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The Effects of the Synthetic Antioxidant, Tempol, on Serum Glucose and Lipid Profile of Diabetic and Non-Diabetic Rats

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Abstract

Background: Hyperlipidemia and low antioxidant levels is one the diabetes side effects. Some studies have indicated the possible effects of nutrients on the improvement of hyperlipidemia, by their antioxidants ingredients.
Objectives: The aim of the present study was to evaluate the effect of the synthetic antioxidant, tempol, on blood lipid profiles and glucose levels in healthy and diabetic rats.
Materials and Methods: Adult Wistar rats were randomly divided to four experimental groups including, healthy control, diabetic control, diabetic receiving tempol and healthy receiving tempol groups. Diabetes was induced by injection of streptozotocin (60 mg/kg, Intraperitoneally (IP)). The rats were then fed saline or tempol (30 mg/kg) by gavage for 60 days. Blood samples were collected by cardiac puncture. Next, glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), cholesterol, triglyceride and HbA1c were measured by specific kits. Also, the coronary risk index was calculated.
Results: The blood glucose level increased following diabetes induction. The level of blood glucose in the diabetic receiving tempol group decreased compared to the control diabetic group. The comparison of LDL, VLDL, cholesterol, triglyceride, HbA1c and coronary risk index among experimental groups indicated the increase of these factors in the diabetic group. High-density lipoprotein in the diabetic groups was lower than the other groups.
Conclusions: It can be concluded that tempol can improve dyslipidemia and may decrease hyperglycemia in diabetes. It seems that antioxidants such as tempol can improve dyslipidemia and may decrease hyperglycemia in diabetes.

Keywords: Tempol, Lipid Profiles, Diabetes, Rat

1. Background

Diabetes mellitus is a major public health problem throughout the world, and is the leading cause of global mortality (1). Diabetic disease is characterized by hyperglycemia, which is the accumulation of free glucose in the blood. Hyperglycemia induces oxidative stress via glucose autoxidation and leads to generation of free radicals due to autoxidation of glucose and glycosylation of proteins (2-4) and has an important role in the development of diabetic complications (5-7). Diabetes is likely to increase the risk of developing various metabolic disorders, including hyperlipidemia, liver-kidney dysfunctions, and hypertension (8).

On the other hand, hyperlipidemia has been observed in diabetic patients (9-11) and experimental diabetic animal models (12-14). Triglyceride levels are enhanced under diabetic conditions (5-7). In experimental models of diabetes, high glucose induced oxidative stress (15), increases in oxidative stress related to lipid, and inhibition of the synthesis of endogenous antioxidants (16, 17). Oxidative stress and triglyceride levels are enhanced in patients with diabetes (2).

In cure of diabetes it is important to prevent diabetes complications. The current evidence suggests that supplementation of antioxidant compounds may protect against diabetic complications (15, 18-21). There are evidences about the protection effect of antioxidant compounds against diabetic problems (15, 18-23).

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a member of a family of nitroxide compounds that is an efficient scavenger of free radicals (24) and improves insulin responsiveness and dyslipidemia in models of diabetes mellitus (25). The anti-inflammatory, neuroprotective effects of tempol have been shown previously (26-30). Furthermore, it is an efficient scavenger of free radicals and improves diabetes-associated dyslipidemia (25) and cardiac fibrosis in rats (31).

2. Objectives

There is no direct study on the efficient activity of tempol on hyperlipidemia in diabetes. Therefore, this study aimed to test the hypothesis that chronic oral administration of tempol could ameliorate hyperlipidemia in a diabetes rat model.

3. Materials and Methods

3.1. Animals

Forty Wistar rats, weighting 200 - 300 g, were supplied by the breeding colony of the Iran Pasteur institute, Tehran. They were maintained at 20 ± 2°C on a 12-hour light/dark cycle (lights on 07:00 am). Water and food were available ad libitum. All rats were acclimatized to the environment for one week prior to initiation of testing. All procedures for the treatment of animals were approved by the research committee of the Hamadan university of medical sciences.

3.2. Induction of Diabetes and Treatment

The animals were divided to the following groups; control (C), diabetic (D), diabetic tempol treatment (D + T), and control group receiving tempol (C + T). The model of type I diabetes was induced by a single dose of intraperitoneal (IP) injection of 60 mg/kg of streptozotocin (32-34). The control rats received IP injections of physiological saline. Blood samples were taken from the tail vein, and glucose levels were determined using a strip-operated blood glucose sensor (Accuchek; Roche, Mannheim, Germany). One week after streptozotocin injection, the rats with blood glucose levels exceeding 250 mg/dL were considered diabetic. Tempol (30 mg/kg; Sigma) was administered to D + T and C + T groups by the gavage process, every day for two months. The control and non-treated diabetes groups received physiological saline with the same volume.

At the end of the treatment period, the rats were anaesthetized with ketamine (100 mg/kg) and blood samples were collected by cardiac puncture. Next, glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol and triglyceride were measured by their specific kits (Biolabo, France). The HbA1c was assessed by its specific kit (Bionik, Iran) and the turbidimetry method. The concentration of very low density-lipoprotein (VLDL) was calculated as TG/5. Also, the coronary risk index was calculated (34-36).

3.3. Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the statistical significant differences between experimental groups, which were followed by Tukey's post hoc test. P values of <0.05 were considered statistically significant. All data were represented as mean ± standard error of the mean (SEM).

4. Results

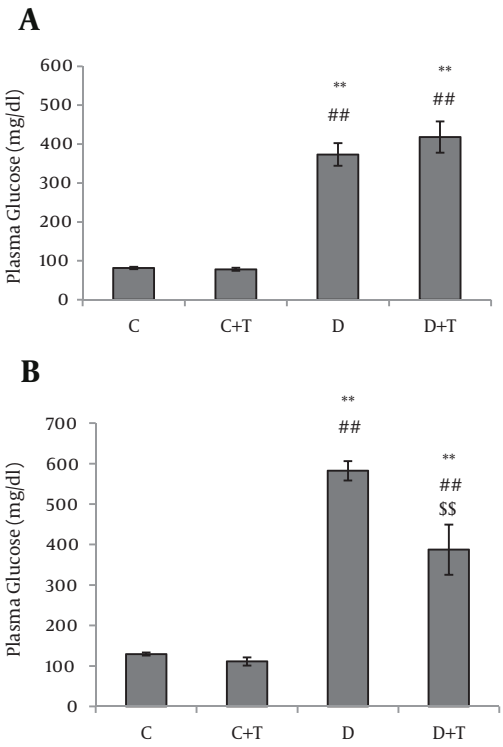
Figure 1 shows the glucose levels of rat groups, after diabetes induction. One-way ANOVA proved that there were significant differences between the groups after induction of diabetes ($P < 0.01$). Tukey's post hoc test revealed that blood glucose levels of diabetic induction groups were significantly higher than non-diabetic groups (Figure 1A, $P < 0.01$). Moreover, one-way ANOVA showed that there were significant differences among the experimental groups of rats after tempol treatment. Tukey's post hoc test revealed that tempol-treated rats had a significant decrease in their plasma glucose compared to untreated diabetic rats at the end of the experiment (Figure 1B, $P < 0.01$).

Figure 2 shows the LDL, VLDL, cholesterol and triglyceride levels in plasma. One-way ANOVA demonstrated that there is a significant difference between the groups in the level of LDL, VLDL, cholesterol and triglyceride in serum plasma. There was a significant increase in the level of LDL in plasma of non-treated diabetic rats in comparison with other groups ($P < 0.05$; Figure 2A). Also, non-treated diabetic rats had a significant increase in their plasma levels of LDL, VLDL, cholesterol and triglyceride in comparison with other groups ($P < 0.01$; Figure 2B - D, respectively). The diabetic rats that received tempol exhibited significantly lower LDL, VLDL, cholesterol and triglyceride compared to the non-treated diabetic group.

Figure 3 shows the HDL level in plasma of the rat groups. One-way ANOVA suggested that there was a significant difference between the groups. The level of plasma HDL in non-treated diabetic rats was significantly lower than other experimental groups ($P < 0.01$).

Figure 4 illustrates the Hb A1c and coronary risk factor in the experimental groups. One-way ANOVA approves that there was a significant difference between the groups ($P < 0.05$). Tukey's post hoc test revealed that Hb A1c and the coronary risk factor in the streptozotocin (STZ)-receiving rats were significantly higher than non-diabetic groups ($P < 0.01$; Figure 4A and B, respectively). The STZ-induced diabetic rats were administrated tempol and showed lower Hb A1c and coronary risk factor when compared to the non-treated diabetic rats ($P < 0.05$).

Figure 1. Blood Glucose Levels in Experimental Groups



A, Blood glucose levels after induction of diabetes; B, Blood glucose levels after induction of diabetes at the end of the study. C, control group; C + T, control group receiving tempol; D, diabetic group; D + T, diabetic group that received tempol. **: ($P < 0.01$) as compared with the control. **: ($P < 0.01$), ##: ($P < 0.01$) and $$: ($P < 0.01$) as compared with the control, control group receiving tempol or diabetic group, respectively. The values represent means ± standard error of the mean (SEM) (n = 10 per group). Each symbol on a column compares the mentioned group with the specified group.

5. Discussion

The present findings demonstrated that administration of tempol for 60 days, improved the blood lipid profiles and hyperglycemia in diabetic rats.

Tempol treated diabetic rats had lower glucose and percentage of HbA1C levels than the diabetic rats. The HbA1c is evaluated in long-term control of diabetes. Glycated hemoglobin reflects the previous two to three months of glycemic control (37). These results confirm previous studies that tempol has hypoglycemic properties (25, 38, 39). Our results are similar to a previous study that demonstrated oral tempol treatment of diabetic mice during eight weeks, reduced plasma glucose (40). Ten weeks of oral tempol administration to rats, that were fed high fat diets, decreased plasma glucose (41), and improved insulin sensitivity in obese diabetic rats (42). Reactive oxygen species (ROS) are involved in many of the complications of diabetes (43, 44), and generation of ROS is prevented with tempol in pancreatic islet cells (45) and in diabetic mice (40).

Tempol acts by several mechanisms such as by alleviation effects on insulin resistance (46), enhancing insulin secretion from rat cultured pancreatic islet cells (38), and increasing the membrane abundance of the glucose transporter-1 and enhanced glucose uptake (47). Oxygen-derived free radicals are easily produced in diabetic disease and have important roles in the development of diabetic complications (5-8, 44, 48). Tempol is a superoxide dismutase mimetic and efficient scavenger of free radicals (49).

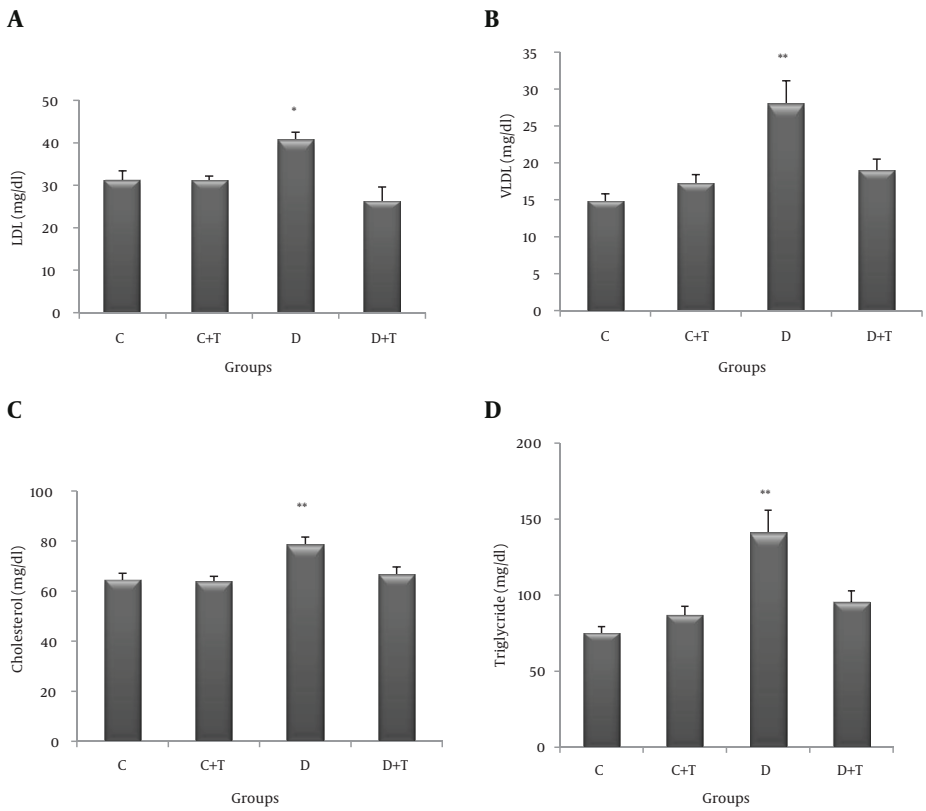
In diabetes, alleviated blood lipid profile is due to increased absorption of cholesterol from the intestine by a carrier of cholesterol acetyltransferase (50, 51). Plasma cholesterol, triglycerides are raised and hyperlipidemia is distinguishable in diabetes (16, 17). Lack of insulin raises free fatty acid mobilization from adipose tissue, which is followed by production of cholesterol rich LDL particles and dyslipidemia (16, 52). On the other hand, production of oxygen free radicals increases in hypercholesterolemia (53).

Antioxidants are a normal defense mechanism of the cell and are involved in the termination of the lipid peroxidation process (4). Tempol decrease VLDL and total cholesterol and increase HDL (40, 54). Antioxidants protected the polyunsaturated fatty acids (a major component of cell membranes) from oxygen free radical attack in diabetes (55) and end the peroxidation events (4).

Another finding of the present study demonstrated that tempol has advantages for coronary risk factor. Increased reactive oxygen species cause oxidative myocardial injury and diabetic cardiomyopathy (56, 57). Evidence has demonstrated specific cardiomyopathy associated with diabetes such as cardiomyopathy, cardiac dysfunction and cardiovascular disease in humans and rats (58-61). The beneficial effect of tempol was shown on blood pressure (62), cardiac fibrosis (63) and amelioration cardiac dysfunction in diabetic rats (31). The potential antioxidant role of tempol was shown in decreasing reactive oxygen species and amelioration of cardiac dysfunction (31).

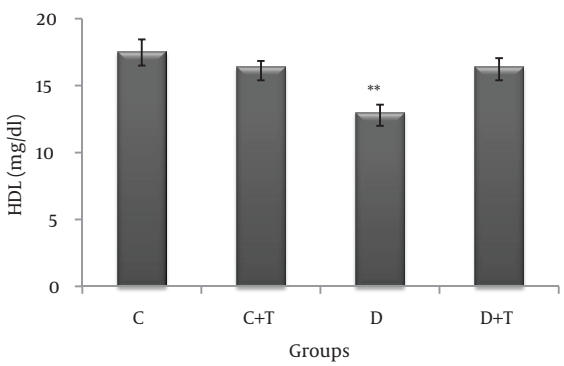
In conclusion, this study confirmed that tempol improved blood lipid profiles, hyperglycemia and coronary risk factor in the diabetic rats. It could prevent the development of diabetic complications. In order to more precisely determine the mechanism of the present findings, measurement of oxidative stress indexes is recommended for the future studies.

Figure 2. The Lipid Profile in Experimental Groups



Effect of 60 days of treatment on A, LDL level in serum; B, VLDL level in serum; C, cholesterol level in serum; D, Triglyceride level in serum. Groups are C (control group), C + T (control group thar recieved temporal), D (diabetic group), D + T (diabetic group that recieved temporal). Groups are C (control group), C + T (control group thar recieved temporal), D (diabetic group),D + T(diabetic group that recieved temporal). *: ($P < 0.05$), and **: ($P < 0.01$) as compared with non-treated diabetic group. The values represent the mean ± SEM (n = 10 per group).

Figure 3. High-Density Lipoprotein Levels at the End of the Study



Groups are C (control group), C + T (control group thar recieved temporal), D (diabetic group), D + T (diabetic group that recieved temporal). **, ($P < 0.01$) as compared with non-treated diabetic group. The values represent mean ± SEM (n = 10 per group).

Footnotes

Authors' Contribution: Study concept and design, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content: Siamak Shahidi, Zahra Jabbarpour, Masoud Saidijam, Rasoul Esmaeili, Alireza Komaki and Nasrin Hashemi-Firouzi; acquisition of data, statistical analysis, and drafting of the manuscript: Siamak Shahidi, Zahra Jabbarpour, Rasoul Esmaeili and Alireza Komaki; administrative, technical and material support: Siamak Shahidi, Zahra Jabbarpour, Masoud Saidijam, Rasoul Esmaeili and Alireza Komaki; study supervision: Siamak Shahidi.

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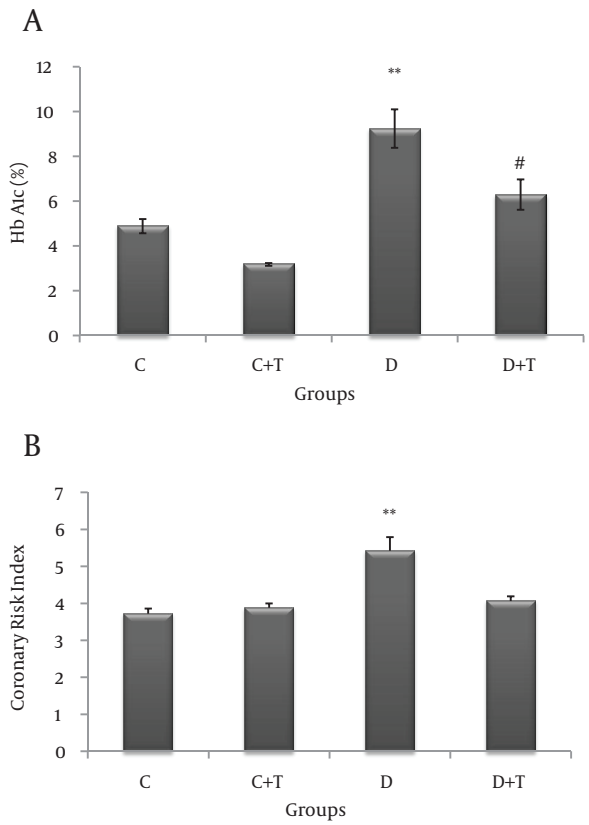
Figure 4. HbA1c and Coronary Risk Index in the Experimental Groups



A, effect of 60 days of treatment on percentage of Hb A1c; B, effect of 60 days of treatment on percentage of coronary risk factotor; Groups are C (control group), C + T (control group thar recieved temporal), D (diabetic group), D + T (diabetic group that recieved temporal). **: ($P < 0.01$) as compared with non-treated diabetic group. #: ($P < 0.05$) as compared to diabetic rats. The values represent mean ± SEM (n = 10 per group).

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