

Research Article

ANTIGLYCATING ACTIVITY OF *ALOE VERA* GEL EXTRACT AND ITS ACTIVE COMPONENT ALOIN

Hina Younus* and Shehwaz Anwar

Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh 202002, India.

Abstract: Glycation plays an important role in diabetes-associated complications. The damage caused by oxidative stress is exacerbated if the antioxidant enzymes, including superoxide dismutase (SOD), themselves are inactivated by glycation. Natural compounds offer advantage over the synthetic drugs and hence are being increasingly preferred in treating diabetes. *Aloe vera* (*A. vera*) has been reported to have various pharmacological activities. The glycation of SOD by glucose or methylglyoxal (MG) and its protection by *A. vera* extract and its key component aloin has been investigated by activity, SDS-PAGE, ELISA, absorbance, fluorescence and far-UV CD measurements. Glycation of SOD resulted in a decrease in enzyme activity, fragmentation/cross-linking, reduced cross-reactivity with anti-SOD antibodies, structural changes, and formation of advanced glycation end products (AGEs) and fibrils. *A. vera* extract or aloin offered protection against glucose or MG induced glycation of SOD. *A. vera* extract has earlier been reported to have anti-diabetic effects, and this along with its antiglycating effect as has been observed in this study, makes it an effective product against diabetes and its complications. The observed antiglycating potential suggests *A. vera* extract as a promising new pharmaceutical lead or dietary supplement to combat diseases associated with diabetic complications, and provides an explanation for the use of *A. vera* extract in traditional medicine.

Keywords: Superoxide dismutase; Glycation; *Aleo vera*; Aloin.

Note: Coloured Figures are available on Journal Website in "Archives" Section

Introduction

Diabetes mellitus is a multifactorial disease which has great impact on health and life expectancy of affected individuals. Hyperglycemia is considered to be an important driving factor of diabetic complications. Protein glycation consists of non-enzymatic series of reactions between reducing sugars and the amino groups of proteins yielding schiff base which form amadori products and eventually advanced glycation end products (AGEs) (Baynes *et al.*, 1989, Neglia *et al.*, 1983). The

rate of formation of AGEs increases under diabetic conditions and glycation is one of the major contributing pathways responsible for the formation of AGEs (Younus and Anwar, 2016). During glycation, various reactive oxygen species (ROS) as well as reactive carbonyl species such as methyl glyoxal (MG), glyoxal etc are also produced that further react and damage the proteins and other biomolecules (Thornalley *et al.*, 1999). MG immediately interacts with amino, guanidine and thiol groups of proteins, resulting in the formation of AGEs and cross-linking and denaturation of proteins (Lo *et al.*, 1994; McLaughlin *et al.*, 1980). MG induces more protein damage as compared to glucose (Khan *et al.*, 2014), and also has been supposed to bring about oxidative DNA damage, strand breakage, and cell apoptosis (Wu and Chan, 2007).

Corresponding Author: Hina Younus
E-mail: hyounus.cb@amu.ac.in

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Glycation plays a significant role in diabetes, antherosclerosis, chronic renal failure, vascular disease, neurodegenerative diseases and aging (Brownlee, 1995). Superoxide dismutase (SOD), an important antioxidant enzyme in the body which counters the deleterious effects of ROS also gets inactivated by glycation (Jabeen *et al.*, 2006; Khan *et al.*, 2014). Therefore, the damage caused by oxidative stress is expected to be greater if the antioxidant enzymes themselves are inactivated. Control of blood sugar level is a very effective and natural method of protection from glycation and AGEs formation (Younus and Anwar, 2016). Furthermore, strategies that inhibit glycation and hence AGEs formation, have been found to significantly reduce diabetic complications (Yamagishi *et al.*, 2008). A number of antiglycating agents with different mechanisms of inhibition of AGEs formation have been described (Younus and Anwar, 2016). Synthetic anti-glycating agents like aminoguanidine often have adverse side effects (Bolten *et al.*, 2004). Therefore, recently much interest has been developed in the search of natural phytochemicals from plants that effectively inhibit glycation and have fewer side effects (Coman *et al.*, 2012; Khan *et al.*, 2014; Meeprom *et al.*, 2013; Anwar and Younus, 2017a; Anwar and Younus, 2017b). For centuries, many plants and their products have been used for their medicinal and remedial values (Abbas *et al.*, 2016).

Aloe vera (*A. vera*) is *Aloe barbadensis*, a member of the liliaceae family is a perennial succulent xerophytes (Fig. 1A). It has been used as a popular folk medicine throughout history. The thick fleshy leaves of *Aloe* plants contain pulp which is used in the food, pharmaceutical, cosmetic and toiletry industries (Nejatzadeh-Barandozi, 2013). The benefits of *A. vera* that have been observed but are not supported by experimental/clinical data are treatment of acne, anemia, haemorrhoids, glaucoma, petit ulcer, tuberculosis and blindness (Wani *et al.*, 2010). Many reports have shown the antioxidant, anti-inflammatory and antibacterial activities of *A. vera* (Lopez *et al.*, 2013; Nejatzadeh-Barandozi, 2013). Anti-cancer effects of a compound of *A. vera* leaves, aloe-emodin has also been reported (Lin *et al.*, 2010). *A. vera* also has antidiabetic and lipid-lowering properties since oral administration of the extract significantly reduced fasting blood glucose level and improved lipid profile status in streptozotocin-induced diabetic rats (Moniruzzaman *et al.*, 2012; Rajasekaran *et al.*, 2006). The phytochemicals present in *A. vera* are tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides anthraquinones, which have medicinal value (Sathyaprakha *et al.*, 2010). Phenolic compounds are the second major substances in *A. vera*. The main active constituent in *A. vera* extract is aloin (Fig. 1B), an anthraquinone heteroside (Zahn *et al.*, 2008). Antioxidant activity

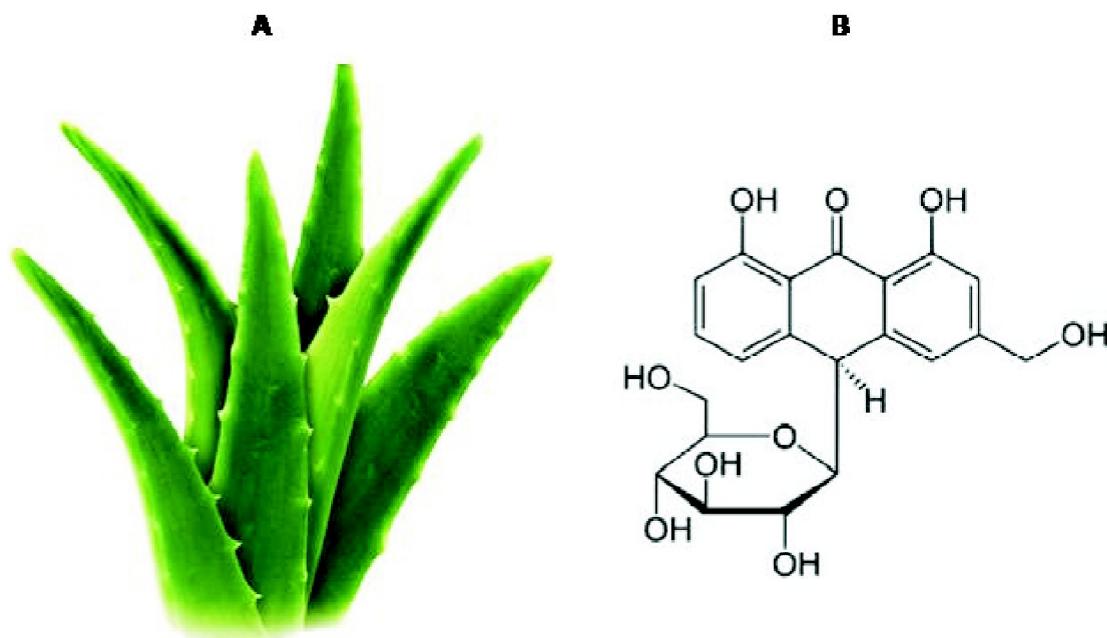


Figure 1: *Aloe vera* plant (A). Chemical structure of aloin (B). Adapted from www.google.com (images)

of aloin has been recently demonstrated (Esmat *et al.*, 2012). Anti-tumour activity of aloin has also been reported (Esmat *et al.*, 2005; Fahim *et al.*, 1997). Its antimicrobial, anti-inflammatory and anticancer activities and positive effects on cardiovascular system, metabolic system as well as on enzyme system have been well documented (Patel and Patel, 2013). However, the potential inhibitory effects of *A. vera* or aloin on protein glycation have not been reported. Therefore, the objective of this study was to determine the anti-glycating potential of *A. vera* extract and its key compound aloin. And the important antioxidant enzyme, SOD was employed for this purpose.

Materials and Methods

Chemicals - Bovine erythrocyte Cu,Zn-SOD (EC 1.15.1.1), MG (40% aqueous solution), aloin, nitro blue tetrazolium (NBT), NADH, phenazine methosulfate (PMS), thioflavin T (ThT), *N,N,N',N'*-tetramethylenediamine (TEMED), bovine serum albumin (BSA), bicinchoninic acid (BCA) and o-phenylenediamine (OPD)/ H_2O_2 were purchased from Sigma, USA. Glucose and sodium dodecyl sulphate (SDS) were the products of Qualigens, India. Acylamide/bisacrylamide, ammonium persulphate and DEAE-cellulose were obtained from SRL, India. Standard molecular weight protein markers (Broad range), Freund's adjuvant and horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG were from Genei, India. All other chemicals used were of analytical grade.

Measurement of SOD concentration and activity - The concentration of SOD was routinely determined by the BCA method using BSA as the standard (Smith *et al.*, 1985). SOD stock (1 mg/ml) was made in 20 mM sodium phosphate buffer, pH 7.4 and stored at -20°C for future use. The activity of SOD was determined spectrophotometrically by employing PMS-NADH-NBT system (Nishikimi *et al.*, 1972). The reaction mixture consisted of 20 mM sodium phosphate buffer (pH 8.2), PMS (1.9 μ M), NBT (184 μ M) and NADH (205 μ M). For assaying, SOD enzyme was pipette into a cuvette at room temperature (25°C) containing freshly prepared NBT and NADH. The reaction was initiated with the addition of freshly prepared PMS and the absorbance at 560 nm was continuously monitored as an index of NBT reduction using a single beam Shimadzu spectrophotometer. Reagent control lacking the enzyme was taken.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) - SDS-PAGE was performed according to the method of Laemmli (1970) (Laemmli *et al.*, 1970) using 15% separating and 5% stacking gels. Protein bands were visualized by overnight staining with 0.1% (w/v) Coomassie Brilliant Blue R250. The gels were destained using 40% (v/v) methanol/10% (v/v) acetic acid.

Enzyme-linked immunosorbent assay (ELISA) - Rabbits were immunized with SOD using Freund's adjuvant, and IgG from the serum of immunized rabbits was purified to homogeneity on DEAE-cellulose matrix after ammonium sulphate precipitation according to the procedure followed in our laboratory (Rehan and Younus, 2006). The cross-reactivity of the antibodies with SOD was determined by ELISA. Ninety six-well microtitre plates (Roll Piove di Sacco, Italy) were coated overnight with 100 μ l of SOD (5 μ g/ml) in 0.05 M carbonate-bicarbonate buffer, pH 9.6 at 4°C. After extensive washing with phosphate buffered saline (PBS)-Tween 20 buffer, 150 μ l of blocking buffer (5% BSA in PBS) was applied to the wells and the plates incubated at 37°C for 2 h. After removal of the blocking buffer 100 μ l of anti-SOD IgG (16 μ g/ml) was added and the binding was allowed to proceed at 37°C for 2 h. The microtitre plates were washed and incubated with 100 μ l of HRP conjugated goat anti-rabbit IgG at 37°C for 1 h. After the usual washing steps, the peroxidise reaction was initiated by the addition of the substrate OPD/ H_2O_2 , arrested by the addition of 1 M H_2SO_4 , and absorbance at 490 nm measured in an ELISA reader.

Preparation of *A. vera* extract - *A. vera* plants were harvested in the month of November from the plant nursery, Aligarh Muslim University, Aligarh, India. The fresh leaves were dissected into two halves and the inner colourless, mucilaginous pulp was homogenized in an electrical blender, centrifuged at 10,000 g at 4°C for 15 min to remove the fibres. The resultant supernatant was filtered, freeze dried at -20°C and lyophilized. To make a stock solution of *A. vera* extract, 30 mg of the lyophilized powder was dissolved in 20 mM sodium phosphate buffer, pH 7.4.

Effect of *A. vera* extract and aloin on the glycation of SOD - The glycation of SOD was induced according to our published procedure (Khan *et al.*, 2014). SOD (0.2 mg/ml) in 20 mM sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl was incubated with 0.5 M glucose or 10 mM MG or

combination of 0.5 M glucose and 10 mM MG for 10 days at 37°C in the presence of 0, 10, 20 and 50 µg/ml of *A. vera* extract or 0, 10, 20 and 50 µM aloin. Aloin was dissolved in DMSO and the final DMSO concentration in the incubation mixture was 1%. The effect of *A. vera* extract and aloin on the glycation of SOD with glucose or MG or a combination of both were assessed by activity, SDS-PAGE, ELISA, absorption, intrinsic fluorescence, AGEs specific fluorescence and thioflavin T (ThT) fluorescence spectroscopic studies. Far-UV circular dichroism (CD) studies were also performed in the presence of *A. vera* extract but not aloin as DMSO in the aloin sample interfered with the measurements in this wavelength range.

Absorption Spectroscopy - Absorbance measurements were carried out on a double beam Perkin Elmer spectrophotometer (Lambda 25). The spectra of SOD (0.2 mg/ml) in absence/presence of glucose or MG and absence/presence of *A. vera* extract or aloin were measured in the wavelength range of 240-500 nm.

Fluorescence Spectroscopy - All fluorescence measurements were carried out on a Shimadzu spectrofluorometer (model RF-5301PC). The intrinsic fluorescence of SOD (0.2 mg/ml) incubated alone, with glucose or with MG in the absence/presence of *A. vera* extract or aloin was monitored with excitation at 280 nm and emission in the range 290-400 nm. The slit widths were 5 nm for both excitation and emission. The formation of fluorescent AGE products was monitored with excitation at 350 nm and emission in the range 400-480 nm. The slit widths were 3 nm for both excitation and emission. The fibrillar state of incubated SOD was determined via ThT, a reagent used for detecting the β-sheet configuration in proteins (Schmitt *et al.*, 2005). The fluorescence of the above incubation mixtures was monitored after adding 6 µM ThT reagent at excitation wavelength of 440 nm and the emission was measured in the range 450-600 nm. The slit widths were 10 nm for both excitation and emission.

CD Spectroscopy - Far-UV CD measurements were carried out with a Jasco spectropolarimeter (J-815) equipped with a Jasco Peltier-type temperature controller (PTC-424S/15). The instrument was calibrated with D-10-camphorsulphonic acid. The spectra were collected in a cell of 0.1 cm with scan speed of 100 nm/min and response time of 1 sec. Each spectrum was the average of 2 scans.

Results and Discussion

In vitro glycation of SOD

SOD is possibly the most important antioxidant enzyme that is specifically involved in the detoxification of superoxide radicals, enabling cells to cope with lethal oxidative environments. SOD itself has been shown to undergo glycation by reducing sugars and MG and hence inactivation (Jabeen *et al.*, 2006; Jabeen and Saleemuddin, 2006; Khan *et al.*, 2014). We have recently reported that glycation of SOD by glucose results in the degradation of the enzyme into small peptides, while by MG high molecular weight cross-linked aggregates of the enzyme are formed (Khan *et al.*, 2014). MG is a highly reactive α-oxoaldehyde that plays an important role in glycation reactions, formation of AGEs and other complications associated with hyperglycemia and related disorders (Jabeen *et al.*, 2006). The purpose of the present study was to evaluate the possible protective effect of *A. vera* extract and its active compound aloin on the glycation of SOD. The glycation of SOD was performed with high non-physiological concentration of glucose (0.5 M) or MG (10 mM) which may serve as an appropriate model for the long-term effects of glucose or MG on the enzyme (Coussons *et al.*, 1997; Jabeen *et al.*, 2006).

Effect of A. vera extract and aloin on the activity and fragmentation/aggregation of SOD

The effect of *A. vera* extract and aloin on the activity of SOD glycated by 0.5 M glucose or 10 mM MG was studied. Fig. 2 shows the remaining activity of SOD incubated for ten days at 37°C alone, and that incubated in the presence of glucose, MG or a combination of both and with increasing concentration of *A. vera* extract (A) or aloin (B). SOD incubated for ten days alone with *A. vera* extract or aloin showed a slight increase in activity with increasing *A. vera* extract or aloin concentration (Fig. 2 Panel 1). The activity increased by 4.4 and 5.4% when the enzyme was incubated with 50 µg of *A. vera* extract or 50 µM aloin, respectively as compared to the control (the sample that had no *A. vera* extract or aloin). This increase in activity of SOD is believed to be due the antioxidant property of *A. vera* extract and aloin. SOD incubated with glucose, MG or a combination of both, and *A. vera* extract or aloin showed a greater increase in activity as compared to the enzyme that was not incubated with glucose or MG. The activity increased by 10.0,

7.4 and 7.0% as compared to the control when the enzyme was incubated with glucose (Fig. 2A Panel 2), MG (Fig. 2A Panel 3) or a combination of both glucose and MG (Fig. 2A Panel 4), respectively, and 50 µg *A. vera* extract. The activity increased by 14.6, 10.1 and 9.8% as compared to the control when the enzyme was incubated with glucose (Fig. 2B Panel 2), MG (Fig. 2B Panel 3) or a combination of both glucose and MG (Fig. 2B Panel 4), respectively, and 50 µM aloin. This observed further increase in activity is believed to be due to the antiglycating activity of *A. vera* extract and aloin. The increase in

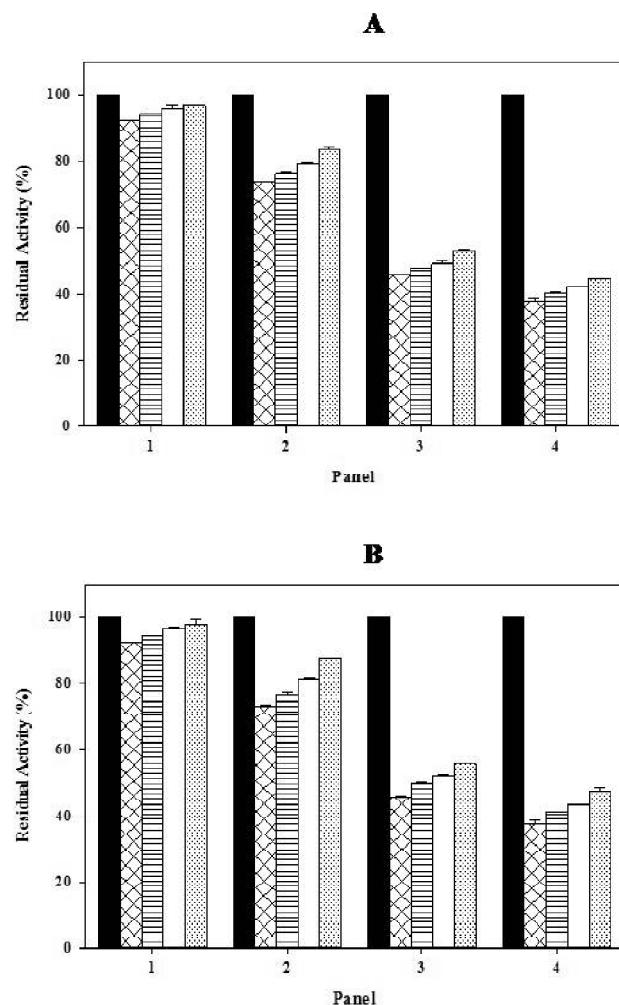


Figure 2: Effect of *A. vera* extract or aloin on the activity of SOD incubated with glucose, MG or both. SOD (0.2 mg/ml) was incubated alone (Panel 1), with 0.5 M glucose (Panel 2) or 10 mM MG (Panel 3) or combination of 0.5 M glucose and 10 mM MG (Panel 4) for 10 days at 37°C in the presence of 0 (▨), 10 (▨), 20 (□) and 50 (▨) µg/ml of *A. vera* extract (A) or 0.2 µM aloin (B). Each Panel also shows native SOD (0.2 mg/ml) alone in buffer that was not incubated at 37°C for any time period (■). The enzyme activity was then determined under standard assay conditions. Each value represents the average for three independent experiments performed in duplicates

activity was more in the case of glucose than for MG or both MG and glucose. Therefore, it appears that *A. vera* extract and aloin is a more effective antiglycating agent for sugars/compounds that are milder glycating agents.

The protective effect of *A. vera* extract and aloin on SOD fragmentation/cross-linking induced by glycation is seen in Fig. 3 and 4, respectively. SDS-PAGE of SOD incubated for ten days in the absence of glucose or MG showed same staining intensity with increasing *A. vera* extract or aloin concentration (Fig. 3A and 4A, respectively). However, the enzyme incubated for ten days with glucose showed a more increase in staining intensity with increasing *A. vera* extract or aloin concentration (Fig. 3B and 4B, respectively). SOD incubated for ten days with MG (Fig. 3C and 4C) or a combination of both glucose and MG (Fig. 3D and 4D) exhibited a decrease in the bands corresponding to the cross-linked aggregates with increasing *A. vera* extract (Fig. 3C and 3D) or aloin (Fig. 4C and 4D) concentration. Infact in the case of MG alone, there was a slight increase in the band corresponding to the native enzyme with increasing *A. vera* extract or aloin concentration.

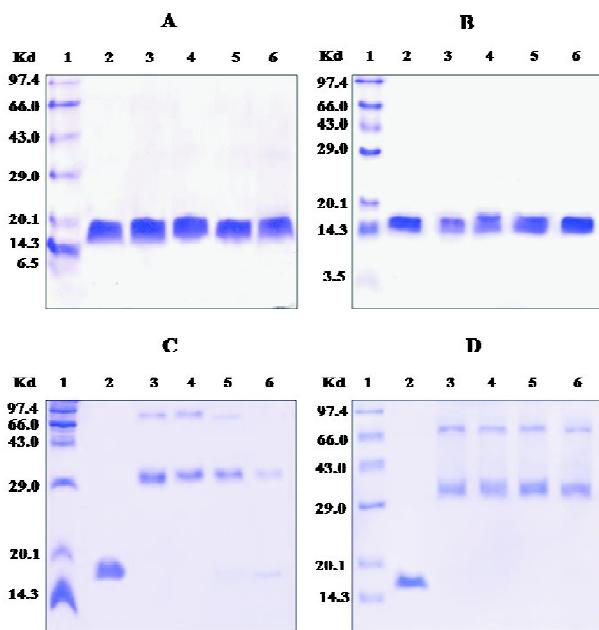


Figure 3: SDS-PAGE of SOD incubated alone (A), with 0.5 M glucose (B), with 10 mM MG (C) or a combination of 0.5 M glucose and 10 mM MG (D) and with varying concentration of *A. vera* extract for 10 days at 37°C. Lane 1 shows molecular weight markers (Genei); Lane 2 shows SOD (10 µg) that has not been incubated with glucose, MG or *A. vera* extract. Lanes 3, 4, 5 and 6 show SOD (10 µg) incubated for 10 days alone or with glucose, MG or a combination of glucose and MG and with 0, 10, 20 and 50 µg/ml *A. vera* extract, respectively

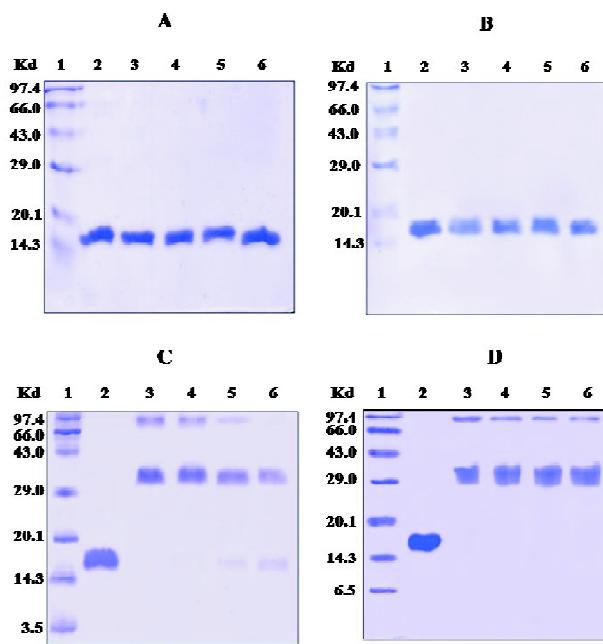


Figure 4: SDS-PAGE of SOD incubated alone (A), with 0.5 M glucose (B), with 10 mM MG (C) or a combination of 0.5 M glucose and 10 mM MG (D) and with varying concentration of aloin for 10 days at 37°C. Lane 1 shows molecular weight markers (Genei); Lane 2 shows SOD (10 µg) that has not been incubated with glucose, MG or aloin. Lanes 3, 4, 5 and 6 show SOD (10 µg) incubated for 10 days alone or with glucose, MG or a combination of glucose and MG and with 0, 10, 20 and 50 µM aloin, respectively

Therefore, it is evident from SDS-PAGE analysis that *A. vera* extract and aloin protected SOD against fragmentation/cross-linking induced by glycation.

Effect of *A. vera* extract and aloin on the cross-reactivity of anti-SOD antibodies with SOD

Incubation of SOD alone or with glucose, MG and both glucose and MG results in a decrease in absorbance at 490 nm in ELISA indicating reduced cross-reactivity with anti-SOD antibodies, which we believe is due to the structural/chemical modification of the epitopes of enzyme due to incubation at 37°C and by glycation. When SOD is incubated for 10 days at 37°C alone or with glucose, MG or both glucose and MG and increasing concentration of *A. vera* extract (Fig. 5A Panel 1, 2, 3 and 4, respectively) or aloin (Fig. 5B Panel 1, 2, 3 and 4, respectively), a progressive increase in absorbance at 490 nm (cross-reactivity with anti-SOD antibodies) with increasing *A. vera* extract or aloin concentration was observed in all the four cases. Therefore, this experiment shows that *A. vera* extract or aloin protected the enzyme to some extent against the structural/chemical changes induced by

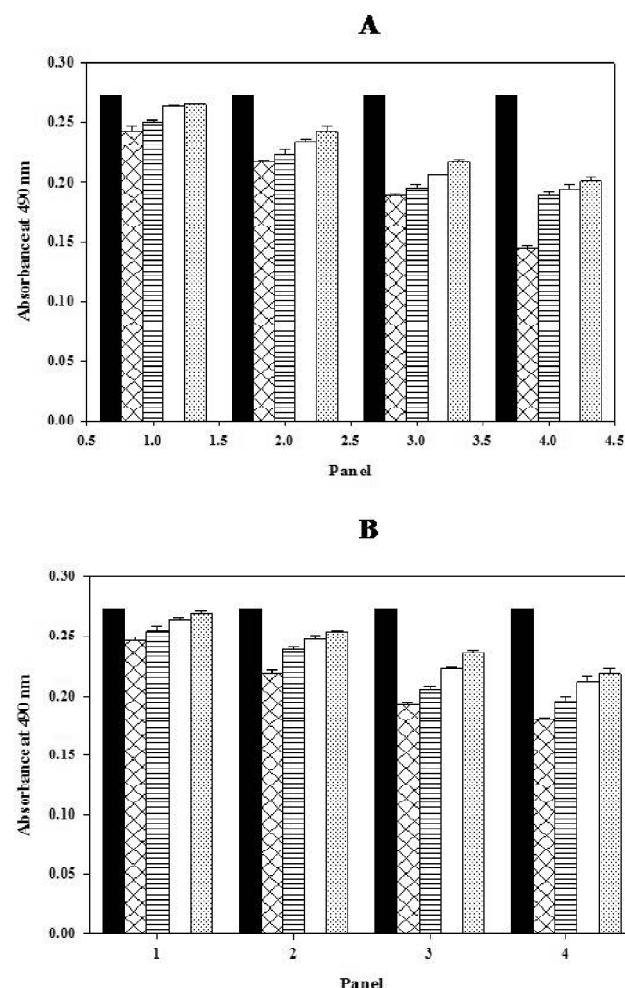


Figure 5: Effect of *A. vera* extract or aloin on the cross-reactivity (ELISA) of anti-SOD antibodies with SOD incubated with glucose, MG or both. SOD (0.2 mg/ml) was incubated alone (Panel 1), with 0.5 M glucose (Panel 2) or 10 mM MG (Panel 3) or combination of 0.5 M glucose and 10 mM MG (Panel 4) for 10 days at 37°C in the presence of 0 (▨), 10 (▨), 20 (□) and 50 (▨) µg/ml of *A. vera* extract (A) or µM aloin (B). Each Panel also shows native SOD (0.2 mg/ml) alone in buffer that was not incubated at 37°C for any time period (■). Each value represents the average for two independent experiments performed in triplicates

incubation at 37°C and glycation with glucose, MG or a combination of both, and the protection increased with increasing concentration of *A. vera* extract or aloin.

Effect of *A. vera* extract and aloin on the conformation of SOD

Bovine erythrocyte Cu,Zn-SOD is a homodimer (Hough *et al.*, 2000) and lacks tryptophan residues and has one tyrosine residue per subunit. Glycation of SOD by glucose, MG and both glucose and MG results in hyperchromicity (structural changes).

When SOD is incubated for 10 days at 37°C with glucose, MG or both glucose and MG and increasing concentration of *A. vera* extract or aloin, a progressive decrease in absorbance at 280 nm with increasing *A. vera* extract (Fig. 6A Panel 2, 3 and 4, respectively) or aloin (Fig. 6B Panel 2, 3 and 4, respectively) concentration was observed in all the three cases. SOD incubated alone for 10 days with increasing *A. vera* extract (Fig. 6A Panel 1) or aloin (Fig. 6B Panel 1) concentration (control) exhibited very slight decrease in absorbance at 280 nm. Therefore, *A. vera* extract or aloin protected the enzyme to some extent against the structural

changes induced by glycation with glucose, MG or a combination of both, and the protection increased with increasing concentration of *A. vera* extract or aloin. However, the enzyme was still far from the structure of the native enzyme even at 50 µg or 50 µM concentration of *A. vera* extract or aloin, respectively, in all the three cases.

Total intrinsic fluorescence of native and glycated SOD was measured by exciting at 280 nm. Native SOD gave an emission peak at 310 nm. Glycation of SOD by glucose, MG and both glucose and MG results in intrinsic fluorescence quenching (structural changes). When SOD is incubated for 10 days at 37°C with glucose, MG or both glucose and MG and increasing concentration of *A. vera* extract (Fig. 7A Panel 2, 3 and 4, respectively) or aloin (Fig. 7B Panel 2, 3 and 4, respectively), a progressive increase in fluorescence at 310 nm with increasing *A. vera* extract or aloin concentration was observed in all the three cases. The control exhibited insignificant increase in fluorescence at 310 nm (Fig. 7A and 7B Panel 1). Therefore, again this experiment shows that *A. vera* extract or aloin protected the enzyme to some extent against the structural changes induced by glycation with glucose, MG or a combination of both, and the protection increased with increasing concentration of *A. vera* extract or aloin. However, it was observed that SOD incubated for 10 days at 37°C with glucose, without *A. vera* extract or aloin but with 1% DMSO showed fluorescence enhancement at 310 nm and not quenching (Fig. 7A and 7B Panel 2 Column 2). Therefore, it appears that in the samples of SOD glycated by glucose in the presence of DMSO, the environment around the aromatic residues of the protein is somehow perturbed which affects their fluorescence.

Far-UV CD studies in the 200-250 nm wavelength range were performed to measure the changes in the secondary structure of SOD upon glycation. Native SOD gave a negative peak at 208 nm. Glycation of SOD by glucose, MG and both glucose and MG results in decrease in the negative ellipticity (secondary structural changes). When SOD is incubated for 10 days at 37°C alone (control) or with glucose, MG or both glucose and MG and increasing concentration of *A. vera* extract, a progressive increase in the negative ellipticity with increasing *A. vera* extract concentration was observed in all the four cases (Fig. 8 Panel 1, 2, 3 and 4, respectively). Therefore, *A. vera* extract protected the enzyme to some extent against the

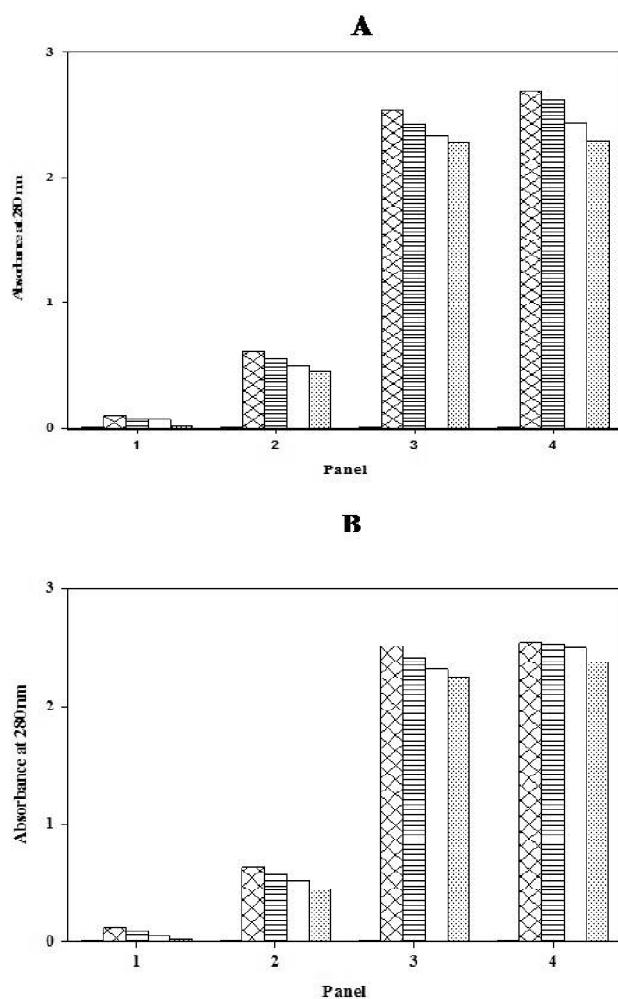


Figure 6: Effect of *A. vera* extract or aloin on the absorption changes induced in SOD due to glycation. Absorbance at 280 nm for samples of SOD (0.2 mg/ml) that were incubated in 20 mM sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl alone (Panel 1) and with 0.5 M glucose (Panel 2) or 10 mM MG (Panel 3) or a combination of 0.5 M glucose and 10 mM MG (Panel 4) for 10 days at 37°C in the presence of 0 (#), 10 (=), 20 (□) and 50 (▨) µg/ml of *A. vera* extract (A) or µM aloin (B). Each Panel also shows native SOD alone in buffer that was not incubated at 37°C for any time period (■).

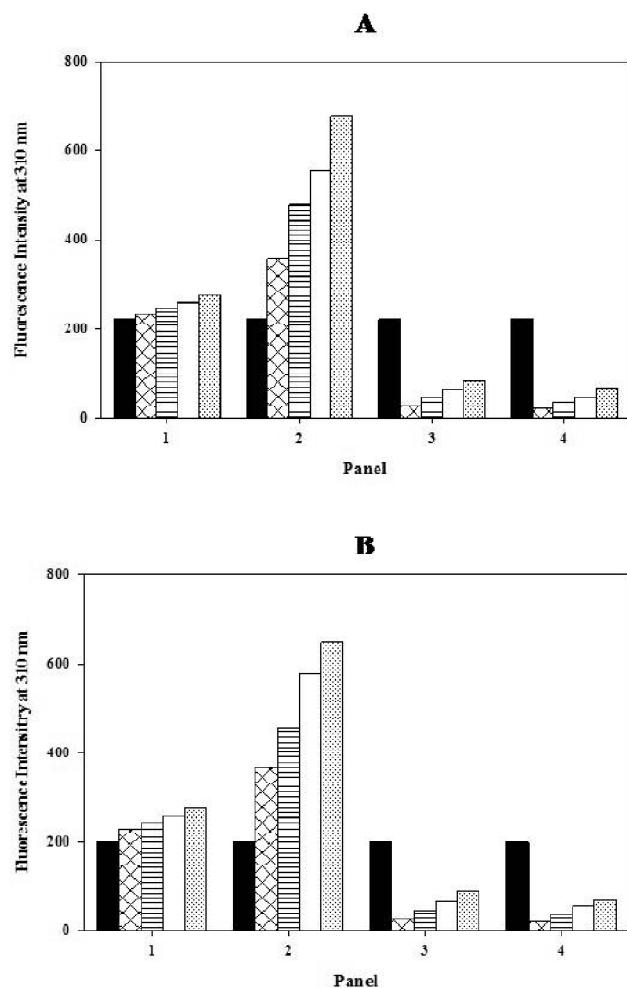


Figure 7: Effect of *A. vera* extract or aloin on the intrinsic fluorescence changes induced in SOD due to glycation. Fluorescence intensity at the excitation/emission wavelengths of 280/310 nm for samples of SOD (0.2 mg/ml) that were incubated in 20 mM sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl alone (Panel 1) and with 0.5 M glucose (Panel 2) or 10 mM MG (Panel 3) or a combination of 0.5 M glucose and 10 mM MG (Panel 4) for 10 days at 37°C in the presence of 0 (#), 10 (=), 20 (□) and 50 (▨) µg/ml of *A. vera* extract (A) or µM aloin (B). Each Panel also shows native SOD alone in buffer that was not incubated at 37°C for any time period (■)

secondary structural changes induced by incubation at 37°C and glycation with glucose, MG or a combination of both, and the protection increased with increasing concentration of *A. vera* extract.

Effect of *A. vera* extract and aloin on the formation of fluorescent AGEs

We have followed the formation of AGEs in the samples via autofluorescence (Schmitt *et al.*, 2005). Fluorescent AGEs specific fluorescence of native and glycated SOD was measured by exciting at 350

nm. SOD incubated with glucose, MG or a combination of both glucose and MG showed AGEs specific fluorescence in the wavelength range 400-480 nm. The spectra of fluorescence intensity versus wavelength (400-480 nm) were rather broad (data not shown), and this probably reflects the presence of a number of different fluorescent compounds being formed during glycation. When SOD is incubated for 10 days at 37°C with glucose, MG or both glucose and MG and increasing concentration of *A. vera* extract (Fig. 9A Panel 2, 3 and 4, respectively) or aloin (Fig. 9B Panel 2, 3 and 4, respectively), a progressive decrease in AGEs specific fluorescence at 450 nm with increasing *A. vera* extract or aloin concentration was observed in all the three cases. The control exhibited insignificant decrease in fluorescence at 450 nm (Fig. 9A and 9B Panel 1). Therefore, *A. vera* extract or aloin protected the enzyme to some extent against formation of AGEs induced by glycation with glucose, MG or a combination of both, and the protection increased with increasing concentration of *A. vera* extract or aloin.

Effect of *A. vera* extract and aloin on the formation of glycation induced fibrils

ThT is a dye that interacts with the fibrillar structure of proteins, upon interaction its fluorescence intensifies, while in its free form is only weakly fluorescent. This quality has been employed in the

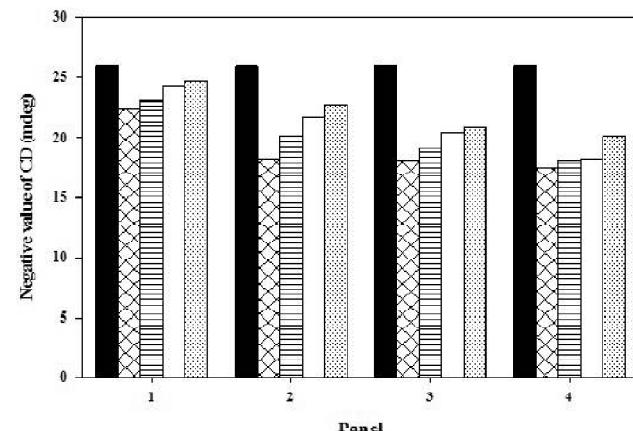


Figure 8: Effect of *A. vera* extract on the far-UV CD changes induced in SOD due to glycation. CD at 208 nm for samples of SOD (0.2 mg/ml) that were incubated in 20 mM sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl alone (Panel 1) and with 0.5 M glucose (Panel 2) or 10 mM MG (Panel 3) or a combination of 0.5 M glucose and 10 mM MG (Panel 4) for 10 days at 37°C in the presence of 0 (#), 10 (=), 20 (□) and 50 (▨) µg/ml of *A. vera* extract. Each Panel also shows native SOD alone in buffer that was not incubated at 37°C for any time period (■)

detection of amyloid fibril structures in proteins (Schmitt *et al.*, 2005). Glycation of SOD by glucose, MG and both glucose and MG results in ThT fluorescence enhancement at 480 nm (formation of fibrils). When SOD is incubated for 10 days at 37°C with glucose, MG or both glucose and MG and increasing concentration of *A. vera* extract (Fig. 10A Panel 2, 3 and 4, respectively) or aloin (Fig. 10B Panel 2, 3 and 4, respectively), a progressive decrease in ThT fluorescence with increasing *A. vera* extract or aloin concentration was observed in all the three cases. The control exhibited insignificant

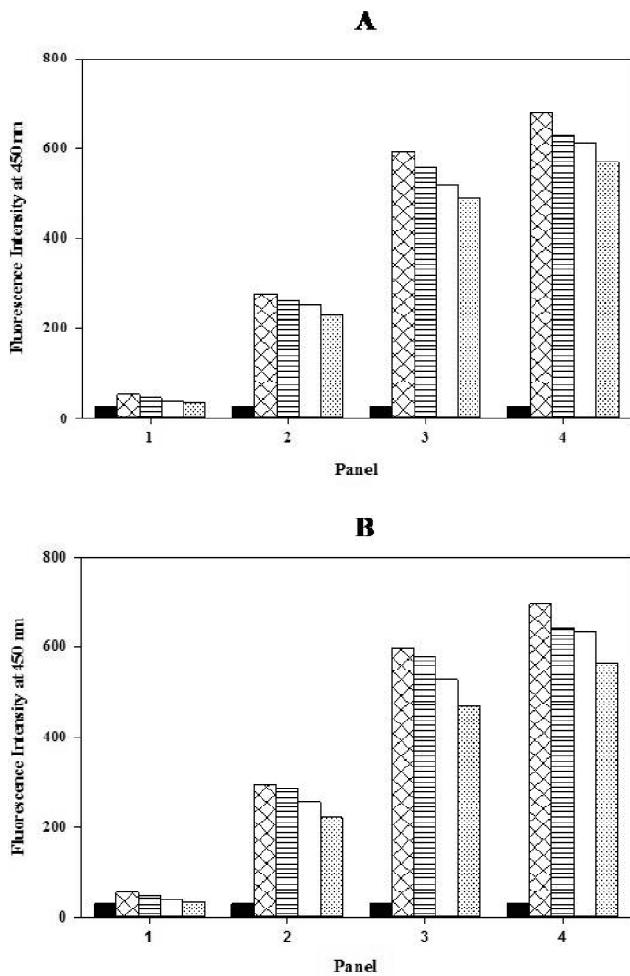


Figure 9: Effect of *A. vera* extract or aloin on the fluorescent AGEs formed of SOD due to glycation. AGEs specific fluorescence intensity at the excitation/emission wavelengths of 350/450 nm for samples of SOD (0.2 mg/ml) that were incubated in 20 mM sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl alone (Panel 1) and with 0.5 M glucose (Panel 2) or 10 mM MG (Panel 3) or a combination of 0.5 M glucose and 10 mM MG (Panel 4) for 10 days at 37°C in the presence of 0 (#), 10 (=), 20 (□) and 50 (●) µg/ml of *A. vera* extract (A) or µM aloin (B). Each Panel also shows native SOD alone in buffer that was not incubated at 37°C for any time period (■).

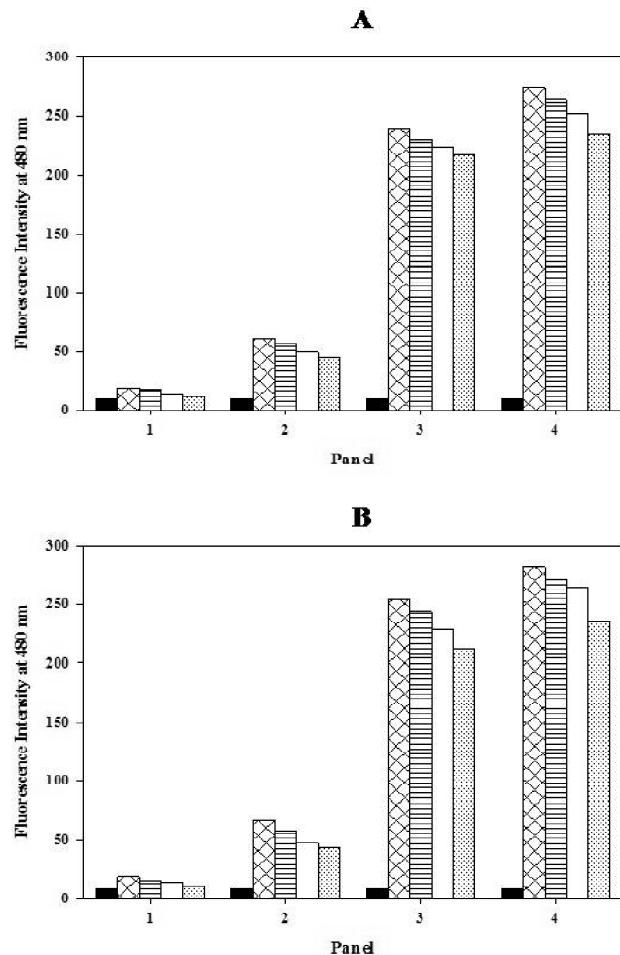


Figure 10: Effect of *A. vera* extract or aloin on the fibrils formed in SOD due to glycation. ThT fluorescence intensity at the excitation/emission wavelengths of 440/480 nm for samples of SOD (0.2 mg/ml) that were incubated in 20 mM sodium phosphate buffer, pH 7.4 containing 0.15 M NaCl alone (Panel 1) and with 0.5 M glucose (Panel 2) or 10 mM MG (Panel 3) or a combination of 0.5 M glucose and 10 mM MG (Panel 4) for 10 days at 37°C in the presence of 0 (#), 10 (=), 20 (□) and 50 (●) µg/ml of *A. vera* extract (A) or µM aloin (B). Each Panel also shows native SOD alone in buffer that was not incubated at 37°C for any time period (■).

decrease in ThT fluorescence at 480 nm (Fig. 10A and 10B Panel 1). Therefore, *A. vera* extract or aloin protected the enzyme to some extent against formation of fibrils induced by glycation with glucose, MG or a combination of both, and the protection increased with increasing concentration of *A. vera* extract or aloin.

Conclusion

This study shows that *A. vera* extract and its active principle component aloin, which have previously reported to have many beneficial pharmacological activities, also have antiglycating activity. *A. vera*

extract has also been reported to have anti-diabetic and lipid-lowering effects (Moniruzzaman *et al.*, 2012; Rajasekaran *et al.*, 2006). This taken together with the antiglycating effect of *A. vera* extract and aloin as observed in this study makes them effective anti-diabetic products which can be used in treating diabetes and its complications. However, more investigations are needed to know their mechanism of inhibition of glycation, as well as their potential against diabetic complications in animal models.

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Conflict of Interest

The authors have no conflict of interest concerning this work.

Abbreviations

AGEs, advanced glycation end products; *A. vera*, *Aleo vera*; BCA, bicinchoninic acid; BSA, bovine serum albumin; CD, circular dichroism; ELISA, Enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; MG, methylglyoxal; NBT, nitro blue tetrazolium; OPD, o-phenylenediamine; PMS, phenazine methosulfate; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SOD, superoxide dismutase; ThT, thioflavin T.

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