JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2022/30085
RESEARCH ARTICLE

Novel Therapeutic Intervention using Coenzyme Q10 and Insulin Sensitizer on Experimentally-Initiated Diabetic Neuropathy

Rajesh A. Maheshwari[1]*, Ramachandran Balaraman[1], Ashim Kumar Sen[1], Umang Shah[2], Kinjal P. Patel[1] and Dhanya B. Sen[1]

[1]Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara – 391760, Gujarat, India; rajpharma2007@gmail.com
[2]Ramanbhai Patel College of Pharmacy, CHARUSAT Campus, Changa – 388421, Gujarat, India

Abstract

Diabetes is one of the main disease having many repercussions due to which there is a huge economic burden globally. Therefore, there is a need of good therapeutic intervention by using some nutraceuticals to combat this dreadful disease especially complications such as diabetic neuropathy where patients suffer from severe pain and disability. Therefore, nutraceuticals like coenzyme Q10 and metformin were used in this study to see how they are effective in alleviating the symptoms of diabetic peripheral neuropathy. The animal experiments were conducted to induce neuropathy by using streptozotocin-nicotinamide. Animals were divided into five groups such as control, diabetic control, coenzyme Q10, metformin, and their combination. The nerve function test was conducted by using paw withdrawal response, tail-flick response, and muscular grip strength. Antioxidant parameters were assessed by estimating such MDA, SOD, and GSH. The sciatica nerve was isolated and a histopathological examination was conducted. Neuropathy was assessed in diabetic control animals which showed a substantial decrease in grip strength, increase in the paw withdrawal, and tail-flick response. It was found that there was a rapid improvement in nerve function test when a combination of coenzyme Q10 and metformin was given together as compared to animals given coenzyme Q10 and metformin alone. From this study, it is shown that combination therapy exhibited a better improvement in the nerve function test and control of the free radical generation which ultimately results in nerve damage.

Keywords: Histopathological Examination, Metformin, Nerve Damage, Nutraceutical, Streptozotocin-nicotinamide

1. Introduction

Diabetes mellitus is one of the global health problems due to which human beings suffer from complications like kidney disease, nerve damage, and eye problem[1,2]. Prolonged hyperglycemia is capable of harming the peripheral nerve which is finally responsible for a foot ulcer and amputation[3,4]. The major symptoms of diabetic peripheral neuropathy are pain and numbness. This kind of symptom usually affects the quality of life and therefore patients suffer from various psychological problems[5,6]. In spite of having an armamentarium of a lot of medications that belong to the group of antidepressants, anti-anxiety, anticonvulsants, and opioid analgesics, they are having their own disadvantage of severe undesirable actions which could not be tolerated by patients[7–9]. In this context, the main culprit which is

*Author for correspondence

Article Received on: 27.04.2022 Revised on: 04.06.2022 Accepted on: 16.06.2022

responsible for this kind of damage is due to an imbalance between the antioxidant activity and oxidative stress. This free radical generation is eventually accountable for peripheral nerve damage (diabetic peripheral neuropathy, DPN)[10,11].

Coenzyme Q10 by the virtue of antioxidant activity has been shown to decrease inflammation, high blood pressure, and glucose levels[12–15]. Metformin (MET) is also having an antioxidant effect through the AMPK pathway and thereby mitigates the diabetic-initiated increase in pain response. In painful DPN, MET has been shown to produce a powerful analgesic effect with minimal adverse reaction[16]. It was thought to evaluate the potential effect of the nutraceutical such as coenzyme Q10 and anti-diabetic drug-like MET against experimentally-initiated DPN.

2. Material and Methods

2.1 Drugs and Chemicals

Zydus Research Centre, Ahmedabad, India provided MET and coenzyme Q10. Himedia (Mumbai, India) supplied streptozotocin and nicotinamide. Kits of the study were purchased from a standard company. The study's other chemicals and reagents were of analytical grade.

2.2 Experimental Animals

This study (SVU/PH/IAEC/11/04/02) was approved by IAEC which is the official authority for the maintenance of experimental animals in all institutions. Wistar rats (200-250 g) were housed in polypropylene cages for the day-night cycle of 12h at 24°C with humidity of 35 to 60%. Water for drinking and a diet for nutrition was provided.

2.3 Induction of Diabetic Neuropathy

The experimentally-initiated DPN was conducted as per the earlier protocol[17]. Overnight starved albino wistar rats (200-250 g) were injected intraperitoneally 65 mg/kg streptozotocin (STZ) and 110 mg/kg (i.p.) nicotinamide (dissolved in normal saline) to induce type 2 diabetes. After 72 and 7 days of STZ-NA administration, the blood sugar level was checked and it was found that all the animals were diabetic.

2.4 Experimental Design

The experimental animals were divided into five groups which are as follows:

Experimental group 1 (a): Normal control group (distilled water 10 ml/kg, p. o.)

Experimental group 2 (b): Diabetic control group

Experimental group 3(c): Coenzyme Q10 (10 mg/kg in 1% aqueous solution of Tween 80, p. o.)[18]

Experimental group 4 (d): MET (500 mg/kg, p. o)[19]

Experimental group 5 (e): Coenzyme Q10 + MET

In experimental group 1, DPN was not conducted, unlike in other groups. On the 7th day after injection of STZ-NA, the treatment schedule of all aforementioned groups was followed for six weeks. Muscle grip strength was measured with the use of Rota-rod apparatus according to the previous method[20]. Paw withdrawal and Tail flick responses were conducted by using a hot plate and hot immersion methods, respectively to check the potency of the abovementioned combination of drugs for the sensation of neurons [21, 22].

2.5 Evaluation of Biomarkers of Antioxidant Activity

After sacrificing the experimental animals, sciatic nerves were isolated and removed for keeping them in cold conditions. Later on, with the use of a surgical scalpel, the nerves were chopped and kept in chilled 0.25 M sucrose. Further, they were chopped into small slices on filter paper. According to the standard method mentioned in the literature[23–25], malondialdehyde (MDA), superoxide dismutase (SOD), and GSH levels were determined for assessing the oxidative stress parameters.

2.6 Histological Study

After sacrificing the experimental animals from the above-mentioned groups, the sciatic nerve was isolated, removed, stored in formalin (10% phosphate buffer), and processed for histopathological examination as mentioned in an earlier report[26].

2.7 Statistical Analysis

One-way ANOVA followed by the Bonferroni multiple comparison test was used to evaluate the level of significance ($P < 0.05$) for all tests. All the data were exhibited in Mean ± SEM.

3. Results

3.1 Effect of Coenzyme Q10 and Metformin on Muscular Grip Strength, Paw Withdrawal Response, and Tail-flick Response

As mentioned in Figure 1 A–C, diabetic untreated rats were shown to cause nerve damage by virtue of its increase in paw withdrawal response, tail-flick response, and decrease in muscular grip strength when compared with untreated normal control rats. Treatment with the neutraceutical or anti-diabetic drug or their combination showed a positive response in which there was a significant decrease in paw withdrawal response, tail-flick response, and increase in muscular grip strength. Treatment of coenzyme Q10 + MET exhibited considerable changes in muscle grip strength and tail-flick

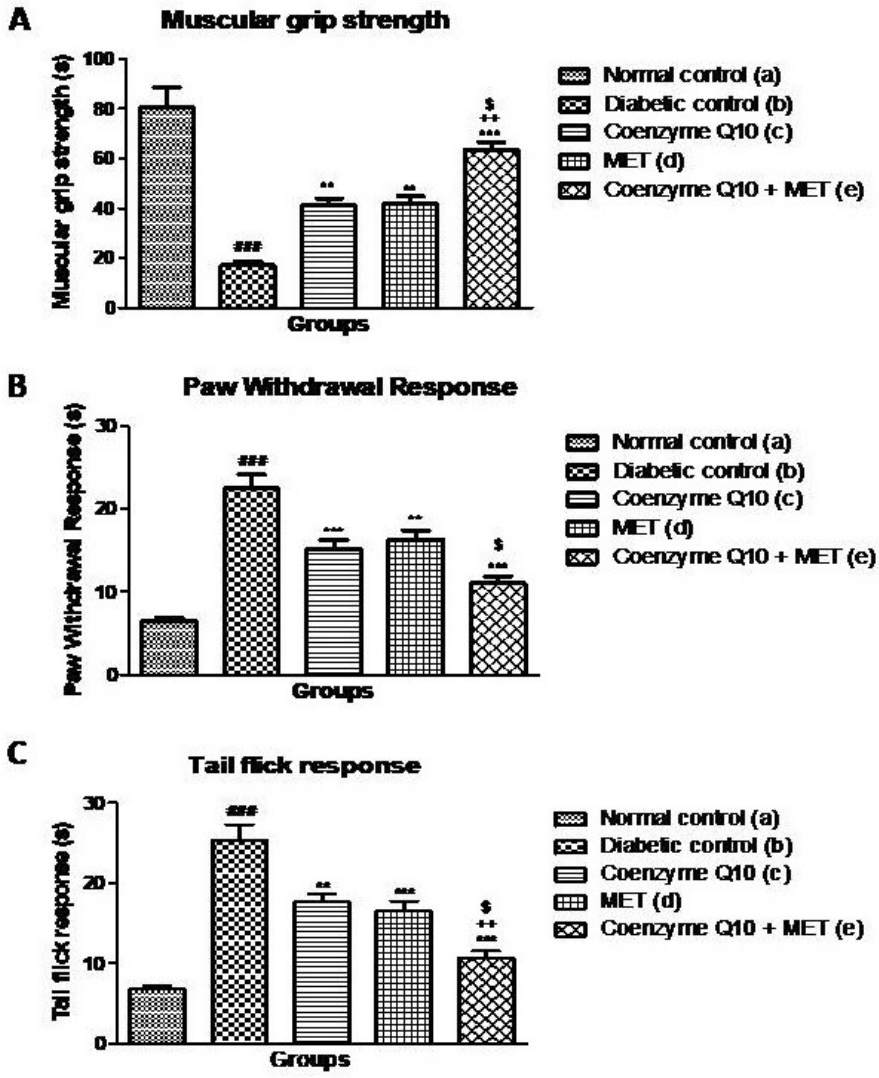

Figure 1. Effect of coenzyme Q10 + MET on **(A)** Muscular grip strength **(B)** Paw withdrawal response and **(C)** Tail flick response.
Values are expressed as mean ± SEM; n=6
$^{###}P < 0.001$ compared with normal control (a)
$^{**}P < 0.01$, $^{***}P < 0.001$ compared with diabetic control rats (b)
$^{++}P < 0.01$ compared with coenzyme Q10 (c)
$^{\$}P < 0.05$ compared with MET (d)

reaction when compared to mono-therapy (coenzyme Q10 or MET). Co-administration of coenzyme Q10 + MET, on the other hand, resulted in a more noticeable alteration in paw withdrawal reaction than MET mono-therapy.

3.2 Effect of Coenzyme Q10 and Metformin on Oxidative Stress Biomarkers of Sciatic Nerve

As mentioned in Figure 2 A–C, diabetic untreated rats were shown to cause nerve damage by virtue of imbalance

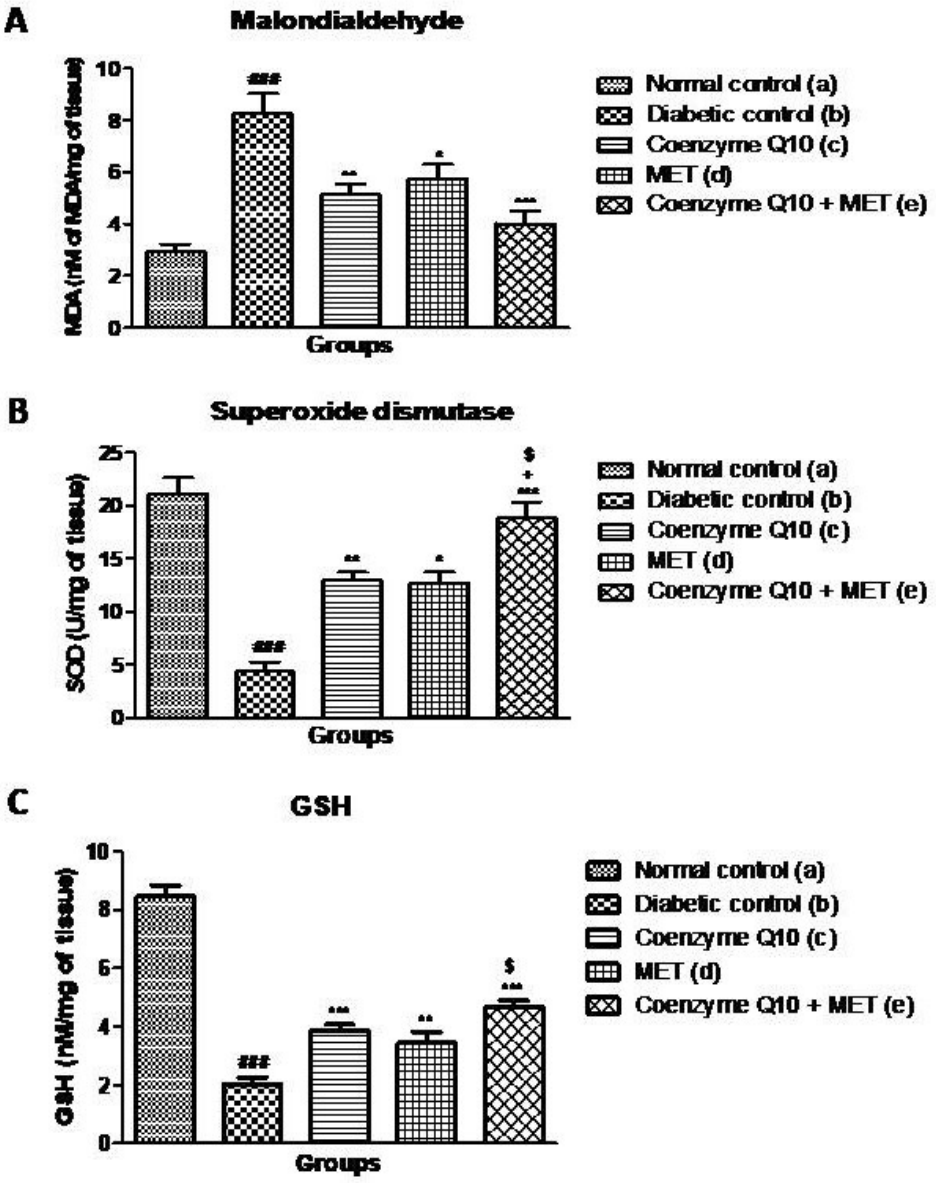

Figure 2. Effect of coenzyme Q10 + MET on **(A)** MDA **(B)** SOD and **(C)** GSH.
Values are expressed as mean ± SEM; n=6.
$^{###}P < 0.001$ compared with normal control (a)
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with diabetic control rats (b)
$^{+}P < 0.05$ compared with coenzyme Q10 (c)
$^{\$}P < 0.05$ compared with MET (d)

between oxidative stress and antioxidant activity. There was an increase in MDA levels and a reduction in SOD and GSH levels when compared with untreated normal control rats. Treatment with the neutraceutical or antidiabetic drug or their combination showed a positive response in which there was a decrease in MDA levels and an elevation in SOD and GSH levels. Treatment of coenzyme Q10 + MET exhibited in considerable changes in SOD and GSH when compared to mono-therapy (MET). Co-administration of coenzyme Q10 + MET, on the other hand, resulted in a more noticeable alteration in SOD than mono-therapy (coenzyme Q).

3.3 Histopathological Studies

In the present study, it was observed that histology of the sciatic nerve of untreated control animals showed a normal structure whereas, nerve in diabetic untreated rats revealed severe damage. There was a significant reduction in a damage of nerve when the animals were treated with coenzyme Q10 or coenzyme Q10 plus

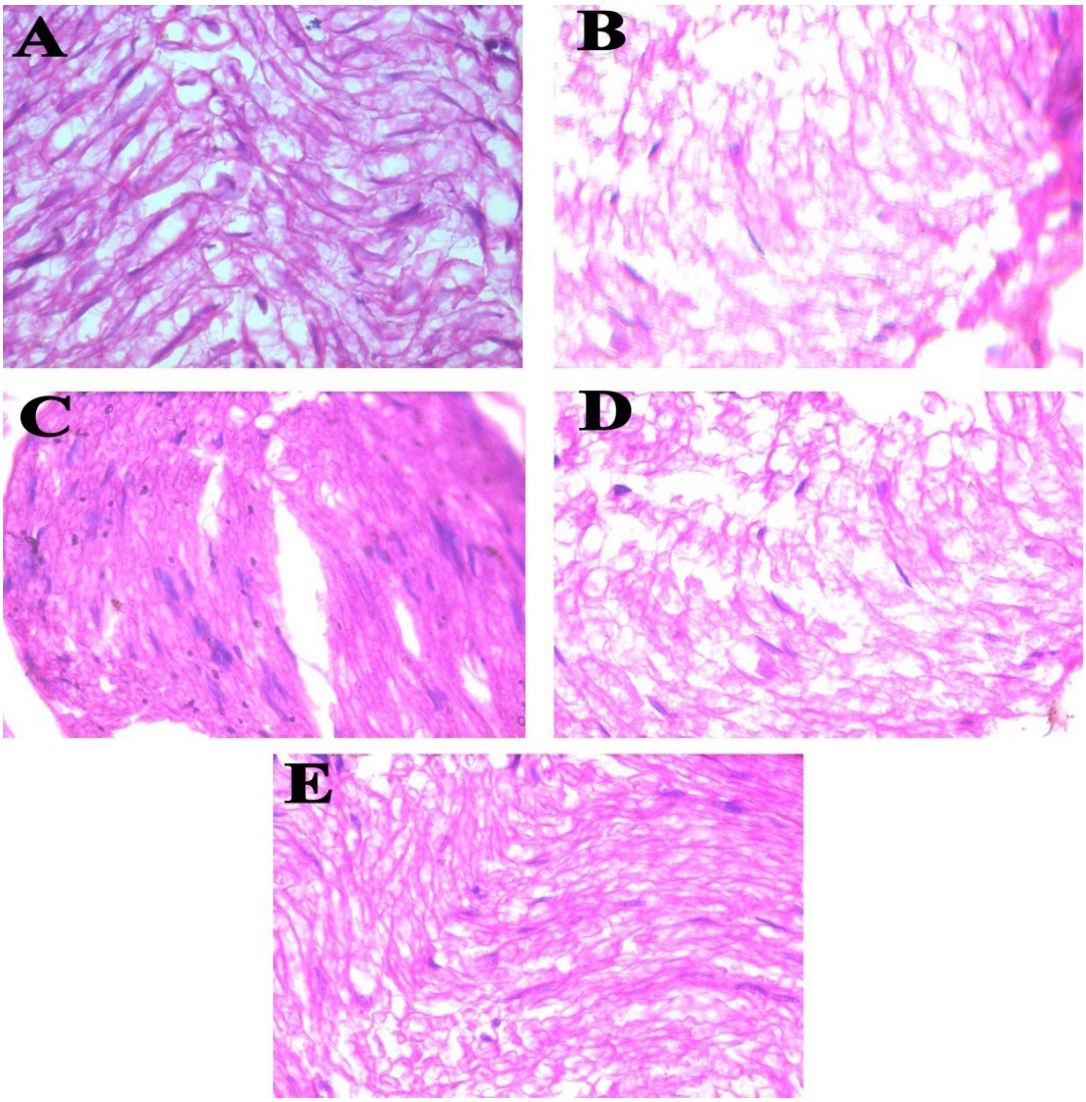

Figure 3. Light microscopy of sciatica nerve from rats **(A)** Normal control group, **(B)** Diabetic control group, **(C)** Coenzyme Q10 **(D)** MET **(E)** Coenzyme Q10 + MET.

MET. Administration of MET *al*one did not bring any histopathological changes in sciatic nerve of diabetic rats. Despite, MET *al*one treatment showed a positive outcome like increase in grip and decrease in nociception, paradoxically there was no change in damage of the sciatic nerve. Increase in grip strength and decrease in nociception by the administration MET might be due to different mechanism of action. Combination of coenzyme Q10 and MET, on the other hand, demonstrated a greater reduction in a damage than mono-therapy (Figure 3 A–E).

4. Discussion

Diabetic untreated rats showed severe DPN when the sciatic nerve was examined by histopathological observation along with changes in muscle grip strength, paw withdrawal, and tail flick response, as well as changes in oxidative stress biomarkers[20]. The untreated animals which was injected STZ-NA exhibited considerable loss in muscle grip strength and a large increase in nociception. These outputs have a comparable effect to that shown in the previous report[27–29]. Treatment with coenzyme Q10 or MET or their combination resulted in a positive outcome like increase in grip and decrease in nociception than diabetic untreated rats. Combination of coenzyme Q10 and MET, on the other hand, resulted in a significant increase in muscular grip and a considerable decrease in tail flick response when compared to coenzyme Q10 or MET given alone and paw withdrawal response was significantly altered in combination therapy compared to MET mono-therapy. As a result, lipid peroxidation products such as MDA was found to be responsible for an alteration in the antioxidant defense system, resulting in nerve injury in diabetic animals. It was shown that elevation of MDA and reduction in the levels of GSH and SOD, have association with the injury of the sciatic nerve in diabetic rats[30–32]. Because of their antioxidant activity, therapy with nutraceutical or MET or their co-administration, reduced the nerve injury produced by STZ-NA injection in the current study. Furthermore, combination of coenzyme Q10 and MET treatment demonstrated in a considerable change in SOD and GSH levels compared to mono-therapy. A histopathological examination of the sciatic nerve of diabetic rats revealed significant nerve tissue damage, whereas combined treatment with nutraceutical such as coenzyme Q10 and MET demonstrated no damage in sciatic nerve.

7. Conclusion

It was concluded that supplementing with coenzyme Q10 and MET can help to prevent nerve damage caused by STZ-NA. However, a combination of coenzyme Q10 and MET is more effective than MET alone in lowering any type of DPN in diabetic individuals. However, this must be demonstrated in a clinical situation.

6. Conflicting Interest

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

7. References

1. Zangiabadi N, Asadi-Shekaari M, Sheibani V, Jafari M, Shabani M, Asadi AR, *et al*. Date fruit extract is a neuroprotective agent in diabetic peripheral neuropathy in streptozotocin-induced diabetic rats: A multimodal analysis. Oxid Med Cell Longev. 2011. https://doi.org/10.1155/2011/976948. PMid:22191015. PMCid:PMC3236446
2. Gallieni M, Aiello A, Tucci B, Sala V, Brahmochary Mandal SK, Doneda A, *et al*. The burden of hypertension and kidney disease in Northeast India: The Institute for Indian Mother and Child noncommunicable diseases project. Sci World J. 2014. https://doi.org/10.1155/2014/320869. PMid:24616621. PMCid:PMC3927758
3. Belchetz PE, Hammond PJ. Mosby's color atlas and text of diabetes and endocrinology. Elsevier Health Sciences; 2003.
4. Houreld NN. Shedding light on a new treatment for diabetic wound healing: A review on phototherapy. Sci World J. 2014. https://doi.org/10.1155/2014/398412. PMid:24511283. PMCid:PMC3913345
5. Gilron I, Coderre TJ. Emerging drugs in neuropathic pain. Expert Opin Emerg Drugs. 2007; 12(1);113–26. https://doi.org/10.1517/14728214.12.1.113. PMid:17355217
6. Clark Jr CM, Lee DA. Prevention and treatment of the complications of diabetes mellitus. N Eng J Med. 1995; 332(18):1210–7. https://doi.org/10.1056/NEJM199505043321807. PMid:7700316

7. Arner S, Meyerson BA. Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain. 1988; 33(1):11–23. https://doi.org/10.1016/0304-3959(88)90198-4
8. Abuaisha BB, Costanzi JB, Boulton AJ. Acupuncture for the treatment of chronic painful peripheral diabetic neuropathy: a long-term study. Diabetes Res Clin Pract. 1998; 39(2):115–21. https://doi.org/10.1016/S0168-8227(97)00123-X
9. Apfel SC. Introduction to diabetic neuropathy. Am J Med. 1999; 107(2):1S. https://doi.org/10.1016/S0002-9343(99)00006-6
10. Baynes JW, Thorpe R. Role of oxidative stress in diabetic complications. A new perspective on an old paradigm. Diabetes. 1999; 48:1–9. https://doi.org/10.2337/diabetes.48.1.1. PMid:9892215
11. Kandhare AD, Raygude KS, Ghosh P. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia 2012; 83:650–9. https://doi.org/10.1016/j.fitote.2012.01.010. PMid:22343014
12. Schmelzer C, Lindner I, Rimbach G, Niklowitz P, Menke T, Döring F. Functions of coenzyme Q10 in inflammation and gene expression. Biofactors. 2008; 32(1–4):179–83. https://doi.org/10.1002/biof.5520320121. PMid:19096114
13. Kohli Y, Suto Y, Kodama T. Effect of hypoxia on acetic acid ulcer of the stomach in rats with or without coenzyme Q10. Jpn J Exp Med. 1981; 51:105–8. PMid:7277789.
14. Lenaz G, Fato R, Formiggini G, Genova ML. The role of Coenzyme Q in mitochondrial electron transport. Mitochondrion. 2007; 7:S8–33. https://doi.org/10.1016/j.mito.2007.03.009. PMid:17485246
15. Alhaider AA, Korashy HM, Sayed-Ahmed MM, Mobark M, Kfoury H, Mansour MA. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through modulation of oxidative stress genes expression. Chem Biol Interact. 2011; 192(3):233–42. https://doi.org/10.1016/j.cbi.2011.03.014. PMid:21457706
16. Ma J, Yu H, Liu J, Chen Y, Wang Q, Xiang L. Metformin attenuates hyperalgesia and allodynia in rats with painful diabetic neuropathy induced by streptozotocin. Eur J Pharmacol. 2015; 764:599–606. https://doi.org/10.1016/j.ejphar.2015.06.010. PMid:26054810
17. 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; 2:893–7.
18. 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. https://doi.org/10.1016/j.ejphar.2011.04.061. PMid:21570962
19. Waisundara VY, Hsu A, Tan BK, Huang D. Baicalin reduces mitochondrial damage in streptozotocin-induced diabetic Wistar rats. Diabetes Metab Res Rev. 2009; 25(7):671–7. https://doi.org/10.1002/dmrr.1005. PMid:19688721
20. Sharma AK, Sharma A, Kumari R, Kishore K, Sharma D, Srinivasan BP, *et al.* Sitagliptin, sitagliptin and metformin, or sitagliptin and amitriptyline attenuate streptozotocin-nicotinamide induced diabetic neuropathy in rats. J Biomed Res. 2012; 26(3):200–10. https://doi.org/10.7555/JBR.26.20110054. PMid:23554750. PMCid:PMC3596070
21. Woolfe G, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther. 1944; 80:300–7.
22. 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. https://doi.org/10.1016/j.lfs.2007.11.031. PMid:18262571
23. 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. https://doi.org/10.1042/bj1230805. PMid:4399399. PMCid:PMC1177080
24. 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. https://doi.org/10.1016/S0021-9258(19)45228-9
25. Moron MS, Depierre JW. Levels of glutathione, glutathione reductase and glutathione S transferase activities in rat lung and liver. Biochim Biophys Acta. 1999; 582(1):67–78. https://doi.org/10.1016/0304-4165(79)90289-7
26. Maheshwari R, Pandya B, Balaraman R, Seth AK, Yadav YC, Sankar VS. Hepatoprotective effect of

Livplus-A polyherbal formulation. Pharmacogn J. 2015; 7(5):311–6. https://doi.org/10.5530/pj.2015.5.11

27. 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(13):1236–44. https://doi.org/10.1016/j.lfs.2006.12.036. PMid:17289084
28. Stevens MJ, Obrosova I, Cao X, Van Huysen C, Greene DA. Effects of DL-alpha-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. Diabetes. 2000; 49(6):1006–15. https://doi.org/10.2337/diabetes.49.6.1006. PMid:10866054
29. 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. https://doi.org/10.1016/j.lfs.2006.02.033. PMid:16581090
30. 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. https://doi.org/10.3177/jnsv.54.321. PMid:18797155
31. 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(1–3):77–83. https://doi.org/10.1016/j.ejphar.2008.08.003. PMid:18768138
32. 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. https://doi.org/10.1016/j.ejphar.2007.04.016. PMid:17521626