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ORIGINAL PAPER

Syzygium jambolanum and *Cephalandra indica* homeopathic preparations inhibit albumin glycation and protect erythrocytes: an *in vitro* study

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Background: **Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia eventually resulting in long-term complications. Increased glycation of proteins is implicated in the pathogenesis of complications. For treatment of diabetes, *Syzygium jambolanum* and *Cephalandra indica* are frequently prescribed in homeopathy. However their role in glycation is not well elucidated. The present study aimed to evaluate the role of these homeopathic preparations in glycation induced structural modifications and further to examine their cellular protection ability.**
Methods: **In human erythrocytes, *in vitro* mother tincture and dilutions of *S. jambolanum* (*Sj* ϕ, *30c, 200c*), *C. indica* (*Ci* ϕ, *30c, 200c*) and standard antiglycator (AG) were compared and their antiglycation potential assessed by the estimating different markers of glycation (frcutosamines, carbonyls, bound sugar), structural modifications (free amino and thiol group). Phytochemical characterization (total phenolic, flavonoids and glycosides contents) was performed.**
Results: **The homeopathic preparations have different mode of action on albumin glycation modifications. *Sj* ϕ preparation demonstrated effective inhibition of all glycation, structural modifications except amino group protection. When dilutions were compared, *Sj* preparations showed reduction of glycation, structural modifications. All preparations showed significant erythrocyte protection. *Sj* ϕ preparation exhibited noteworthy antiglycation and cell protection ability as compared to AG.**
Conclusion: **These homeopathic preparations especially *Sj* ϕ prevented glycation induced albumin modifications and subsequent toxicity in human eryrthrocytre *in vitro*. Further investigation of their potential as antiglycators is justified.** *Homeopathy* (2015) **104**, 197–204.

Keywords: Glycation; Human erythrocytes; Homeopathic preparations; *Syzygium jambolanum*; *Cephalandra indica*

Introduction

Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, nerves, blood vessels, skin, and kidneys. The prevalence of diabetes amongst all age-

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groups worldwide is estimated to increase from 2.8% in 2000 to 4.4% in 2030.[1] Increased glycation of proteins and accumulation of advanced glycation end products (AGEs) have been implicated in the pathogenesis of diabetic complications. Glycation and AGE formation are also accompanied by formation of free radicals via auto-oxidation of glucose and glycated proteins. Further, AGEs bind to their cell surface signaling receptor i.e. receptor of AGE causes a diverse set of consequences like generation of oxidative stress, vascular dysfunction and inflammation – all of which synergize to trigger diabetic complications.[2] Hyperglycemia-induced increase in oxidative stress, leads to changes in erythrocyte structure and function. This results in decreased levels of antioxidant enzymes and increased fragility due to lipid peroxidation.[3]

Currently available allopathic therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and glinides.[4] Due to the side effects associated with these oral drugs there is growing interest in herbal remedies. Several species of herbal drugs having antidiabetic activity are described in the scientific and popular literature.[5] Due to their perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are being used increasingly.[6] Biological actions of the plant products used as alternative medicines to treat diabetes are related to their chemical composition. Compounds with combined antiglycation and antioxidant properties have high therapeutic potential. Herbal products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which show reduction in blood glucose levels, insulin resistance and lipid peroxidation, increase in plasma insulin level, hexokinase activity, anti-inflammatory and antioxidant activity.[7]

Homeopathy is a therapeutic system that has been in use for more than 200 years. It is estimated that the number of patients using homeopathy in United States increased by 500% between 1990 and 1997.[8] Homeopathy is a holistic method of treatment that uses small doses of natural substances originating from plants, minerals, or animal parts. *Syzygium jambolanum* (Jamun) and *Cephalandra indica* (Ivy gourd) in herbal form have been investigated for antidiabetic effects both in preclinical and human studies.[9,10] Homeopathic preparations of these are used to treat patients with diabetes related symptoms. *S. jambolanum* and *C. indica* are officially covered by Homeopathic pharmacopoeia of India.[11,12]

As the molecular mechanism of action of these preparations are not known, an attempt was made in the present study to investigate the antiglycation potential of *S. jambolanum* and *C. indica* and their possible protective role in glycated albumin mediated toxicity in human erythrocytes. Various glycation markers i.e. fructosamines, protein carbonyls, protein bound sugar and structural modifications such as free amino and thiol groups are estimated. In cellular studies, hemolysis and intracellular antioxidant activity were examined.

Materials and methods

Chemicals

Aminoguanidine (AG), Bovine serum albumin (BSA) [fraction V], nitro blue tetrazolium (NBT), Di-nitro Phenyl Hydrazine (DNPH), and 2, 2-azobis-(2-methylpropiona midine) dihydrochloride (AAPH) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Dimethyl sulphoxide (DMSO), 2,3,5-triphenyltetrazolium chloride (TPTZ), p-Benzoquinone, Urea, Trichloroacetic acid (TCA), Dithiobis Nitrobenzoic acid (DTNB) and all other chemicals of analytical grade were obtained from other commercial sources.

Procurement of homeopathic preparations

The homeopathic preparations, namely the mother tinctures of *C. indica* (*Ci* ϕ) and *S. jambolanum* (*Sj* ϕ), along with their liquid dilutions viz., *C. indica 200* (*Ci 200c*), *S. jambolanum 30* (*Sj 30c*) and *S. jambolanum 200* (*Sj 200c*) were obtained from SBL, India and *C. indica 30* (*Ci 30*c) was obtained from Dr. Willmar Schwabe India Pvt. Ltd.

In vitro albumin glycation

Albumin glycation was performed as per the method of McPherson *et al.*[13] with some modifications. Fatty acid-free BSA, 1 ml, (10 mg/ml), was incubated with D-fructose, 1 ml, (250 mM), in phosphate buffer saline (PBS), 1 ml, (200 mM, pH 7.4 containing 0.02% sodium azide) along with homeopathic preparations, 1 ml. BSA and fructose solutions were prepared in PBS and were filter sterilized using 0.22 μm membrane filters under aseptic condition. AG was used as standard inhibitor of glycation. Appropriate controls i.e. positive (BSA + Fructose+ homeopathic diluent), negative (BSA + diluent) and standard inhibitor (AG + diluent) were prepared and maintained under similar conditions. All the reactions were performed in triplicates and reaction tubes were incubated at 37°C for 5 days. After incubation, unbound fructose was removed by dialysis against distilled water and the glycated protein samples were stored at 4°C for further analysis.

Determination of glycation markers

Estimation of fructosamine: The concentration of frutosamine, the amadori product in glycated albumin samples and controls, was determined by using NBT assay as described by Baker *et al.*[14] NBT solution (0.75 mM) was prepared in carbonate buffer (0.1 M, pH 10.35). Glycated samples, 40 μl, were incubated with NBT solution, 0.8 ml, at 37°C for 30 min. The absorbance was measured at 530 nm and the % inhibition of fructosamine by homeopathic preparations was calculated using the following equation:

Inhibitory activity (%) = $[(A_0 - A_1)/A_0] \times 100$, Where A_0 is the absorbance value of the positive control at 530 nm and A_1 is absorbance of the glycated albumin samples co-incubated with homeopathic preparations at 530 nm.

Estimation of protein-carbonyls: Protein carbonyls were estimated according to Uchida *et al.*[15] Glycated protein