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The Effects of Combined Vitamins C and E Treatment on Pain Sensitivity in Diabetic Mice

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ABSTRACT

Introduction: Neuropathic pain is a common and debilitating complication of diabetes, significantly affecting their quality of life. This study investigates the effects of combined vitamins C and E treatment on pain sensitivity in diabetic mice, aiming to elucidate their therapeutic potential for managing diabetic neuropathic pain.

Methods: Adult male BALB/c mice (25-30g) were used to induce animal model of diabetes by intraperitoneal injection of streptozotocin (60 mg/kg). The pain sensitivity was evaluated by several behavioral tests such as hot plate, tail flick and formalin test.

Results: Our results demonstrated that glucose levels in diabetic mice were significantly elevated compared to controls, and Vitamin E treatment notably reduced glucose levels, while vitamin C alone did not show significant changes. Pain behavior was assessed using the formalin test, where both vitamins significantly reduced licking time during the acute phase and chronic phase with combined treatment exhibiting an additive effect. Additionally, the tail flick test revealed prolonged latency in response to thermal stimuli with both vitamins, indicating enhanced analgesic effects, particularly when administered together. The hot plate test further confirmed increased latency times with vitamin supplementation.

Conclusion: These findings suggest that vitamins C and E co-supplementation ameliorates pain sensitivity and may improve metabolic outcomes in diabetic conditions, highlighting the benefits of these antioxidants and their potential as therapeutic agents to improve clinical outcomes for patients suffering from diabetes-related pain.

Keywords:

Diabetes, Neuropathic Pain, Antioxidants, Vitamin C, Vitamin E.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels, resulting from either insufficient insulin production by the pancreas or the body's inability to effectively utilize insulin. This condition can lead to serious health complications affecting various organs, including the heart, kidneys, eyes, and nerves (1). Diabetic neuropathy, particularly painful diabetic peripheral neuropathy (PDPN), affects a significant proportion of individuals with diabetes, leading to debilitating pain, sensory loss, and reduced quality of life (2). The pathophysiology of diabetic neuropathy involves complex mechanisms, including oxidative stress, inflammation, and nerve damage, which contribute to heightened pain sensitivity (3).

Oxidative stress plays a pivotal role in the development and progression of diabetic neuropathy, a common complication of diabetes characterized by nerve damage and pain (4). In diabetes, chronic hyperglycemia leads to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, resulting in oxidative damage to neuronal tissues. This oxidative stress is not only

associated with direct neuronal injury but also contributes to inflammatory processes that exacerbate neuropathic pain (5). The overproduction of ROS can impair cellular functions, disrupt energy metabolism, and lead to the activation of pathways that promote neuroinflammation, further aggravating symptoms, neutralize ROS effectively (6).

Several Studies have shown that targeting oxidative stress through antioxidant therapies may offer new avenues for alleviating pain and improving nerve function in diabetic patients, highlighting the importance of managing oxidative damage as part of comprehensive diabetic care (7). Preclinical and clinical research increasingly recognized the therapeutic potential of vitamins C and E as antioxidants in managing pain sensitivity in diabetes (8). These vitamins play a crucial role in combating oxidative stress, a key contributor to the development of diabetic neuropathy. Vitamin C, known for its ability to scavenge reactive oxygen species (ROS), has been shown to alleviate hyperalgesia and promote nerve regeneration in diabetic models by reducing oxidative damage and enhancing neuroprotective mechanisms (9, 10). Similarly, vitamin E contributes to cellular protection by neutralizing free radicals and improving endothelial function, which is often compromised in diabetic patients (11). Together, these vitamins not only mitigate oxidative stress but also may have potential to enhance pain modulation through their analgesic properties. The aim of this study is to explore the combined effects of vitamins C and E on pain sensitivity and glucose level in sterptozotocin induced diabetic mice, an area that has received limited attention in existing literature. While both vitamins have individually demonstrated beneficial effects as antioxidants in mitigating oxidative stress and improving nerve function, their synergistic potential remains underexplored. The findings from this study could pave the way for novel therapeutic strategies that leverage the combined antioxidant properties of vitamins C and E, ultimately improving clinical outcomes and quality of life for individuals suffering from diabetes-related pain.

METHODS

Animals

The experiments were carried out on 60 male BALB/c mice weighing 25-30 grams (n=48) that obtained from the animal care and breeding center of Babol University of Medical Sciences. During the experiment, the animals were housed in plastic cages (n=8) under standard lighting conditions (12 hours light, 12 hours dark) at a temperature of 22°C and humidity of around 60% in the animal house of Babol University of Medical Sciences and were given food and water ad libitum. Animal care and handling procedures were in accordance with the guidelines for the care and use of laboratory animals approved by the Ethics Committee of Babol University of Medical Sciences with code: IR.MUBABOL.HRI.REC. 1396.204.

The animals were randomly divided into 6 groups (n=8) : 1- Control group (control), 2- Diabetic animals without any treatments (DM), 3- Diabetic animals receiving an equal volume of sesame oil (vitamin E solvent) and normal saline (vitamin C solvent) (DM+Veh), 4- Diabetic animals treated with vitamin C at a dose of 50 mg/kg/p.o. (DM+VitC) (12), 5- Diabetic animals treated with vitamin E at a dose of 100 mg/kg/p.o. (DM+VitE) (12), 6- Diabetic animals treated with both vitamins E and C at the above doses (DM+VitC+VitE)

Diabetes Model Induction

The animal model of diabetes in mice was induced by intraperitoneal (i.p.) injection of 60 mg/kg streptozotocin (STZ) (Sigma, Germany), dissolved in distilled water (12, 13). Three days post-STZ injection, tail vein blood glucose levels were measured using a laboratory glucometer. Mice with fasting blood glucose levels exceeding 250 mg/dL were classified as diabetic, while those with levels below this threshold were considered non-diabetic and were excluded from the study.

Vitamin Treatment

Following the confirmation of diabetes induction, vitamin treatment was administered daily for 20 days according to the group assignments. Vitamin C was dissolved in normal saline at a dose of 50 mg/kg, while Vitamin E was dissolved in sesame oil at a dose of 100 mg/kg. Both vitamins were administered to the mice via daily oral gavage (12, 14).

Hot plate

Three weeks after diabetes induction, the duration of limb tolerance to heat-related pain in mice will be evaluated using the hot plate test. The procedure is as follows: the temperature of the plate is set to 52 ± 2°C, and a pain response is

recorded when the mice begin to jump or lick their paws. To prevent tissue damage, the maximum duration of the test is capped at 60 seconds(15).

Tail flick

Three weeks after diabetes induction, a spotlight is focused on the dorsal surface of the mice's tails, and the tail flick latency is recorded. The light intensity is adjusted so that the tail flick duration for all mice prior to diabetes induction is between 2 and 4 seconds. The maximum cut-off time for the test is set at 10 seconds(16).

Formalin test

Three weeks after diabetes induction, the formalin test is conducted in an open Plexiglas cage equipped with a mirror below, allowing for an unobstructed view of the mice's paws. The mice are placed in the test cage for 15 minutes to acclimate before receiving the formalin injection. Each mouse is injected intraplantarly with 20 microliters of 2.5% formalin in the right paw. After the injection, the mice are observed in pairs during two phases: Phase 1 (0-5 minutes) and Phase 2 (20-30 minutes). The time spent licking the injected paw is recorded during these phases. The interval between the two phases reflects a period of weak activity in the antinociceptive sensory response(16).

RESULTS

The effect of vitamin E and C on glucose level

Our data indicated that the glucose levels in mice treated with streptozotocin (60 mg/kg) (DM group) were significantly higher compared to the control group ($p < 0.00001$, n=8). In the Vitamin E treatment group (DM + Vit E), there was a notable reduction in glucose levels compared to the DM group ($p<0.05$, n=8). However, glucose levels in the other treated groups did not show significant changes compared to the DM group.

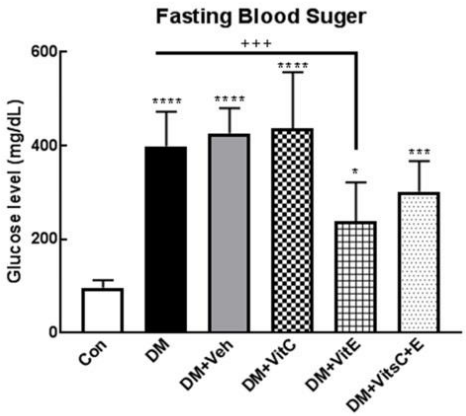

Figure 1. The fasting blood glucose of diabetic mice under treatment of vitamin E, C and combination. *comparison with control ($^{****}p<0.0001$, $^{***}p<0.001$, $^{*}p<0.05$). +Comparison with DM ($^{+++}p<0.001$, n=8).

Vitamin E and C modulating pain responses in formalin test

In our study, we assessed pain behavior using the formalin test by measuring licking time as an index of pain in both acute (1-5 minutes) and chronic (20-30 minutes) phases. Our results indicated that licking time was significantly increased in the diabetic (DM) and DM + Vehicle (DM + Veh) groups compared to the control group ($p < 0.05$), suggesting heightened pain sensitivity in these groups.

In the acute phase, supplementation with Vitamin C and Vitamin E, both individually and in combination, led to a significant reduction in licking time compared to the DM group ($p < 0.01$ and $p < 0.001$, respectively). This finding indicates that these vitamins may have analgesic effects in the context of acute pain.

In the chronic phase, licking time did not show significant differences between the control group and either the DM or DM + Veh groups. However, treatment with Vitamins C and E-both individually and together resulted in a significant decrease in licking time compared to the DM group ($p < 0.0001$). Notably, the combined effect of Vitamin C and E was more pronounced than that of either vitamin alone ($p < 0.05$), highlighting an additive effect that enhances their pain-relieving properties. These findings suggest that Vitamin C and E may play a crucial role in modulating pain responses in diabetic conditions.

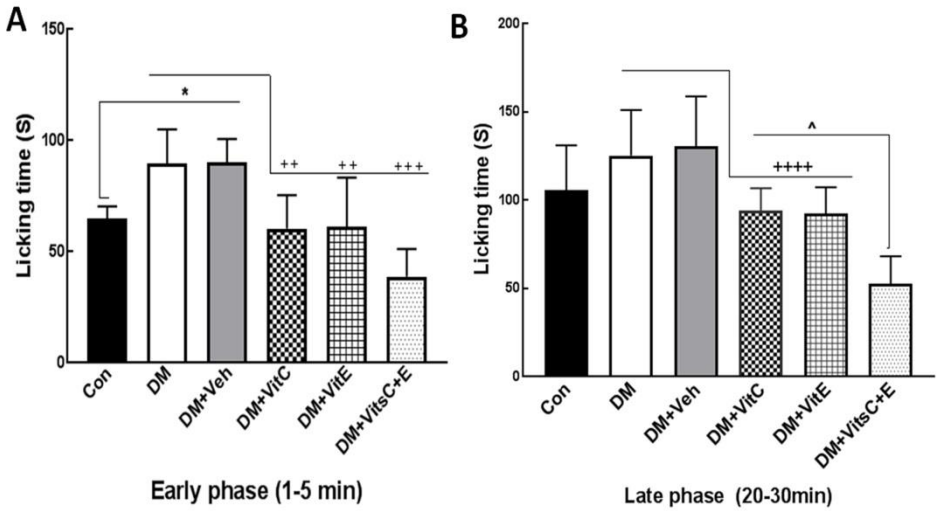

Figure 2. The anti-nociceptive effect of combination of vitamin E and C on pain score in formalin test in two early (1-5 min) and late (20-30 min) phase. The number of animal in each group was eight.
*comparison with control ($^{*}p<0.05$). +Comparison with DM($^{++++}p<0.0001$,$^{+++}p<0.001$, $^{++}p<0.01$). . ^ comparison with DM+Vit E and DM+Vit C ($^{\wedge}p<0.05$).

The anti-nociceptive effect of vitamin E and C in tail-flick

The tail flick test is an effective method for evaluating acute thermal pain responses in animals. In this study, we measured the latency of the reflexive withdrawal response to a painful heat stimulus applied to the tail. Our findings revealed a significant decrease in tail flick latency in both the diabetic (DM) and DM + Vehicle (DM + Veh) groups compared to the control group ($p < 0.01$). Conversely, treatment with Vitamin C and Vitamin E, administered separately and in combination, significantly prolonged the latency time for tail flicking when compared to the DM groups ($p < 0.01$ and $p < 0.0001$, respectively). Importantly, the combined administration of Vitamin C and E produced a more substantial effect than either vitamin alone ($p < 0.05$), indicating a synergistic interaction that enhances their analgesic properties.

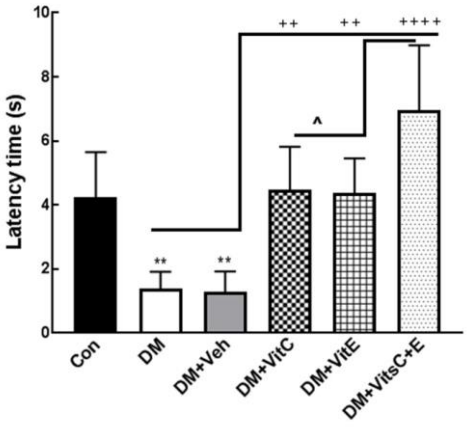

Figure 3. The anti-nociceptive effect of combination of vitamin E and vitamin C in tail flick test (n=8). *comparison with control ($^{**}p<0.01$). +Comparison with DM ($^{++}p<0.01$, $^{++++}p<0.0001$). ^ comparison with DM+Vit E and DM+Vit C ($^{\wedge}p<0.05$).

The effect of combination vitamin E and C in hot plate test

The hot plate test evaluates pain sensitivity by placing an animal on a heated surface measuring the time it takes for the animal to exhibit a nociceptive response, such as licking its paw or jumping off the plate. Our findings revealed that the latency to exhibit a nociceptive response was significantly reduced in the both diabetic (DM) group and the DM + Vehicle (DM + Veh) group compared to the control group ($p < 0.01$ and $p < 0.05$, respectively). This suggests that diabetes may heighten pain sensitivity in these animals. In contrast, treatment with Vitamin C and Vitamin E-both individually and in combination-significantly increased the latency time compared

to DM groups ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively). These results indicate that both vitamins have potential analgesic effects, helping to alleviate pain sensitivity associated with diabetes.

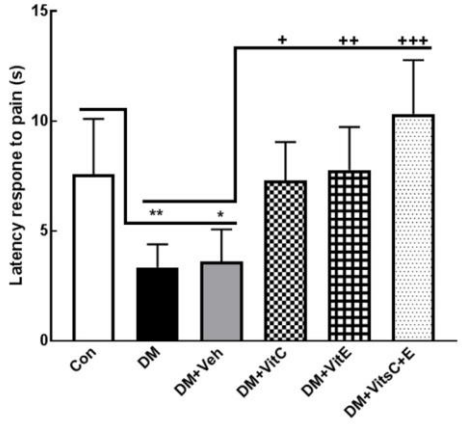

Figure 4. Hot plate latency response to pain in different experimental group (n=8).
*comparison with control ($^{*}p<0.05$, $^{**}p<0.01$). +Comparison with DM ($^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$) .

DISCUSSION

The present study investigated the effects of Vitamins C and E on glucose levels and pain responses in diabetic mice, utilizing various behavioral tests to assess both metabolic and nociceptive outcomes. Our findings contribute to the growing body of evidence supporting the beneficial roles of these antioxidants in managing diabetes-related complications.

Our data demonstrated that streptozotocin-induced diabetic mice exhibited significantly elevated glucose levels compared to the control group, this aligns with previous studies indicating that oxidative stress and impaired insulin signaling contribute to hyperglycemia in diabetic models. Notably, treatment with Vitamin E resulted in a significant reduction in glucose levels, suggesting its potential role in enhancing glycemic control. This finding is consistent with meta-analyses showing that Vitamin E supplementation can improve glycemic indices and insulin resistance, particularly in short-term interventions (17, 18). However, it is important to note that other treatment groups did not show significant changes compared to the diabetic group, indicating that while Vitamin E may have beneficial effects, its efficacy with Vitamin C and their combined effects may vary based on dosage and duration of treatment**.** Research indicates that oxidative stress is a critical factor in the pathogenesis of diabetes, contributing to tissue damage and metabolic dysregulation (4). In a study examining the effects of vitamin C and E supplementation on oxidative stress markers in diabetic rats, it was found that these vitamins effectively reduced levels of malondialdehyde (MDA), a marker of lipid peroxidation, while simultaneously enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (19, 20).

In assessing pain sensitivity through the formalin test, our results indicated significantly increased licking times in both the diabetic and DM+Vehicle groups compared to controls, highlighting heightened pain sensitivity associated with diabetes. This finding is supported by literature suggesting that diabetic neuropathy can lead to altered pain perception due to peripheral nerve damage and central sensitization (21). In the acute phase, supplementation with Vitamins C and E significantly reduced licking times, indicating their analgesic properties. This aligns with studies demonstrating that antioxidants can mitigate oxidative stress-induced pain pathways, thereby providing relief from acute nociceptive stimuli (22). In the chronic phase, while licking times did not differ significantly between controls and diabetic groups, both vitamins administered individually or together resulted in a marked decrease in licking time compared to the DM group. The additive effect observed when combining Vitamins C and E suggests that these antioxidants may work together to enhance their analgesic effects, potentially through complementary mechanisms that target oxidative stress and inflammation (23).

The tail flick test further supported our findings regarding pain modulation, revealing a significant decrease in latency for reflexive withdrawal responses in diabetic groups that is consistent with existing literature that highlights how diabetes can lead to peripheral neuropathy resulting in increased pain perception (24). Treatment with Vitamins C and E significantly prolonged this latency, reinforcing their role as effective analgesics. Previous studies have shown that Vitamin C can attenuate hyperalgesia and peripheral nerve degeneration through mechanisms involving the reduction of oxidative stress and enhancement of nerve function (9). The mechanism may be by inhibiting the central mechanism of pain through prevention of oxidation since enhanced (25). Furthermore, the

additive effects observed when combining Vitamins C and E may be attributed to their complementary antioxidant properties, which together may enhance neuroprotection and analgesia. In this context, a study has suggested that vitamin C and E potentially can alleviate pain symptoms experienced by individuals with endometriosis (26).

The hot plate test corroborated these results, showing reduced nociceptive response latencies in diabetic groups but increased latencies following vitamin treatment. These tests collectively indicate that both vitamins have potential therapeutic effects on pain sensitivity associated with diabetes. The combined use of these antioxidants appears to provide a multifaceted approach to managing diabetic neuropathy by addressing both metabolic dysregulation and nociceptive pathways. Future studies should explore optimal dosing regimens and long-term effects of Vitamin C and E co-supplementation on diabetic complications to fully elucidate their therapeutic potential.

CONCLUSION

In conclusion, this study highlights the significant effects of vitamins C and E on glucose regulation and pain sensitivity in streptozotocin-induced diabetic mice. The observed additive effects of vitamins C and E indicate that their combined use may enhance neuroprotection and alleviate pain through complementary mechanisms targeting oxidative stress and inflammation. While our findings are promising, further research is necessary to determine optimal dosages, treatment durations, and the long-term efficacy of vitamin C and E co-supplementation in diabetic models. Understanding the mechanisms underlying these effects will be crucial for translating these findings into clinical practice. Ultimately, the integration of vitamins C and E into therapeutic strategies may offer a multifaceted approach to improving metabolic health and reducing pain sensitivity in individuals with diabetes, potentially enhancing their overall quality of life.

DECLARATION

The author declares no conflicts of interest

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