Original Article

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Attenuation of Hyperlipidemia in Diabetic and Triton x-100 Induced Hyperlipidemic Rats by *Thymus daenensis* Celak Extract

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Abstract

Hyperlipidemia is one of the vital complications in diabetes. The leaves of *Thymus daenensis* Celak are being widely used to reduce hyperlipidemia in Persian folk medicine. Present study is based upon the investigation of total phenol content and antioxidant activity of *Thymus daenensis* Celak extract (Td) as well as the effect of Td on some biochemical parameters (glucose, total cholesterol, HDL, LDL and Triglyceride level) in normal, streptozotocin-induced (STZ-induced) and Triton x-100 induced hyperlipidemic male Sprague Dawley rats. Moreover, synergic effect of this extract with atorvastatin was evaluated in mentioned groups. Animals were divided into five groups: healthy control group (sham), diabetic and hyperlipidemic control group (STZ+ Tri), diabetic and hyperlipidemic groups treated with Td extract (Ex 1000); diabetic and hyperlipidemic groups treated with atorvastatin (Ator 10), diabetic and hyperlipidemic groups treated with Td extract + atorvastatin (Ex 1000 + Ator 10). Treatment of diabetic rats with Td (1000 mg/kg) significantly decreased glucose, total cholesterol, triglyceride and LDL level as compared with the diabetic hyperlipidemic control group. Nevertheless, there were no synergistic effects between Td and atorvastatin on reducing lipid indications. The total phenol content of Td was 76 mg/g and the result of nitric oxide radical scavenging assay showed IC_{50} of 451.14±44.4 and 486.94±8.91 µg/ml for Td and ascorbic acid respectively. The results from this study indicate that Td is a potent antioxidant and hypoallergenic, which may be proper to prevent coronary heart disease in diabetes in future clinical studies.

Keywords: Diabetes, Hyperlipidemia, Hyperglycemia, STZ, *Thymus daenensis* Celak.

1. Introduction

Patients with type two diabetes mellitus suffer from several health complications especially atherosclerotic cardiovascular disease. This condition related to increasing risk factors of dyslipidemia such as elevated plasma triglyceride (TG) levels, low levels of high-density lipoprotein (HDL), cholesterol and low-density lipoprotein (LDL) particles (1). So Hyperglycemia and hyperlipidemia are two vital characteristics of diabetes mellitus (2, 3). During past decays there is great interest to traditional medicine in all over the world. According to their potential therapeutic effects, there are more researches done on different plant species to evaluate therapeutic effects (4). Despite the progress of synthetic drugs for diabetes complications, therapeutic effects of herbal medicine have been considered because of minimal/no side effects, Furthermore, WHO extensive-

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ly recommended to assessment of herbal medicine efficiency for diabetes treatment (5). *Thymus daenensis* Celak (Td) known as an Iranian endemic plant belongs to Lamiaceae family with different pharmacological characteristics (6, 7), possesses wide application in folk medicine. Antibacterial, antifungal, antioxidant and radical-scavenging activities and immunomodulatory effects are some reported properties of this plant (6, 8). In Persian folk medicine, Td known as an antihyperlipidemic and antidiabetic agent (based on ethnopharmacology study). The aim of this study was evaluation of the phenolic content and antioxidant activity of this extract by nitric oxide radical scavenging assay. Moreovere, the effects of Td on hyperlipidemia and hyperglycemia in Triton x-100 and STZ injected rats and of synergic effect of this aqueous extract with atorvastatin on hyperlipidemia are other purposes of this study.

2. Materials and methods

2.1. Extract preparation

Thymus daenensis Cleak was harvested on September 2015, from Daran mountain (Isfahan province) were authenticated (voucher no.628) by Dr. Ziba Jamzad in the museum of medicinal plants, department of Pharmacognosy, Shiraz University of Medical Sciences, Shiraz, Iran.

The leaves were dried at room temperature then grounded to fine powder by mixer grinder.

The samples were extracted with boiling water at 1:20 ratio, keep in 70-80 ºC for 30 minutes then keep in dark for 24 h. The extracts were filtered to remove redundant residues. The extract was concentrated by rotary evaporator then frozen and lyophilized. Yield of extraction was 14.5%.

2.2. Determination of total phenolic content

Total phenolic content of Td extract was evaluated by modified Folin Ciocalteu method (9). Five hundred µL of different galic acid concentration (0.024, 0.075, 0.105 and 0.3 mg/ml) were mixed with 2.5 ml Folin Ciocalteu reagent and 2 ml (75 g/l) sodium carbonate. The mixture was kept at 20 ºC for 30 min and the absorption was read at 765 nm. Plant extract was mixed with the same reagents as described previously. All samples were assayed in triplicate. Total content of phenolic compounds in plant were expressed as gallic acid equivalents (GAE) and were calculated by the following formula:

C= c. V/m

C: total content of phenolic compounds

c: the concentration of gallic acid established from the calibration curve (mg/ml)

v: the volume of extract (ml)

m: the weight of plant extract (g)

2.3. Nitric Oxide Radical Scavenging Assay

To determine nitric oxide radical scavenging agent the Griess reaction was applied with slight modification (10).

Fifty µL of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH: 7.4) was mixed with 50 µL of each sample and incubated 150 min at 27 ºC. Then 100 µl of Griess reagent was added to each sample and incubated at a room temperature for 5 min, absorbance of mixture was measured at 542 nm. Radical scavenging was determined by the follow formula: $[(A_{control}-A_{sample})/A_{control}]\times100$

Blank: Absorbance of extract without any reagent

Control: Absorbance of control without sample

2.4. Pharmacological study

2.4.1. Animal

The study was conducted on Sprague Dawley rats in both gender (n=25) with initial approximate body mass of 180-220 g purchased from animal house of Shiraz University of Medical Sciences, Shiraz, Iran. Animals were kept in 12/12 h light/dark cycle, 22-25 ºC temperatures and fed with accessible normal diet.

Twenty five rats were divided randomly into 5 groups of five as described bellow:

group1: treated by normal saline in three steps (sham);

group 2: treated by streptozotocin,Tritonx-100 and normal saline;

group 3: treated by streptozotocin, Tritonx-100 and atorvastatin (10 mg/kg/day) (Ator 10);

group 4: treated by streptozotocin, Tritonx-100 and Td extract (1000 mg/kg/day))(Ex 1000);

group 5: treated by streptozotocin, Tritonx-100 and Td extract (1000 mg/kg/day) and atorvastatin (10 mg/kg/day) (Ex 1000+ Ator 10);

Diabetes was induced in 16 h fasted rat by single dose of fresh streptozotocin (STZ) solution (60 mg/Kg, i.p.) in 4 groups whereas sham group received equivalent amount of normal saline.

After one week, hyperglycemia was confirmed by measuring the blood glucose. All groups except sham received Triton x-100 (100 mg/Kg, i.p.) after 16 hours of fasting. Three days after Triton x-100 treatment, rats were given other treatments (Td extract and atorvastatin) by means of gavage for 7 days.

After passing this time, all groups were kept fasting 16 h before blood sampling.

Then rats were sacrificed under thiopental anesthesia. Thereafter 3-5 ml of blood was taken from heart. Collected blood was permitted to clot and the serum was separated by centrifugation at 4500 ×g for 15 min for further analysis such as blood glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG) and Total cholesterol (TC).

2.5. Statistical analysis

Significant differences among groups were analyzed by student t-test or one-way ANOVA with Tukey's post-hoc test (SPSS version 22). A statistical P value less than 0.05 was considered significant.

3. Results

The results of Nitric Oxide scavenging test showed no significant differences between IC_{50} of Td extract and ascorbic acid (451.14±44.4 and 486.94± 8.91 µg/ml respectively).

Phenol content was calculated based on gallic acid in one gram of dried extract.

Ability of STZ to evoke the immune system against langerhans islets beta cells leads to diabetes emergence after STZ injection (11) (shouldn't be written in the Results section). In this study, diabetes was induced in rats by a single IP injection of STZ 60 (mg/k). According to Figure 1 glucose increased in all groups significantly in comparison to healthy control (sham), so diabetes was successfully induced in them.

Our findings confirmed that treatment of diabetic rats with Td extract at a dose of 1000 mg/kg reduce serum glucose levels of diabetic rats considerably in comparison to STZ+Tri group. Co-treatment of diabetic rats with Td extract and atorvastatin, reduce glucose level significantly in comparison to STZ+Tri group (P<0.001).

Hyperlipidemia was induced in diabetic rats by single IP injection of Triton X-100 (100 mg/kg). Data of cholesterol measurement suggested that cholesterol reduced in group received only atorvastatin (P<0.001), extract (P<0.01) and group treated with extract and atorvastatin (P<0.001) in comparison to STZ+Tri group considerably. It seems that combination of atorvastatin with Td extract is more effective than sole extract (Figure 2).

As evident in Figure 3, significant relationships between Td extract treatment and reduction of LDL so, there was considerable difference between the LDL level of atorvastatin, Td extract and Td extract + atorvastatin treated groups in

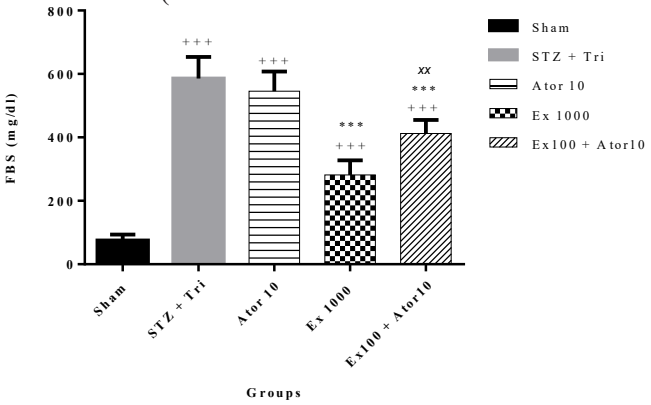

Figure 1. Glucose levels (mean±SD) in different groups.
+++: P<0.001 comparison to Sham.
***: P<0.001 comparison to STZ+Tri.
xx: P<0.01 comparison to Ex 1000.

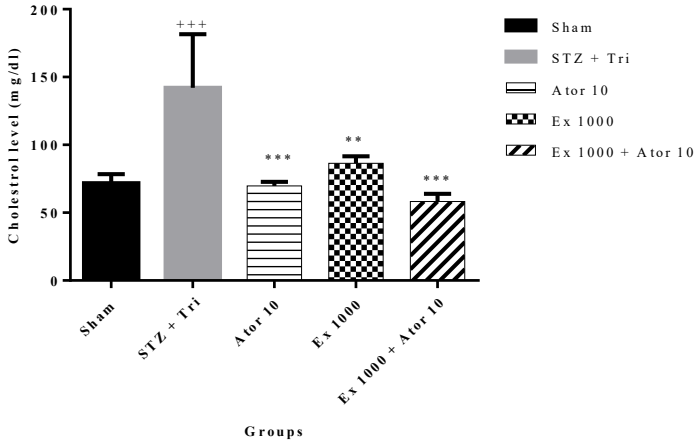

Figure 2. Cholesterol levels (mean±SD) in different groups.
+++: *P*<0.001 comparison to Sham
***: *P*<0.001 comparison to STZ+Tri.

comparison to STZ+Tri group. Also Td extract plus atorvastatin caused more reduction in LDL level in comparison to Td extract alone (*P*<0.05). Similarly the results in Figure 4 show that STZ + Triton-x 100 induced hypertriglyceridemia significantly (*P*<0.001). Atorvastatin, Td extract and atorvastatin in combination with Td extract declined TG significantly (*P*<0.001) in comparison to STZ+Tri. It seems that combination of atorvastatin with Td extract is more effective than sole extract (Figure 4).

The results of HDL level showed no significant change in groups receiving treatment (data not shown).

4. Discussion

Thymus daenensis Celak belonging to Lamiaceae family is extensively used in Persian folk medicine as antidiabetic and anti hyperlipidemic agent (based on ethnopharmacology study).

Antibacterial and antifungal effect, antioxidant and radical-scavenging activities, tyrosinase inhibitory and immunomodulatory effects are also its therapeutic effects (6).

In this research total phenolic content of Td extract was 76±5 mg/g. This value is close to phenolic content of Td seeds methanolic extract (70.56±0.04 mg/g) collected from Daran (Isfahan, Iran). However phenolic content was estimated 31.38±0.03 and 34.37±0.02 mg/g for samples col-

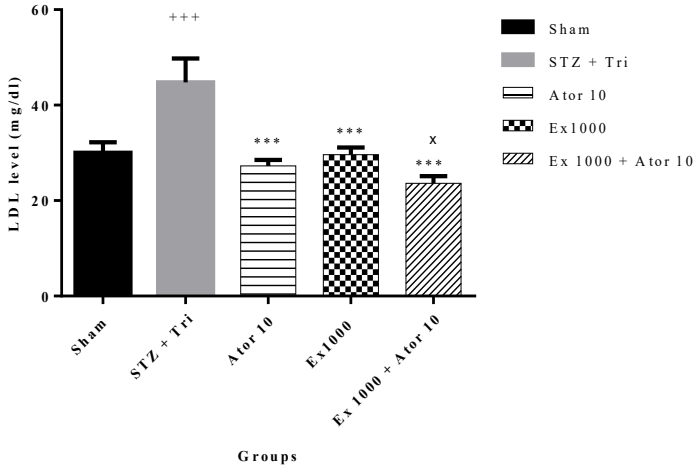

Figure 3. LDL levels (mean±SD) in different groups.
+++: *P*<0.001 comparison to Sham
***: *P*<0.001 comparison to STZ+Tri
x: *P*<0.05 comparison to Ex 1000.

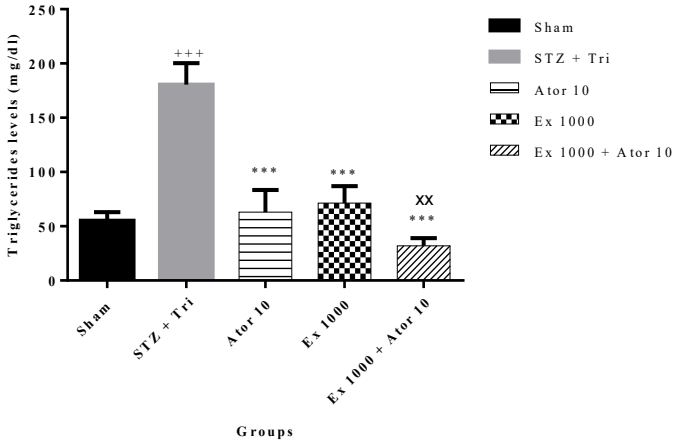

Figure 4. Triglycerides levels (mean±SD) in different groups.
+++: P<0.001 comparison to Sham
***: P<0.001 comparison to STZ+Tri
xx: P<0.01 comparison to Ex 1000 .

lected from Khoram Abad, Lorestan, and Fereydonshahr, Isfahan in Iran, respectively. (12).

This value is more than some total phenol content reported for other thymus species, for instance in methanolic extracts of *Thymus vulgaris* phenol compound was about 19.65 mg GAE/g dw (13). According to a previous study rosmarinic acid was reported as the most abundant phenolic compound while, ferulic, chlorgenic, p-cumaric acid; naringenin, eriodictyol, epigenin, quercetin and rutin were other determined phenolic compounds in *Thymus* sp.(14). Also, in water /ethanol extract of wild thymus aerial parts rosmarinic acid, caffeic acid, luteoline and apigenin were also present in the mentioned extract (15). Phenolic content of Td may be similar to these reported compounds but it is clear that certain variability between these compounds in various plants are associated with the species, geographic situation, storage condition and procedure of experiment (16, 7). Phenolic compounds are famous secondary plant metabolites, with different structure and effects. They are known as potent antioxidants by different mechanisms including radical scavenging, metal chelating and enzyme inhibition involved in oxidative stress (16, 7). In our study, nitric oxide radical scavenging assay show IC_{50} of Td extracts 451.14±44.74 µg/ml while IC_{50} of ascorbic acid was 486.94±8.91 µg/ ml. Comparable IC_{50} of Td and ascorbic acid might indicate that thymus may have potent nitric oxide scavenging activity and it might be a proper candidate as an anti-inflammatory agent due to role of NO in pathogenesis of neurodegenerative diseases and inflammation (18). Furthermore, this extract as potent antioxidant was able to reduce LDL oxidation, atherosclerosis progress (17) and diabetes complications related to oxidative stress (18).

In diabetes, risk of coronary heart disease and congestive heart failure is more than non-diabetic cases so dyslipidemia is a remarkable risk factor which should be controlled as soon as possible (19, 20).There is strong relationship between insulin resistance and abnormalities of plasma lipid and lipoprotein (20).TG, HDL, cholesterol and small dense LDL particles alter in diabetic dyslipidemia (19, 20).

In this study, Triton X-100 (100 mg/kg) used to induce acute hyperlipidemia model by blocking clearance of TG-rich lipoprotein, reducing VLDL and LDL catabolism and increase plasma cholesterol and TGs (21). Glucose, TG, LDL (P<0.001) and total cholesterol (P<0.01) in STZ and Triton X- injected group increased in comparison to control group which means successful induction of diabetes and hyperlipidemia in study rats. The results showed that Td extract was able to modify serum glucose level in treated rats like other thymus specious such as *Thymus vulgaris* (23, 24) and *Thymus serphyllum* as antidiabetic treatment to reduce glucose (24).

In Td extract receiving group (1000 mg/

ml), level of LDL, TG and total cholesterol decreased significantly compared to STZ and Triton X- injected group. There was no improvement on HDL level of all treated group compared to STZ and Triton X- received group. Also, there was no synergic effect between Td extract and atorvastatin in different factors of hyperlipidemia.

Td extract effects on LDL may be associated with reduction of cholesterogenesis and fatty acid synthesis (24). Moreover, activity of cholesterol reduction was related to decrease of LDL catabolism, which is a potent risk factor of cardiovascular complications and a target of many hypocholesterolemic treatments. Also Td extract may increase LDL-C catabolism by its hepatic bile acid receptors (25, 26). Previous studies revealed the important role of TG and lipoprotein interaction in lipid metabolism (27). Td extract may increase induction of lypolitic activity of plasma lipoprotein in order to reduce TG (28).

Hyperlipidemia can be responsible of oxidative stress, it seems that Td extract is rich of water soluable compounds because of extraction method. Some previous studies reported the efficacy of this type of extract in treatment of hyperlipidemia and reduction of cardiovascular disease (25, 29). Antioxidants are able to reduce lipid peroxidation and hyperlipidemia by radical scavenging (26) so, as mention previously, Td extract may reduce these side effects by their antioxidant activities.

It seems that Td extract is rich of water soluable compounds because of extraction method. Some previous studies reported the efficacy of this type of extract in treatment of hyperlipidemia and reduction of cardiovascular disease (25, 29). Like previous studies, polar herbal extracts are beneficial in treatment of hyperlipdeidmia, being effective in reduction of cardiovascular disease progress (25, 29).

Extract of other herbal medicine was shown to be effective in developed hyperlipidemia in diabetic studies such as aqueous extract of *Tamarindus indica* seed (2), aqueous extract of *Punica granatum* flower (30), flavonoid rich extract of *Eugenia jambolana* seed (31), aqueous extract of *Artemisia campestris* (32) and ethanol flower extract of *Hibiscus rosa sinensis* (33).

There are various synthetic hypolipidemic drugs available in the market with different side effects. These side effects and incompliance of some patients lead to revealing necessity of natural alternative therapy with less or no side effects (34, 35). The results of this study suggested that Td extract might be a proper candidate in hyperlipidemia therapy which is a serious diabetes complication.

5. Conclusion

Results of this study show *Thymus daenensis* Celak extract might be a valuable natural sources of nitric oxide radical scavenger and rich in phenolic contents. Furthermore, this extract was able to reduce serum lipids in diabetic rats. As dyslipidemia is a serious complication in diabetes, these results reveal potential of this herbal medicine in future clinical application, so detection of active compounds should be investigated for further possible mechanisms in reducing the risk of dyslipidemia.

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Conflict of Interest

None declared.

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