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Dill tablet: A potential antioxidant and anti-diabetic medicine

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ABSTRACT

Objective: To evaluate the antiglycation and antioxidant properties of the dill tablet, an herbal product used in Iran as a hypolipidemic medicine.

Methods: In this descriptive study, the antioxidant and antiradical properties of dill tablet at different concentration (0.032, 0.065, 0.125, 0.25, 0.5 and 1 mg/mL) were measured. The total phenolic, flavonols and flavonoid, alkaloids, anthocyanin, tannin and saponin contents in dill tablet were determined. Furthermore, antiglycation properties of dill tablet were assayed. In the *in vivo* experiments, male rats were randomly divided into three groups (n = 6): Group 1: normal rats; Group 2: diabetic rats; Group 3: diabetic rats + 300 mg/kg dill tablet, and Group 4: diabetic rats + 100 mg/kg dill tablet. After 2 months, the blood glucose was measured enzymatically and advanced glycation end-products (AGEs) formation was determined using a fluorometric method.

Results: Our results illustrated that different concentrations of dill tablet had significant antioxidant activity. Dill tablet markedly declined AGEs formation and fructosamine levels ($P < 0.001$) compared with glycated sample. Oxidation of protein carbonyl and thiol group was significantly reduced by dill tablet in a dose dependent manner ($P < 0.001$). Formation of amyloid cross-β and fragmentation were markedly inhibited by dill tablet ($P < 0.001$) compared with glycated sample. After 2 months, fasting blood glucose levels ($P < 0.001$) and AGEs formation ($P < 0.05$) were significantly reduced by dill tablet in diabetic animals.

Conclusions: Dill tablet exhibited significant antiglycation and antioxidant activities. This study provides a scientific basis for using dill in treatment of diabetic patients.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder which is recognized by declining or dysfunction of insulin leading to hyperglycemia [1]. The World Health Organization estimated that in 2030 about 366 million people will suffer from diabetes mellitus [2]. The chronic complications of diabetes include microvascular damage, kidney failure, heart disease, nerve damage, and blindness [1]. One of the main ways triggering diabetes complication is a chronic rise of blood glucose which causes advanced glycation end-products (AGEs) formation [3,4]. Proteins glycation is accepted as the central molecular basis of several complications including diabetic nephropathy, retinopathy, neuropathy and heart diseases [4,5].

Studies have confirmed that natural antioxidants play a significant role in the prevention and treatment of metabolic disorders [6–10]. Subsequently, drugs of herbal origin are now getting an exceptional interest for this issue, because of its low cost, low adverse effect and many useful properties [10,11]. *Anethum graveolens* (dill) is used in the traditional medicine for treatment of digestive disorders, bad breath, lactation motivation, and also as hypolipidemic agent. Currently, many experiments have shown that dill has many useful properties such as anticancer, antimicrobial, antigastric irritation, anti-inflammatory, and antioxidant activities [12]. Dill tablet (DT) used in Iran as hypolipidemic agent consists of *Anethum graveolens* (68%), *Cichorium intybus* (5%), *Fumaria*

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parviflora (5%) and *Citrus aurantifolia* sp. (4%) [13]. Dill is a rich source of flavonoid, phenolic, terpene, saponin, tannins and cardiac glycosides, which are responsible for beneficial effects of this plant [12]. Administration of dill in the animal models, diabetic patients, hyperlipidemic patients and patients with metabolic syndrome markedly reduced blood glucose, total cholesterol, triglyceride, very-low-density lipoprotein cholesterol, low-density lipoprotein cholesterol; also it increased high density lipoprotein cholesterol levels [9,14–16]. However, the antioxidant and antiradical properties and also inhibitory effect of DT on protein glycation have not been reported. Therefore, this present study was conducted to evaluate the antiglycation and antioxidant properties of DT.

2. Materials and methods

All reagents were from Sigma–Aldrich and Merck. DT was purchased from Iran Darouk Company (Iran). A spectrophotometer (JENWAY 6105 UV/Vis) was used for all colorimetric assays.

2.1. Total antioxidant capacity

2.1.1. Ferric reducing antioxidant power (FRAP)

The FRAP of DT was tested using method described by Setorki *et al.* with slight modifications [17].

2.1.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay

The antioxidant activity of DT was determined via assay of the stable DPPH free radical scavenging activity according to the previously published study [18].

2.1.3. Superoxide anion and hydrogen peroxide scavenging activity

Superoxide anion scavenging activity was evaluated using nitro blue tetrazolium according to method of Dadashpour *et al* [19]. The hydrogen peroxide scavenging activity of DT was determined according to the method described by Güder and Korkmaz [20].

2.1.4. Reducing power and ferrous ions chelating capacity

Reducing power and ferrous ions chelating capacity was tested according to a previously published report [18].

2.1.5. Nitric oxide (NO) scavenging activity

This activity of DT was determined spectrophotometrically using Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% of *N*-1-naphthylethylenediamine dihydrochloride) [21].

2.2. Phytochemical properties

The amounts of total alkaloids and total tannins, phenolic, flavonoids and flavonols, saponin and anthocyanin were determined according to the methods of previously published papers [22–24].

2.3. Glycation of bovine serum albumin (BSA)

Glycation of BSA was performed according to a published paper [25]. Briefly, DT in different concentration (0.25–2 mg/mL) was added to the solution containing: BSA (10 mg/mL), fructose (200 and 500 mmol/L), sodium azide (0.02%) in 0.1 mol/L phosphate buffer (pH 7.4). The mixture was incubated at 37 °C at dark for 1, 2, 3, and 4 weeks. After that, glycated samples were dialyzed for 48 h and the fluorescence intensity was determined at 335 nm excitation and 460 nm emission wavelength using a spectrofluorometer (Jasco FP-6200). Aminoguanidine was used as a positive control for glycation inhibition.

2.4. Determination of fructosamine

The formation of fructosamine (Amadori product) was determined by using nitroblue-tetrazolium dye, after 1, 2, 3, and 4 weeks of incubation at 37 °C [25].

2.5. Determination of protein carbonyl content

Carbonyl content of prepared glycated protein which known as a marker of protein oxidative damage was measured using 2,4-dinitrophenylhydrazine according to the previously reported method [25].

2.6. Determination of the thiol group

The assay was based on Ellman's reaction with small modifications using 5, 5′-dithiobis (2-nitrobenzoic acid) according to method of Caengprasath *et al* [25].

2.7. Protein aggregation

Amyloid cross-β structure which is known as an indicator of protein aggregation was measured by colorimetric method using Congo red dye according to a prior published paper [26].

2.8. Protein fragmentation

The analysis of protein fragmentation was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Briefly, 1 mg of DT was added to the mixture which contained 200 mmol/L fructose, 10 mg/mL of BSA and 100 μmol/L copper ions (as a catalyst) at 37 °C for 1 week [27]. A sample of the prepared solution was subjected to SDS-PAGE, and visualized using Coomassie brilliant blue.

2.9. In vivo studies

Twenty four male rats with average weight between 240 and 260 g were used for this experiment. The rats were kept at 12 h light/12 h dark cycles at temperature of (22 ± 1) °C. Following 10 days of acclimatization in animal house, animals were randomly divided into 4 groups: Group 1: control animals; Group 2: diabetic animals; Group 3: diabetic rats +300 mg/kg DT; and Group 4: diabetic rats +100 mg/kg DT [1,5]. Diabetes in rats was induced according to the previously published method using streptozotocin (65 mg/kg dissolved in citrate buffer, *i.p.*) [1,5]. After 8 weeks, the rats were anesthetized with diethyl ether and then sacrificed. Blood samples were collected from the heart of animal and allowed to clot, and then centrifuged at 5 800 r/min for 10 min [1]. This study was approved by the Research Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran.

2.10. Blood glucose and AGEs assay

The level of fasting blood glucose was measured by glucose oxidase method according to the manufacture protocol (Pars Azmoon Co.). The AGEs formation in diabetic rats was determined according to the previously published method [4]. The fluorescence intensity of the samples was determined using Jasco FP-6200 spectrofluorometer at 335 nm and 460 nm wavelength, for excitation and emission, respectively [4].

2.11. Statistical analysis

Data are expressed as mean ± SEM (n = 3). The comparison of the obtained data was performed using One-way ANOVA (followed by Tukey and Dunnett's test). $P < 0.05$ was regarded statistically significant. IC_{50} value (50% inhibition) was determined for antioxidant tests via analysis of regression in Microsoft Excel.

3. Results

DT presented significant DPPH radical scavenging activity with an IC_{50} value of 0.03 mg/mL. At the dose of 1 mg/mL DT showed significant DPPH radical scavenging activity similar to butylated hydroxytoluene (BHT) and ascorbic acid (Figure 1). In this experiment DT at 1 mg/mL had FRAP value of 2.72 mmol/L equivalent to $FeSO_4.7H_2O$, while ascorbic acid showed 2.65 mmol/L equivalent to $FeSO_4.7H_2O$ (Figure 1). As shown in Figure 1, DT has strong O_2^- and H_2O_2 scavenging activity at a dose of 0.5 and 1 mg/mL compared with BHT and ascorbic acid. DT showed significant O_2^- scavenging activity with an IC_{50} of 0.075 mg/mL (IC_{50} of BHT and ascorbic acid was 0.058 and 0.03 mg/mL, respectively). DT also showed significant H_2O_2 scavenging activity with an IC_{50} of 0.065 mg/mL. In this experiment, DT showed significant reducing activity which increased in higher dose (1 mg/mL); DT showed 96% activity, more than BHT which showed 93% capacity. The reducing power of the DT increased in a dose dependent manner, and at dose of 1 mg/mL it was similar to that of BHT (Figure 1). DT showed NO scavenging activity in a dose dependent manner. The IC_{50} value of DT, BHT and ascorbic acid was 0.069, 0.022 and 0.023 mg/mL respectively (Figure 1).

3.1. Phytochemical analysis

Total phenols, flavonoids and flavonols content of DT was found to be (190.0 ± 4.4), (151.0 ± 3.5) and (135 ± 3) mg equivalent of gallic acid (for phenolic) and quercetin (flavonoid and flavonols) equivalents per gram, respectively. The total alkaloid of DT was (80 ± 2) mg atropine equivalent per gram; total anthocyanin content of DT was (28.0 ± 2.2) mg/g; the total tannins content of DT was (71.0 ± 3.2) mg equivalent of tannic acid per gram. The total saponin content of DT was (51 ± 2) mg/g.

3.2. Anti-glycation properties

Incubation of BSA with fructose and DT at the different doses significantly reduced the AGEs formation. Interestingly, inhibitory effect of 1 and 2 mg of DT was more stronger than that of aminoguanidine (positive control for anti-glycation effect) at the 1, 2, 3 and 4 weeks of incubation (Figure 2). Our study also established that DT markedly reduced fructosamine levels (Figure 3). Significant rise in the carbonyl content and

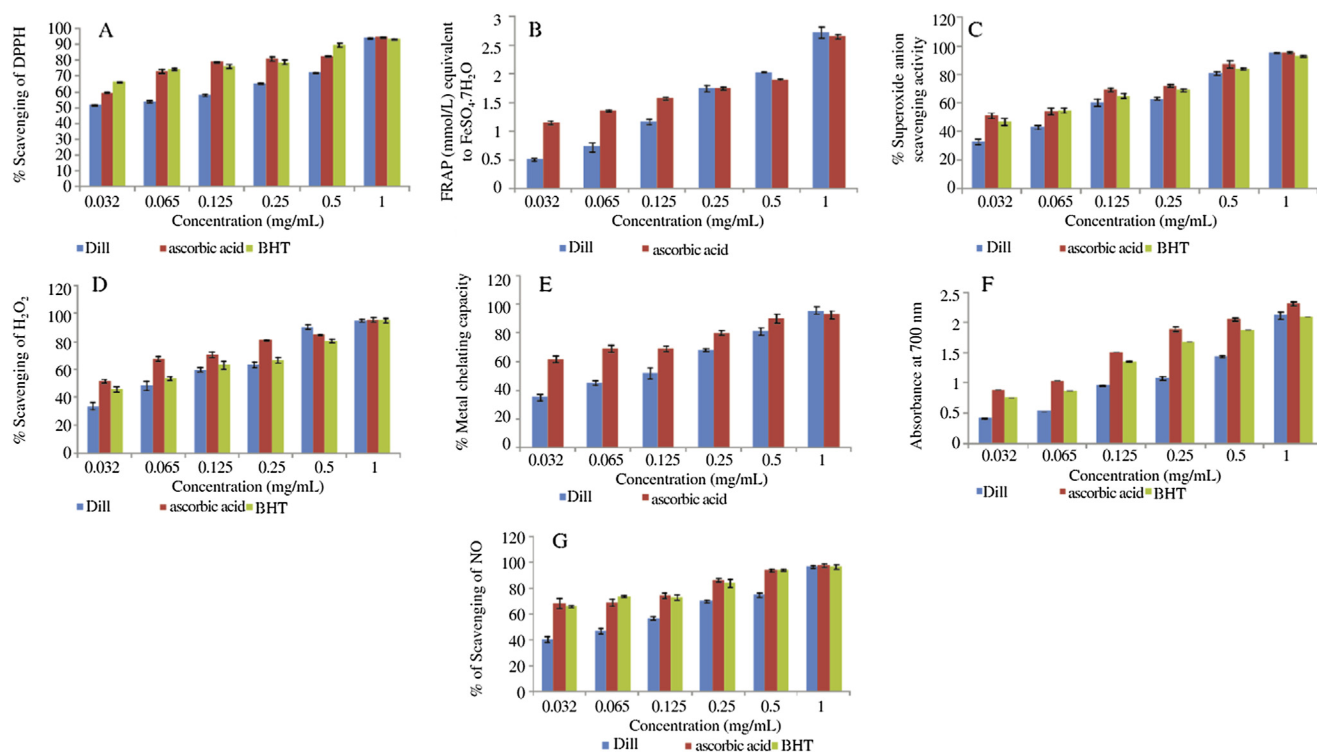

Figure 1. Antioxidant and antiradical activity of DT.

A: DPPH radical scavenging activity; B: FRAP assays; C: Superoxide radical scavenging activity; D: Hydrogen peroxide radical scavenging activity; E: Metal chelating activity; F: Reducing power activity; G: NO scavenging activity. Values are the average of triplicate experiments and expressed as mean ± SEM.

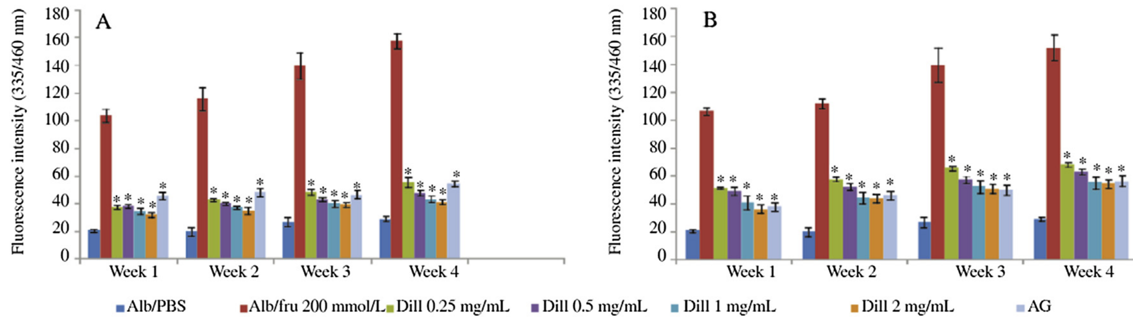

Figure 2. The effect of DT on fluorescent AGEs formation in BSA incubated with 200 mmol/L (A) and 500 mmol/L fructose (B).
Data are expressed as mean ± SEM ($n = 3$). *: $P < 0.05$ when compared to BSA/fructose at the same incubation time. AG: Aminoguanidine; Alb: Albumin; Alb/Fru: Albumin + fructose.

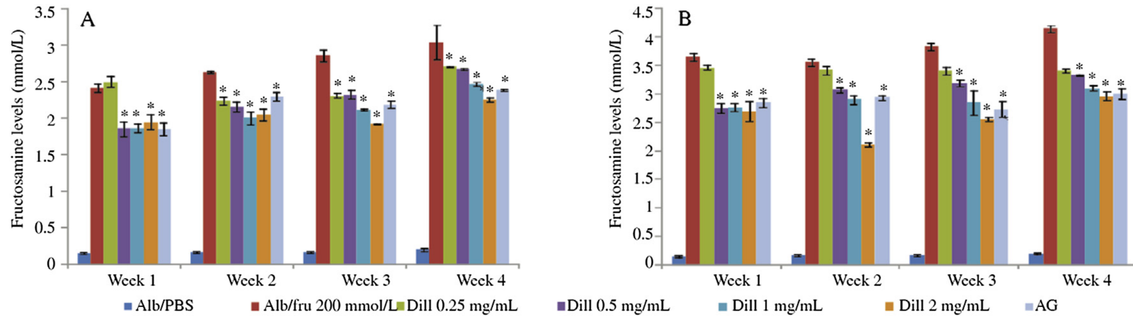

Figure 3. The effect of DT on fructosamine levels in BSA incubated with 200 mmol/L (A) and 500 mmol/L fructose (B).
Data are expressed as mean ± SEM ($n = 3$). *: $P < 0.05$ when compared to BSA/fructose at the same incubation time. AG: Aminoguanidine; Alb: Albumin; Alb/Fru: Albumin + fructose.

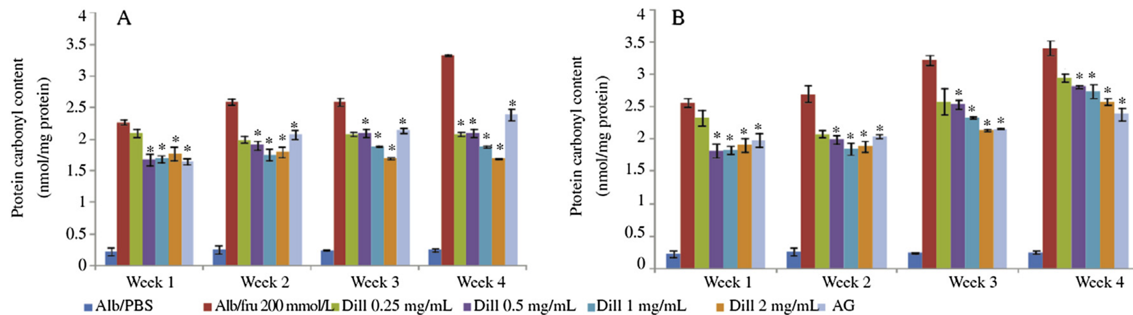

Figure 4. The effect of DT on protein carbonyl content in BSA incubated with 200 mmol/L (A) and 500 mmol/L fructose (B).
Data are expressed as mean ± SEM ($n = 3$). *: $P < 0.05$ when compared to BSA/fructose at the same incubation time. AG: Aminoguanidine; Alb: Albumin; Alb/Fru: Albumin + fructose.

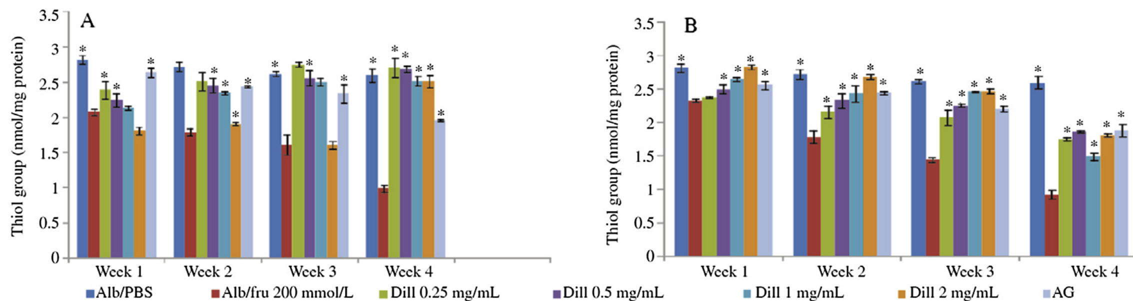

Figure 5. The effect of DT on the level of thiol group in BSA incubated with 200 mmol/L (A) and 500 mmol/L fructose (B).
Data are expressed as mean ± SEM ($n = 3$). *: $P < 0.05$ when compared to BSA/fructose at the same incubation time. AG: Aminoguanidine; Alb: Albumin; Alb/Fru: Albumin + fructose.

also thiol groups oxidation were observed when the albumin was incubated with fructose after 1, 2, 3 and 4 weeks. Aminoguanidine and DT at different doses normalized these markers (Figures 4 and 5). In this experiment the amyloid cross-β conformation significantly increased in glycated samples. DT and aminoguanidine markedly inhibited amyloid formation in a dose-dependent manner. However, reduction by DT was more than that by aminoguanidine (Figure 6). In this experiment, the

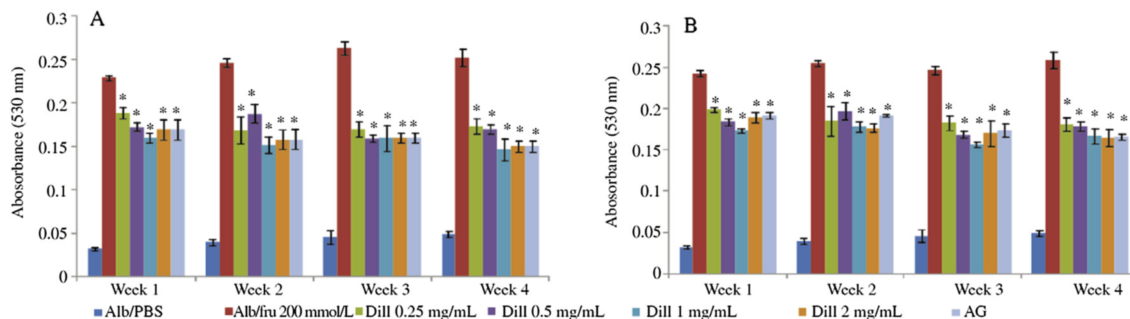

Figure 6. The effect of DT on the formation of amyloid cross-β structure in BSA incubated with 200 mmol/L (A) and 500 mmol/L fructose (B). Data are expressed as mean ± SEM (n = 3). *: $P < 0.05$ when compared to BSA/fructose at the same incubation time. AG: Aminoguanidine; Alb: Albumin; Alb/Fru: Albumin + fructose.

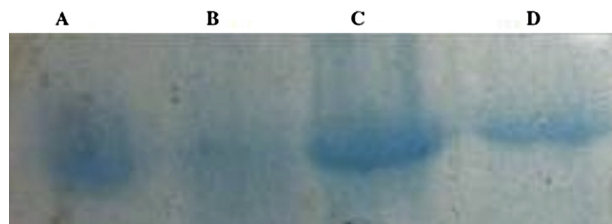

Figure 7. Protein fragmentation in BSA incubated with 200 mmol/L fructose in the presence of Cu^{+2} ion, aminoguanidine and DT for one week, detected by SDS-PAGE. Protein fragmentation was reduced by aminoguanidine and DT compared with BSA/fructose.
Lane A: 10 mg/mL BSA; Lane B: 10 mg/mL BSA + 200 mmol/L fructose; Lane C: 10 mg/mL BSA + 200 mmol/L fructose + aminoguanidine; Lane D: 10 mg/mL BSA + 200 mmol/L fructose + DT.

fragmentation of protein was inhibited when glycated BSA was incubated with aminoguanidine or DT. Fragmentation of albumin was detected by SDS-PAGE (Figure 7).

3.3. In vivo study

Fasting blood sugar significantly increased in diabetic rats compared with normal group [(97.0 ± 6.1) *vs.* (340.0 ± 7.1) mg/mL, $P < 0.001$]. Administration of 100 mg/kg of DT significantly declined fasting blood sugar levels compared with untreated diabetic rats [(210.26 ± 6.38) *vs.* (340.0 ± 7.1) mg/mL, $P < 0.01$]. DT at the concentration of 300 mg/kg significantly caused a reduction in blood glucose levels [(150.26 ± 6.38) *vs.* (340.0 ± 7.1) mg/mL, $P < 0.001$]. After 2 months of treatment, AGEs formation considerably increased in diabetic animals compared with normal group [(6.87 ± 0.53) *vs.* (3.49 ± 0.36) AGEs unit, $P < 0.01$], while treatment with 300 mg/kg DT significantly reduced AGEs formation compared to untreated diabetic animals [(5.00 ± 0.45) *vs.* (6.87 ± 0.53) AGEs unit, $P < 0.05$]. Reduction of AGEs formation in the animal which received 100 mg/kg DT was not significant compared with diabetic animals [(6.55 ± 0.85) *vs.* (6.87 ± 0.53) AGEs unit].

4. Discussion

4.1. DPPH free radical scavenging activity

In this experiment, DT showed significant DPPH radical scavenging activity. DPPH is known as a model of lipophilic radical which is applied for estimation of antioxidant activity based on ability to act as a hydrogen donor [28]. In the study of Ramadan *et al.* [8], water extract of dill at the concentration of 400 μg/mL showed 89.7% DPPH free radical scavenging activity. In our study DT at the concentration of 0.125, 0.25, 0.5 and 1 mg/mL showed 58.14%, 65.22%, 72.33% and 93.98% DPPH free radical scavenging activity respectively.

4.2. FRAP assay

FRAP assay is a colorimetric method and measures the reducing ability of antioxidant agents, which reacts with a complex of colorless oxidized Fe^{3+} and reduces it to colored Fe^{2+}-tripyridyltriazine [17]. The data of a study conducted by Bahramikia and Yazdanparast showed that ethyl acetate fraction of dill had the maximum and the water extract had the minimum IC_{50} values [9]. In this experiment, DT at 1 mg/mL had FRAP value near to that of ascorbic acid.

4.3. Superoxide anions radical and hydrogen peroxide scavenging activity

Superoxide anion is the strongest reactive oxygen species which involves in the pathogenesis of several disorders due to converting to more reactive species such as hydroxyl radical and hydrogen peroxide which make oxidative damage and provoke oxidation of DNA, lipids and proteins [29]. Hydrogen peroxide itself is not very reactive; nevertheless it can occasionally be poisonous to body cells since it may form hydroxyl radical in the cells. Formation of hydroxyl radicals is the origin of many toxic effects of hydrogen peroxide [21]. Hydrogen peroxide also directly inhibits several enzymes by oxidation of thiol groups. DT showed markedly superoxide radical and hydrogen peroxide scavenging activity. The results of Dadashpour *et al.* showed that essential oils of dill had the IC_{50} value of 0.013 mg/mL [19]. In our study dill showed O_2^- scavenging activity with IC_{50} values of 0.075 mg/mL.

4.4. Ferrous ions chelating capacity

Fe (II) is recognized as a strong pro-oxidant agent. Transition of Fe^{2+} to Fe^{3+} can motivate reactive oxygen species production via Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$) [28]. The ability of DT to chelate Fe^{2+} is recognized as a precious antioxidant activity and may provide defense against oxidative damage by reduction of free radicals production and consequently lipid peroxidation. In our study, DT showed iron chelating ability with IC_{50} values of 0.120 mg/mL.

4.5. Reducing power

Presence of antioxidants in DT is responsible for the reduction of Fe^{3+} to Fe^{2+} by donation of electron. Experiments stated that reducing power ability of natural components is commonly correlated with the presence of reductones. Reductones providing hydrogen atoms lead to breaking of the free radical chain [18]. These agents are able to react with certain peroxide precursors and subsequently inhibit the peroxide formation [18].

4.6. NO scavenging activity

In this study, DT showed significant NO scavenging activity. Excess generation of NO is associated with several disorders [30]. Dadashpour *et al.* revealed that essential oils of dill had the IC_{50} value of 0.001 4 mg/mL and in the concentration of 0.002 mg/mL showed 57.5% ± 3.3% NO scavenging activity [19]. In our experiment, DT showed NO scavenging activity with IC_{50} values of 0.069 mg/mL.

4.7. Phytochemical analysis

The beneficial effects of dill may be attributed to the high amount of total phenolics, flavonoids and flavonols [31]. Polyphenol, flavonoids and flavonols in nutrient or herbal medicine may be accountable for their antioxidant activity. Due to redox properties, these agents play a vital role in adsorption and neutralization of free radicals, reducing singlet and triplet oxygen or decaying peroxides. The previous study also showed that flavonoids have antioxidant activity through scavenging or chelating procedure [31].

Alkaloids have different physiological effect in mammalians. These agents have hypoglycemic effect by stimulating glucose transports and subsequently increasing glucose transfer to peripheral tissue and also stimulation of insulin secretion. Alkaloids have also antioxidant activity by rises of glutathione content in the liver [32].

Anthocyanins are water soluble pigments which belong to the flavonoids and were found in dill. These components have an important role in the color quality of plants. Anthocyanins have many pharmacological and biological activities including antioxidant, anti-carcinogenic, and anti-inflammatory activities [33].

Tannin has been considered as a secondary component in plant kingdoms, which is found in dill and have many valuable properties such as anti-inflammatory, anti-carcinogenic, antimutagenic, antiradical, anti-hyperglycemic, cardio-protective, and antioxidant activity. Tannins by inhibition of reactive oxygen species and AGEs formation play a vital role in remission of diabetes complications. Chronic use of tannins in diabetic animals considerably declined blood glucose and improved lipid profile [34].

Saponin is another biological active component, which is found in dill and has several pharmacological properties including motivation of insulin and C-peptide secretion, antioxidant activity, inhibition of AGEs formation and also normalizing diabetic nephropathy [35].

In the study by Priecina and Karklina [10], the alkaloids, flavonoids, tannins, and saponins in the seed of dill were 2.8% ± 0.1%, 11.05% ± 0.07%, 19.71% ± 0.28%, and 0.55% ± 0.04% respectively. In the study of Stankevičius *et al.* [22], the total phenol content in dried dill which grown in Latvia was (1399.8 ± 2.4) mg/100 g, and total flavonoid content was (650.1 ± 4.8) mg/100 g. In our study, these components in DT were more than what has been reported by of Priecina and Karklina [10] and Stankevičius *et al.* [22].

4.8. Anti-glycation properties

Proteins glycation through a non-enzymatic reaction in diabetic patients causes AGEs formation which involves in the diabetic pathogenesis and ageing. In the blood stream, high levels of glucose lead to glycation of albumin and proposed as a target to explain harmful effects of hyperglycemia. Some drugs have been assessed as inhibitors of AGEs formation [36]. Intracellular glycation by fructose takes place in higher rate compared to that of glucose. Therefore, fructose and its metabolites are known to be important precursors for formation of AGEs in the body [37]. Hence in this study, we used fructose for glycation of BSA. In the early stage of protein glycation, unstable Schiff's base is converted into fructosamine (Amadori product). This metabolite is clinically used for monitoring of hyperglycemia in short time. Decrease in fructosamine has a beneficial effect for reduction of vascular complications [37]. Our study established that DT markedly reduced AGEs formation and fructosamine levels.

Other intermediaries such as reactive carbonyl contents affect proteins modification that generally makes it prone to the oxidative reaction and consequently stimulates oxidative damage [37,38]. reactive oxygen species generation during glycation process causes oxidation of amino acid residues in proteins and forms carbonyl derivatives and also reduces oxidative defense by declining thiol groups. Oxidation of thiol group in the protein structure causes conformational modification which forms disulfide bonds, and generates high levels of free radicals [38].

Major molecular changes in the protein structure can be determined by assessment of carbonyl content and loss of protein thiol groups, which reflected the high levels of free radical generation [37]. Major rise in the carbonyl content and also thiol group's oxidation was observed when the BSA was incubated with 200 and 500 mmol/L fructose. Aminoguanidine and DT normalized these markers. These properties may be due to antioxidant and antiradical activity of DT. Other properties such as iron chelating activity are established as the chief actions that mediate anti-glycation property. Iron chelating affects decline of reactive oxygen species production, glycoxidation and autoxidative glycosylation, and also reduces AGE cross-link formation [39]. In this experiment, DT displayed significant iron chelating activity. Other approaches proposed for anti-glycation activity are breaking the cross-linking structure, decreasing carbonyl groups, Amadori products or Schiff's bases, and also late-stage Amadori products [39].

We showed antiglycation properties of DT, while previous studies showed hypolipidemic properties of dill extract and dill tablet [9,14–16]. Mirhosseini *et al.* in the randomized clinical trial study reported that administration of DT for 8 weeks in hyperlipidemic patients declined total cholesterol by 18% and triglyceride by 7.38% compared with control group [40]; interestingly the patients did not report any side effects. Therefore, DT can be used as a hypoglycemic and hypolipidemic agent.

Sushruta *et al.* [7] and Mishra [1] showed that aqueous extract of dill significantly declined blood glucose in diabetic animals.

In the *in vivo* study, we showed that administration of DT significantly reduced blood glucose and AGEs formation in diabetic rats. Mobasseri *et al.* reported that dill has useful effects on insulin sensitivity and also normalized lipid profiles in diabetic patients [11]. An antidiabetic property of dill has been reported by Setorki *et al.* [17], Madani *et al.* [5] and Panda [6] in animal models and also in diabetic women by Rashidlamir and Gholamian [3] and Mobasseri *et al* [11]. Inhibition of AGEs formation by DT in diabetic animals and *in vivo* condition is reported for the first time in this experiment.

4.9. Determination of protein aggregation and fragmentation

Glycation of proteins directly related to protein aggregation. The aggregated protein is insoluble and can form amyloid cross-β structure and alter protein stability and structure. Formation of amyloid is involved in many disorders such as Alzheimer, Parkinson, and diabetes. In the majority of diabetic patients, amyloid accumulates in the pancreatic islets which can directly destroy β-cell and decrease insulin secretion. In diabetic patients, formation of amyloid islet is known as a serious problem and the medical goal is to prevent amyloidosis-induced toxicity [26].

Incubation of glycated protein with cupper ions was accompanied by the decrease of protein-bound glucose, proposing that fragmentation of glycated BSA happened at the expense of BSA-bound glucose [23,27]. Incubation of BSA with fructose leads to the production of fragments.

Sakai *et al.* reported that incubation of BSA with 200 mmol/L fructose and cupper ions significantly increased fragmentation of protein, while aminoguanidine significantly inhibited this process [27]. Ahmad *et al.* reported that aged garlic extract and S-allyl cysteine considerably reduced AGEs formation [23], fructosamine levels and also inhibited protein fragmentation [23]. The limitation of this research was the measurement of the other group of AGE products which are non-florescent compounds.

DT with high antiglycation, antiradical and antioxidant properties may offer significant prospects for the prevention and treatment of AGE-mediated complications in diabetic patients.

Conflict of interest statement

The authors declare no conflict of interest.

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