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ORIGINAL ARTICLE

Molsidomine ameliorates diabetic peripheral neuropathy complications in Wistar rats

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Abstract

Diabetic neuropathy is a disorder which affects various regions of the nervous system and there is no specific treatment available for it. So, the present study evaluated protective effect of molsidomine in diabetic neuropathy in rats. Diabetes was induced in male Wistar rats by administrating streptozotocin (52 mg/kg i.p). Diabetic rats were administered with molsidomine 5 mg/kg p.o, and 10mg/kg p.o. as treatment. After 8 weeks of treatment, Motor coordination, Mechanical allodynia, Mechanical hyperalgesia, Nerve conduction velocity, Glycosylated hemoglobin were assessed. Thereafter animals were sacrificed and sciatic nerve was isolated. It was used for reduced glutathione, lipid peroxidation and for histopathology. Treatment with molsidomine significantly improved motor coordination, paw withdrawal threshold, mechanical threshold and nerve conduction velocity. Furthermore, molsidomine treatment also reduced malondialdehyde levels and prevented depletion of reduced glutathione in sciatic nerve homogenate. Histopathology shows molsidomine treatment maintained normal architecture of sciatic nerve. The results of our study strengthens the alterative use of molsidomine in diabetic neuropathy.

KEYWORDS

diabetic neuropathy, molsidomine, streptozotocin, Wistar rats

1 | INTRODUCTION

Diabetes mellitus (DM) prevalence is increasing at an alarming rate as it was 381 million people globally in 2013 and is estimated to be 463 million people in 2019 rising to 578 million by 2030. It is a disease with high rate of complications such as neuropathy, nephropathy, retinopathy, erectile dysfunction etc.[1,2]

Diabetic neuropathy is a family of disorders that damage the different regions of the nervous system, either individually or in combination. It affects pain fibres, motor neurons and autonomic nervous system.[3] It results in large economic costs for its care.[4-6] The various kind of neuropathies include peripheral neuropathy, proximal neuropathy, autonomic neuropathy and focal neuropathy.[7] There are a number of reasons for the pathogenesis of diabetic neuropathy and polyol pathway of glucose metabolism is thought as one of the major mechanism.[8] Peripheral neuropathy is a type of nerve damage that usually affects feet and legs and sometimes hands and arms.[9] It is proved that reactive oxygen species (ROS) plays a significant role in the pathophysiology of neuropathic pain in diabetes.[10] Out of all diabetic patients, 50% of patients develop neuropathy and painful neuropathy ranges from 10% to 20% in patients with diabetes. Diabetic patients can experience nerve problems at any time and the problem increases with age, weight and duration.[5] The complications across various countries varies from 10% to 30% and it is higher in developed countries than in developing countries. These complications can lead to painful symptoms and can affect quality of life of the patient. The treatment for the painful diabetic

neuropathy is mainly focused on pain control. The treatments include a number of antidepressants, anticonvulsants, topical agents such as capsaicin cream, lidocaine patches and isosorbide dinitrate topical spray have also been tried but the benefits were uncertain. Since there is lack of exact medication for neuropathy, research is still carried out.[2]

Nitric oxide is an endogenous vasodilator which act as a neurotransmitter which is produced from L-arginine using an enzyme nitric oxide synthase. In diabetic patients, hyperglycemia stimulates the over production of ROS such as superoxide anion which reacts rapidly with nitric oxide radicals forming peroxynitrite anion, which is a toxic oxidant capable of damaging neurons leading to neuronal injury.[11,12]

Molsidomine is a vasodilating and antianginal agent. Since it is a prodrug, it is converted into its metabolite 3-morpholinosydnonimine (SIN-1), which spontaneously provides nitric oxide. Nitric oxide has a significant impact on tissue injury, inflammation, vasodilation, cell defence as well as for regulating cerebrovascular hemodynamics. Nitric oxide also has following characters like antioxidant, antiapoptotic and anti-inflammatory activity. It can be used to treat the eye tissue damage caused due to ionising radiation.[13-15] Till now, there is no data of molsidomine acting against diabetic peripheral neuropathy. So, the present study was designed to evaluate the protective effect of molsidomine against diabetic peripheral neuropathy.

2 | METHODS

2.1 | Chemicals

Streptozotocin (STZ; MP Biomedical Pvt. Ltd), ketamine hydrochloride (Neon Laboratories Limited, India), xylazine (Indian Immunologicals Limited, India). Molsidomine was gift samples from Taj Pharmaceutical Pvt. Ltd, India. All other chemicals and reagents used were of analytical grade.

2.2 | Animals

Wistar rats of appropriate age of either sex weighing about 250-300g were used for the study. The animals were housed in large propylene cages in an air-conditioned room at 24 ± 1°C with a 12 hr light/dark cycle and allowed ad libitum access to water and standard diet. Paddy husk was used as bedding material. The use of animals for the experiments were approved by Institutional Animal Ethics Committee (IAEC, reference number: AACP/IAEC/Dec2016/05) and Committee for the Purpose of Control and Supervision of Experimental Animals guidelines were followed.

2.3 | Induction of diabetes

STZ was administered at a dose of 52mg/kg body weight intraperitoneally to induce diabetes. Glucose solution was given a day after STZ injection. After 72 hours of STZ injection, blood samples (from tail) were collected after overnight fasting. Animals with fasting blood sugar >250mg/dL was considered as diabetic and divided into the following groups.

Non diabetic rats were assigned as the normal control group (Group 1).

1. Group 1: Normal Control (n = 6)
2. Group 2: Vehicle treated diabetic control (n = 6)
3. Group 3: Diabetic rats treated with molsidomine (5 mg/kg,p.o.) for 8 weeks (n = 6)
4. Group 4: Diabetic rats treated with molsidomine (10 mg/kg,p.o.) for 8 weeks (n = 6)

2.4 | Motor coordination

Motor coordination was evaluated by Rota-Rod tread mill. Rats were initially trained to remain themselves on the rotating rod for more than 2 minutes. In the test session the rats were placed in the rotating rod and the latency to fall was recorded.[16]

2.5 | Mechanical allodynia

Mechanical allodynia was performed as per the method of Yamamoto H et al using Von Frey apparatus.[17]

2.6 | Mechanical hyperalgesia

The measurement of mechanical nociceptive threshold was measured using fabricated Randall Selitto paw pressure device which applies a linearly increasing mechanical forcing to the dorsum of the rat's hind paw.[18]

2.7 | Nerve conduction velocity

Non-invasive nerve conduction velocity (NCV) was measured using power lab data acquisition system. The rats were anaesthetized with ketamine:xylazine(80-100 mg/kg :5-10 mg/kg i.p.) and during the experiment the body temperature of animal was maintained. The sciatic nerve was stimulated with supramaximal stimuli (8V) at 20 Hz. The latencies of the compound muscle action potentials were recorded via bipolar electrodes from the first interosseous muscle of the hind paw and measured from the stimulus artifact to the onset of the negative M-wave deflection. Motor NCV was calculated by subtracting the distal latency from the proximal latency and the result were divided by the distance between the stimulating and recording electrode.

2.8 | Biochemical studies

2.8.1 | Estimation of glycosylated hemoglobin

Blood was withdrawn from retro orbital of rat and collected in EDTA tubes. The glycosylated hemoglobin (GHb) was determined by using commercially available kits.[19]

2.8.2 | Sciatic nerve homogenate preparation

The rats were sacrificed by overdose of anaesthesia. A segment of sciatic nerves was isolated. Sciatic nerve samples were rinsed with ice cold saline (0.9%w/v sodium chloride) and homogenised in chilled phosphate buffer (pH 7.4). The homogenate thus obtained was used for measurement of reduced glutathione and lipid peroxidation.

2.8.3 | Estimation of reduced glutathione

The sciatic nerve was dissected out and washed with saline, chopped over ice and homogenates were prepared with 0.1 mol/L phosphate buffer. Glutathione was quantified by the method of Moron et al.[20]

2.8.4 | Measurement of lipid peroxidation

This parameter was accessed by the method of Ester bauer and Cheeseman to find out the extent of lipid peroxidation in terms of thiobarbituric acid reactive substances formation. Thiobarbituric acid reacts with malondialdehyde (MDA), a secondary product of lipid peroxidation.[21]

2.9 | Histopathology

Sciatic nerve was used for histopathology to observe the changes in the cell architecture using H&E (Hematoxylin and Eosin) stain.

2.10 | Statistical analysis

All data were expressed as mean ± SEM and analysed with one-way analysis of variance between the groups and followed by Tukey's Multiple Comparison Test were used to assess differences between the groups. Probability values $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ were considered significant.

3 | RESULTS

3.1 | Effect of molsidomine on motor coordination

The diabetic rats showed a significant decrease in the motor coordination as compared to normal rats ($P < .001$). Pre-treatment with molsidomine improved the retention time as compared to diabetic rats. (Figure 1).

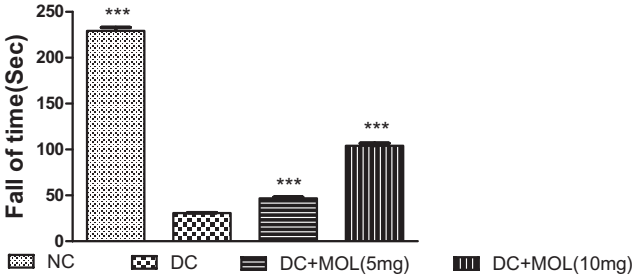

FIGURE 1 Effect of Molsidomine treatment on motor coordination in diabetic rats. Values are represented as mean ± SEM (n = 6). One way ANOVA followed by Dunnet's test. $^{***}P < .001$ Vs diabetic control group. DC, diabetic control; Mol, molsidomine; NC, normal control

3.2 | Effect of molsidomine on mechanical allodynia

The diabetic rats showed a significant reduction in paw withdrawal threshold as compared to normal rats ($P < .001$). Pre-treatment with molsidomine improved paw withdrawal threshold as compared to diabetic rats (Figure 2).

3.3 | Effect of molsidomine on mechanical hyperalgesia

Diabetic rats showed significant reduction in mechanical threshold as compared to diabetic rats ($P < .001$). Pre-treatment with molsidomine improved mechanical threshold as compared to diabetic rats (Figure 2).

3.4 | Effect of molsidomine on sciatic nerve conduction velocity

The nerve conduction velocity was significantly reduced in diabetic control rats when compared to normal rats ($P < .001$). Pre-treatment with molsidomine shows significantly improved in NCV when compared with diabetic rats (Figure 3).

3.5 | Effect of molsidomine on glycosylated hemoglobin, reduced glutathione and lipid peroxidation

Diabetic rats showed a significant increase in percentage of GHb and MDA levels as compared to normal rats ($P < .001$). Pre-treatment with molsidomine significantly reduced percentage of GHb and MDA levels as compared to the diabetic control rats ($P < .001$). Whereas diabetic rats showed a significant reduction in sciatic nerve reduced glutathione content as compared to normal rats ($P < .001$).

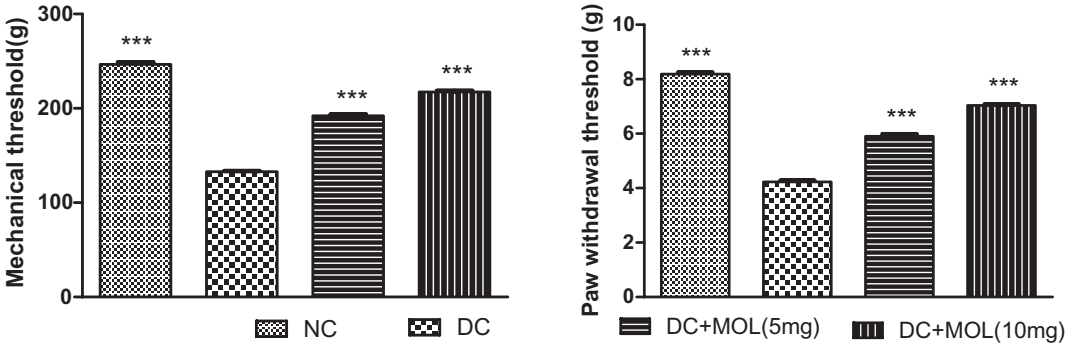

FIGURE 2 Effect of Molsidomine on mechanical threshold and paw withdrawal threshold in diabetic rats. Values are represented as mean ± SEM (n = 6). One-way ANOVA followed by Dunnet's test. ***$P < .001$ Vs diabetic control group. DC, diabetic control; Mol, molsidomine; NC, normal control

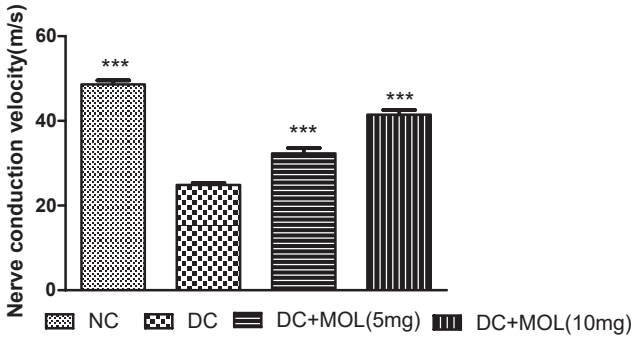

FIGURE 3 Effect of Molsidomine on sciatic nerve conduction velocity in diabetic rats. Values are represented as mean ± SEM (n = 6). One way ANOVA followed by Dunnet's test. ***$P < .001$ Vs diabetic control group. DC, diabetic control; Mol, molsidomine; NC, normal control

Pre-treatment with molsidomine significantly improved glutathione levels as compared to diabetic rats ($P < .001$) (Table 1).

3.6 | Histopathology

Sciatic nerve of normal rats stained with hematoxylin-Eosin(40X) shown longitudinal section showing the elongated Schwann cell nuclei and longitudinally oriented axons with myelin sheath (Figure 4A). Sciatic nerve of diabetic rats stained with hematoxylin-Eosin(40X) has shown mainly axonal swelling observed with intact myelin (Figure 4B). Sciatic nerve of rats treated with molsidomine 5 mg/kg showed nuclear degeneration in focal areas with few fibre arrangements (Figure 4C) whereas rats treated with molsidomine 10 mg/kg showed nuclear degeneration in few areas with few fibre arrangements (Figure 4D).

4 | DISCUSSION

Diabetic neuropathy has to be identified in early stages to prevent secondary complications such as neuropathic pain and diabetic foot.[22] The most commonly used animal for painful diabetic neuropathy is STZ induced diabetic rats. Mechanical allodynia and mechanical hyperalgesia are the common endpoints used to assess analgesic activity of drug in animal model. In our study mechanical allodynia and mechanical hyperalgesia were employed to assess the withdrawal threshold of the rat hind paw. The withdrawal threshold was improved following the treatment with molsidomine showing the analgesic activity of the drug.[17]

In STZ induced diabetic rats causing hyperalgesia is often accompanied by motor incoordination and reduced muscle strength. Therefore muscle coordination and nerve strengths were evaluated by measuring grip strength in STZ induced diabetic rats. Diabetic animals showed a significant decrease in grip strength compared to normal rats showing muscle weakness. The present study reveals that the treatment with nitric oxide donor molsidomine exerts a positive effect on muscle strength and motor coordination by stimulating soluble guanyl cyclase.[23,24]

Diabetic neuropathy is also accompanied with the functional changes in the sciatic nerve and can be evaluated by NCV. In the present study, reduction in NCV in STZ treated groups confirmed the abnormal functions of nerve due to diabetes. Molsidomine treated groups showed a significant improvement in NCV because sodium ions are pumped from extracellular fluid (ECF) to axoplasm and potassium ions are pumped from axoplasm to ECF and normalization of nerve speed due to decrease in oxidative stress at which an electrochemical impulse propagates down a neural pathway.[25,26]

Excessive generation of ROS and reactive nitrogen species with decreased antioxidant activity leads to develop diabetic neuropathy. Diabetes produces a significant oxidative damage in sciatic nerve as indicated by an increase in lipid peroxidation and nitric oxide concentration as well as depletion of antioxidant enzymes.[27]

Previous experimentations have shown that the oxidative stress induced by diabetes causes damage to protein, lipid, DNA and also causes damages myelin sheath surrounding the nerve. In the present study, increase in MDA levels and depletion in reduced glutathione was seen in sciatic nerve homogenate. Treatment with nitric oxide donor significantly attenuated the oxidative stress markers in sciatic nerve homogenate of diabetic rats.[28]

Molsidomine after biotransformation by esterases converts to its metabolite SIN-1 which then liberates nitric oxide and superoxide anion

TABLE 1 Effect of Molsidomine on glycosylated Hb, GSH and MDA levels in diabetic rats

Sl. No.	Groups	% Glycosylated Hb	GSH (mcg/mg)	MDA (nmol/mg)
1.	Normal control	2.76 ± 0.16***	104.2 ± 02.32***	3.28 ± 0.17***
2.	Diabetic control	14.71 ± 0.19	62.89 ± 1.99	7.44 ± 0.55
3.	Diabetic + MOL (5mg/kg)	10.53 ± 0.93***	73.77 ± 0.93***	5.06 ± 0.04***
4.	Diabetic + MOL (10mg/kg)	6.58 ± 0.28***	89.94 ± 0.87***	3.74 ± 0.16***

Note: Values are represented as mean ± SEM (n = 6). One way ANOVA followed by Dunnet's test.

Abbreviations: GSH, glutathione; Hb, hemoglobin; mcg, microgram; MDA, malondialdehyde, mg, milligram; MOL-molsidomine; nmol, nanomole.

***$P < .001$ vs diabetic control group.

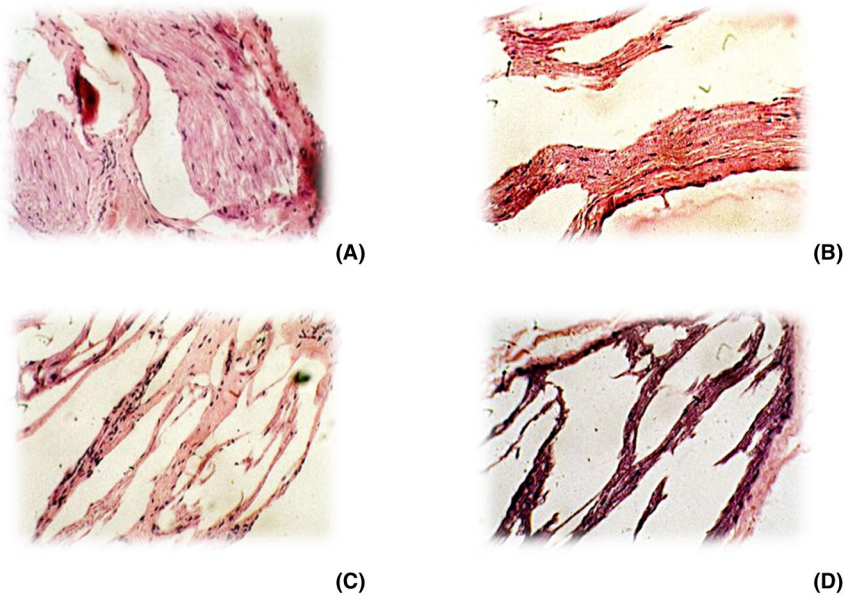

FIGURE 4 Histopathology of sciatic nerve (10X). A, Sciatic nerve of normal rats showing elongated Schwann cell nuclei and longitudinally oriented axons with myelin sheath. B, Sciatic nerve of diabetic rats has shown mainly axonal swelling observed with intact myelin. C, Sciatic nerve of molsidomine (5 mg) treated rats showing nuclear degeneration in focal areas with few fibre arrangements. D, Sciatic nerve of molsidomine (10 mg) treated rats showing nuclear degeneration in few areas with few fibre arrangements

radical. This superoxide anion radical rapids reacts to form peroxynitrite. Literature shows that treatment with molsidomine increases peroxynitrite concentration and decreases superoxide anion radical.[29]

Histopathological analysis of sciatic nerves of diabetic rat showed mainly axonal swelling observed with intact myelin. Treatment with molsidomine significantly improved fibre arrangements and is dose dependent.

CONFLICT OF INTEREST

There is no conflict of interest.

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REFERENCES

1. Kakrani AL, Gokhale VS, Vohra KV, Chaudhary N. Clinical and nerve conduction study correlation in patients of diabetic neuropathy. *J Assoc Phys India*. 2014;62(1):24-27.
2. Saeedi P, Petersohn I, Salpea P, et al. 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*. 2019;157:107843.
3. Haroon HB, Murali A. Antihyperglycemic and neuroprotective effects of Wattakaka volubilis (L.f.) Stapf root against streptozotocin induced diabetes. Braz. *J Pharm Sci*. 2016;52(3):413-424.
4. Cvijanovic M, Simic S, Kopitovic A, Raicevic R. Neurophysiological evaluation of short term outcome of pharmacological treatment of diabetic neuropathy. *Vojnosanit Pregl*. 2016;20:261.
5. Mohammed F. The pharmacological evaluation of epigallocatechin-3-gallate (EGCG) against diabetic neuropathy in wistar rats. *Int J Sci Res Rev*. 2012;1(3):75-87.
6. Viswanathan V, Snehalatha C, Seena R, Ramachandran A. Early recognition of diabetic neuropathy: evaluation of a simple outpatient procedure using thermal perception. *Postgrad Med J*. 2002;78(923):541-542.
7. Said G. Diabetic neuropathy- a review. *Nat Clin Pract Neurol*. 2007;3(6):331-340.
8. Maladkar M, Saggu NJS, Moralwar P, Mhate AA, Zemse D. Evaluation of efficacy and safety of epalrestat and epalrestat in combination with methylcobalamin in patients with diabetic neuropathy in a randomized, comparative trial. *Sci Res*. 2013;3(1):22-26.
9. Bansal V, Kalita J, Misra UK. Diabetic neuropathy. *Postgrad Med J*. 2006;82(964):95-100.
10. Morani AS, Bodhankar SL. Neuroprotective effect of early treatment with pioglitasone and vitamin E acetate in alloxan induced diabetes in rats. *Pharmacologyonline*. 2007;3:308-322.

11. Assmann TS, Brondani LA, Boucas AP, Rheinheimer J, Canani LH. Nitric oxide levels in patients with diabetes mellitus: a systematic review and meta-analysis. *Nitric Oxide*. 2016;61:1-9.
12. Chan MM, Mattiacci JA. Nitric oxide: actions and roles in arthritis and diabetes. *Foot*. 2001;11(1):45-51.
13. Ozer MA, Polat N, Ozen S, Parlakpinar H, Ekici K. Effects of molsidomine on retinopathy and oxidative stress induced by radiotherapy in rat eyes. *Curr Eye Res*. 2016;42(5):803-809.
14. Durak MA, Parlakpinar H, Polat A, Vardi N, Ekici K. Protective and therapeutic effects of molsidomine on radiation induced neural injury in rats. *Biotech Histochem*. 2017;92(1):68-77.
15. Nathani M, Razdan R, Pathak L. Repositioning of molsidomine for its efficacy in diabetes induced erectile dysfunction in rats: In silico, In vitro and In vivo approach. *Pharmacol Rep*. 2018;70(2):309-315.
16. Salim S, Abuohashish HM, Ahmed MM, Arrejaie AS, Aleisa AM. Telmisartan inhibits hyperalgesia and inflammatory progression in a diabetic neuropathic pain model of wistar rats. *Neurosciences (Riyadh)*. 2015;20(2):115-123.
17. Yamamoto H, Shimoshige Y, Yamaji T, Murai N, Aoki T. Pharmacological characterization of standard analgesics on mechanical allodynia in streptozotocin induced diabetic rats. *Neuropharmacology*. 2009;57(4):403-408.
18. Santos-Nogueira E, Castro E, Mancuso R, Test R-S. A new approach for the detection of neuropathic pain after spinal cord injury. *J Neurotrauma*. 2012;29(5):898-904.
19. Nathan D, Singer D, Hurxthal K, Goodson J. The clinical information value of the glycosylated haemoglobin assay. *N Engl J Med*. 1984;310(6):341-346.
20. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione-s transferase activities in rat lungs and liver. *Biochem Biophys Acta*. 1979;582:67-78.
21. Jiang Y, Guo C, Vasko M, Kelly M. Implications of apurinic/apyrimidic endonuclease in reactive oxygen signalling response after cisplatin treatment of dorsal root ganglion neurons. *Cancer Res*. 2008;68(15):6425-6434.
22. Balbinot LF, Canani LH, Robinson CC, Achaval M, Zaro MA. Plantar thermography is useful in the early diagnosis of diabetic neuropathy. *Clinics (Sao Paulo)*. 2012;67(12):1419-1425.
23. Van Schie C, Vermigli C, Carrington A, Boulton A. Muscle weakness and foot deformities in diabetes: relationship to neuropathy and foot ulceration in Caucasian diabetic men. *Diabetes Care*. 2004;27(7):1668-1673.
24. Anderson H. Motor dysfunction in diabetes. *Diabetes Metabol Res Rev*. 2012;28(Suppl 1):89-92.
25. Wuarin-Bierman L, Zahnd G, Kaufmann F, Burckel L, Adler J. Hyperalgesia in spontaneous and experimental animal models of diabetic neuropathy. *Diabetologia*. 1987;30(8):653-658.
26. Cendelin J, Korelusova I, Vozeh F. The effect of repeated rotarod training on motor skills and spatial learning ability in lurcher mutant mice. *Behav Brain Res*. 2008;189(1):65-74.
27. Toplu Y, Parlakpinar H, Sapmaz E, Karatas E, Polat A. The protective role of molsidomine on the cisplatin induced ototoxicity. *Indian J Otolaryngol Head Neck Surg*. 2014;66(3):314-319.
28. Serizawa K, Yogo K, Aizawa K, Tashiro Y, Ishizuka N. Nicorandil prevents endothelial dysfunction due to antioxidative effects via normalization of NADPH oxidase and nitric oxide synthase in streptozotocin diabetic rats. *Cardiovasc Diabetol*. 2011;10:105.
29. Marković SD, Vukajlović MDJ, Ognjanović BI, et al. A comparative study of the effects of molsidomine and 3-morpholinosydnonimine on the redox status of rat erythrocytes and reticulocytes. *Cell Biochem Funct*. 2007;25(3):251-258.

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