

RESEARCH ARTICLE

The Effects of Aqueous Extract of *Raphanus sativus* on Blood Glucose, Triglyceride and Cholesterol in Diabetic Rats

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ABSTRACT

The probability of cardiovascular diseases in diabetic patients increases due to hyperlipidemia. *Raphanus sativus* is a traditional plant which is used to lower plasma lipid. The aim of this study was to evaluate the effect of aqueous extract of *Raphanus Sativus* on plasma glucose, triglyceride, and cholesterol in diabetic rats. For this purpose, 30 male rats were selected, maintained in standard conditions and divided randomly into 3 groups. Streptozotocin (65 mg/kg) was given IP to induce diabetes in 14 days. The aqueous extract of *Raphanus sativus* was given orally to the experimental groups at doses of 800 and 1600 mg/kg once daily for 14 days by a feeding needle. At days 1, 14, 28 and 35, blood samples were taken from the tail vein of all the groups. After this period, the animals were sacrificed under deep anesthesia by ether and their pancreases were removed, fixed in 10% formaldehyde, processed with paraffin and stained with hematoxyline and eosin for histopathological examination. Results showed that, there was a significant decrease in plasma triglyceride in the 1600 mg/kg group compared with group 1 in 28 and 35 days. No significant difference was observed in blood cholesterol and glucose in all days. In conclusion, it seems that aqueous extract of *Raphanus sativus* can lower the plasma triglyceride, but it has no effect on the plasma glucose or cholesterol.

Keywords: *Raphanus sativus*, Glucose, Triglyceride, Cholesterol, Diabetes

Diabetes mellitus is one of the metabolic diseases that results in carbohydrate, lipid, and protein metabolic dysfunction. Due to insulin deficiency, lipolysis decreases which, in turn, results in hyperlipidemia and an increase in blood cholesterol and triglyceride, eventually leading to atherosclerosis and increase in the risk of heart attack [1-3]. Therefore, although controlling the blood glucose in these patients is very important, prevention of long term side effects should be taken into account very seriously. Any treatment which could control triglyceride and cholesterol in diabetic patients is very useful. In some cases, phytotherapy is used for treatment of hyperlipidemia. The fibers in diets can retard the absorption of carbohydrate and decrease blood lipids [4,5]. Researches have shown that plants such as *Eugenina jambolana seed krne*, *Tamarindus indica* and *Fenugreek leaves* can decrease blood lipids in diabetic rats [6-8]. One of these plants is *Raphanus sativus* which increases the serum HDL in normal rats [9]. In rats with a high-fat diet, the use of extract of the *Raphanus*

Sativus causes epithelium repair and an increase in enteroecyts [10]. Therefore, it seems that *Raphanus Sativus* can decrease blood lipids. The aim of this study was to evaluate the effect of aqueous extract of *Raphanus Sativus* on the blood glucose, cholesterol and triglyceride and also to study the possible changes in the microscopic structure of the liver and pancreas in diabetic rats.

MATERIALS AND METHODS

Ethics

All animal experiments were approved by the Animal Ethics Committee of Shiraz University of Medical Sciences.

Extraction method

Raphanus sativus was collected from the rural areas of the city of Shiraz in winter and scientifically identified by expert botanists of Yasuj University as

Table 1. Effect of aqueous extract of *Raphanus sativus* on blood glucose level between different groups in streptozotocin -induced diabetic rats

Diabetic rats	Days			
	1	14	28	35
Group 1 (saline solution) N=10	135.2 ± 13.35	411.0 ± 10.1	434.8 ± 9.3	415.9 ± 21.3
Group 2 (800mg/kg RS) N=10	113.8 ± 16.86	392.7 ± 16.4	418.2 ± 13	405.4 ± 10.6
Group 3 (1600mg/kg RS) N=10	1001 ± 11.2	388.2 ± 26.3	405.8 ± 39.7	396.6 ± 21.9

The Data are expresses as Mean ± SD.

Table 2. Effect of aqueous extract of *Raphanus sativus* on blood cholesterol level between different groups in streptozotocin -induced diabetic rats

Diabetic rats	Days			
	1	14	28	35
Group 1 (saline solution) N=10	78.1 ± 20.2	128.2 ± 2.8	119.12 ± 1.8	114.34 ± 7.9
Group 2 (800mg/kg RS) N=10	65.3 ± 33	102.7 ± 4.5	105.6 ± 5.8	98.1 ± 6.2
Group 3 (1600mg/kg RS) N=10	59.4 ± 3.2	99.7 ± 4.5	100.31 ± 9.9	107.0 ± 6.3

The Data are expresses as Mean ± SD.

Raphanus sativus. Extraction was performed by the percula tion method. For this purpose, about 230 g of *Raphanus sativus* was grinded and mixed with 300 ml of water, using a blender. The mixture was kept at dark and room temperature After 24 hrs, the mixture was filtered through a Wattman filter no. 40. The filtrate was evaporated by a rotary evaporator and the remaining extract was dried in a desiccator. At the end, 10 g of semi-solid extract was obtained. For preparing the dosage form, an appropriate amount of the extract was mixed with water to obtain 800 mg/ml and 1600 mg/ml preparations.

Animals and treatment

In this study, 30 male rats weighting 200-250 g were selected. They were habituated with standard experimental conditions (temperature of 20-22 °C, 12 hour period of light and dark and free access to food and water). Diabetes was induced by a single interaperitoneal injection of streptozotocin (Sigma, USA) at a dose of 65 mg/kg body weight in 1 ml sodium citrate buffer PH 4.5. The animals were kept for the occurrence of diabetes for 2 weeks. Thirty diabetic rats were randomly divided into 3 groups: In group 1, the rats received saline solution orally by gavage for 3 weeks. In groups 2 and 3, *Raphanus sativus* was dissolved in saline and given orally by gavage for 3

weeks at a daily dose of 800 and 1600 mg/kg respectively.

Biochemical analysis

Before starting the experiments, a blood sample was taken via the tail vein. After 14 days, another blood sample was taken to ensure the induction of diabetes. At the end of the experiments, another blood sample was taken and centrifuged at 3000 rpm for 10 min. The plasma was separated and used for determination of blood glucose, cholesterol and triglyceride.

Histopathological analysis

The animals were deeply anesthetized and fixed by cardiac perfusion with formaldehyde buffer. Their pancreases were removed, fixed (10% formaldehyde for 72 hrs), processed and stained with Hematoxylin-Eosin. Histological evaluation was performed to determine the possible effects of the extract.

Histopathological changes of each pancreas were evaluated by a scoring system. In this system, histpathological evaluation was performed by examination of at least 30 islets from each group. Each pancreas was graded for cell infiltrations, cell necrosis and atrophy of islets.

Statistical analysis

The Wilcoxon-Friedman test was applied and $p < 0.05$ was considered as statistically significant.

Table 3. effect of aqueous extract of *Raphanus sativus* on blood triglyceride level between different groups in streptozotocin -induced diabetic rats

Days	1	14	28	35
Diabetic rats				
Group 1 (saline solution) N=10	89.6 ± 11.9	147.2 ± 15.1	134.13 ± 7.7	142.0 ± 15.8
Group 2 (800mg/kg RS) N=10	76.5 ± 6.1	131.9 ± 5.4	124.1 ± 7.4	126.8 ± 4.9
Group 3 (1600mg/kg RS) N=10	66.1 ± 9.52	139.6 ± 7.8	81.8 ± 8.3*	69.6 ± 10*

The Data are expresses as Mean ± SD.

* $p < 0.05$ significant difference relative to group 1

Table 4. Histopathologic changes of pancreatic islets in diabetic rats after treatment with *Raphanus sativus*

lesion	Cell infiltration	Cell necrosis	Island atrophy
Groups			
Group 1	8	70	81
Group 2	11	65	78
Group 3	3	59	70

0: no reaction

1-25: minimal reaction

26-50: mild reaction

51-75: moderate reaction

76-100: marked reaction

RESULTS

Blood glucose level

Table 1 shows that in group 1, Streptozotocin significantly increased the blood glucose level, at 14, 28 and 35 days compared to 1 day ($p < 0.05$). However, administration of different dose of *Raphanus sativus* extract did not change this parameter in 28 and 35 days compared to 14 days.

Blood cholesterol level

In Table 2, it can be seen that in group 1, Streptozotocin significantly increased blood cholesterol level in 14 days ($p < 0.05$). However, administration of different dose of *Raphanus sativus* extract did not change this parameter in 28 and 35 days compared to 14 days.

Blood triglyceride level

Table 3 shows that in group 1, Streptozotocin significantly increased blood triglyceride level in the rats in 14, 28 and 35 days compared to 1 day ($p < 0.05$). However, administration of different dose of *Raphanus sativus* extract did not change this parameter in 28 and 35 days compared to 14 days in the dose of 800 mg/kg. There is a significant decrease in plasma triglyceride in the 1600 mg/kg of hydroalcoholic extract of *Raphanus sativus* in 28 and 35 days compared to 14 day ($p < 0.05$).

Histopathological evaluation

Table 4 shows the histopathological evaluation of pancreas structure by a scoring system. In the histopathological study of the pancreas in group 1, islets atrophy was seen or in some lobules they had disappeared completely. Necrotic cells were observed in most of the islets and mild lymphocyte infiltration was seen in some lobules. In groups 2 and 3 the structure of pancreas was similar to these in group 1 (Fig 1). Fig 1 also shows normal pancreatic islet.

DISCUSSION

Metabolic dyslipidemia is the most common complication of insulin resistance and type 2 diabetes [1]. This is characterized by distinct changes from a normal plasma lipid which includes elevated triglyceride, cholesterol and LDL [1-3]. These parameters are major factors for cardiovascular diseases. Streptozotocin is a drug which experimentally causes diabetes in experimental animals. This drug also causes hyperlipidemia in the animals including increase in triglyceride, cholesterol and plasma free fatty acids [7]. However, some studies indicated that Streptozotocin increases the plasma triglyceride and glucose but has no effect on cholesterol [11]. In this study, Streptozotocin increased glucose and triglyceride but had no effect on the cholesterol level. Some reports show that in the case of hyperlipidemia, the activity of lipoprotein lipase decreases [12]. The control of the activity of this enzyme is dependent on insulin levels.

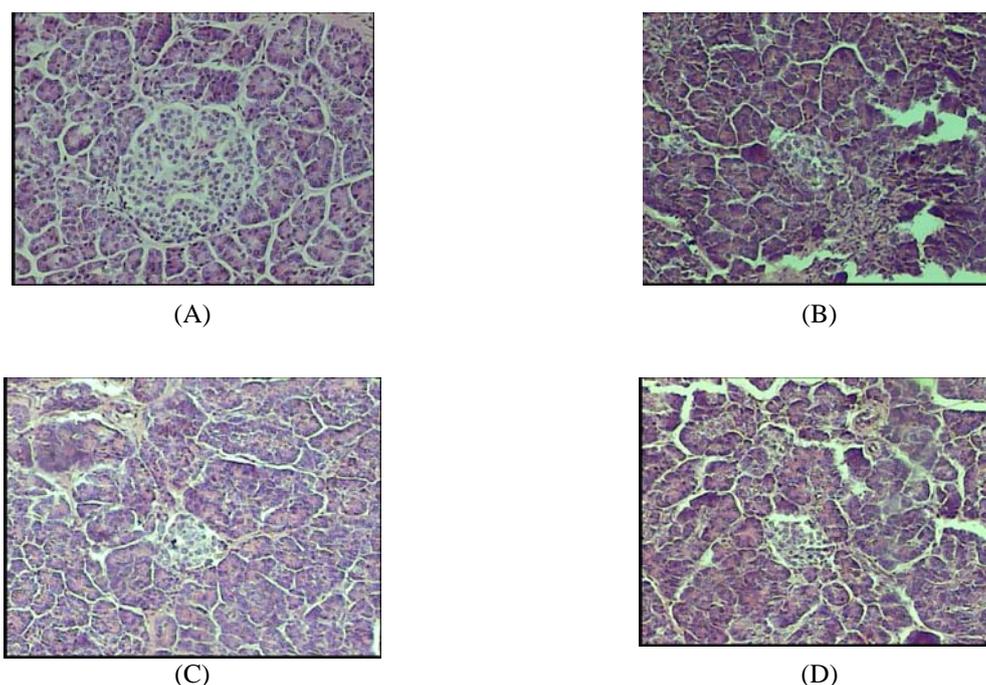


Fig 1. Histopathological changes of pancreas in various groups. (A) Normal pancreatic islet (100×), (B) pancreatic islet in diabetic rats receiving normal saline (100×), (C) pancreatic islet in diabetic rats receiving 800 mg/kg of *Raphanus sativus* extract (100×) (D) pancreatic islet in diabetic rats receiving 1600 mg/kg of *Raphanus sativus* extract (100×).

Reduction of insulin will lead to lipo-protein lipase inhibition. This enzyme is responsible for the removal of most of the plasma triglyceride [13]. Therefore, it seems that in diabetic patients, the reduction of lipo-protein lipase activity causes an increase in the plasma triglyceride levels. So, the use of drugs which are able to lower plasma lipids in diabetic patients is necessary. Statins, fibrates and niacin have clearly been demonstrated to reduce hyperlipidemia and are required in prevention of cardiovascular diseases [14]. However, for various reasons in recent years, popularity of traditional and complementary medicine had a significant increase for treatment of different diseases [15]. Some medicinal plants such as *Eugenia jambolana seed krne*, *Tamarindus indica* and *Fenugreek leaves* were shown to increase fat metabolism and decrease plasma lipids in diabetic animals [6-8]. *Raphanus sativus* is one of medicinal plants that prevent injuries to the colon in high fat diet rats [10]. Some reports show that *Raphanus sativus* can decrease the plasma cholesterol, triglyceride and phospholipids in normal rats [9,16]. It seems that *Raphanus sativus* increases the lipid metabolism and lowers the lipid plasma by increasing the activity of lipoprotein lipase. Other researchers indicated that the secretion of lipid peroxidase was increased in Streptozotocin-induced diabetic rats [17]. Oxidative stress develops from complication in diabetes and the balance between free radicals and antioxidants deteriorates [18]. So, diabetes might lead to increased free radical production. Researchers show that free radicals are higher in hyperlipidemic rats compared to

normal rats [19]. Therefore, in these cases, the antioxidant enzyme and the free radical scavenging capacity were lower. Several studies have indicated that daily treatment by insulin and vitamin E inhibits the release of lipid peroxides and decrease the free radicals [17]. Black radish juice is shown to increase antioxidant enzymes, resulting in a significant improvement of hyperlipidemia [19]. This extract has antioxidant properties and decreases free radicals [20]. Therefore, perhaps the use of *Raphanus sativus* in these who have hyperlipidemia leads to reduction of free radicals.

In conclusion, it seems that *Raphanus sativus* decreases plasma triglycerid, but it has no role in controlling plasma glucose and cholesterol.

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REFERENCES

1. Avramoglu RK, Basciano H, Adeli K. Lipid and lipoprotein dysregulation in insulin resistant states. *Clin Chim Acta* 2006; 368:1-19.
2. krauss RM. Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care* 2004; 27: 1496-504.
3. Taskinen MR. Type 2 diabetes as a lipid disorder. *Curr Mol Med* 2005; 5:297-308.
4. Pereira MA, O'Reilly E, Augustsson K, et al. Dietary fiber and risk of coronary heart disease. A pooled analysis of cohort studies. *Arch Intern Med* 2004; 164:370-6.

5. Fernandez ML. Soluble fiber and nondigestible carbohydrate effects on plasma lipids and cardiovascular risk. *Curr Opin Lipidol* 2001; 12:35–40.
6. Ravi K, Rajasekaran S, Subramanian S. Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats. *Food Chem Toxicol* 2005; 43:1433-9.
7. Maiti R, Das UK, Ghosh D. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of *Tamarindus indica*. *Biol Pharm Bull* 2005; 28:1172-6.
8. Annida B, Stanely Mainzen Prince P. Supplementation of fenugreek leaves lower lipid profile in streptozotocin-induced diabetic rats. *J Med Food* 2004; 7:153-6.
9. Taniguchi H, Muroi R, Kobayashi-Hattori K, Uda Y, Oishi Y, Takita T. Differing effects of water-soluble and fat-soluble extracts from Japanese radish (*Raphanus sativus*) sprouts on carbohydrate and lipid metabolism in normal and streptozotocin-induced diabetic rats. *J Nutr Sci Vitaminol* 2007; 53:261-6.
10. Sipos P, Hagymási K, Lugasi A, Fehér E, Blázovics A. Effects of black radish root (*Raphanus sativus* L. var niger) on the colon mucosa in rats fed a fat rich diet. *Phytother Res* 2002; 16:677-9.
11. Gerrity RG, Natarajan R, Nadler JL, Kimsey T. Diabetes-induced accelerated atherosclerosis in swine. *Diabetes* 2001; 50:1654-65.
12. Veiraiah A. Hyperglycemia, lipoprotein glycation, and vascular disease. *Angiology* 2005; 56:431-8.
13. Robinson DS, Cryer A, Davies P. The role of clearing factor lipase (lipo protein lipase) in the transport of plasma triglycerides. *Proc Nutr Soci* 1975; 34:211-5.
14. Courville KA, Lavie CJ, Milani RV. Lipid-lowering therapy for elderly patients at risk for coronary events and stroke. *Am Heart Hosp J* 2005; 3:256-62.
15. Kameswara RB, Renuka SP, Rajasekhar MD, Nagaraju N, Appa RC. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. *J Ethnopharmacol* 2003; 85:169-72.
16. Taniguchi H, Kobayashi-Hattori K, Tenmyo C, Kamei T, Uda Y, Sugita-Konishi Y, Oishi Y, Takita T. Effect of Japanese radish (*Raphanus sativus*) sprout (Kaiware-daikon) on carbohydrate and lipid metabolisms in normal and streptozotocin-induced diabetic rats. *Phytother Res* 2006; 20:274-8.
17. Yoshida M, Kimura H, Kyuki K, Ito M. Effect of combined vitamin E and insulin administration on renal damage in diabetic rats fed a high cholesterol diet. *Biol Pharm Bull* 2005; 28:2080-6.
18. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40:405-12.
19. Lugasi A, Blázovics A, Hagymási K, Kocsis I, Kéry A. Antioxidant effect of squeezed juice from black radish (*Raphanus sativus* L. var niger) in alimentary hyperlipidaemia in rats. *Phytother Res* 2005; 19:587-91.
20. Barillari J, Cervellati R, Costa S, Guerra MC, Speroni E, Utan A, Iori R. Antioxidant and choleric properties of *Raphanus sativus* L. sprout (Kaiware Daikon) extract. *J Agric Food Chem* 2006; 54:9773-8.

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