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Aldose Reductase Inhibitory Activity of *Butea monosperma* for the Management of Diabetic Complications

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ABSTRACT

Background: Aldose Reductase (AR) enzyme and advanced glycation end products (AGEs) play an important role in diabetic complications. The AR and AGEs inhibitors are beneficial in prevention of diabetic complications. **Objective:** Aim of present study was to evaluate the *in vitro* and *in vivo* AR and AGEs inhibitory activity of an antidiabetic plant *Butea monosperma.* **Methods:** The methanolic extract, standardized extract of flower and the major constituent butein were studied for their inhibitory activity against Rat Lens AR (RLAR), rat kidney AR and generation of AGEs. In addition, *in vivo* inhibition of lens galactitol accumulation in galactose-fed rat model was studied. **Results:** The plant extracts and butein were shown to possess AR inhibitory activity in both *in vitro* and *in vivo* assays with equal potency to that of standard quercetin. In case of inhibited AGEs formation, the plant extracts showed insignificant activity where as butein was found to be potent. **Conclusion:** The results obtained in this study provide a new dimension to the hitherto unknown activity of the plant as possible protective agent against long-term diabetic complications.

Key words: Aldose reductase, antidiabetic, galactose rat model, advanced glycation end products

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INTRODUCTION

Hyperglycemia plays an important role in the pathogenesis of long term diabetic complications and diabetic patients with poor blood-glucose control are particularly at risk. Persistent hyperglycemia induces abnormal changes such as the formation of Advanced Glycation Endproducts (AGEs) and activation of Aldose Reductase (AR) which is implicating the diabetic or other pathogenic complications. The AR is a cytosolic enzyme and is small monomeric protein composed of 315 aminoacid residues. It is the key enzyme of the polyol pathway (POP) which catalyzes the conversion of glucose and galactose to the sugar alcohols sorbitol and galactitol, respectively[1].

Under hyperglycemia, because of the saturation of hexokinase with ambient glucose, there is an increase in flux of glucose through the POP accounts for as much as one-third of the total glucose turnover [2]. This leads to overflow of the products of the POP along with depletion in reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and the oxidized form of Nicotinamide Adenine Dinucleotide (NAD), the cofactors in the pathway. The acceleration of the POP thus elicits various metabolic imbalances in those tissues that undergo insulin independent uptake of glucose. Such metabolic perturbation provokes the early tissue damage in the "Target" organs of diabetic complications, such as ocular lens, retina, peripheral nerve and renal glomerulus[3-4].

Increased extracellular glucose levels as well as fructose from the POP leads to the formation of AGEs, due to non-enzymatic glycation reactions such as Maillard's reaction. The Maillard's reaction takes place between the sugars, tissue proteins and aminoacids. This leads to the formation of reactive compounds through Schiff's base formation and amodari rearrangement. The compounds resulted are finally converted to fluorescent compounds that are further more reactive to the tissue proteins and aminoacids in the body. These reactive, fluorescent substances are known as AGEs. These are responsible for subsequent damage of the tissues and severe complications of DM[5]. Therefore, there is growing interest in drugs that inhibit AR and AGEs.

Butea monosperma (Fabaceae) is commonly found throughout India, except in the arid regions. Flowers are typically papilinaceous, the stigma is wet papillate and

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the style is hollow. They are reported to possess astringent, diuretic, depurative, aphrodesiac, tonic properties, anticonvulsive and antihepatotoxic properties. Antidiabetic activities of flowers of *B. monosperma* are also documented. The flowers are reported to contain several flavonoids of which butein is more active and possesses antidiabetic, hepatoprotective, anti cancer and antioxidant properties[6-12]. The present study is aimed to know whether *B. monosperma or* its active constituent can be useful in preventing the diabetic complications in a animal model.

MATERIALS AND METHODS

Materials: Aminoguanidine, DL-glyceraldehyde, NADPH, quercetin and phenylisocyanate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). *B. monosperma* standardized extract was obtained as gift sample from Amsar extracts Pvt. Ltd., Indore. The active constituent butein procured from Tokyo chemical Industries, Japan.

Animals: Wistar albino rats purchased from Mahaveer Enterprises, Hyderabad, India, were housed under standard laboratory conditions and fed commercial rat feed and tap water *ad libitum*. All the experiments were conducted in accordance with the internationally accepted laboratory animal use, care and guideline (ILAR, 1996). Ethical clearance was obtained from the institutional animal ethical committee of the university (IAEC/12/UCPSC/KU/2012).

In vitro AR inhibitory activity using rat lens homogenate

Preparation of lens homogenate: The crude rat lens AR was obtained by standard method of Hayman and Kinoshita[13,14].

The protein content, enzyme activity and specific activity of the enzyme preparation were determined using reported methods[15,16].

Inhibitory effect of dimethylsulfoxide (DMSO) on AR activity: The effects of dimethylsulfoxide (DMSO) on AR were measured by changing DMSO concentration. The DMSO concentrations ranging between 5-100% prepared in water were used for the determination of enzyme inhibitory activity.

Measurements of rat lens enzyme activity: The AR inhibitory activity was assayed spectrophotometrically. Quercetin and butein each at concentrations of 1.0, 5.0 and 10.0 μg mL^{-1} and *B. monosperma* Methanolic Extract (BME) and *B. monosperma* Standardized Extract (BSE) at concentrations of 10, 50 and 100 μg mL^{-1} were prepared in 10% dimethyl sulfoxide (DMSO). The ARI activity of each sample was calculated by using the reported formula[14]. Quercetin was used as a reference standard.

In vitro AR inhibitory activity using rat kidney homogenate

Measurements of rat kidney enzyme activity: Rats were dissected ventrally and both kidneys of each rat were collected and cut into small pieces[17]. The kidney pieces were homogenized in the same way described for the preparation of lens homogenate. Similar procedure was followed as in the case of lens homogenate, where instead of lens enzyme preparation, kidney enzyme preparation was used.

Inhibition of AGEs formation: Inhibition of AGEs formation was determined according to the modified method of Vinson and Howard[18]. The percentage inhibition of the AGE formation was determined by reported formula. The nucleophilic hydrazine aminoguanidine was used as reference in AGE assay.

***In vivo* galactosemic animal model:** *In vivo* galactosemic animal study performed according to the repotted method.

The quercetin and butein were given at a dose of 10 mg kg^{-1} body weight. BME and BSE were given at a dose of 200 mg kg^{-1} body weight. All the animals were sacrificed on the 15th day by spinal nerve dislocation, both eye balls were collected from each rat and lens was enucleated through posterior approach. After washing with saline, the pair of lenses of each rat homogenized with 1 mL of ice cold water, individually. The proteins were precipitated with ethanol (70% of the final volume) and removed by centrifugation (30 min at 16,000 rpm). Centrifugation was done at 4°C and the supernatant was collected as described by Kato *et al*.[19].

Estimation of lens galactitol levels by RP-HPLC: Lyophilized samples of all groups of rats were derivatized by adding 250 μL of pyridine and 500 μL of phenylisocyanate and then incubated for 1 h at 55°C in water bath with mechanical shaking. After incubation period, reactor flasks were cooled and 250 μL of methanol was added to remove excess of phenylisocyanate which otherwise could react with water of the eluent. The clear solutions obtained were

then diluted twice with pyridine to decrease the interferences due to absorption of the reagents[20].

Derivatized samples of each group were analyzed by HPLC with Photo Diode Array Detector (Schimadzu LC-10 AT, Japan), Hamilton Rheodyne injector syringe (Sigma Aldrich), Chemsil ODS-2 C18 HPLC-column, 250 mm-4.6 mm (Chem India Pvt. Ltd., Hyderabad). A mixture of 60% acetonitrile and 40% of double distilled water was used as mobile phase. Flow rate was adjusted to 1 mL min^{-1} and the injection volume was 20 µL. Detector wavelength was adjusted to 240 nm. A standard graph was plotted by analyzing solutions of different concentrations of galactitol using glucose as internal standard.

Statistical analysis: The data was presented as a Mean±SD. The significance of the difference was analyzed by one-way analysis of variance.

RESULTS

***In vivo* inhibition of AR:** The RLAR and RKAR inhibitory activities of the plant extracts and the active constituent were compared with quercetin. The RLAR enzyme protein concentration, enzyme activity and specific activity of the lens homogenate were found to be 1.24 mg mL^{-1}, 3.58 U mL^{-1} and 2.91 U mg^{-1}, respectively. The AR Inhibitory values (ARI%) and IC_{50} values (mg mL^{-1}) of the test compounds against lens homogenate were given in Table 1.

The RKAR enzyme protein concentration, enzyme activity and specific activity of the lens homogenate were found to be 1.76 mg mL^{-1}, 22.04 U mL^{-1} and 12.52 U mg^{-1}, respectively. The AR Inhibitory values (ARI%) and IC_{50} values (mg mL^{-1}) of the test compounds against kidney homogenate were given in Table 1.

Inhibition of AGEs formation: The results of inhibitory activity of AGEs formation and IC_{50} values of the plant extracts and the active constituent were presented in Table 2.

***In vivo* inhibition of AR:** The derivatized lens homogenates of each test as well as control group rats were analyzed by HPLC. The retention time of galactitol was found to be 7.1 min and that of glucose 4.5 min. Galactitol concentration was calculated using a standard graph. Administration of quercetin or BME or BSE significantly ($p<0.05$) reduced the galactitol levels in the lens when compared to control group. However, administration of butein produced a more significant ($p<0.01$) change in galactitol levels when compared to control groups (Fig. 1).

Table 1: *In vitro* AR inhibitory activity of the plant extracts and active constituent on RLAR and RKAR

		RLAR		RKAR	
Test samples	Concentration (µg mL^{-1})	Inhibitory activity (%)	IC_{50} value (µg mL^{-1})	Inhibitory activity (%)	IC_{50} value (µg mL^{-1})
Quercetin	1	9.79±1.81	5.72±0.10	10.98±1.80	7.08±0.23
	5	40.30±1.30		25.07±1.79	
	10	90.50±1.19		76.60±1.55	
Butein	1	5.19±0.51	9.21±0.32	14.92±1.80	7.89±0.19
	5	24.80±1.60		33.39±1.48	
	10	55.17±1.60		62.19±1.42	
BME	10	5.01±0.23	66.88±1.43	32.17±0.90	46.99±0.47
	50	29.96±1.75		56.68±0.26	
	100	80.60±1.30		68.75±0.25	
BSE	10	10.02±1.98	65.10±1.42	33.30±1.13	44.84±0.81
	50	36.19±0.43		57.40±0.72	
	100	77.17±1.04		69.33±0.58	

All values were expressed as Mean±S.D, n = 3, IC_{50}: 50% inhibitory concentration, SD: Standard deviation

Table 2: *In vitro* AGEs formation inhibitory activity of plant active constituent

Test sample	Concentration (µg mL^{-1})	Inhibitory activity (%)	IC_{50} value (µg mL^{-1})
Aminoguanidine	1	14.86±1.30	8.54±0.35
	5	41.39±1.39	
	10	53.35±1.47	
Butein	1	37.10±0.98	16.61±1.94
	5	39.42±0.68	
	10	44.80±1.60	

All the values were expressed as Mean±SD, n = 3, IC_{50}: 50% inhibitory concentration, SD: Standard deviation

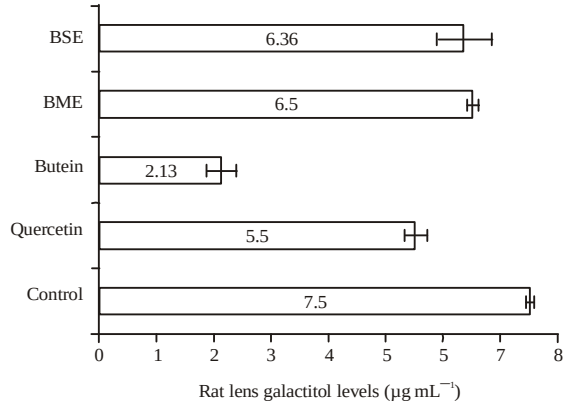

Fig. 1: *In vivo* rat lens galactitol levels of active constituent and plant extracts determined by RP-HPLC. Column: ODS-2 C18, 250 mm-4.6 mm, Detector: Photo Diode Array, Mobile phase: 60% acetonitrile and 40% double distilled water; Flow rate: 1 mL min^{-1}, Injection volume: 20 µL, BSE: *B. monosperma* standardized extract, BME: *B. monosperma* methanolic extract

DISCUSSION

The AR activity of the plant extracts and active constituent were compared with quercetin, a potent natural AR inhibitor. Specific activity was calculated from the protein concentration and enzyme activity. This concentration influences the optimization condition for AR by taking saturation concentration of NADPH and glyceraldehydes, i.e., 0.15 mM and 10 mM, respectively.

As shown in Table 1, the plant extracts and the active constituent displayed AR activity although the active constituent showed potent activity that can be compared with that of quercetin. The values indicate that there is not much difference which reflects equal potency of butein with that of quercetin. So, butein is also considered as a potent AR inhibitor according to the *in vitro* results.

Due to the close link between AGE formation and AR-related polyol pathway, the *in vitro* AGEs formation inhibitory activities of the plant extracts and the active constituent were studied. As shown in Table 2, the active constituent exhibited good inhibition of generation of AGEs whose potency is comparable with that of aminoguanidine. Interestingly the plant extracts showed insignificant activity towards inhibition of AGEs formation.

In view of the powerful *in vitro* AR inhibitory effects of the tested compounds, their *in vivo* inhibitory activity was studied by estimation of rat lens galactitol levels in galactose fed rat model using RP-HPLC. The accumulation of polyols such as galactitol was considered to be responsible for the development of cataracts[21]. Lens changes occur more quickly under galactosemic conditions because glucose is converted to sorbitol by AR and then to fructose by sorbitol dehydrogenase in polyol pathway. However, since galactitol is not further metabolized, accumulation of galactitol will take place in a relatively short period of time. Genesis of such galactosemic conditions was reported to result in more speedy onset and progression of retinal change than other diabetic models [22]. As shown in Fig. 1, quantification of galactitol by RP-HPLC indicated that all the studied compounds significantly suppressed galactitol accumulation.

However, the active constituent butein, showed more activity than the standard compound, quercetin in the *in vivo* study. Literature reveals that, poly phenol could elicit AR inhibition by preventing the enzymatic conversion of glyceraldehyde to glycerol and also glucose to sorbitol thereby increasing the levels of NADPH, a coenzyme highly involved in protecting against the toxicity of reactive oxygen species[23]. This is in agreement with the present findings. Butein (3,4,2',4'-tetrahydroxychalone), is a polyphenolic compound which revealed the action in both *in vitro* and *in vivo* assays.

CONCLUSION

The present study proved that the BME and BSE and its active constituent butein possess ARI activity. As a chief constituent with powerful AR and AGEs inhibitory activity butein appears to be responsible for the activity of the plant. In conclusion, it can be said that *B. monosperma* has a potential for the complications of diabetes.

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