Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 1, 2015

Original Article

EFFECT OF COENZYME Q10 ALONE AND ITS COMBINATION WITH ROSUVASTATIN ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC NEUROPATHY IN RATS

RAJESHKUMAR A MAHESHWARI*, RAMACHANDRAN BALARAMAN, ASHIM KUMAR SEN, VIKAS R CHANDRAKAR

Department of Pharmacy, Sumandeep Vidyapeeth, Piparia, Vadodara 391760, Gujarat, India.
Email: rajpharma2007@gmail.com

Received: 03 Nov 2014 Revised and Accepted: 31 Nov 2014

ABSTRACT

Objectives: This study was aimed to investigate the effect of coenzyme Q10 and its combination with rosuvastatin on STZ-nicotinamide induced diabetic neuropathy.

Methods: Diabetic neuropathy in rats were induced with streptozotocin-nicotinamide. The diabetic rats were treated with coenzyme Q10 or rosuvastatin or their combination. Various parameters like muscular grip strength, paw withdrawal response, tail flick response and markers of oxidative stress such as malondialdehyde (MDA) level, superoxide dismutase (SOD) and reduced glutathione (GSH) in the sciatic nerve were measured. All treated animal was subjected to histopathological changes of sciatica nerve.

Results: In diabetic control group, muscular grip strength was significantly decreased and increased paw withdrawal response, tail flick response as compared to normal control rats. In addition, STZ-nicotinamide caused nerve cell damage with a higher MDA level, depletion of SOD and GSH level along with marked degeneration of the nerve cell. The treatment of diabetic rats with coenzyme Q10 or rosuvastatin or their combination ameliorate STZ-nicotinamide induced nerve damage due to improvement in the muscular grip strength, paw withdrawal response, tail flick response, reduction in oxidative stress along with histopathological changes.

Conclusion: This finding suggests that treatment with coenzyme Q10 or rosuvastatin showed significant neuroprotective effect against STZ-nicotinamide induced diabetic neuropathy. However, concomitant administration of both showed a better neuroprotective effect than coenzyme Q10 or rosuvastatin alone treatment.

Keywords: Diabetic neuropathy, Coenzyme Q10, Rosuvastatin, Muscular grip strength, Oxidative stress.

INTRODUCTION

Diabetic neuropathy is most common long-term complications of diabetes affecting 50% of the patient worldwide [1–3]. Diabetic nephropathy occurs as a result of damage to the nervous system due to persistent hyperglycemia can affect many parts of the body. The symptoms of diabetic neuropathy include pain, numbness, tingling. Neuropathic pain is usually considered to be one of the most upsetting complications affecting diabetic patients [4,5]. Current treatment for neuropathic pain includes antidepressant (duloxetine, citalopram, venlafaxine), anticonvusants (pregabalin, gabapentin, carbamazepine) and opioid and opioid-like drugs (tramadol, oxycodone). Pain relief with existing therapy is associated with many side effects [6-8]. It was previously reported that persistent and chronic hyperglycemia responsible for generation of reactive oxygen species and yield in the oxidative stress due to depletion of antioxidant defense system and damage to the peripheral neurons [9-11].

Coenzyme Q10 or ubiquinone has a potent antioxidant, scavenging free radicals and antidiabetic effect [12-14]. The 3-hydroxy-3-methylgluraryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) have pleiotropic effects on cerebrovascular, cardiovascular, and micro-vascular diseases independent of their cholesterol-lowering effect [15].

Therefore, it was thought to combine antioxidant like coenzyme Q10 and rosuvastatin to study their neuroprotective effect in experimentally induced neuropathy. Hence, the present study was aimed to investigate the protective effect of coenzyme Q10 alone and its combination with rosuvastatin on STZ-nicotinamide induced diabetic neuropathy.

MATERIALS AND METHODS

Drugs and chemicals

Rosuvastatin and coenzyme Q10 were obtained from Zydus Cadila, Ahmedabad, India. Streptozotocin and nicotinamide were purchased from Himedia (Mumbai, India). All other chemicals and reagents used in the study were of analytical grade.

Experimental animals

The experimental protocol in the present study was approved by the Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experiment was carried out on healthy adult Wistar rats weighing 200-250 g of either sex. Rats were housed in polypropylene cages, maintained under standardized condition (12-h light/dark cycle, 24°C, 35 to 60% humidity) and allowed free access to diet (Nav Maharashtra Oil Mills Pvt. Ltd., Pune) and purified drinking water *ad libitium*.

Induction of diabetic neuropathy

Type 2 diabetes was induced in overnight fasted adult albino Wistar rats (200-250g) by a single intraperitoneal (i. p.) Injection of 65 mg/kg streptozotocin (dissolved in citrate buffer, pH 4.5), followed by the i. p. administration of 110 mg/kg of nicotinamide (dissolved in normal saline) [16]. Hyperglycemia was confirmed by elevated blood glucose levels at 72 h and then on day 7 after injection. Those animals with fasting blood glucose level greater than 200 mg/dl were considered as diabetic and were used for diabetic neuropathy studies.

Experimental design

Diabetic rats were randomly divided into five groups each consisting of six animals.

Group I: Normal control rats (distilled water10 ml/kg, p. o.).

Group II: Diabetic control rats.

Group III: Diabetic rats treated with 10 mg/kg coenzyme Q10 (1% aqueous solution of Tween 80, p. o.) [17].

Group IV: Diabetic rats treated with rosuvastatin (10 mg/kg, p. o) [18].

Group V: Diabetic rats treated with the combination of coenzyme Q10 and rosuvastatin.

All the aforementioned treatments were started one week (7 days) after injection of streptozotocin-nicotinamide and treatments continued for 42 days.

At the end of experiments, muscle grip strength was evaluated using the Rota-rod apparatus. The test was used to evaluate muscle strength in rodents. The apparatus consists of 3 cm diameter horizontal metal rod coated with rubber and attached to motor with speed adjusted to 25 rpm/min. the rod is 92 cm in length and is divided into six compartments, thereby allowing simultaneous testing of six rats. Only those animals which showed the ability to remain on the rotating rod for at least 1 min were used for the test. The fall off time was measured. This can be read from the timer which was stopped by the fall of an animal.

The sensory function was evaluated by hot plate test and hot immersion test. The hot plate test and hot immersion test were carried out according to the previous method described by Eddy's et al. and Sharma et al, respectively [19,20]. In hot plate test, animals were placed on hot plates maintained at 55±1°C, withdrawal response was measured. The cut-off time for hot plate test was 10 s. In hot immersion test, the tail of the rat was immersed in hot water maintained 55±1°C, tail flick responses (tail withdrawal response) were observed. The cut-off time 15 s.

Estimation of biomarkers of oxidative stress

Sciatic nerve was removed and kept in cold conditions. The tissues were cross chopped with surgical scalpel into fine slices in chilled 0.25 M sucrose, quickly blotted on a filter paper. They were minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10 %w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 10,000 ×g at 0 C using the Remi cooling centrifuge. The clear supernatant obtained was used for assay of lipid peroxidation (MDA content), endogenous antiperoxidative enzymes such as superoxide dismutase (SOD) and GSH. Lipid peroxidation or malondialdehyde (MDA) formation was estimated by the method of Slater and Sawyer [21]. SOD was determined by the method of Mishra and Fridovich [22]. GSH was determined by the method of Moron and Depierre [23].

Histopathology

After sacrifice, Sciatic nerve tissues of each group were rapidly dissected out and washed immediately with saline and fixed in 10% phosphate buffered formalin. Paraffin-embedded specimens were cut into 5 µm-thick sections and stained with hematoxylin and eosin (H&E). The sections were examined under the light microscope (Olympus BX10, Tokyo, Japan) for the presence of histopathological changes and photomicrographs (Olympus DP12 camera, Japan) were taken. The observer performing histopathological evaluation was blinded to the animal treatment group.

Statistical Analysis

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using computer based fitting program (Prism, Graphpad). The significance level was set at $P < 0.05$ for all tests.

RESULTS

Effect of coenzyme Q10, rosuvastatin or combination of both on muscular grip strength, paw withdrawal response and tail flick response

Measurement of muscular grip strength was used to evaluate diabetic neuropathy after streptozotocin-nicotinamide injection. In diabetic control group, muscular grip strength was significantly ($P < 0.001$) decreased as compared to normal control rats. The treatment of diabetic rats with coenzyme Q10 or rosuvastatin or their combination showed a significant ($P < 0.01$; $P < 0.05$; $P < 0.001$) increase in muscular grip strength as compared to diabetic control rats. On the other hand, co-administration of coenzyme and rosuvastatin showed a significant ($P < 0.05$) increase in muscular grip strength than when coenzyme Q10 or rosuvastatin administered singly (fig. 1A).

In diabetic rats, there was a significant ($P < 0.001$) increase in paw withdrawal response and tail flick response as compared to normal control rats showing significant nerve damage in diabetic animals. The treatment of diabetic rats with coenzyme Q10 or rosuvastatin or coenzyme Q10 + rosuvastatin showed a significant decrease in paw withdrawal response ($P < 0.001$; $P < 0.01$; $P < 0.001$) and tail flick response ($P < 0.01$; $P < 0.01$; $P < 0.001$) as compared to diabetic control rats. Moreover, treatment with coenzyme Q10 + rosuvastatin did not show any significant alterations in paw withdrawal response as compared to mono-therapy (coenzyme Q10 or rosuvastatin) (fig. 1B-C).

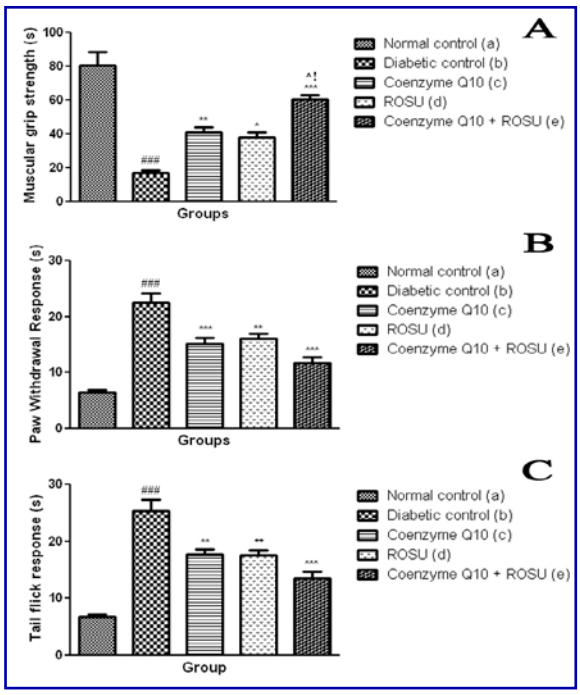

Fig. 1: Effect of coenzyme Q10, rosuvastatin or combination of both on (A) Muscular grip strength (B) Paw withdrawal response (C) Tail flick response
Values are expressed as mean ± SEM ; n=6, a vs.b, ### P< 0.001; b vs. c, b vs. d, b vs. e, *P < 0.05; **P < 0.01; ***P < 0.001, c vs. e,^P < 0.05; d vs. e, !P < 0.05.

Effect of coenzyme Q10, rosuvastatin or combination of both on markers of oxidative stress in sciatic nerve tissue

The content of MDA, end product of lipid peroxidation and marker of oxidative stress was significantly ($P < 0.001$) increased in sciatic nerve tissue of diabetic control rats as compared to non diabetic rats after six weeks of study. There was a significant ($P < 0.001$) decrease in the levels of GSH, an endogenous antioxidant and antiperoxidative enzymes (SOD) in sciatic nerve tissue as compared to normal control group.

The treatment of diabetic rats with coenzyme Q10 or rosuvastatin or their combination showed a significant decrease in the levels of MDA ($P < 0.01$; $P < 0.01$ and $P < 0.001$) and increase GSH ($P < 0.001$), SOD ($P < 0.01$; $P < 0.05$ and $P < 0.001$) as compared to diabetic control rats (fig. 2A-C).

Histopathological studies

Histopathology of sciatic nerve in normal control rats showed normal structure, while sciatica nerve revealed that the nerve cells of the diabetic control rats showed marked degenerations. However, the treatment with coenzyme Q10, rosuvastatin or combination of both showed a significant increase in tissue regeneration capacity. In

contrast, co-administration of coenzyme Q10 and rosuvastatin showed more tissue regeneration capacity when compared to diabetic control group as well as mono-therapy (coenzyme Q10 or rosuvastatin) (fig. 3).

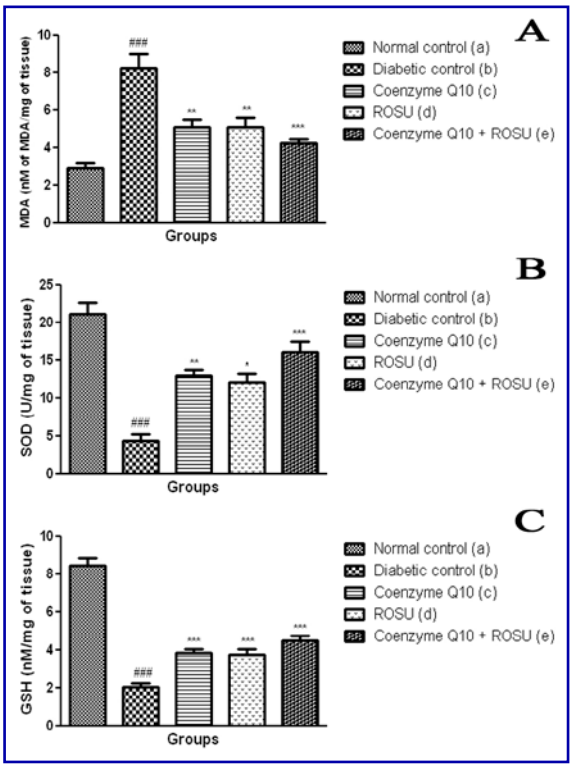

Fig. 2: Effect of coenzyme Q10, rosuvastatin or combination of both on (A) MDA (B) SOD (C) GSH in sciatic nerve
Values are expressed as mean ± SEM ; n=6, a vs.b, ### P< 0.001; b vs. c, b vs. d, b vs. e, *P < 0.05; **P < 0.01; ***P < 0.001.

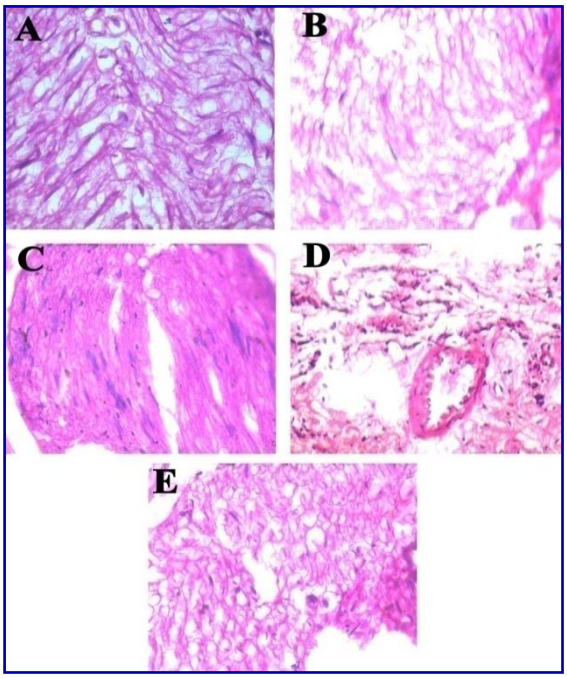

Fig. 3: Light microscopy of sciatic nerve from rats (A) Control group, (B) Diabetic control group, (C) Coenzyme Q10 (D) Rosuvastatin (E) Coenzyme Q10 + Rosuvastatin

DISCUSSION

It was previously reported that streptozotocin is most commonly used to induce diabetes in experimental animals and administration of streptozotocin-nicotinamide caused diabetic neuropathy [24,25]. Development of diabetic neuropathy was evident from alteration in muscle grip strength, nociception (paw withdrawal and tail flick response) and biochemical changes including oxidative stress. In the present study, it was shown that after six weeks of streptozotocin-nicotinamide treatment, muscular grip strength in diabetic rats was found to be reduced as compared to normal control rats. Nociception was evaluated by increased in paw withdrawal response and tail flick response (hyperalgesia). These results are in accordance with the earlier study in which it was shown that metformin alone produced a beneficial effect on diabetic nephropathy [26-28]. However, the treatment of diabetic rats with coenzyme Q10 or rosuvastatin or their combination showed a significant increase in muscular grip strength as compared to diabetic control rats. On the other hand, co-administration of coenzyme and rosuvastatin showed a significant increase in muscular grip strength than when coenzyme Q10 or rosuvastatin administered singly. Coenzyme Q10 or rosuvastatin or coenzyme Q10 + rosuvastatin treated rats showed a significant decrease in paw withdrawal response and tail flick response when compared to diabetic control rats. In this study, there was a significant increase MDA level and decrease in the level of GSH, an endogenous antioxidant and antiperoxidative enzymes (SOD) in the untreated diabetic rat sciatic nerve. Thus, it was concluded that the elevated level of MDA might be responsible for the decrease in enzymatic and non-enzymatic antioxidant of defense systems in diabetic rats. Similarly, in an earlier study, it was shown that increased MDA level and depletion of GSH and SOD have been found in sciatic nerve of diabetic rats [29-31]. In our study, it was shown that treatment with coenzyme Q10 or rosuvastatin or their combination prevented the increased in the levels of MDA and decreased GSH, SOD in sciatic nerve. Histopathological study of sciatic nerve of rats in diabetic group showed a significant degeneration of nerve tissue, while combined treatment of coenzyme Q10 and rosuvastatin showed normal sciatic nerve growth.

CONCLUSION

These results indicate that treatment with coenzyme Q10 or rosuvastatin showed significant neuroprotective effect against STZ-nicotinamide induced diabetic nephropathy. However, concomitant administration of both showed a better neuroprotective effect than coenzyme Q10 or rosuvastatin alone treatment by virtue of amelioration of lipid peroxidation as well as due to improvement in muscular grip strength, paw withdrawal response and tail flick response along with histopathological changes. Finally, it was concluded that adjuvant therapy of coenzyme Q10 with antidiabetic drug might prevent or delay the diabetic neuropathy.

CONFLICTING INTEREST

The Author(s) declare(s) that they have no conflicts of interest to disclose

REFERENCES

1. Kumar S, Ashe HC, Parnell LN, Fernando DJ, Tsigos C, Young RJ, *et al.* The prevalence of foot ulceration and its correlates in type 2 diabetes: a population-based study. Diabet Med 1994;11:480–4.
2. Cabezas-Cerrato J. The prevalence of diabetic neuropathy in Spain: a study in primary care and hospital clinic groups. Diabetol 998;41:1263–9.
3. Ghosh P, Kandhare AD, Gauba D, Raygude KS, Bodhankar SL. Determination of efficacy, adverse drug reactions and cost effectiveness of three triple drug regimens for the treatment of H. pylori infected acid peptic disease patients. Asian Pac J Trop Biomed 2012;2:S783–S9.
4. Gilron I, Coderre TJ. Emerging drugs in neuropathic pain. Expert Opin Emerg Drugs 2007:12:113–26.
5. Clark CM, Lee DA. Prevention and treatment of the complications of diabetes mellitus. N Eng J Med 1995;332:1210–7.

6. Arner S, Meyerson BA. Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 1988;33:11–23.
7. Abuaisha BB, Costanzi JB, Boulton AJM. Acupuncture for the treatment of chronic painful peripheral diabetic neuropathy: a long term injury. Diabetes Res Clin Pract 1998;39:115–21.
8. Apfel SC. Introduction to diabetic neuropathy. Am J Med 1999;107:1S.
9. Baynes JW, Thorpe R. Role of oxidative stress in diabetic complications. A new perspective on an old paradigm. Diabetes 1999;48:1–9.
10. Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia 2012;83:650–9.
11. The Diabetes Control Complications Trial Research Group. The effect of intensive diabetes therapy on the development and progression of neuropathy in the diabetes control and complications trial. Ann Int Med 1995;122:561–8.
12. Niklowitz P, Menke T, Andler W, Okun JG. Simultaneous analysis of coenzyme Q10 in plasma, erythrocytes and platelets: comparison of the antioxidant level in blood cells and their environment in healthy children and after oral supplementation in adults. Clin Chim Acta 2004;342(1-2):219-26.
13. Ahmadvand H. Effects of coenzyme Q10 on hemoglobin A1C, serum urea and creatinine in alloxan-induced type 1 diabetic rats. Iranian J Pharmacol Ther 2012;11:64-7.
14. Visnagri A, Kandhare AD, Shiva Kumar V, Rajmane AR, Mohammad A, Ghosh P, *et al*. Elucidation of ameliorative effect of Co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomed Aging Pathol 2012;2:157-72.
15. McFarlane SI, Muniyappa R, Francisco R, Sowers JR. Pleiotropic effects of statins: lipid reduction and beyond. J Clin Endocrinol Metab 2002;8(4):1451-8.
16. Maheshwari RA, Khatri K, Sailor GU, Balaraman R. Antidiabetic activity of Dibolin (a polyherbal formulation) in streptozotocin-nicotinamide induced type 2 diabetic rats. Int J Pharm Pharm Sci 2014;Suppl 2:893-7.
17. Garjani A, Andalib S, Biabani S. Combined atorvastatin and coenzyme Q10 improve the left ventricular function in isoproterenol-induced heart failure in rat. Eur J Pharmacol 2011;666(1-3):135-41.
18. Maheshwari RA, Sailor GU, Patel L, Balaraman R. Amelioration of cisplatin-induced nephrotoxicity by statins. Indian J Pharmacol 2013;45(4):354-8.
19. Woolfe G, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther 1944;80:300-7.
20. Sharma SS, Kumar A, Kaundal R. 4-Amino 1,8-napthalimide: a potent PARP inhibitor, its neuroprotective role in experimental diabetic neuropathy. Life Sci 2008;82:570–6.
21. Slater TF, Sawyer BC. The stimulatory effect of carbon tetrachloride and other halogenalkane or peroxidative reaction in the rat liver functions in vitro. Biochem J 1971;123(5):805-15.
22. Mishra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay of superoxide dismutase. J Biol Chem 1972;247(10):3170-5.
23. Moron MS, Depierre JW. Levels of glutathione, glutathione reductase and glutathione S transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582(1):67-78.
24. Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, *et al*. Pharmacokinetics and pharmacody-namics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther 2005;78:675-88.
25. National Prescribing Service, August 2010. Sitagliptin for Type 2 Diabetes.http://www.nps.org.au/consumers/publications/medicine_update/issues/sitagliptin.
26. Kumar A, Kaundal RK, Iyer S, Sharma SS. Effects of resveratrol on nerve functions, oxidative stress and DNA fragmentation in experimental diabetic neuropathy. Life Sci 2007;80:1236–44.
27. Stevens MJ, Obrosova I, Cao X, Van Huysen C, Greene DA. Effects of DLalpha-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. Diabetes 2000;49:1006–15.
28. Sayyed SG, Kumar A, Sharma SS. Effects of U83836E on nerve functions, hyperalgesia and oxidative stress in experimental diabetic neuropathy. Life Sci 2006;79:777–83.
29. Cui XP, Li BY, Gao HQ, Wei N, Wang WL, Lu M. Effects of grape seed proanthocyanidin extracts on peripheral nerves in streptozocin-induced diabetic rats. Nutr Sci Vitaminol 2008;54(4):321-8.
30. Arora M, Kumar A, Kaundal RK, Sharma SS. Amelioration of neurological and biochemical deficits by peroxynitrite decomposition catalysts in experimental diabetic neuropathy. Eur J Pharmacol 2008;596:77–83.
31. Saini AK, Kumar HAS, Sharma SS. Preventive and curative effect of edaravone on nerve functions and oxidative stress in experimental diabetic neuropathy. Eur J Pharmacol 2007;568:164–72.