Original Article

Advanced glycation end product inhibitory assay- 'Achyranthes Aspera leaf'

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ABSTRACT

Introduction: Achyranthes aspera leaf is a species of plant in the Amaranthaceae family. It is used as an Ayurvedic herb to make a special medicine that is used extensively in surgical procedures to treat fistula, and as oral medicine for obesity, tumours etc. Materials and Methods: Advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. The formulation was evaluated for its activity on AGEs formation. Amino guanidine was used as positive control. The percentage activity was calculated with respect to solvent control. Result: AGEs accumulation in body tissues has been implicated in the pathogenesis of diabetes associated complications which is attributed to the formation of free radicals via auto oxidation of glucose and glycated proteins. In our study, Achyranthes has potentially inhibited the AGEs formation with an IC_{50} of 61.06µg/mL relative to standard amino guanidine which showed IC50 of 72.66µg/ml. Conclusion: According to the results obtained, we know that Achyranthes had potentially inhibited the AGEs formation and it can be used in the synthesis of drugs for diabetic patients. AGEs play an important role in the pathogenesis of diabetic complications during long standing hyperglycaemia state in diabetes mellitus protein glycation and formation of advanced glycation end products.

Keywords: Advanced glycation end-products, diabetic complications, achyranthes aspera, hyperglycaemia, proteins.

Introduction

Achyranthes aspera leaf is a species of plant in the Amaranthaceae family. It is used as an Ayurvedic herb to make a special medicine that is used extensively in surgical procedures to treat fistula, and as oral medicine for obesity, tumours etc. Yunani practitioners and Kabirajes use different parts of the plant to treat leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, renal and cardiac dropsy, kidney stone, diabetes, dermatological disorders, gynecological disorders, gonorrhea, malaria, pneumonia, fever, cough, pyorrhea, dysentery, rabies, hysteria, toothache etc. [1, 2]. The weed is found in many found in many other countries of Asia as well as several other places around the world [3, 4]. Diabetic complications appear to be multifactorial in origin, but in particular, the biochemical process of advanced glycation, which is accelerated in diabetes as a result of chronic hyperglycaemia and increased oxidative stress, has been postulated to play a central role in these disorders [5]. AGEs are a complex group of compounds formed via a nonenzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids. The major AGEs in vivo appear to be formed from highly reactive intermediate carbonyl groups, known as α-dicarbonyls or oxoaldehydes, including 3-deoxyglucosone, glyoxal, and methylglyoxal [6, 7]. AGEs often accumulate intracellularly [8] as a result of their generation from glucose-derived dicarbonyl precursors [6]. It is likely that these intracellular AGEs play important roles as stimuli for activating intracellular signaling pathways as well as modifying the function of intracellular proteins [6, 9].

Diabetes and its complications are rapidly becoming the world's most significant cause of morbidity and mortality [10, 11]. Protein glycation and formation of advanced glycation end products play an important role in the pathogenesis of diabetic complications like retinopathy, nephropathy, neuropathy, cardiomyopathy along with some other diseases such as rheumatoid arthritis, osteoporosis and aging [12]. Retinopathy is a serious

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microvascular complication of diabetes and is the leading cause of blindness in individuals between the ages of 30 and 70 years [13, 14]. AGEs play an important role in the progression of diabetic retinopathy, and leads to dysfunction and death of various retinal cells [15]. Diabetic nephropathy is defined as a progressive decline in glomerular filtration rate, accompanied by proteinuria and other end-organ complications such as retinopathy [16]. It is suggested that AGEs play an important role in the pathogenesis of diabetic nephropathy through interacting with RAGE, which activate a series of intracellular signalling pathways [17]. Experimental studies have shown that the pharmacological interventions capable of interfering with AGEs produce beneficial effects in various diabetic complications [12].

This study investigates Advanced glycation end product by conducting an inhibitory assay on Achyranthes aspera extract. During long standing hyperglycaemia state in diabetes mellitus protein glycation and formation of advanced glycation end products (AGEs) play an important role in the pathogenesis of diabetic complications.

Materials and Methods

Plant Material

Achyranthes aspera extract used for the study were obtained from Green Chem Herbal extract and Formulations, Bengaluru as a gift sample.

Chemicals

Albumin, sodium phosphate, sodium benzoate, fructose, glucose, methanol and amino guanidine were purchased from Sigma-Aldrich. Bovineserum was purchased from GIBCO/BRL Invitrogen.

Inhibition of Advanced Glycation End (AGE) products formation

Advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. The formulation was evaluated for its activity on AGEs formation. AGE reaction solution was constituted as follows; 10mg/mL bovine serum, albumin in 50mM, sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2 M fructose and 0.2 M glucose. The reaction mixture (2.75 mL) was treated with Achyranthes extract (10, 20, 40, 60, 80, 100, 120, 140ug/ml in methanol). Amino guanidine was used as positive control. After incubating at 37oC for 7 days, the fluorescence intensity of the reaction was determined at excitation and emission wavelengths of 350 nm and 450 nm, respectively, using a multi-mode reader (PerkinElmer Enspire, USA). The percentage activity was calculated with respect to solvent control.

Results and Discussion

AGEs accumulation in body tissues has been implicated in the pathogenesis of diabetes associated complications which is attributed to the formation of free radicals via auto oxidation of glucose and glycated proteins. In our study, Achyranthes has potentially inhibited the AGEs formation with an IC_{50} of 61.06µg/mL relative to standard amino guanidine which showed IC_{50} of 72.66µg/ml. A graph plotting the percentage of inhibition of AGEs for different concentrations of Achyranthes aspera and for the standard amino guanidine is shown in Fig.1.

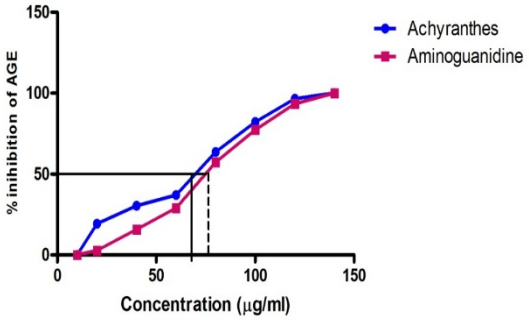

Figure 1. The graph shows the percentage of the inhibition of AGEs at different concentrations of the extract and the standard

Achyranthes aspera is an annual, stiff-erect herb found commonly as a weed throughout India [18]. It possesses medicinal properties and used in treatment of cough, bronchitis and rheumatism, malarial fever, dysentery, asthma, hypertension, and diabetes [19]. Achyranthes aspera contains triterpenoid saponins which possess oleanolic acid as the aglycone.

Ecdysterone and long chain alcohols are also found in Achyranthes aspera. Achyranthes aspera has expressed antioxidant activity, antimicrobial, anti-cancerous, hypoglycaemic, anti-inflammatory and anti-spasmodic [20, 21]. Advanced glycation end products (AGEs) are proteins or lipids that become glycated after exposure to sugars. AGEs are prevalent in the diabetic vasculature and contribute to the development of atherosclerosis [22].

In a similar study that was conducted by Xiofang Peng and Zongping Zheng advanced glycation end products were inhibited using mung bean extract. Vitexin and isovitexin both showed inhibitory activities against AGEs formation induced by glucose but failed to inhibit formation in another assay [23].

In another study Cuminum cyminum was used to inhabit the formation of AGEs in comparison to glibenclamide. The study was conducted using streptozotocin induced diabetic rats. Significant reduction in renal oxidative stress and AGE was observed with CC when compared to diabetic control and glibenclamide [24].

A study conducted by Kentaro Tsuji-Naito, Hiroshi Saeki and Miyuki Hamano on the inhibitory effects of Chrysanthemum species extract on the AGEs, it was found that Chrysanthemum species strongly inhibited the formation of AGEs and Nε-(carboxymethyl)lysine (CML). C.morifolium R, not C. indicum L, also acted to inhibit the formation of fluorescent AGEs, including pentosidine [25].

The results obtained from the study conducted found that Achyranthes aspera extract potentially inhibits the formation of the AGEs. The standard that was used was amino guanidine. The results that were obtained from the studies similar to this one were found to be similar to this study.

be obtained.

Conclusion

According to the results obtained, we know that Achyranthes had potentially inhibited the AGEs' formation and it can be used

in the synthesis of drugs for diabetic patients. During long standing hyperglycaemia state in diabetes mellitus protein glycation and formation of advanced glycation end products (AGEs) play an important role in the pathogenesis of diabetic complications. This study will be useful in the synthesis of drugs from natural sources as well in the pathogenesis.

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