International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.862.871

Research Article
Aesculus hippocastanum Alleviates Diabetic Neuropathy by Reducing MMP-9 and MMP-10 Levels

[1]Edip Gonullu, [2]Gozde Dagistan, [3]Yakup Ozsezer, [4]Mumin Alper Erdogan and [5]Oytun Erbas

[1]Department of Anesthesiology and Reanimation, Faculty of Medicine, İzmir Bakirçay Üniversitesi, 35665 İzmir, Turkey
[2]Department of Anesthesiology and Reanimation, Faculty of Medicine, Akdeniz University, Antalya, Turkey
[3]Biomedical Engineering Program, Department of Electric and Electronic Engineering, Faculty of Engineering and Natural Sciences, Isik University, 34980 İstanbul, Turkey
[4]Department of Physiology, Faculty of Medicine, Izmir Katip Çelebi University, 35620 Izmir, Turkey
[5]Department of Physiology, Faculty of Medicine, Bilim University, 34394 Istanbul, Turkey

Abstract

Background and Objective: Diabetic neuropathy (DN) is a prevalent complication of diabetes, characterized by neuropathic pain and motor dysfunction. The role of oxidative stress and inflammation in DN pathophysiology is well-documented. This study aims to evaluate the therapeutic potential of *Aesculus hippocastanum* (AH) in mitigating DN symptoms, focusing on its antioxidant and anti-inflammatory properties. **Materials and Methods:** The study utilized 40 adult male Wistar rats, divided into four groups: Control, diabetic and two AH-treated groups (receiving 10 mg kg^{-1} and 20 mg kg^{-1} AH, respectively). Diabetes was induced using streptozotocin (STZ) injections. Evaluations included lipid peroxidation (via malondialdehyde levels), matrix metalloproteinases (MMP-9 and MMP-10) levels, electrophysiological records (assessing compound muscle action potential), histopathological examination of the sciatic nerve and motor function tests (using an inclined plane). **Results:** The AH-treated groups exhibited a significant reduction in MDA levels, indicating decreased lipid peroxidation. Plasma MMP-9 and MMP-10 levels were also lower in these groups, suggesting reduced inflammation. Electrophysiological records showed increased CMAP amplitudes and decreased distal latency in AH-treated rats, indicative of improved nerve conduction. Histopathological examination revealed reduced perineural thickness in the sciatic nerve of AH-treated rats, suggesting less fibrosis. In motor function tests, AH-treated rats demonstrated enhanced performance, implying improved muscle strength and motor capacity. **Conclusion:** The findings indicate that AH treatment effectively reduces oxidative stress and inflammation in a rat model of diabetic neuropathy, leading to improved neuropathic symptoms. These results suggest that AH could be a promising therapeutic agent for managing DN, warranting further investigation for its potential clinical application.

Key words: Diabetic neuropathy, *Aesculus hippocastanum*, MMP-9 and MMP-10, oxidative stress, anti-inflammatory treatment, neuropathy management

Citation: Gonullu, E., G. Dagistan, Y. Ozsezer, M.A. Erdogan and O. Erbas, 2023. *Aesculus hippocastanum* alleviates diabetic neuropathy by reducing MMP-9 and MMP-10 levels. Int. J. Pharmacol., 19: 862-871.

Corresponding Author: Mumin Alper Erdogan, Department of Physiology, Faculty of Medicine, Izmir Katip Çelebi University, 35620 Izmir, Turkey
Tel: +905433818677

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes is a disease of high prevalence globally, significantly increasing mortality and morbidity. According to the International Diabetes Federation, there are currently 425 million people worldwide living with diabetes, a number expected to rise to 578 million by 2023[1,2]. Neuropathy is one of the most common and distressing complications of diabetes, contributing substantially to healthcare costs[3,4].

Literature indicates that the prevalence of neuropathy ranges from 1-4%, with 40-55% of neuropathic cases attributed to diabetes[5-8]. The rate of type 2 diabetes stands at 42.2%, which is higher than the 29.1% observed in type 1 diabetes patients[4].

Approximately 75% of diabetic neuropathies (DN) are characterized as distal symmetric polyneuropathy, a condition that progresses slowly and insidiously. This type of neuropathy often leads to neuropathic pain, significantly diminishing patients' quality of life and functional capacity[9].

The primary mechanisms causing DN are inflammation and oxidative stress due to hyperglycemia. Regular monitoring of blood pressure can help reduce these factors, thereby decreasing the risk of diabetes-related neuropathy.

Aesculus hippocastanum (AH) contains a compound known as aescin, which includes alpha and beta triterpene saponins, distinguished by their water solubility and melting points. Additionally, AH comprises bioflavonoids (quercetin and kaempferol), proanthocyanidin A2 and coumarins (fraxin and aesculin). The antiedematous, anti-inflammatory, venoprotective and venotonic effects of aescin are primarily due to its triterpene saponin content[10,11]. Proanthocyanidin A2, also found in AH, is believed to possess antioxidant properties[12].

The AH has demonstrated antioxidant capabilities, inhibiting free radicals, lipid peroxidation and lysosomal enzymes[12]. Moreover, AH has shown beneficial effects on complications associated with diabetes, such as nephropathy and cardiac autonomic neuropathy, due to its anti-inflammatory properties[10,11]. This study aims to investigate the impact of AH on diabetic neuropathy.

MATERIALS AND METHODS

Study area: The study was carried out at the Experimental Animals Application and Research Center of Demiroglu Bilim Üniversitesi, located in Istanbul, Turkey. The research spanned a period from September, 2022 to January, 2023.

Animals: About 40 adult male Wistar rats, weighing 200-210 g, were obtained from the Demiroğlu Science University Experimental Animal Research Center for the study. Animals were housed in cages and maintained under standard conditions with 12 hrs light/dark cycles at room temperature (22±2°C). They were fed by standard pellet diet and tap water *ad libitum* throughout the study. The protocol employed in the study has been approved by the Institutional Animal Care and Ethical Committee of the University of Demiroğlu Science University (Ethical Number: 1623030906). All chemicals have been obtained from Sigma-Aldrich Inc. unless otherwise noted.

Experimental protocol: In the study, diabetes mellitus (DM) was induced in 24 out of 40 Wistar rats. This was achieved through an intraperitoneal injection of a single dose of streptozotocin (STZ) from Sigma-Aldrich, Inc., Saint Louis, Missouri, USA, at a concentration of 60 mg kg^{-1} in 0.9% NaCl, adjusted to a pH of 4.0 using 0.2 M sodium citrate. In contrast, 10 rats were designated as the control group, receiving no chemical treatment and maintaining normal blood glucose levels below 120 mg dL^{-1}. The DM induction was confirmed after 24 hrs by measuring blood glucose levels, which were above 250 mg dL^{-1} in diabetic rats, using glucose oxidase reagent strips from Boehringer Mannheim, Indianapolis.

Subsequently, the diabetic rats were segregated into three groups for further treatment. The first group, comprising 10 rats, was termed the 'diabetes and saline group' and received a 1 mL kg^{-1} saline treatment. The second group, also consisting of 10 rats, was labeled the 'diabetes and *Aesculus hippocastanum* (AH) extract-treated group' and was administered AH at a dose of 10 mg/kg/day through oral gavage using Venotrex (50 mg, Abdi Ibrahim). The third group, identical in number, received a higher dose of AH at 20 mg/kg/day via oral gavage for a duration of 4 weeks. The study concluded with Electromyography (EMG) and inclined plane testing. Afterward, the animals were humanely euthanized, with blood samples collected via cardiac puncture for biochemical analysis. Additionally, the sciatic nerve was extracted for histopathological examination.

This experimental procedure is adapted and modified from the protocol described in the previously published article[13].

Three Electromyography (EMG) recordings were made from the right sciatic nerve of each subject. For stimulation, a high intensity of 10 V was used, with a brief duration of 0.05 ms and a frequency of 1 Hz, covering a range from 0.5 to 5000 Hz. The EMG signals were sampled at a rate of 40 kHz/sec. This process was facilitated using a Biopac bipolar

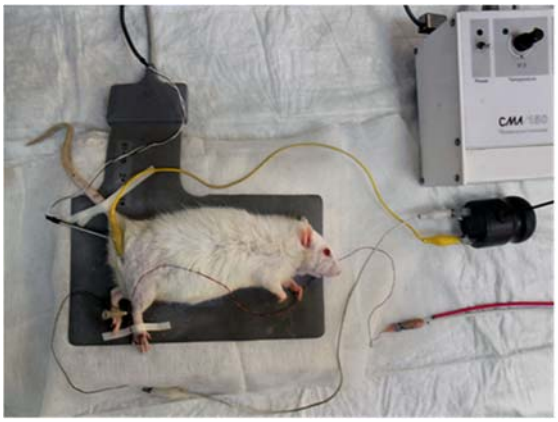

Fig. 1: EMG recording system

Fig. 2: Inclined plane test system

subcutaneous needle stimulation electrode, a product of BIOPAC Systems, Inc., based in Santa Barbara, California, USA. The point of stimulation was the Achilles tendon[14] (Fig. 1). Compound muscle action potentials (CMAPs) and changes in motor Nerve Conduction Velocity (NCV) were recorded by unipolar needle electrodes located in the 2-3 interosseous muscle. Data were evaluated using Biopac Student Lab Pro version 3.6.7 software (BIOPAC Systems, Inc.), with distal latency, duration and amplitude of CMAP as the parameters. During the EMG recordings, rectal temperatures of the rats were monitored by a rectal probe (HP Viridia 24-C; Hewlett-Packard Company, Palo Alto, California, USA) and the temperature of each rat was kept at approximately 36-37°C by heating pad.

Histopathological examination of the sciatic nerve: Sections of the sciatic nerve, which were fixed in formalin and cut into 4-micrometer slices, underwent staining using Hematoxylin and Eosin (H&E). Perineural thickness of the sciatic nerve was measured with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope.

Inclined plane test: Motor performance in rats was assessed using a modified version of the sliding apparatus, as previously described by Rivlin and Tator and further modified by Yilmaz *et al.*[15]. This evaluation was conducted one month following the induction of STZ. The apparatus consisted of a stainless steel plane measuring 50 cm by 30 cm, as shown in Fig. 2. We determined the maximum angle at which a rat's limb began to slip while attempting to maintain its body position. This test was replicated three times for each orientation of the rat's head, with the results being averaged. A one-minute break was allowed between each trial[15].

Evaluation of lipid peroxidation: Lipid peroxidation was determined in plasma samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substance (TBARS). Briefly, trichloroacetic acid and TBARS reagent were added to the plasma samples, then mixed and incubated at 100°C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. The MDA levels were expressed as nM and tetraethoxypropane was used for calibration.

Measurement of plasma MMP-9 and MMP-10

Statistical analysis: Data analyses were performed using SPSS version 15.0 for Windows. The groups of parametric variables were compared by Student's t-test and Analysis of Variance (ANOVA). The groups of nonparametric variables were compared by the Whitney U test. Results were given as Mean±Standard error of mean (SEM). A value of $p<0.05$ was accepted as statistically significant. The $p<0.001$ was accepted as statistically highly significant.

RESULTS

Evaluation of lipid peroxidation: In the evaluation of lipid peroxidation, the levels of malondialdehyde (MDA) were measured. The control group exhibited MDA levels of 53.6±2.5 nM, while the group with diabetes receiving saline showed significantly higher levels at 195.3±10.1 nM. In comparison, diabetic rats treated with 10 mg kg^{-1} of *Aesculus hippocastanum* (AH) had MDA levels of 94.2±7.8 nM and those treated with 20 mg kg^{-1} AH had levels of 90.6±4.5 nM. These findings indicate that MDA levels in diabetic rats were significantly reduced upon AH treatment compared to those not receiving AH (Table 1).

Furthermore, Matrix Metalloproteinase-9 (MMP-9) levels were assessed. In the control group, MMP-9 levels were found

to be 31.5±1.7 pg mL^{-1}. Diabetic rats receiving saline had higher levels at 44.9±1.4 pg mL^{-1}. Notably, diabetic rats treated with 10 mg kg^{-1} AH showed a decrease in MMP-9 levels to 36.2±0.6 pg mL^{-1} and those treated with 20 mg kg^{-1} AH had levels of 34.4±0.2 pg mL^{-1}. These results demonstrate that both MMP-9 and MMP-10 levels were elevated in diabetic rats compared to the control group, but significantly decreased in those receiving AH treatment (Table 1).

Evaluation of electrophysiological records: The evaluation of electrophysiological records focused on the compound muscle action potential (CMAP) and distal latency measurements. In the control group, CMAP was recorded at 14.2±0.9 mV (Fig. 3a). For the diabetic group receiving saline, the CMAP was significantly lower, at 7.4±0.7 mV (Fig. 3b). Diabetic rats treated with 10 mg kg^{-1} of *Aesculus hippocastanum* (AH) showed an improved CMAP of

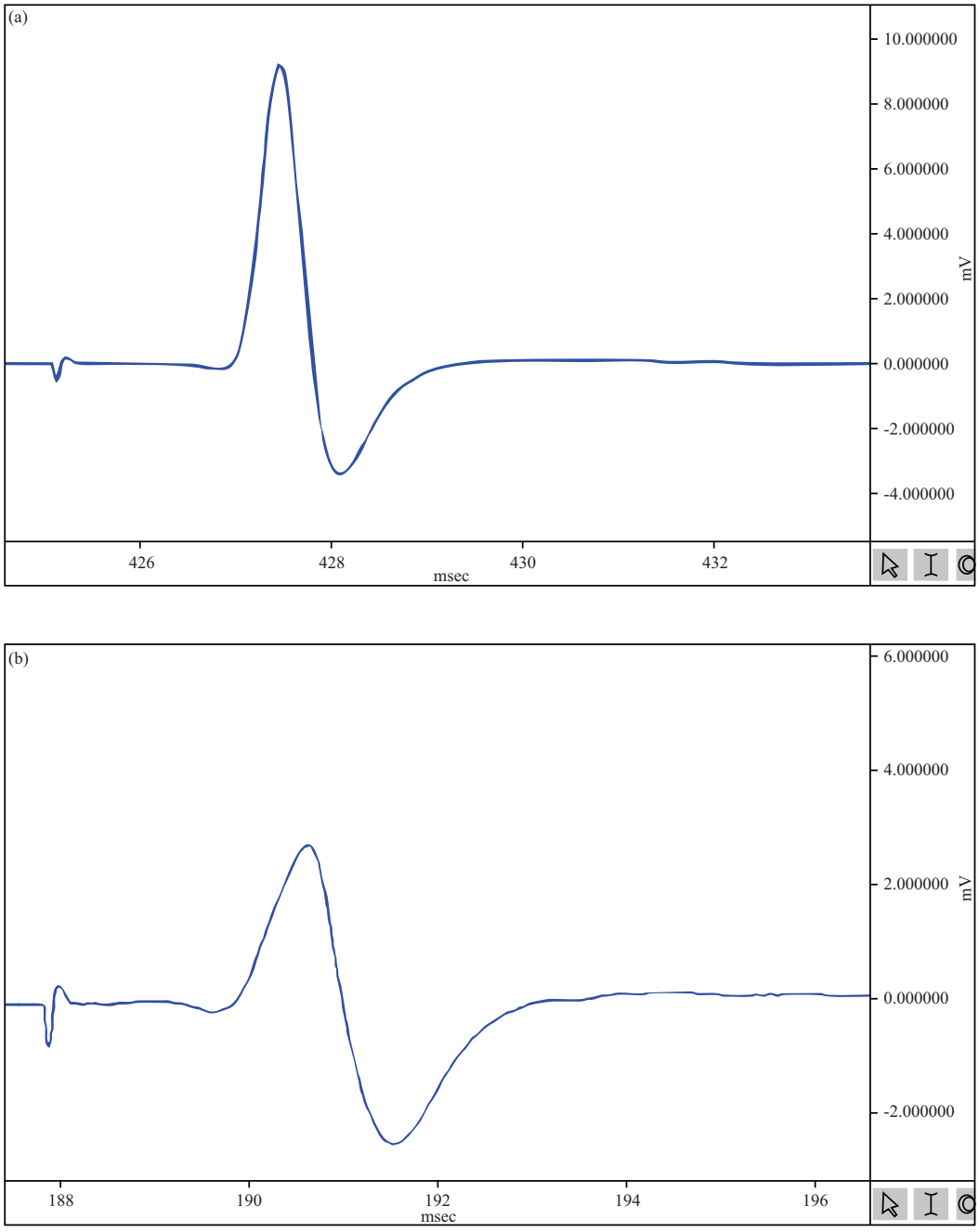

Fig. 3(a-d): Continue

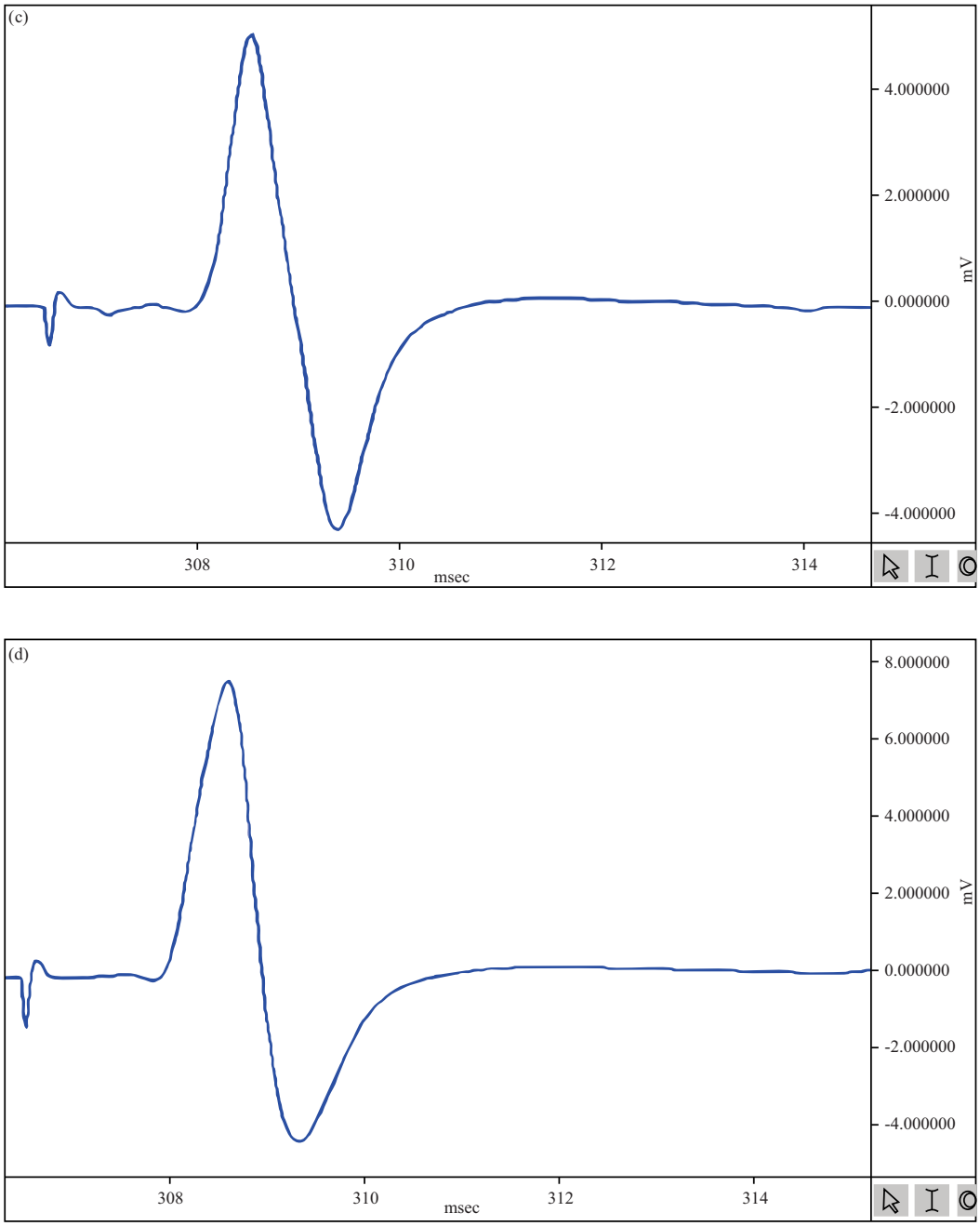

Fig. 3(a-d): (a) EMG of control group, (b) EMG of diabetic rats with saline treatment, (c) EMG of diabetic rats treated with 10 mg kg^{-1} AH and (d) EMG of diabetic rats treated with 20 mg kg^{-1} AH

Table 1: Comparative analysis of different treatment groups in a diabetic rat model

	Control group	Diabetes and saline treatment	Diabetes and 10 mg kg^{-1} AH treatment	Diabetes and 20 mg kg^{-1} AH treatment
Perineural thickness (μm)	4.1±0.2	17.2±4.6*	8.3±1.9$^{\#}$	7.7±1.1$^{\#\#}$
Plasma MMP-9 (pg mL^{-1})	31.5±1.7	44.9±1.4*	36.2±0.6$^{\#}$	34.4±0.2$^{\#}$
Plasma MMP-10 (pg mL^{-1})	48.3±2.08	67.5±1.3*	56.1±2.2$^{\#}$	53.7±0.8$^{\#}$
Plasma MDA (nM)	53.6±2.5	195.3±10.1**	94.2±7.8$^{\#\#}$	90.6±4.5$^{\#\#}$

Data are expressed as mean±SEM, *p<0.01 (different from control), **p<0.001, $^{\#}$p<0.05 and $^{\#\#}$p<0.01 (different from diabetes+saline)

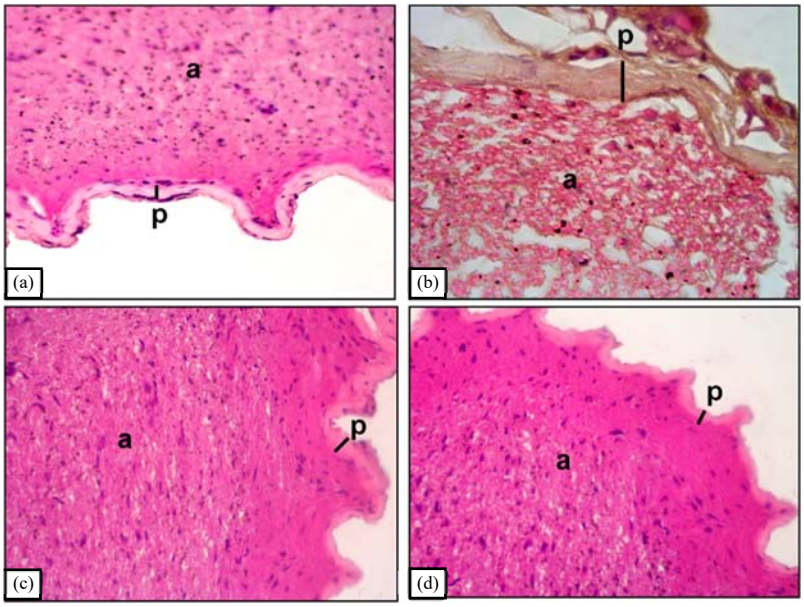

Fig. 4(a-d): Histological sections of sciatic nerve, H&E staining (×40 magnification), (a) Control group, (b) DM and saline treated group was shown increased perineural thickness, (c) DM and 10 mg kg^{-1} AH group was shown decreased perineural thickness and (d) DM and 20 mg kg^{-1} AH group was shown decreased perineural thickness
p: Perineurium and perineural thickness and a: Axon

Table 2: Impact of AH treatment on CMAP amplitude and distal latency in diabetic rats

	Control group	Diabetes and saline treatment	Diabetes and 10 mg kg^{-1} AH treatment	Diabetes and 20 mg kg^{-1} AH treatment
CMAP amplitude (mV)	14.2±0.9	7.4±0.7*	11.5±0.4$^{\#}$	13.5±0.6$^{\#\#}$
Distal latency (ms)	1.33±0.08	1.91±0.3*	1.58±0.1$^{\#}$	1.51±0.2$^{\#}$

Data are expressed as mean±SEM, *p<0.05 (different from control), $^{\#}$p<0.05 and $^{\#\#}$p<0.01 (different from diabetes+saline)

11.5±0.4 mV (Fig. 3c), while those receiving 20 mg kg^{-1} AH exhibited further improvement, with a CMAP of 13.5±0.6 mV (Fig. 3d, Table 2).

Regarding distal latency, the control group showed a latency of 1.33±0.08 ms (Fig. 3a). This was prolonged in the diabetic group receiving saline, with a latency of 1.91±0.3 ms (Fig. 3b). In the diabetic groups treated with AH, there was a notable reduction in distal latency: 1.58±0.1 ms for the 10 mg kg^{-1} AH group (Fig. 3c) and 1.51±0.2 ms for the 20 mg kg^{-1} AH group (Fig. 3d, Table 2).

A comparison between the control group and the diabetic group not receiving AH revealed significant differences in sensory nerve conduction studies. Specifically, the diabetic rats not treated with AH had a considerably lower CMAP amplitude and prolonged distal latency in the sciatic nerve. In contrast, diabetic rats treated with AH demonstrated a significant increase in CMAP amplitudes. Notably, the increase in amplitude was more significant in the group receiving 20 mg kg^{-1} AH compared to the group receiving 10 mg kg^{-1} AH. Additionally, AH treatment was associated with shorter distal latencies in diabetic rats compared to those not receiving AH treatment (Fig. 3, Table 2).

Histological evaluation of the sciatic nerve: The histological examination focused on measuring the perineural thickness of the sciatic nerve. In the control group, the perineural thickness was found to be 4.1±0.2 µm (Fig. 4a). In contrast, diabetic rats receiving saline exhibited a significantly increased perineural thickness of 17.2±4.6 µm (Fig. 4b). Diabetic rats treated with 10 mg kg^{-1} of *Aesculus hippocastanum* (AH) showed a reduced perineural thickness of 8.3±1.9 µm (Fig. 4c), while those treated with 20 mg kg^{-1} AH demonstrated a further reduction to 7.7±1.1 µm (Fig. 4d, Table 1).

The histological sections revealed that the perineural thickness in the sciatic nerves of diabetic rats receiving saline was markedly increased compared to the control group. Notably, the decrease in perineural thickness was more significant in rats receiving 20 mg kg^{-1} AH compared to those receiving 10 mg kg^{-1} AH (Fig. 4, Table 1).

Table 3: Inclined plane performance and plasma glucose levels in diabetic rats under different treatments

	Control group	Diabetes and saline treatment	Diabetes and 10 mg kg^{-1} AH treatment	Diabetes and 20 mg kg^{-1} AH treatment
Maximum angle of inclined plane test (degree)	87.2±4.2	57.1±6.5*	74.3±5.3#	78.8±4.6#
Plasma glucose (mg dL^{-1})	94.2±7.8	442.5±18.8*	389.2±14.5	378.3±9.4#

Data are expressed as mean±SEM, *p<0.001 (different from control) and #p<0.05 (different from diabetes+saline)

Inclined plane test: The inclined plane test was conducted to assess motor function, measuring the maximum angle at which the rats could maintain their position without slipping. In the control group, the maximum angle achieved was 87.2±4.2°. For the group with diabetes receiving saline, the angle was significantly lower at 57.1±6.5°. Rats with diabetes treated with 10 mg kg^{-1} of *Aesculus hippocastanum* (AH) managed a higher angle of 74.3±5.3° and those treated with 20 mg kg^{-1} AH showed further improvement, achieving an angle of 78.8±4.6° (Table 3).

The results indicate that the angle at which the diabetic rats receiving saline began to slide was markedly lower compared to the control group, suggesting reduced motor capability.

Plasma glucose level: The study measured plasma glucose levels across different groups. In the control group, the glucose level was found to be 94.2±7.8 mg dL^{-1}. By contrast, diabetic rats receiving saline showed significantly elevated glucose levels at 442.5±18.8 mg dL^{-1}. Diabetic rats treated with 10 mg kg^{-1} of *Aesculus hippocastanum* (AH) exhibited a glucose level of 389.2±14.5 mg dL^{-1}, while those treated with 20 mg kg^{-1} AH had a reduced level of 378.3±9.4 mg dL^{-1} (Table 3).

The findings indicate that blood glucose levels in diabetic rats receiving saline were substantially higher than those in the control group. Although there was no significant difference between the glucose levels of diabetic rats treated with 10 mg kg^{-1} AH and those receiving saline, the group receiving 20 mg kg^{-1} AH displayed a significantly lower glucose level compared to the diabetic rats receiving saline.

DISCUSSION

The DN is such a heterogeneous disease whose mechanism is not fully known, it has a complex pathophysiology and affects both autonomic and somatic components of the nervous system[4].

The most significant mechanism emphasized in the pathophysiology of DN; Chronic hyperglycemia which causes an increase in free radical formation in the body and endogenous antioxidants are insufficient to balance toxic reactive oxygen products. Due to oxidative damage, the glycosylation products on the myelin sheath and nerve cell biomolecules increase and there has been occurred neuropathy findings[16].

In addition, in hyperglycemia, they carry the lipid molecules which became prone to oxidation due to glycosylation of plasma lipoproteins. The increase in plasma oxidized LDL levels of diabetic patients confirms this outcome situation. Lipid peroxides have been shown to increase endothelial damage and stimulate the development of vasculitis. The vasculitis accompanying DN negatively affects nerve blood flow, which triggers neuropathy. In the study which has been conducted by Sasaki *et al.*[17] in diabetic rats, it has been shown that sciatic nerve blood flow decreased. The decrease in the blood flow in peripheral nerves can lead to neuronal ischemia and therefore oxidative stress, which can stimulate the development of sensory neuropathy[12].

It has been reported that the lipid peroxidation end product malondialdehyde (MDA), which one of the most significant markers of oxidant, stress, increases in rats[11]. In the animal studies from the literature, the decrease in MAD, is an indicator of oxidative stress and an increase in the amounts of reduced glutathione, superoxide dismutase and catalase, which are known to have antioxidant activities that were observed in rats receiving AH[10,11,18].

In a different study, it has been reported that aescin has significantly increased apoptosis, which has a significant role in the clearance of damaged cells[19]. Considering the role of oxidative stress and apoptosis in inflammation, the antioxidant and apoptotic effects of aescin bring value[12].

The current research study also supports the above information; We have observed that MDS levels in diabetic rats have been significantly higher than in the control group. The MDA level in diabetic rats which were given AH, was significantly lower than in diabetic rats which were not given AH.

Moreover, due to oxidative stress, it is known that several molecular processes such as activation of the polyol pathway, activation of protein kinase C and advanced glycation end product formation are associated with functional disorders in the nerves and pathological changes in the neurons. Distal

symmetrical depletion has been observed in the peripheral end terminals of nociceptors and intraepidermal nerve fibers. More proximally, some changes have occurred in peripheral nerves, such as demyelination of myelinated nerve fibers, axonal degeneration, necrosis, schwannopathy and microangiopathy[12].

Nerve conduction abnormalities are present in 29-70% of the patients with type-1 diabetes and 45-60% of the patients with type-2 diabetes at the of diagnosis and electromyographic improvement has been reported after the treatment for hyperglycemia[20,21].

The DN is an axonal disease and manifests itself with reduced amplitude in nerve conduction studies. Slowing of nerve conduction velocity and prolongation of distal latency can be considered indicators of demyelination[22].

In this research study, when the control group and the diabetic rat group that did not receive AD, were compared in terms of sensory nerve conduction studies, the CMAP amplitude of the sciatic nerve was significantly lower and the distal latency was obtained to be prolonged in the diabetic rat group that did not receive AD. When the diabetic rat group that did not receive AH was compared with the groups that received AH, a significant increase was obtained in the CMAP amplitudes of the groups that received AH. The amplitude increase rate in the group receiving 20 mg kg^{-1} AH was significantly higher than in the group receiving 10 mg kg^{-1}. It has been observed that the distal latency was shorter in diabetic rats receiving AH compared to the group not receiving AH.

It has been observed that AH has provided significant improvement in the conduction abnormalities such as the decrease in CMAP amplitude and an increase in distal latency caused by diabetic neuropathy of DN. The increase in CAMP amplitude suggested that the number of stimulated muscle units and the number of stimulation axons have increased. Current findings may be explained by increased axonal regeneration and remyelination in neurons.

It has also been reported that escin has positive effects on neuropathic pain through the mechanism of reducing the concentration of inflammatory cytokines in neuropathic pain due to sciatic nerve damage, suppressing the signal pathway of toll-like receptor 4 and nuclear factor KB and reducing the levels of glial fibrillary acidic protein and nerve growth factor[23].

The inclined plane test, used to measure the muscle strength and motor capacity of rats, is widely used. This test is the inclination of the plane on which the rat stands is gradually increased and the angle of inclination at which the rat slide is objectively evaluated in rats with neuropathy[24].

Although the majority of neural deficits have been observed in diabetes which are sensory deficits and significant motor dysfunction that has been also reported as 1-6%[25]. Atrophy of the extensor digitorum brevis (EDB) muscle is the earliest motor out finding. Involvement of motor fiber axons may cause changes in foot posture and morphology, foot ulcers and may result in amputation[26,27]. In our research study, the angle of inclination in which diabetic rats slid was much lower than the control group. A significant effect was observed in the motor functions of diabetic rats. The angle of inclination at which rats receiving AH shifted was found to be significantly higher than diabetic rats. These data reveal that there was a significant improvement in the motor functions of rats receiving AH.

Matrix metalloproteinases (MMPs) are involved in the inflammation and remodeling process in the tissue. They regulate the degradation of extracellular matrix proteins, cytokines and chemokines in both neurons and glial cells in the nervous system. The MMPs are involved in the regulation neuroinflammatory process in response to peripheral and central injuries. It has been shown that after nerve root injury in rats, MMPs cause an increase in the release of pro-inflammatory cytokines and cause myelin basic protein destruction and demyelination. The MMP-9 contributes to neurological disorders by disrupting neurovascular barriers, enabling migration of immune cells and demyelination[28]. The MMP-9 has also been shown to play a role in the development of neuropathic pain after peripheral nerve injury. One study showed that MMP-9 plays a critical role in DN and contributes to myelin abnormalities and central spinal sensitization-induced DN. It has been observed that allodynia is also suppressed by inhibition of MMP-9[29].

In our research study, MMP-9 and -10 levels in diabetic rats were higher than in the control group. The MMP-9 and 10 have been obtained to be lower in diabetic rats who were receiving AH treatment than in diabetic rats who were not receiving AH. This outcome will contribute positively to preventing the development of DN, as inflammation and demyelination will decrease with the decrease in enzyme levels.

The main responsible for DN are several reactions initiated by hyperglycemia. Literature studies have shown the hypoglycemic effect of aescin[30]. It has been reported that aescin increases the insulin level in the blood and some saponin-containing plants increase insulin secretion from the pancreas and glucose utilization by tissues[31].

In our research study, the blood sugar level of diabetic rats was found to be much higher than the control group. Although there was no significant difference between the

10 mg kg^{-1} AH and the blood sugar levels of rats that were not receiving AH, a significant decrease has been detected in the blood sugar levels of the rats which were receiving 20 mg kg^{-1} AH. However, in a study comparing the blood sugar levels of diabetic rats that did not receive any treatment and diabetic rats that did not receive any treatment and diabetic rats that received 50 mg/kg/day aescin, contrary to our findings, the blood sugar levels of the group receiving AH is higher than the diabetic rats that did not receive any treatment[10].

In the histopathological examination of the nerves of the rats with diabetic neuropathy, axon atrophy and degeneration, myelin degeneration and looseness and increased perineural thickness due to perineural fibrosis are observed[32]. In this study, in the histopathological examination of the sciatic nerve, perineural thickness has been obtained to significantly increased in diabetic rats which did not receive the treatment compared to the control group. Perineural thickness has been obtained to be decreased in rats receiving AH treatment. The decrease in perineural thickness of rats receiving 20 mg kg^{-1} AH was significantly greater than the rats which were receiving 10 mg kg^{-1} AH. This can be explained by the fact that AH reduces fibrosis by reducing inflammation and the resulting increase in perineural thickness is less in rats which were receiving AH.

CONCLUSION

This research establishes that *Aesculus hippocastanum* (AH) significantly alleviates diabetic neuropathy symptoms in rats. Treatment with AH improved nerve conduction, evidenced by higher CMAP amplitudes and shorter distal latencies. Histologically, AH reduced perineural thickness, suggesting decreased nerve fibrosis. Biochemically, AH treatment corresponded with lowered levels of oxidative stress markers, particularly MDA, indicating its antioxidant capacity. Additionally, reductions in MMP-9 and MMP-10 levels highlight AH's anti-inflammatory effects. These findings support the potential of AH as a promising therapeutic agent for diabetic neuropathy, warranting further investigation in clinical applications.

SIGNIFICANCE STATEMENT

The escalating global prevalence of diabetes highlights the urgency of addressing its complications, notably diabetic neuropathy (DN), which significantly impairs quality of life due to neuropathic pain and motor dysfunction. This study is pivotal in exploring the therapeutic potential of *Aesculus hippocastanum* (AH) against DN, focusing on its antioxidant and anti-inflammatory properties. By methodically investigating the effects of AH on oxidative stress markers and inflammatory mediators in a controlled animal model, our research sheds light on AH's role in reducing MMP-9 and MMP-10 levels, thereby alleviating DN symptoms. The findings offer promising insights for clinical application, potentially enhancing the management and treatment strategies for DN and paving the way for future human clinical trials.

ACKNOWLEDGMENT

The Authors would like to thank Scribendi for the English language editing during the preparation of this manuscript.

REFERENCES

1. Saeedi, P., I. Petersohn, P. Salpea, B. Malanda and S. Karuranga *et al*., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9th edition. Diabetes Res. Clin. Pract., Vol. 157. 10.1016/j.diabres.2019.107843.
2. Sun, H., P. Saeedi, S. Karuranga, M. Pinkepank and K. Ogurtsova *et al*., 2022. IDF diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res. Clin. Pract., Vol. 183. 10.1016/j.diabres.2021.109119.
3. Robinson, C.C., R.P.G. Barreto and R.D.M. Plentz, 2018. Effects of whole body vibration in individuals with diabetic peripheral neuropathy: A systematic review. J. Musculoskeletal Neuronal Interact., 18: 382-388.
4. Pfannkuche, A., A. Alhajjar, A. Ming, I. Walter, C. Piehler and P.R. Mertens, 2020. Prevalence and risk factors of diabetic peripheral neuropathy in a diabetics cohort: Register initiative "diabetes and nerves". Endocr. Metab. Sci., Vol. 1. 10.1016/j.endmts.2020.100053.
5. do Nascimento, O.J.M., C.C.B. Pupe and E.B.U. Cavalcanti, 2016. Diabetic neuropathy. Rev Dor. São Paulo, 17: S46-S51.
6. Katulanda, P., P. Ranasinghe, R. Jayawardena, G.R. Constantine, M.H.R. Sheriff and D.R. Matthews, 2012. The prevalence, patterns and predictors of diabetic peripheral neuropathy in a developing country. Diabetology Metab. Syndr., Vol. 4. 10.1186/1758-5996-4-21.
7. Bharucha, N.E., A.E. Bharucha and E.P. Bharucha, 1991. Prevalence of peripheral neuropathy in the Parsi community of Bombay. Neurology, 41: 1315-1315.
8. Callaghan, B.C., K.A. Kerber, L.L. Lisabeth, L.B. Morgenstern and R. Longoria *et al*., 2014. Role of neurologists and diagnostic tests on the management of distal symmetric polyneuropathy. JAMA Neurol., 71: 1143-1149.

9. Weng, Y.C., S.S. Tsai, R.K. Lyu, C.C. Chu and L.S. Ro *et al*., 2020. Diabetic distal symmetrical polyneuropathy: Correlation of clinical, laboratory, and electrophysiologic studies in patients with type 2 diabetes mellitus. J. Diabetes Res., Vol. 2020. 10.1155/2020/6356459.
10. Elmas, O., O. Erbas and G. Yigitturk, 2016. The efficacy of *Aesculus hippocastanum* seeds on diabetic nephropathy in a streptozotocin-induced diabetic rat model. Biomed. Pharmacother., 83: 392-396.
11. Suryavanshi, S.V. and Y.A. Kulkarni, 2021. Attenuation of cardiac autonomic neuropathy by escin in diabetic rats. Pharmacology, 106: 211-217.
12. Ezberci, F. and E. Ünal, 2018. *Aesculus hippocastanum* (aescin, horse chestnut) in the management of hemorrhoidal disease: Review. Turk. J. Colorectal. Dis., 28: 54-57.
13. Erbaş, O., F. Oltulu, M. Yılmaz, A. Yavaşoğlu and D. Taşkıran, 2016. Neuroprotective effects of chronic administration of levetiracetam in a rat model of diabetic neuropathy. Diabetes Res. Clin. Pract., 114: 106-116.
14. Pala, E.E., H.G. Pala, S. Ekmekci and O. Erbas, 2022. Vitamin C (ascorbic acid) protects from neuropathy caused by cisplatin, through enhanced heat shock protein-70 and reduced oxidant effect. Rev. Assoc. Med. Bras., 68: 1017-1022.
15. Yilmaz, M., H. Aktug, F. Oltulu and O. Erbas, 2016. Neuroprotective effects of folic acid on experimental diabetic peripheral neuropathy. Toxicol. Ind. Health, 32: 832-840.
16. Babizhayev, M.A., I.A. Strokov, V.V. Nosikov, E.L. Savel'yeva, V.F. Sitnikov, Y.E. Yegorov and V.Z. Lankin, 2015. The role of oxidative stress in diabetic neuropathy: Generation of free radical species in the glycation reaction and gene polymorphisms encoding antioxidant enzymes to genetic susceptibility to diabetic neuropathy in population of type I diabetic patients. Cell Biochem. Biophys., 71: 1425-1443.
17. Sasaki, H., J.D. Schmelzer, P.J. Zollman and P.A. Low, 2002. Neuropathology and blood flow of nerve, spinal roots and dorsal root ganglia in longstanding diabetic rats. Acta Neuropathol., 93: 118-128.
18. Küçükkurt, I., S. Ince, H. Keleş, E.K. Akkol, G. Avcı, E. Yeşilada and E. Bacak, 2010. Beneficial effects of *Aesculus hippocastanum* L. seed extract on the body's own antioxidant defense system on subacute administration. J. Ethnopharmacol., 129: 18-22.
19. Güney, G., H.M. Kutlu and A. İşcan, 2013. The apoptotic effects of escin in the H-*Ras* transformed 5RP7 cell line. Phytother. Res., 27: 900-905.
20. Young, R.J., D.J. Ewing and B.F. Clarke, 1983. A controlled trial of sorbinil, an aldose reductase inhibitor, in chronic painful diabetic neuropathy. Diabetes, 32: 938-942.
21. Akbar, D.H., S.A. Mira, T.H. Zawawi and H.M. Malibary, 2000. Subclinical diabetic neuropathy. A common complication in Saudi diabetics. Saudi Med. J., 21: 433-437.
22. Partanen, J., L. Niskanen, J. Lehtinen, E. Mervaala, O. Siitonen and M. Uusitupa, 1995. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. New Engl. J. Med., 333: 89-94.
23. Zhang, L., X. Chen, L. Wu, Y. Li and L. Wang *et al*., 2021. Ameliorative effects of escin on neuropathic pain induced by chronic constriction injury of sciatic nerve. J. Ethnopharmacol., Vol. 267. 10.1016/j.jep.2020.113503.
24. Chang, M.W., M.S. Young and M.T. Lin, 2008. An inclined plane system with microcontroller to determine limb motor function of laboratory animals. J. Neurosci. Methods, 168: 186-194.
25. Dyck, P.J., K.M. Kratz, J.L. Karnes, W.J. Litchy and R. Klein *et al*., 1993. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: The rochester diabetic neuropathy study. Neurology, Vol. 43. 10.1212/WNL.43.4.817.
26. Andersen, H., 2012. Motor dysfunction in diabetes. Diabetes Metab. Res. Rev., 28: 89-92.
27. Zochodne, D.W., 2007. Diabetes mellitus and the peripheral nervous system: Manifestations and mechanisms. Muscle Nerve, 36: 144-166.
28. Oliveira, A.L., S.L.G. Antunes, R.M. Teles, A.C.C. da Silva and T.P. da Silva *et al*., 2010. Schwann cells producing matrix metalloproteinases under *Mycobacterium leprae* stimulation may play a role in the outcome of leprous neuropathy. J. Neuropathol. Exp. Neurol., 69: 27-39.
29. Deng, X., P. Ma, M. Wu, H. Liao and X.J. Song, 2021. Role of matrix metalloproteinases in myelin abnormalities and mechanical allodynia in rodents with diabetic neuropathy. Aging Dis., 12: 1808-1820.
30. Avci, G., I. Küçükkurt, E.K. Akkol and E. Yeşilada, 2010. Effects of escin mixture from the seeds of *Aesculus hippocastanum* on obesity in mice fed a high fat diet. Pharm. Biol., 48: 247-252.
31. Choudhary, N., G.L. Khatik and A. Suttee, 2021. The possible role of saponin in type-II diabetes-A review. Curr. Diabetes Rev., 17: 107-121.
32. Cavusoglu, T., T. Karadeniz, E. Cagiltay, M. Karadeniz and G. Yigitturk *et al*., 2015. The protective effect of losartan on diabetic neuropathy in a diabetic rat model. Exp. Clin. Endocrinol. Diabetes, 123: 479-484.