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Efficiencies of Low-Level Laser Therapy (LLLT) and Gabapentin in the Management of Peripheral Neuropathy: Diabetic Neuropathy

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Abstract Diabetic neuropathy (DN) is the highly occurred complication of diabetes mellitus; it has been defined as an event of peripheral nerve dysfunction characterized by pain, allodynia, hyperalgesia, and paraesthesia. The current study was conducted to evaluate the efficacy of low-level laser therapy (LLLT) in the management of neuropathy in diabetic rats. The used animals were divided into the following groups: negative control, streptozotocin-induced diabetic rats, and diabetic rats with peripheral neuropathy (DNP) and DNP treated with gabapentin or with LLLT. Behavioral tests were carried out through hotplate test for the determination of pain sensations and the Morris water maze test for spatial reference memory evaluation. Blood samples were collected at the end of treatment for biochemical determinations. In the current study, the latency of hind-paw lick decreased significantly when DNP are treated with gabapentin or LLLT. The Morris water maze test showed that LLLT treatment improved memory that deteriorated in DNP more than gabapentin do. The results of the biochemical study revealed that LLLT could not affect the level of beta-endorphin that decreased in DNP but significantly decreased S100B that rose in DNP. PGE2 and cytokines IL-1β, IL-10, and TNF-α showed significant increase in DNP compared with control group. The gabapentin administration or LLLT application significantly reversed the levels of the mentioned markers towards the normal values of the controls. Levels of serum MDA and nitric oxide increased significantly in the DNP but rGSH showed significant decrease. These markers were improved significantly when the DNP were treated with gabapentin or LLLT. The treatment with gabapentin or LLLT significantly decreased the raised level in total cholesterol in DNP but could not decrease the elevated level of triglycerides, while LDL cholesterol decreased significantly in DNP treated with gabapentin but not affected by LLLT.

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Values of serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), urea, and creatinine increased significantly in the DPN and diabetic rats without peripheral neuropathy (PN) compared with control group. The treatment of DNP with gabapentin induced significant increases in ALAT and ASAT activities but LLLT treatment induced significant decreases in ALAT and ASAT activities as compared with DNP group. Neither gabapentin nor LLLT could improve the elevated levels of serum urea and creatinine in the DNP. It could be concluded that LLLT is more safe and effective than gabapentin in the management of neuropathy in diabetic rats.

Keywords Low-level laser therapy · Diabetes · Neuropathy · Gabapentin

Introduction

Diabetes mellitus (DM) is considered as a complex metabolic disease because it affects the metabolism of glucose and other metabolites [1]. It is characterized by chronic hyperglycemia associated with many pathophysiological deteriorations like polyuria, polydipsia, blindness, polyphagia, sore heals, weight loss or gain, and burning and tingling sensation [2]. The most common and debilitating microvascular complication of diabetes is diabetic peripheral neuropathy (DPN), affecting 50–90% of people with diabetes [3].

Neuropathy is a nerve damage. Nerve cells are vulnerable to damage from disease or anything that impairs the body's ability to turn nutrients into energy, to process waste products, to circulate oxygen, or to make cellular repair. Diabetes does create the nerve cells vulnerable to damage, but there are many ways in which nerves can get damaged [4].

DPN is a widespread disorder comprising damage of peripheral nerves. DPN develops as a complication of hyperglycemia as well as metabolic disturbance, mainly due to oxidative stress [5]. Pathologically axonal damage and segmental demyelination can be seen with diabetic neuropathies. Management of diabetic neuropathy should begin at the initial diagnosis of diabetes and mainly requires tight and stable glycemic control [6]. Extremely long axons originating in the small neuronal body are vulnerable on the most distal side as a result of malnutritional axonal support or environmental insults [7]. Sparse vascular supply with impaired autoregulation is likely to cause hypoxic damage in the nerve. Such dual influences exerted by long-term hyperglycemia are critical for peripheral nerve damage, resulting in distal-predominant nerve fiber degeneration [7].

Peripheral neuropathy involves changes in sensory and motor nerves, as well as autonomic nerves [8]. One or more types of nerves may be affected. Muscle weakness, cramps, spasms, and loss of balance are the main symptoms associated with damage to the motor nerve [9], whereas tingling, numbness, and a burning pain are associated with sensory nerve damage [9]. Disturbance in involuntary and bladder functions and abnormal blood pressure and heart rate are caused in autonomic nerve damage [9]. Until now, there is no strategy to completely treat or reverse nerve injury although hyperglycemic treatments decelerate diabetic neuropathy and decrease the development of symptoms. The obtainable treatments of neuropathy are only sedative, with the purpose of minimizing pain.

Laser is a noninvasive, nonionizing, monochromatic electromagnetic high concentrated light beam [10]. Low-level laser therapy (LLLT) has a large spread of usage in medicine. During the previous decades, LLLT has been used in the treatment of a variety of pathological conditions such as musculoskeletal complications, wounds, and pain controls [11]. It has been

found that LLLT also induces collagen synthesis, protein synthesis, tissue repair, and pain relief and accelerates the healing of injured nerve fiber [12, 13]. Laser has also been admitted as a noninvasive therapy that was added to medicine and physiotherapy. It is suggested that the bio-effect of LLLT on animal and human tissues is through photochemical cellular reactions that mediated by photochemical actions at the cellular level [14]. It is used also for the improvement of tissue hypoxia/ischemia and inflammation in nerve entrapment neuropathy, as well as for the promotion of nerve regeneration [15]. Therefore, the current study aimed to evaluate the efficacy of low-level laser therapy (LLLT) in amelioration of diabetic peripheral neuropathy-induced rat model.

Materials and Methods

Chemicals and Kits

Streptozotocin (STZ) and the anti-neuropathy drug (Gabapentin®) produced by Sigma Co. Kits for the determination of alanine aminotransferase (ALAT) and aspartate transaminase (ASAT) activities as well as for lipid peroxidation (MDA), nitric oxide (NO), glutathione (GSH), urea, creatinine, cholesterol, triglycerides, and LDL cholesterol and HDL cholesterol levels in the animal sera were obtained from Leader Trad Co. ELISA kits for the determination of serum S100B, beta-endorphin, PGE2, TNF-α, IL-1β, and IL10 were purchased from ASSAY Pro, USA. Other chemicals, disposables, plastics, glasses, small equipment, and solvents were obtained from the stores on the National Research Centre.

Animals and Treatments

One hundred adult male albino rats (weighting 150–200 g) were obtained from the animal house, in the National Research Centre. The animals were housed in suitable plastic cages for 1 week for acclimation with the new room conditions. Fresh tap water and standard rodent food pellets (proteins, lipids, fibers, NaCl, lysine, methionine, vitamins, salts, and wheat) were always available. All animals received human care in compliance with the standard institution's criteria for the care and use of experimental animals according to the National Research Centre, ethical committee (committee number FWA 00014747).

After acclimation, the animals were randomly divided into main two main groups. The first one is the negative control (*control group*, *ten rats*) which comprised of healthy male rats and did not receive any treatments, while the second one is the diabetic group. Animals were made diabetic by intraperitoneally injecting with streptozotocin (55 mg/kg b.w.) dissolved in ice cold 0.1 M sodium citrate buffer (20 ml of 0.1 M sodium citrate with 30 ml of 0.1 M citric acid, pH = 4.0) followed by oral administration of 2–3 ml sucrose solution 10% (*w*/*v*) for 1 day. Animals were fasted overnight and one drop blood sample was obtained by nicking the lateral tail vein using a sterile surgical scissors and immediately the blood glucose level was determined using GlucoDr Super Sensor AGM-2200, Korean glucometer, and its test strips. Animals with blood glucose level above 240 mg/dl were considered to be diabetic [16]. After 1 week, ten subjects from the diabetic rats served as diabetic without peripheral neuropathy (*D without PN group*) and the rats of the control group were subjected to hotplate and maze tests as behavioral estimation then were fasted overnight, and following diethyl ether anesthesia, blood specimens from each animal were withdrawn from the retro-orbital plexus using

heparinized and sterile glass capillary (single draw vacutainer needle) into open vacutainer collecting tubes. Immediately, blood samples were centrifuged and blood sera were separated in aliquots and frozen for further biochemical investigations.

The remaining diabetic rats were feed high-fat diet for 12 weeks for establishing peripheral neuropathy and then subdivided into three groups. One group of which served as diabetic with peripheral neuropathy (*DPN group*) and was subjected to hotplate and maze tests then fasted overnight and their blood samples were collected for biochemical analysis. The second group (peripheral neuropathy-induced animals) was treated daily for 8 weeks with pharmaceutical drug (*gabapentin 20 mg/kg) diluted with sterilized water (*diabetic with PN+ Gab*) [17]. The third group of peripheral neuropathy-induced animals was treated: three sessions per week, with LLLT (gallium arsenide 808-mW laser) at three anatomical points (sciatic notch, popliteal fossa, planter of foot) for 30 s each point at a dose of 3 J/s each point (*diabetic with PN+ LLLT group*) [18]. After 8 weeks of treatment, the last two groups were subjected to hotplate and maze tests then fasted overnight and their blood samples were collected for biochemical analysis.

Latency or Hotplate Test

Hotplate was manipulated because it has previously been suggested that pain sensations are elicited by different thermal stimuli. It may initially recruit Aδ or C fibers. The hotplate test was conducted during the subjects light period on an IITC Export model 35-D Analgesia meter in a dimly lit room. A Plexiglas box without a lid 25 cm high enclosed the hotplate.

Latency to the first hind-paw lick or escape from the cylinder which rarely occurred was used as the behavioral endpoint. Sessions were terminated after 60 s when tests were conducted at 45 °C to avoid tissue damage. The maximum latency was assigned to any subject that failed to emit the hind-paw lick or to escape [19].

The Morris Water Maze Test

Spatial reference memory was evaluated using this test. In brief, the apparatus was a plastic round tub (120 cm in diameter) filled with room temperature water containing a submerged platform (10 cm in diameter). The platform remained in a fixed location across all days and trials, testing spatial reference memory; the test consisted of six trials/day for 3 days. Animals were dropped off at different starting points (north, south, east, or west) for each trial, varying semi-randomly. Animals had 60 s to locate the platform where they remained for 15 s before being placed back into a heated cage awaiting the next trial. The inter-trial interval was approximately 5–8 min. To evaluate whether animals spatially localized the platform, a seventh probe trial was given on the third day of testing, during which the platform was removed and animals were given 60 s to swim freely in the maze [20].

Serum Biochemistry, Oxidative Stress, and Inflammatory Markers

Using spectrophotometry analysis, serum ALAT and ASAT activities as well as lipid peroxidation (MDA), nitric oxide, GSH, urea, creatinine, total cholesterol, triglycerides, and LDL cholesterol and HDL cholesterol levels were assayed, while ELISA technique was used in the determination of serum S100B,beta-endorphin, PGE2, TNF-α, IL-1β, and IL10 levels.

Statistical Analysis

The obtained data were subjected to one way analysis of variance (ANOVA) followed by the Duncan test at level of $P \leq 0.05$ [21], using statistical analysis system (SAS) program software (copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA).

Results

Peripheral thermal sensitivity was assessed by paw withdrawal threshold and hind-paw lifting duration, as an index of heat hyperalgesia. The latency of hind-paw lick (measured by seconds) increased significantly in diabetic rats with PN (DPN) but did not affected in diabetic rats without PN compared with the control group. The latency of hind-paw lick decreased significantly when DPN treated with gabapentin or LLLT compared with the DPN (Table 1). The Morris water maze test showed that LLLT treatment improved the deteriorated memory in DPN more than gabapentin does (Table 1).

In comparison with the control group, serum β-endorphin decreased significantly in diabetic rats without PN but S100B was not affected. In DPN, β-endorphin showed significant decrease and S100B showed significant increase in their levels compared with the control values. Neither gabapentin nor LLLT could improve the reduction in β-endorphin level which occurred in DPN. On the other hand, gabapentin could increase significantly the raised level of S100B in DPN; in contrast, LLLT could decrease it (Table 2).

PGE2 and the cytokines IL-1β, IL-10, and TNF-α showed significant increase in DPN and diabetic rats without PN. The administration of gabapentin or LLLT application significantly decreased the levels of the mentioned markers towards the normal values of the controls (Table 3).

Levels of serum lipid peroxidation (MDA) and nitric oxide (NO) increased significantly in DPN and the diabetic rats without PN but reduced glutathione (rGSH) showed significant decrease in DPN. MDA and NO were improved significantly when DPN treated with gabapentin or LLLT but rGSH was not affected (Table 4).

Levels of serum cholesterol, triglycerides, and LDL cholesterol showed significant increase in the DPN and without PN. The treatment with gabapentin or LLLT significantly decreased the raised level in total cholesterol in DNP but could not decrease the elevated level of triglycerides, while as LDL cholesterol decreased significantly in DNP treated with gabapentin

Table 1 Serum fasting blood glucose, latency of hind-paw lick (seconds), and the Morris water maze test of STZ diabetic animal groups with peripheral neuropathy treated with either gabapentin or LLLT

Group	Fasting blood glucose (mg/dl)	Hotplate test (seconds)	The Morris water maze test
Control	99 ± 21^{B}	33 ± 2.1^{A}	6/6 healthy memory
D without PN	406 ± 37.3^{A}	36 ± 2.3^{A}	6/6 healthy memory
D with PN	424 ± 22.8^{A}	54 ± 2.4^{B}	4/6 deteriorated memory
D with PN + Gab.	402 ± 24.6^{A}	30 ± 2.2^{A}	2/6 deteriorated memory
D with PN + LLLT	409 ± 20^{A}	39 ± 2.1^{A}	1/6 deteriorated memory

All data are expressed as mean ± standard deviation for ten animals per group. Data were subjected to one way ANOVA and the Duncan test. Means with different superscript letters are significantly different at $p \leq 0.05$

D diabetic, *PN* peripheral neuropathy, *Gab.* gabapentin, *LLLT* low-level laser therapy

Table 2 Levels of serum beta-endorphin and S100B of STZ diabetic animal groups with peripheral neuropathy treated with either gabapentin or LLLT

Group	β-endorphin (pg/ml)	S100B (pg/ml)
Control	51.9 ± 3.89^{A}	51 ± 4.2^{C}
D without PN	45.1 ± 6.8^{B}	48 ± 3.5^{C}
D with PN	33.4 ± 5.1^{C}	113 ± 9.5^{B}
D with PN + Gab.	37.1 ± 5.6^{C}	126 ± 10.5^{A}
D with PN + LLLT	36.6 ± 5.5^{C}	78 ± 9.2^{D}

All data are expressed as mean ± standard deviation for ten animals per group. Data were subjected to one way ANOVA and the Duncan test. Means with different superscript letters are significantly different at $p \leq 0.05$

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but not affected by LLLT. On the other hand, serum HDL cholesterol showed insignificant changes among the different studied groups (Table 5).

Activities of serum ALAT and ASAT increased significantly in DNP and D without PN. The treatment of DNP with gabapentin induced insignificant increase in ALAT activity and significant increase in ASAT activity but LLLT treatment induced significant decreases in ALAT and ASAT activities as compared with DNP group (Table 6). On the other hand, levels of serum urea and creatinine showed significant increase in the DNP and D without PN. Neither gabapentin nor LLLT could improve the elevated levels of serum urea and creatinine in the DPN (Table 7).

Discussion

In the current study, we used the hotplate test to investigate the pain response. In our results, we observed that the latency of hind-paw lick decreased significantly when diabetic rats with PN are treated with gabapentin or LLLT. This indicated improvement in pain response. Hyperglycemia is the major pathophysiological factor in the development of diabetic neuropathy. It triggers a number of mechanisms that lead to diabetic neuropathy [22] such as generation of free radicals [23], activation of the polyol pathway [24], stray regulation of neurotrophic factors [25], and formation of glycation end products [26]. More details on the mechanisms leading to neuropathy are described by Yagihashi et al. and Bierhaus et al. [7, 27]. Neuronal dysfunction that leads to loss of pain perception triggers sequence of events which in

Table 3 Levels of serum PGE2 and the cytokines of STZ diabetic animal groups with peripheral neuropathy treated with either gabapentin or LLLT

Group	PGE2 (ng/l)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-10 (pg/ml)
Control	196 ± 16^{E}	19 ± 6.70^{D}	715 ± 66^{E}	184 ± 23^{D}
D without PN	344 ± 29^{D}	32 ± 19.2^{C}	1072 ± 95^{C}	263 ± 23^{B}
D with PN	877 ± 56^{A}	98 ± 25.4^{A}	1275 ± 98^{A}	326 ± 29^{A}
D with PN + Gab.	727 ± 60^{B}	73 ± 31.0^{B}	1142 ± 88^{B}	231 ± 21^{C}
D with PN + LLLT	660 ± 55^{C}	72 ± 23.0^{B}	849 ± 79^{D}	221 ± 19^{C}

All data are expressed as mean ± standard deviation for ten animals per group. Data were subjected to one way ANOVA and the Duncan test. Means with different superscript letters are significantly different at $p \leq 0.05$

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Table 4 Levels of serum lipid peroxidation (MDA), nitric oxide (NO), and rGSH of STZ diabetic animal groups with peripheral neuropathy treated with either gabapentin or LLLT

Group	MDA (μmol/l)	NO (μmol/l)	rGSH (nmol/ml)
Control	1.76 ± 0.15^{D}	169 ± 21^{D}	8.2 ± 1.9^{A}
D without PN	2.19 ± 0.18^{C}	245 ± 32^{C}	7.2 ± 1.7^{A}
D with PN	3.60 ± 0.30^{A}	318 ± 41^{A}	6.4 ± 1.5^{B}
D with PN + Gab.	3.21 ± 0.26^{B}	285 ± 36^{C}	5.8 ± 1.2^{B}
D with PN + LLLT	2.24 ± 0.19^{C}	266 ± 55^{BC}	6.7 ± 1.4^{B}

All data are expressed as mean ± standard deviation for ten animals per group. Data were subjected to one way ANOVA and the Duncan test. Means with different superscript letters are significantly different at $p \leq 0.05$

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turn results in diabetic ulcers, which is considered the major cause of morbidity in diabetic patients [28].

LLLT which accelerate the healing of injured tissue is a noninvasive therapy that was added to medicine and physiotherapy for treatment of rheumatoid arthritis and osteoarthritis for joint pain control and functional improvement [29]. It was suggested that the effects of laser radiation are initiated at cellular and subcellular levels that cause electronic excitation of the photoacceptor molecules [30]. This occurs by binding with the corepressor carboxyl-terminal binding protein (CtBP) that is involved in transcriptional pathways important for cell cycle regulation [31]. It was suggested that four possible events follow electronic excitation of the photo acceptors including redox regulation by NAD+ and NADH [31], the changes in formation of superoxide and single oxygen, and the change in biochemical activity [32]. The electron transport in the mitochondrial membrane enhances metabolism and proliferation of cells including generation of ATP from ADP, which could manifest itself as increased DNA and syntheses of protein [11] and hence accelerate the healing of injured nerve cells and improve pain perception. In addition to the significant reduction in the latency of hind-paw lick by LLLT, the improved pain perception in the present work is further confirmed by the significant reduction in S100B level that increased in DPN. This indicates the regeneration of injured nerve cells. S100B is glial-specific protein; it is usually elevated in the adult organism due to nervous system damage, which makes it a potential clinical marker [33]. On the other hand, LLLT could not significantly affect the level of β-endorphin that had significantly decreased in diabetic rats with PN. Others found that LLLT treatments increase peripheral β-endorphin precursor mRNA expression in blood cells of the rats by 830-nm Ga-

Table 5 Levels of some markers of lipid profile in serum of STZ diabetic animal groups with peripheral neuropathy treated with either gabapentin or LLLT

Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL C (mg/dl)	HDL C (mg/dl)
Control	80 ± 11^{D}	52 ± 5.9^{B}	38 ± 8^{C}	29 ± 11^{A}
D without PN	297 ± 42^{C}	292 ± 52^{A}	143 ± 30^{B}	24 ± 9^{A}
D with PN	321 ± 46^{A}	270 ± 48^{A}	220 ± 33^{A}	23 ± 8^{A}
D with PN + Gab.	263 ± 37^{B}	264 ± 46^{A}	165 ± 26^{B}	23 ± 6^{A}
D with PN + LLLT	260 ± 44^{B}	269 ± 47^{A}	209 ± 32^{A}	24 ± 9^{A}

All data are expressed as mean ± standard deviation for ten animals per group. Data were subjected to one way ANOVA and the Duncan test. Means with different superscript letters are significantly different at $p \leq 0.05$

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Table 6 Activities of serum ALAT and ASAT of STZ diabetic animal groups with peripheral neuropathy treated with either gabapentin or LLLT

Group	ALAT (U/l)	ASAT (U/l)
Control	88 ± 7^{D}	155 ± 11^{D}
D without PN	308 ± 25^{B}	233 ± 17^{B}
D with PN	336 ± 28^{A}	248 ± 18^{B}
D with PN + Gab.	351 ± 29^{A}	295 ± 22^{A}
D with PN + LLLT	282 ± 24^{C}	210 ± 15^{C}

All data are expressed as mean ± standard deviation for ten animals per group. Data were subjected to one way ANOVA and the Duncan test. Means with different superscript letters are significantly different at $p \leq 0.05$

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Al-Ar Laser irradiation [34]. This discrepancy is returned to the variation in the wavelength, dosage, duration of LLLT treatment, and site of application.

Several evidences suggest that DM might have also negative effects on cognitive performance [35]. Deteriorated memory in DM appears to be caused by various factors [36] that strongly correlate with cardiovascular disease, such as hypertension and cerebral vascular complications [37]. Honardoost et al. and Reagan et al. [38, 39] suggested that diabetes-related cognitive impairment is attributed to disrupted insulin signaling and glucose homeostasis in the central nervous system. It is found, in another study, by Wessels et al. [40] that hyperglycemia-associated microvascular changes in the brain triggered cognitive deficits in diabetic patients and reduced the risk of microvascular complication. The Morris water maze test, in the current study, showed that LLLT treatment improved the deteriorated memory in diabetic rats with PN more than gabapentin do.

PGE2 and the cytokines IL-1β, IL-10, and TNF-α showed significant increase in serum of DPN rats. A large epidemiological studies showed that inflammation and insulin resistance are both common in several chronic diseases such as diabetes mellitus [41]. Several reports have shown that the accumulation of advanced glycation end products results from hyperglycemia in tissues from diabetic patients, binding with a cellular receptor and initiates a signaling cascade leading to an increase of the nuclear transcription factor (NF-κB) which cause an additional increase in oxidative stress and production of pro-inflammatory cytokines [42]. The inflammatory reaction results in increased levels of TNF-α and IL-1 β [43] which are found to be elevated following nerve injury [44]. IL-10 cytokines also might play a role in the pathogenesis of nerve fiber damage or represent a compensatory or neuroprotective mechanism. Elevated IL-10 level was found to associate with signs of motor nerve demyelination and

Table 7 Levels of serum urea and creatinine of STZ diabetic animal groups with peripheral neuropathy treated with either gabapentin or LLLT

Group	Urea (mg/dl)	Creatinine (mg/dl)
Control	22 ± 6^{B}	0.99 ± 0.08^{D}
D without PN	45 ± 12^{A}	2.03 ± 0.16^{C}
D with PN	47 ± 13^{A}	2.65 ± 0.21^{B}
D with PN + Gab.	50 ± 13^{A}	2.95 ± 0.23^{B}
D with PN + LLLT	48 ± 13^{A}	2.76 ± 0.22^{B}

All data are expressed as mean ± standard deviation for ten animals per group. Data were subjected to one way ANOVA and the Duncan test. Means with different superscript letters are significantly different at $p \leq 0.05$

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associated with large nerve fiber damage but not to small fiber function [45]. The administration of gabapentin or LLLT application significantly reversed the levels of the mentioned markers towards the normal values of the controls. This indicates that LLLT can reduce inflammation. Our present results are in analogue with the results of Yamaura et al. [46] which indicated that LLLT treatment reduce pro-inflammatory cytokines such as TNF-alpha, IL-1 beta, and IL-8 produced by synoviocytes from RA patients. Moreover, reduced prostaglandin concentrations with LLLT therapy were reported [47, 48].

In our study, levels of serum lipid peroxidation (MDA) and nitric oxide (NO) increased significantly in the DPN but rGSH showed significant decrease. This study results are agreed with the results of Mourad et al. [49].These markers were improved significantly when the DPN rats were treated with gabapentin or LLLT. It is established that diabetes is associated with oxidative stress due to autoxidation of glucose and glycosylation of proteins [50].The persistent increase in reactive oxygen species and reactive nitrogen species concomitant with a decrease in antioxidant activity leads to the occurrence of oxidative and nitrosative stress which can cause endothelial dysfunction, insulin resistance, and alterations in number and functions of pancreatic 훽 cells [51, 52] and the beneficial effect of LLLT therapy in reducing this oxidative stress may be attributed to the anti-inflammatory activity of LLLT which was demonstrated by several studies [46, 47].

Levels of serum cholesterol, triglycerides, and LDL-cholesterol showed significant increase in the diabetic rats with PN. These results are agreed with the results of Kou et al. [53]. The EURODIAB study found a significant association between the elevated serum cholesterol and triglycerides and the development of diabetic neuropathy [54] and cardiac autonomic neuropathy [55]. Vincent et al. [56] suggested that oxy low-density lipoproteins are one notable “lipid factor” responsible for nervous system injury. Systemic oxidative stress results in the modification of these lipoproteins, which is well characterized in atherosclerosis [57]. The treatment with LLLT significantly decreased the raised level in cholesterol in diabetic rats with PN but could not decrease the elevated level of triglycerides and LDL cholesterol.

Activities of serum ALAT and ASAT increased significantly in DPN group. ALAT and ASAT are released into blood after cellular damage so the increase in these enzymes in serum may be attributed to the damage in the structural integrity of the liver [58]. Liver disease is an important cause of death in type 2 diabetes [59]. This attributed to protein glycation and glucose autoxidation which may generate free radicals, which in turn enhance lipid peroxidation [60, 61] and induce disturbances of antioxidant defense systems [62]. The treatment of diabetic rats with PN with gabapentin induced significant increases in ALAT and ASAT activities but LLLT treatment induced significant decreases in ALAT and ASAT activities as compared with diabetic rats with PN group which is still significantly higher than the control values. These data indicate the toxic effect of gabapentin on hepatic tissue.

Levels of serum urea and creatinine showing significant increase in the diabetic rats with PN indicated the progressive nephrotoxicity. Neither gabapentin nor LLLT could improve the elevated levels of serum urea and creatinine in the diabetic rats with PN. Diabetic nephropathy is one of the most prevalent and serious microvascular complications of diabetes mellitus [63]. Several studies suggest that oxidative stress plays a major role in the pathogenesis of diabetic nephropathy in both type 1 and type 2 diabetes mellitus [64, 65]. Various reports suggest that inflammation is a key pathophysiological mechanism in diabetic nephropathy and that kidney inflammation is crucial in promoting the development and progression of diabetic nephropathy [66, 67]. Furthermore, previous studies have shown that many inflammatory cytokines that play a pivotal role in diabetic nephropathy, such as TGF-β1, TNF-α, and interleukin (IL)-1β,

can also induce tubular epithelial cell-myofibroblast transdifferentiation, which is an important event in diabetic nephropathy [68, 69].

It could be concluded that LLLT is independently effective in the management of DPN and its complications as proven by the biochemical tests. It could be used as an important adjunct modality above or in addition to pharmacological agents like gabapentin.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Sajak, A., Mediani, A., Maulidiani, Ismail, A., & Abas, F. (2017). Metabolite variation in lean and obese streptozotocin (STZ)-induced diabetic rats via 1H NMR-based metabolomics approach. *Applied Biochemistry and Biotechnology, 182*(2), 653–668.
2. Sandireddy, R., Yerra, V. G., Areti, A., Komirishetty P., & Kumar, A. (2014). A neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *International Journal of Endocrinology, 2014*, Article ID 674987, 10 pages.
3. Almuhannadi, H., Ponirakis, G., Khan, A., & Malik, R. A. (2018). Diabetic neuropathy and painful diabetic neuropathy: Cinderella complications in South East Asia. *The Journal of the Pakistan Medical Association, 68*(1), 85–89.
4. Daino, C.(2017). Is there any difference between diabetic neuropathy and other forms of neuropathy?.mcvitamins.com/health%20articles/diabetes-non-diabetic-neuropathy.htm.
5. Hosseini, A., & Abdollahi, M. (2013). Diabetic neuropathy and oxidative stress: therapeutic perspectives. *Oxidative Medicine and Cellular Longevity, 2013*, Article ID 168039, 15 pages.
6. Pasnoor, M., Dimachkie, M. M., Kluding, P., & Barohn, R. J. (2013). Diabetic neuropathy part 1: overview and symmetric phenotypes. *Neurologic Clinics, 31*(2), 425–445.
7. Yagihashi, S., Mizukami, H., & Sugimoto, K. (2011). Mechanism of diabetic neuropathy: where are we now and where to go? *Journal of Diabetes Investigation, 2*(1), 18–32.
8. Pirart, J. (1978). Diabetes mellitus and its degenerative complications: a prospective study of 4400 patients observed. *Diabetes Care, 1*(3), 168–188.
9. Kasper, D., Fauci, A., Hauser, S., Longo, D., Jameson, J. L., & Loscalzo, J. (2015). *Harrison's principles of internal medicine* (19th ed.). United States: McGraw-Hill Education.
10. Dogan, S. K., Saime, A. Y., & Evcik, D. (2010). The effectiveness of low laser therapy in subacromial impingement syndrome: a randomized placebo controlled double-blind prospective study. *Linics, 65*(10), 1019–1022.
11. Lin, D., Huang, M., & Chai, C. (2006). Effects of helium-neon laser on the mucopolysaccharide induction in experimental osteoarthritic cartilage. *Osteoarthritis Cartilage, l4*(4), 377–383.
12. Enwemeka, C. S., Parker, J. C., Dowdy, D. S., Harkness, E. E., Sanford, L. E., & Woodruff, L. D. (2004). The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. *Photomedicine and Laser Surgery, 22*(4), 323–329.
13. Dundar, U., Evcik, D., Samli, F., Pusak, H., & Kavuncu, V. (2007). The effect of gallium arsenide aluminum laser therapy in management of cervical myofacial pain syndrome: a double blind, placebo-controlled study. *Clinical Rheumatology, 26*, 930–934.
14. Brosseau, L., Welch, V., Wells, G., Tugwell, P., de Bie, R., Gam, A., Harman, K., Shea, B., & Morin, M. (2000). Low level laser therapy for osteoarthritis and rheumatoid arthritis: a meta-analysis. *The Journal of Rheumatology, 8*, 1961–1969.
15. Hsieh, Y. L., Chou, L. W., Chang, P. L., Yang, C. C., Kao, M. J., & Hong, C. Z. (2012). Low-level laser therapy alleviates neuropathic pain and promotes function recovery in rats with chronic constriction injury:

possible involvements in hypoxia-inducible factor 1α (HIF-1α). *The Journal of Comparative Neurology, 520*(13), 2903–2916.
16. Kim, H. J., Kong, M. K., & Kim, Y. C. (2008). Beneficial effects of Phellodendri Cortex extract on hyperglycemia and diabetic nephropathy in streptozotocin-induced diabetic rats. *BMB Reports, 41*(10), 710–715.
17. Hamidi, G. A., Jafari-Sabet, M., Abed, A., Mesdaghinia, A., Mahlooji, M., & Banafshe, H. R. (2014). Gabapentin enhances anti-nociceptive effects of morphine on heat, cold, and mechanical hyperalgesia in a rat model of neuropathic pain. *Iranian Journal of Basic Medical Sciences, 17*(10), 753–759.
18. Joensen, J., Gjerdet, N. R., Hummelsund, S., Iversen, V., Lopes-Martins, R. A., & Bjordal, J. M. (2012). An experimental study of low-level laser therapy in rat Achilles tendon injury. *Lasers in Medical Science, 27*(1), 103–111.
19. Mikołajczak, P. Ł., Kędzia, B., Ożarowski, M., Kujawski, R., Bogacz, A., Bartkowiak-Wieczorek, J., Białas, W., Gryszczyńska, A., Buchwald, W., Szulc, M., Wasiak, N., Górska-Paukszta, M., Baraniak, J., Czerny, B., & Seremak-Mrozikiewicz, A. (2015). Evaluation of anti-inflammatory and analgesic activities of extracts from herb of Chelidonium majus L. *Central European Journal of Immunology, 40*(4), 400–410.
20. Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*(5868), 681–683.
21. Steel, R. G., & Torrie, G. H. (1980). *Principles and procedures of statistics: a biometrical approach* (p. 633). New York: McGraw-Hill.
22. Vinik, A. I., Park, T. S., Stansberry, K., & Pittenger, G. L. (2000). Diabetic neuropathies. *Diabetologia, 43*(8), 957–973.
23. Greene, D. A., Stevens, M. J., Obrosova, I., & Feldman, E. L. (1999). Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. *European Journal of Pharmacology, 375*(1-3), 217–223.
24. Oates, P. J. (2008). Aldose reductase, still a compelling target for diabetic neuropathy. *Current Drug Targets, 9*(1), 14–36.
25. Sahenk, Z. (2006). Neurotrophins and peripheral neuropathies. *Brain Pathology, 16*(4), 311–319.
26. Schmid, U., Stopper, H., Heidland, A., & Schupp, N. (2008). Benfotiamine exhibits direct antioxidative capacity and prevents induction of DNA damage in vitro. *Diabetes/Metabolism Research and Reviews, 24*(5), 371–377.
27. Bierhaus, A., Haslbeck, K. M., Humpert, P. M., Liliensiek, B., Dehmer, T., Morcos, M., Sayed, A. A., Andrassy, M., Schiekofer, S., Schneider, J. G., Schulz, J. B., Heuss, D., Neundörfer, B., Dierl, S., Huber, J., Tritschler, H., Schmidt, A. M., Schwaninger, M., Haering, H. U., Schleicher, E., Kasper, M., Stern, D. M., Arnold, B., & Nawroth, P. P. (2004). Loss of pain perception in diabetes is dependent on a receptor of the immunoglobulin superfamily. *The Journal of Clinical Investigation, 114*(12), 1741–1751.
28. Boulton, A. J. (1998). Lowering the risk of neuropathy, foot ulcers and amputations. *Diabetic Medicine, 15*(4), 57–59.
29. Helewa, A. (1996). Physical therapy management of patients with rheumatoid arthritis and other inflammatory condition. In J. M. Walker & A. Helewa (Eds.), *Physical therapy in arthritis* (p. 245e65). Philadelphia: W.B. Saunders.
30. Hecht, J. (1992). *Understanding lasers*. New York: IEEE Press.
31. Zhang, Q., Piston, D. W., & Goldmann, R. H. (2002). Regulations of corepressor function by nuclear NADH. *Science, 295*(5561), 1895–1897.
32. Karu, T. (1989). *Photobiology of low-power laser therapy*. New York: Harwood.
33. Yardan, T., Erenler, A. K., Baydin, A., Aydin, K., & Cokluk, C. (2011). Usefulness of S100B protein in neurological disorders. *The Journal of the Pakistan Medical Association, 61*(3), 276–281.
34. Hagiwara, S., Iwasaka, H., Hasegawa, A., & Noguchi, T. (2008). Pre-irradiation of blood by gallium aluminum arsenide (830 nm) low-level laser enhances peripheral endogenous opioid analgesia in rats. *Anesthesia and Analgesia, 107*(3), 1058–1063.
35. Biessels, G. J., Deary, I. J., & Ryan, C. M. (2008). Cognition and diabetes a lifespan perspective. *Lancet Neurology, 7*(2), 184–190.
36. Kodl, C. T., & Seaquist, E. R. (2008). Cognitive dysfunction and diabetes mellitus. *Endocrine Reviews, 29*(4), 494–511.
37. Elias, M. F., Elias, P. K., Sullivan, L. M., Wolf, P. A., & D'Agostino, R. B. (2005). Obesity, diabetes and cognitive deficit: the Framingham heart study. *Neurobiology of Aging, 26*(1), 11–16.
38. Honardoost, M., Sarookhani, M. R., Arefian, E., & Soleimani, M. (2014). Insulin resistance associated genes and miRNAs. *Applied Biochemistry and Biotechnology, 174*(1), 63–80.
39. Reagan, L. P., Grillo, C. A., & Piroli, G. G. (2008). The As and Ds of stress: metabolic, morphological and behavioral consequences. *European Journal of Pharmacology, 585*(1), 64–75.
40. Wessels, A. M., Scheltens, P., Barkhof, F., & Heine, R. J. (2008). Hyperglycaemia as a determinant of cognitive decline in patients with type 1 diabetes. *European Journal of Pharmacology, 585*(1), 88–96.

41. Piya, M. K., McTernan, P. G., & Kumar, S. (2013). Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. *The Journal of Endocrinology, 216*, 1–15.
42. Schmidt, A. M., Yan, S. D., Wautier, J. L., & Stern, D. M. (1999). Activation of receptor for advanced glycation end products—a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circulation Research, 84*(5), 489–497.
43. Garcia, F. A., Rebouças, J. F., Balbino, T. Q., da Silva, T. G., de Carvalho-Júnior, C. H., Cerqueira, G. S., Brito, G. A., & Viana, G. S. (2015). Pentoxifylline reduces the inflammatory process in diabetic rats: relationship with decreases of pro-inflammatory cytokines and inducible nitricoxide synthase. *Journal of Inflammation, 12*, 33.1–3310.
44. Stemkowski, P. L., Noh, M. C., Chen, Y., & Smith, P. A. (2015). Increased excitability of medium-sized dorsal root ganglion neurons by prolonged interleukin-1β exposure is K+ channel dependent and reversible. *The Journal of Physiology, 593*(16), 3739–3755.
45. Magrinelli, F., Briani, C., Romano, M., Ruggero, S., Toffanin, E., Triolo, G., Peter, G. C., Praitano, M., Lauriola, M. F., Zanette, G., & Tamburin, S. (2015).The association between serum cytokines and damage to large and small nerve fibers in diabetic peripheral neuropathy. *Journal of Diabetes Research*, *2015*, Article ID 547834, 7 pages.
46. Yamaura, M., Yao, M., Yaroslavsky, I., Cohen, R., Smotrich, M., & Kochevar, I. E. (2009). Low level light effects on inflammatory cytokine production by rheumatoid arthritis synoviocytes. *Lasers in Surgery and Medicine, 41*(4), 282–290.
47. Mizutani, K., Musya, Y., Wakae, K., Kobayashi, T., Tobe, M., Taira, K., & Harada, T. (2004). A clinical study on serum prostaglandin E2 with low-level laser therapy. *Photomedicine and Laser Surgery, 22*(6), 537–539.
48. Bjordal, J. M., Lopes-Martins, R. A., & Iversen, V. V. (2006). A randomised, placebo controlled trial of low level laser therapy for activated Achilles tendinitis with microdialysis measurement of peritendinous prostaglandin E2 concentrations. *British Journal of Sports Medicine, 40*(1), 76–80.
49. Mourad, H. H., EL-Kassaby, M. I., El-Hussieny, E. A., Esmail, R. S., Mannaa, F. A., & Abdel-Wahhab, K. G. (2017). Role of soy protein concentrate on oxidative stress and DNA fragmentation in streptozotocin-induced diabetic rats. *Journal of Innovations in Pharmaceutical and Biological Sciences, 4*, 16–25.
50. Al-Faris, N. A., Al-sawadi, A. D., & Alokail, M. S. (2010). Effect of samh seeds supplementation (Mesembryanthemum forsskalei Hochst) on liver enzymes and lipid profiles of streptozotocin (STZ)-induced diabetic Wistar rats. *Saudi Journal of Biological Sciences, 17*(1), 23–28.
51. Lee, S. H., Park, M. H., Park, S. J., Kim, J., Kim, Y. T., Oh, M. C., Jeong, Y., Kim, M., Han, J. S., & Jeon, Y. J. (2012). Bioactive compounds extracted from Ecklonia cava by using enzymatic hydrolysis protects high glucose-induced damage in INS-1 pancreatic β-cells. *Applied Biochemistry and Biotechnology, 167*(7), 1973–1985.
52. Shrivastava, A., Chaturvedi, U., Sonkar, R., Khanna, A. K., Saxena, J. K., & Bhatia, G. (2012). Antioxidant effect of *Azadirachta indica* on high fat diet induced diabetic Charles Foster rats. *Applied Biochemistry and Biotechnology, 167*(2), 229–236.
53. Kou, L., Du, M., Zhang, C., Dai, Z., Li, X., & Zhang, B. (2017). The hypoglycemic, hypolipidemic, and anti-diabetic nephritic activities of zeaxanthin in diet-streptozotocin-induced diabetic Sprague Dawley rats. *Applied Biochemistry and Biotechnology, 182*(3), 944–955.
54. Tesfaye, S., Chaturvedi, N., Eaton, S. E., Ward, J. D., Manes, C., Ionescu-Tirgoviste, C., Witte, D. R., & Fuller, J. H. (2005). Vascular risk factors and diabetic neuropathy. *The New England Journal of Medicine, 352*(4), 341–350.
55. Kempler, P., Tesfaye, S., Chaturvedi, N., Stevens, L. K., Webb, D. J., Eaton, S., Kerenyi, Z., Tamas, G., Ward, J. D., & Fuller, J. H. (2002). Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM complications study. *Diabetic Medicine, 19*(11), 900–909.
56. Vincent, A. M., Hayes, J. M., McLean, L. L., Vivekanandan-Giri, A., Pennathu, S., & Feldman, E. L. (2009). Dyslipidemia-induced neuropathy in mice: the role of oxLDL/LOX-1. *Diabetes, 58*(10), 2376–2385.
57. Tsuzura, S., Ikeda, Y., Suehiro, T., Ota, K., Osaki, F., Arii, K., Kumon, Y., & Hashimoto, K. (2004). Correlation of plasma oxidized low-density lipoprotein levels to vascular complications and human serum paraoxonase in patients with type 2 diabetes. *Metabolism: Clinical and Experimental, 53*(3), 297–302.
58. Recknagel, R. O., GlendeJr, E. A., & Briton, R. S. (1991). Free radical damage and lipid peroxidation. In R. G. Meeks (Ed.), *Hepatotoxicology* (pp. 401–436). Florida: CRC press.
59. de Marco, R., Locatelli, F., Zoppini, G., Verlato, G., Bonora, E., & Muggeo, M. (1999). Cause-specific mortality in type 2 diabetes: the Verona diabetes study. *Diabetes Care, 22*(5), 756–761.
60. Mullarkey, C. J., Edelstein, D., & Brownlee, L. (1990). Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochemical and Biophysical Research Communications, 173*(3), 932–939.

61. Baynes, J. W. (1991). Role of oxidative stress in development of complications in diabetes. *Diabetes, 40*(4), 405–412.
62. McLennan, S. V., Heffernan, S., Wright, L., Rae, C., Fisher, E., Yue, D. K., & Turtle, J. R. (1991). Changes in hepatic glutathione metabolism in diabetes. *Diabetes, 40*(3), 344–348.
63. Liu, L., Gao, C., Chen, G., Li, X., Li, J., Wan, Q., & Xu, Y. (2013). Notch signaling molecules activate TGF- in rat mesangial cells under high glucose conditions. *Journal of Diabetes Research*, *2013*, Article ID979702, 8 pages.
64. Meenakshi, P., Bhuvaneshwari, R., Rathi, M. A., Thirumoorthi, L., Guravaiah, D. C., Jiji, M. J., & Gopalakrishnan, V. K. (2010). Antidiabetic activity of ethanolic extract of *Zaleya decandra* in alloxan-induced diabetic rats. *Applied Biochemistry and Biotechnology, 162*(4), 1153–1159.
65. Chen, H. C., Guh, J. Y., Chang, J. M., Hsieh, M. C., Shin, S. J., & Lai, Y. H. (2005). Role of lipid control in diabetic nephropathy. *Kidney International. Supplement, 94*, S60–S62.
66. Tuttle, K. R. (2005). Linking metabolism and immunology: diabetic nephropathy is an inflammation disease. *Journal of the American Society of Nephrology, l16*, 1537–1538.
67. Lim, A. K., & Tesch, G. H. (2012). Inflammation in diabetic nephropathy. *Mediators of Inflammation, 2012*, 146154.
68. Doerner, A. M., & Zuraw, B. L. (2009). TGF-beta1 induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1beta but not abrogated by corticosteroids. *Respiratory Research, 10*(1), 100–114.
69. Kamitani, S., Yamauchi, Y., Kawasaki, S., Takami, K., Takizawa, H., Nagase, T., & Kohyama, T. (2011). Simultaneous stimulation with TGF-β1 and TNF-α induces epithelial mesenchymal transitioning bronchial epithelial cells. *International Archives of Allergy and Immunology, 155*(2), 119–128.