

Combined therapeutic effects of low power laser (980nm) and CoQ10 on Neuropathic Pain in adult male rat

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Abstract

Background: Neuropathic pain (NP) is one of the most suffering medical conditions that often fail to respond to certain pain therapy. Although its exact etiology is still unknown the role of reactive oxygen species (ROS) and oxidative stress were explored by many researchers. Neuropathies either central or peripheral lead to painful condition as well as social and economic isolation, thus various therapies were used to treat or reduce the pain. Laser therapy and antioxidant drugs have separately considered as treatment for NP, but the combination of them have not been used yet. In order to study the combination effects of Low Level Laser Therapy (LLLT) and Coenzyme Q₁₀ (CoQ₁₀) the present study was designed.

Methods: Sixty adult male rats (230-320g) were used in this experimental study that divided into six groups (n=10). Chronic constriction injury (CCI) was used to induce neuropathic pain. The CoQ₁₀ or vehicle, a low level laser of 980nm was used for two consecutive weeks. Thermal and mechanical paw withdrawal thresholds were assessed before and after surgery on 7th and 14th days.

Results: As we expected CCI decreased the pain threshold, whereas CoQ₁₀ administration for two weeks increased mechanical and thermal threshold. The same results obtained for laser therapy using the CCI animals. Combination of laser 980nm with CoQ₁₀ also showed significant differences in CCI animals.

Conclusion: Based on our findings the combination of CoQ₁₀ with LLLT showed better effects than each one alone. In this regard we believe that there might be cellular and molecular synergism in simultaneous use of CoQ₁₀ and LLLT on pain relief.

Keywords: Low Level Laser Therapy, Neuropathic pain, Chronic Constriction Injury, Coenzyme Q₁₀.

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Introduction

Various agents including direct nerve and spinal cord trauma; viral infections and metabolic diseases such as diabetes may trigger neuropathic pain (NP). As clinical laboratory examination shows that the neuropathic pain is often independent of any obvious signs of inflammation; it is sometimes described as 'non-inflammatory pain'. The International Association for the

Study of Pain (IASP) defines NP as pain 'initiated or caused by a primary lesion or dysfunction in the nervous system' (1). Despite of this definition that refers to mainly the cause, the exact mechanism of the events following the injury or trauma remain poorly understood. There are certain evidences that confirm cellular events following injury that mainly emphasize on the role of cellular organelles such as mito-

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chondria (2). The first mitochondrial dysfunction described in the 1960s and during the last two decades the role that it plays in health, disease, and aging have been reported by others. Several studies showed that free radical; oxidative stress and inflammation have major role in the pathogenesis of neurodegenerative diseases, such as amyotrophic lateral sclerosis, epilepsy, migraine headaches, strokes, Alzheimer and Parkinson's diseases and NP resulting in mitochondrial dysfunction (3-5). Mitochondrial dysfunction has been shown in rats with painful peripheral neuropathies (6). It is reported that the major reason for mitochondrial dysfunction is reactive oxygen species (ROS) production thus antioxidant could be a good candidate and therapeutic strategy for decreasing the severity of the damage to mitochondria. Ubiquinone CoQ₁₀ is a vital cofactor in complexes I to III of the mitochondrial electron transport chain, which acts as an electron acceptor and also a key component of the mitochondrial respiratory chain for adenosine triphosphate synthesis (7-9). In addition to its unique role in mitochondria, CoQ₁₀ is a potent antioxidant and scavenging free radicals and inhibiting lipid per oxidation (10, 11). The CoQ₁₀ has bioenergetics and anti-inflammatory effects that protect against apoptosis of neurons and cells from oxidative stress in vivo (12-15). The previous studies carried out by Kohli et al. and El-Abhar et al. showed that CoQ₁₀ possesses anti-ulcer potential as well (16,17). Ghule et al. have documented that CoQ₁₀ provides protection against isoproterenol-induced cardiotoxicity and cardiac hypertrophy pre-clinically, and Burke et al. showed that it could be used as a treatment for systolic hypertensive patient (18, 19). CoQ₁₀ treatment improves endothelial function and blood flow; thus, long-term treatment may be effective by improving oxygenation of the peripheral nerves (20). Hernandez-Ojeda et al. reported that a 12-wk treatment with ubiquinone significantly improves diabetic polyneuropathy in patients with type II diabetes (21).

As drug therapy it may lead to unwanted and undesirable side effects hence other therapeutic procedures including physical methods have been improved due to the absence of side effects (22). Low level laser therapy (LLLT) is well known for its anti-inflammatory, analgesic and tissue repair effects (23-28). It seems that some mechanisms of low level laser involved in mitochondrial respiratory chain and oxidative stress biomarkers (29). According to Karu et al. laser exposure can lead to an increase in mitochondrial electrochemical activity and ATP synthesis (30). Eells et al. showed that cytochrome c oxidase is the main photo acceptor of laser light (31). Other researchers postulated that laser therapy influences oxidative stress parameters such as changes in antioxidant enzyme activity and the production of ROS (32-35). The absorption of laser light have shown to accelerate the transfer of electrons (respiratory chain) and induces an initial ROS production, specifically increasing the production of superoxide anion (32). Despite the known clinical effects of LLLT and CoQ₁₀, to our knowledge there are no study on combined effects on NP. Thus the present study designed first to compare the effects of CoQ₁₀ and LLLT on neuropathic pain model and explore the combination of these two procedures on same model.

Methods

Animals

Sixty adult male Wistar rats (250-320 g) were used in this study with food and water ad libitum. The animals were divided into six groups (n=10) as follows:

- CCI group: animals that were subjected to surgical procedure, without undergoing treatment.
- Coenzyme Q₁₀ group: received 200mg/kg/day intraperitoneal (i.p) injection of Co Q₁₀ (Tishcon, NY, USA)
- Vehicle group: received 200mg/kg/day (i.p) injection vehicle of CoQ₁₀
- Laser therapy group (980nm): laser irradiation with energy density of 4 J/cm² and intensity of 0.248 (W/cm²)

- Laser 980nm+CoQ₁₀ group: received 200mg /kg/day (i.p) injection of CoQ₁₀ and laser 980nm.

- Laser 980nm+Vehicle group: received 200mg /kg/day (i.p) injection vehicle of CoQ₁₀ and laser 980nm.

All animals were subjected to the behavioral evaluation before surgery. To induce NP, the sciatic nerve injury model described by Bennett and Xie was used (36).

Treatment

The day after surgery, vehicle and CoQ₁₀ groups received 200 mg/kg (i.p) injection of CoQ₁₀ (CoQ₁₀ is in LiQsorb form) for 14 days.

A CW diode laser emitter with following specification was used in this study. A laser with wavelength of 980 nm, power of 70mW (Aixiz; model: AH980-6015AC), the energy density of 4 J/cm², power density of 0.248 W/cm² and beam area ~ 0.238 cm² was used. The irradiation was as follows: two points on two ends of surgical incision and another at the mn time 11.3s for visible wavelength and 16.13s for NIR one.

Laser calibration was done prior to use. Three points of the surgical incision were irradiated transcutaneously with no direct skin conidpoint. Treatment was started on the first day after the surgery and was continued for two weeks daily at the same time between 10-12 AM.

Functional analysis

Behavioral study was carried out before and after surgery on 14th day.

Thermal withdrawal threshold

Using a Plantar Test apparatus (Ugo-Basile, Italy) thermal hyperalgesia, the latency to withdrawal of the hind paws from a focused beam of radiant heat applied to the plantar surface. The animals were placed in an acrylic box with glass floor and the plantar surface of their hind paw exposed to a beam of infrared radiant heat. The paw withdrawal latencies were recorded at infrared intensity of 50 and three trials

for the right hind paws were performed and for each reading, the apparatus was set at a cut-off time of 25s. Each trial separated by an interval time of 5 minutes.

Mechanical withdrawal threshold

Mechanical paw withdrawal thresholds were assessed with the Randall–Selitto method using an Analgesy-meter apparatus (UgoBasile, Italy). This instrument exerts a force that was increased at a constant rate. The force was applied to the hind paw of the rat, which was placed on a small plinth under a cone shaped pusher with a rounded tip (1.5 mm in diameter).The rat was held upright with the head and limb to be tested free, but most of its body cradled in the hands of the experimenter. The paw was then put under the pusher until the rat withdrew the hind paw. Each hind paw was tested twice, with a 10 min interval between the measurements and mechanical paw withdrawal thresholds were calculated as the average of two consecutive measurements.

Statistical analysis

Using SPSS 19.0 statistical analysis was done, and the results presented as means ± SD, and p-value less than 0.05 was considered to be significant.

Results

For functional evaluation of gait we used the Plantar Test and Randall–Selitto method preoperatively and those recorded on the 14th day after surgery. Our results were as follow:

Plantar Test

The thermal withdrawal threshold of the control group was, on average, 18.91±4.08 sec of the data recorded prior to the injury. For the CCI group 12.42±4.82 sec and 10.70±5.02 sec on the 7th day and the 14th day respectively after surgery.

For the CoQ₁₀ group, the mean values were 18.91±4.09 sec and 16.15±4.52 sec on the 7th and the 14th day respectively after surgery. For the Vehicle group, the mean values were 14.67±5.64 sec and 13.86±4.91

sec on the 7th and the 14th respectively day after surgery. For the LLLT 980nm group, the mean values were 16.13±4.11 sec and 14.18±3.35 sec on the 7th and the 14th day respectively after surgery. For the LLLT 980nm+CoQ₁₀ group, the mean values were 19.02±3.02 sec and 19.11±4.61 sec on the 7th and the 14th day after surgery. For the LLLT 980nm+Vehicle group, the mean values were 17.64±5.22 sec and 17.54±4.96

sec on the 7th and the 14th day respectively after surgery. By using ANOVA, the results among the CCI and treatment groups considered significant. However, there were no significant difference between the 7th, 14th post-surgery days of the CoQ₁₀ group and the control group; but there was significant difference between the 7th, 14th post-surgery days in the LLLT 980nm group and the control group ($p < 0.01$, $p < 0.001$) re-

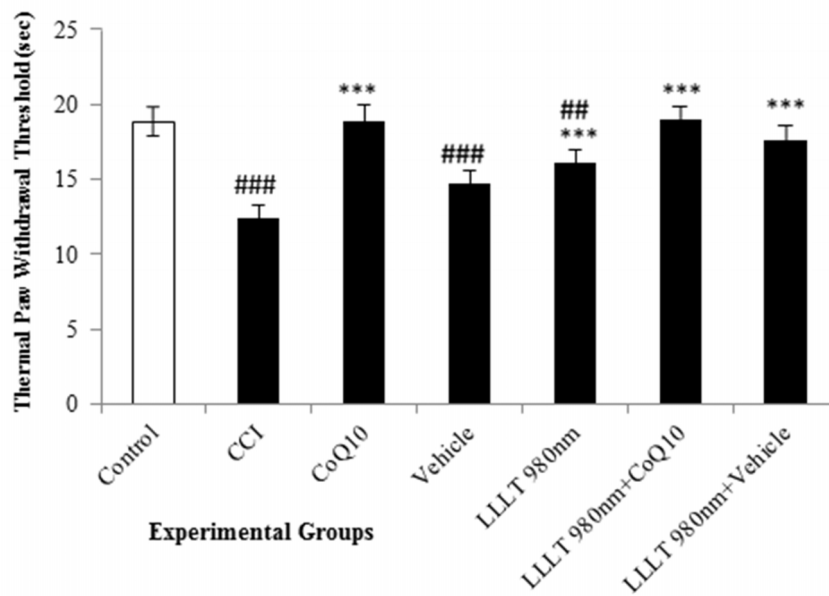


Fig. 1. Mean values of the Thermal Paw Withdrawal Threshold obtained from the groups during the study period (before surgery (control), the 7th day after surgery). Asterisks represent significant differences from CCI group (** $p < 0.01$, *** $p < 0.001$) and (### $p < 0.001$, ## $p < 0.01$) represent significant differences from control group.

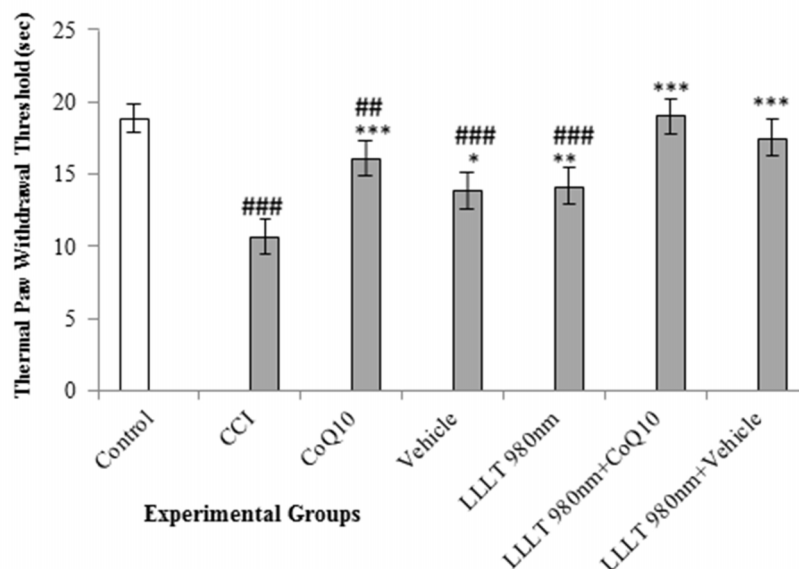


Fig. 2. Mean values of the Thermal Paw Withdrawal Threshold obtained from the groups during the study period (before surgery (control), the 14th day after surgery). Asterisks represent significant differences from CCI group (** $p < 0.01$, *** $p < 0.001$, * $p < 0.05$) and (### $p < 0.001$, ## $p < 0.01$) represent significant differences from control group.

spectively; and there were no significant difference between the 7th, 14th post-surgery days in the LLLT 980nm+CoQ₁₀ group and the Control group; also between LLLT 980nm+vehicle group and the control group. The comparison between the LLLT 980nm+CoQ₁₀ group and LLLT 980nm+Vehicle group showed there was no significant difference in both values. There was no significant difference between the 7th post-surgery days in the LLLT 980nm+CoQ₁₀ group and the CoQ₁₀ group and there was significant difference

between the 7th post-surgery day of the LLLT 980nm+Q₁₀ group and the LLLT 980nm group at (p< 0.01). Comparison of the results among the LLLT 980nm+CoQ₁₀ group and treatment groups (CoQ₁₀ and LLLT 980nm) illustrated significant difference (p< 0.05, p< 0.001) on the 14th day after surgery (Fig 1&Fig 2).

Randall–Selitto method

The mean of mechanical withdrawal threshold of the control group was 19.18±4.66 g before surgery. The means

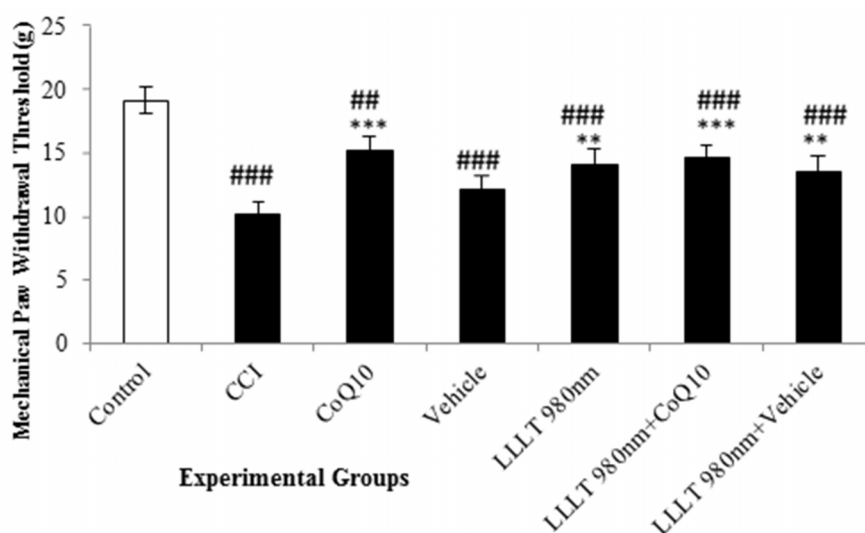


Fig. 3. Mean values of the Mechanical Paw Withdrawal Threshold obtained from the groups during the study period (before surgery (control), and 7th day after surgery).Asterisks represent significant differences from CCI group (** p<0.01, *** p<0.001) and (### p< 0.001, ## p< 0.01) represent significant differences from control group.

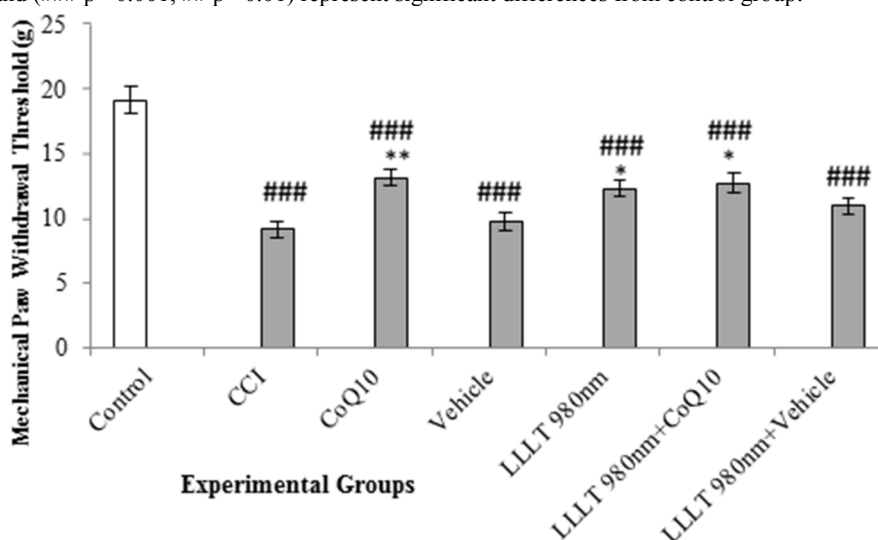


Fig. 4. Mean values of the Mechanical Paw Withdrawal Threshold obtained from the groups during the study period (before surgery (control), and 14th day after surgery).Asterisks represent significant differences from CCI group (** p<0.01, *** p<0.001, * p< 0.05) and (### p< 0.001) represent significant differences from control group.

for the CCI group were 10.17 ± 4.18 g and 9.15 ± 4.20 g on the 7th and the 14th day respectively after surgery. For the CoQ₁₀ group, the mean values were 15.29 ± 5.50 g and 13.19 ± 4.12 g on the 7th and the 14th day after surgery. For the Vehicle group, the mean values were 12.09 ± 3.92 sec and 9.75 ± 3.15 sec on the 7th and the 14th day respectively after surgery. For the LLLT 980nm group, the mean values were 14.15 ± 4.25 g and 12.35 ± 5.28 g on the 7th day and the 14th day respectively after surgery. For the LLLT 980nm+CoQ₁₀ group, the mean values were 14.65 ± 5.77 sec and 12.72 ± 4.63 sec on the 7th and the 14th day respectively after surgery. For the LLLT 980nm+Vehicle group, the mean values were 13.6 ± 3.50 sec and 10.98 ± 2.99 sec on the 7th and the 14th day respectively after surgery. Statistical analysis indicated that the difference between control group and the CCI group on the 7th, 14th post-surgery days, was significant at ($p < 0.001$); also difference between CoQ₁₀ group and the CCI group on the 7th, 14th post-surgery days, was significant ($p < 0.001$, $p < 0.01$); and difference between LLLT 980nm group and the CCI group on the 7th, 14th post-surgery days, was also significant ($p < 0.01$, $p < 0.05$). Moreover, difference between LLLT 980nm+CoQ₁₀ group and the CCI group on the 7th, 14th post-surgery days, was significant ($p < 0.001$, $p < 0.05$). Difference between LLLT 980nm+Vehicle group and the CCI group on the 7th post-surgery day was significant ($p < 0.01$) but there was no significant difference on 14th day post-surgery. Also, there was significant difference between the 7th, 14th post-surgery days of the treatment groups and the control group ($p < 0.001$). In a comparison between the LLLT 980nm+CoQ₁₀ and LLLT 980nm+Vehicle groups, there were no significant differences in the values on the 7th, 14th post-surgery days and comparison between the LLLT 980nm+CoQ₁₀ group and treatment groups (CoQ₁₀ and LLLT 980nm), showed no significant difference on the 7th, 14th days (Fig 3 & Fig 4).

Discussion

Our finding showed that LLLT and CoQ₁₀ alone and combined after 1 week increase thermal and mechanical sense thresholds compared to the CCI animals. The results were the same for two weeks of intervention.

To explain the cellular mechanisms that led to these findings, the nature and pathology of NP should be considered. It is generally accepted that neuropathic pain hyperalgesia depends on an increase in proinflammatory cytokines (37). In addition, NO a diffusible multifunctional transcellular messenger also contribute to hyperalgesia. It acts directly by sensitizing peripheral nerve or indirectly by influencing the local inflammatory process. NO is also involved in the transmission and modulation of nociceptive information at the periphery, spinal cord and supraspinal level (38). Other factors including cytokines, such as tumor necrosis factor alpha (TNF- α) and interleukin-10 (IL-10) are important for pain behavior following nerve injury. It is shown that these factors are associated with mitochondrial dysfunction and lead to increasing ROS production and oxidative stress generation (39). In addition to their pronociceptive action, TNF- α and NO act as proapoptotic messengers (43, 44). TNF- α is responsible for a cascade of cellular events that result in mitochondrial dysfunction. It acts via reducing complex III activity, increasing ROS production and causing damage to mtDNA (40-41).

The LLLT is widely used for its cellular therapeutic effects, which lead to wound healing, pain relief, reduction of edema and inflammation. During the period of LLLT, absorption of red or near-infrared photons by cytochrome c oxidase in the mitochondrial respiratory chain causes an increase in cellular respiration (42). Silveira et al. evaluated the effects of low level laser therapy (904 nm) with varied irradiation intensity on mitochondrial respiratory chain activity and some oxidative stress markers. They showed LLLT reduces the complex II activity of the mitochondrial respiratory

chain and they concluded that LLLT could protect the cell against oxidative damage to membrane lipids, due to the decreases in both superoxide anion production and oxidative stress. (43). The reduction of oxidative damages has been postulated as one of the main mechanisms following using LLLT which induces an increase in SOD activity, thus lead to decrease in tissue damages and the maximization of the healing process (44-46). Various mechanisms for therapeutic efficacy of low level laser irradiation have been proposed, including increases in mitochondrial activity and ATP levels, production of low levels of reactive oxygen species, induction of transcription factors NF- κ B, and inhibition of apoptosis (47). Khalil et al. have shown that N-type Ca^{2+} channel activation plays a role in nerve repair (48). Following increased ATP and protein synthesis after LLLT, the expressions of growth factors and cytokines increase and activation of calcium channels resulting in increased intracellular calcium concentration, ultimately lead to cell survival (49-55).

Increases in pain relief factor such as beta-endorphins, blocked depolarization of C-fiber afferent nerves (56), axonal sprouting and nerve cell regeneration (57), decreased bradykinin levels, ion channel normalization (58), stabilization of the cell membrane (59), enhancement of ATP synthesis (60), stimulated vasodilation along with release histamine, NO and serotonin (61), reduction in interleukin-1 β levels (62), increasing angiogenesis (63), enhancing superoxide dismutase (64), decreasing C-reactive protein and neopterin levels (65) are other reported mechanisms for reducing pain by red and near infrared light .

Regarding CoQ10 it is reported that it can decrease neurological symptoms in patients with Parkinson and Huntington diseases. It is also shown that CoQ₁₀ may play an important role in neuroprotection against diabetic neuropathy and other neurodegenerative disorders (66, 67). Zhang et al. demonstrated the potential benefits of CoQ₁₀ as a potent antioxidant and its ability to relieve

neuropathic pain in the type I diabetic mouse model (68). Also, Shi et al. reported that the CoQ₁₀ may represent a promising therapeutic strategy for type II diabetic neuropathy (69). The CoQ₁₀ neuroprotection may also leads to functional improvement of respiratory chain activity and prevention of neuronal apoptosis (68). It also acts throughout inhibiting oxidative stress and reducing inflammation by down-regulating of proinflammatory factors (11). The CoQ₁₀ intensely reduced apoptotic cell death, attenuated ATP decrease, and hindered DNA fragmentation elicited by all apoptotic stimuli that is accompanied by inhibition of mitochondrial depolarization, cytochrome c release (70). Tsai et al. showed that CoQ₁₀ significantly reduced the activation of NF- κ B, suppressed the expression of P₅₃ and the expression of Bax and led to a significant increase in expression of the antiapoptotic protein Bcl-2 and suppressing oxidative stress-related responses by modulating NO-related signaling (71). The role of CoQ₁₀ is to reduce hypertension-mediated oxidative damage (72), increases the antioxidant capacity of glutathione reductase and superoxide dismutase (SOD) also reported (73). Nonetheless the CoQ₁₀ treatment improves endothelial function and blood flow; thus, long-term treatment may be effective by improving oxygenation of the peripheral nerves (74). An increase in the concentration of CoQ₁₀ might affect mitochondrial respiratory function and early supplementation should be administrated in cases of deficiency (77). Since these events are due to mitochondrial PTP opening, Papucci et al. suggested the antiapoptotic activity of CoQ₁₀ could be related to its ability to prevent PTP opening and thus apoptosis (70). It is reported by Singh et al. that CoQ₁₀ supplements could increase the levels of vitamins A, C, and E (75, 76), hence some of its effects might be related to this function.

Since better results was obtained in combined therapy (CoQ₁₀+LLLT 980nm) and based on our knowledge from literature we believe that there might be separated or

synergetic mechanisms for this phenomenon. It is possible that each of these modalities that we used acts in its own way to reduce pain, prevents apoptosis or inhibits the inflammation process. There are numerous evidences presented that support this possibility. It is also possible that they acted together with same or other mechanisms. From this point of view it is shown that LLLT and CoQ₁₀ under different or same pathway are simultaneously able to inhibit proinflammatory process (11, 23, 25), enhancing mitochondrial respiratory chain (11, 42) inhibiting or down regulating the apoptotic cascade (43, 68, 71) and decreasing the effects of oxidative stress (72-74, 43-46). The specific mechanisms for these events are still unknown and more studies needed to explain them, and the possibility of adverse effects of LLLT and CoQ₁₀ should be considered in future.

Conclusion

The CoQ₁₀ can prevent deleterious effects of nerve injury and laser application at 980nm was also effective in promoting early functional recovery. The combination of CoQ₁₀ with LLLT showed better effects than each one alone. There might be cellular and molecular synergism in simultaneous use of CoQ₁₀ and LLLT. Under careful guide clinical application of these modalities may be used in treatment of the NP.

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