

RESEARCH ARTICLE

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Attenuation of glycation-induced multiple protein modifications by Indian antidiabetic plant extracts

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ABSTRACT

Context: Protein glycation is the major contributing factor in the development of diabetic complications. The antiglycation potential of medicinal plants provides a promising opportunity as complementary interventions for complications.

Objective: To investigate the antiglycation potential of 19 medicinal plants extracts using albumin by estimating different indicators: (1) glycation (early and late), (2) albumin oxidation, and (3) amyloid aggregation.

Materials and methods: The effect of aqueous plant extracts (1% w/v) on protein glycation was assessed by incubating albumin (10 mg/mL) with fructose (250 mM) for 4 days. Degree of protein glycation in the absence and presence of plant extracts was assessed by estimating fructosamine, advanced glycation end products (AGEs), carbonyls, free thiol group and β -amyloid aggregation.

Results: *Petroselinum crispum*, *Boerhavia diffusa*, *Terminalia chebula*, *Swertia chirayita* and *Glycyrrhiza glabra* showed significant antiglycating activity. *P. crispum* and *A. barbadensis* inhibited the carbonyl stress and protected the thiol group from oxidative damage. There was significant correlation between protein thiols and amyloid inhibition ($R = -0.69$, $p < .001$).

Conclusion: *P. crispum*, *B. diffusa* and *T. chebula* had the most potent antiglycation activity. These plant exerted noticeable antiglycation activity at different glycation modifications of albumin. These findings are important for identifying plants with potential to combat diabetic complications.

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KEYWORDS

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Introduction

The prevalence of diabetes and its complications are rapidly rising all over the world. High blood glucose levels for a longer period of time leads to dysfunction and failure of the multiple organs causing diabetic secondary complications (De Zeeuw & Raz 2008). Non-enzymatic glycation reaction between the aldehyde group of sugars and the amino group of proteins is one of the reasons behind complications in diabetes (Wu et al. 2009). Glycation reaction is accelerated in diabetes and is involved in post translational modifications of proteins, which can alter their functions in living organisms. This reaction is complicated cascade of condensations, rearrangement, fragmentations and oxidative modifications, which leads to the plethora of compounds collectively called advanced glycation end products (AGEs) (Ashraf et al. 2015a; Tabrez et al. 2015). A number of studies have revealed that cell and tissue damage by AGEs comes from protein modifications like conformational changes and functional impairments (De Zeeuw & Raz 2008; Adeshara et al. 2016). Inhibitors of the glycation cascade have therapeutic potential for prevention of diabetic or pathogenic complications in aging (Ahmad et al. 2013a). Hence, it is of concern to discover medicines for targeting steps of glycation cascade and its harmful intermediate molecules, thereby controlling and preventing the conformational changes of glycated proteins. Numerous synthetic compounds with antiglycation properties have been investigated with some encouraging results, but none has received approval as

drugs for the treatment of glycation induced complications because of the observed side effects in clinical trials (Abbas et al. 2016; Younus & Anwar 2016).

Traditional and complimentary medicines like herbal therapies are gaining importance over conventional therapies for the treatment of different diseases especially in non-communicable chronic diseases (Tupe et al. 2015a). They are used for prevention of the disease, health promotion and health maintenance. Complimentary herbal therapies are becoming popular day by day as they are proving to be more cost effective with least side effects (WHO traditional medicine strategy 2013). Functional molecules that are present in natural compounds are responsible for their curative potential. Culinary herbs with high polyphenol content and antioxidant activity represent promising approach (Tupe et al. 2013b, 2015b). Recent studies have shown the beneficial effects of medicinal plants and bioactive compounds in AGEs mediated pathogenesis using experimental diabetic animal models (Elosta et al. 2012; Sadowska-Bartosz & Bartosz 2015; Younus & Anwar 2016). The Indian flora is rich in a vast variety of medicinal plants, which are used for anti-diabetic therapy from a long period of time (Gupta et al. 2007; Tupe et al. 2015c). The investigation of natural products for the treatment and prevention of diabetes and associated complications is a promising opportunity for complementary interventions that may be more acceptable to high-risk populations in search of non-pharmaceutical alternatives. In the current study, by employing complementary *in vitro* assays, we comprehensively and comparatively

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investigated antiglycation activity of extracts from 19 antidiabetic plants. The selections of 19 plants were based on their traditional usage and known antidiabetic, antioxidant properties (Tupe et al. 2013a). To validate this bovine serum albumin (BSA) as a model protein was subjected to glycation by fructose and glycation induced alteration were assessed with and without extract at three stages: (1) glycation reaction (early and late), (2) albumin oxidation and (3) amyloid aggregation.

Materials and methods

Bovine serum albumin (BSA, Fraction V, catalogue no: A2153, purity $\geq 96\%$), sodium azide, D-fructose, 2,4-dinitrophenylhydrazine (DNPH), 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), Congo red and thioflavin T were obtained from Sigma (St. Louis, MO). All other reagents were of analytical grade. Table 1 indicates the plant species used along with their common names, family, and part used. They were obtained from the local herbal and Ayurvedic medicine store (Ambadas Vanaushadhalaya, Pune, India). The authenticated plant parts were collected, dehydrated (in a chamber below 40 °C for 48 h), powdered with a mechanical grinder and stored in airtight containers.

Preparation of plant extracts

Extracts were prepared according to the method described by Deetae et al. (2012) with some modifications. Aqueous extracts (1% w/v) of the plant parts were prepared in deionized water with stirring at room temperature (150 rpm, 3 h). Insoluble residues were removed by centrifugation at 3000 rpm followed by filtration. All extracts were placed in plastic vials, stored at 4 °C until use and all experiments were performed in triplicates.

In vitro glycation of albumin

BSA was glycated in accordance with the method described by McPherson et al. (1988) with some modifications. For evaluation of antiglycating property of plant extracts, BSA (1 mL, 10 mg/mL) was incubated along with aqueous plant extracts (1 mL) and

fructose (1 mL, 250 mM) in potassium phosphate buffer saline (PBS), (2 mL, 200 mM, pH 7.4) containing 0.02% sodium azide, in the dark at 37 °C for 4 days in sealed tubes under sterile conditions (0.22 μ filter), hereafter referred to as 'glycated sample'. Negative control (1 mL BSA + 3 mL PBS) and positive control (1 mL BSA + 1 mL fructose + 2 mL PBS) were maintained under similar condition. After incubation, unbound fructose was removed by dialysis against PBS and dialysate was used for further analysis. All additions and analysis were performed in triplicates.

Determination of fructosamine level

The concentration of fructosamine, the Amadori product in glycated albumin samples and controls, was determined by using nitroblue tetrazolium (NBT) assay (Ahmad et al. 2013b). NBT solution (0.75 mM) was prepared in carbonate buffer (0.1 M, pH 10.35). Glycated samples, 40 μ L, were incubated with NBT solution, 0.8 mL, at 37 °C for 30 min. The absorbance was measured at 530 nm (Genesys 10S UV-Visible, Thermo Scientific). Fructosamine concentration was calculated using standard 1-deoxy-1-moepholino-fructose curve ($Y = 0.00X + 0.017$, $r = .981$) and was expressed in μ M/mg of protein.

Measurement of AGEs by fluorescence

The formation of AGEs in glycated albumin samples was assessed by the method given previously (Tupe & Agte 2010). AGE fluorescence of glycated samples was measured at an excitation and emission wavelength of 370 nm and 440 nm, respectively, on Perkin Elmer Luminescence spectrometer (LS 50 B) (Perkin Elmer Ltd., Beaconsfield, Buckinghamshire, England). The results were expressed as arbitrary units (AU).

Determination of protein carbonyl groups

Carbonyl group in glycated samples was assayed according to Ashraf et al. (2015b). DNPH (10 mM) was prepared in 2.5 M HCl. Glycated sample, 500 μ L, was incubated with DNPH solution, 500 μ L, for 1 h at room temperature followed by precipitation with TCA, (1.0 mL, 20%). The precipitate was washed with ethanol: ethyl acetate mixture (1:1 v/v, 1 mL), and the pellet dissolved in urea (1 mL, 6 M), and absorbance was read at 365 nm. The protein carbonyl group conc Asentration was calculated by using molar extinction coefficient (ϵ at 365 nm = 21 mM⁻¹ cm⁻¹) and was expressed in nM/mg of protein.

Thiol group estimation

Free thiol groups in glycated albumin samples were estimated according to the method given by Ellman (1959). Glycated sample, 250 μ L, was incubated with DTNB (0.5 mM, pH 7.4) for 15 min. Absorbance was measured at 410 nm. Free thiol content was calculated by using the molar extinction coefficient (ϵ at 410 nm = 13.6 mM⁻¹ cm⁻¹) and the results were expressed as nM/mg of protein.

Determination of amyloid β -aggregation by congo red

Aggregation in glycated sample was measured using amyloid specific Congo red dye according to the method described previously (Tupe et al. 2013b). Congo red (100 μ M), was prepared in PBS (pH 7.4) containing ethanol (10%, v/v). Glycated sample

Table 1. Plant species used along with common names, family and part used^a.

Scientific name	English name	Family	Parts used
<i>Aegle marmelos</i>	Bael	Rutaceae	Leaf
<i>Aloe barbadensis</i>	Aloe vera	Aloaceae	Latex
<i>Andrographis paniculata</i>	Indian echinacea	Acanthaceae	Leaf
<i>Bacopa monnieri</i>	Bacopa	Scrophulariaceae	Leaf
<i>Boerhavia diffusa</i>	Spreading hogweed	Nyctaginaceae	Root
<i>Caesalpinia bonducella</i>	Bonducella nut	Cesalpiniaceae	Fruit
<i>Commiphora mukul</i>	Salaitree	Burseraceae	Leaf
<i>Glycyrrhiza glabra</i>	Liquorice	Fabaceae	Root
<i>Gymnema sylvestre</i>	Gymnema	Asclepiadaceae	Leaf
<i>Hemidesmus indicus</i>	Indian sarsaparilla	Asclepiadaceae	Root
<i>Ocimum basilicum</i>	Common basil	Lamiaceae	Seed
<i>Ocimum sanctum</i>	Holy basil	Lamiaceae	Leaf
<i>Ocimum sanctum</i>	Holy basil	Lamiaceae	Seed
<i>Petroselinum crispum</i>	Parsley	Apiaceae	Leaf
<i>Salacia reticulata</i>	Salacia	Celastraceae	Root
<i>Silybum marianum</i>	Milk thistle	Asteraceae	Stem
<i>Swertia chirayita</i>	Indian gentian	Gentianaceae	Leaf
<i>Terminalia arjuna</i>	Arjuna	Combretaceae	Bark
<i>Terminalia chebula</i>	Myrobalan	Combretaceae	Fruit
<i>Withania somnifera</i>	Winter cherry	Solanaceae	Root

^aThe specific parts of plants were obtained from the local herbal and Ayurvedic medicine store (Ambadas Vanaushadhalaya). The authenticated plant parts were collected, dehydrated (in a chamber below 40 °C for 48 h), powdered with a mechanical grinder and stored in air-tight containers.

(500 μ L), was incubated with Congo red solution (100 μ L) and absorbance was measured after incubation for 20 min, at 530 nm.

Determination of amyloid β -aggregation by thioflavin T

For determination of β amyloid aggregation, Thioflavin T, a marker for the amyloid cross β structure was used (Tupe & Agte 2010). Thioflavin (32 mM), was dissolved in glycine-NaOH buffer, (50 mM, pH 8.5). Glycated samples (100 μ L), were incubated with Thioflavin T solution (3 mL) for 1 h, and fluorescence was measured at an excitation and emission wavelength of 435 nm and 485 nm, respectively (slit, 10 nm), with appropriate blanks devoid of Thioflavin T. The results were expressed as AU (Arbitrary Unit).

Statistical analysis

Data were expressed as the mean and standard deviations of triplicate values. The statistical analysis was carried out using the Microsoft Excel software package (Microsoft Corp.). The Pearson correlation matrix was applied to the analytical data to find the relationships between the different analytical methods, which were expressed as the correlation coefficient 'R'.

Results

The current study evaluated the antiglycation potential of 19 anti-diabetic medicinal plant extracts used in India to treat symptoms of diabetes and complications. Each extract has been analyzed for the antioxidant potential in our previous report (Tupe et al. 2013). The potential of plant extracts to inhibit the glycation reaction were analyzed at three levels: (1) glycation reaction (early and late), (2) albumin oxidation and (3) amyloid aggregation.

Effects of extracts on early and late stages of protein glycation

As depicted in Figure 1, after four days of incubation of albumin with fructose the content of fructosamine in glycated albumin

(48.23 μ M/mg protein) were significantly increased as compared to native albumin (3.53 μ M/mg protein). The presence of *G. glabra* and *W. somnifera* significantly inhibited the level of fructosamine by 69 and 60%, respectively ($p < .001$). In addition, other herbs extracts suppressed the elevation of fructosamine by $\sim$ 10–57%. Whereas *Andrographis paniculata*, *Commiphora mukul*, *O. sanctum* (leaf), *Salacia reticulata*, *Silybum marianum* extracts did not inhibit the fructosamine formation during glycation.

AGEs are heterogeneous molecules and based on their properties, they are categorized as: (a) fluorescent and cross-linking AGEs, such as pentosidine, crossline, 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole, glyoxal-lysine dimer, and methyl-glyoxal-lysine dimer (MOLD); and (b) nonfluorescent and non-cross-linking agents such as *N*-carboxymethyl lysine (CML), *N*3-(carboxyethyl) lysine, and pyrroline (Ahmed 2005; Palimeri et al. 2015). The formation fluorescent AGEs after albumin glycation is generally assessed by monitoring their fluorescence at excitation and emission maxima of 370 and 440 nm, respectively. The fluorescence intensity was highly increased ($p < .001$) in glycated albumin (806.77 AU) against native albumin (57.12 AU) indicating progressive formation of glycated AGEs products. As evident from Figure 2 presence of all medicinal plant extracts significantly decreased formation of AGEs except *Ocimum basilium* and *C. bonducella*. Comparatively, *T. chebula* and *P. crispum* showed significant reduction in AGEs formation by reducing the fluorescent intensity by 91 and 90%, respectively ($p < .001$).

Effects of extracts on glycation induced protein oxidation

Oxidative modifications of BSA during glycation were demonstrated using a combination of carbonyl and thiol group detection. Extent of protein oxidation during this incubation period was determined with the help of DNPH reagent. As shown in Table 2, the highest percentage of carbonyl groups were detected in glycated BSA (positive control). However, addition of the plant extracts during glycation significantly reduced the carbonyl

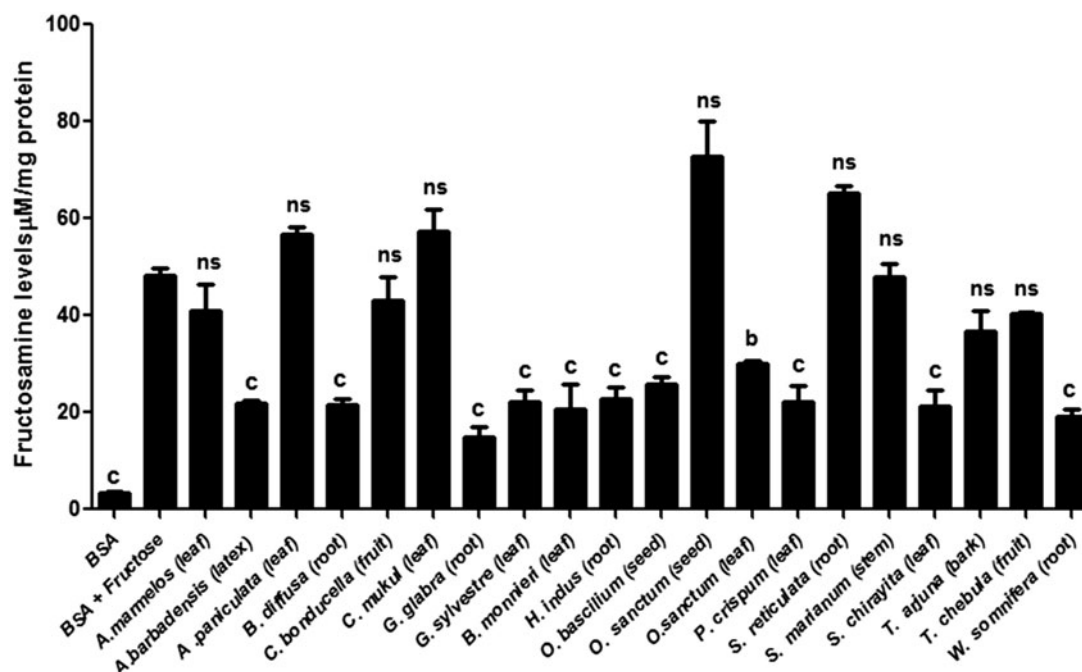


Figure 1. Effect of plant extracts on *in vitro* formation of fructosamine in albumin glycation. Mean values were significantly different from that of positive control (BSA + fructose): ^a $p < .05$, ^b $p < .01$, ^c $p < .001$, ns: non significant (One way ANOVA).

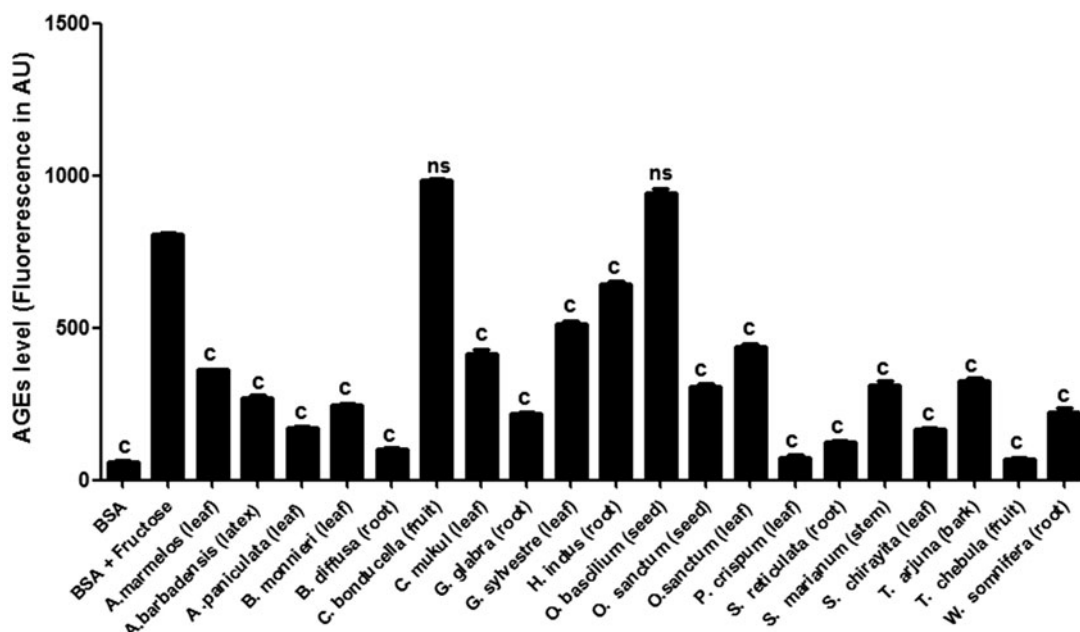


Figure 2. Effect of plant extracts on *in vitro* formation of fluorescent AGEs in albumin glycation. Mean values were significantly different from that of positive control (BSA + fructose): ^a $p < .05$, ^b $p < .01$, ^c $p < .001$, ns: non significant (One way ANOVA).

group formation, indicating their antioxidant activities. Among the 19 plant extracts tested, maximum reduction in carbonyl groups was observed in the presence of *P. crispum* (52%) followed by *A. barbadensis* (43%), *S. marianum* (34%), *O. sanctum-seed* (34%) and *Withania somnifera* (33%).

Evaluation of free thiol groups in BSA after glycation was performed with the help of DTNB reagent. Incubation of BSA with fructose reduced free thiol groups significantly ($p < .05$) by 96% when compared with negative control (0.05 and 1.46 mM/mg protein, respectively). As shown in Table 2, presence of *B. diffusa* and *T. chebula* during glycation showed significant decrease in thiol group oxidation ($p < .001$). Glycation in presence of *B. diffusa* and *T. chebula* extract increased free thiol groups by 32 and 30.8%, respectively. Least protection was observed in presence of *A. barbadensis*, which showed only 4% increase in thiol groups.

Effect of plant extracts on β -amyloid aggregation

The level of β -amyloid cross structure in albumin was determined by using amyloid specific Congo red and Thioflavin T, which specifically binds with fibrous structure (Table 3). The fluorescent intensity of Thioflavin T was observed to be significantly elevated in glycated albumin as compared with albumin without fructose, suggesting protein glycation gradually induced the formation of amyloid structure in BSA. Results obtained from present study showed, presence of all plant extracts effectively inhibited the β -amyloid aggregation by 74–85% except for *Terminalia arjuna* (61%) and *Caesalpinia bonducella* (67%).

Analogous results were obtained when amyloid structure was analyzed with Congo red dye as illustrated in Table 3. Maximum absorbance was observed when BSA was incubated along with fructose. Both *P. crispum* and *T. chebula* reduced absorbance by 51% when incubated with BSA and fructose. Whereas presence of the remaining plant extracts showed inhibition in the range from 26–48% in terms of Congo red absorbance.

Table 2. Effects of plant extracts on protein oxidation measured as protein carbonyl groups and free thiol groups in glycated albumin samples.

	Free carbonyl groups (nM/mg of protein)	Free thiol groups (nM/mg of protein)
BSA	32.88 $\pm$ 5.13 ^c	1.46 $\pm$ 0.10 ^c
BSA + fructose	58.30 $\pm$ 0.71	0.05 $\pm$ 0.00
<i>A. marmelos</i>	45.00 $\pm$ 3.10 ^c	0.19 $\pm$ 0.01 ^c
<i>A. barbadensis</i>	32.84 $\pm$ 1.83 ^c	0.06 $\pm$ 0.02 ^{ns}
<i>A. paniculata</i>	43.76 $\pm$ 0.32 ^c	0.12 $\pm$ 0.02 ^a
<i>B. monnieri</i>	43.10 $\pm$ 0.05 ^c	0.10 $\pm$ 0.01 ^{ns}
<i>B. diffusa</i>	42.84 $\pm$ 5.51 ^c	0.47 $\pm$ 0.05 ^c
<i>C. bonducella</i>	41.93 $\pm$ 0.25 ^c	0.20 $\pm$ 0.01 ^b
<i>C. mukul</i>	44.95 $\pm$ 0.48 ^c	0.10 $\pm$ 0.01 ^{ns}
<i>G. glabra</i>	42.32 $\pm$ 0.70 ^c	0.10 $\pm$ 0.01 ^{ns}
<i>G. sylvestre</i>	41.78 $\pm$ 0.58 ^c	0.15 $\pm$ 0.02 ^c
<i>H. indus</i>	44.82 $\pm$ 0.37 ^c	0.08 $\pm$ 0.02 ^{ns}
<i>O. basilium</i>	42.84 $\pm$ 0.5 ^c	0.16 $\pm$ 0.03 ^c
<i>O. sanctum</i> (seed)	40.46 $\pm$ 0.88 ^c	0.14 $\pm$ 0.02 ^b
<i>O. sanctum</i> (leaf)	38.30 $\pm$ 2.88 ^c	0.18 $\pm$ 0.01 ^c
<i>P. crispum</i>	27.87 $\pm$ 2.50 ^c	0.45 $\pm$ 0.04 ^c
<i>S. reticulata</i>	43.43 $\pm$ 0.27 ^c	0.10 $\pm$ 0.01 ^b
<i>S. marianum</i>	38.30 $\pm$ 3.87 ^c	0.19 $\pm$ 0.01 ^c
<i>S. chirayita</i>	41.80 $\pm$ 0.45 ^c	0.16 $\pm$ 0.01 ^c
<i>T. arjuna</i>	47.11 $\pm$ 0.01 ^c	0.094 $\pm$ 0.02 ^{ns}
<i>T. chebula</i>	40.25 $\pm$ 0.63 ^c	0.45 $\pm$ 0.21 ^c
<i>W. somnifera</i>	38.55 $\pm$ 1.91 ^c	0.19 $\pm$ 0.01 ^c

Values are mean $\pm$ standard deviation ($n = 3$). Mean values were significantly different from positive control (BSA + Fructose):

^a $p < .05$,

^b $p < .01$,

^c $p < .001$, ns: nonsignificant (One way ANOVA).

Correlation analysis between various antiglycation potential of plant extracts

A correlation analysis was used to determine the relationship between the different antiglycation potential of plant extracts. The very good correlation was observed between protein thiols and amyloid by Congo red ($R = -.69$, $p < .001$). On the contrary, Thioflavin T exhibited a good correlation with AGEs and carbonyls with R values .57 and .49, respectively. Thus, the results suggest

Table 3. Effects of plant extracts on β -amyloid fibril formation on fructose modified albumin by using Congo Red and Thioflavin T.

	Detection of β -amyloid fibril formation	
	Congo red (Absorbance at 560nm)	Thioflavin T (Fluorescence in AU)
BSA	0.12 $\pm$ 0.01 ^a	6.77 $\pm$ 2.38 ^a
BSA + fructose	0.68 $\pm$ 0.01	285.49 $\pm$ 5.17
<i>A. marmelos</i>	0.50 $\pm$ 0.02 ^a	42.63 $\pm$ 1.92 ^a
<i>A. barbadensis</i>	0.44 $\pm$ 0.02 ^a	50.56 $\pm$ 1.50 ^a
<i>A. paniculata</i>	0.40 $\pm$ 0.01 ^a	67.69 $\pm$ 2.51 ^a
<i>B. monnieri</i>	0.46 $\pm$ 0.03 ^a	40.90 $\pm$ 1.91 ^a
<i>B. diffusa</i>	0.35 $\pm$ 0.05 ^a	47.20 $\pm$ 3.21 ^a
<i>C. bonducella</i>	0.44 $\pm$ 0.02 ^a	95.27 $\pm$ 4.98 ^a
<i>C. mukul</i>	0.42 $\pm$ 0.01 ^a	63.88 $\pm$ 1.96 ^a
<i>G. glabra</i>	0.45 $\pm$ 0.04 ^a	49.89 $\pm$ 2.57 ^a
<i>G. sylvestre</i>	0.39 $\pm$ 0.02 ^a	48.62 $\pm$ 1.69 ^a
<i>H. indus</i>	0.49 $\pm$ 0.02 ^a	74.47 $\pm$ 4.04 ^a
<i>O. basilium</i>	0.44 $\pm$ 0.04 ^a	69.61 $\pm$ 3.05 ^a
<i>O. sanctum</i> (seed)	0.50 $\pm$ 0.02 ^a	40.96 $\pm$ 1.94 ^a
<i>O. sanctum</i> (leaf)	0.36 $\pm$ 0.02 ^a	43.51 $\pm$ 2.59 ^a
<i>P. crispum</i>	0.33 $\pm$ 0.04 ^a	36.08 $\pm$ 2.55 ^a
<i>S. reticulata</i>	0.41 $\pm$ 0.01 ^a	44.69 $\pm$ 1.55 ^a
<i>S. marianum</i>	0.48 $\pm$ 0.01 ^a	40.60 $\pm$ 3.00 ^a
<i>S. chirayita</i>	0.44 $\pm$ 0.01 ^a	46.07 $\pm$ 3.04 ^a
<i>T. arjuna</i>	0.46 $\pm$ 0.02 ^a	111.06 $\pm$ 3.60 ^a
<i>T. chebula</i>	0.33 $\pm$ 0.01 ^a	37.70 $\pm$ 3.07 ^a
<i>W. somnifera</i>	0.49 $\pm$ 0.03 ^a	42.98 $\pm$ 2.37 ^a

Values are mean $\pm$ standard deviation ($n = 3$). Mean values were significantly different from positive control (BSA + fructose):

^a $p < .001$ (One way ANOVA).

that plant extracts, which can reduce thiol oxidation (high thiol value) can inhibit the further amyloid formation (low Congo red reading). Secondly, their protein glycation and oxidation inhibition potential is similar to amyloid reduction potency.

Total antiglycation potential of plant extracts

The total antiglycation potential of plant extracts has given according to their total performance in the antiglycation activity, i.e., inhibition to fructosamine, AGEs, Congo Red, Thioflavin T, carbonyl groups and protection to thiol group. Accordingly, ranking is given to each plant extract as mentioned in Table 4.

The average antiglycation potential was in the range from 32.35 to 61.14. *P. crispum* surpassed all the other plants as it obtained first ranking in overall performance (61.14). This was followed by *B. diffusa* (55.64) and *T. chebula* (51.26). *B. diffusa* showed great effect in protecting thiol group and *T. chebula* outperformed in inhibition to β -amyloid aggregation. *S. chirayita* and *G. glabra* obtained fourth and fifth ranking respectively by inhibiting fructosamine and AGEs. They moderately inhibited β -amyloid aggregation, but failed to protect thiol group from oxidation. Sixth and seventh position obtained by *W. somnifera* and *Aloe barbadensis* respectively, both of them moderately inhibited fructosamine, AGEs and β -amyloid formation, however *A. barbadensis* greatly inhibited the carbonyl formation. *Bacopa monnieri*, *O. sanctum* (Leaf) and *G. sylvestre* also reasonably have antiglycation potential. All the remaining plant extracts haven't shown significant antiglycation potential as their average values were less than 40.41.

Discussion

Protein glycation involves a cascade of complex reactions that occur between sugars (here fructose) and amino acids, producing

an unstable Schiff base and then forming Amadori products like fructosamine. The determination of fructosamine levels after BSA glycation is used to monitor the accumulation of early glycation products. Results from the present investigation demonstrated that reduced level of fructosamines by *G. glabra* and *W. somnifera* was associated with the decreased formation of AGEs indicating their inhibitory effect on Amadori production and conversion into AGEs. *G. glabra* has glycyrrhizin, a triterpenoid saponin, major water soluble component. Sen et al. (2011) have reported capability of glycyrrhizin against hyperglycemia, hyperlipidemia and associated oxidative stress. Sil et al. (2013) has demonstrated inhibitory activity of glycyrrhizin on hemoglobin glycation, hemoglobin mediated iron release and iron mediated free radical reactions. The pharmacological effects of the roots of *W. somnifera* are mainly due to the presence of withanolides, a group of steroidal lactones. Tripathi et al. (1996) reviewed the antidiabetic activities of *W. somnifera*. Moreover hypoglycemic effects of *W. somnifera* have also been reported (Udayakumar et al. 2009). Thus, additional prevention of glycation at early stage by *G. glabra* and *W. somnifera* can definitely show beneficial effects in diabetes and related complications. Based on fluorescence property, we studied the influence of plant extracts on formation of total AGEs. Our results demonstrated that plant extracts efficiently inhibited AGEs formation. According to data obtained from our study, *P. crispum* and *T. chebula* were the strongest inhibitor of the AGEs formation among the other plants tested. A similar finding has been reported by Ramkissoon et al. (2012) where they have shown antiglycation activity of *P. crispum*. Flavonols (kaempferol and quercetin) and flavones (apigenin and luteolin), which occur in glycosidic form, are major flavonoids found in *P. crispum* (Peterson et al. 2006). Kaempferol and quercetin has been shown to possess antioxidant and anti-inflammatory activities (Blonska et al. 2003; Abo-Salem & Osama 2014). Thus, promising effects shown by *P. crispum* can be attributed to these components.

T. chebula has been reported to have a variety of biological activities including antidiabetic activities. Ethanol extracts of *T. chebula* have shown hypoglycemic action on streptozotocin induced diabetic rats. It also showed reduction in glycosylated hemoglobin with concomitant increase in hemoglobin level in diabetic rat (Kumar et al. 2006). Antiglycation activity of *T. chebula* is also evident from studies reported by Lee et al. (2011). In their study, they showed inhibition of AGEs formation by chebulic acid, the major constituent of *T. chebula*, comparable to aminoguanidine. Additionally, they also reported a significant reduction in the adhesion of monocytes to endothelial cells treated with AGEs in the presence of chebulic acid (Lee et al. 2011). Moreover, *T. chebula* is reported to contain more phenolic compounds than any other plants which may be the reason why this plant showed strong antioxidant activity (Gupta 2012).

Besides *P. crispum* and *T. chebula*, *Swertia chirayita*, *B. diffusa* and *O. sanctum* (leaf) have shown promising inhibitory action against both the early glycation product i.e., fructosamine and late AGEs. *S. chirayita* contains swerchirin, mangiferin and swertiamarin, which are having antidiabetic activities (Saxena et al. 1996; Saleh et al. 2014). Additionally, it has a high content of phenols reflecting its known antioxidant and antidiabetic activity (Singh & Ambika Chauhan 2012; Phoboo et al. 2013) and this may contribute to its observed antiglycation potential. *B. diffusa* has also reported to possess antioxidant and antidiabetic activity (Pari & Amarnath, 2004; Satheesh & Pari, 2004). Its major active principles are boeravinones, which are responsible for its antioxidant and anti-inflammatory activity (Aviello et al. 2011;

Table 4. Summary of the antiglycation activities of medicinal plant extracts at three different stages and their relative rankings.

Samples	Inhibition TO glycation		Inhibition TO protein oxidation		Inhibition TO amyloid aggregation	
	Average value (Fructosamines and AGEs)	Rank	Average value (Protein carbonyls and Thiols)	Rank	Average value (Congo red and Thioflavin T)	Rank
<i>A. marmelos</i>	201.16	14	22.79	19	21.57	6
<i>A. barbadensis</i>	144.90	10	16.53	2	25.50	14
<i>A. paniculata</i>	113.96	6	22.08	15	34.05	16
<i>B. monnieri</i>	133.42	9	21.71	13	20.69	4
<i>B. diffusa</i>	60.71	3	22.13	16	23.78	11
<i>C. bonducella</i>	513.70	20	21.27	10	47.86	19
<i>C. mukul</i>	236.29	16	22.63	18	32.15	15
<i>G. glabra</i>	116.15	7	21.32	11	25.18	13
<i>G. sylvestre</i>	267.68	17	21.13	8	24.51	12
<i>H. indus</i>	332.59	18	22.54	17	37.48	18
<i>O. bascilum</i>	485.32	19	21.66	12	35.03	17
<i>O. sanctum</i> (Seed)	190.26	13	20.45	6	20.74	5
<i>O. sanctum</i> (leaf)	233.70	15	19.44	3	21.94	8
<i>P. crispum</i>	47.85	1	14.61	1	18.21	1
<i>S. reticulata</i>	94.34	5	21.87	14	22.55	9
<i>S. marianum</i>	180.59	11	19.44	4	20.54	3
<i>S. chirayita</i>	93.01	4	21.15	9	23.26	10
<i>T. arjuna</i>	181.88	12	23.70	20	55.76	20
<i>T. chebula</i>	55.63	2	20.81	7	19.02	2
<i>W. somnifera</i>	121.46	8	19.57	5	21.74	7

Bairwa et al. 2014). In our study, we also found this preventive activity of *B. diffusa* as it showed maximum protection of thiol group from oxidation.

In our previous studies (Tupe et al. 2013a), *O. sanctum* leaves were found to contain four-fold higher phenolic content than seeds which might be the reason for observed higher antiglycation activity in leaves than seeds. Similar studies conducted by Kaewnarin et al. (2013) have shown that *O. Sanctum* contains high amount of phenolic compounds like rosmarinic acid and luteolin, which might be responsible for antiglycation activity. *S. reticulata* contains number of phytochemicals like mangiferin, kotalanol and salacinol, which are responsible for the inhibition to glycation reaction as was shown by Li et al. (2010).

In an attempt to evaluate the mode of action, we examined the effect of plant extracts on oxidative parameters. Oxidative processes plays major role in the formation of AGEs (Fu et al. 1994). This process can take place by two mechanisms: (1) through autooxidation of free sugars in the presence of oxygen to form reactive dicarbonyl compounds, which reacts with proteins forming highly reactive ketoamines, (2) oxidation of Amadori products, which gives rise to highly reactive protein enediols and protein dicarbonyls generating AGEs. Formation of carbonyl protein through oxidation is also accompanied by loss of free thiols in albumin (Ardestani & Yazdanparast 2007). *P. crispum* showed marked reduction in carbonyl levels along with an increase in free thiol groups. Though in previous studies *P. crsipum* did not show strong antioxidant properties, it prevented albumin oxidation in the presence of fructose indicating antiglycating potential by reducing the carbonyl group generation and inducing protection to free thiol groups. Though some plant extracts, like *A. barbadensis*, *B. monnieri*, *C. mukul*, *G. glabra*, *G. sylvestre*, *Hemidesmus indicus*, *T. arjuna* did not exert protection to free thiol groups, but significantly prevented generation of carbonyl groups. Inhibitory effects on the AGEs formation by these plant extracts was thus through scavenging carbonyl radicals.

In previous studies, while evaluating antioxidant potential, *B. monnieri*, *C. mukul*, *G. glabra*, *G. sylvestre*, *H. indicus*, *T. arjuna* showed antioxidant potential on various radicals (Tupe et al. 2013a). Extracts of *B. monnieri* have already shown protective effects on tissue antioxidant defense systems and lipid

peroxidative status in streptozotocin induced diabetic rats (Kapoor et al. 2009). Guggulsterone, a major chemical constituent of *C. mukul* is also reported to prevent oxidation of lipoproteins and generation of hydroxyl radicals in the nonenzymatic system (Singh et al. 1997). Visavadiya et al. have shown considerable *in vitro* antioxidant and protective properties of ethanolic extracts of *G. glabra* against the human lipoprotein oxidative system (Visavadiya et al. 2009). *G. sylvestre*, a rich source of gymnemic acid, is well known for its antidiabetic properties (Kanetkar et al. 2007). Protections from carbonyl generation during glycation by *G. sylvestre* can definitely contribute to its antidiabetic potential. Ravishankara et al. (2002) have evaluated radical scavenging properties of ethanolic extract of *H. indicus*, which can be one of the mechanisms through which it reduced generation of free carbonyl groups. Antidiabetic effects of *T. arjuna* in fat fed and streptozotocin induced diabetic rat has already been documented by Kehkashan et al. (2011) where they observed reduction in the tissue protein carbonyl level on treatment with *T. arjuna*.

Panaskar et al. (2013) have demonstrated the antiglycation and antioxidant activity of *A. marmelos* leaf extract and its effectiveness in the prevention of diabetic nephropathy and cataract in experimental diabetic rats. In present study, similar inhibition to the AGEs formation have been shown by this plant however it failed to inhibit further protein modifications such as oxidation and β -fibril formation. According to Premnath and Nanjaiah (2015), *A. paniculata* possesses antidiabetic and antioxidant activities. Likewise, in our study *A. paniculata* showed significant inhibition of AGEs formation and moderately to protein aggregation, however it showed poor inhibition to protein oxidation.

A study conducted by Shin et al. (2015) has shown that *S. marianum* has silibinin as its active ingredient which inhibits the accumulation of AGEs in *in vitro* model as well as in the human skin explants model. This antiglycation potential of *S. marianum* is also evident in the present study as it inhibited the AGEs fluorescence as well as carbonyl formation. There are a number of reports about antidiabetic and antioxidant activities of *C. bonducella* and *O. bascilum* (Chakrabarti et al. 2003; Shukla et al. 2009; El-Beshbishy & Bahashwan 2012). However, they haven't shown significant antiglycation potential in this study,

which may explain the contribution of other mechanisms, e.g., gut absorption of glucose and stimulation of liver glycogen synthesis for having hypoglycemic activities.

Glycation induces conformational changes in protein by increasing the level of amyloid cross β -structure, which plays a major role in the protein aggregation. Protein aggregation was evaluated with the help of two β -amyloid specific dyes – Congo red and Thioflavin T. Though results obtained from these two studies were not correlated, *P. crispum* and *T. chebula* showed maximum reduction in aggregation by both dyes. This difference in results by both dyes may be due to difference in their binding modes to amyloid fibrils. According to Groenning (2010), Thioflavin T binds in channels running parallel to the long axis of the fibril either a monomeric or dimeric form, whereas Congo Red may bind in grooves formed along the β -sheets as a planar molecule in either a monomeric or supramolecular form. Additionally, Thioflavin T fluorescence can be bias as polyphenolics directly interact with Thioflavin T and therefore another method using Congo red is suggested by Hudson et al. (2009). In the present study, this may justify the observed differences among both these aggregation marker's levels in the presence of plant extracts.

Conclusions

Nineteen different plant extracts were evaluated for their antiglycation activity using the albumin-fructose model. Among which *P. crispum*, *B. diffusa* and *T. chebula* had the most potent antiglycation activity. These plant exerted noticeable antiglycation activity at different glycation modifications of albumin. Positive correlation was observed between amyloid versus AGEs formation and carbonyl groups versus amyloid formation and a negative correlation was observed in amyloid and thiol levels. The present study provides valuable information and better understanding of how these medicinal plants inhibit albumin glycation, oxidative modifications and amyloid aggregation. Inclusion of these plants as complementary therapy could minimize albumin modifications and may prevent the pathogenesis of diabetic vascular complications.

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