International Journal of Phytomedicine 9 (2017) 628-633
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

The effect of troxerutin on alterations of lipid profile and biochemical enzymes in blood of rats with chronic diabetes

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Received: 12 Jul 2017
Accepted: 17 Oct 2017
Published: 28 Dec 2017

Abstract

Diabetes Mellitus (DM) is a progressive disease that leads to complex disorders such as biochemical changes in the blood. The use of medicinal plants are superior to synthetic drugs because of the few side-effects in disease prevention. In this study, we examined the effect of troxerutin on lipid profile and biochemical enzymes in the blood of type 1 diabetic rats.

32 male Wistar rats (200-250) were randomly divided into four groups: control, diabetes, control+ troxerutin, diabetes+ troxerutin. Type 1 diabetes was induced by i.p injection of streptozotocin (50 mg/kg) in animals in diabetic groups. Lasted for 4 weeks, oral administration of troxerutin (150 mg/kg) was carried daily for 4 weeks. At the end of study, anesthesia was induced intraperitoneally with sodium pentobarbital (mg / kg 60). Blood samples was collected for measuring lipid profile and biochemical enzymes in blood of rats.

Diabete significantly increased LDL, COL, TG and significantly decreased HDL compared to the control group. Treatment diabetic rats with troxerutin for 4 weeks significantly decreased LDL, COL, TG and significantly increased HDL. Furthermore, Diabetes significantly increased ALT, AST, LDH, and CPK in blood of rats. Treatment diabetic rats with troxerutin for 4 weeks significantly decreased ALT, AST, LDH, and CPK in blood of rats compared to the control group.Troxerutin improve the lipid profile and reduce biochemical enzymes in blood of diabetic rats.In this way could be useful in reducing the complications of diabetes.

Keywords: Diabetes, Troxerutin, biochemical enzymes, lipid profile, rat.

Introduction

Diabetes mellitus (DM) is a syndrome characterized by disordered metabolism, hyperglycemia, resulting from low levels of insulin production by beta cells of pancreass [1]. Approximately 95% of diabetics are suffering from dyslipidemia and have changes in their total blood protein [2]. Blood levels of liver enzymes in diabetic subjects are changed compared with healthy subjects (non-diabetic) [3]. Prospective studies have found that high levels of hepatic enzymes, including alanine amino transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) are associated with later development diabetes [4]. It is reported that increased levels of ALT is precursor to type 2 diabetes [4]. Also, elevation of serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) concentration has been observed in patients with diabetes mellitus [5].

Hypoglycemic agents are useful in the treatment of DM, but have many side effects and use of these drugs restricted by the pharmacokinetic properties and secondary failure rates. So, there is a need to look for more efficacious agents with fewer side effects[6]. Troxerutin, a trihydroxyethylated derivative of the natural bioflavonoid rutin, has been reported to possess important biological activity, including antioxidative, anti-inflammatory, anti-hiperlipidemia and anti-thrombolytic activity [7]. It is shown that troxerutin decrease advanced glycation end products and oxidative stress in mice. Many studies have shown that troxerutin has a variety of biological activities [8-11].Therefore, due to the possible effects of troxerutin in control of diabetes, this study is designed to investigate the effects of troxerutin on alterations of lipid profile and biochemical enzymes in blood of rats with chronic diabetes.

Methods

Animals and study design

This research was carried out in accordance with the National Research Council's protocol for the care and use of laboratory animals. We obtained 32 Wistar male rats (200 to 250 g) from the laboratory animal house of Tabriz University of Medical Sciences. The animals were housed in standard conditions (temperature 22 C, light from 8.00 am to 8.00 pm) and had free access to tap

DOI:10.5138/09750185.2165

water and food pellets. The animals were divided into into four groups (n = 7):
1-Control (Con): animals that have no intervention and received placebo for 4 weeks.
2-Diabetes (Dia): animals that were diabeticand received placebo for 4 weeks.
3-Troxerutin (Trx): animals that received troxerutin (150 mg/kg/day) for 4 weeks.
4-Diabetes + troxerutin (Dia-Trx): diabetic rats that received troxerutin for 4 weeks.
Troxerutin (150 mg/kg) was gavaged 6 days a week for 4 weeks.

Induction of diabetes

Type 1 diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg) (Sigma, St. Louis, Missouri, USA) to animals [12]. Streptozotocin was dissolved in 10mM sodium citrate, pH 4.5, with 0.9% NaCl. Diabetes was verified 72 hours later by evaluating blood glucose levels using a glucometer (Elegance, model CT-X10, Frankenberg, Germany). Rats with blood glucose levels 300 mg/dL (16.67 mmol/L) were considered to have diabetes [13].

Biochemistry Analysis

At the end of the treatment period of 4 weeks, the animals were sacrificed after overnight fasting and blood for serum was collected. The blood collected without EDTA was centrifuged at 3500 rpm during 15 minutes to obtain the serum that was stored at -20ºC. The serum concentration of liver enzymes (alanine aminotransferase - ALT, aspartate aminotransferase - AST, and alkaline phosphatase - ALP) were determined by colorimetric assay using commercially available kits according to the manufacturer's instructions (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil).
Biochemical parameters including total cholesterol (TC), triglycerides (TGs), high density lipoprotein-cholesterol (HDLc), and low density lipoproteincholesterol (LDLc) that were determined enzymatically on a COBAS FARA analyzer Roche Diagnostics, Switzerland) [14].

Statistical analysis

All values were analyzed by one-way analysis of variance (ANOVA), and the Tukey test was used to compare quantitative data. Values less than 0.05 were considered statistically significant in all cases. Results are expressed as means ±SEM.

Tabel 1: Effect of 4 weeks troxerutine treatment on lipid profile in the serum of the tested groups

متغیرهاگروهها	Triglyceride (mg/dl)	Colostrol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Con	82/2 ± 10/2	65 ± 2/5	5/5 ± %2	%30 ± %02
Dia	100 ± 1/18**	105 ± 2/5 **	7/5 ± 0/6 **	%24 ± 0/ 07**
Con-Trx	75 ± 9/5 *	6 ± 5/5 +++	4/2 ± %78 **+++	%38 ±%05 **+++
Dia-Trx	91 ± 5/6*++##	78 ± 3/6 *+++##	6/8 ±%10 *++##	%29 ±%05 +###

Data are expressed as mean± SEM for 7 animals. * p<0.05, ** p<0.01, *** p<0.001 vs the Con group. + p<0.05, ++ p<0.01, +++ p<0.001 vs the Dia group. ## p<0.01, ### p<0.001 vs the **Con**-Trx group.

LDL: Low density lipoprotein ،HDL: High density lipoprotein.
Con: Control, Dia: Diabetes, Con-Trx:Control-Troxerutin, Dia-Trx: Diabetes- Troxerutin.

Result

Effects of troxerutin on lipid profile (TG, COL, LDL, and HDL)

Tabel 1 shows that diabetes significantly (p< 0/01) increase TG, COL, LDL and significantly (p< 0/01) decrease HDL in blood in comparison to the control group. After 4 weeks of treatment of diabetic rats with troxerutin, the level of TG (p< 0/01), COL (p< 0/001), LDL (p < 0/01) and HDL (p < 0/05) significantly reversed in blood in comparison to the diabetes group. Also, in the Trx group, TG (p < 0/05), LDL (p < 0/01) significantly decreased and HDL (p < 0/01)
Significantly increased in blood in comparison to the control group.

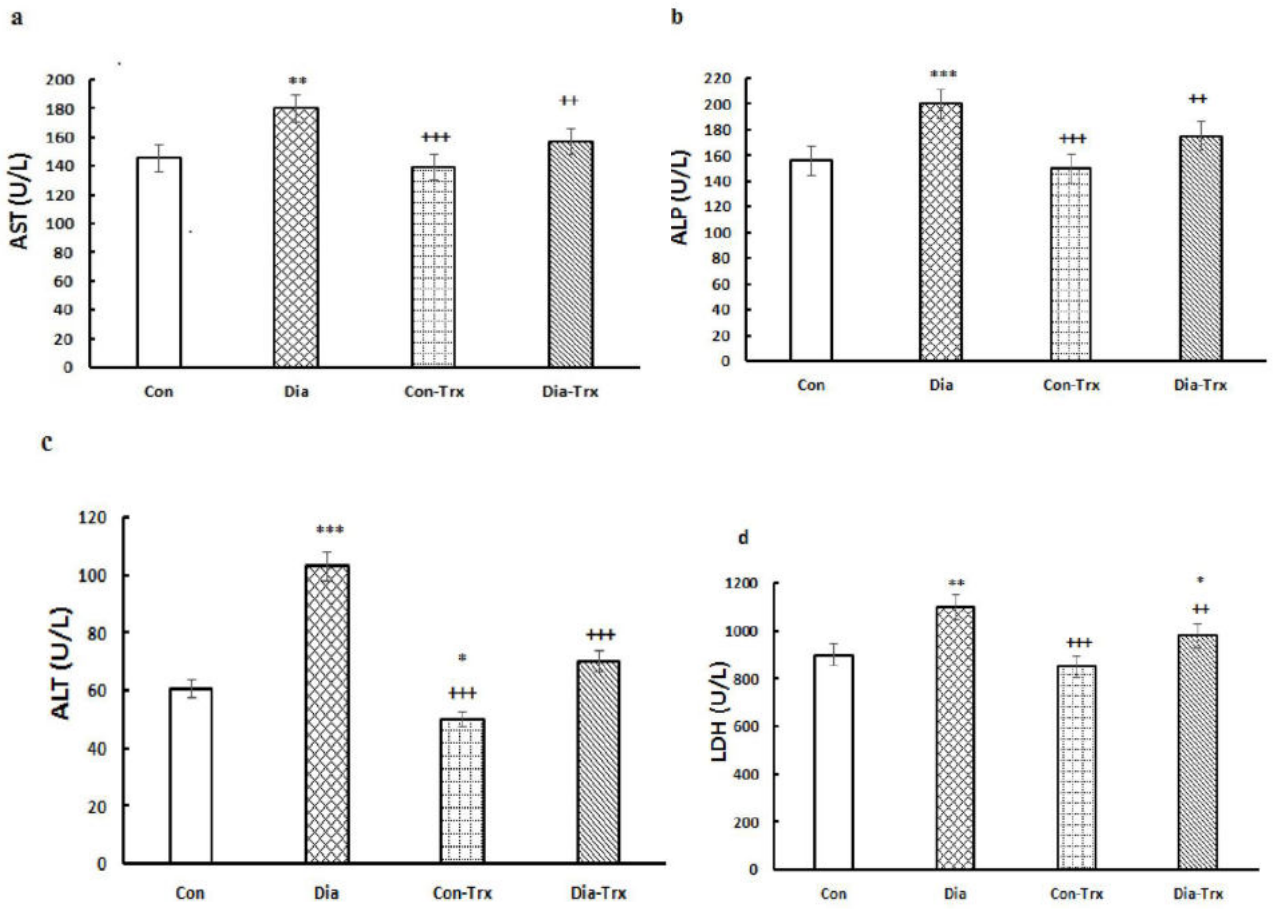

Figure 1. A) Effect of 4 weeks troxerutine treatment on AST levels in the serum of the control and diabetes groups B) Effect of 4 weeks troxerutine treatment on ALP levels in the serum of the control and diabetes groups C) Effects of 4 weeks troxerutine on ALT levels in the serum of the control and diabetes groups D) Effects of 4 weeks troxerutine on LDH levels in the serum of the control and diabetes groups. Data are expressed as mean ± SEM for 7 animals. *p<0.05, *** p<0.001 vs the Con group. ++ p<0.01 , +++ p<0.001 vs the Dia group.

Effects of troxerutin on blood biochemical enzymes (ALT, AST, ALP, CKP, LDH)

One-way ANOVA showed that diabetes significantly(p<0/01) increase AST in blood in comparison to the control group. After 4 weeks of treatment of diabetic rats with troxerutinsignificantly (p<0/01) decrease AST in blood in comparison to the diabetes group.As shown in figure b, diabetes significantly (p<0/001) increase ALP in blood of Dia group in comparison to the control group. Treatment of diabetic rats with troxerutin significantly (p<0/01) decrease this enzyme. One way ANOVA in figure C shows that diabetes significantly (p<0/001) increase ALT in blood in comparison to the control group. Also, treatment with troxerutin significantly decrease ALT in control group (p<0/05) and diabetic rats (p<0/001). As shown in figure d, diabetes significantly (p<0/01) increase LDH in blood of diabetes group in comparison to the control group. Also, treatment of diabetic rats with troxerutin significantly decrease LDH in blood in comparison to the control group (p<0/05) and diabetes group (p<0/01).

Discussion

This study is the first to investigate the effect of troxerutin on lipid profile and biochemical enzymes in blood of rats with type 1 diabetes. In this study we shown that 4 weeks treatment of diabetes and control groups with troxerutin significantly decrease triglyceride, cholesterol, LDL and increase HDL in blood in comparison to the control group and diabetes group, respectively. Also, our findings showed that treatment of diabetic rats with troxerutin significantly decrease blood of biochemical enzymes in comparision to control group.

Diabetes mellitus is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced HDL cholesterol, a predominance of small dense LDL particles, and

elevated triglycerides[15]. There is an evidence that each of these dyslipidemic features is associated with increased risk of arteriosclerosis, cardiovascular disease and renal failure leading cause of death in patients with diabetes[16-18]. In this study, we demenstrated that diabetes leads to dyslipidemia in rats and treatment with troxerutin for 4 weeks reverse this abnormality in blood of diabetic rats.

In order to our study , it have been shown that total cholesterol, LDL, and triglyceride levels in children and adolescents with type 1 diabetes to be higher than the control group [19-21].

Insulin affects the metabolism of lipoproteins through a variety of metabolic pathways [22]. Studies indicate that production of VLDL in the gut is controlled by insulin [23]. Several recent studies have suggested that insulin resistance may be a factor in causing dyslipidemia [22-24]. It has been shown that troxerutin increases the level of insulin and its receptor in diabetes type 2 [25].One of the reasons for reducing insulin in diabetes is the increase of apoptosis in the Langerhans Island cells. In 2011, Lu and colleagues showed that troxerutin activates the PI3K / Akt pathway [26]. The PI3K / Akt signaling pathway plays an important role in the survival, apoptosis, metabolism and proliferation of cells [27]. Probably, troxerutrin decreases the rate of apoptosis in -pancreatic cells by activation of PI3K / Akt signaling pathway.

Also, troxerutin increases the amount of GLUT4 in the membrane of skeletal muscle cells and reduces blood glucose level and reduces complications from hyperglycemia [25]. In order to our work, troxerutrin reduces total blood plasma cholesterol in high-fat rats, which is likely to result from inhibitory effect of troxerutrin on endogenous cholesterol production by suppressing HMGCoA reductase [28]. Chylomicrons and VLDL, as carriers of endogenous and exogenous TGs, are substrates of the lipoprotein lipase system (LPL) [29]. The level of activity of this enzyme decreases under insulin deficiency conditions; which can be the beginning of hyperlipidemia in diabetic patients [29]. It has been reported that troxerutrin increases the activity of LPL and so decreases plasma triglyceride levels [30].

It is thought that troxerutrin improve dyslipidemia in stropotozocin - induced diabetic rats by activating the PI3K / Akt pathway and decreasing the amount of apoptosis in -pancreatic cells, increasing the amount of insulin produced, suppressing the HMGCoA reductase enzyme, and enhancing LPL activity.

The study also showed that diabetes significantly increases the blood biochemical enzymes and treatment of diabetic rats with troxerutin for 4 weeks resulted in a significant decrease in liver enzymes. It has been reported that, high levels of liver enzymes including ALT, AST and ALKp are seen in rats with diabetes [31].

In order to confirm our work, it has also been shown that adding troxerutin to coumarin in the treatment of patients with varicose veins reduces ALT, LDH, and AST enzymes [32].It is shown that functional impairment of liver tissue in diabetic patients increases the activity of liver enzymes [33]. Acute hepatocellular necrosis have been reported in 80% of diabetic patients [34],therefore, increased activity of ALT, AST, ALP, LDH, and CPK in diabetes mellitus may be due to the leakage of these enzymes from liver cytosol to the bloodstream [35]. It has been reported that troxerutin reduces necrosis in the skin of the rats [36]. It is likely that troxerutin can reduce the amount of blood biochemical enzymes by reducing the liver necrosis of diabetic rats.

It is reported that, production of free radicals and lipid peroxidation of the hepatocytes increase in type 1 diabetes [37]. Increased ROS formation in diabetes cause mitochondrial function impairment [38]. Hypertrophy of hepatocytes, which is characterized by a significant increase in the number of mitochondria and a clear reduction in glycogen granules, is one of the characteristics found in type 1 diabetes mellitus. Following such changes, the permeability of the membrane increases and cytoplasmic enzymes of hepatocytes leak from the cells into the blood stream [38]. It has been reported that troxerutin reduces oxidative stress in the kidneys and brain of diabetic rats [39]. Therefore, it seems that troxerutin can reduce the amount of blood biochemical enzymes by reducing the oxidative stress in the liver of diabetic rats.

Also, insulin suppresses the genes that produce gluconeogens enzymes, as the level of insulin decreases in diabetes, the amount of ALT as a gluconeogeneic enzyme, is increased.

In conclusion, troxerutin, a rutoside derivative, diminish blood biochemical enzymes and improve dyslipidemia in STZ-diabetic rats. Potential mechanisms underlying the effect of troxerutin in the rats with diabetes might be: (i) reducing the oxidative stress in the liver (ii) reducing the liver necrosis by activation of PI3K / Akt signaling pathway and (iii) increasing insulin production.

Acknowledgments

Support of this investigation by the Drug Applied Research Center, Tabriz University of Medical Sciences, through a grant, is gratefully acknowledged.

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