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Protective effects of thymoquinone on behavioral and biochemical changes associated with diabetic neuropathic pain

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Diabetic neuropathy is a common and debilitating complication of diabetes mellitus, often marked by chronic pain that significantly impairs quality of life. This study aimed to evaluate the protective effects and mechanisms of action of thymoquinone (TQ), a compound with neuroprotective, antioxidant, hypoglycemic, anti-inflammatory, and antinociceptive properties, on behavioral and biochemical changes associated with diabetic neuropathic pain, compared with pregabalin (PGB). Male Wistar Albino rats were randomly divided into four groups: Control, Diabetes, Diabetes + TQ, and Diabetes + PGB. Diabetes was induced using streptozotocin (45 mg/kg, i.p.). The Diabetes + TQ group received 50 mg/kg TQ, and the Diabetes + PGB group received 30 mg/kg PGB via oral gavage for 14 days. Blood glucose levels were monitored, and nociceptive responses were assessed using tail flick and hot plate tests. Serum and tissue samples were analyzed for TNF-α, IL-1β, IL-6, cGMP, TBARS, SOD, and CAT levels. Hot plate and tail flick latencies were significantly reduced in the diabetes group ($P< 0.05$) but were preserved in both treatment groups, indicating delayed onset of neuropathic pain. TNF-α, IL-1β, IL-6, cGMP, and TBARS levels were significantly elevated in diabetic rats and were significantly attenuated following treatment. In contrast, while PGB treatment significantly increased SOD and CAT levels, the improvements observed with TQ treatment did not reach statistical significance. TQ significantly decreased blood glucose levels, and PGB showed a modest reduction. TQ demonstrated comparable efficacy to PGB in alleviating diabetic neuropathic pain, likely through its hypoglycemic, anti-inflammatory, and antinociceptive effects. These findings support TQ as a promising alternative or adjunctive therapy for managing diabetic neuropathic pain.

Keywords: Diabetic Neuropathic Pain, Hot Plate, Pregabalin, Tail Flick, Thymoquinone

Diabetes is a disease that poses a major challenge to healthcare systems worldwide due to its significant health, social, and economic burdens[1]. Additionally, it is one of the most common chronic diseases. The most frequent complication of diabetes is neuropathy. When neuropathic pain is not adequately treated, it can lead to sleep disturbances and mood disorders, hindering individuals' work and social lives and ultimately reducing their quality of life. As a result, its significance in public health continues to grow[2] .

Reactive oxygen species play a crucial role in diabetes, with hyperglycemia-induced free radical formation being a key factor in the pathogenesis of diabetic neuropathy[3]. Furthermore, oxidative stress has a significant impact on neuronal structural and functional damage[4]. Studies have demonstrated that reducing reactive oxygen molecule levels is effective in alleviating neuropathic pain[5]. On the other hand, optimal glycemic control is known to be the most effective strategy in slowing the progression of diabetic neuropathic pain. However, in the symptomatic treatment of neuropathic pain, medications such as antidepressants (duloxetine, amitriptyline, imipramine, *etc*.), anticonvulsants (pregabalin, gabapentin, phenytoin, carbamazepine, *etc*.), and opioids (tramadol and oxycodone) are frequently used[6].

Thymoquinone (TQ) is a monoterpene and the major bioactive component of *Nigella sativa* (Ranunculaceae) seeds, a medicinal plant with numerous therapeutic properties[7]. *Nigella sativa* seeds (black cumin) have been used in medicine since ancient times, and interest in this compound continues to grow[8]. The U.S. Food and Drug Administration has labeled black cumin seed oil as "Generally Recognized as Safe"[9].

The chemical composition of black cumin seed oil is highly diverse, containing proteins and carbohydrates, with TQ ($C_{10}H_{12}O_2$) as its main

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bioactive constituent (54%)[10]. TQ (2-isopropyl-5-methyl-1,4-benzoquinone) was first synthesized in its pure form in 1910 through the oxidation of thymol (2-isopropyl-5-methylphenol) with hydrogen peroxide, appearing as bright yellow crystals[8,11].

Numerous preclinical studies have demonstrated that TQ exhibits antitussive, gastroprotective, anti-inflammatory, antinociceptive, antihistaminic, antibacterial, anthelmintic, antioxidant, immunomodulatory, anticancer, hepatoprotective, cardioprotective, antidiabetic, ototoxicity-protective, antihypertensive, antiasthmatic, and nephroprotective properties[8,12]. Recent studies have highlighted its neuropharmacological properties, including its anti-inflammatory and antioxidative roles in neurological models. Additionally, TQ has been shown to alleviate neuropathy in an experimental peripheral diabetic model[12].

The neuroprotective effects of TQ, owing to its anti-inflammatory and antioxidant activities, have also been observed in various neurological disorder models, including Alzheimer's disease, Parkinson's disease, autoimmune encephalomyelitis, stroke, schizophrenia, epilepsy, and neuropathic pain[12,13]. TQ appears to be a promising neuroprotective agent capable of preventing neuronal damage associated with trauma, ischemia, and pathological processes in Alzheimer's and Parkinson's disease. Its neuroprotective properties make it particularly intriguing in clinical neurology[8].

This study aims to investigate the protective effect and mechanism of action of TQ in the development of diabetic neuropathic pain, considering its antinociceptive, antioxidant, anti-inflammatory, antidiabetic, and neuroprotective properties. The study uses Pregabalin, a neuroprotective drug with an established indication for neuropathic pain, as a reference.

Materials and Methods

Animal Preparation

This study utilized 24 male Wistar Albino rats, each weighing between 245–330g, obtained from the Experimental Animals and Research Laboratory of Sivas Cumhuriyet University Faculty of Medicine. The animals were housed in plastic cages under a 12:12 h light/dark cycle at a controlled temperature of 22±3°C with 65–70% humidity. They were provided with unlimited access to standard laboratory chow and water. All animals were maintained under conditions compliant with the "Principles of Laboratory Animal Care". Experiments were conducted between 14:00 and 16:00.

Induction of the Diabetes Model

To induce diabetes, a single intraperitoneal (i.p.) dose of 45 mg/kg streptozotocin (STZ), prepared in 0.1 M citrate buffer (pH=4.5), was administered to the designated diabetic groups. Blood glucose levels were measured 48 h after injection. To mitigate hyperinsulinemia and hypoglycemic shock following STZ injection[14], cages were supplemented with water bottles containing 5mmol/L glucose solution[15–17]. Since STZ was prepared in citrate buffer, control groups received an equivalent volume of citrate buffer via intraperitoneal injection (Fig. 1).

Blood Glucose Level Measurements

Forty-eight h after STZ injection, the day on which diabetes was confirmed was designated as day 0 of the study. Blood glucose levels were measured in all groups on days 0 and 15. To collect blood samples, the tails of the animals were cleaned with warm water, and a puncture was made in the tail vein, approximately 5–6cm from the base, using an insulin needle. The first blood drop was wiped away, and the second drop was collected for measurement using a glucometer (Smart Check®) with a test strip. Animals with blood glucose levels exceeding 250 mg/dL[18] were considered diabetic (Fig. 1).

Chemical Substances and Experimental Group Formation

Streptozotocin (STZ; Sigma Aldrich), Thymoquinone (TQ; Sigma 274666), and Pregabalin (Neurica 75 mg Capsule) were purchased for the study. Pregabalin was dissolved in 0.9% isotonic NaCl solution, while TQ was dissolved in corn oil and freshly prepared daily for each experiment. The doses of both compounds were selected based on previously published studies and a review of the relevant literature[1,12,19–23].

- The Control group received an intraperitoneal injection of citrate buffer, followed by 14 days of oral gavage administration of corn oil.
- The Diabetes group received an intraperitoneal injection of STZ, and after diabetes confirmation, they were administered corn oil via oral gavage for 14 days.
- The Diabetes + TQ group received an intraperitoneal injection of STZ, and after diabetes confirmation, they were administered

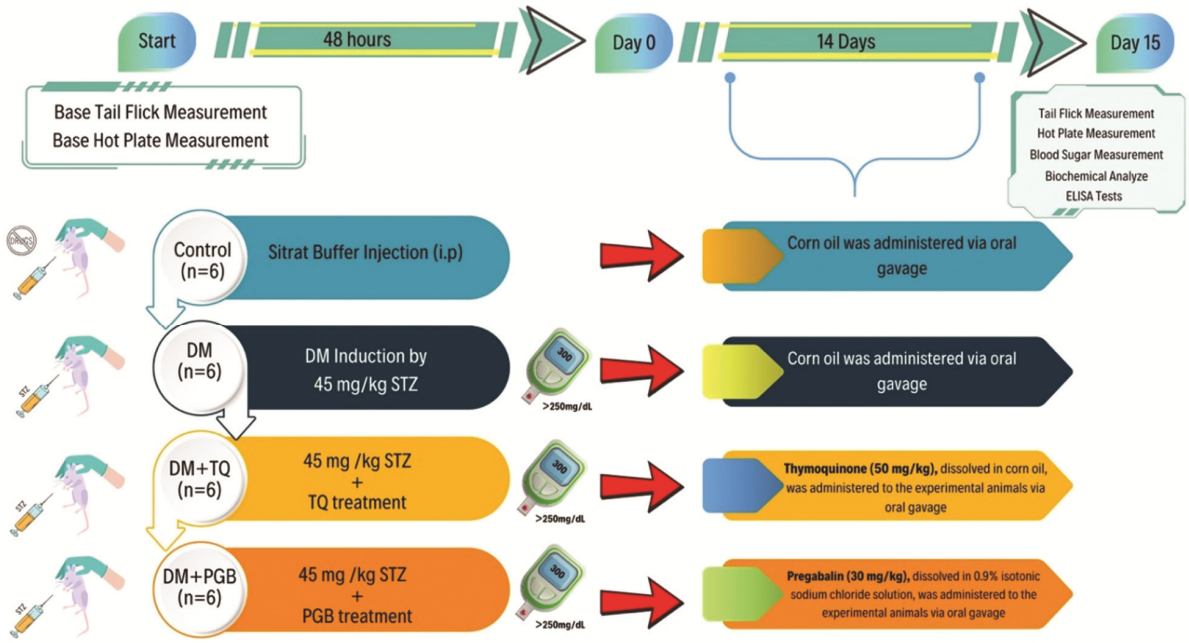


Fig. 1 — Experimental Group Formation and Experimental Process

50 mg/kg of TQ dissolved in corn oil via oral gavage for 14 days.

- The Diabetes + PGB group received an intraperitoneal injection of STZ, and after diabetes confirmation, they were administered 30 mg/kg of Pregabalin dissolved in 0.9% isotonic NaCl solution via oral gavage for 14 days (Fig. 1).

Pain assessment experiments

Tail flick test

Before the pain assessment, all rats were placed in the tail flick device (May TF 0703 Tail Flick Unit, Commat, Ankara, Turkey) without measurement to allow adaptation to the environment. Thermal stimulation was applied to the tail at a point 3 cm from the distal end. The time between the start of heat application and tail withdrawal was recorded as the tail flick latency. To prevent tail injury, the cut-off time was set to 15 sec[24]. Measurements were taken at baseline and after treatment administration (on day 0 and day 15) at 0, 15, 30, 60, 90, and 120 min. All measurements were conducted by the same researcher and analyzed as the mean of repeated measurements. Blinding was not implemented during the behavioral experiments.

Hot plate test

Before the pain assessment, all rats underwent a learning trial without measurement in the hot plate device (May AHP 0603 Analgesic Hot Plate, Commat, Ankara, Turkey). The hot plate surface was set to 52°C to induce thermal hyperalgesia. If the animal did not respond within 60 sec, it was removed from the plate to prevent tissue damage, which was considered the cut-off time[25]. One minute after the tail flick measurements, all animals underwent hot plate testing at the same time intervals. The time until hind paw licking or jumping was recorded using a stopwatch. Measurements were taken at baseline and after treatment administration (on day 0 and day 15) at 0, 15, 30, 60, 90, and 120 min. All measurements were performed by the same researcher and analyzed as the mean of repeated measurements. Blinding was not implemented during the behavioral experiments.

Biochemical Analysis

Serum separation and analysis

At the end of the experiment, blood samples were collected from the hearts of the animals into tubes containing ethylenediaminetetraacetic acid (EDTA) and placed on ice before centrifugation. The samples were centrifuged at 3000 rpm for 15 min. Plasma samples were stored in Eppendorf tubes at -20°C until analysis.

Levels of TBARs, SOD, IL-1β, IL-6, CAT, cGMP, and TNF-α were measured using rat ELISA kits (Sinogeneclon, TBARs catalog no: SG-20510; SOD

catalog no: SG-20188; IL-1β catalog no: SG-20260; IL-6 catalog no: SG-20267; CAT catalog no: SG-20737; cGMP catalog no: SG-20601; TNF-α catalog no: SG-20127), following the kit protocols.

Absorbance was read spectrophotometrically at 450 nm using a Thermo Scientific™ Multiskan™ FC Microplate Photometer ELISA reader. Results were expressed in pg/mL. The final values were calculated by multiplying the dilution factor, considering the dilution ratio. The measurement range was 20 pg/mL–1000 pg/mL, with a sensitivity of 2.5 pg/mL.

Statistical analysis of the experiment

A power analysis was conducted, and with $\alpha = 0.05$, $\beta = 0.20$, and $1-\beta = 0.80$, it was determined that six rats per group were required. The power of the test was found to be $p = 0.80086$.

For statistical analysis, IBM SPSS Statistics 20.0 software was used. Data were transferred to the program, and statistical differences among more than two independent groups were analyzed using One-Way ANOVA and the Tukey HSD multiple comparison test for normally distributed variables. For non-normally distributed variables, the Kruskal-Wallis multiple comparison test was used. Differences within the same group at different time points were analyzed using the Paired Samples *t*-Test.

All values were expressed as mean ± standard deviation (SD). Statistical significance was set at $P< 0.05$. Graphs were created using GraphPad PRISM 6.01.

Results

Mean blood glucose levels in the groups

The mean blood glucose levels of the experimental groups at day 0 and on day 15 are presented in (Fig. 2). Blood glucose levels in the control group remained stable throughout the experimental period, with values of 97.33 ± 12.20 mg/dL at baseline and 97.50 ± 8.96 mg/dL on day 15 ($P> 0.05$). Similarly, the diabetic group maintained persistently elevated blood glucose levels, increasing slightly from 331.66 ± 10.44 mg/dL at baseline to 336.50 ± 11.52 mg/dL on day 15 ($P> 0.05$). In the Diabetes + TQ group, blood glucose levels were significantly reduced from 329.60 ± 11.07 mg/dL at baseline to 163.00 ± 9.36 mg/dL after treatment ($P< 0.05$ compared with day 0). The Diabetes + PGB group also showed a significant decrease in blood glucose levels, from 366.28 ± 10.41 mg/dL at baseline to 314.00 ± 9.61 mg/dL on day 15 ($P< 0.05$ compared with day 0), although glucose levels remained within the hyperglycemic range (Fig. 2).

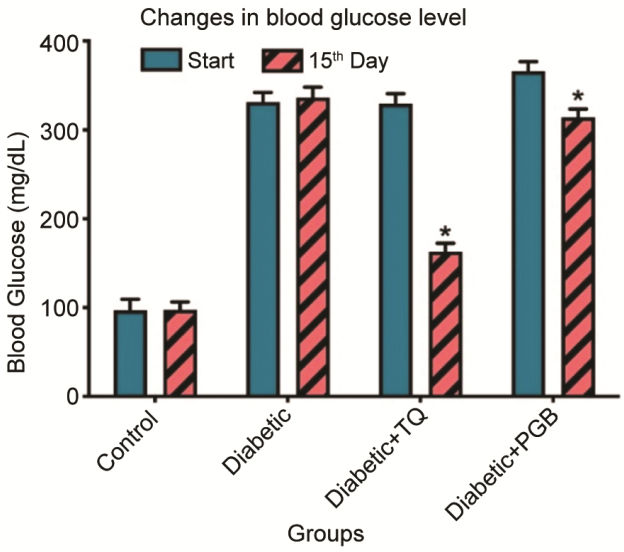


Fig. 2 — Comparison of mean blood glucose levels at the Start and on Day 15 across the groups. $P< 0.05$ (statistically significant compared to Day 0)

Changes in tail flick latency in the groups

At the end of the 14-day treatment period, comparison of pre- and post-treatment values within each group showed a statistically significant decrease in tail flick latency in the Diabetes group, indicating the development of hyperalgesia ($P< 0.05$). In the groups treated with TQ and PGB, no significant change in tail flick latency was observed, supporting a potential protective effect of the treatments (Fig. 3), (Table 1).

Baseline latency values were compared among groups using one-way ANOVA. No statistically significant differences were observed among the groups at baseline ($P> 0.05$), confirming the comparability of the experimental groups before treatment (Table 1). Subsequently, treatment effects were evaluated based on the changes from baseline within each group, allowing assessment of the treatment-induced changes over time.

Changes in hot plate latency in the groups

Following the 14-day treatment period, within-group comparisons of pre- and post-treatment values showed a statistically significant decrease in hot plate latency in the Diabetes group ($P< 0.05$). In

Table 1 — Mean change in tail flick latency (sec) across the groups

Groups	Baseline (Mean ± SD)	Day 15 (Mean ± SD)	n
Control Group	4.60 ± 0.26	4.60 ± 0.21	6
Diabetes Group	4.43 ± 0.18	2.60 ± 0.11	6
Diabetes + TQ Group	4.24 ± 0.20	4.26 ± 0.17	6
Diabetes + PGB Group	4.48 ± 0.24	4.38 ± 0.15	6

Table 2 — Mean change in hot plate latency (sec) across the groups

Groups	Baseline (Mean ± SD)	Day 15 (Mean ± SD)	n
Control Group	8.93 ± 0.15	8.90 ± 0.23	6
Diabetes Group	7.53 ± 0.11	5.30 ± 0.16	6
Diabetes + TQ Group	7.32 ± 0.16	7.10 ± 0.17	6
Diabetes + PGB Group	7.00 ± 0.18	7.32 ± 0.20	6

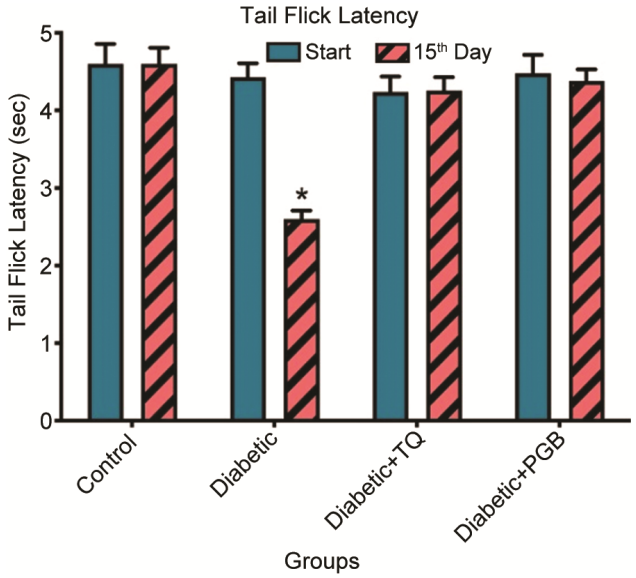


Fig. 3 — Comparison of mean tail flick latency at the Start and on Day 15 across the groups. $P< 0.05$ (statistically significant compared to baseline)

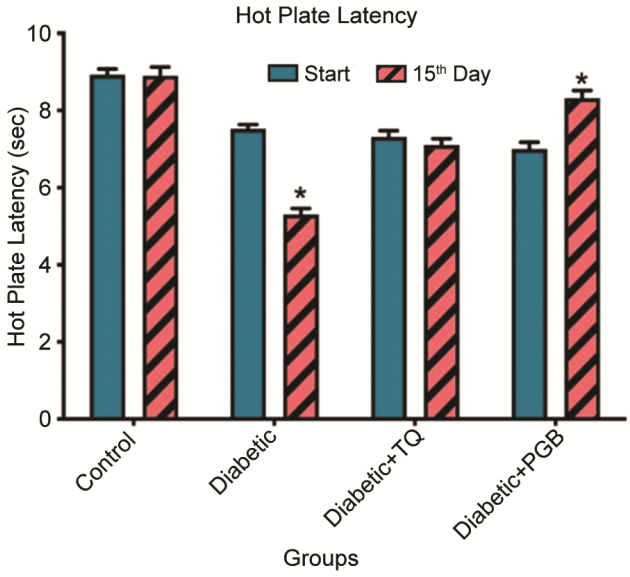


Fig. 4 — Comparison of mean hot plate latency at the Start and on Day 15 across the groups. $P< 0.05$ (statistically significant compared to baseline)

the Diabetes + TQ group, no statistically significant change in latency was observed, supporting a potential protective effect. In contrast, the Diabetes + PGB group exhibited a statistically significant increase in hot plate latency ($P< 0.05$), suggesting a delayed onset of neuropathic pain compared to the diabetic group. No statistically significant difference was observed between the TQ and PGB groups (Fig. 4), (Table 2).

Baseline (Day 0) latency values were compared among groups using one-way ANOVA. Baseline latency values differed significantly among the groups ($P< 0.05$) (Table 2). Therefore, treatment effects were assessed by comparing the changes from baseline within each group rather than the absolute latency values, thereby minimizing the influence of baseline differences among the groups.

Evaluation of ELISA Results

Measurement of TNF-a, IL-1β, and IL-6

In parallel with the development of diabetes and associated inflammation, levels of TNF-α, IL-6, and IL-1β were found to be significantly increased in the diabetic groups compared to the control group ($P< 0.05$, n=6).

IL-1β levels were highest in the Diabetes group ($P< 0.05$), and significantly reduced in the Diabetes + TQ and Diabetes + PGB groups compared to the diabetic group ($P< 0.05$). However, levels remained

elevated compared to the control group ($P< 0.05$). The reduction observed in the Diabetes + PGB group was greater than that in the Diabetes + TQ group ($P< 0.05$).

IL-6 levels were also highest in the Diabetes group ($P< 0.05$), and significantly decreased in the Diabetes + TQ and Diabetes + PGB groups relative to the diabetic group ($P< 0.05$), although they remained elevated compared to the control group ($P< 0.05$). Again, the reduction in the Diabetes + PGB group was greater than that in the Diabetes + TQ group ($P< 0.05$).

TNF-α levels followed a similar pattern: The highest levels were observed in the Diabetes group ($P< 0.05$), with significant reductions in the Diabetes + TQ and Diabetes + PGB groups compared to the diabetic group ($P< 0.05$), though still higher than those in the control group ($P< 0.05$). The decrease in the Diabetes + PGB group was greater than in the Diabetes + TQ group ($P< 0.05$) (Fig. 5).

Measurement of cGMP

cGMP levels were found to be highest in the Diabetes group ($P< 0.05$). Although there was a relative decrease in the Diabetes + TQ and Diabetes + PGB groups compared to the diabetic group, this reduction was not statistically significant. No significant difference was observed between the Diabetes + TQ and Diabetes + PGB groups (Fig. 6).

Measurement of TBARS

TBARS levels were found to be highest in the Diabetes group ($P< 0.05$). In the Diabetes + TQ and Diabetes + PGB groups, TBARS levels showed a significant decrease compared to the diabetic group ($P< 0.05$) and approached those of the Control group ($P< 0.05$). Although the reduction in the Diabetes + TQ group was greater than that in the Diabetes + PGB group, this difference was not statistically significant ($P> 0.05$) (Fig. 7).

Measurement of Antioxidant Parameters (SOD and CAT)

SOD levels were found to be lowest in the Diabetes group ($P< 0.05$). In the Diabetes + PGB group, SOD levels increased slightly compared to the diabetic group ($P< 0.05$), but remained significantly lower than those in the Control group ($P< 0.05$). The increase observed in the Diabetes + TQ group was not statistically significant compared to the diabetic group (Fig. 7).

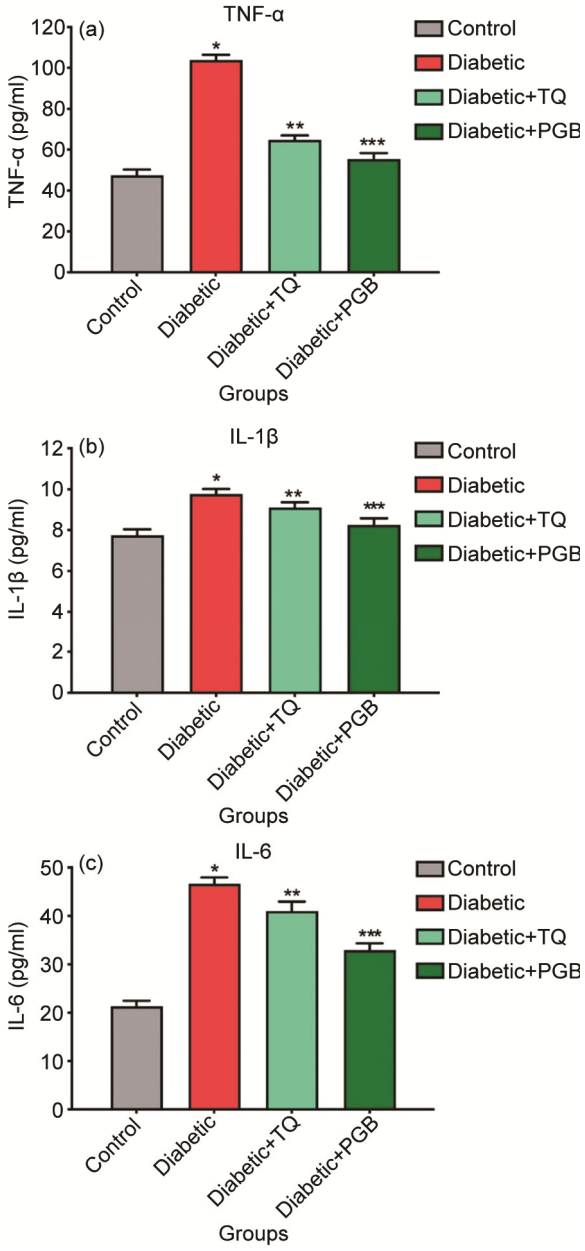


Fig. 5 — Comparison of mean IL-1β, IL-6, and TNF-α levels across the groups (a) Comparison of mean TNF-α levels across the groups; (b) Comparison of mean IL-1β levels across the groups türkçe; and (c) Comparison of mean IL-6 levels across the groups türkçe

CAT levels were also lowest in the Diabetes group ($P< 0.05$). In the Diabetes + PGB group, CAT levels were moderately increased compared to the diabetic group ($P< 0.05$), yet still significantly lower than those in the Control group ($P< 0.05$). The increase in CAT levels in the Diabetes + TQ group was not statistically significant compared to the diabetic group (Fig. 8).

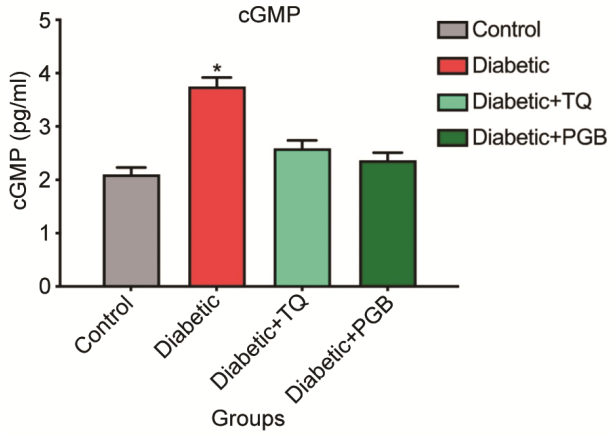


Fig. 6 — Comparison of mean cGMP levels across the groups, *P< 0.05 (statistically significant compared to the control group), **P< 0.05 (statistically significant compared to the diabetes group). *** P< 0.05 (statistically significant compared to all groups)

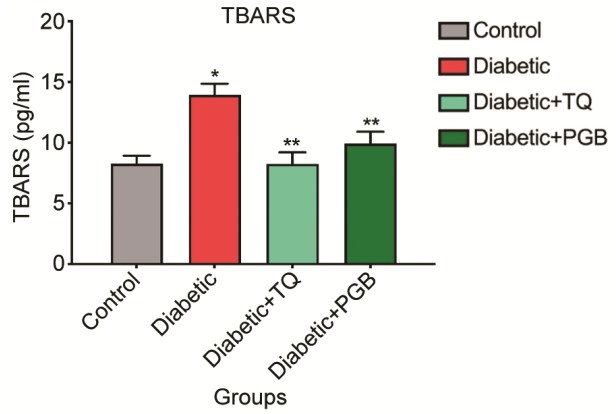


Fig. 7 — Comparison of mean TBARS levels across the groups, *P< 0.05 (statistically significant compared to the control group), **P< 0.05 (statistically significant compared to the diabetes group). ***P< 0.05 (statistically significant compared to all groups)

Discussion

Diabetic neuropathy is a multifactorial disorder of the peripheral nervous system based on neurochemical, neurotrophic, or neuromuscular dysfunctions induced by the metabolic consequences of insulin deficiency and hyperglycemia. Studies have shown that increased oxidative stress and alterations in glucose-sensitive nerve signaling pathways are involved in the pathogenesis of experimental diabetic neuropathy[19].

Several studies have been conducted to investigate the glucose-lowering effects and mechanisms of TQ in diabetes, a metabolic disease characterized by uncontrolled blood glucose levels. TQ administration at doses of 50 mg/kg and 80 mg/kg has been reported to

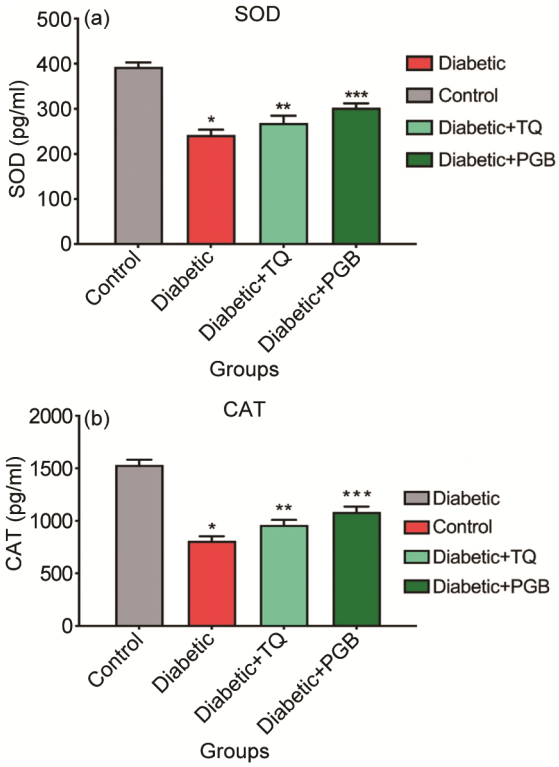


Fig. 8 — Comparison of mean SOD and CAT levels across the groups, (a) Comparison of mean SOD levels across the groups; and (b) Comparison of mean CAT levels across the groups

significantly reduce elevated serum glucose levels[19,26–28]. Moreover, TQ has been shown to enhance the antidiabetic effect of metformin by inhibiting hyperglycemia and oxidative damage[1]. In alignment with previous findings, our study demonstrated that TQ significantly reduced blood glucose levels (P< 0.05). Additionally, PGB was found to slightly reduce blood glucose levels; however, this reduction was less pronounced than that observed with TQ.

In this study, tail flick and hot plate tests were performed to evaluate STZ-induced diabetic neuropathic pain. Numerous studies in the literature have demonstrated that a single dose of STZ induces early-onset allodynia and hyperalgesia, making it a widely used model[21]. In STZ-induced diabetes, thermal pain is initially observed as hyperalgesia followed by hypoalgesia[29], and hyperalgesia is expected around the second and fourth weeks[29–31]. TQ has been reported to increase pain threshold in experimental diabetic neuropathy[32]. Abdel-Fattah *et al.* (2000) demonstrated that TQ (4 mg/kg, i.p.) exerts antinociceptive effects through indirect activation of supraspinal μ1 and δ-opioid receptor subtypes in mice[33]. Amin *et al.* (2014) reported

that TQ, administered at 5 mg/kg i.p. for 14 days immediately after nerve injury, exhibited antinociceptive properties likely due to its antioxidant effects[34]. In another study, Javidi *et al.* (2016) showed that TQ significantly reduced behavioral signs of neuropathic pain and had antinociceptive effects[35]. In a study by Çelik *et al.* (2014), TQ was administered via nasogastric route at doses of 100, 200, and 400 mg/kg for two weeks in a spinal cord injury (SCI)-induced neuropathic pain model using Wistar Albino rats. No dose-dependent difference was found between TQ groups[36]. In our study, comparison of the control and diabetes groups revealed statistically significant thermal hyperalgesia in the diabetic rats after 14 days ($P< 0.05$). In line with previous reports on the antinociceptive effects of TQ, our tail flick and hot plate results demonstrated that TQ suppressed nociceptive responses and significantly prolonged latency. No significant difference was found between the Diabetes + PGB and Diabetes + TQ groups in either pain test, indicating delayed onset of neuropathic pain compared to the diabetic group. Our findings suggest that TQ may be as protective as PGB in preventing diabetic neuropathic pain.

Several studies have shown that TQ modulates inflammatory molecules such as interferons, TNF-α, interleukins, and oxidative stress markers[9,13]. Superoxide dismutase (SOD) and catalase (CAT) are major antioxidant defense mechanisms against the toxic effects of oxygen metabolism. In oxidative stress, superoxide (O_2^-) is rapidly cleared from healthy tissues by SOD[37]. These antioxidant enzymes regulate the concentration of reactive oxygen species, thereby reducing their toxic effects[38,39]. TQ exhibits antidiabetic effects by reducing reactive oxygen species production and preventing β-cell damage. TQ treatment has been reported to reduce malondialdehyde (MDA) levels and increase SOD activity in the pancreatic tissues of diabetic rats[20].

In diabetes, increased lipid peroxidation leads to elevated oxidative stress and cellular damage. Due to the lack of an efficient antioxidant system in β-cells, these cells are more vulnerable to oxidative stress than others[40]. Products of lipid peroxidation (lipid hydroperoxides and aldehydes) are collectively referred to as Thiobarbituric Acid Reactive Substances (TBARS), which can be measured spectrophotometrically or fluorometrically as MDA equivalents in tissue or body fluids[41]. TQ has been reported to reduce elevated MDA levels[42–45]. Sayed-Ahmed and Nagi (2007) demonstrated that TQ (50 mg/L in drinking water) reduced TBARS and increased GSH, GPx, CAT, and ATP levels[46]. In line with these findings, our study showed that antioxidant enzyme levels (CAT and SOD) decreased with diabetes, and this reduction was prevented by PGB treatment. However, no significant improvement was observed with TQ treatment. TBARS levels were significantly elevated in the diabetic group and were significantly attenuated following treatment with both TQ and PGB. Nevertheless, no statistically significant difference was observed in the reduction of TBARS levels between the TQ- and PGB-treated groups ($p > 0.05$).

Studies investigating the effects of TQ on neurotoxicity induced by toxic metals like $AlCl_3$ and arsenic exposure have shown that TQ significantly reduces oxidative stress and mitigates associated adverse effects[47]. TQ also provided neuroprotection against acrylamide-induced neurotoxicity in rats, possibly by attenuating lipid peroxidation[48].

Recent studies have explored the role of cGMP in neural tissues, suggesting that the cGMP pathway plays a crucial role in neuronal development and may be implicated in neuropathies and diabetic retinopathy. Dysregulation of the cGMP pathway is believed to contribute to the development of atherosclerosis and vascular complications in hypertension and diabetes. In experimental models, moderate acute increases in cGMP do not lead to apoptosis, whereas prolonged elevation may induce apoptosis in pancreatic β-cells in both rats and humans[49]. TQ has been shown to reduce NO production in macrophages, potentially benefiting the treatment of inflammatory and autoimmune diseases[50]. Consistent with the literature, our study found no statistically significant differences in elevated cGMP levels following TQ and PGB treatments. This result may be attributed to the use of serum rather than tissue samples in the ELISA analysis.

Numerous studies have investigated the anti-inflammatory properties of TQ. It has been shown to reduce pro-inflammatory cytokines such as IL-1β and TNF-α[51,52]. In a study by Alkharfy *et al.* (2011), mice treated with TQ had relatively lower levels of TNF-α and IL-2[53]. In another study by Çelik *et al.* (2014), TQ treatment resulted in lower levels of TNF-α, NO, MDA, and IL-1β in SCI-induced neuropathic pain[36]. Gökce *et al.* (2016) compared the neuroprotective effects of TQ and methylprednisolone in spinal cord ischemia-reperfusion (I/R) injury in Wistar Albino

rats, reporting significant reductions in tissue MDA, NO, and TNF-α level with TQ. TQ also restored SOD and CAT levels reduced by spinal cord I/R injury[48]. Similarly, Kazancı *et al.* (2017) investigated the effects of PGB pre-treatment on spinal cord I/R injury and found that PGB and methylprednisolone both increased GPx, SOD, and CAT levels, with no significant difference between the two. Both treatments significantly reduced MDA, NO, TNF-α, and IL-1β levels[54]. Kassab and El-Hennamy (2017) also reported that TQ (10 mg/kg) reduced MDA, NO, and TNF-α levels while increasing CAT and SOD in an arsenic-induced neurotoxicity model[55]. Consistent with these findings, our study showed that inflammatory markers (IL-1β, IL-6, and TNF-α) increased in diabetes and were reduced by TQ and PGB treatments, although these reductions were not statistically significant.

Conclusion

In the current study our findings indicate that thymoquinone (TQ), through its hypoglycemic, anti-inflammatory, and antinociceptive properties, may be as effective as pregabalin (PGB) in providing protection against diabetic neuropathic pain.

As the prevalence of diabetes continues to rise globally, neuropathic pain remains one of its most common and debilitating complications. Considering the side effects of current pharmacological treatments, our study may contribute to the development of new agents aimed at preventing or delaying the onset of diabetic neuropathic pain.

To our knowledge, this is the first study to compare the prophylactic effects of TQ and PGB in preventing diabetic neuropathic pain. Although promising results were obtained, further studies are needed to confirm these findings and to elucidate the underlying mechanisms. Future research should consider different treatment durations and combinations of TQ, and incorporate electrophysiological methods such as EMG to evaluate nerve conduction — a limitation in our current study.

Acknowledgement

This study was conducted with the ethical approval of the Local Ethics Committee for Animal Experiments of Cumhuriyet University, under decision number 65202830-050.04.04-52 dated 04.05.2017. It was supported by the Scientific Research Projects Unit of Cumhuriyet University (project number T-742).

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of interest

All authors declare no conflict of interests.

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