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The Safety Profiles of *Nigella sativa* Oil as a Supplement in Diabetic Neuropathy Patients: Assessment on Hematology and Hepatorenal Functions

(Profil Keselamatan Minyak *Nigella sativa* sebagai Tambahan pada Pesakit Neuropati Diabetik: Penilaian ke atas Fungsi Hematologi dan Hepatorenal)

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ABSTRACT

The potential effectiveness of *Nigella sativa* oil (NSO) has been shown in patients with diabetic neuropathy. However, its safety profiles in this group of patients remain underexplored. This study aimed to evaluate the hematological and biochemical safety profiles of NSO in this population. A single-center clinical trial was conducted involving 24 patients with diabetic neuropathy who received NSO at a dose of 2 mL/day for 30 days. Hematological and biochemical parameters were assessed before and after intervention. The hematological parameters included hemoglobin (HGB), white blood cell (WBC) count, red blood cell (RBC) count, hematocrit (Hct), and platelet (Plt) count. Meanwhile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine were measured representing hepatorenal parameters. Other adverse events accompanying NSO supplementation were also recorded. The results showed no significant changes in hematological parameters or hepatorenal function markers. A slight increase in mean corpuscular hemoglobin concentration ($p = 0.0483$) was observed, but values remained within normal physiological limits. Other adverse events reported were categorized non-serious, except one transient case of tachycardia that resolved without clinical consequences. The findings suggest that NSO is safe for patients with diabetic neuropathy. Further long-term studies with larger sample sizes are recommended to confirm these results.

Keywords: Diabetic neuropathy; hematological parameters; liver and kidney function; *Nigella sativa*; safety evaluation

ABSTRAK

Keberkesanan potensi minyak *Nigella sativa* (NSO) telah ditunjukkan pada pesakit dengan neuropati diabetik. Walau bagaimanapun, profil keselamatannya dalam kumpulan pesakit ini masih kurang diterokai. Penyelidikan ini bertujuan untuk menilai profil keselamatan hematologi dan biokimia NSO dalam populasi ini. Percubaan klinikal pusat tunggal telah dijalankan melibatkan 24 pesakit dengan neuropati diabetik yang menerima NSO pada dos 2 mL/hari selama 30 hari. Parameter hematologi dan biokimia dinilai sebelum dan selepas intervensi. Parameter hematologi merangkumi hemoglobin (HGB), kiraan sel darah putih (WBC), kiraan sel darah merah (RBC), hematokrit (Hct) dan kiraan platelet (Plt). Sementara itu, aspartate aminotransferase (AST), alanine aminotransferase (ALT) dan kreatinin diukur mewakili parameter hepatorenal. Kejadian buruk lain yang mengiringi suplementasi NSO juga direkodkan. Keputusan menunjukkan tiada perubahan ketara dalam parameter hematologi atau penanda fungsi hepatorenal. Sedikit peningkatan dalam min kepekatan hemoglobin korpuskular ($p = 0.0483$) telah diperhatikan, tetapi nilai kekal dalam had fisiologi normal. Kejadian buruk lain yang dilaporkan dikategorikan sebagai tidak serius, kecuali satu kes sementara takikardia yang diselesaikan tanpa kesan klinikal. Keputusan menunjukkan bahawa NSO adalah selamat untuk pesakit dengan neuropati diabetik. Kajian lanjut jangka panjang dengan saiz sampel yang lebih besar disyorkan untuk mengesahkan keputusan ini.

Kata kunci: Fungsi hati dan buah pinggang; neuropati diabetik; *Nigella sativa;* parameter hematologi; penilaian keselamatan

INTRODUCTION

Diabetic neuropathy is one of the most common complications associated with diabetes mellitus, affecting up to 50% of diabetic patients over their lifetime. It is characterized by nerve damage resulting from chronic hyperglycemia, leading to various symptoms, predominantly in the extremities, including pain, numbness, tingling, and weakness (Hicks & Selvin 2019).

This condition diminishes the quality of life and increases the risk of foot ulcers and amputations due to loss of sensation and poor wound healing. The progressive nature of diabetic neuropathy and the limited efficacy of current treatment options highlight the urgent need for alternative therapeutic strategies that are both effective and safe (Akkus & Sert 2022).

Nigella sativa, commonly known as black seed or black cumin, has been used for centuries in traditional medicine systems across the Middle East, Africa, and Asia for its purported health benefits. It has managed various ailments, including respiratory issues, gastrointestinal disturbances, and inflammatory conditions (Hannan et al. 2021). In the context of diabetes, *Nigella sativa* has gained attention due to its potential to modulate blood glucose levels, improve insulin sensitivity, and reduce oxidative stress, pivotal factors in the pathophysiology of diabetic complications (Hamdan, Haji Idrus & Mokhtar 2019). Recent preclinical and clinical studies suggest that bioactive compounds in *Nigella sativa*, such as thymoquinone, may offer neuroprotective effects, potentially mitigating the progression of diabetic neuropathy (Khodaie et al. 2024; Syuhada, Kurniati & Akrom 2023). However, despite these promising findings, comprehensive safety evaluations, particularly in diabetic neuropathy patients, remain limited.

The composition of *Nigella sativa* seeds includes oils, proteins, carbohydrates, fibers, and minerals. The oil content (36-38%) primarily consists of linoleic (50-60%), oleic (20-23.4%), palmitic (12.5%), dihomolinoleic (10%), and eicosadienoic (3%) acids, along with beta-sitosterol, sterols, and minor lipids (Ahmad et al. 2013; Al-Jassir 1992; Gholamnezhad, Havakhah & Boskabady 2016). The therapeutic effects are attributed to thymoquinone (TQ), dithymoquinone, nigellidine, nigellicine, thymol, and carvacrol (Ahmad et al. 2013). The essential oil contains TQ (30-48%), p-cymene (7-15%), carvacrol (6-12%), longifolene (1-8%), and α-pinene (Ahmad et al. 2013) while trace components include alkaloids (nigellidine, nigellicine), alpha-hederin, saponins, B vitamins, vitamin E, and minerals such as calcium and iron (Nergiz & Ötleş 1993). However, detailed phytochemical profiles have been limited in clinical studies (Heshmati & Namazi 2015).

Nigella sativa oil (NSO) is traditionally considered safe when used appropriately. Preclinical studies show that acute and subchronic administration of thymoquinone, its primary active compound, causes no significant toxicity in animal models with stable liver and kidney function (Ong et al. 2016). *In vivo*, toxicities of *Nigella sativa* include acute toxicity characterized by behavioral disorders. Subacute toxicity may increase gamma-glutamyl transferase (γ-GT) and prothrombin time (PT) while decreasing activated partial thromboplastin time (APTT) and thrombin time (TT). Subchronic toxicity elevates aspartate aminotransferase (AST), alanine aminotransferase (ALT), and mean corpuscular volume (MCV) while reducing red blood cells (RBC), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), and packed cell volume (PCV). Chronic toxicity decreases platelet count (Plt), hematocrit (Hct), mean globular volume (MGV), mean corpuscular hemoglobin (MCH), and MCHC. Clinical trials in type 2 diabetic patients report no adverse hepatic or renal effects, and meta-analyses confirm the absence of significant hepatotoxicity or nephrotoxicity. Doses of up to 5 mL/day of NSO for 26 days and 3 g/day of seeds for three months show no significant side effects in humans, including diabetics. However, high doses (5 g/day) inhibit CYP2D6 and CYP3A4 enzymes, necessitating cautious use (Mashayekhi-Sardoo, Rezaee & Karimi 2020).

While a growing body of evidence supports the therapeutic potential of NSO in diabetes management, concerns about its safety profile, especially in vulnerable populations like diabetic neuropathy patients, necessitate rigorous investigation. The complex interplay between diabetes, neuropathy, and the pharmacological properties of NSO warrants a thorough assessment to ensure that its use does not exacerbate existing conditions or introduce unforeseen adverse effects (Alberts et al. 2024). Evaluating the safety of NSO in this specific patient population is critical to establishing its role as a nutraceutical intervention, providing healthcare professionals and patients with reliable data to guide its use.

The primary objective of this study was to evaluate the safety profile of NSO supplementation in patients with diabetic neuropathy. It is hypothesized that NSO could demonstrate a favorable safety profile without causing significant adverse effects in diabetic neuropathy patients. Additionally, it is expected that NSO contributes to the amelioration of neuropathic symptoms, supporting its potential as a complementary approach to diabetes management.

MATERIALS AND METHODS

STUDY DESIGN

This study was conducted as part of a clinical trial evaluating the safety profile of NSO supplementation in diabetic neuropathy at a district general hospital in North Kalimantan, Indonesia. Each participant received a daily oral dose of 2 mL of NSO, administered as two 500 mg capsules taken twice daily for 30 days. The NSO used in this trial was produced by a registered manufacturer and authorized by the Indonesian National Agency for Drug and Food Control (BPOM) as a traditional medicine. The formulation comprised a pure of oral extract of *Nigella sativa* seeds obtained through cold-press extraction without additives. Quality assurance was supported by a Certificate of Analysis, confirming compliance with microbial safety standards (total plate count and fungal contamination <10 CFU/g), appropriate organoleptic properties, and a thymoquinone content of 11.70% area in chloroform, as determined by gas chromatography-mass spectrometry (GC-MS).

PARTICIPANTS

Participants were selected based on stringent inclusion and exclusion criteria to ensure consistency within the study population and to maintain a clear focus on safety outcomes. Inclusion criteria required patients to have a confirmed diagnosis of diabetes mellitus with neurological complications, verified by a physician. Eligible participants were between 20 and 60 years old and provided written informed consent. Exclusion criteria included pregnancy, known allergies to *Nigella sativa* or its components, autoimmune diseases, and regular use of corticosteroids.

The sample size of 24 was calculated using a two-sample mean comparison formula based on previous studies evaluating *Nigella sativa*'s effects on pain scores (Visual Analogue Scale), glycemic markers, and oxidative stress (Kaatabi et al. 2015; Mousavi et al. 2024). These values informed sample size computation using the standard formula for comparing two means (Gogtay 2010; Wellek & Blettner 2012).

$$N = \frac{\left(Z_{\frac{\alpha}{2}} + Z\beta\right)^2 . (2.\sigma_e^2)}{\tau^2}$$

where $Z_{\frac{\alpha}{2}}$ =1.96 (significance level α = 0.05); $Z\beta$ = 0.84 (power = 80%); σ_e^2 is the estimated variance of the outcome parameter; τ is the expected treatment effect.

In total, 24 subjects were finally included to account for possible dropouts and ensure adequate power to detect significant outcomes.

INTERVENTION

The study evaluated the safety of NSO, administered in capsule form at 2 mL per day for 30 days. Hematological and biochemical parameters were assessed before and after the intervention to identify potential adverse effects. This approach provided a comprehensive analysis of the safety profile of NSO, ensuring that any observed changes were directly linked to the supplementation.

DATA COLLECTION AND PARAMETERS

The analyses were conducted at the RSUD Dr. Jusuf SK clinical laboratory following standardized protocols with blinded analysts to ensure unbiased results. Safety assessments involved a comprehensive evaluation of hematological and biochemical parameters to detect potential changes associated with NSO consumption. The hematological assessment included HGB, WBC count, RBC count, Hct levels, Plt count, MCV, MCH, and MCHC measurements. Biochemical assessments focused on liver and kidney functions by measuring AST, ALT, and creatinine levels. Other events accompanying NSO supplementation were also recorded.

ETHICAL CONSIDERATIONS

The Ethics Committee of Dr. Jusuf SK hospital reviewed and approved the study protocol, by the guidelines set by the Council for International Organizations of Medical Sciences (CIOMS 2016). The trial was registered with the Indonesia Registry of Clinical Trials Ethic (Registration Number: 00102165713111520221108030) and granted the protocol approval (Approval Number: 114/KEPK-RSUD-drHJSK/XI/2023). Before enrollment, all participants provided written informed consent after receiving comprehensive information about the study's objectives, procedures, potential risks, and benefits. Participants were also explicitly informed about their right to withdraw from the study at any time without any repercussions.

STATISTICAL ANALYSIS

Statistical analyses were conducted using GraphPad Prism software. Safety data were evaluated using paired *t*-tests to compare pre-and post-intervention values within each group. Additionally, data visualization in the form of diagrams was generated to facilitate further analysis and interpretation. This comprehensive statistical approach ensured a robust evaluation of NSO's safety profile within the study population.

RESULTS

This study involved 24 participants, each receiving 2 mL/day of NSO. To evaluate its effects, analysis was conducted before and after 30 days of supplementation. The following section outlines the study findings.

PARTICIPANT DEMOGRAPHIC CHARACTERISTICS

Participants aged 20 to 60, all diagnosed with diabetic neuropathy, were recruited for the study. Inclusion criteria required a confirmed diagnosis and the presence of symptoms such as pain or motor dysfunction. Table 1 summarizes the demographic and clinical characteristics of the participants.

Majority of the participants were female (79.17%), suggesting a higher prevalence of healthcare-seeking behavior among women with diabetic neuropathy. Most had been diagnosed with diabetes for less than five years (37.5%), with balanced representation across longer durations. A high rate of medication adherence (66.67%) was observed, although 8.33% were non-adherent, posing a risk for complications. While 70.83% never used traditional medicine, occasional use was noted in 25%, indicating some interest in complementary therapies. The population was predominantly homemakers (79.17%), reflecting possible socioeconomic influences on disease management. Common comorbidities included dyslipidemia (41.67%) and hypertension (29.17%), emphasizing the need for integrated care to address multiple risk factors in diabetic neuropathy management.

TABLE 1. Demographic and clinical characteristics

Characteristic	Subject (n)	Percentage (%)
Gender		
▪ Male	5	20.83
▪ Female	19	79.17
Time since diabetes diagnosis		
▪ Less than 5 years	9	37.5
▪ 5-10 years	8	33.33
▪ More than 10 years	7	29.17
Adherence to medication		
▪ Non-adherent	2	8.33
▪ Occasionally adherent	6	25
▪ Fully adherent	16	66.67
Use of traditional medicine		
▪ Never	17	70.83
▪ Occasionally	6	25
▪ Regularly	1	4.17
Occupation		
▪ Homemaker	19	79.17
▪ Fisherman	1	4.17
▪ Private sector employee	1	4.17
▪ Entrepreneur	1	4.17
▪ Others	2	8.33
Comorbidities		
▪ Dyslipidemia	10	41.67
▪ Hypertension	7	29.17
▪ Gastropathy	4	16.67
▪ Pure Hyperglyceridemia	2	8.33
▪ Coronary artery disease	2	8.33
▪ Hyperuricemia	2	8.33
▪ Peptic ulcer	2	8.33
▪ Others	13	54.17

THE EFFECT OF NSO ON HEMATOLOGY

Hemoglobin

The HGB levels showed no significant changes following NSO supplementation, with a p-value of 0.1781. Although there was a slight upward trend in HGB levels post-supplementation, most values remained within the normal physiological range (Figure 1).

A closer examination of the data showed that the number of physiological outliers was the same in pre- and post-supplementation measurements. Additionally, several data points showed low HGB levels in the pre-supplementation group, indicating mild baseline anemia in some individuals. Despite these outliers, most HGB levels in both groups remained within normal limits, indicating that NSO does not significantly affect HGB concentration.

White blood cell counts

The WBC counts showed no significant changes following NSO supplementation, with a p-value of 0.5482. Although there was a slight upward trend in WBC levels post-supplementation, most values remained within the normal physiological range (Figure 2).

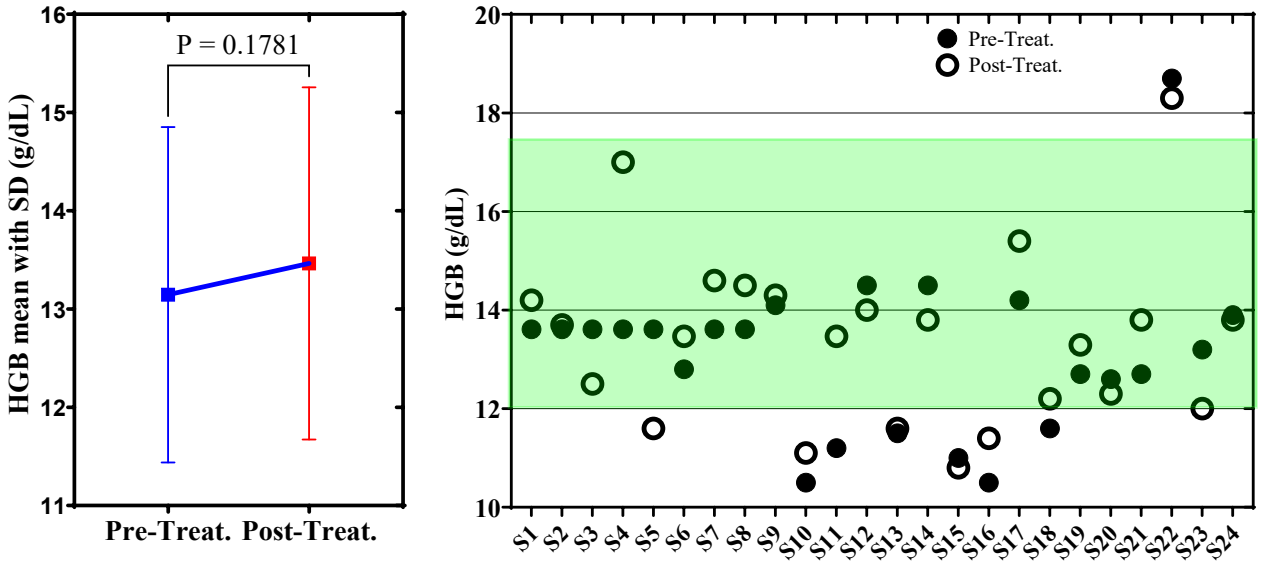

FIGURE 1. Comparison of pre-and post-treatment HGB levels. The normal HGB range is 13.5 to 17.5 g/dL for men and 12.0 to 16 g/dL for women (Dean 2005)

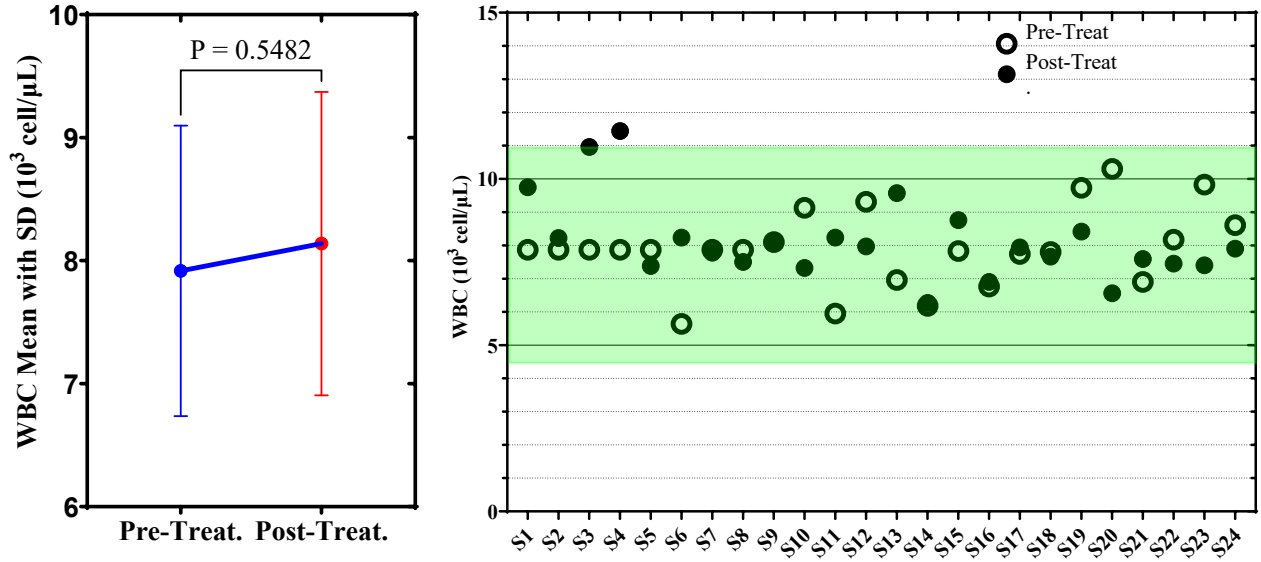

FIGURE 2. Comparison of pre- and post-treatment WBC. The normal WBC ranges from 4.5 to 11.0 × 10^3 cells/µL (Dean 2005)

A closer examination of the data points relative to the physiological range showed that none of the pre-supplementation WBC counts fell outside this range, indicating stable baseline immune parameters. However, after NSO supplementation, two data points exceeded the upper limit of the physiological range, suggesting a mild increase in WBC levels in specific individuals. Despite these outliers, most post-supplementation WBC counts remained within normal limits.

Red blood cell counts

The RBC counts showed no significant changes following NSO supplementation, with a p-value of 0.3380. Although there was a slight upward trend in RBC levels post-supplementation, most values remained within the normal physiological range (Figure 3).

A closer examination of the data showed some outliers, both pre- and post-supplementation. Still, most RBC counts remained within normal limits, indicating that NSO does not significantly affect RBC concentration.

Hematocrit

The Hct showed no significant changes following NSO supplementation, with a p-value of 0.3240, although there was an upward trend post-supplementation (Figure 4).

A closer look at the data showed similar outliers in pre- and post-supplementation measurements. Additionally, the data showed several cases of low Hct levels in the pre-supplementation group, indicating mild baseline anemia in some individuals. Despite these outliers, most Hct levels remained within normal limits, indicating that NSO does not significantly affect Hct concentration.

Platelet counts

The Plt counts showed no significant changes following *Nigella sativa* supplementation, with a p-value of 0.2852. Although Plt levels trended downward post-supplementation, most values remained within the normal physiological range (Figure 5).

A closer examination of the data showed one extreme outlier in the pre-supplementation group, where Plt counts exceeded the upper physiological limit. In contrast, Plt levels appeared more stable post-supplementation, suggesting a trend toward normalized Plt counts after NSO intake. Despite these differences, most Plt levels in both groups remained within normal limits, indicating that NSO does not significantly affect Plt concentration.

MCV, MCH and MCHC

The MCV and MCH showed no significant changes following NSO supplementation, with p-values of 0.9459 and 0.3195, respectively (Figure 6). This indicates that NSO does not significantly affect red blood cell dimension or the amount of hemoglobin per cell. However, MCHC showed a statistically significant increase post-supplementation ($p = 0.0483$), suggesting improved hemoglobin concentration within red blood cells, which may enhance their oxygen-carrying capacity.

THE SAFETY OF NSO ON LIVER AND KIDNEY

The creatinine, AST, and ALT levels showed no significant changes following NSO supplementation, with p-values of 0.7047, 0.2185, and 0.7509, respectively (Figure 7). These results suggest NSO does not significantly affect kidney or liver function. Although some individual values exceeded the normal physiological range, most measurements remained within normal limits, indicating no adverse effects on renal or hepatic health.

ADVERSE EVENTS

No significant adverse events or side effects were reported throughout the study period among NSO supplementation participants. Continuous monitoring ensured participant safety and well-being, and the data highlighted a robust safety profile for NSO. The absence of hepatotoxicity, nephrotoxicity, or hematological disturbances further supports the tolerability of this supplement. Notably, only one participant with a pre-existing tachycardia condition experienced a transient episode, which resolved without lasting clinical implications, indicating that such events were isolated and non-severe. Overall, these findings confirm that NSO can be safely administered, particularly in populations with chronic conditions like diabetic neuropathy, without increasing the risk of adverse health outcomes.

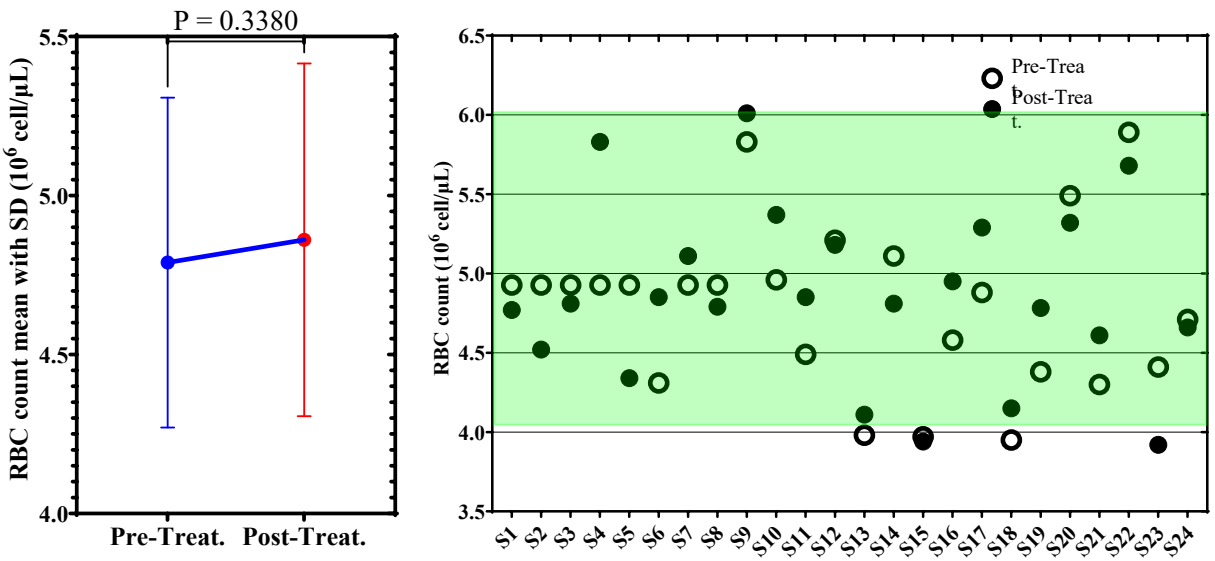

FIGURE 3. Comparison of pre-and post-treatment RBC counts. The normal RBC count ranges from 4.7 to 6.1 × 10^6 cells/µL for men and 4.2 to 5.4 × 10^6 cells/µL for women (Dean 2005)

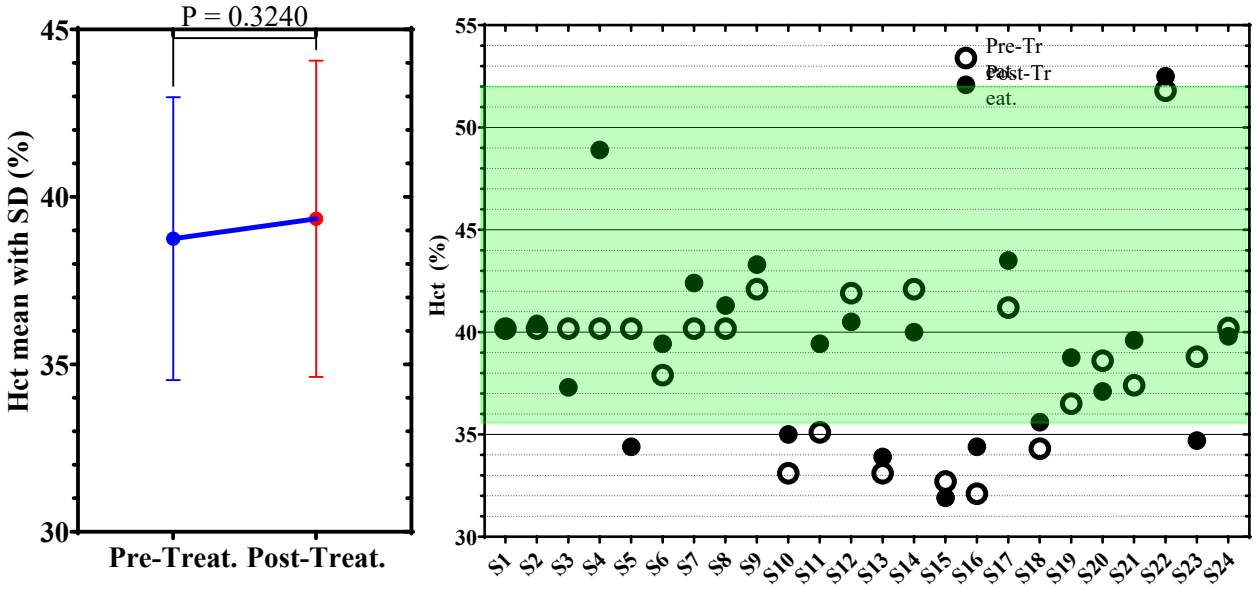

FIGURE 4. Comparison of pre-and post-treatment Hct levels. The normal Hct range is 41% to 53% for men and 36% to 46% for women (Dean 2005)

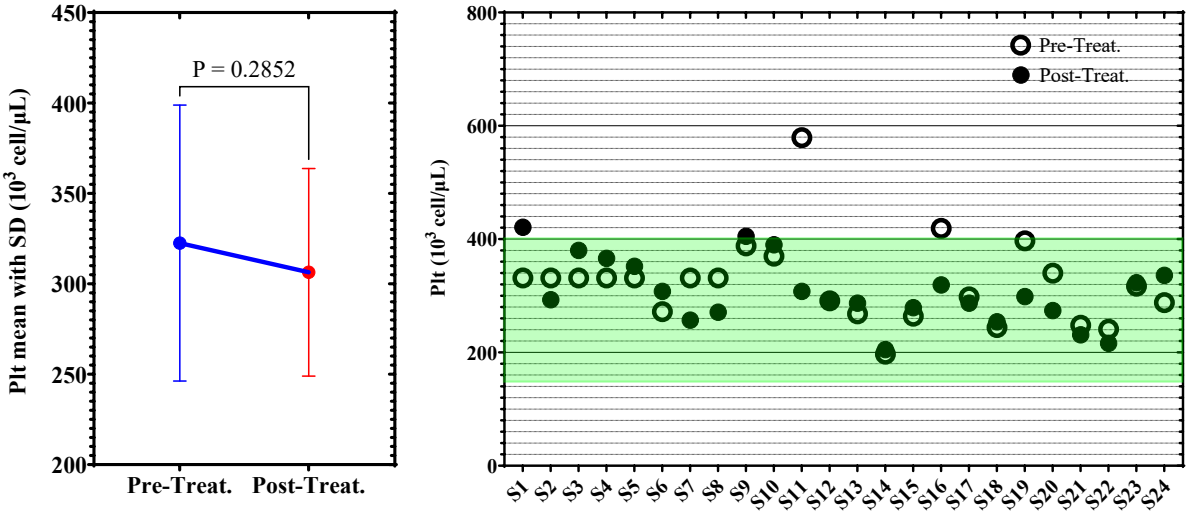

FIGURE 5. Comparison of pre-and post-treatment Plt counts. The normal Plt count ranges from 150 to 400 × 10^3 cells/µL (Dean 2005)

DISCUSSION

The majority of participants were female (79.17%), aligning with existing literature suggesting that women with diabetic neuropathy are more proactive in seeking healthcare services. This trend might be due to gender-specific factors influencing pain perception and coping mechanisms, as highlighted in another study, which emphasizes that women often report higher pain levels and are more likely to pursue medical consultations (Ciarambino et al. 2022).

Regarding the duration since diabetes diagnosis, the sample showed a balanced representation across different disease progression stages. Participants diagnosed within the last five years constituted 37.5% of the sample, while those with a diagnosis of 5-10 years and more than 10 years accounted for 33.33% and 29.17%, respectively. This distribution reflects a comprehensive overview of diabetic neuropathy across various disease stages. Most participants (66.67%) demonstrated full adherence to their prescribed medication regimens. However, 8.33% reported

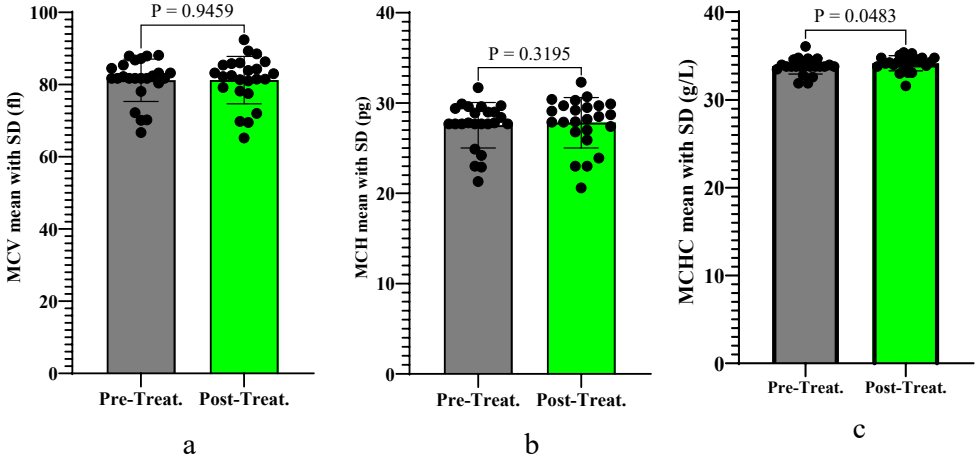

FIGURE 6. Comparison of pre-and post-treatment. a. MCV, b. MCH, and c. MCHC Levels. Normal Ranges, MCV 80-100 fL; MCH 25.4-34.6 pg; MCHC 31-36 g/L (Dean 2005)

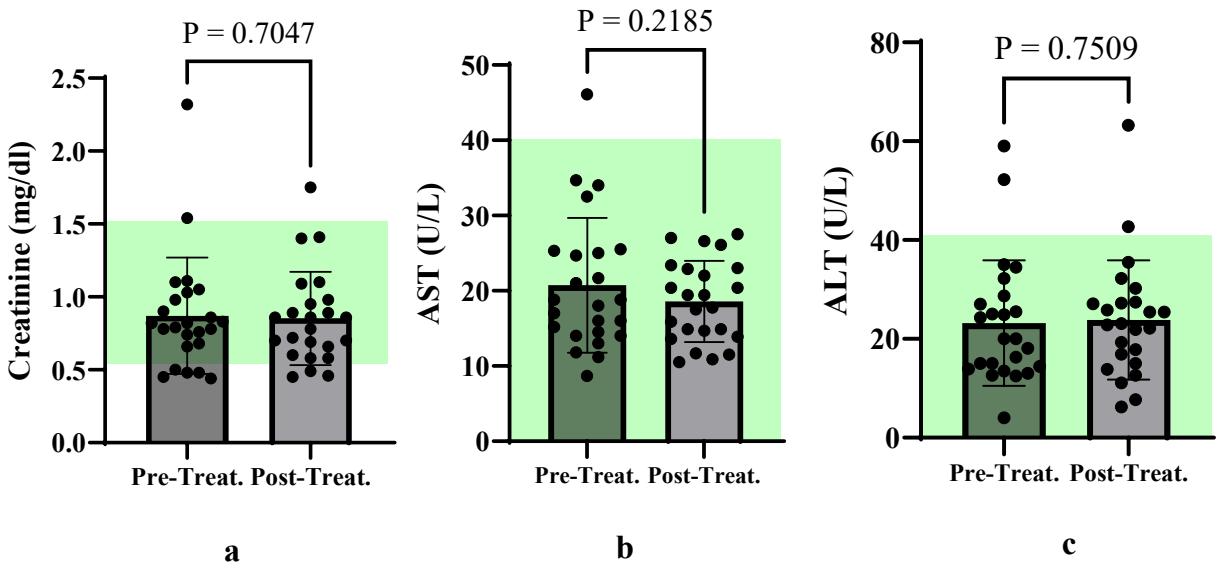

FIGURE 7. The NSO supplementation on kidney and liver function markers (a) creatinine, (b) AST, and (c) ALT

non-adherence, a factor associated with increased risks of complications and suboptimal glycemic control. This finding is consistent with other studies emphasizing the importance of adherence in managing chronic conditions like diabetes (Pop-Busui et al. 2016).

Interestingly, while most participants (70.83%) did not use traditional medicine, 25% reported occasional use, and 4.17% were regular users. This suggests a degree of cultural openness to complementary therapies, which could influence future integrative treatment approaches (Ng et al. 2023). The socioeconomic profile, dominated by homemakers (79.17%), indicates potential limitations in healthcare access and financial resources, which could impact overall disease management and outcomes (McMaughan, Oloruntoba & Smith 2020). Comorbidities were prevalent, with dyslipidemia (41.67%) and hypertension (29.17%) being the most common. These conditions are known to exacerbate diabetic neuropathy symptoms and complicate disease management, highlighting the necessity for integrated care models that address multiple risk factors concurrently (Abdulghani et al. 2018).

This evaluation of hematological parameters suggests that NSO supplementation does not exert significant adverse effects on blood components, supporting its hematological safety in individuals with diabetic

neuropathy. The HBG levels showed no significant changes following supplementation ($p = 0.1781$). The RBC counts also remained statistically unchanged ($p = 0.3380$). Similarly, Hct levels exhibited no significant differences ($p = 0.3240$), indicating that NSO does not influence the overall circulating volume of red blood cells. Although a slight upward trend in hemoglobin and Hct levels was noted, all values remained within the normal physiological range, confirming that NSO does not adversely affect hemoglobin concentration. These findings are consistent with preclinical studies, which reported that NSO did not significantly affect hematological parameters (Sanpinit et al. 2023).

WBC counts showed no significant changes post-supplementation ($p = 0.5482$). However, two data points exceeded the upper physiological limit, indicating a mild, non-clinically considerable increase in specific individuals. This may suggest a potential immunomodulatory effect of NSO, warranting further investigation. Another study reported that NSO significantly reduced WBCs, macrophages, and eosinophils in ovalbumin-sensitized mice, accompanied by decreased Th2 cytokines (IL-4, IL-5, IL-13) and increased IFN-γ levels, leading to improved airway inflammation (Ciesielska-Figlon et al. 2023). These variations in results may reflect context-dependent immunomodulatory effects of NSO, reducing inflammation in allergic models while supporting immune stability in different immunological conditions (Falcon & Caoili 2023).

The Plt counts did not significantly change after supplementation ($p = 0.2852$). However, a slight downward trend was observed, with post-supplementation levels appearing more stable. This may suggest a potential normalization effect of NSO on Plt homeostasis (Hosseini & Hosseinzadeh 2020). Further supporting this, thymoquinone, the active compound in NSO, minimally affected normal coagulation but significantly reversed inflammation-induced thrombosis ($p < 0.001$). It reduced TNF-α-mediated NF-κB activation ($p < 0.001$), suggesting a role in stabilizing Plt counts by modulating inflammation-thrombosis interactions (Muralidharan-Chari et al. 2016).

While MCV and MCH remained unchanged ($p = 0.9459$ and $p = 0.3195$, respectively), a significant increase was observed in MCHC following supplementation ($p = 0.0483$). This elevation indicates an enhanced hemoglobin concentration within RBCs, potentially improving their oxygen-carrying capacity (Sarma 1990). The observed increase in MCHC may be attributed to the antioxidant properties of NSO, mainly due to the presence of thymoquinone, a bioactive compound known for its protective effects on red blood cell membranes against oxidative damage (Jrah-Harzallah et al. 2013). Additionally, study results suggest that thymoquinone may enhance nitric oxide (NO) production in red blood cells and provide protection against ischemia-reperfusion injury (Gunay et al. 2024). These findings highlight the potential clinical applications of thymoquinone's red blood cell-protective properties. Previous studies have also demonstrated the positive effects of NSO consumption. The ingestion of 2 g/day of NSO seeds for 12 weeks in patients with type 2 diabetes exerted a favorable impact on hemodynamic parameters (Lebda et al. 2012).

Serum creatinine levels, a key indicator of renal function, showed no significant changes post-supplementation ($p = 0.7047$), though some individuals experienced improvements. This stability suggests that NSO does not impair kidney function, even in those at risk for diabetic nephropathy. Additionally, studies have shown that *Nigella sativa* supplementation can improve liver and kidney parameters, reducing ALP, AST, and blood urea nitrogen (BUN) levels with normal dosages over longer durations. However, higher doses were associated with increased BUN, indicating potential dose-dependent effects. While these results highlight its beneficial impacts, larger-scale, long-term studies are needed to determine the optimal dose and duration for kidney and liver health (Razmpoosh et al. 2020).

Hepatic markers, including AST ($p = 0.2185$) and ALT ($p = 0.7509$), remained within normal physiological ranges throughout the study, with some individuals improving liver enzyme levels. The absence of significant changes suggests that NSO does not exert hepatotoxic effects. This aligns with prior research by Azizi et al. (2021) which reported that NSO supplementation over several weeks led to stable liver enzyme levels, further supporting its hepatic safety in diabetic and non-diabetic populations.

NSO supplementation was well-tolerated throughout the study period, with no significant adverse events reported among participants. This high level of tolerability underscores the supplement's safety profile in individuals with diabetic neuropathy. The only notable incident involved a participant with a pre-existing history of tachycardia, who experienced a transient episode of increased heart rate during the supplementation period. Importantly, this episode was resolved without any lasting clinical implications or the need for medical intervention. While this isolated case does not suggest a direct causal relationship between NSO and tachycardia, it highlights the need for cautious monitoring in individuals with underlying cardiovascular conditions. Supporting these findings, studies assessing the safety of NSO generally classify it as a safe medicinal herb, in tandem to which another clinical trial study reported no severe adverse effects following NSO consumption (Mashayekhi-Sardoo, Rezaee & Karimi 2020).

These findings support the clinical integration of NSO as a complementary supplement in managing diabetic neuropathy. While the primary objective of this study was to evaluate safety, the observed hematological stability, absence of hepatorenal toxicity, and improved MCHC values suggest that NSO may confer additional physiological benefits, particularly through enhanced oxygen-carrying

capacity and red blood cell integrity. These properties are highly relevant in diabetic neuropathy, where oxidative stress and tissue hypoxia play pivotal roles in symptom exacerbation and nerve degeneration. Furthermore, the favorable safety profile and absence of significant adverse effects position NSO as a potential adjunct in symptom management strategies, especially in resource-limited settings where conventional neuroprotective treatments may be less accessible. Future clinical protocols may consider incorporating NSO as part of an integrative approach to enhance patient quality of life and delay the progression of neuropathic complications.

However, this study had certain limitations. The small sample size may limit the generalizability of the findings to broader populations, and the short intervention duration may not capture the long-term effects or sustainability of NSO's therapeutic benefits. Although carryover effects were minimal, they could still influence outcomes and warrant cautious interpretation. To address these limitations, future research should include larger sample sizes, longer intervention periods, and more diverse populations to validate and expand upon these results.

CONCLUSION

The administration of NSO at a dose of 2 mL/day over 30 days demonstrated a strong safety profile in patients with diabetic neuropathy, with no significant adverse effects observed on hematological or biochemical parameters. Hematological markers, including HGB, WBCs, RBCs, Hct, and Plt, remained stable throughout the intervention, indicating that NSO does not disrupt blood cell production or function. A slight but statistically significant increase in MCHC was observed; however, it remained within normal physiological limits, suggesting no pathological implications. Similarly, biochemical markers of liver and kidney function, such as AST, ALT, and creatinine, remained within normal ranges, with no evidence of hepatotoxicity or nephrotoxicity detected. These findings support the potential of NSO as a safe complementary therapy for managing diabetic neuropathy. However, additional studies are recommended to validate these results further, including longer-term trials with larger and more diverse populations, dose-response investigations to determine the optimal therapeutic range, and evaluations in populations with pre-existing hepatic or renal conditions.

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