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Potential of C*assia auriculata* and *Saraca asoca* standardized extracts and their principal components for alleviating diabetic complications

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Abstract

Aldose reductase (AR) enzyme and advanced glycation endproducts (AGEs) play an important role in diabetic complications such as cataracts. The purpose of this study was to look into two standardized plant extracts used in Ayurvedic medicine for the treatment of diabetes, and their principal components for AR and AGEs inhibitory activities, and to evaluate their potential in combating the various pathological consequences of diabetes. *Cassia auriculata* Linn and *Saraca asoca* (Roxb.) De Wild and their respective major constituents, proanthocyanidin B_1, and leucocyanidin were studied for their inhibitory activity against rat lens AR (RLAR), rat kidney AR (RKAR), human recombinant AR (HRAR), and generation of AGEs. In addition, *in vivo* inhibition of lens galactitol accumulation by the major constituents of the plants in galactose-fed rat model has been studied. The results show that both plant extracts and their principal components possess AR inhibitory actions in both *in vitro* and *in vivo* assays, and also inhibited AGEs formation significantly. In all assays carried out, proanthocyanidin B_1 was found to be the most potent showing comparable or better effect than the reference compounds used. In both RP-HPLC and GLC analyses, rat lens galactitol concenration of procyanidin B_1 (1.5, 1.6 μg/ml, respectively) displayed a slightly better activity than the reference compound quercetin (1.65, 1.63 μg/ml, respectively). The results obtained in this study give a new dimension to the hitherto unknown activity of the plants as possible protective agents against long-term diabetic complications.

Keywords: aldose reductase, advanced glycation endproducts, galactitol accumulation, *Cassia auriculata*, *Saraca asoca*, proanthocyanidin B_1

Introduction

According to World Health Organization reports, around 300 million or more people will be affected by diabetes by the year 2025. The predictable number of diabetic patients in 2030 will be more than two fold than in 2005. Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia and changes in lipid and protein metabolism. Further, long-term diabetic patients who are treated unsuccessfully suffer from complications of retinopathy, nephropathy, and peripheral neuropathy. The risks of acquiring cardiovascular disease, stroke, and cancer are also higher in diabetic patients [1].

Aldose reductase (AR) and advanced glycation endproducts (AGEs) may play an important role in the pathogenesis of diabetic complications. Although numerous synthetic AR and AGE formation inhibitors show potent effects, either their use is inadequate or they have been remote from clinical trials because of relatively low efficacy, poor pharmacokinetics and intolerable safety [2]. To date, epalrestat is the only synthetic AR inhibitor (ARI) available in the market, approved only in Japan for the improvement of subjective neuropathy symptoms associated with diabetic peripheral neuropathy [3]. Thus, there is a growing interest in the benefits of dietary supplements as nutraceuticals, as well as herbal medicines because in most cases they lack toxic and side effects [4].

In traditional medicine the flowers of *Cassia auriculata* Linn (Caesalpiniaceae) are widely used as a cure for rheumatism, conjunctivitis and diabetes [5]. Being the main constituent of Kalpa herbal tea in Ayurvedic medicine, *C. auriculata* has been investigated extensively. Pari & Latha, 2002 have shown that its flower extracts possess genuine blood sugar lowering effect in streptozocin-induced diabetic mice [6]. Similarly, in folk medicine extracts prepared from the various parts of *Saraca asoca* (Roxb.) De Wild (Caesalpiniaceae) are employed for the management of diabetes, and their antihyperglycemic and antioxidants effects have been confirmed by Sunil et al., 2012 [7].

This study was initiated with the aim of evaluating the inhibitory effects of the standardized flower extracts of C. auriculata and S. asoca, and their respective major constituents, procyanidin B1,

leucocyanidin on AR and generation of AGEs which play an important role in diabetic complications.

Material and Methods

According to World Health Organization reports, around 300 million or more people will be affected by diabetes by the year 2025. The predictable number of diabetic patients in 2030 will be more than two fold than in 2005. Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia and changes in lipid and protein metabolism. Further, long-term diabetic patients who are treated unsuccessfully suffer from complications of retinopathy, nephropathy, and peripheral neuropathy. The risks of acquiring cardiovascular disease, stroke, and cancer are also higher in diabetic patients [1].

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This study was initiated with the aim of evaluating the inhibitory effects of the standardized flower extracts of *C. auriculata* and *S. asoca*, and their respective major constituents, procyanidin B_1, leucocyanidin on AR and generation of AGEs which play an important role in diabetic complications.

Results

RLAR enzyme protein concentration, enzyme activity and specific activity of the lens homogenate were found to be 2 mg/ml, 14.11 U/ml and 7.06 U/mg, respectively. Similarly, RKAR crude enzyme protein concentration, enzyme activity and specific activity of the rat kidney homogenate were 2.2 mg/ml, 9.68 U/ml and 4.39 U/mg, respectively. As shown in Table 1, both the standardized extracts showed comparable RLAR and RKAR inhibitory activity in a concentration dependant manner with maximum activity obtained at the highest concentration (100 µg/ml) employed. This has prompted us to assess the activity of their respective major constituents namely, procyanidin B_1 and leucocyanidin, against these enzymes and human recombinant AR (HRAR) *in vitro*. In all the three assays procyanidin B_1 was found to be twice as active as leucocyanidin in inhibiting AR. As shown in Table 1, the AR inhibitory activity of procyanidin B_1 with IC_{50} values of 11.00, 12.47 and 9.68 µM, in RLAR, RKAR and HRAR assays, respectively, was either comparable or better than the reference compound quercetin (IC_{50} = 14.89, 18.67 and, 9.26 µM, respectively).

The results of inhibitory activity of AGEs formation for the standardized extracts and their principal components are presented in Table 2. It is evident from the table that the extracts displayed maximum activity at the highest dose (100 µg/ml) tested. Both the extracts displayed a moderate activity with *C. auriculata* (IC_{50} = 65.63 ± 0.25) showing better inhibition than *S. asoca* (IC_{50} = 76.60 ± 0.74).

In this study, the derivatized lens homogenates of control group rats and rats treated with the test substances were analyzed by HPLC and GLC. In HPLC analysis the retention times (RTs) of galactitol and the internal standard glucose were found to be 6.804 and 4.292 min, respectively. In GLC analysis the RTs of galactitol and the internal standard methyl- -D-mannopyranoside were 22.11 min and 6.41 min, respectively. In both analyses the concentration of galactitol was calculated using standard graphs. The graphs which compare the test groups' galactitol concentrations with that of the control as determined by RP-HPLC and GLC are depicted in Figures 1 and 2, respectively.

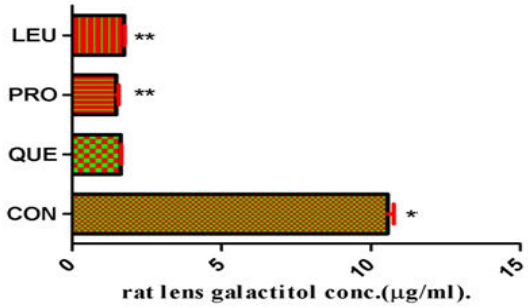

Figure 1. *In vivo* rat lens galactitol levels determined by reverse phase high pressure chromatography (RP-HPLC) (CON: control, PRO: procyanidin B_1, LEU: leucocyanidin, QUE: quercetin). Asterisk(**) designates statistical insignificance ($p>0.05$); (*) designates statistical significance ($p<0.05$); in comparison to quercetin group, n=6.

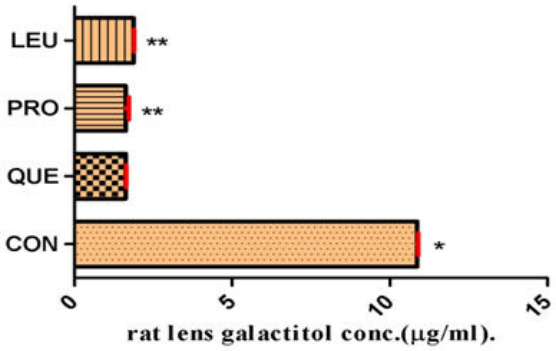

Figure 2. *In vivo* rat lens galactitol levels measured by gas liquid chromatography (GLC) (CON: control, PRO: procyanidin B_1, LEU: leucocyanidin, QUE: quercetin). Asterisk(**) designates statistical insignificance ($p>0.05$); (*) designates statistical significance ($p<0.05$); in comparison to quercetin group, n=6.

Discussion

In the present study standardized flower extracts of *C. auriculata* and *S. asoca*, widely used in Ayurvedic medicine for the treatment of a variety of ailments including diabetes were assessed for their possible AR inhibitory activities on rat lens AR (RLAR), rat kidney (RKAR) and HRAR. Several studies have revealed that hyperglycemia has important role in the pathogenesis of diabetic complications by increasing AR related polyol pathway and increase in AGEs formation. The polyol pathway which has two energy-dependent and enzymatically catalyzed steps involves the conversion of glucose to sorbitol, and oxidation of NADPH to NADP followed by the formation of fructose from sorbitol by sorbitol dehydrogenase during which NAD is reduced to NADH. Such increased activity of the polyol pathway could have potentially deleterious consequences: such as decrease in cellular NADPH levels, resulting in decreased concentrations of glutathione and nitric oxide leading to oxidativestress and vasodilatation. Moreover, in diabetic condition, sufficient glucose can enter into tissue, increasing levels of the AR-related polyol pathway which augments intracellular concentration of sorbitol and its metabolites, followed by accumulation in cells due to their poor penetration across membrane and inefficient metabolism. This results in the development of diabetic complications [16-18]. Genetic and biochemical data have also suggested a strong link between raised AR activity and strongly altered risk of diabetic complications such

Table 1. *In vitro* aldose reductase (AR) inhibitory activities of the standardized plant extracts and their active constituents on rat lens aldose reductase (RLAR), rat kidney aldose reductase (RKAR) and human recombinant aldose reductase (HRAR) enzymes.

No.	Test sample	Conc. (µg/ml)	RLAR			RKAR			HRAR		
			Inhibitory activity (%)	IC_{50} (µg/ml)	IC_{50} (µM)	Inhibitory activity (%)	IC_{50} (µg/ml)	IC_{50} (µM)	Inhibitory activity (%)	IC_{50} (µg/ml)	IC_{50} (µM)
1.	*Cassia auriculata*	10	17.52 ± 0.31	64.62± 0.24		12.03 ± 0.15	63.96 ± 0.45	-			
		50	51.33 ± 0.76			55.33 ± 0.76					
		100	64.03 ±0.15			64.70 ± 0.31					
2.	Procyanidin B_1	1	6 .84 ± 0.5	6.36 ± 0.71	11.00	06 .84 ± 0.50	7.21 ± 0.71	12.47	15.04 ± 0.80	5.60 ± 0.27	9.68
		5	35.30 ± 1.43			27.30 ± 1.43			27.00 ± 0.20		
		10	75.33 ± 0.56			66.33 ± 0.56			82.12 ± 0.50		
3.	*Saraca asoca*	10	11.33 ±0.07	67.23± 0.19		9.31 ± 0.07	74.03 ± 0.37	-			
		50	46.42 ± 0.82			40.80 ± 0.82					
		100	66.13 ±0.07			63.17 ± 0.30					
4.	Leucocyanidin	1	2.84 ± 0.04	6.77 ± 0.79	21.12	03.84 ± 0.04	7.16 ± 0.38	23.39	14.02 ± 0.77	6.37 ± 0.77	20.16
		5	40.10 ± 0.9			30.10 ± 0.90			30.14 ± 0.20		
		10	68.18 ± 0.25			63.18 ± 0.25			77.20 ± 0.50		
5.	Quercetin	1	25.00±1.73	4.5 ± 0.05	14.89	15.00	5.64±0.02	18.67	26.33 ± 0.57	2.80 ± 0.21	9.26
		5	57.33±0.57			51.00			51.66 ± 1.52		
		10	85.00			77.66 ± 1.15			97.00		

All values are expressed as mean ± S.D, n = 3.IC_{50}, 50% Inhibitory concentration; SD, Standard deviation.

Table 2. *In vitro* advanced glycation endproducts (AGEs) formation inhibitory activities of standardized plant extracts and their active constituents.

No.	Test sample	Concentration (μg/ml)	AGEs formation inhibitory activity (%)	IC_{50} (μg/ml)	IC_{50} (μM)
1.	*Cassia auriculata*	10	14.02 ± 0.77	65.63 ± 0.25	
		50	37.14 ± 0.20		
		100	74.20 ± 0.50		
2.	Procyanidin B_1	1	22.04 ± 0.80	5.48 ± 0.27	9.48
		5	44.00 ± 0.20		
		10	81.12 ± 0.50		
3.	*Saraca asoca*	10	12.01 ± 0.12	76.60 ± 0.74	
		50	33.30 ± 0.57		
		100	64.20 ± 0.70		
4.	Leucocyanidin	1	15.10 ± 0.12	7.70 ± 0.52	25.16
		5	37.40 ± 0.54		
		10	62.00 ± 0.10		
5.	Aminoguanidine	1	23.00 ± 0.00	5.58 ± 0.33	50.47
		5	51.20 ± 0.36		
		10	78.87 ± 0.71		

All values are expressed as mean ± S.D, n=3.$IC_{50,}$ 50% inhibitory concentration; SD, standard deviation.

as cataract, nephropathy, retinopathy and neuropathy [19]. It has been reported that suppressing the metabolism of glucose via the polyol pathway by inhibiting AR is a potential way to prevent the complications noted above [20]. Thus, the results of the present study suggest that both extracts possess AR inhibitory activity of different magnitude thereby suppressing glucose metabolism, with their major components significantly contributing to their activities.

Hyperglycemia can also lead to glycation, a non enzyme Maillard reaction responsible for the pathogenesis of diabetic complications. Amadori products are formed in the later stages of the Maillard reaction by reaction between reducing sugars such as sorbitol and protein and then converted to dicarbonyl moiety such as glucosones, glyoxal and methylglyoxal, followed by the formation of cross linking AGEs, including pentosidine and crosslines [21-22]. Therefore, antioxidant agents with inhibitory effects on AR and AGE formation would be of great value in the prevention of complications caused by polyol pathway [18]. The results of inhibitory activity of AGEs formation, both the extracts displayed a moderate activity, *C. auriculata* showing better inhibition than *S. asoca*. On the other hand, the inhibitory activities of the phytoconstituents were remarkable with procyanidin B_1 (IC_{50} = 9.88 μM) being about 5 times as active as the reference compound aminoguanidine (IC_{50} = 50.47 μM). Both these constituents have the basic skeleton of flavonoids with hydroxyl groups at positions 3',4',5 and 7, which have been reported to possess potent AGEs inhibitory activity when they have such substitution pattern [23].

It has long been observed that accumulation of high concentration of polyol in the lens results in an increase in the intracellular ionic strength resulting in excessive hydration, eventually loss of membrane integrity and leakage of compounds such as free amino acids, glutathione and myo-inositol leading to the formation of cataract [24]. It has also been shown that lens changes occur more quickly under galactosemic conditions because glucose is converted to fructose by AR and sorbitol dehydrogenase in the sorbitol pathway, but galactitol is not further metabolized by sorbitol dehydrogenase. Because of this, the onset and progression of retinal changes are more rapid in galactosemia than other diabetic models [14].

The findings demonstrate that both procyanidin B_1 and leucocyanidin suppress the accumulation of galactitol in rat lens significantly. In both RP-HPLC and GLC analyses, rat lens galactitol concentration of procyanidin B_1 (1.5, 1.6 μg/ml, respectively) displayed a slightly better activity than the reference compound quercetin (1.65, 1.63 μg/ml, respectively).

The results of the present study indicate that the standardized flower extracts of *C. auriculata* and *S. asoca* and their respective major components, procyanidin B_1 and leucocyanidin possess AR and AGEs formation inhibitory activities. Looking at the powerful AR and AGES inhibitory effects of the major components, it can be concluded that these constituents significantly contribute to the activities of the plant extracts, and also could be potentially useful in the treatment of diabetic complications. Although the blood glucose lowering effect of these plant extracts has been known

previously, to the best of our knowledge, this is the first report on their AR and AGEs inhibitory activities. It is, therefore, suggested that the above plants should be evaluated further for combating the various pathological consequences of diabetes.

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