

Effects of *Thymus Vulgaris L.* and *Thymbra Spicata L.* on diabetes mellitus associated cognitive impairment and neuropathy: *Thymus Vulgaris* and Cognitive Function Improvements

Zafer Akan¹, Melek Dikilidal², Hulya Ozdemir², Gokhan Oto², Ahmet Yilmaz³

Abstract

Aim: Diabetes mellitus (DM) is a metabolic disease due to increased blood glucose, with multiple organ involvement. Although various oral drugs are used to treat DM, they do not prevent the development of DM related diseases such as cognitive disorder, neuropathy and vascular diseases. Thus novel strategies for the prevention and treatment of DM are urgently needed. This research aimed to reveal the effects of *Thymus Vulgaris Lamiaceae* (TVL) and *Thymbra Spicata Lamiaceae* (TSL) on the damaging effects of DM.

Materials and Methods: Therefore, prepared TVL and TSL aqueous extracts were studied in the streptozocin induced experimental diabetic rat model. Blood glucose, body weight, and cognitive functions were examined. Morris water maze test was used to define the effect of TVL and TSL on DM related cognitive dysfunction.

Results: Briefly, impaired blood glucose, and cognitive dysfunction of Diabetic rats were significantly improved by TVL in dose dependent manner ($P<0.01$). Impaired Blood Glucose significantly improved and adjusted to the control group values ($P<0.01$)

Conclusion: Our findings strongly recommend the usage of TVL treatments for DM control by the DM patients

Keywords: Diabetes mellitus, Streptozocin, Diabetic Rat Model, *Thymus Vulgaris L.*, *Thymbra Spicata L.*, Morris Water Maze, Cognitive Dysfunction

Introduction

Diabetes mellitus (DM) is the common metabolic disease referring to hyperglycemia due to the corruption of insulin secretion, insufficient insulin sensitivity, or both. DM can affect almost every organ system in the body, and the level of the damage is particularly related to the severity and duration of the disease [1, 2]. Type 1 DM (T1D) is an autoimmune disease characterized by the destruction of pancreatic β -cells.

Exposure of environmental harmful substances during neonatal period is accused of leading to initiation of immune

process underlying the destruction of β -cells and the development of disorder [3]. Patients with Type 1 Diabetes (T1D) are presented with absolute insulin deficiency, and multiple types of insulin formulations have been developed for the treatment of T1D in the last three decades [4]. Type 2 Diabetes (T2D), non-insulin-dependent type of DM, is mainly an adult disease and associated with insulin resistance. The incidences of T2D rapidly increase due to the decrease in physical activity, sedentary lifestyle, and aging population.

Received: 16 Jun 2014, **Revised** 24 Jun 2014, **Accepted** 24 Jun 2014, **Available Online** 30 Jun 2014

¹Celal Bayar University, School of Medicine, Department of Biophysics, Manisa-Turkey

²Yuzuncu Yil University, School of Medicine, Department of Pharmacology, Van-Turkey

³Dicle University, School of Medicine, Department of Family Medicine, Diyarbakir-Turkey

*Corresponding Author: Zafer Akan, zafer.akan@cbu.edu.tr

T2D is characterized by insulin insensitivity as a result of insulin resistance, decreased insulin production, and finally pancreatic β -cell failure. Patients with T2D should receive lifestyle alteration recommendations and diet modification.

Karabas Kekik or Zahter) (TSL) is also a member of the Lamiaceae family, and the leaves of this plant have recently gained much popularity as a remedy to combat hypercholesterolaemia [8].

TVL and TSL have been arbitrarily and widely used in the west region of Turkey

Table 1: The effects of different doses of aqueous extract of TVL and TSL on fasting blood glucose level (mg/dl) in streptozocin induced diabetic rats. Values given represent the Mean \pm SD; One-way Analysis of Variance (ANOVA) was applied to results *P<0.05, **P<0.01 and ***P<0.001.

	Blood glucose (mg/dl)				
	1 st week	2 nd week	3 rd week	4 th week	5 th week
CRs	86,70 \pm 10,82	94,40 \pm 14,72	94,70 \pm 14,11	96,00 \pm 16,81	94,80 \pm 11,22
DRs	425,22 \pm 133,01	409,56 \pm 80,43	423,67 \pm 108,99	436,43 \pm 68,62	416,63 \pm 110,64
DRs+Gli	340,50 \pm 89,37	410,00 \pm 118,89	341,00 \pm 107,59	332,33 \pm 191,85	345,11 \pm 140,73
DRs+TVL100	312,10 \pm 53,71	286,11 \pm 66,04	298,78 \pm 85,85	237,56 \pm 100,37	322,75 \pm 182,56
DRs+TVL200	299,10 \pm 83,55	293,70 \pm 52,23	278,40 \pm 118,16	288,40 \pm 116,48	261,67 \pm 144,76
DRs+TSL100	303,50 \pm 76,57	431,40 \pm 91,14	367,00 \pm 140,82	345,50 \pm 115,92	425,33 \pm 156,48
DRs+TSL200	406,80 \pm 81,68	395,89 \pm 110,72	355,22 \pm 113,39	359,88 \pm 166,87	348,25 \pm 143,99
CRs/versus	P value				
DRs	***	***	***	***	***
DRs+Gli	***	***	***	**	**
DRs+TVL100	***	***	**	*	*
DRs+TVL200	***	***	**	P>0.05	P>0.05
DRs+TSL100	***	***	***	***	***
DRs+TSL200	***	***	***	***	**

Although novel drugs are being developed, no cure is currently available for DM related diseases [5, 6]. DM may lead to the microstructural complications, such as neuropathy, retinopathy. Diabetic neuropathy is characterized by distal symmetric polyneuropathy. The most common symptoms and findings are fatigue, paresthesia, plantar burning, loss of sense and pain. As a result, some pathology, such as diabetic foot and a morbid condition, may occur due to diabetic neuropathy [7]. *Thymus vulgaris* L. (Local name: Izmir Kekigi) (TVL) is a species of flowering plant in the mint family Lamiaceae and native to the west of Turkey. *Thymbra Spicata* L (Local name:

for the treatment of various diseases, such as diabetes mellitus, diabetes dependent retinopathy, neuropathy, urinary system disorders, and cardiovascular diseases, in folk medicine. However, no previous scientific report is available regarding the influences of TVL and TSL on DM related damage and impairment in cognitive systems. Therefore, the present study was created to examine the possible effects of TVL and TSL on the DM associated neuropathy and cognitive improvements.

Material and Methods

Animals and experimental groups

Male Wistar albino rats weighing 305.2 ± 4.33 g, 60 days old, were obtained from the Yuzuncu Yil University experimental animal unit. Rats were maintained on 12-h dark/light cycle at 22°C , housed in groups of seven, and fed with a standard commercial rodent chow. The care of the animals and this experimental animal study were conducted with the approval of the Institutional Animal Care and Use Committee of the Yuzuncu Yil University Experimental Animal Unit and Ethic Committee (YUHADYEK).

DM induction

To examine the effects of TVL and TSL treatment on DM, fifty six Wistar albino rats were divided into seven equal groups. Group 2, 3, 4, 5, 6 and 7 were induced to DM. Severe DM was induced in the animals by intra-peritoneal injection of Streptozotocin (STZ; Sigma–Aldrich, St. Louis, MO) that was dissolved in 0.1 M citrate buffer solution (0.1 M, pH 4.5) at the dose of 50 mg/kg body weight (BW). Animals were fasted overnight for 12 h

blood glucose levels were recorded once a week throughout the study.

Extraction of TVL and TSL:

The species of TVL and TSL were collected from Aegean region (Aydın and İzmir cities). Taxonomic identification was performed by Associate Prof. Dr. Fevzi Ozgokce. The collected plants were dried in an oven at 40°C and then ground into a powder. For extraction, the decoction method and distilled water as solvent were used [8]. For the decoction method, 20 g of dried powder was extracted with 100 ml of distilled water at 100°C for 30 min in a water bath. Subsequently, it was filtered, and the water was evaporated to dryness. The residue was weighed to obtain the extractive yield, and it was in air tight bottle at 4°C . The yield of dried extract were found % 5.9 and 5.85, respectively. The extracts were prepared daily. Resolved in 100 and 200 mg/ml distilled water and orally administered to the rats daily.

The creation of groups and the assessment of the effects of TVL and TSL treatment on blood glucose level (BGL) and body weight changes:

Diabetic rats were treated by the agents of Glibenclamide, TVL and TSL aqueous

Table 2: Effects of different doses of aqueous extract of TVL and TSL on spatial learning latency (minute) of streptozocin induced diabetic rats. Values given represent the mean and S.E.M. One-way Analysis of Variance (ANOVA) was applied to results.

	1 st day	2 nd day	3 rd day	4 th day
CRs	0,60±0,080	0,19±0,02	0,22±0,050	0,14±0,02 ^a
DRs	0,80±0,089	0,55±0,082**	0,37±0,064*	0,34±0,064*
DRs+Gli	0,80±0,081	0,47±0,073	0,52±0,074	0,29±0,055
DRs+TVL100	0,71±0,089	0,36±0,066	0,36±0,072	0,14±0,037 ^a
DRs+TVL200	0,78±0,084	0,50±0,075*	0,33±0,054	0,12±0,017 ^{aa}
DRs+TSL100	0,91±0,085	0,52±0,078*	0,50±0,094*	0,30±0,076
DRs+TSL200	0,76±0,088	0,47±0,086	0,36±0,064	0,22±0,046

^aP<0,05 and ^{aa}P<0,01 compromising between DRs versus CRs, TVL100 and TVL200

prior to STZ administration. Water and food were available immediately after dosing. The development of DM was determined by observing hyperglycemia (>300 mg/dl) [10] as measured by an Accu-Chek Go glucometer (Roche, Mannheim, Germany). Body weights and

extracts. Both BGL and body weight alterations were compared with the result of control group. The groups were created as; (1) no additive; (2) 50 mg/kg STZ only; (3) 50 mg/kg STZ plus 5 mg/kg glibenclamide; (4) 50 mg/kg STZ plus 100 mg/kg TVL; (5) 50 mg/kg STZ plus 200

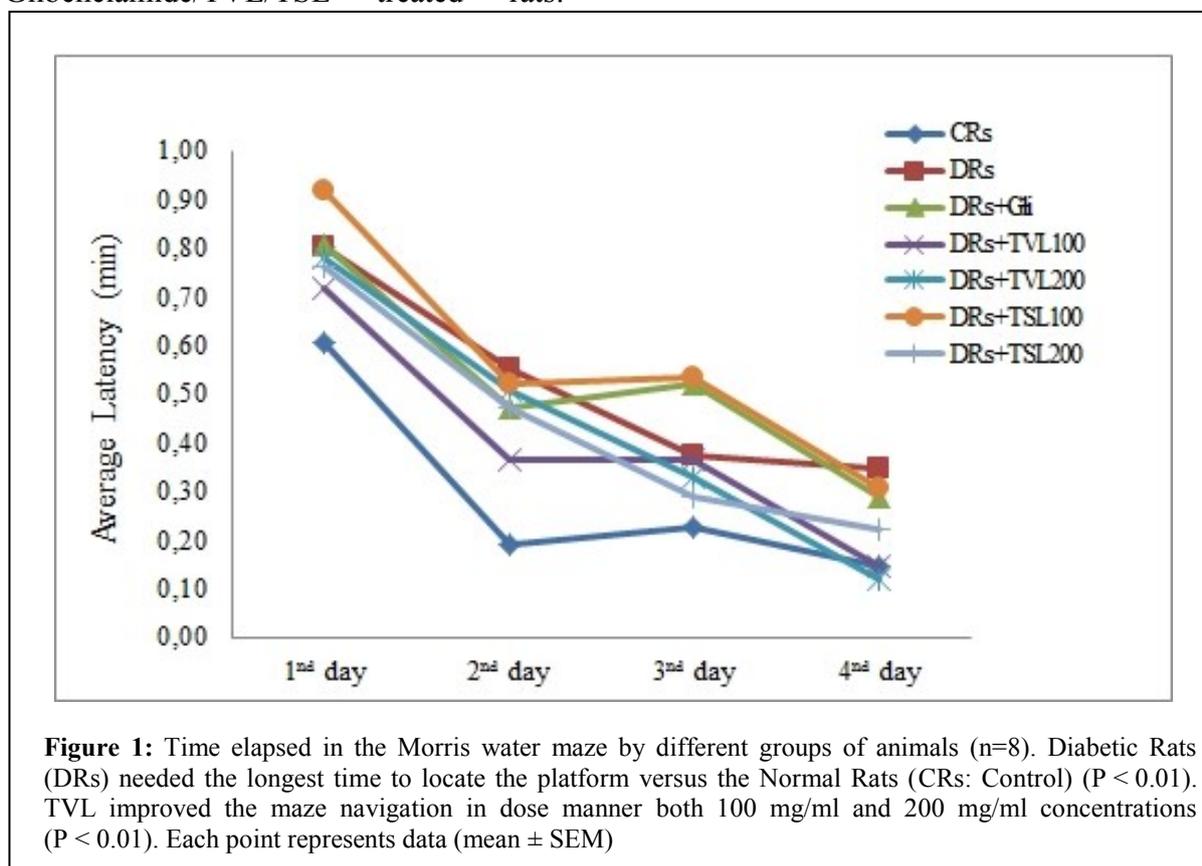
mg/kg TVL; (6) 50 mg/kg STZ plus 100 mg/kg TSL; (7) 50 mg/kg STZ plus 200 mg/kg TSL. Body weight and were measured once in a week through 5 weeks. Rats were treated with glibenclamide/TVL/TSL single daily dose.

The assessment of TVL and TSL treatment on spatial learning of diabetic rats by Morris water maze test:

The Morris water maze (MWM) is a test for spatial learning examination for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform [9]. We tested spatial learning of control, diabetic, and Glibenclamide/TVL/TSL treated rats.

Statistical Methods

For spatial learning tests; a total of 56 rats including 8 rats in each group were subjected to the study. Each experiment was repeated at least 3 times, and the results of a representative experiment were shown. One-way Analysis of Variance (ANOVA) and Tukey-Kramer Multiple Comparisons tests were applied to the variables, and each result was reported as mean \pm S.E.M. and a, p value less than 0.05 was accepted as statistically significant. (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$)



Present protocols for performing variants of the MWM test, from which results can be obtained from individual animals in as few as 4 days end of the study.

All tissue and blood samples were collected to search for different tissue involvements under anesthetic conditions (200 U/kg Ketalar).

Results

Control group blood glucose levels were measured in an average of 86.7 mg/dl. BGL were chronically elevated to the average of 422.3 mg/dl by the single (Acute) dose STZ (50 mg/kg) administration. BGL remained stable in both Control and STZ groups for 5 weeks.

BGL were slightly decreased by the Glibenclamide treatment in the Glibenclamide group. The most significant results over the increased BGL were obtained with TVL100 mg/kg and TVL 200 mg/kg treatments ($p < 0.01$) (Table 1). The mean body weight was 305.2 gr, and it was decreased to 208.3 gr by the administration of STZ at the end of 5th week. TVL treatment reduced body weight loss in STZ administrated diabetic rats ($p < 0.01$). As to spatial learning MWM test results; figure 1 and table 2 showed that the escape latency to locate the hidden platform was statistically significantly shorter in the groups of control, TVL 100 and 200 mg/kg treated diabetic rats compared with the diabetic vice-versa glibenclamide, TSL100 and 200 mg/kg treated groups ($*p < 0.05$, $**p < 0.01$). The performance at fourth day of the control, TVL 100 and 200 mg/kg groups was not statistically different ($p > 0.05$). Spatial learning performances of diabetic rats were improved by the TVL 100 and 200 mg/kg treatments (Figure 1)

Discussion

TVL and TSL are known with their antimicrobial and antiviral effects, thus the use is common among the population for the treatment of DM and various diseases. They are characterized by the large scale component of free radical scavenger essential oils such as thymol, carvacrol, 8-terpinene, p-cymene and α -pinene. In addition, the chemical composition of TVL and TSL essential oils varies in a wide range. Carvacrol and thymol are the main components of the mint family Lamiaceae plants. The rate of these components is due to the environment and ecology [8]. The aim of the present study was to analyze the impact of TVL and TSL in terms of blood glucose, vascular, renal and cognitive systems in diabetic rats. Streptozotocin is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. It is used in medical research to produce an

animal model for Type 1 DM in single large dose as well as Type 2 DM with multiple low doses. In a long period of time, diabetic rats are characterized by high blood glucose and body weight loss. In our study, we produced Type 1 DM by a single large dose of STZ administration (50 mg/kg) in the Wistar albino rats. The diabetic rats were treated by glibenclamide, TVL and TSL aqueous extracts. It was seen that TVL was significantly effective in the avoidance of the increase in blood glucose, weight loss, the decline in cognitive functions, and also in the improvement of them.

DM is associated with slowly progressive end-organ damage in the brain. Mild to moderate deteriorations of cognitive functions have been declared in patients with both type 1 and in type 2 DM [10]. Many of DM related clinical complications are commonly caused by the vessel disorders. The peripheral microvascular complications of DM occurring outside the brain seem to be associated with corresponding microvascular changes in the brain. For instance, diabetic retinal microvascular abnormalities were associated with various MRI findings including small focal white matter hyperintensities and lesions [11]. In the present study, it was determined that a significant improvement in cognitive functions of diabetic rats was provided by the TVL treatment. It was supposed that this positive impact of TVL on the cognitive function was due to its positive effects on blood glucose level and microvascular system.

In conclusion, it was supposed that both TVL and TSL had dose dependent protecting and healing effects against the damaging effects of DM in terms of blood glucose cognitive systems moreover, diabetic neuropathy.

Our finding strongly recommends the usage of TVL treatments for DM control by the DM patients

Conflict of Interest

The authors declared that they had no conflicts of interest.

Acknowledgements

This project was supported by Yuzuncu Yil University Scientific Research Project Funding (YYU-BAP/2013-SBE-YL051). Thanks to Associate Prof. Dr. Ozgokce F for herbal taxonomy and Associate Prof. Dr. Keskin S for statistical analysis

References

1. Rehman HU, Mohammed K. Perioperative management of diabetic patients. *Current surgery*. 2003;60(6):607-11.
2. Krishnan B, Babu S, Walker J, Walker AB, Pappachan JM. Gastrointestinal complications of diabetes mellitus. *World journal of diabetes*. 2013;4(3):51-63.
3. Majeed AA, Mea, Hassan K. Risk Factors for Type 1 Diabetes Mellitus among Children and Adolescents in Basrah. *Oman medical journal*. 2011;26(3):189-95.
4. Chen W, Xie A, Chan L. Mechanistic basis of immunotherapies for type 1 diabetes mellitus. *Translational research : the journal of laboratory and clinical medicine*. 2013;161(4):217-29.
5. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. *Oman medical journal*. 2012;27(4):269-73.
6. Germino FW. Noninsulin treatment of type 2 diabetes mellitus in geriatric patients: a review. *Clinical therapeutics*. 2011;33(12):1868-82.
7. Aring AM, Jones DE, Falko JM. Evaluation and prevention of diabetic neuropathy. *American family physician*. 2005;71(11):2123-8.
8. Akkol EK, Avci G, Kucukkurt I, Keles H, Tamer U, Ince S, et al. Cholesterol-reducer, antioxidant and liver protective effects of *Thymbra spicata* L. var. *spicata*. *Journal of ethnopharmacology*. 2009;126(2):314-9.
9. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols*. 2006;1(2):848-58.
10. van den Berg E, Dekker JM, Nijpels G, Kessels RP, Kappelle LJ, de Haan EH, et al. Cognitive functioning in elderly persons with type 2 diabetes and metabolic syndrome: the Hoorn study. *Dementia and geriatric cognitive disorders*. 2008;26(3):261-9.
11. Luchsinger JA. Type 2 diabetes and cognitive impairment: linking mechanisms. *Journal of Alzheimer's disease : JAD*. 2012;30 Suppl 2:S185-98.