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RESEARCH ARTICLE

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Observation of the neuroprotective efficacy of vitamin K in a streptozocin-induced diabetes model in chick embryos

Ayhan Vurmaz[1] | Emre Atay[2] | Usame Rakip[3] | Tülay Koca[4]

[1]Department of Medical Biochemistry, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

[2]Department of Anatomy, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

[3]Department of Neurosurgery, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

[4]Department of Physiology, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

Correspondence

Ayhan Vurmaz, Department of Medical Biochemistry, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey.
Email: ayhan.vurmaz@gmail.com

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Abstract

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to insulin deficiency and/or resistance. Vitamin K (VK) is a group of fat-soluble molecules, including naturally occurring vitamin K1 (phylloquinone). vitamin K2 (menaquinone), and synthetic vitamin K3 (menadione). Beyond coagulation, the health benefits of VK have been described to play different roles in both physiological and pathological processes such as inflammation, energy metabolism, neuroprotection, cellular growth, and survival. It was aimed to observe the antioxidant and/or neuroprotective activity of vitamin K1 in our model of chick embryo diabetic neuropathy (DN) induced by streptozotocin (STZ). Ninety White Leghorn, fertile and 0-day-old SPF (specific pathogen-free) eggs (57 ± 4 gr) were used in the study. Chick embryo blood brain tissues were taken for biochemical evaluation. Plasma insulin and glucose levels were measured. In addition, brain tissue total antioxidant level (TAS), total oxidant level (TOS), malondialdehyde (MDA), and vascular endothelial growth factor (VEGF) levels were measured. Plasma glucose levels were higher in the STZ-treated groups and lower in the treatment groups. Plasma insulin levels were observed to be higher in STZ groups in groups treated with high VK. Low TAS, high MDA, TOS, and VEGF levels were recorded in brain tissue STZ groups. Low VEGF, TOS, and MDA levels were recorded in the group treated with the highest VK, while high TAS levels were observed. In our STZ-induced chick embryo diabetic neuropathy model, we observed that VK1 reduced oxidant damage by showing antioxidant properties or by modulating antioxidant enzymes.

KEYWORDS

chick embryo, diabetic neuropathy, total antioxidant, total oxidant, vitamin K

1 | INTRODUCTION

Diabetes is a chronic disease, about 400 million people worldwide and 60 million people in Europe suffer from this disease. Type 2 diabetes patients account for 90% of cases worldwide. This disease is recognized as the leading lifestyle disease. However, it is estimated that approximately 600 million patients will be reached in the world by 2035. The burden of diabetes has generally been defined in terms of its impact on working-age adults. In recent years, it has been reported that diabetes causes higher mortality and impaired

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functional status in older adults. Pharmaceutical treatment, education, and self-care principles are taken into consideration in diabetes management.[1–3]

Diabetes mellitus (DM) is a metabolic disorder that characterized by high blood sugar levels, and has two types: type 1 diabetes mellitus (DM-T1) and type 2 diabetes mellitus (DM-T2).[4] DM is characterized by long-term disorders of protein, fat, and carbohydrate metabolism and dysfunction in many systems and organs.[5]

Approximately 60%–70% of patients with DM have mild or moderate cognitive impairment. This occurs more slowly in information processing, with learning and memory deficits and impaired walking function.[5,6] Several published studies show that the structural changes in the brain contribute to cognitive decline in patients with DM. DM may accelerate brain atrophy and cognitive impairment in elderly patients.[4]

It has been reported that DM causes significant differences in brain metabolite levels in both human studies and animal model studies. Therefore, investigating brain metabolite levels in DM studies can determine how DM mechanically causes neurodegenerative disorders. However, studies focusing on brain metabolites in patients with diabetes are limited in the literature.[7]

Vitamin K (VK) is divided into two: VK1 (phylloquinone) and VK2 (menaquinones). VK1 is a compound that forms the main dietary source of VK and carries a side chain of four isoprene residues, three of which are saturated. The relationship between VK and coagulation is widely known. It acts as a cofactor for the enzyme that activates vitamin K–K-dependent factors (II, VII, IX, X, protein S, and protein C). In addition, VK is involved as a cofactor in synthesizing sphingolipids, which are important components of the brain cell membrane.[8–10]

Besides the naturally occurring phylloquinones and menaquinones, a synthetic form of VK, VK3 (menadione, 2-methyl-1, 4-nafoquinone core), exists, which represents the basic structure common to phylloquinones and menaquinones.[11,12] VK2 is found in dairy and fermented foods. Menaquinone-4, a homolog of VK2, is the main form of VK in animal tissues and is converted from ingested VK1 and some other menaquinones. In studies evaluating the postmortem storage status of VK in tissues (heart, brain, liver, kidney, pancreas, and lung human tissues), although VK1 is stored in most tissues and organs, it is relatively low in the brain, kidneys, liver, heart, and pancreas compared with other tissues and organs reported to be stored at a higher rate.[13,14]

In addition to its functions in blood coagulation and bone metabolism, VK has also special functions in the regulation of glycemic status. Hence, it is thought that high levels of VK in the blood will help in reducing the risk of DM. Menadione (VK3), a redox-cycled polycyclic aromatic ketone, is an over-the-counter drug used for treating hypoprothrombinemia, abdominal cramps, cancer, osteoporosis, colitis, hay fever, bleeding, diarrhea, and joint pain.[14,15]

It should not be overlooked that oxidative stress is among the factors that cause diabetic neuropathy (DN). Therefore, it is not surprising to note that antioxidants have played an important role in the investigation of effective treatment of nerve dysfunction in diabetes in recent years. However, antioxidants and agents that mimic antioxidants have been tested in in vitro and in vivo animal experimental models.[16]

Existing approaches to treat DM and/or investigate DM-related damage have been demonstrated by studies on animal models.[17] These studies allow the identification of both treatments and potential side effects/toxicities, which helps protect healthy volunteers and patients participating in clinical studies.[16] Today, the chemical model of DM is performed with streptozotocin (STZ) and alloxan.[18] STZ is a nitrosourea derivative drug isolated from StrePtomyces acromogens with broad-spectrum antibiotic and antineoplastic activities. STZ is also a potent alkylating agent that has been shown to interfere with multiple DNA strand breaks, glucokinase function, and glucose transport.[19] Animal model studies have been conducted on DM created using STZ.[20]

This study aimed to observe the antioxidant and/or neuroprotective activity of VK1 in our chick embryo DN model induced by STZ.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

Necessary permissions were obtained from Afyon Kocatepe University Animal Experiments Ethics Committee (Decision No: 49533702/146) for this study. The experimental process was carried out in accordance with animal research protocols. The experimental process of the study and the analysis of chemical parameters were carried out in Afyonkarahisar Health Sciences University, Medicine Faculty, Department of Medical Biochemistry and Anatomy.

2.2 | Experimental animals and incubation conditions

In this study, 90 fertilized specific pathogen-free (SPF) White Leghorn chicken eggs obtained from Izmir Bornova Veterinary Control Institute (Izmir/Turkey) were used. The weight of these eggs ranged from 55 to 60 g. SPF eggs were placed in the incubator in the Anatomy laboratory and incubated at 65%–70% RH and 37.5 ± 20°C. The control of the incubator and vital follow-ups of the embryos were carried out by the researchers.

2.3 | Chemical and drug doses

STZ was used to create the DM model. A stock solution was prepared by dissolving STZ (*N*-(methylnitrosocarbamoyl)-α-D-glucosamine, CAS Number: 18883–66-4; Sigma-Aldrich Chemie GmbH) in saline. Two different doses (0.15 and 0.30 mg/egg) of STZ (50 μL) were administered. Three different doses (0.05, 0.025, and 0.005 mg/egg) of VK (Konakion 10 mg/mL; Roche) were administered.

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TABLE 1 Study procedure.

Group	Agent	Dose	Volume	Frequency	Time
Control (Group 1)	PBS (pH:0.7)	0.1 mL	0.1 mL	Once	On 12th and 18th days
STZ-1 (Group 2)	STZ	0.15 mg/egg	0.1 mL	Once	On 12th and 18th days
STZ-2 (Group 3)	STZ	0.30 mg/egg	0.1 mL	Once	On 12th and 18th days
STZ-1 + VK-1 (Group 4)	STZ + VK	0.15 + 0.05 mg/egg	0.1 + 0.1 mL	Once	On 12th and 18th days
STZ-1 + VK-2 (Group 5)	STZ + VK	0.15 + 0.025 mg/egg	0.1 + 0.1 mL	Once	On 12th and 18th days
STZ-1 + VK-3 (Group 6)	STZ + VK	0.15 + 0.005 mg/egg	0.1 + 0.1 mL	Once	On 12th and 18th days
STZ-2 + VK-1 (Group 7)	STZ + VK	0.30 + 0.05 mg/egg	0.1 + 0.1 mL	Once	On 12th and 18th days
STZ-2 + VK-2 (Group 8)	STZ + VK	0.30 + 0.025 mg/egg	0.1 + 0.1 mL	Once	On 12th and 18th days
STZ-2 + VK-3 (Group 9)	STZ + VK	0.30 + 0.005 mg/egg	0.1 + 0.1 mL	Once	On 12th and 18th days

Abbreviations: PBS, phosphate-buffered saline; STZ, streptozocin; VK, vitamin K1.

2.4 | Study procedure

A total of nine groups were formed, with 10 SPF eggs in each group randomly (Table 1). On the 12th day, sterilization was achieved by applying 70% ethyl alcohol on the eggshell under sterile conditions in the groups that would develop type 1 diabetes with two different doses of STZ (0.15 and 0.30 mg/egg).[21,22] Then, a 0.5-mm-diameter hole was drilled through the upper part of the air sac, which the Hamilton needle could pass through. STZ was administered to the first group at a dose of 0.15 mg/egg (STZ1, 40 units), and STZ was administered to the second group at a dose of 0.30 mg/egg STZ (STZ2, 40 units). After STZ injections, the hole opened in the eggshell was closed with tape, maintaining the sterile conditions so that the egg would not get air. Groups 2 and 3 (10 + 10 = 20 units), which were administered only STZ1 and STZ2 doses, were put back into the incubator to be opened on the 18th day.

The groups to which VK1 was to be administered were opened from the same area 3 h after the STZ application, and VK1 was administered (0.05, 0.025, and 0.005 mg/egg)[23,24] via a Hamilton injector. The opened hole was closed with the tape, keeping the sterile conditions so that the egg would not get air. The closed eggs were turned 180° and placed back in the incubator to open on the 18th day.

On the 18th day of incubation, the eggs were opened from the upper part of the air sac under sterile conditions. The inner shell membrane was carefully removed, and 200 µL of chicken blood was taken from the thickest vein under it with a 30GX 13-mm-diameter mesotherapy needle attached to the tip of the insulin injector. The blood samples taken were centrifuged without delay and stored at −20°C to study insulin levels.

2.5 | Study groups

Group 1: Phosphate-Buffered Saline, (PBS, pH 7.4);
Group 2: STZ (0.15 mg/egg);
Group 3: STZ (0.30 mg/egg);
Group 4: 0.15 mg/egg STZ + 0.05 mg/egg VK1;
Group 5: 0.15 mg/egg STZ + 0.025 mg/egg VK1;
Group 6: 0.15 mg/egg STZ + 0.005 mg/egg VK1;
Group 7: 0.30 mg/egg STZ + 0.05 mg/egg VK1;
Group 8: 0.30 mg/egg STZ + 0.025 mg/egg VK1;
Group 9: 0.30 mg/egg STZ + 0.005 mg/egg VK1, injected as a solution.

2.6 | Preparation of brain samples

For preparing samples, 0.1 g of brain tissues were removed from the embryos (Figure 1), placed in 1 mL of 0.1 M phosphate buffer, pH 7.4, and homogenized for 1 min at 24,000 rpm on ice using an Ultra Turrax homogenizer (IKA Works). The homogenates were ultrasonicated at 20,000 cycles/s for 1 min using a compact ultrasonic laboratory device (UP100H; Hielscher) sonicator. The homogenates were centrifuged at 10,000*g* for 15 min, and the supernatant was collected and stored at −20°C until the day of analysis.

2.7 | Total antioxidant status (TAS) and total oxidant status (TOS) measurement

Brain tissue TOS and TAS were evaluated colorimetrically using a total oxidant and antioxidant status assay kit (Mega Medicine) following the manufacturer's protocols. A ChemWell 2910 microanalyzer (Awareness Technology) was used for absorbance tests. Brain tissue TAS and TOS results were expressed as mmol Trolox Equiv./g tissue and nmol H_2O_2 Equiv./g tissue, respectively.

2.8 | Measurement of malondialdehyde (MDA) levels

Brain tissue MDA levels were measured using a thiobarbituric acid reagent assay kit (Cayman Chemical Company) following the

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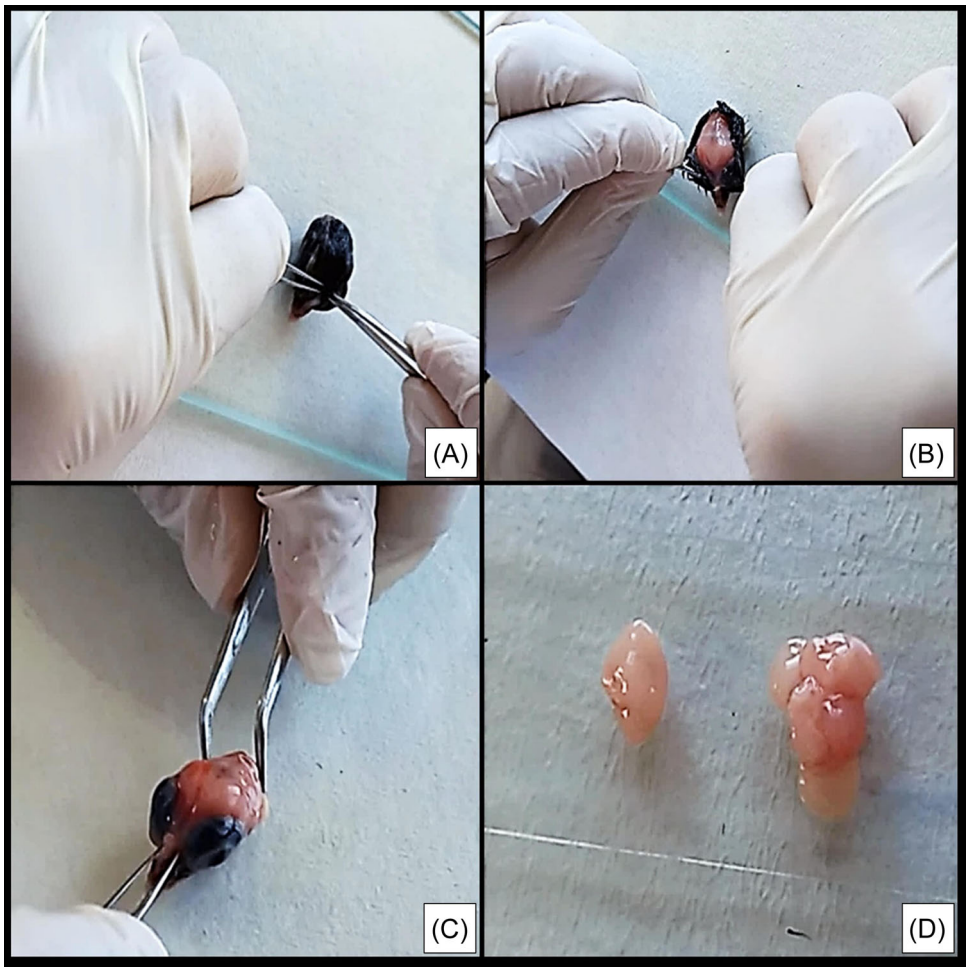

FIGURE 1 Obtaining brain samples. (A) Chick embryo skull dissection. (B) Chick embryo skull skin exfoliation. (C) Chick embryo skull bone removal. (D) Chick embryo brain tissue.

manufacturer's protocols. The ChemWell 2910 microanalyzer was used for absorbance tests. MDA levels were expressed as µmol/g tissue.

2.9 | Measurement of vascular endothelial growth factor (VEGF) levels

Brain tissue VEGF levels were measured using a thiobarbituric acid reagents assay kit (eBioscience Inc.) following the manufacturer's protocols. The ChemWell 2910 microanalyzer was used for absorbance tests. VEGF levels were expressed as ng/g tissue.

2.10 | Plasma glucose and insulin level measurement

A suitable vessel was found under the inner shell membrane, and a blood glucose strip used for glucose measurement was placed under the vessel without damaging the structures. Afterward, the vein was gently lifted with a strip to allow blood circulation in the vein. The vein on the strip was fragmented through the mesotherapy needle tip. The blood was dispersed on the strip, and the glucose level was recorded as mg/dL with an Accu-Chek blood glucose meter (Roche Diagnostics), (Figure 2). Blood was drawn from the vein on the strip using an insulin injector to determine the plasma insulin level.

2.11 | Statistical analysis

Statistical analyses were performed using SPSS software version 22.0 (SPSS Inc.). Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as frequency and percentage. Differences between the groups were analyzed with the Kruskal–Wallis test and the Dunn test within the group. The statistical significance level was expressed as $p \leq 0.05$.

3 | RESULTS

3.1 | Plasma glucose and insulin levels

The chick embryo plasma glucose levels are shown in Figure 3. The lowest plasma glucose level was observed in Group 4 and the highest in Group 3. High glucose levels were observed in Groups 3 and 9 and

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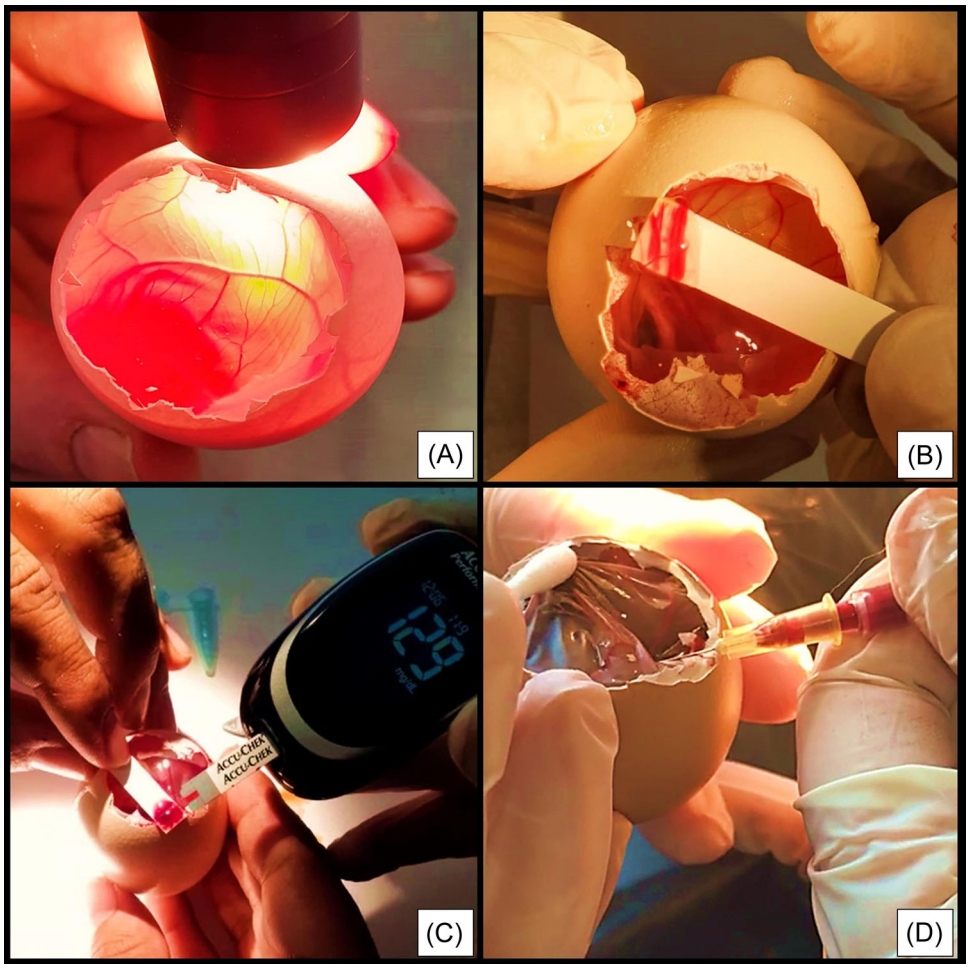

FIGURE 2 Measurement of plasma glucose and insulin levels. (A) Detection of the thickest vein. (B) Dissection of the blood vessel on the strip with a tuberculin needle and blood collection from the vein. (C) Blood glucose measurement. (D) Blood collection with an insulin injector to determine the insulin level.

statistically significantly lower glucose levels in Group 4 compared with the control group (Figure 3). Statistically significantly lower glucose levels were observed in Group 4 compared with Group 2. Statistically significantly higher plasma glucose levels were observed in Groups 5 and 6 compared with Group 4. Statistically significantly higher plasma glucose levels were observed in Group 7 compared with Group 4. Statistically significantly higher plasma glucose levels were observed in Group 9 compared with Group 6 (Figure 3).

The chick embryo plasma insulin levels are shown in Figure 4. Statistically significantly lower insulin levels were observed in Groups 2, 3, and 9 compared with the control group (Figure 4). Statistically significantly higher plasma insulin levels were observed in Group 4 compared with Group 2. Statistically significant higher plasma insulin levels were observed in Groups 7 and 8 compared with Group 3 (Figure 4).

3.2 | Brain tissue TAS, TOS, MDA, and VEGF levels

The brain tissue TAS levels are summarized in Figure 5. Statistically significant higher levels were observed in Group 4 compared with Group 2 in which STZ was applied (Figure 5). They were statistically significantly lower in Groups 5, 6, 7, 8, and 9 compared with Group 4 (Figure 5).

The chick embryo brain tissue MDA levels are shown in Figure 6. Statistically significantly higher results were observed in Groups 2 and 3 compared with the control group (Figure 6). Statistically significantly lower levels were observed in Group 4 compared with Group 2. Statistically significantly lower levels were observed in Groups 7 and 8 compared with Group 3 (Figure 6).

The TOS levels in chick embryo brain tissues are summarized in Figure 7. Although high levels were observed only in the groups in which STZ was applied, statistically significant differences were not observed compared with the other groups in which vitamin K was administered (Figure 7).

The chick embryo brain tissue VEGF levels are shown in Figure 8. Statistically significant higher VEGF levels were observed in Groups 2, 3, 8, and 9 compared with the control group (Figure 8). Statistically significantly lower levels were recorded in Group 4 compared with Group 2. Statistically significantly higher VEGF levels were observed in Group 6 compared with Group 4. Statistically significant higher levels were observed in Group 7 compared with Group 3 (Figure 8).

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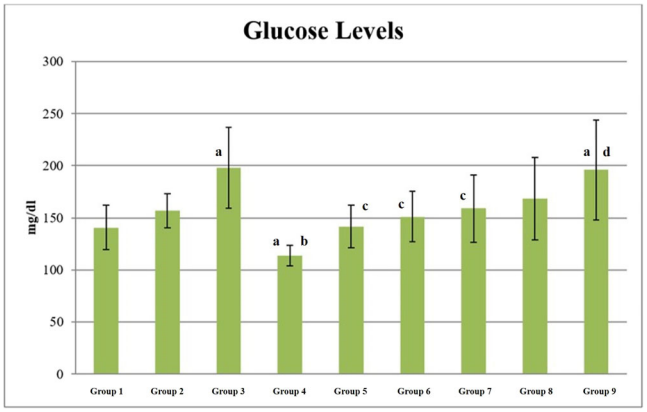

FIGURE 3 Statistical analysis between groups of plasma glucose level. [a]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with the Control group, $p < 0.05$. [b]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 2, $p < 0.05$. [c]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 4, $p < 0.05$. [d]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 6, $p < 0.05$.

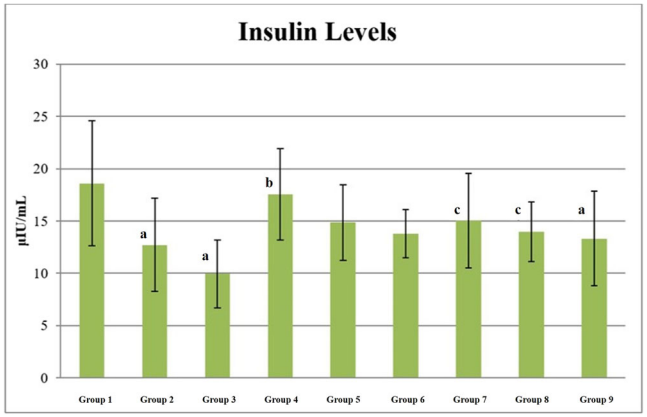

FIGURE 4 Statistical analysis between groups of plasma insulin levels. [a]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with the Control group, $p < 0.05$. [b]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 2, $p < 0.05$. [c]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 3, $p < 0.05$.

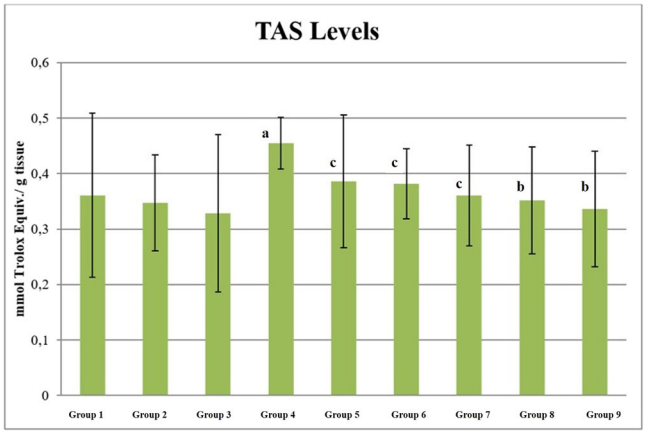

FIGURE 5 Statistical analysis between groups of brain tissue TAS levels. [a]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 2, $p < 0.05$. [b]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 3, $p < 0.05$. [c]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 4, $p < 0.05$. TAS, total antioxidant level.

4 | DISCUSSION

DM is a disease closely related to dementia and cognitive dysfunction. While this affects quality of life, it places a great burden on patients, their families, and society. Unfortunately, there is currently no effective treatment for DM-induced cognitive dysfunction.[2]

Oxidative stress is defined as the deterioration of the oxidative balance as a result of the insufficiency of antioxidants that detoxify them with the increase of reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, and hydrogen peroxide formed during cellular metabolism. ROS, these products that can be beneficial or harmful to the cell, are known as highly unstable species. Oxidative stress is responsible for the pathogenesis of many diseases such as cardiovascular diseases, diabetes, and neurological diseases. Examples of ROS include superoxide ($\bullet O_2^-$), peroxyl ($\bullet RO_2^-$), hydroxyl ($\bullet HO$), hydrogen peroxide (H_2O_2), and hydroperoxyl ($\bullet HRO_2^-$).[1,16,25]

Antioxidants can be obtained endogenously as a normal defense mechanism of the cell or exogenously from the diet. Examples include enzymatic antioxidants such as glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase, and nonenzymatic antioxidants such as vitamins C, E, and K and reduced glutathione (GSH). In general, antioxidants work to achieve two main aims: to reduce the harmful effects of free radicals and/or to strengthen the body's natural defense systems by inducing the activities of antioxidant enzymes.[16,26] Increases in the levels of biomarkers of lipid peroxide products (MDA and isoprostanes), proteins (protein carbonyls and nitrosylated proteins), and DNA-related oxidative stress have been observed in various in vivo and in vitro experimental diabetes models.[16]

An important feature of diabetes is hyperglycemia, which underlies the various mechanisms involved in generating oxidative stress that eventually leads to DN. Oxidative stress plays a role in the initiation and development of impaired insulin secretion and insulin resistance, which are the two main mechanisms involved in DM. Oxidative stress caused by hyperglycemia remains the most understandable way diabetes progresses to DN. Therefore, therapies based on combating hyperglycemia and oxidative stress can serve as safe, cost-effective solutions for the prevention/treatment of DM and DN.[16,27,28]

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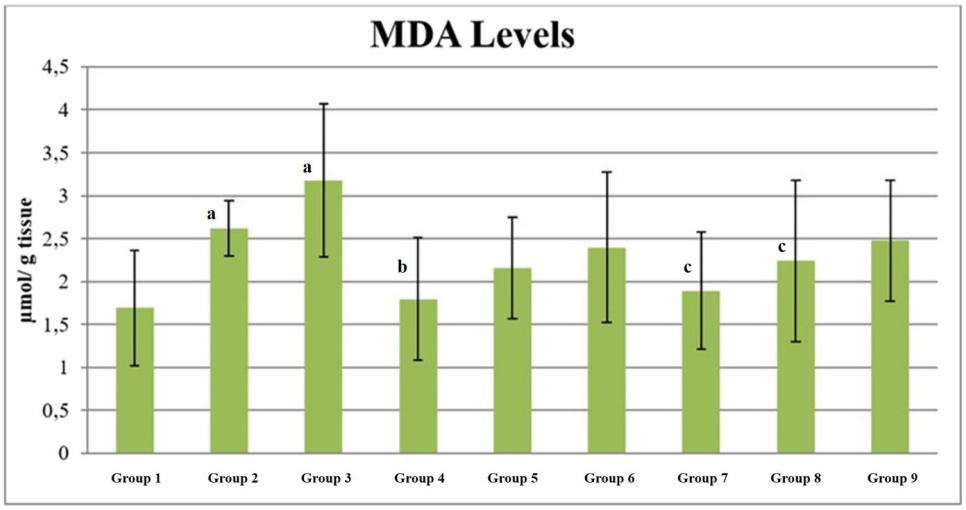

FIGURE 6 Statistical analysis between groups of brain tissue MDA levels. [a]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with the Control group, $p < 0.05$. [b]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 2, $p < 0.05$. [c]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 3, $p < 0.05$. MDA, malondialdehyde.

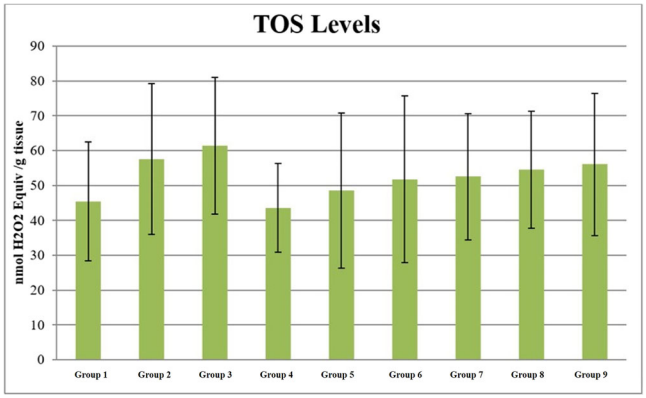

FIGURE 7 Statistical analysis between groups of brain tissue TOS levels. TOS, total oxidant level.

Many complications (microvascular and macrovascular) may occur in the body due to DM. One of these complications, DN, is due to dysfunctions of the peripheral nervous system.[26,29]

The brain is the most vulnerable tissue to oxidative damage due to its high oxygen consumption rate, abundant lipid content, and low levels of enzymatic and nonenzymatic antioxidants.[27] Increased ROS and reactive nitrogen species may damage the lipids in the myelinated structures of the nerves, causing axon loss and disruption of the microvasculature in the peripheral nervous system.[30] Oxidative damage to peripheral nerves causes hyperexcitability in afferent nociceptors and central neurons, resulting in spontaneous impulses in nerve axons and dorsal root ganglia that contribute to DN-related neuropathic pain. In addition, a high glucose level causes an increase in superoxide anion and peroxynitrite ion levels, which can damage nerves in DN.[27,31]

The health benefits of VK go beyond coagulation; it has been shown to have an extra-hepatic role, ensuring the proper function of several extra-hepatic VK–VK-dependent proteins (VKDPs). These have been described to play different roles in both physiological and pathological processes such as energy metabolism, cellular growth and survival, neuroprotection, and inflammation. More recently, a novel role of VK as an antioxidant and anti-inflammatory agent has been described, independent of its efficacy as a cofactor for γ-glutamyl carboxylase. VK acts as an anti-inflammatory agent by suppressing nuclear factor κB (NF-κB) signal transduction. It has a protective effect against oxidative stress by blocking the formation of ROS.[14,32]

In our chick embryo model, in which we applied two different doses of STZ, high plasma glucose and low plasma insulin levels were observed as in type-1 diabetes. The protective role of VK1 was investigated by applying three different doses. Low plasma glucose and high plasma insulin levels were recorded in the groups in which the highest VK1 was applied, compared with those in which STZ was applied (Figures 3 and 4). At low doses of VK1, statistically significantly higher plasma glucose levels were observed in Groups 5 and 6 compared with Group 4, while statistically significantly higher levels were observed in Group 9 compared with Group 6. High plasma glucose levels induced by STZ were effectively reduced at high doses of VK1 (Figure 3). The lowest plasma insulin levels were recorded in Group 3, in which the high dose of STZ was administered. Statistically significantly lower levels were recorded compared with the groups that received STZ at high doses of VK1 (Figure 4). These results indicated that VK1 reduced pancreatic β-cell destruction and/or played a role in regulating blood glucose hemostasis.

We believed that VK exhibited antioxidant properties and/or modulated the activity of antioxidant enzymes in response to oxidant stress caused by hyperglycemia. Varsha et al.[33] showed that the

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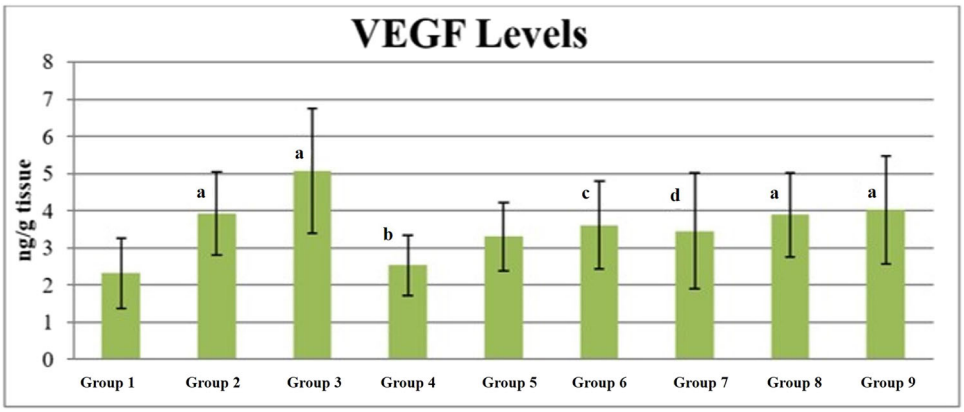

FIGURE 8 Statistical analysis between groups of brain tissue VEGF levels. [a]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with the Control group, $p < 0.05$. [b]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 2, $p < 0.05$. [c]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 3, $p < 0.05$. [d]Kruskal–Wallis test and post hoc Dunn test, it is statistically significant when compared with Group 4, $p < 0.05$. VEGF, vascular endothelial growth factor.

plasma glucose level tended to decrease in the high-VK intake group and the insulinogenic index increased 30 min after glucose loading, suggesting that VK intake improved acute insulin response in relation to glucose tolerance. It suggested that VK intake might have a beneficial effect on glucose homeostasis in adult men and women. Varsha et al.[33] further observed that phylloquinone treatment (subcutaneously, 5 mg/kg body weight, twice weekly) reduced cataract formation in the STZ-treated rats by regulating blood glucose homeostasis and minimizing subsequent oxidative stress. They reported a significant reduction in the levels of free radicals and inflammatory markers. They also reported a significant increase in antioxidant activities and the levels of SOD, GSH, CAT, and anti-inflammatory markers. This study suggested a beneficial role of phylloquinone in the regulation of glucose homeostasis in diabetic animals treated with STZ.[33]

In another study, the dietary intake of both VK1 and VK2 for 10.3 years was associated with a reduced risk of type-2 DM in adult male (9.740) and female (28.354).[34]

Many factors contribute to inflammation, angiogenesis, and neuropathy. High blood sugar levels via various enzymatic and nonenzymatic mechanisms lead to ROS production and promote oxidative stress. Some studies indicated that the administration of VEGF-C and VEGF-A and neurotrophic factors in animal models contributed to the improvement in blood flow in damaged nerves. VK1 and VK2 have been shown to have antioxidant properties in cultured neurons and oligodendrocytes, independent of their role in the carboxylation of VKDPs.[35–37]

Evidence suggests that VK can reduce insulin response and glycemic status through the inhibition of inflammation. An early study demonstrated the survival-promoting role of VK in maintaining the survivability of neurons of the central nervous system.[14]

The first relevant mechanism discovered was the polyol pathway and increased polyol pathway flux. According to the metabolic theory of DN pathogenesis, glucose is converted into sorbitol by the enzyme aldose reductase in tissues exposed to hyperglycemia, especially those affected by diabetic complications. Sorbitol is then oxidized to fructose, which accumulates in the surrounding tissues. As a result, the myoinositol levels decrease in nerve and Schwann cells, while protein kinase C subunit levels increase. Increased intracellular glucose and sorbitol levels are associated with the development of oxidative stress, which eventually leads to the pathogenesis of long-term diabetic complications.[38]

However, VK has been shown to prevent oxidative stress and its complications in different ways: (a) VK can suppress hyperglycemia; (b) VK antioxidant potential can elevate the levels of SOD and suppress ROS production, including O2− and OH radicals, and increase GSH levels; and (c) VK can inhibit NF-κB production.[33,39]

VEGF is the most potent endothelial-specific vascular growth factor. The VEGF signaling pathway maintains the formation and stability of blood vessels. ROS can affect this signaling pathway.[38,40] Exogenous ROS can increase VEGF expression in different cell types such as macrophages, smooth muscle, and endothelial cells. It has been reported that vascular factors play a role in the pathogenesis of DN as well as metabolic disorders.[41] VEGF induces endothelial cell migration and proliferation through endogenous ROS production. The role of VEGF has been recognized as a mechanistic link between hyperglycemia and DM. In addition, diabetic conditions such as hypoxia, high glucose concentration, and oxidative stress can be considered as the strongest stimulators affecting VEGF gene expression.[40–42]

Numerous studies have proven this positive association. Hydrogen peroxide-induced VEGF expression in endothelial and vascular smooth muscle cells, encouraging angiogenesis. Taking diabetic retinopathy (DR) and its vascular pathological process as an example, ROS-mediated angiogenesis is closely related to VEGF expression. VEGF supports ROS production by activating Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase in endothelial cells. Angiogenesis is more affected by oxidative stress in tumors, retinopathy, and atherosclerotic diseases.

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Oxidative stress-induced angiogenesis is an important factor in chronic diseases.[40,43]

It has been reported that high VEGF level in the corpus vitreum is associated with the development of DR. Previous studies have shown that the increased expression of VEGF in the retina of diabetic patients or animals is caused by the induction of oxidative stress and inflammation.[13–15] Addition of ROS and inflammatory cytokines to vascular cells has also been shown to induce VEGF expression.[44]

Numerous studies have associated the changes in oxidative stress and inflammation with various retinal changes, animal models of diabetes, including elevated VEGF expression in DR, or retinal vascular cells exposed to high glucose levels.[44]

Moghadam et al.[45] studied pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and SOD nitrite/nitrate levels in their study in which they applied menaquinone-4 (MK-4) in a rat brain ischemia/reperfusion (I/R) model. The behavioral analysis was performed to measure cognitive function and surviving neuronal assessment 7 days later. The results showed that the administration of MK-4 following I/R injury improved anxiety-like behavior, short-term and spatial learning, and memory impairment induced by I/R. The study reported that high nitrate/nitrite, IL-6, IL-1β, and TNF-α levels induced by I/R decreased after MK-4 administration and SOD activity increased.[45]

5 | CONCLUSION

The present study recorded low levels of MDA, a lipid peroxidation product, in the groups in which VK1 was administered against the effects of STZ-induced neurodegeneration. Although low TOS levels were observed in VK1-applied groups, the results were not statistically significant. High TAS levels were recorded in VK1-applied groups. These results indicated that oxidative stress was induced by STZ in brain tissues, resulting in high VEGF levels. Low VEGF levels were recorded in the groups in which VK1 was administered, especially in the high-dose VK1 groups. It was speculated that VK1 reduced and/or inhibited oxidative stress, resulting in lower VEGF levels. These results agreed with the study result of Moghadam et al.[45]

VK may assist in developing a new therapy to achieve better control of glycemia and/or improve the lives of the diabetic patient population. The mechanism for the positive effect of VK on insulin sensitivity and glucose metabolism has not been fully elucidated in the literature. Therefore, studies are needed to explain the underlying cause and to examine the molecular mechanism.

AUTHOR CONTRIBUTIONS

Ayhan Vurmaz: Conceptualization; methodology; formal analysis; investigation; writing-original draft preparation; writing-review and editing. **Emre Atay**: Conceptualization; formal analysis; statistical analysis and counseling. **Usame Rakip**: Validation; investigation; resources; data curation. **Tülay Koca**: Resources; data curation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ORCID

Ayhan Vurmaz http://orcid.org/0000-0002-1840-2900
Emre Atay http://orcid.org/0000-0002-2378-1183
Usame Rakip http://orcid.org/0000-0001-7494-0335
Tülay Koca http://orcid.org/0000-0002-6222-315X

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