patients with deficient primary and/or secondary haemostasis (Huri et al. 2010b; Ozseker et al. 2012). ABS has been used in gastrointestinal bleeding (Kurt et al. 2010), lung bleeding (Arslan et al. 2009; Uzun et al. 2014), Urologic surgery (Istanbulluoglu et al. 2013), tonsillectomy (Teker et al. 2009), partial nephrectomy (Yalcinkaya et al. 2011), radical prostatectomy (Huri et al. 2009), acute anterior epistaxis (Meric Teker et al. 2010), management of bleeding GI tumours (Heller et al. 2010) and dentistry (Pamuk et al. 2016; Vezeau 2016). The mechanism of action of ABS is the formation of an encapsulated protein network providing focal points where vital erythrocytes to aggregate (Goker et al. 2008). According to these reports, ABS is a well established topical haemostatic agent. However, in some cases, neuronal dysfunctions were reported in patients who have received ABS applications (Pampu et al. 2013). ABS is directly applied in all the cases mentioned above without any dilution.

Peripheral nerve damage is a severe public health problem associated with motor, sensory and autonomic deficiencies. Ineffective treatment of such nerve injuries can cause partial or complete loss of sensory, motor and autonomic functions, resulting in a limited activity or lifelong disability (Araujo-Filho et al. 2016). Toxin exposure, genetic mutation and metabolic impairment can also be detrimental to axonal

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connectivity by hindering cargo transport along axons or dismantling cytoskeletal structures, finally leading to axon loss (Shin and Cho 2017). Peripheral neuropathies (PNs) are injuries or diseases of the nerves which result from varied aetiology, including metabolic syndrome, trauma and drug toxicity. The clinical manifestation of PNs depends on the type of neuropathy. It may include the loss of motor, sensory and autonomic functions or develop debilitating neuropathic pain away from the site of injury. An important manifestation of axonopathy in the PN causes degeneration of the terminal ends of the peripheral nerves, loss of epidermal nerve fibres and inadequate nerve endings (Landowski et al. 2016). Peripheral neuropathies can be a severe impediment to quality-of-life and survival (Landowski et al. 2016). Peripheral nerves are comprised of myelinated and unmyelinated fibres, with myelinated nerves surrounded by the specialized Schwann cells to provide insulation (Sullivan et al. 2016). The myelin provides the structural basis for saltatory action potential propagation, which accelerates nerve conduction 20–100-fold compared with non-myelinated axons of the same diameter (Nave and Werner 2014). A neuron develops a single axon that transmits electrical signals from the cell body to the target tissue over long distances (Shin and Cho 2017).

We planned this study because of the complaints of neuronal dysfunctions in the clinical use of ABS. In this context, we wanted to test whether the direct exposure of peripheral nerve fibres to ABS would cause neuromuscular dysfunction using the mouse sciatic nerve model under *in vivo* conditions.

Materials and methods

Mice (animals)

All animal protocols were approved by the local animal experiment ethics commission, in accordance with the European Community Council Directive 86/609/ECC for the care and use of laboratory animals. Experiments were performed on 20 Male 6–8-week-old Balb-C mice purchased from the Van Yüzüncü Yıl University Experimental Animal Production Centre. The animals were housed in an air-conditioned room (temperature = 21 °C ± 2 °C), under a 12-h light/dark cycle, with free access to food and water. Every effort was made to minimize the suffering of animals and to reduce the number of animals used.

Drug administration

The following drugs were used in this study: Xylazine (2%, Bayer, Germany), Ketamine (100 mg/ml, Hexal, Germany), Ankaferd Blood Stopper (2 ml ampul, İmmun Drug, Istanbul, Turkey) and Physiological Saline (PS) (0.9% sterile saline solution, 3 ml, Hudson RCI, USA).

Surgical procedure

Before the sciatic nerve surgery, ketamine (100 mg/kg) and xylazine (10 mg/kg) were used to minimize pain in the mouse and to provide deep anaesthesia. The surgical procedure was performed by a single surgeon using standard aseptic techniques on the sciatic nerve of the left rear limb. The non-operated right limb sciatic nerve served as a healthy comparison. The sciatic nerve was revealed with a skin incision over the glutaeal region. A muscle splitting approach was utilized to reach the nerve. In the experimental group, 0.3 ml was administered from the ABS in ampoule form to the muscle space where the sciatic nerve was present. The sciatic nerve was exposed to the ABS solution for 5 min. In the control group, 0.3 ml of PS was administered, the sciatic nerve was exposed to the sterile PS solution for 5 min. The incisions of skin and muscles were sutured (Figure 1). The animals were kept in a warm environment to wake. Before surgery, behaviour tests were performed in all the mice. Seven days after surgery and treatment, the behaviour tests and the electrophysiological investigation was carried out in both groups. At the end of this study, animals were sacrificed by cervical transection under deep anaesthesia (Nijhuis et al. 2013).

Sciatic function index (SFI)

The SFI was used to evaluate the motor function capacity of the sciatic nerve treated with ABS or PS. The sciatic function index (SFI), which is an analysis of the walking pattern via the recording of footprints, is a well-established and widely used method for the assessment of motor healing after nerve damage. Walk trail analysis was performed on both groups before treatment and 7 days after treatment, based on the protocol described by Inserra et al. (1998). First, the mice' rear feet were painted with Indian ink. Then the mice walked across a tunnel to record the footprints on white paper paved on the bottom of the tunnel (48 cm × 4.5 cm). The pawprints were analysed considering two different parameters: toe spread (TS), the distance between the first and fifth toes; and print length (PL), the distance between the third toe and the rear pad (Figure 2).

These measures were calculated according to the sciatic function index (SFI) formula by Inserra et al. (1998):

$$\mathrm{SFI} = \frac{118.9(\mathrm{ETS} - \mathrm{NTS})}{\mathrm{NTS}} - \frac{51.2(\mathrm{EPL} - \mathrm{NPL})}{\mathrm{NPL}} - 7.5$$

where TS = toe spread in mm, PL = print length in mm; E and N indicate the experimental and normal hind foot, respectively. In general, an SFI value indicates around 0 for normal nerve function, whereas an around –100 SFI value indicates total dysfunction (Inserra et al. 1998; Goulart et al. 2014).

Rota-rod (motor coordination) test

The rota-rod test was used to evaluate the motor coordination abilities of mice treated with ABS or PS. According to the method described by Kuribara et al. (1977), the rotating rod test was performed on an accelerating Rota-Rod Treadmill using a 3.8-cm-diameter rubberized rod. The rota-rod apparatus (Ugo Basile, Comerio VA, Italy) consisted of a bar with a diameter of 3 cm that was sub-divided into five compartments. The test was performed before treatment and 7 days after treatment. The mice were allowed a trial run of

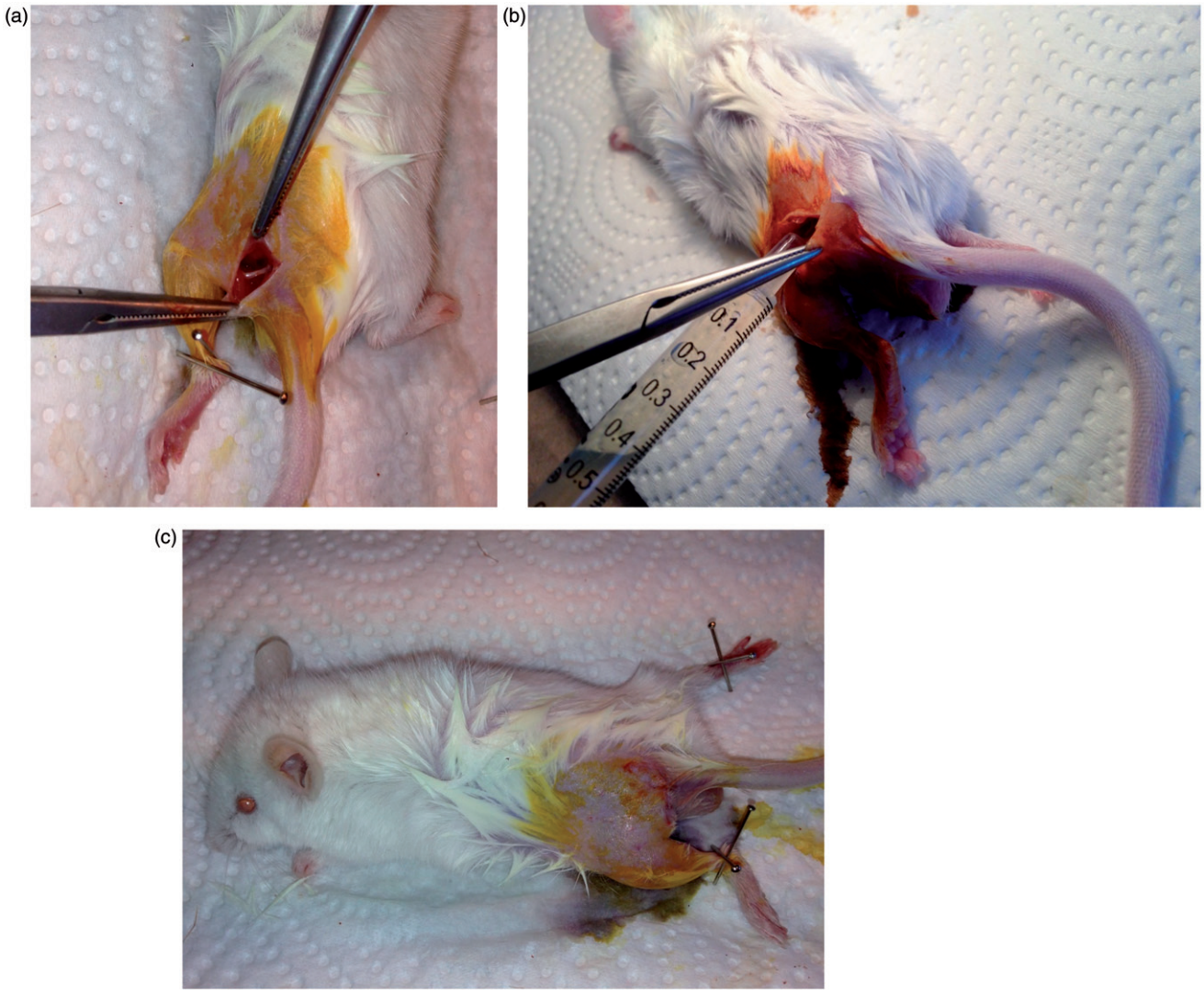

Figure 1. Surgical procedure and treatment application in mice. The expose of the sciatic nerve (a). Treatment with ABS or PS to the sciatic nerve (b). Suture of skin and muscles (c).

5 min on the rotor set at 20 rpm on the day preceding the measurement. On the next morning, the rotation speed was set to rise from 4 rpm to 40 rpm over 5 min. The mice were walked on the rotor to assess. The test was repeated three times at 45-min intervals for each animal. The time taken to fall off the rota-rod was recorded as the latency (s). Mice were allowed to walk a maximum of 300 s (cut-off time) on the rod. The average of the three tests was recorded as the 'Rotarod Value' of that animal (Kuribara et al. 1977; Harauma et al. 2015; Zhang et al. 2016).

Heat hyperalgesia (hotplate test)

The hotplate test is a standard technique used to measure the reaction latency to acute thermal stimuli and to evaluate the degree of pain in mice. The thermal hyperalgesia was tested in mice by using a hot-plate apparatus (May, Turkey) as described previously (Parvathy and Masocha 2013). The test was performed before the treatment and at day 7 after the treatment. Briefly, mice were individually placed on a hot plate apparatus with the temperature adjusted to 55 ± 1 °C. Thermal nociception latency was described as the time in seconds passed from the time when the animal was placed on the hot-plate surface until it either licked/shook/lifted its left (experimental) hind paw or jumped onto the plexiglass wall around the metal hot plate surface. A cut-off time was set at 30 s to avoid tissue damage to the animal (Menendez et al. 2002; Gong et al. 2016).

Motor nerve conduction tests

Electrophysiological tests provide a quantitative measure of nerve activity. We carried out electrophysiology tests to assess motor nerve function, myelination and innervation of muscle. Nerve conduction tests are used to evaluate the arrival of degenerated axons to distant nerves and muscles by sizeable myelinated nerve fibres (Navarro and Udina 2009). At 7 days post-surgery and treatment, nerve conduction tests were performed as previously described (Wang et al. 2016). Briefly, mice were provided deep anaesthesia by intraperitoneal injection of xylazine/ketamine. The dorsal sides of the mice's rear feet were shaved and cleaned using

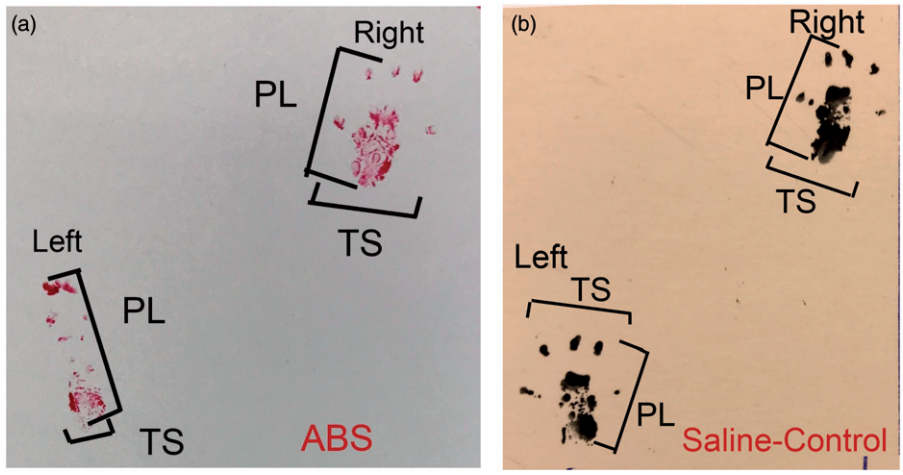

Figure 2. Footprints of the mice treated with ABS (a) and saline (b) on the 7th day after surgery and treatment. Measurements of print length (PL) and toe spread (TS), exemplifying the values used to measure the footprint.

a damp cotton plug. A longitudinal incision was made under sterile conditions in the posterolateral part of both legs; the skin and muscles were separated layer by layer to expose the sciatic nerve fully. So, sciatic nerves on both sides were re-exposed. Sciatic nerves treated (experimental) and untreated (contralateral-healthy) were stimulated (5–10 V, 0.05 ms single square-wave pulses) with a pair of small electrodes, first at the sciatic notch and then at the ankle. Responses were recorded from the plantar muscles using a platinum needle electrode and The BIOPAC hardware, software, connection cables, needle electrodes and conductive gel (Aswar et al. 2014; Morrison et al. 2015). Besides, to record compound muscle action potentials (CMAPs), the recording electrode was placed in the gastrocnemius muscle. The sciatic nerve was stimulated at the level of the sciatic notch. The CMAP peak amplitude was measured. Motor nerve conduction velocity (MNCV) was conducted as previously described (Alvarez et al. 2013). MNCV was calculated by subtracting the ankle distance from the notch distance. The resultant length was then divided by the difference in the ankle and notch latencies for MNCV (Yuan et al. 2010). The amplitude of the CMAP is determined by the number of muscle fibres innervated and it is the most useful indicator for nerve degeneration or regeneration studies. The latency to the CMAP includes the conduction time of the impulse along the nerve and also the transmission time in the neuromuscular junction. MNCV can be calculated only in the nerve segment between two sites of stimulation. The animal rectal temperature was kept at $37 \pm 1.0\,^{\circ}C$ during the measurements. The platinum needle electrodes were cleaned with 70% alcohol between animals.

Wet muscle weight

The muscle atrophy, correlating with denervation, was evaluated with measure wet weight of target muscles. After the behavioural tests and electrophysiological investigation were completed, the mice were euthanized with an overdose of ketamine administered intravenously. The muscles of gastrocnemius on both sides were harvested. Wet muscle weights were measured immediately using a digital scale. The wet muscle weight of the treated (experimental) side was divided into the wet muscle weight of the untreated (healthy) side. Thus, the wet muscle weight ratio was calculated (Nijhuis et al. 2013). The mass of muscles distal to a degenerated and regenerated nerve is considered proportional to the degree of innervation, given that denervated muscles suffer progressive atrophy and, thus, is proposed as a parameter for functional recovery or functional deterioration (Wang et al. 2016).

Statistical analysis

Descriptive statistics for the features studied; Median, Mean, Standard Deviation, Minimum and Maximum values. Mann-Whitney U-test was used to compare the application and control groups. Also, Wilcoxon test was used to compare pre- and post-treatment and control measurements. Statistical significance level was considered as 5% and the SPSS statistical package program was used for all the calculations.

Results

Severe complications, such as anesthesia-related lethalities and postoperative infections, were not observed during procedures on mice.

Sciatic function index (SFI)

The sciatic nerve motor function assessment was performed according to SFI. Before treatment, SFI values in both groups were near zero (Figure 3). Seven days after therapy, while the mean SFI value was calculated to be −12.82 in the control animals, the mean SFI value was calculated as −54.34 in the animals in the ABS group. The results showed that SFI values were significantly decreased in the ABS group compared to the control group on day 7 ($p \leq .05$) (Figure 3).

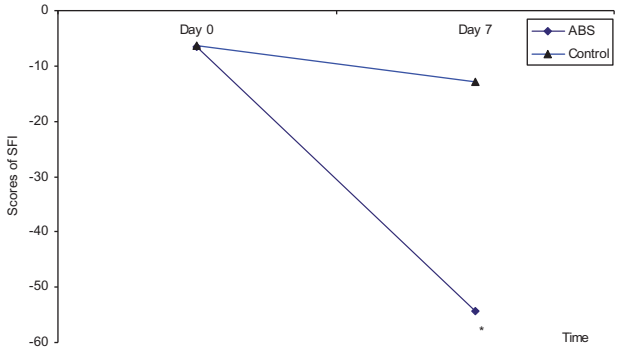

Figure 3. Quantitative analysis of the sciatic function index (SFI). SFI was calculated before treatment (day 0) and on the 7th day following treatment using two different parameters: toe spread (TS) and print length (PL). *$p \leq .05$ indicates a significant difference from the control group of the ABS group.

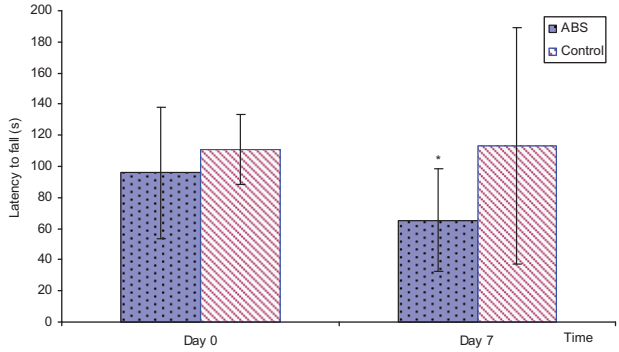

Figure 4. Effect of ABS and saline on the motor performance of mice in the rota-rod test. The sciatic nerves of mice were treated with ABS or saline after the initial rota-rod test. Latency is represented as rota-rod staying time. *$p \leq .05$ indicates a significant difference from the PS group of the ABS group.

Motor co-ordination test (Rota-rod)

We performed a rota-rod test in the control group and ABS group to determine whether ABS affected motor coordination and balance of mice. Before surgery and treatment, there was no significant difference in rota-rod values in both groups. However, 7 days post-treatment, while the mean rota-rod value was 113.36 s in the animals in control group, the mean rota-rod value was 65.45 s in the ABS treated group. These results show that rota-rod values were decreased by 42% in the ABS treated animals compared to the control animals at post-operative day 7. The difference between the groups was statistically significant ($p \leq .05$) (Figure 4).

Heat hyperalgesia (hotplate test)

We carried out a hotplate test to determine whether ABS affected peripheral sensory nerves. This analysis was performed before treatment and at day 7 post-treatment with ABS. Before surgery, the hotplate values were 4.45 s in the control group and 4.29 s in the ABS group. On the 7th day of treatment, the values were 8.45 s in the control group and 26.45 s in the ABS group. These post-treatment results showed that reaction latency to acute thermal stimuli was prolonged significantly in the ABS group compared to the control group ($p \leq .05$) (Figure 5).

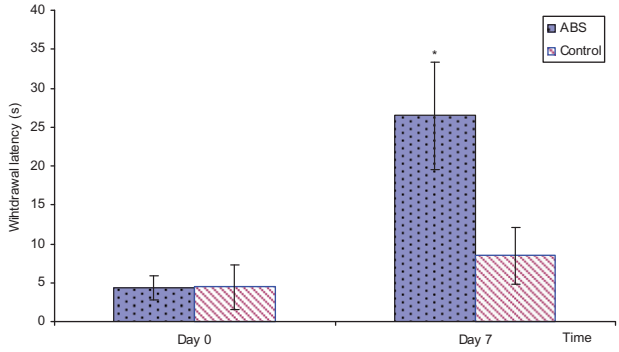

Figure 5. Effect of ABS on thermal hyperalgesia in the hotplate test. The thermal hyperalgesia effect of ABS and saline administration on the sciatic nerve in BALB C mice was tested on the hotplate device (55 °C) before treatment and on the 7th day after treatment. ABS-treated mice were compared with saline-treated mice before treatment (day 0) and 7 days after treatment. The difference between the ABS group and the control group indicates a significance on day 7, but not significant on day 0 (*$p \leq .05$).

Electromyography (EMG)

Axon degeneration, myelin damage and muscle fibre innervation were assessed via analysis of evoked EMG responses in the muscles (Figure 6). Direct muscle (M) responses were measured electrically at day 7 post-treatment. Proximal stimulation (PS) was applied to the notch point of the sciatic nerve. Distal stimulation was applied to tibial branches wrapping around the ankle. The proximal recording was measured from the centre of the gastrocnemius muscle. The distal record was measured from the plantar side of the foot. In the experimental leg; the CMAP peak amplitude was determined to be 9.60 mV in the control group and 5.07 mV in the ABS group (Table 1). The proximal onset latency was measured to be 2.36 ms in the control group and 2.92 ms in the ABS group (Table 1). The MNCV was determined to be 34.83 m/s in the control group and 18.13 m/s in the ABS group (Table 1). ABS caused significant decreases in the CMAP (–47%) and in the MNCV (–52%) groups, causing elongation in the proximal onset latency (+30%).

The difference between the groups was significant ($p \leq .05$). On the other hand, ratios of the CMAP, latency and MNCV of the experimental leg to the healthy leg (intact contralateral) were calculated and compared to the values between the two groups. The ratio of the CMAP was determined to be 0.60 in the control group and 0.94 in the ABS group (Figure 7(A)). The rate of the latency was calculated to be 0.98 in the control group and 1.24 in the ABS group (Figure 7(B)). The ratio of the MNCV was calculated to be 0.53 in the control group and 1.94 in the ABS group (Figure 7(C)). The rates of three parameters showed a statistically significant difference between the groups ($p \leq .05$).

Evaluation of gastrocnemius muscle atrophy

Gastrocnemius muscle weights were also measured to assess the muscle atrophy and their denervation between the experimental and control groups. The ratio of experimental muscle weight to the healthy (intact contralateral) muscle

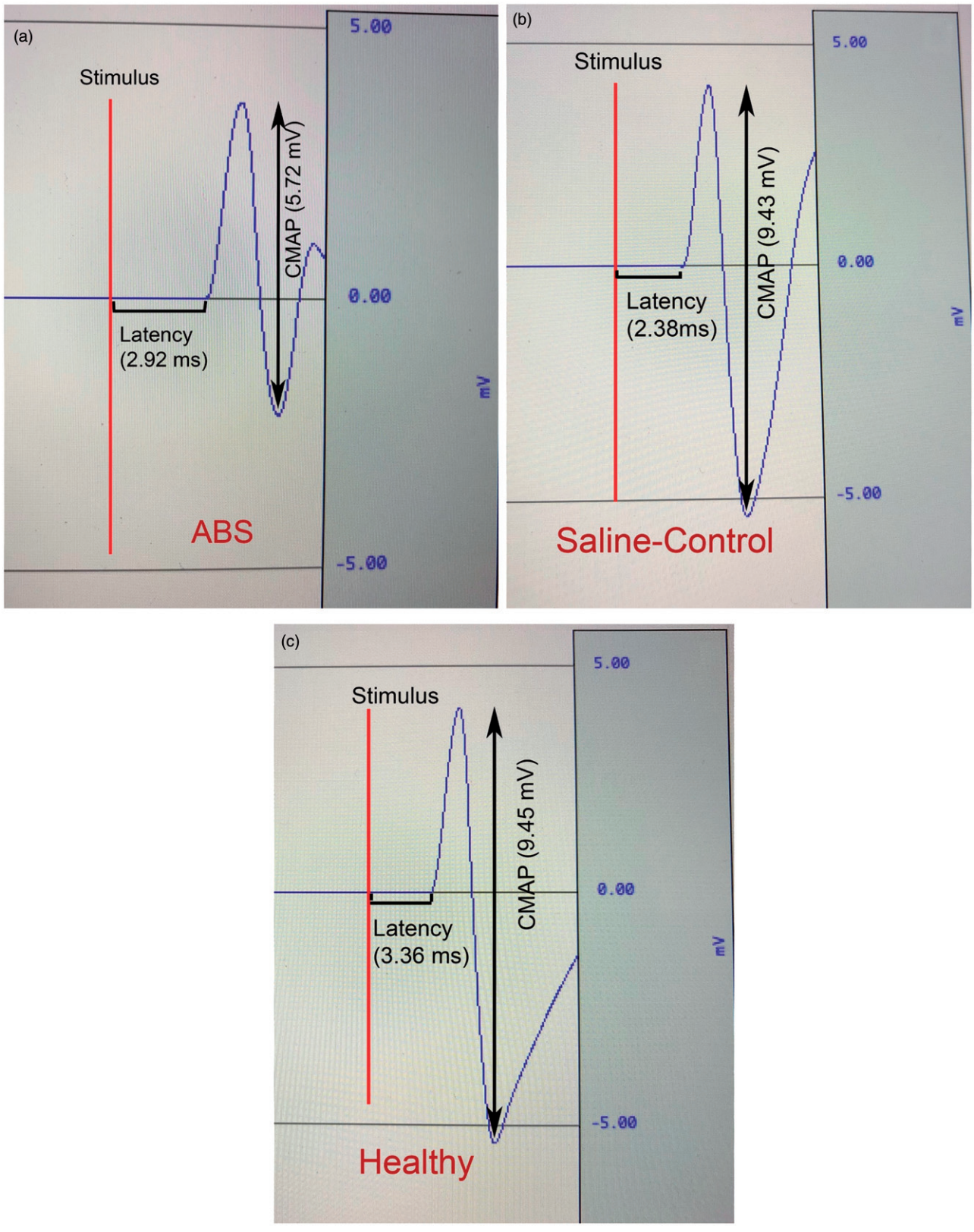

Figure 6. ABS reduced CMAP, extended onset latency. Representative CMAP and latency responses from ABS leg (a), Saline leg (b) and Healthy leg (c).

weight was calculated. The muscle weight ratio was determined to be 0.88 in the control group and 0.79 in the ABS group (Figure 8). The gastrocnemius muscle weight ratio of the ABS group was significantly lower than that of the control group ($p \leq .05$).

Discussion

Haemostasis is a compulsory process to avert blood loss and post-operative bleeding. Achieving haemostasis during the course of neurosurgical procedures presents an important clinical challenge (Gazzeri et al. 2017). Topical haemostatic

Table 1. Electrophysiological data of ABS and control groups.

	ABS group		The saline (control) group	
Groups ($n = 10$)	Experimental	Healthy	Experimental	Healthy
Peak amplitude of CMAP (mV)	5.07* ± 2.44	8.94 ± 1.64	9.60 ± 0.79	9.70 ± 0.88
Onset latency (ms)	2.92* ± 1.02	2.37 ± 0.43	2.36 ± 0.44	2.43 ± 0.16
Nerve conduction velocity (m/s)	18.13* ± 12.82	41.58 ± 12.49	34.83 ± 10.08	37.64 ± 8.34

*Compared with Control group, $p \leq 0.05$. Values are the means ± SEM ($n = 10$). $p \leq 0.05$ indicates significant differences from the control group of the ABS group.

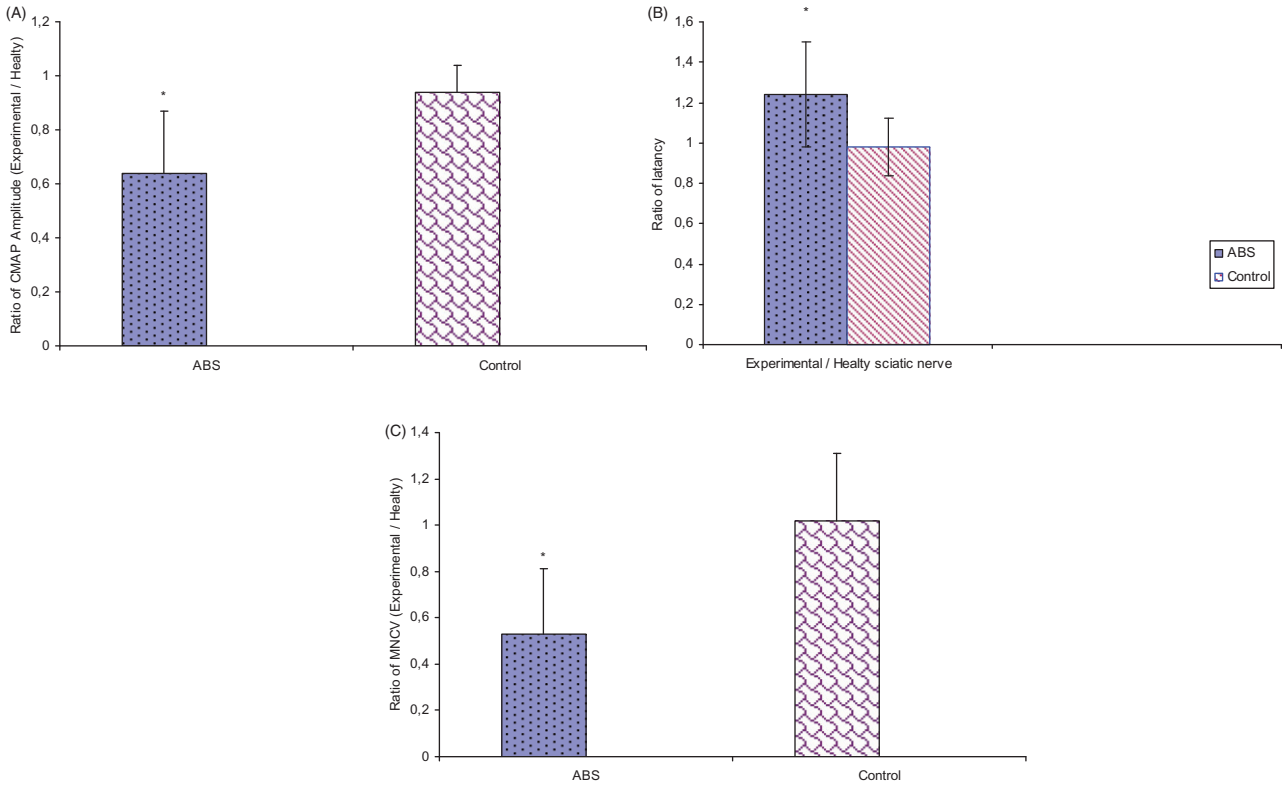

Figure 7. The effects of ABS on electrophysiological parameters in mice. The ratio of CMAP (A), the ratio of latency (B) and the ratio of MNCV (C) were calculated at 7 days after treatment. * $p \leq 0.05$ indicates significant differences from the control group of the ABS group.

agents have long been used in all surgical disciplines. The ideal haemostatic agent should be free of significant cytotoxicity (Vezeau 2016). Although the commercial formulation of ABS claims no adverse effects (Goker et al. 2008), in some cases, neuronal dysfunctions were reported due to ABS applications (Pampu et al. 2013). ABS has been proven to be useful as a topical haemostatic agent on patients in clinical settings, surgeries, and dentistry.

The peripheral nervous system consists of a mixture of sensory, motor and autonomic neurons which are required for the functioning of the limbs and organs (Zhang et al. 2015). The most widely used model for assessment of motor and sensory nerve function is the rat and mouse sciatic nerve model. The primary purpose of this study was to investigate the degenerative effects of mouse peripheral myelinated nerve (sciatic) treated with topical ABS in the mouse sciatic nerve model. The experimental design of this research allowed the functional evaluation of degenerative effects of ABS on the mouse sciatic nerve. We investigated the motor function with the sciatic function index, the motor coordination with the rotarod test, thermal hyperalgesia with the hotplate test, motor axon, myelin and nerve-muscular junction damage with electrophysiological experiments and denervation of target muscles with the muscle wet weight ratio.

The SFI value is a reliable index for examining the motor function of ABS-treated peripheral nerves (Varejão 2003). Following topical ABS or Saline treatment of the sciatic nerve, the analysis of walking tracks, quantified by the sciatic function index, has proven to be a reliable method for assessing functional degeneration. Walking is a coordinated activity involving sensory input, motor response and cortical integration. For this reason, the sciatic function index (walking track analysis) has been used as a comprehensive test (Varejão 2003). At day 7 post-surgery and treatment, our SFI outcomes showed that ABS-treated animals exhibited poorer functional performance compared to the control group. These results indicate the functional decline of lower limb muscles and nerves. The SFI decrease is caused by significant motor nerve damage and loss of sciatic nerve function.

The Rotarod test evaluates locomotion and co-ordination (Nascimento et al. 2011). Animals treated with ABS showed significantly lower (42%) functional performance than control animals. Therefore, it is evident that ABS-treated animals had

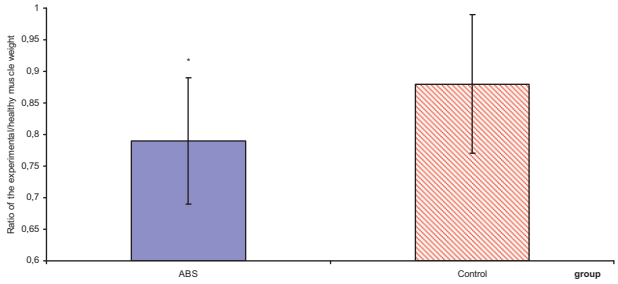

Figure 8. Weight ratios comparison of the muscle of groups. The ratio of the experimental muscle weight to the healthy (contralateral) muscle weight in the gastrocnemius muscles. $^{*}p \leq 0.05$ indicates a significant difference from the control group of the ABS group.

a reduced function in motor co-ordination, balance, motor function and affected motor systems.

To further assess the sensory function of mice, an acute nociceptive response was evoked by a painful heat stimulus, the hotplate assay was carried out. In this study, we measured the nociceptive reactivity to thermal stimuli in mice using the classical hotplate test method. The findings of the hotplate test showed that treatment with ABS prolonged the response latency (313%) to thermal stimuli compared with the control group. The results showed that topical ABS treatment impaired the nociceptive pain sensation (thermal hyperalgesia) by causing sensory nerve damage in the sciatic nerve.

Peripheral nerve function depends on nerve fibre organization (Ivanovic et al. 2012; Gordon et al. 2014). We carried out electrophysiology tests to assess axon, muscle innervation and myelination. The amplitude of the CMAP is directly proportional to the number of nerve fibres innervating the muscle and CMAP values represent a significant functional index of peripheral nerves (Shen et al. 2011). MNCV is directly associated with the functional integrity of the myelin sheath and the nerve–muscle functional conjunction (Aminoff 2004). In mice, myelination of the peripheral nervous system starts at the pre-natal period and it is complete several days post-partum (Ariza et al. 2014). Latency is an indirect parameter, which refers to the maturation of nerve fibres (Wolthers et al. 2005). Axon loss is characterized electromyographically by signs of denervation. Demyelination, in contrast, is manifested by markedly slowed nerve conduction velocities (Aminoff 2004). The results of electrophysiological assessment demonstrated a significant increase in onset latency (prolonged conduction time), a substantial decrease in the CMAP amplitude and a considerable reduction of MNCV in ABS-treated mice compared to the control group. The slower NCV is related to reflected demyelination on nerve fibres, and the lower CMAP amplitude demonstrated the number of active axons and reduced muscle denervation (Dai et al. 2012). These results highlight the degenerative effect of ABS on the healthy peripheral nerves. Pampu et al. (2013) previously reported on rat sciatic nerves with ABS. In the ABS group, 2 mL ABS was soaked in a sterile sponge and then exposed to the nerve region for 3 min. The sponges were taken away from the area, nerve conduction velocity data were assessed at 30-min, 120-min and 3-week post-exposure. A similar process was performed for the control group with sterile saline solution. According to the baseline values, the MNCV values measured at different times increased by 5% in the saline group and decreased by 20% in the ABS group. However, a significant difference between the ABS group and saline group was not found ($p < .05$) (Pampu et al. 2013). In our study, the MNCV value in the ABS group was significantly reduced by 52% compared to the control (saline) group ($p < .05$). In our work, the sciatic nerve was exposed directly to the ABS liquid for 5 min, whereas Pampu et al. (2013) performed their studies using an ABS soaked sponge and exposed the nerves for 3 min. Therefore, in our study, the sciatic nerve exposure to ABS was direct and longer and, thus, had advanced degeneration and functional impairment.

When a muscle is denervated as a result of nerve injury, the shift to degradation tendency leads to decreased muscle cell size, muscle weight loss and hyperplasia of connective tissues (Wang et al. 2008). In our study, muscle mass was measured in both experimental groups 7 days after surgery and treatment. Topical ABS therapy on the sciatic nerve caused a significant decrease in the wet weight of the gastrocnemius muscle when compared to that of control mice. The gastrocnemius muscles were almost atrophied in the ABS group. The conservation of muscle mass is managed by a balance between protein synthesis and protein degradation pathways (Cirillo et al. 2014). Furthermore, these results were also confirmed by the weight ratio of experimental muscles to healthy muscles, indicating indirect evidence of damage (Wallerian degeneration) of end-organ innervation. The electrophysiologic alterations in peripheral nerve degeneration were accompanied by persistent muscle atrophy and muscle innervation loss.

Kaya et al. (2016) previously studied the degenerative effect of ABS on cartilage tissues. Twenty-one albino Sprague Dawley rats were randomly divided into three groups: 0.1 mL of saline was injected in the first group, 0.1 mL of ABS was injected in the second group and 0.1 mL of blood and 0.1 mL of ABS were injected in the third group. A month later, all the rats were sacrificed. Specimens were taken for histopathological evaluations. As a result, ABS had toxic effects on knee cartilage. Evren et al. (2015) investigated the histopathological effects of ABS treatment on rabbit ear cartilage; 0.2 ml ABS ($n = 30$) and 0.2 ml saline ($n = 30$) were subcutaneously syringed into the right auricle and left auricle, respectively. After 14 days, the results indicated that ABS administration, into a covered space, led to a significantly raised fibrosis and necrosis in the ear cartilage. Consistent with these studies, our results support the degenerative effect of ABS on the sciatic nerve. Our study demonstrated for the first time that topical application of ABS to the sciatic nerve led to degeneration in both the myelin sheath and the axons. Sufficient axonal regeneration, reinnervation, myelination and functional recovery, unfortunately, often fail due to complex requirements (Cirillo et al. 2014).

Conclusion

Our studies presented here have demonstrated that ABS exposure resulted in functional impairments in the mice

peripheral myelinated nerves model. The nociceptive pain sensation in the experimental foot is diminished. The motor coordination and the motor functions of mice are weakened. The number of active axons is reduced and the demyelination increased in the sciatic nerve. The denervation and atrophy of the target muscles are increased. The functional decline of ABS treated-motor nerves and lower extremity muscles has occurred. Therefore, the topical ABS administration to areas with peripheral nerves in clinical settings can lead to sensory, motor and autonomic dysfunctions. In this context, there is a need for further experimental and clinical studies to determine ultrastructural and functional effects of ABS on myelinated peripheral nerves.

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