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 IC₅₀ 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...
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. Moreover, 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:

\[ C = \frac{c \cdot V}{m} \]

\[ C: \text{total content of phenolic compounds} \]
\[ c: \text{the concentration of gallic acid established from the calibration curve (mg/ml)} \]
\[ v: \text{the volume of extract (ml)} \]
\[ m: \text{the weight of plant extract (g)} \]

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:

\[ \frac{(A_{\text{control}}-A_{\text{sample}})/A_{\text{control}} \times 100} \]

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 re-
cived equivalent amount of normal saline.

After one week, hyperglycemia was con-
firm 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 Tri-
ton x-100 treatment, rats were given other treat-
ments (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 ANO-
VA 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 sin-
gle IP injection of STZ 60 (mg/k). According to
Figure 1 glucose increased in all groups signifi-
cantly 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 suggest-
ed 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 ex-
tract is more effective than sole extract (Figure 2).

As evident in Figure 3, significant rela-
relationships between Td extract treatment and reduc-
tion 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.](image-url)
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-
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lected from Khoram Abad, Lorestan, and Fereydonsahr, 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, chlorogenic, 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 IC50 of Td extracts 451.14±44.74 μg/ml while IC50 of ascorbic acid was 486.94±8.91 μg/ml. Comparable IC50 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 species 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 lipolytic 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 soluble 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 soluble 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 hyperlipidemia, 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 Tammarindus 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.

6. References


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