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Inhibition of aldose reductase by *Aegle marmelos* and its protective role in diabetic cataract

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ABSTRACT

Ethnopharmacological relevance: *Aegle marmelos* (L.) Corr. Serr. (*Aegle marmelos*) leaves were extensively used in the Ayurvedic, Unani and Siddha systems of Indian medicine as an anti-diabetic agent, which serves as hypoglycemic agent. However, the significance of this plant on secondary complications of diabetes such as cataract remained unknown. The aim of the study was to investigate the possible anti-cataractous activity of *Aegle marmelos* against streptozotocin (STZ) induced diabetic cataract in rats.

Materials and methods: *Aegle marmelos* leaf extract was prepared using three different solvents (petroleum ether, ethyl acetate and methanol) and tested for inhibition against rat lens aldose reductase (AR), a key enzyme of polyol pathway. Furthermore, the pharmacological potential of *Aegle marmelos* extract was investigated against osmotic stress induced opacification of lens in *ex vivo* organ culture and streptozotocin (STZ) induced diabetic cataract in rats.

Results: Ethyl acetate extract of *Aegle marmelos* inhibited rat lens AR *in vitro* with an IC_{50} value of ~15 μg/ml. This extract also prevented the hyperglycemia induced increase in AR activity, sorbitol accumulation and opacification of rat lens in *ex vivo* lens organ culture. Supplementation of ethyl acetate extract of *Aegle marmelos* to STZ-induced diabetic rats decreased the blood glucose levels due to hyperglycemia and inhibited the AR activity and delayed cataract progression in dose dependent manner. α-crystallin isolated from diabetic rats fed with *Aegle marmelos* showed improved chaperone activity than that of isolated from rats naïve to *Aegle marmelos*.

Conclusion: This study indicates that ethyl acetate extract of *Aegle marmelos* has pharmacologically active components with a potential to inhibit rat lens AR and consequential decrease in osmotic stress. Besides this, the present study also demonstrates that the extract prevented loss of antioxidants contributing to the integrity of α-crystallin's chaperone activity and thereby delaying cataract.

1. Introduction

Cataract, opacification of normal transparent eye lens, is the leading cause of blindness worldwide and diabetes is a major predisposing factor in cataract formation. The transparency and necessary refractive index of the lens is achieved by short-range order of concentrated solutions of structural proteins called crystallins. In mammals they comprise of three families: α-, β- and γ-crystallins. α-crystallin, a molecular chaperone of eye lens plays an important role in protecting lens against cataract formation by preventing non-specific aggregation of sub-lenticular proteins via its chaperone activity (Kumar and Reddy, 2009). Studies suggest that decrease in the chaperone activity of α-crystallin in diabetes may predispose to cataract formation (Cherian et al., 1997; Finley et al., 1998; Gupta and Srivastava, 2004; Kumar et al., 2004, 2007; Kumar and Reddy, 2009). Chronic elevation of blood glucose in diabetes plays a critical role in the etiology of complications including cataract. During hyperglycemic conditions, the bulk of glucose disposal occurs via polyol pathway, where glucose is converted into sorbitol by aldose reductase (AR; EC 1.1.1.21) and sorbitol dehydrogenase catalyzes sorbitol to fructose (Chung et al., 2003).

Since polyol pathway is implicated in the pathogenesis of diabetic cataract and AR is a rate-limiting enzyme of the polyol pathway, it has been suggested that inhibition of aldose reductase

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(AR) could be a pharmacological target in the management of diabetic cataract (Srivastava et al., 2005, 2011; Anil and Bhanuprakash, 2007). Although many synthetic and semi-synthetic AR inhibitors (ARI) have been developed, the majority of them have not received significant consideration either due to limited efficacy or adverse effects. Nevertheless, many studies suggest that inhibition of AR could be effective in combating oxidative stress and helps in the prevention of diabetic cataract (Drel et al., 2008; Tang et al., 2012). In recent times, there is an increased incidence of exploring natural products of plant origin with a potential to inhibit AR. Natural products as pharmacological agents may be useful in bio-safety issues such as greater assimilation and reduced toxicity. Several groups reported that some common dietary sources such as fruits and spices have been explored as ARIs (Suryanarayana et al., 2004; Saraswat et al., 2008; Muthenna et al., 2009; Akileshwari et al., 2012).

Aegle marmelos (L.) Corr. Serr (*Aegle marmelos*) of Rutaceae family, known as Bael (or Bel), is a moderate sized, slender and aromatic plant that grows in several parts of India and South East Asia. This plant has been used as herbal medicine in Ayurvedic, Unani and Siddha systems of Indian medicine for the management of diabetes mellitus. Leaf extracts of *Aegle marmelos* were evaluated for antidiabetic properties (Seema et al., 1996; Anandharajan et al., 2006; Gacche and Dhole, 2011; Ram et al., 2012). The leaves are bitter and are used as a remedy for ophthalmia, ulcers, dropsy, cholera and beriberi. Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* are reported to have a cardiotonic effect. An aqueous decoction of the leaves has been shown to possess significant hypoglycemic effect (Karunanayake et al., 1984). *Aegle marmelos* leaf extract has been reported to regenerate damaged pancreatic cells in diabetic rats (Das et al., 1996) and is found to be as effective as insulin in the restoration of blood glucose to normal levels (Seema et al., 1996). However, the mechanism by which *Aegle marmelos* prevents diabetic complications has not yet been studied. In the present study, *Aegle marmelos* leaf extracts were evaluated for AR inhibitory potential in addition to its hypoglycemic and antioxidant property in hyperglycemic induced lens organ culture and STZ induced diabetic rats.

2. Material and methods:

2.1. Materials

DL-glyceraldehyde, Lithium sulfate, β-mercaptoethanol, NADPH, Quercetin, 2,4-dinitrophenylhydrazine, TC-199 medium, reduced glutathione, pyrogallol, and Sephacryl S-300HR were procured from Sigma Chemicals (MO, USA). Insulin, DTT, and Tris were obtained from SRL (Mumbai, India). All other chemicals and solvents were of analytical grade and obtained from indigenous companies in India.

2.2. Preparation of Aegle marmelos extracts

Aegle marmelos plant was identified and authenticated (Deposited Number: Bot/184/OU/A.0101/HYD) by Prof. Ramchandra Reddy, a taxonomist at the Department of Botany, Osmania University Hyderabad, India. *Aegle marmelos* leaves were collected from Nallamalla forest, Kurnool district, Andhra Pradesh, India. The plant leaves were air dried for 8 days, and then grounded using a mechanical grinder at room temperature and made into coarse powder. The dried powder (150 g) of *Aegle marmelos* was extracted sequentially using different organic solvents in increasing order of polarity (petroleum ether, ethyl acetate and methanol) as per standard protocols (Anandharajan et al., 2006; Dheeba et al., 2010; Amit, 2011; Kothari et al., 2011). Extraction mixtures were kept in a dark room for 72 h at room temperature in sterilized bottles wrapped with aluminum foil to avoid evaporation. After 72 h, mixtures were filtered through Whatman no. 1 filter paper. This step was continued three to four times to remove any undissolved plant material which was subsequently extracted with another solvent. These filtered extracts were concentrated using a vacuum rotary evaporator (Superfit™) and kept at 37 °C to remove any traces of solvent. Concentrated preparations of *Aegle marmelos* were stored at −20 °C until further use. The yield for 150 g of dried powder was 16 g, 13 g and 18 g of petroleum ether, ethyl acetate and methanol extracts respectively.

2.3. Aldose reductase inhibition studies

Partial purification of rat lens AR was performed essentially as described earlier (Hayman and Kinoshita, 1965). Eyeballs were removed from 9 weeks old SD male rats obtained from the National Center for Laboratory Animal Sciences, Hyderabad. Lenses were then separated from the eye ball and homogenized with 10 volumes of 100 mM potassium phosphate buffer pH 6.2. The homogenate was centrifuged at 12,000*g* at 4 °C for 30 min. The supernatant was precipitated with ammonium sulfate at 40%, 50% and 75% salt saturations. After which pellet from the previous step, possessing AR activity, was dispersed in 75% ammonium sulfate and stored at −80 °C in small aliquots.

AR activity was determined as described previously (Suryanarayana et al., 2004). The assay mixture of 1 ml consisted of 50 mM phosphate buffer (pH 6.2), 0.4 M lithium sulfate, 5 mM 2-mercapto ethanol, 10 mM DL-glyceraldehyde, 0.1 mM NADPH and rat lens AR preparation. The reaction mixture was incubated at 37 °C for 5 min and the reaction was initiated by adding NADPH. Change in the absorbance at 340 nm due to NADPH oxidation was measured by double beam spectrophotometer (Hitachi U-2910). For inhibition studies three concentrated stock preparations of *Aegle marmelos* leaf extracts were prepared in water. Various concentrations (1–25 μg) of these extracts were added to the reaction mixture and AR activity was assayed as described above. The percent inhibition with *Aegle marmelos* extracts was calculated considering AR activity as 100% in the absence of inhibitor. Quercetin, a known AR inhibitor, was used as a reference (Bendicht et al., 1982; Suryanarayana et al., 2004; Hyun et al., 2008; Bhanuprakash Reddy et al., 2011; Patel et al., 2012). The concentration of each extract resulting in 50% inhibition (IC50) was determined by non-linear regression analysis of log concentration of extract versus percentage inhibition.

2.4. Lens organ culture

Lens organ culture was performed as described earlier (Moghaddam et al., 2005). Eyeballs from 2 months old SD (Sprague Dawley) rats were collected and were dissected under a flow hood by an anterior approach. Individual lens devoid of any damage was incubated in 2 ml TC-199 medium at 37 °C, 5% CO_2 and 50 mM glucose in the presence and absence of ethyl acetate extract of *Aegle marmelos* (15 μg/ml). Lens incubated in the medium containing 5.5 mM glucose and 30 mM fructose was treated as osmotic controls. Lenses were maintained for 24 h for sorbitol estimation and for 10 days to study lens morphology. At the end of the incubation visual inspection was conducted by placing lenses on a transparent glass slide with grids.

2.4.1. Estimation of sorbitol in the lens

Lenses that were maintained in organ culture for 24 h were terminated and homogenized in 9 volumes of 0.8 M perchloric acid. The homogenate was centrifuged at 5000*g* at 4 °C for 10 min