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ORIGINAL ARTICLE

Potential Role of *Tribulus terrestris* Fruit Extract in Inhibition of Advanced Glycation End Products

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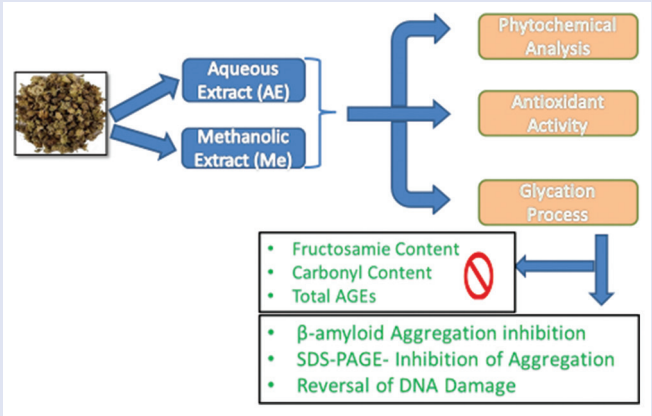
ABSTRACT

Background: Glycation refers to the interaction between sugars and proteins leading to the production of harmful advanced glycation end-products (AGEs). Many different diseases such as neurodegenerative, cardiovascular, and secondary complications in diabetic patients have reported the involvement of these products. Diabetes is one such disease, wherein the accumulation of AGEs causes secondary complications. **Objectives:** This study inclines to investigate the antiglycation and antioxidant potential of aqueous and methanolic extracts of *Tribulus terrestris* (TT). **Materials and Methods:** The *in-vitro* glycation system (bovine serum albumin [BSA] and glucose) was incubated with aqueous and methanolic extracts of TT for 28 days at 37°C. Standard methods such as browning, nitroblue tetrazolium (NBT) assay, 2,4-dinitrophenylhydrazine (DNPH) assay, and assessment of fluorescent AGEs were carried out spectroscopically to check the number of glycation products. The antioxidant activity was also assessed for both extracts. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and agarose gel electrophoresis were also performed. **Results:** The presence of aqueous extracts (AE) of TT showed the inhibition of carbonyl content (41.74%) and total AGE generation (40.14%). Reduction in β-amyloid aggregation was observed in SDS-PAGE analysis and thioflavin-T assay. The conformation of DNA was maintained in the presence of aqueous extract in glycated DNA in the presence of Fe^{+}. **Conclusion:** The extracts of fruits of TT exhibit potential antiglycation and antioxidant activity *in-vitro* in protein and DNA. It may be used as a therapeutic agent for the management of diabetes and its complications.

Key words: Advanced glycation end product, aggregation, antioxidant, diabetes, *Tribulus terrestris*.

SUMMARY

- *Tribulus terrestris* extracts showed an evident effect in thwarting the early and advanced glycation end products. The significant presence of antioxidants and phytoconstituents in the aqueous extract imparts anti glycating and anti aggregation properties and prevention of glycoxidation in DNA. Understanding the mechanism and action of the extracts to thwart the glycation process and its related complications in diabetes may help design new therapeutics.



Abbreviations used: AE1: *Tribulus terrestris* fruit extracts (aqueous) – 0.5 mg/mL; AE2: *Tribulus terrestris* fruit extracts (aqueous) – 1 mg/mL; AGEs: Advanced glycation end products; BSA: Bovine Serum Albumin; DPPH: 2,2-diphenyl-1-picrylhydrazyl; Glu: Glucose; Lys: Lysine; MG: Methyl gloxal; ME1: *Tribulus terrestris* fruit extracts (Methanol) – 0.5 mg/mL; ME2: *Tribulus terrestris* fruit extracts (Methanol) – 1 mg/mL; NBT: Nitroblue tetrazolium; TT: *Tribulus terrestris*.

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INTRODUCTION

Diabetes is a type of metabolic disorder, characterized by an increase in glucose level in the blood, termed hyperglycemia, which is associated with various complications. Because the rates of morbidity and the mortality of diabetes are crossing all the ordinary boundaries globally, both prevention and control programs have become necessary to stem the rising rates of the epidemic and its complications.[1] Several processes, namely, oxidation, glycation, and nitration are responsible for causing structural modifications to biomolecules such as lipids, proteins, and nucleic acids, thus curtailing their potential functions.[2]

Among these processes, the most often studied process is glycation. Alternatively, it is also termed non-enzymatic glycosylation. It is a spontaneous deleterious reaction between the free carbonyl groups of reducing sugars and amino groups of nucleic acids, proteins, and lipids, thus causing modifications and altering their functions.[3] It is often referred to as an unavoidable and deleterious non-enzymatic reaction responsible for the pathogenesis of diabetes and its secondary

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complications, cardiovascular and neurodegenerative disorders, cancer, and physiological aging.[2]

Glycation leads to the generation of AGEs that alters or modifies the structures of biomolecules, thus inhibiting the potential function of proteins via the production of certain reactive free radicals, cross-linkages, aggregation, or precipitation.[2] Glycation is further promoted by forming free radicals (oxyradicals), in turn inducing oxidative stress. The rise in cellular carbonyl stress and oxidative stress cause tissue damage through various mechanisms. The oxidative changes along with protein glycation bring about a hyperglycemic condition in cells and tissues.[4] Different studies have reported the significance of glycation in aging and its diseases such as diabetes, neurodegenerative diseases, cardiovascular diseases, rheumatoid arthritis, and renal diseases.[2,4] Therefore, glycation and oxidation can be used to study the beneficial and detrimental effects on normal aging and its related complications. Aminoguanidine, benfotiamine, pyridoxamine, and others act as the most promising glycation inhibitors.[5] Also, to combat the severe oxidative stress in the human body, the innate defense system is not enough; thus, to balance the reactive oxygen species (ROS)/reactive nitrogen species (RNS) amounts, a certain quantity of exogenous antioxidants is required.[6]

Certain medicinal plants that produce natural compounds have recently become a novel source for the production of glycation inhibitors and antioxidants with their unique structures and modes of action.[5,6] Generally, the inhibition of both protein oxidation and glycation is the most studied property for investigating therapeutic purposes in any of these plants. It is being reported that plants used for pharmaceuticals have been continuously studied for their antioxidant and anti-glycation activities and their anti-diabetic properties. Various Arabian plant extracts such as *Tribulus terrestris, Rosa indica, Glycyrrhiza glabra, Sida cordifolia*, and *Plumbago zeylanica* have been reported as active glycation inhibitors *in-vitro*.[7]

T. terrestris (Gokhru, and several other common names such as devil's thorn, puncture vine, bull's head, caltrop), is an annual herbaceous plant belonging to a Family named Zygophyllaceae that grows in a dry climate worldwide. It produces an axe-shaped, hard textured, polymorphic fruit having radially arranged spines on its outer surface [Figure 1]. It has been known to have a tonic for diuretics and a strong aphrodisiac.[6,8-10] It has also been used to treat infertility, erectile dysfunction, decreased libido, increased muscular resistance, urinary disorders, and cardiovascular diseases.[8,10] Other than fruits, various other parts of the plant such as leaves, flowers, and seeds have been used extensively at the time of hemorrhages, kidney stones, and in treating various ailments, such as heart diseases and urinary and gastrointestinal disorders.[10] Thus, the plant has been reported for providing anti-tumor, anti-inflammatory, anti-urolithic, anti-diabetic, and antioxidant effects.[9] Several reports have studied the antioxidant activities of TT;[6] however, very few studies have reported its role in MG-mediated glycation.[7] Further, these research studies have mainly focused only on the measurement of total AGEs, which are fluorescent. The purpose of the current study is to investigate the antioxidant property and antiglycation potential of the aqueous and methanolic extracts of fruit of the plant extensively in model protein (BSA) and DNA. We also studied the role of *Tribulus terrestris* extracts in the process of glycation at early, advanced, and glycation-induced processes such as aggregation and glycoxidation.

Figure 1: Dry fruits of *Tribulus terrestris* (TT)

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA), D-dextrose (Glu), thioflavin-T (ThT) were purchased from Sigma-Aldrich. Di-nitrophenyl hydrazine (DNPH), nitro blue tetrazolium (NBT), and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) were procured from Hi-media. All other chemicals used were of high-grade quality.

Collection of plant material

The collected plant *T. terrestris* was identified by Dr. Fahad, Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia with the certification number FMA-128 (KSUSH). The fruits were dried in a hot air oven in the laboratory at 60°C for 4 to 5 days and then crushed to a fine powder.

Preparation of plant extract

The method used to extract soluble chemical constituents from the fruits of the plant was decoction.

a. Aqueous extract (AE): The crude extract was prepared by dissolving 2.5 g of fine powder of TT to 50 mL of distilled water. The mixture was boiled at 100°C in a water bath for 15 min (until the color changed to a darker shade of brown).
b. Preparation of methanolic extract (ME): The crude extract was prepared by dissolving 2.5 g of fine powder of TT with 50 mL of methanol. The mixture was boiled at 60°C in a water bath for 15 min (until the color changed to a darker shade of emerald green).

The flasks were kept covered to reduce solvent evaporation during extraction. These extracts were then filtered using the Whatman filter paper no. 45 until the supernatant was obtained in the conical flask. The volume of obtained supernatant was reappropriated with the respective solvent and stored in the refrigerator for further use after ensuring the absence of contaminants in the extracts.[11]

Determination of total phenolic (TPC) and flavonoid content (TFC)

The phenolic and flavonoid contents were estimated for both AE and ME extracts using the well-established conventional Folin–Ciocalteu method and the aluminum chloride method.[12] Then, 100 µL of both AE and ME extracts (20 mg/mL) was used for estimation. For TPC and TFC measurements, the standard curves of gallic acid (0–250 µg/mL) and quercetin (0–100 µg/mL) were used for result analysis, respectively.

Determination of antioxidants activity (DPPH assay)

The antioxidant potential of both the extracts of TT was estimated with some necessary modifications.[13] Several concentrations (0.125 mg/mL–2 mg/mL) of both the extracts were set as a reaction mixture (1 mL) with 100 µL of DPPH (1 mM) in methanol incubated at 37°C for 30 min in dark, and absorbance was measured at 517 nm. The scavenging property was measured spectrophotometrically and calculated using the following formula:

Inhibition (%) = $(A_0 - A_t)/A_0 \times 100$

A_0 refers to the absorbance of the control, and A_t refers to the absorbance of the sample.

Preparation of *in-vitro* glycation samples

In-vitro glycation activity of different concentrations of aqueous and methanolic extracts of TT was examined according to the method mentioned by Rubab *et al.*[11] with some modifications. The glycated samples were prepared by incubating 10 mg/mL aqueous solution of BSA along with glucose (100 mg/mL) with or without both concentrations (0.5 and 1 mg/mL) of aqueous and methanolic extracts of TT at 37°C for 28 days. Sodium azide (3 mM) was added to each tube to prevent bacterial contamination due to prolonged incubation.

Measurement of browning

The extent of the formation of glycated products can be analyzed by measuring browning in incubated samples spectrophotometrically (Shimadzu UV 1800) at 420 nm.[14] The inhibition percentage was calculated using a formula applied while calculating the antioxidant activity.

Measurement of fructosamine content

The formation of fructosamine or Amadori products was measured by NBT assay as mentioned by Meeprom *et al.*[15] with some minor modifications. In brief, 100 µL of NBT (0.5 mmol/L) in a carbonate buffer was incubated with 10 µL of glycated sample in a water bath at 37°C for 15 min. Later, the volume was made sufficient up to 1 mL using distilled water to measure spectrophotometrically at 530 nm. The percentage of inhibition of the formation of fructosamine was calculated. The inhibition percentage was calculated using a formula applied for calculating the antioxidant activity.

Estimation of carbonyl content

The carbonyl content was estimated using the DNPH method mentioned by Meeprom *et al.*[15] with slight modifications. The glycated samples were incubated with 400 µL of DNPH (10 mmol/L) in 2.5 mol/L HCl in the dark at 37°C for 1 h. After incubation, 500 µL of 20% (w/v) TCA was added to the incubated samples and kept for 5 min in ice for the proteins to precipitate out. Centrifugation for 10 min at 10,000 rpm at 4°C was performed, and the pellet was washed three times using ethanol: ethyl acetate mixture (1:1). The obtained pellet was resuspended in 250 µL of 6 M guanidine HCl after ethanol/ethyl acetate evaporation. The volume was raised to 1 mL with distilled water and measured spectrophotometrically at 370 nm. The inhibition percentage was calculated using the formula applied for calculating the antioxidant activity.

Estimation of total fluorescent AGEs

The formation of total fluorescent AGEs was measured using the Cary Eclipse Fluorescence spectrophotometer (Varian) at an excitation wavelength of 370 nm and an emission wavelength of 440 nm.[2] The inhibition percentage was calculated using the formula applied for calculating the antioxidant activity.

Measurement of β-amyloid structure by ThT method

The aggregates of the β-amyloid structure are the markers for aggregation that can be measured in the glycated sample using the Cary Eclipse Fluorescence spectrophotometer (Varian) at an excitation wavelength of 440 nm and emission wavelength of 490 nm.[16] To determine protein aggregation in the glycated samples, 20 µM of ThT was added to the glycated samples. The inhibition percentage was calculated using the formula applied for calculating the antioxidant activity.

SDS-PAGE analysis

SDS–PAGE was performed to check the role of TT extracts (AE2 and ME2) in the glucose-mediated glycation system according to the method described by Zhu *et al.*[17] The native and glycated samples of BSA in the presence and absence of TT were incubated for 28 days. These glycated BSA (25 µg) samples and tracking dye were loaded on gels (4% stacking and 7% separating) and electrophoresed at 100 V. On completion of electrophoresis, gels were stained and destained using the standard procedure and then visualized for analysis.

Agarose gel electrophoresis analysis

The pBR322 plasmid (0.25 µg) was incubated with lysine (20 mM), MG (20 mM), and $FeCl_3$ (100 µM) in the presence and absence of AE and ME extracts (AE2 and ME2: 10 µg/µL) at 37°C for 3 h. Agarose gel electrophoresis was performed and analyzed as per the methods described by Rubab *et al.*[11]

Statistical analysis

The statistical analysis was performed with the Microsoft Excel software package (Microsoft Corp.). The correlation coefficient (R) of two sets in triplicate (n = 3) was obtained from analytical data of various assays for relationships between these methods by applying the Pearson correlation matrix. The result of analytical methods is represented in relative percentage (%)/inhibition (%) and P value ≤ 0.05 was considered statistically significant.

RESULTS

Determination of total phenolic (TPC) and flavonoid content (TFC)

The TPC content was calculated to be 35.02 ± 0.63 and 38.13 ± 0.39 mg gallic acid equivalent per gram aqueous (AE) and methanolic (ME) extract of TT, respectively. Similarly, the TFC amount was quantified to be 24.02 ± 0.38 and 29.13 ± 0.02 mg quercetin equivalent per gram of AE and ME extracts, respectively. From these calculations, both aqueous and methanolic extracts of TT possessed a high amount of phenolic content than flavonoid content. However, the methanolic extract had more TPC and TFC than the aqueous extract.

Antioxidant activity (DPPH method)

DPPH assay was performed to check the antioxidant property of aqueous extract and the methanolic extract of TT. On incubation, along with extracts of TT, it showed a decrease in the coloration due to its scavenging property. It was observed that the presence of aqueous extract (AE) and methanolic extract (ME) showed gradual scavenging of free radicals on the increasing concentration gradient of extracts. The AE and ME extracts of TT resulted in free radical inhibition in the range of 8.33 to 22.72% and 4.44 to 21.02% at concentrations ranging from 250 to 2000 µg for both the extracts [Figure 2]. At

lower concentrations, the AE extract showed better antioxidant activity than the ME extract. However, the scavenging activity of the AE extract was slightly better than that of the ME extract at higher concentrations.

Measurement of browning

The measurement of browning was observed as its inhibition, shown by 36.33% at 0.5 mg/mL concentration of AE and 55.03% at 1 mg/mL concentration of AE compared to glycated BSA. Similarly, the methanolic extract showed inhibition of browning by 30.51% (ME1) and 48.63% (ME2) [Figure 3].

Measurement of fructosamine content

The formation of Amadori products/fructosamine content or early glycation products was evaluated using the fructosamine assay. The inhibition of fructosamine content was observed as 22.03% (AE1), 43.12% (AE2), 27.63% (ME1), and 34.45% (ME2) in a glucose-mediated glycation system in the presence of both aqueous and methanolic extracts of TT than control as glycated BSA [Figure 4].

Measurement of carbonyl content

The carbonyl content was inhibited by 34.41% and 41.74% in aqueous extracts AE1 and AE2 in the glycation system, respectively. The decrement inferred by methanolic extract ME1 and ME2 was 30.08% and 39.32%, respectively, in comparison with the glycation system as control [Figure 5].

Estimation of total fluorescent AGEs

The formation of early and late-stage glycation end-products showed inhibition in the presence of TT extract, as seen from the results of browning, fructosamine content, carbonyl content. Further, the evaluation of the generation of AGEs was carried out fluorometrically in the presence and absence of TT extracts in the glycation system. The formation of total AGEs was reduced by 34.17% and 40.14% in the presence of AE1 and AE2 extracts, respectively. Similarly, the reduction of formation of total AGEs was calculated to be 19.53% (ME1) and 30.54% (ME2) in the presence of methanolic extract than in the glucose-mediated glycation system [Figure 6]. The spectrum indicated that in the formation of total AGEs, the fluorometric emission of BSA in the presence of both individual extracts was similar to the fluorometric emission of native BSA alone. Thus, the formation of total AGEs in glycated protein was significantly reduced in the presence of both aqueous and methanolic extracts.

Measurement of β-amyloid structure by thioflavin-T method

The generation of AGEs may lead to the accumulation of glycated proteins. The accumulation of β-amyloid structures was evaluated with thioflavin-T assay fluorometrically. Glycated BSA aggregation was inhibited in the presence of AE1 (11.73%), AE2 (23.68%), ME1 (6.37%), and ME2 (17.25%) than glycated BSA as control. It was noticed that glycation and aggregation prevented the presence of both aqueous and methanolic extracts significantly ($P < 0.5$) [Figure 7].

SDS-PAGE analysis

The SDS-PAGE was performed and analyzed for the aggregation of glycated proteins in the presence/absence of both aqueous and methanolic extract of TT. The visualization of gel showed that the parental band of monomer, dimer, and trimer molecules of BSA were seen more intensely in glycated BSA [Figure 8; Lane 2]. The band positions of glycated BSA in the presence of AE2 [Figure 8; Lane 5] was observed the same band positions and intensity as of native BSA [Figure 8; Lane 1]. ME2 showed methanolic precipitation in Lane

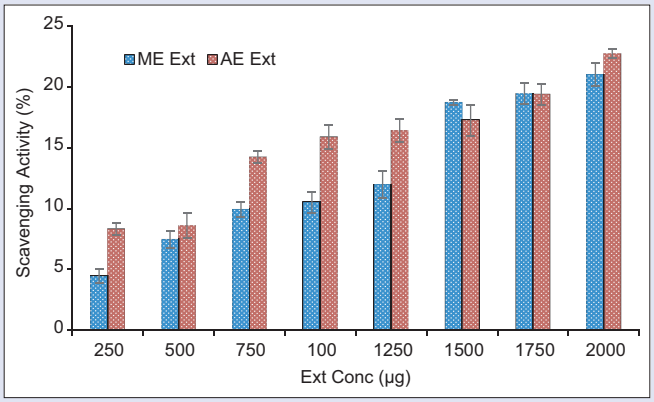

Figure 2: Scavenging of free radicals by TT. (aqueous extract [AE] and methanolic extract [ME]). ($n = 3$ and P value < 0.5)

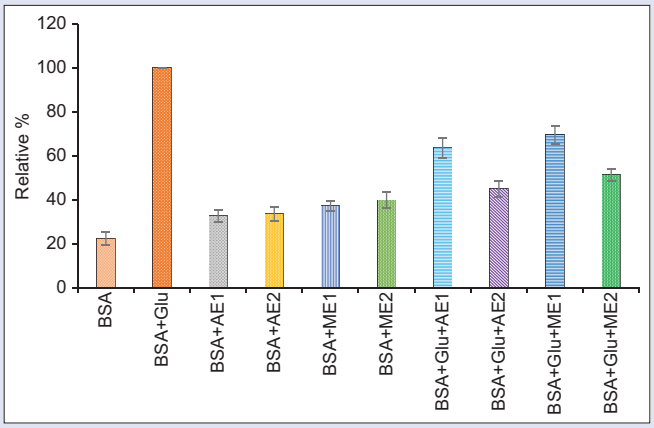

Figure 3: Measurement of browning in glucose-mediated glycation system in the presence/absence of TT extracts. ($n = 3$ and P value < 0.5; BSA- bovine serum albumin, Glu- glucose, aqueous extract (AE1- 0.5 mg/mL and AE2- 1 mg/mL), and methanolic extract (ME1- 0.5 mg/mL and ME2- 1 mg/mL)

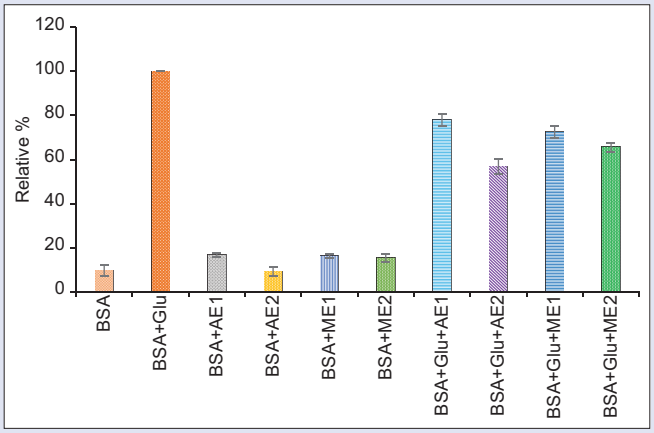

Figure 4: Measurement of fructosamine content. ($n = 3$ and P value < 0.5; BSA- bovine serum albumin, Glu- glucose, aqueous extract (AE1- 0.5 mg/mL and AE2- 1 mg/mL), and methanolic extract (ME1- 0.5 mg/mL and ME2- 1 mg/mL)

4 and Lane 6 [Figure 8]. However, ME2 showed anti-aggregation and antiglycation effects in all assays.

Agarose gel electrophoresis analysis

The glycation system in the presence of ferric ions (Fe^{3+}) enhanced the breakage of DNA strands with the conversion of supercoiled into circular conformations [Figure 9; Lane 2]. The presence of aqueous extract (AE2: 10 µg/µL) of TT helps in maintaining the supercoiled and circular structure of DNA in the glycation system [Figure 9; Lane 5]. The methanolic extract (ME2: 10 µg/µL) also maintained the conformation in the glycation system [Figure 9; Lane 6]; however, ME2 alone caused minor damage to circular conformation [Figure 9; Lane 4].

Correlation analysis between various antiglycation potentials of plant extract

The Pearson correlation analysis was applied to analyze the relationship between the independent analytical methods for antiglycation and anti-aggregation potentials of plant extracts. The generation of total AGEs was strongly correlated with carbonyl content and fructosamine content inhibition. The results of browning and β-amyloid aggregation also showed a strong correlation with the formation of total AGEs [Table 1]. Thus, protein glycation and analytical aggregation parameters suggested that plant extracts inhibited the glucose-mediated glycation and aggregation process.

DISCUSSION

Hyperglycemia and oxidative stress lead to the production of free radicals and in turn, cause the formation of glycation end products that lead to cell and tissue damage. This causes various diseases such as neurodegenerative (Parkinson's, Alzheimer's disease), cancer, diabetes, and its vascular complications and early onset of aging.[2,4,6] Modifications in proteins lead to alteration in their structures, biological activities, and half-lives, thus causing mutations in nucleic acids and affecting transport and signaling processes by causing damage to lipids in the membrane.[13] Many synthetically made AGE inhibitors have shown promising results *in vitro* and in diabetic animals; however, unfortunately, due to their side effects, they are still in the phase of clinical trials. Thus, the demand for more specific and safer herbal and nutritional interventions has increased in patients, inclining toward alternative medicinal practices.

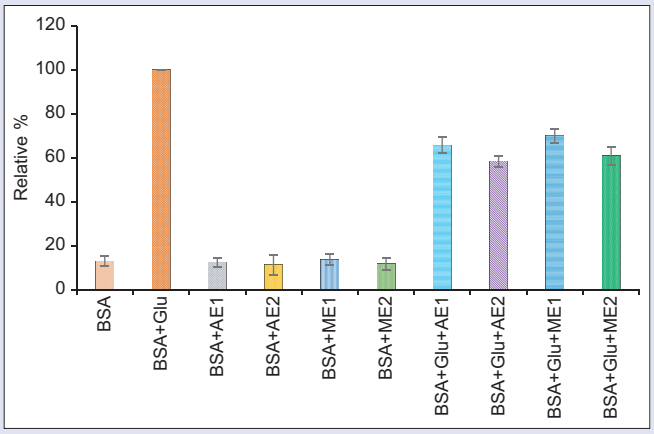

Figure 5: Measurement of carbonyl content. (n = 3 and P value < 0.5; BSA- bovine serum albumin, Glu- glucose, aqueous extract (AE1- 0.5 mg/mL and AE2- 1 mg/mL), and methanolic extract (ME1- 0.5 mg/mL and ME2- 1 mg/mL)

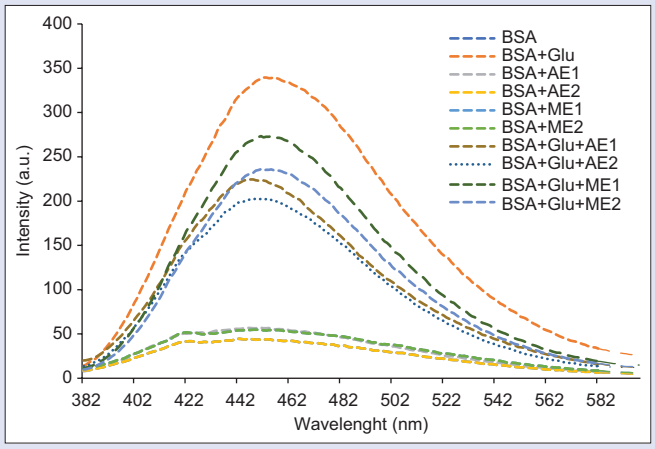

Figure 6: Measurement of total AGEs (excitation- 370 nm, emission- 440 nm). (n = 3 and P value < 0.5; BSA- bovine serum albumin, Glu- glucose, aqueous extract (AE1- 0.5 mg/mL and AE2- 1 mg/mL), and methanolic extract (ME1- 0.5 mg/mL and ME2- 1 mg/mL)

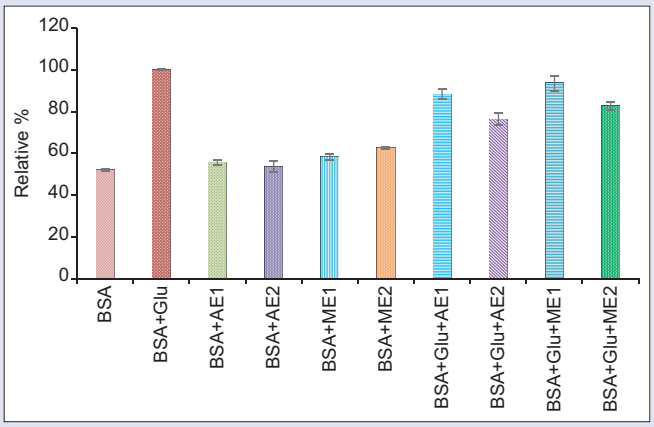

Figure 7: Measurement of β-amyloid structure (excitation- 440 nm, emission- 490 nm). (n = 3 and P value < 0.5; BSA- bovine serum albumin, Glu- glucose, aqueous extract (AE1- 0.5 mg/mL and AE2- 1 mg/mL), and methanolic extract (ME1- 0.5 mg/mL and ME2- 1 mg/mL)

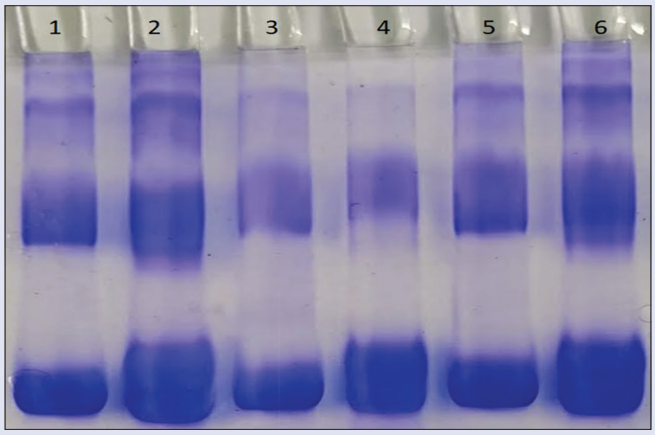

Figure 8: SDS-PAGE analysis. (Lane description: L1: native BSA, L2: BSA + Glu, L3: BSA + AE2, L4: BSA + ME2, L5: BSA + Glu + AE2, and L6: BSA + Glu + ME2)

Table 1: Pearson's correlation coefficient between browning, fructosamine content, carbonyl content, total AGEs, and β-amyloid aggregation

	Browning	Fructosamine content	Carbonyl content	Total AGEs	β-amyloid aggregation
Browning	1.000				
Fructosamine content	0.917	1.000			
Carbonyl content	0.919	0.991	1.000		
Total AGEs	0.875	0.989	0.980	1.000	
β-amyloid aggregation	0.929	0.983	0.973	0.982	1.000

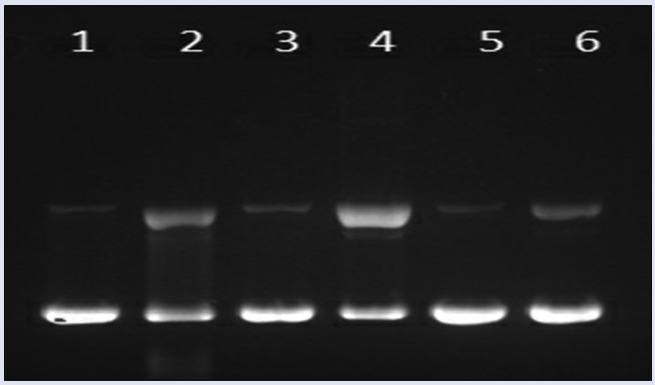

Figure 9: Agarose gel electrophoresis analysis. (Lane description: L1: pBR 322 alone, L2: pBR 322 + lysine (20 mM) + MG (20 mM) + FeCl3 (100 μM), L3: pBR 322 + AE2 (10 μg/10 μL), L4: pBR 322 + ME2 (10 μg/10 μL), L5: L2 + AE2 (10 μg/10 μL), and L6: L2 + ME2 (10 μg/10 μL)

Recent studies have demonstrated the ability of medicinal plants to inhibit the glycation of protein (formation of AGEs) and prevent any alterations in protein activity.[11,13,16]

TT is one such plant that has shown several pharmacological properties, namely, antioxidant, anti-inflammatory, and anti-glycation activities, and is used as a folk medicine to treat hypertension, kidney stones, impotency, and edema.[7] The TT extract is a good source of antioxidants that help decrease the activity of free radicals due to the presence of phenols, saponins, and alkaloids as their active constituents.[7,18] The extracts of TT have shown a significant decrease in the generation of H_2O_2, thus implying that it can be used as a powerful natural antioxidant.[19] The yield of extraction depends on various physiochemical factors and the polarity of the solvent. Do *et al.*[20] found more yield of TPC and TFC in *Limnophila aromatica* in methanol than in the aqueous solvent. Methanol has been considered an efficient solvent for low molecular weight polyphenols.[20] The presence of phenolic and flavonoid content is considered to be involved in imparting antioxidant activity.[18] Further, the multi-step process of glycation is known to generate free radicals, reactive oxygen species (ROS), and dicarbonyl compounds that cause the weakening of the antioxidant defense mechanisms and enhance the formation of AGEs.[11,16,21] The inhibition of glycation of proteins is a complex process and various mechanisms are followed by glycation inhibitors at every step that might slow down or prevent the process; these include a) the scavenging of hydroxyl radicals and others at an early stage and the reduction in the formation of dicarbonyls, b) blocking the monocarbonyls and dicarbonyls in sugars, Schiff's base, or Amadori products during the formation of glycation products, c) the inhibition of AGE formation by metal–ion chelation, d) inhibition of Amadori products at late stages of glycation, e) breaking the crosslinks in the AGEs (formed), and f) the blocking of receptors of AGEs.[13]

A series of experiments were performed to elucidate the effect of TT at different concentrations according to physiological relevance on albumin glycation. The inhibition of early and late products of glycation was observed using the aqueous and methanolic extracts of TT. In a glucose-mediated glycation system, a significant decrease in the formation of glycation products was observed that inhibited the activity of both aqueous and methanolic extracts of TT. The increase in the inhibitory activity was noticed with an increase in the concentration of extracts. However, the aqueous extract showed better inhibitory activity. Methylglyoxal, a dicarbonyl is more reactive than glucose, it interacts with albumin at a faster rate than glucose.[11] Thus, the formation of Schiff's base, Amadori products, and carbonyls or dicarbonyls is induced by hyperglycemia and oxidative stress in diabetic patients and should be taken care of by treating with medicinal plants having antiglycation and antioxidant properties.[11,13] The band intensity of glycated proteins in the presence of aqueous extract, which is equivalent to that of the native protein, has also supported the results of all analytical methods of antiglycation and anti-aggregation properties of TT. Moreover, the reversal of supercoiled and circular conformations of DNA in the glycation system shows the antioxidant and antiglycation potentials of TT.

The medicinal value of plants is also dependent on the antioxidant and radical scavenging properties of significant compounds known as polyphenols present in the plants that help in fighting against diseases caused due to hyperglycemia, glycation, and oxidative stress.[19] There are reports on antioxidants, diabetic activity, etc., but no reports on glucose-mediated protein glycation have been reported yet. TT's aqueous and methanolic extracts showed antiglycation and antiglycation properties under *in-vitro* conditions. Thus, studying antiglycation and antioxidant properties of many valuable medicinal plants has become the basis for the treatment of various diseases due to their safety and efficacy.[7,13,18-19]

CONCLUSION

Under oxidative stress, the glycated products and AGEs are formed in many diseases, especially in diabetes mellitus and its complications. Recently, herbal or medicinal plants have shown therapeutic potential in identifying the lead compounds for the treatment of hyperglycemia-induced vascular complications in diabetic patients. The present study suggests that TT's methanolic and aqueous extracts showed noticeable antioxidant or free radical scavenging activity and potent antiglycation activity in an *in-vitro* glucose-mediated glycation system. However, the aqueous extract of the plant showed strong antioxidant and antiglycation inhibitory potential. Thus, it can be concluded that the extracts of the TT plant can be used as a herbal medicine for diabetic patients. Further, these plant extracts need to be investigated phytochemically and pharmacologically to identify the lead compounds or active constituents for their use as therapeutics against diabetes-induced pathologies.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Chiang JL, Kirkman MS, Laffel LM, Peters AL; Type 1 Diabetes Sourcebook Authors. Type 1 diabetes through the life span: A position statement of the American Diabetes Association. Diabetes Care 2014;37:2034-54.
2. Ali A, More TA, Hoonjan AK, Sivakami S. Antiglycating potential of acesulfame potassium: An artificial sweetener. Appl Physiol Nutr Metab 2017;42:1054-63.
3. Kim CS, Park S, Kim J. The role of glycation in the pathogenesis of aging and its prevention through herbal products and physical exercise. J Exerc Nutrition Biochem 2017;21:55-61.
4. Kim HY, Kim K. Protein glycation inhibitory and antioxidative activities of some plant extracts *in vitro*. J Agric Food Chem 2003;51:1586-91.
5. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 2006;114:597-605.
6. Hammoda HM, Ghazy NM, Harraz FM, Radwan MM, ElSohly MA, Abdallah II. Chemical constituents from *Tribulus terrestris* and screening of their antioxidant activity. Phytochemistry 2013;92:153-9.
7. Siddiqui MA, Rasheed S, Saquib Q, Al-Khedhairy AA, Al-Said MS, Musarrat J, *et al. In-vitro* dual inhibition of protein glycation, and oxidation by some Arabian plants. BMC Complement Altern Med 2016;16:276.
8. Shaibany A, Habori M, Tahami B, Massarani S. Anti-hyperglycaemic activity of *Tribulus terrestris* L. aerial part extract in glucose-loaded normal rabbits. Trop J Pharm Res 2015;14:2263-8.
9. Zhu W, Du Y, Meng H, Dong Y, Li L. A review of traditional pharmacological uses, phytochemistry, and pharmacological activities of *Tribulus terrestris*. Chem Cent J 2017;11:60.
10. Amin A, Lotfy M, Shafiullah M, Adeghate E. The protective effect of *Tribulus terrestris* in diabetes. Ann N Y Acad Sci 2006;1084:391-401.
11. Rubab U, Kumar D, Farah MA, Al-Anazi KM, Ali MA, Ali A. Inhibitory roles of *Nigella sativa* seed extracts on *in vitro* glycation and aggregation. Phcog Mag 2021;17:220-4.
12. Ali A, Shahu R, Balyan P, Kumari S, Ghodmare R, Jobby R, Jha P. Antioxidation and antiglycation properties of a natural sweetener: Stevia rebaudiana. Sugar Tech. 2022;24:563-75. doi: 10.1007/s12355-021-01023-0.
13. Tupe RS, Sankhe NM, Shaikh SA, Phatak DV, Parikh JU, Khaire AA, *et al.* Aqueous extract of some indigenous medicinal plants inhibits glycation at multiple stages and protects erythrocytes from oxidative damage-an *in vitro* study. J Food Sci Technol 2015;52:1911-23.
14. Rondeau P, Armenta S, Caillens H, Chesne S, Bourdon E. Assessment of temperature effects on β-aggregation of native and glycated albumin by FTIR spectroscopy and PAGE: Relations between structural changes and antioxidant properties. Arch Biochem Biophys 2007;460:141-50.
15. Meeprom A, Sompong W, Chan CB, Adisakwattana S. Isoferulic acid, a new anti-glycation agent, inhibits fructose- and glucose-mediated protein glycation *in vitro*. Molecules 2013;18:6439-54.
16. Kumar D, Desa A, Chougle S, Bhatkalkar SG, Sachar S, Selvaa Kumar C, Ali A. Evaluation of the antiglycating potential of thymoquinone and its interaction with BSA. J Biomol Struct Dyn. 2021. doi: 10.1080/07391102.2021.1912642. [Online ahead of print].
17. Zhu Z, Bassey AP, Khan IA, Huang M, Zhang X. Inhibitory mechanism of catechins against advanced glycation end products of glycated myofibrillar protein through anti-aggregation and anti-oxidation. LWT-Food Sci Technol. 2021;147:111550. doi: 10.1016/j.lwt.2021.111550, PMID 111550.
18. Dakshayini PN, Mahaboob Basha P. Phytochemical screening and *in vitro* antioxidant potential of *Tribulus terrestris* fruit and *Mesua ferrea* flower extracts: A comparative study. Int J Pharm Sci 2018;10:70-5.
19. Mahaboob Basha P, Dakshayini PN. *Tribulus terrestris* fruit extract improves antioxidant defense in female reproductive tract: A comprehensive study in diabetic rats. J Innov Pharm Biol Sci 2018;5:101-10.
20. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al.* Effect of extraction solvent on total phenol content, total flavonoids content, and anti-oxidant activity of *Limnophila aromatica*. J Food Drug Anal 2014;22:296-302.
21. Ahmad S, Khan MS, Akhter F, Khan MS, Khan A, Ashraf JM, *et al.* Glycoxidation of biological macromolecules: A critical approach to halt the menace of glycation. Glycobiology 2014;24:979-90.