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Effect of Aqueous Extract of *Cyclotrichium niveum* on the Lipophilic Vitamins, Cholesterol and Fatty Acids Profile in the Brain and Kidney of Streptozotocin-induced Diabetes in Rats

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Diabetes, *Cyclotrichium niveum*, Fatty acids, Lipophilic vitamins, Brain, Kidney

ABSTRACT

This study examined the influence of *Cyclotrichium niveum* (CN) extract on fatty acids, ADEK vitamins, cholesterol, and malondialdehyde levels in the brain and kidney of streptozotocin (STZ)-induced diabetes in rats. Thirty-two adults female Wistar albino rats weighing between 250 and 300 g were divided in four groups of eight animals each: control (C) group, diabetic (D) group, CN group, and D + CN group. The control group was fed a standard diet. The diabetic groups received 55 mg/kg of STZ by intraperitoneal (ip) route. The D + CN group received STZ (ip) and 4 ml/kg CN orally. The CN group was administered 20mg/kg of a CN plant extract prepared by prepared by using decoction method. The levels of Alfa-linolenic acid (18:3, n-3) and retinol decreased in the brain ($p<0.001$) andincreased in the kidney ($p<0.01$) compared to the control group. , significantly higher vitamin K2 levels were detected in the D+ CN group ($p< 0.001$) compared to the control group. Vitamin D3 level was drastically lower in the D and CN groups ($p<0.001$, $p<0.01$).

INTRODUCTION

Diabetes is a growing global health issue largely attributed to lifestyle changes and environmental factors, including sedentary lifestyles, unhealthy dietary habits, and exposure to stress and pollutants. According to the International Diabetes Federation (IDF), the worldwide number of people with diabetes was estimated to be 463 million in 2019, projected to rise to 592 million by 2035 (Guariguata *et al*., 2014).

Diabetes, a metabolic disorder with various causes, is characterized by chronically elevated blood sugar levels and complications in the body's use of carbohydrates, fats, and proteins. These complications may stem from issues with insulin production, insulin action, or defects. Notably, the brain can uptake glucose independently of insulin, whereas the kidneys, like most other tissues in the body, depend on insulin to facilitate glucose uptake (Giugliano *et al*., 1995).

Diabetes, characterized by glucose homeostasis issues,inadequate insulin synthesis and resistant is closely linked to lipid metabolism changes, impacting diabetes etiology, development, and therapy beyond mere association (Ma *et al*., 2021). Insulin plays a pivotal role in facilitating the uptake of glucose by cells for energy production and simultaneously reducing fat synthesis, in the presence of insulin resistance and with diabetes these mechanisms can be compromised, leading to disruptions in lipid metabolism (Samuel and Shulman, 2012; Kahn *et al*., 2014).

The dysregulation of lipid metabolism in diabetes gives rise to a distinctive pattern of lipid abnormalities, including elevated triglycerides, diminished high-density lipoprotein cholesterol. These lipid disturbances contribute to the complications of diabetes. It has been established that hyperglycemia and hyperlipidemia are typical features of STZ-induced diabetes in rats. The prooxidant/antioxidant equilibrium is disrupted by hyperglycemia, which raises free radical levels and lowers antioxidant levels (Dashtban *et al*., 2016; Furse, 2022).

Traditional medicine has consistently emphasized the significance of endemic plants, those exclusive to a small, constrained region. In Turkey, one of the noteworthy native plants is *Cyclotricium niveum*, belonging to the

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Labiatae family. Recognized as mountain mint, this annual plant not only boasts high antioxidant levels but also features a potent aroma (Gulcin *et al*., 2008). *Cyclotricium niveum* has been found to contain chemical molecules such as isomenthone, pulegone, triterpenoids, and flavonoids (Tümen *et al*., 1998; Baser, 2002). *In vitro* antioxidant, antibacterial, and antifungal properties of this indigenous plant have been documented (Gulcin *et al*., 2008). According to Gerich (2010), the kidneys can function independently of insulin due to the continued functioning of GLUT-2 glucose transporters, even in the absence of insulin. The structural lipids of the mammalian brain differ from those of other tissues due to their significant concentration of polyunsaturated fatty acids, specifically arachidonic (20:4 n-6) and docosahexaenoic (22:6 n-3) fatty acids. These fatty acids are essential for brain growth and function. Additionally, they increase the number of insulin receptors in brain cell membranes, which play a crucial role in cognitive activities such as learning and memory (Ozkan *et al*., 2005). This study investigated the impact of *Cyclotrichium niveum* extract on ADEK vitamins and fatty acids (FA) in the brain and kidney tissues of diabetic rats.

MATERIALS AND METHODS

Thirty-two adults female Wistar albino rats, weighing between 250 and 300 g, were supplied from Adıyaman University Experimental Animals Unit. Approval for this study was obtained from Adıyaman University Animal Experiments Local Ethical Committee and the reference number is ADYU-HADYEK-2018/033. Each group comprising eight rats. The groups were categorized as follows: Control (C), Diabetes (D), *Cyclotricium niveum* (CN), and Diabetes+*Cyclotricium niveum* (D+CN). The control group received a standard diet and 0.9% saline. The induction of diabetes in the diabetes group was conducted following the method established by Guvenç *et al*. (2009). A single intraperitoneal injection of 55 mg/kg Streptozotocin (STZ)dissolved in a solution of 55 ml citric acid and 35 ml disodium hydrojen phosphate buffer was administered (ARAS *et al*., 2022) blood glucose levels were measured 72 h post-STZ administration, considering values above 200 mg/dl as indicative of diabetes (Dashtban *et al*., 2016). For the *Cyclotricium niveum* (CN) extract, a modified version of the procedure by Pandanaboina *et al*. (2012) was employed. Dried CN leaves (20 g) were infused in 100 ml hot water. After cooling, the extract was obtained through filtration and stored in closed containers at 4°C. It was orally administered at a dosage of 20mg/kg.

After the 6th week, the animals were euthanized. Tissue samples from the total brain and kidneys were meticulously wrapped in aluminum foil, labelled, and stored at -80 degrees until examination (Ozkan *et al*., 2005). The quantification of malondialdehyde (MDA) was conducted using high-performance liquid chromatography (HPLC) based on the concentration of thiobarbituric acid reactive species, serving as a marker of lipid peroxidation (Karatepe, 2004). In addition, FA was performed by GC by converting total lipid samples to methyl esters in tissues. For total lipid extraction from tissues, a 3:2 v/v ratio of hexane/isopropanol mixture method was used (Hara and Radin, 1978). The amounts of saturated (SA) and unsaturated fatty acids (USFA) in the total lipid were converted to volatile forms of FA and determined by gas chromatography (GC) (Gunstone, 1994). HPLC was used to detect levels of cholesterol and ADEK vitamins (Bragagnolo and Rodriguez-Amaya, 2003).

Extraction of lipids

Furthermore, fatty acid analyses were conducted using gas chromatography (GC) by converting total lipid samples to methyl esters in tissues. For the extraction of total lipids from tissues, a method involving a 3:2 v/v ratio of hexane/isopropanol mixture was employed. The technique developed by Hara and Radin (1978) was used to extract lipids from both brain and renal tissues. One g of brain and kidney tissue was placed in a homogenizer with 5 ml of hexane-isopropanol solution and mixed for 30 sec. Before homogenizing each sample, the homogenization vessel and homogenizer were cleaned by washing with 2 ml of hexane-isopropanol solution. Subsequently, the supernatant part of the tissue samples was obtained through centrifugation at 5000 rpm for 10 min and transferred to closed test tubes.

Preparation of fatty acid methyl esters

The method, as outlined by Christie (1990), was employed to transform the fatty acids within the lipid structure into stable methyl ester derivatives suitable for gas chromatographic analysis. Subsequently, the quantities of saturated (SAF) and unsaturated fatty acids (USFA) present in the total lipid were converted into volatile forms of FA and analysed using GC, following the methodology described by Gunstone (1994).

Identification of fatty acid methyl esters by GC

The evaluation of methyl esters, created during the conversion of fatty acids (FA) in the lipid extract, was performed using the Shimadzu GC-2010 Plus Series. A 60-meter-long capillary column from Machery-Nagel (Germany) with a 0.25-mm inner diameter and a 25-mm PERMABOND film thickness was used for this study. The column detector and injection temperature were

maintained according to the specifications outlined by Guvenç *et al*. (2009). Nitrogen gas served as the carrier gas throughout the analysis. The results were expressed as the percentage of each fatty acid in the total fatty acid content.

The analysis method of ADEK vitamins

One gram of weighed brain and kidney tissues was collected for ADEK vitamin analysis. To this, 10 ml of a 3:2 (600/400, v/v) hexane/isopropanol solution was added, followed by homogenization. The homogenized tissues were then centrifuged at 5000 rpm at 4 ºC for 10 min, and the supernatant was separated from the tissue pellet. The solvent in the supernatant was evaporated with liquid nitrogen flow and dissolved in 1 ml of a methanol/acetonitrile mixture (1:1) before being transferred to autosampler vials and analysed by HPLC. For the HPLC analysis of vitamins A, D, E, and K, a 3:2 (600/400) acetonitrile/methanol solution was prepared as the mobile phase. The flow rate of the mobile phase was set at 1 ml/min. The column, a Supelcosil LC 18 (150 x 4.6 mm, 5 µm), was maintained at a temperature of 40°C following the method by Bragagnolo and Rodriguez-Amaya (2003). The analysis employed a UV detector with wavelengths set for retinol (326 nm) (vitamin A) and vitamin E (202 nm). The amounts of cholesterol and ADEK vitamins determined from the analysis were calculated as µg/g (Guvenc *et al*., 2017).

Statistical analysis

Statistical evaluations were conducted using the SPSS 22.0 for the Windows package program. Group comparisons were performed using the ONE-WAY ANOVA test, and differences between groups were assessed using the LSD test, with standard error considered as the standard deviation. $p<0.05$ values were considered statistically significant.

RESULTS

Kidney fatty acid parameters

The levels of elaidic acid (18:1, n-9t) significantly decreased in the D+CN and CN groups ($p < 0.01$) (Table I). No statistically significant change was observed in the D group ($p > 0.05$). Similarly, linoleic acid (18:2, n-6t) showed no statistically significant difference in the D and D+CN groups ($p > 0.05$), and the ratio of omega-3 to omega-6 in the D and CN groups did not exhibit any meaningful change ($p > 0.05$). Linoleic acid (18:2, n-6t) levels decreased in the CN group ($p < 0.01$). On the other hand, linoleic acid (18:2, n-6c) increased in the D and CN groups ($p < 0.05$), with no statistically significant change observed in the D+CN combination group ($p > 0.05$). The levels of gamma-linolenic acid (18:3, n-6), arachidic acid (20:0), alpha-linolenic acid (18:3, n-3), eicosatrienoic acid

Table I. Effect of *Cyclotrichium niveum* extract (CN) on fatty acid composition (%) of non-insulin treated diabetic rat kidney.

Fatty acids	Control	CN	D	D + CN
14:0	0.39±0.05	0.52±0.10 [a]	0.52±0.06 [a]	0.57±0.06 [a,b]
15:0	0.47±0.03	0.45±0.05	0.48±0.04	0.49±0.05
16:0	19.98±0.38	21.31±0.85	20.14±0.47[a]	21.52±0.75
16:1, n-9	1.08±0.23	1.57±0.39 [a]	1.21±0.21	1.67±0.10 [a]
17:0	0.92±0.04	0.83±0.07	0.87±0.02[a]	0.82±0.06
17:1	0.32±0.01	0.37±0.05	0.37±0.03	0.39±0.05
18:0	16.09±0.43	14.87±1.03[a]	15.63±1.01	14.42±0.99[a]
18:1, n-9t	0.64±0.02	0.44±0.04 [a]	0.57±0.06[a]	0.41±0.01[a,c]
18:1, n-9c	14.64±0.69	15.13±1.01	15.75±0.78	16.28±0.99
18:2, n-6t	0.11±0.00	0.08±0.01[a]	0.10±0.00	0.10±0.00
18:2, n-6c	18.67±0.08	20.03±0.29 [a]	20.10±0.30 [a]	19.64±0.16
18:3, n-6	0.02±0.02	0.13±0.00 [a]	0.15±0.00 [a]	0.14±0.00 [a]
20:0	0.16±0.01	0.17±0.01	0.19±0.01	0.18±0.02
18:3, n-3	0.50±0.03	0.62±0.03 [a]	0.63±0.03 [a]	0.66±0.05 [a]
20:1, n-9	0.22±0.02	0.00±0.00[a,c,]	0.12±0.01[a,b,d]	0.01±0.01[a,b,c]
21:0	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
20:2	0.37±0.04	0.35±0.04	0.38±0.02[b,d]	0.33±0.04 [a,c]
20:3, n-3	0.80±0.09 [d]	0.75±0.06	0.80±0.07	0.75±0.15 [a,c]
20:3, n-6	0.14±0.02 [b,c,d]	0.20±0.03[a]	0.23±0.02[a]	0.22±0.02[a]
20:4, n-6	19.05±0.52	18.14±1.27	17.58±1.30	17.01±1.35
22:1, n-9	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00
23:0	0.00±0.00 [b,c,d]	0.03±0.00[a,c,d]	0.02±0.00[a,b,d]	0.05±0.01[a,b,c]
22:2	0.15±0.00	0.14±0.01	0.15±0.00	0.14±0.01
20:5, n-3	0.13±0.01 [b,c,d]	0.10±0.01 [a]	0.13±0.01[b,d]	0.14±0.01 [a,c]
24:0	0.11±0.05[b,c,d]	0.33±0.03[a,c,d]	0.29±0.02[a]	0.29±0.02[a]
24:1, n-9	0.04±0.01 [b,c,d]	0.08±0.01[a,d]	0.10±0.00[a,b,d]	0.11±0.00[a,b,c]
22:6, n-3	2.15±0.18	1.94±0.14	1.83±0.15	1.93±0.17
MONO-SAT	16.98±0.86	17.60±1.35	18.14±0.92	18.93±1.10[a]
POLİSAT	42.07±0.80	42.52±1.04	42.01±0.77	40.88±1.13
Omega-3	3.59±0.27	3.45±0.15	3.40±0.18	3.28±0.26
Omega-6	38.00±0.59	38.59±0.85	38.19±0.62	37.13±0.94
Omega-3/ omega-6	0.09±0.00	0.08±0.00	0.08±0.00	0.19±0.11[a,b,c]

$p < 0.05$, a, significant from control; b, significant from CN; c, significant from D; d, significant from D + CN. CN, *Cyclotrichium niveum* extract group; D, diabetes group; D + CN, Diabetes+ *Cyclotrichium niveum* extract group.

Table II. Effect of *Cyclotrichium niveum* extract (CN) on fatty acid composition (%) of brain of non-insulin (STZ) treated diabetic rat.

Fatty acids	Control (C)	CN	D	D +CN
14:0	0.12±.00	0.15±0.01^{a,d}	0.16±0.01^{a,d}	0.12±.00^{b,c}
15:0	0.06±0.06	0.05±0.02^{c}	0.00±0.00^{b}	0.02±0.01
16:0	23.92±0.21	24.14±0.16	23.89±0.16	24.10 ±0.16
16:1, n-9	0.77 ±0.30	0.56 ±0.08	0.46±0.01	0.46 ±0.01
17:0	0.39 ±0.04	0.36±0.01	0.38±0.01	0.41±0.01
17:1	0.07±0.07	0.03±0.02	0.00±0.00	0.00±0.00
18:0	23.72±2.11	26.07±0.37	26.5±0.15	26.41 ±0.10
18:1, n-9t	0.75±0.06^{b,d}	0.01±0.00^{a,c,d}	0.67±0.02^{a,d}	0.22±0.05^{a,c}
18:1, n-9c	17.72±0.37^{d}	16.76±0.33^{c}	15.72±0.16^{a,b}	15.87±0.46^{a}
18:2, n-6	0.67±0.02^{b,c,d}	0.90±0.02^{a}	0.92±0.04^{a}	1.04±0.03^{a}
18:3, n-6	0.00±0.00	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
20:0	0.28±0.00^{c,b}	0.32±0.01^{a}	0.31±0.00^{a}	0.30±0.00
18:3, n-3	0.15±0.14^{b,c,d}	0.03±0.00^{a}	0.02±0.00^{a}	0.03±0.00^{a}
20:1, n-9	1.22±0.04^{b,c,d}	2.37±0.07^{a,c,d}	1.90±0.09^{a,b,d}	2.14±0.05^{a,b,c}
21:0	0.07 ±0.01^{b,d}	0.00 ±0.00^{a}	0.02±0.01^{a}	0.00 ±0.00^{a}
20:2	0.10±0.02	0.11±0.01^{c}	0.05±0.01^{b,d}	0.13±0.01^{c,a}
20:3, n-3	0.36±0.02	0.19±0.02^{d}	0.31±0.03^{a}	0.13±0.01^{d}
22:0	0.26±0.01^{a,b}	0.33±0.02^{a,c,d}	0.22±0.00^{a,b}	0.21±0.00^{a,b}
20:3, n-6	0.06±0.00^{c,d}	0.31±0.00^{a,c}	0.47±0.02^{a,d}	0.32±0.01^{a,c}
20:4, n-6	12.01±0.10	11.88±0.04^{c}	12.29±0.09^{b,d}	11.94±0.12^{c}
22:1, n-9	0.12±0.00^{c,d}	0.11±0.01^{c,d}	0.09±0.00^{a,b,d}	0.07±0.00^{a,b,c}
23:0	0.13±0.01^{d}	0.00±0.00^{a,c}	0.13±0.00^{d}	0.03±0.01^{a,c}
22:2	0.06±0.01^{b,c,d}	0.13±0.01^{a,d}	0.14±0.01^{a,d}	0.16±0.01^{a}
20:5, n-3	0.00±0.00^{b}	0.02±0.00^{a,c}	0.01±0.00^{b}	0.02±0.00^{a,c}
24:0	0.33 ±0.02	0.34 ±0.01^{d}	0.34 ±0.00^{d}	0.29±0.00^{a,b,c}
24:1, n-9	0.11 ±0.01^{b,c,d}	0.40 ±0.02^{a,c}	0.25 ±0.01^{a,b,d}	0.39 ±0.02^{a,c}
22:6, n-3	14.55± 0.05^{c,d}	14.74 ±0.08^{a}	14.99 ±0.07^{a}	14.74±0.08^{a}
MONOSAT	21.00±0.59^{c,d}	20.35± 0.42^{c}	19.06 ±0.14^{a}	19.22±0.51^{a}
POLİSAT	28.01±0.25	28.35±0.22	29.26 ±0.11	28.72±0.32
Omega3t	15.08±0.15	14.99±0.17	15.34±0.09	15.19±0.18
Omega6t	12.76±0.10	13.09±0.14	13.70 ±.09	13.23±0.11
Omga3t/bom6t	2.61 ±1.42	1.14 ±0.02	1.12 ±0.01	1.14±0.01

For statistical detail and abbreviations see, Table I.

(20:3, n-6), tricosanoic acid (23:0), lignoceric acid (24:0), and nervonic acid (24:1, n-9) increased in the D, D+CN, and CN groups ($p < 0.001$, $p < 0.01$, $p < 0.05$, $p < 0.001$, $p < 0.01$, $p < 0.001$, $p < 0.01$). Arachidonic acid (20:4, n-6) levels decreased in all groups compared with the control ($p < 0.01$). Additionally, the ratio of omega-3 to omega-6 increased in the D and CN groups (Table I).

Brain fatty acid parameters

Comparison with the control group revealed that while 14:0 and 20:0 levels increased in the D and CN groups ($p < 0.05$, $p < 0.01$), no significant change was observed in the D+CN group ($p > 0.05$) (Table II). Levels of 18:1, n-9t increased in the D+CN and CN groups ($p < 0.001$) but did not exhibit a meaningful change in the D group ($p > 0.05$). Additionally, 18:2, n-6c, 20:1, n-9, 20:3, n-6, 20:1, n-9, 22:2, and 24:1 significantly increased in all groups ($p < 0.001$). Eicosadienoic acid (20:2, n-6) levels decreased in the D group ($p < 0.01$). Observations also showed that alpha-linolenic acid (18:3, n-3), heneicosanoic acid (21:0), and tricosanoic acid (23:0) decreased in all groups compared to the control group ($p < 0.001$, $p < 0.01$,

$p < 0.001$). Eicosatrienic acid (20:3, n-3) decreased in the D+CN and CN groups. The levels of polyunsaturated fatty acids (PUFA) and Omega-6 fatty acid levels increased in the D and D+CN groups ($p < 0.01$, $p < 0.05$, $p < 0.001$, $p < 0.05$). Behenic acid (22:0) levels significantly decreased in the D+CN group ($p < 0.05$) and increased in the CN group ($p < 0.01$). Erucic acid (22:1, n-9) and monounsaturated fatty acids (MUFA) levels decreased in D and D+CN groups ($p < 0.01$, $p < 0.001$, $p < 0.01$, $p < 0.05$). While lignoceric acid levels did not change in the D and CN groups ($p > 0.05$), they decreased in the D+CN group ($p < 0.05$) (Table II).

Kidney lipophilic vitamin, cholesterol and MDA parameters

All groups were compared to the control group. Notably, retinol levels significantly increased in the D+CN group compared to the control group ($p < 0.001$) (Table III), while no statistically significant change was observed in the D and CN groups ($p > 0.05$). In the D group, the detector did not detect a value when compared with the retinol acetate control group, and no statistically significant change was observed in the D+CN and CN groups ($p > 0.05$).

Vitamin K2 levels were elevated in the D group ($p < 0.05$), with no statistically significant change in the D+CN and CN groups ($p > 0.05$). Comparing RTOK vitamin levels to the control group, the D group exhibited a statistically significant increase ($p < 0.001$), while the D+DN and CN groups showed no change ($p > 0.05$). For vitamin D3 levels compared to the control group, no statistically significant change was observed in the D, D+CN, and CN groups ($p > 0.05$).

Alpha-tocopherol levels, when compared with the control group, significantly decreased in the D, D+CN, and CN groups ($p < 0.05$). No statistically significant change in cholesterol levels was observed in the D, D+CN, and CN groups compared to the control group ($p > 0.05$). Stigmasterol levels showed a significant increase in the D and CN groups ($p < 0.05$, $p < 0.01$), but no statistically significant increase in the D+CN group ($p > 0.05$). Comparing MDA levels with the control group, a significant increase was noted in the D group, while no statistically significant increase was observed in the D+CN and CN groups ($p < 0.001$, $p > 0.05$) (Table III).

Brain lipophilic vitamin, cholesterol and MDA parameters

All groups were compared to the control groups. Notably, retinol levels exhibited a decrease in the D, D+CN, and CN groups ($p < 0.001$, $p < 0.01$) (Table IV).

Table III. Effect of *Cyclotrichium niveum* extract (CN) on lipophilic vitamin, cholesterol, stigmasterol, MDA parameters of kidney's of streptozotocin (STZ) treated diabetic rats.

Parameters	Control (C)	CN	D	D + CN
Retinol (µg/g)	$0.11\pm0.01^{b,c,d}$	0.81 ± 0.10^{a}	$0.71\pm0.12^{a,d}$	$3.83\pm0.34^{a,c,d}$
Retinol ast (µg/g)	$1.99\pm1.04^{b,c,d}$	$0.08\pm0.01^{a,c,}$	$0.00\pm0.00^{a,b,d}$	$3.57\pm0.00^{a,b,c}$
K2 (µg/g)	2.11 ± 0.17^{c}	1.99 ± 0.08^{c}	$2.80\pm0.16^{a,b,d}$	1.88 ± 0.20^{c}
RTOK (µg/g)	0.12 ± 0.06^{c}	0.12 ± 0.01^{c}	$0.32\pm0.03^{a,b,d}$	0.11 ± 0.01^{c}
Vitamin D_3 (µg/g)	13.60±0.57	12.98±0.22	14.21±0.32	14.02±0.31
Alfa-tocoferol (µg/g)	$19.49\pm2.43^{c,d}$	15.68 ± 0.29^{a}	15.65 ± 0.77^{a}	13.42 ± 0.69^{a}
Cholesterol (mg/g)	$32.53\pm1.52^{c,d}$	34.02±0.79	29.20 ± 1.18^{a}	31.28 ± 0.67^{a}
Stigmasterol (µg/g)	$4.26\pm0.23^{b,c}$	$17.22\pm1.66^{a,d}$	$14.34\pm3.88^{a,d}$	$5.81\pm0.30^{b,c}$
MDA (nMol/g)	6.56 ± 0.02^{c}	6.25 ± 0.01^{c}	$33.93\pm1.37^{a,b,d}$	6.25 ± 0.01^{c}

For statistical detail and abbreviations, see Table I.

Table IV. Effect of *Cyclotrichium niveum* extract (CN) on lipophilic vitamin, cholesterol, stigmasterol and MDA parameters of non-insulin treated diabetic rat brain.

Parameters	Control	CN	D	D+CN
Retinol (µg/g)	$0.89\pm0.17^{b,c,d}$	$0.32\pm0.07^{a,c,d}$	$0.04\pm0.01^{a,b,d}$	$0.63\pm0.09^{c,b}$
Retinol ast. (µg/g)	0.56±0.05	0.54±0.13	$0.20\pm0.00^{a,b,d}$	$0.27\pm0.00^{a,b,c}$
K_2 (µg/g)	2.88 ± 0.22^{d}	3.12±0.09	2.58±0.14	3.64 ± 0.07^{a}
Vitamin D_3 (µg/g)	$8.61\pm1.02^{b,c}$	$7.31\pm0.22^{a,c}$	$5.70\pm0.31^{a,b}$	8.06±0.12
Alfa-tocoferol (µg/g)	9.59 ± 1.40^{c}	9.30±0.41	6.77 ± 0.48^{a}	9.33±0.30
Cholesterol (µg/g)	$49.52\pm0.27^{b,c}$	45.54 ± 0.09^{a}	43.58 ± 0.37^{a}	48.35±0.18
Stigmasterol (µg/g)	$35.96\pm10.04^{c,d}$	29.15±1.97	24.57 ± 5.40^{a}	52.49 ± 4.37^{a}
MDA (nMol/g)	$14.57\pm0.21^{b,c,d}$	$16.71\pm0.27^{a,c}$	$22.39\pm0.51^{a,b,d}$	$16.96\pm0.8^{a,c}$

For statistical detail and abbreviations, see Table I.

Significant reductions were observed in retinol acetate levels in the D and D+CN groups ($p < 0.001$). When vitamin K2 levels were compared to the control group, neither the D nor the CN groups showed a statistically significant change ($p > 0.05$), but the D+CN group's vitamin K2 levels significantly rose ($p < 0.001$). The vitamin D3, alpha-tocopherol, and cholesterol levels of the D+CN groups showed no statistically significant change ($p > 0.05$). Vitamin K2 and stigmasterol levels were increased in the D and CN groups ($p < 0.001$, $p < 0.01$). A significant decrease was detected in vitamin D3 and cholesterol levels of the D and CN groups ($p < 0.001$, $p < 0.05$, $p < 0.001$). When the alpha-tocopherol level and stigmasterol levels were compared with the control group, a significant decrease was observed in the D group ($p < 0.01$), but no statistically significant change was observed in the CN group ($p > 0.05$). MDA levels were increased in the D, D+CN, and CN groups ($p < 0.001$, $p < 0.05$) (Table IV).

Significant reductions were observed in retinol acetate levels in the D and D+CN groups ($p < 0.001$). When vitamin K2 levels were compared to the control group, neither the D nor the CN groups showed a statistically significant change ($p > 0.05$), but the D+CN group's vitamin K2 levels significantly rose ($p < 0.001$). The vitamin D3, alpha-tocopherol, and cholesterol levels of the D+CN groups showed no statistically significant change ($p > 0.05$). Vitamin K2 and stigmasterol levels were increased in the D and CN groups ($p < 0.001$, $p < 0.01$). A significant decrease was detected in vitamin D3 and cholesterol levels of the D and CN groups ($p < 0.001$, $p < 0.05$, $p < 0.001$). When the alpha-tocopherol level and stigmasterol levels were compared with the control group, a significant decrease was observed in the D group ($p < 0.01$), but no statistically significant change was observed in the CN group ($p > 0.05$). MDA levels were increased in the D, D+CN, and CN groups ($p < 0.001$, $p < 0.05$) (Table IV).

DISCUSSION

The prevalence of diabetes is rapidly increasing worldwide, and in the advanced stages of the disease, it can cause various damage to tissues. Numerous studies indicate that oxidative stress is associated with diabetes. Many studies also suggest that there are changes in the fatty acid composition of tissues such as the brain, kidneys and muscles that are affected by diabetes-related oxidative stress. We aimed to examine the influence of *Cyclotrichium niveum* (CN) extract on the levels of fatty acids (FA), ADEK vitamins, cholesterol, and MDA in the brain and kidney tissues of rats with STZ-induced diabetes.

The process of producing unsaturated fatty acids involves the use of fatty acid desaturases, while monounsaturated fatty acids are synthesized by a specific enzyme called stearoyl CoA desaturase (SCD), as stated by Ntambi (1995). SCD performs a crucial rate-limiting step by inserting a double bond in the cis configuration at the delta-9 position (Δ9) of the fatty acid substrates. Polyunsaturated fatty acids play an important role as structural components, contributing to the fluidity and selective permeability of membranes (Douillet and Ciavatti, 1995; Ozkan *et al*., 2008).

The fatty acid composition in the brain tissue was analyzed, and it was found that the amount of oleic acid decreased significantly in the diabetic groups compared to the control group. The reduction in these fatty acids is caused by a deficiency in the Stearoyl CoA Desaturase (SCD) enzyme (also called Δ9 desaturase) activation. This enzyme uses palmitic acid (16:0) and stearic acid (18:0) as substrates to form palmitoleic acid and oleic acid (Douillet and Ciavatti, 1995; Nitambi and Miyazaki, 2004).

Essential fatty acid metabolism is responsible for the synthesis of unsaturated fatty acids, in addition to de novo fatty acid synthesis. The metabolism of essential fatty acids begins with two specific fatty acids, namely linoleic acid (18:2, n6) and linolenic acid (18:3, n3). These two fatty acids are found in all tissues. The metabolism of these fatty acids continues with the activity of enzymes that provide chain extension and double-bond entry into the hydrocarbon chain. Since mammals lack the Delta 12 desaturase and Delta 15 desaturase enzymes, they cannot synthesize 18:2, n6, and 18:3, n3 fatty acids. Mammals obtain these types of fatty acids from their diet, and they are referred to as essential fatty acids (Douillet and Ciavatti, 1995; Ozkan *et al*., 2008).

The delta 6 desaturation pathway is a metabolic process using linoleic acid and linolenic acid as substrates, facilitated by delta 6 and delta 5 desaturase enzymes, leading to chain extension. The presence of diabetes has been associated with a reduction in the production of D-6 and D-5 desaturase enzymes (Brenner, 2003; Ozkan *et al*., 2008).

As a result of diabetes, the levels of arachidonic acid decrease, and linoleic acid increases. The study conducted by Ozkan *et al*. (2005) revealed that the levels of palmitic acid and arachidonic acid increased in diabetic groups, while the level of oleic acid decreased in rat brain tissue. However, in the current research, the changes in the amounts of palmitic acid and arachidonic acid in brain tissue were not statistically meaningful.

Omega-3 fatty acids have been examined more than any other kind of fatty acid in the brain. Extremely high levels of omega-3 and omega-6 have been proven to be

crucial for the development and operation of cultured brain cells (Bourre *et al*., 1984). Reduced levels of omega-3 have been linked to mild cognitive impairments in animal models and subsequently in humans (Fontani *et al*., 2005). We found that the alpha-linolenic acid was increased in all groups compared to the control in brain and kidney tissue, but the increases of alpha-linolenic levels in the D+CN group of brain tissue when compared to the diabetic group may be due to the phenolic content of CN that contains hydroxybenzoic acid, salicylic acid, syringic acid, acetohydroxamic acid, and luteolin. Hydroxybenzoic acid is a phenylpropanoid compound that acts as an insulin secretagogue (Rosile *et al*., 2022; Guzel, 2023).

An increase in linoleic acid levels was observed in all brain tissue groups compared to the control group. The same results were also reported by other researchers (Ozkan *et al*., 2005; Demir *et al*., 2020). Kidney tissue is the most important cardiovascular cause of death resulting from the progressive complications of diabetes (Mohanty et al., 2005; Alberti and Zimmet, 1998). The amounts of arachidonic and docosahexaenoic acid in the kidney decrease as a result of insufficient insulin levels which can lead to reduced levels of D-6 and D-5 desaturases. The decrease in the CN group is thought to be due to pulegone, isomenthone, triterpenoids, and flavonoids present in the structure of *Cyclotrichium niveum.*

The mammalian central nervous system contains a high concentration of polyunsaturated fatty acids (PUFAs), and diabetes-induced oxidative stress can lead to changes in brain lipid composition (Ozkan et al., 2005). Docosahexaenoic acid, a vital component for neural tissue growth and maintenance, is derived from (n-3) fatty acids. The availability of docosahexaenoic acid (DHA), a prominent structural fatty acid in the retina and central nervous system, plays a crucial role in brain development (Singh, 2005). In our study, the levels of docosahexaenoic acid increased in the D, D+CN, and CN groups, but when compared to the diabetic group, the D+CN group showed a decrease in docosahexaenoic acid levels.

MDA levels in kidney tissue did not significantly alter, according to research by Hunka *et al*. (2002). However, another study (Zararsiz *et al*., 2006) discovered a decline in MDA levels in renal tissue. High quantities of MDA, stearic acid, linoleic acid, arachidonic acid and docosahexaenoic acid were detected in kidney tissue by Demir *et al*. (2020). Our findings suggest that diabetes results in elevated levels of MDA in diabetic kidney and brain tissues, but treatment with CN effectively mitigates this effect, possibly due to its antioxidant properties and its ability to scavenge reactive oxygen species (Ulusu *et al*., 2003; Gulcin *et al*., 2008; Cardoso *et al*., 2013).

Serum retinol-binding protein has binding sites in the choroidal epithelium's choroid plexus, cuboidal, and brain microvasculature endothelial cells (MacDonald *et al*., 1990). While vitamin A levels are stable in patients with non-insulin-dependent diabetes, individuals with type I diabetes exhibit lower levels (Basu and Basualdo, 1997). Our study's findings, showing a decrease in the amount of vitamin A in brain tissue, are consistent with previous research.

Vitamin D has a crucial role in managing the intracellular calcium balance, regulating the internal and external calcium balance in the cell, promoting the expression of insulin receptors in the tissues, and assisting the insulin response (Zhang *et al*., 2008). In research examining the impact of vitamin D3 on brain development, it has been demonstrated that rats with a deficiency in vitamin D3 exhibited significant abnormalities in their brains. Specifically, it has been discovered that these rats had a longer cortex, bigger lateral ventricles, proportionately thinner cortex, and greater cell proliferation throughout the brain. Research suggests that adequate intake of vitamin D3 during pregnancy is important for the developing brain of a child (Eyles *et al*., 2003). The mechanism of the positive effect of CN on the levels of vitamin D3 in the combination of D and CN has not been elucidated.

The increase in blood glucose levels in diabetes due to insufficient insulin correlates significantly with increased cholesterol levels. The reduction of cholesterol in peripheral tissues, particularly in the heart and kidney, has been associated with the development of diabetic nephropathy and diabetic cardiomyopathy. Furthermore, some reports have shown that the depletion of cholesterol can suppress insulin secretion (Hao *et al*., 2007; Larsson *et al*., 2008). Type 1 diabetes causes a decrease in the levels of both free and total cholesterol in peripheral tissues (Wang *et al*., 2012). In our study, we found that cholesterol levels decreased in the diabetic group, and the application of CN stabilized these decreases in brain and kidney tissue. Additionally, CN decreased cholesterol levels when compared to the control group due to its sitosterol content (Fadzelly *et al*., 2006).

The beneficial effect of vitamin E on diabetes lies in its ability to reduce hyperglycaemia, which can be achieved by suppressing hepatic gluconeogenesis and reducing glucose output from the liver. This suppression is linked to the inhibition of lipolysis in adipose tissue. Vitamin E helps maintain blood glucose balance by reducing the levels of circulating glucagon, which plays an important role in blood glucose regulation and is part of the hepatic metabolic activity in the body. Vitamin E levels were found to be decreased in both the kidney and brain tissues of the diabetic groups. Although the administration

of CN normalized Vitamin E levels in the brain tissue, this increase was attributed to the antioxidant property of CN. However, its effectiveness in the kidney tissue may have been decreased due to inadequate insulin levels resulting from diabetes.

CONCLUSION

Upon evaluating the results of the analysis of the aqueous extract of *Cyclotrichium niveum* on the brains and kidneys of rats with diabetes, notable findings emerged. The extract demonstrated a significant antioxidant effect, particularly influencing the levels of vitamin E, vitamin D, cholesterol, and stigmasterol in diabetic brain tissue. Notably, the extract exhibited a positive impact on reducing cholesterol levels while increasing the levels of malondialdehyde in diabetic kidney tissue.

Moreover, the study revealed intriguing nuances in the influence of *Cyclotrichium niveum* on the levels of fatty acids, synthesized as a consequence of desaturase enzyme activity in fatty acid biosynthesis, showcasing distinct patterns in brain and kidney tissue. These observations contribute insights into the multifaceted effects of *Cyclotrichium niveum* on different biochemical parameters in tissues affected by diabetes.

DECLARATIONS

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Ethical statement and IRB approval

This study and included experimental procedures were approved by the institutional animal care and use committee of Adıyaman University Animal Experiments Local Ethical Committee Ethics Approval Number 2018/033).

Statement of conflict of ınterest

The authors have declared no conflict of interest.

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