Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Inhibitory effect of two Indian medicinal plants on aldose reductase of rat lens *in vitro*

Rajesh Kumar, Dinesh Kumar Patel, Damiki Laloo, Krishnamurthy Sairam, Siva Hemalatha*

Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, India

ARTICLE INFO

Article history:
Received 2 May 2011
Received in revised form 11 June 2011
Accepted 15 July 2011
Available online 20 September 2011

Keywords:
Caesalpinia digyna
Alangium lamarckii
Aldose reductase
Diabetic cataract

ABSTRACT

Objective: To assesse the inhibitory effect of alcoholic extract of two Indian medicinal plants namely *Ceasalpinia digyna* Rottler and, *Alangium lamarckii* Thwaits on aldose reductase (AR) of rat lens. **Methods:** Rats lens were enucleated through posterior approach and their homogenate was prepared and centrifuged to obtain a clear supernatant for the determination of AR activity and protein content. **Results:** The alcoholic extract of *Ceasalpinia digyna* and *Alangium lamarckii* had a potent inhibitory effect on the lens AR enzyme. The IC_{50} values of alcoholic extract of the selected plants were calculated and were (46.29±11.17) and (106.00±5.11) μg/mL, respectively. Quercetin was used as a positive control and its IC_{50} value was (2.95±1.53) μg/mL. **Conclusions:** Thus, it is concluded that alcoholic extracts of the selected plant exhibit significant inhibitory effects on AR in the rat lens *in vitro*.

1. Introduction

'Cataract' the leading cause of blindness worldwide is associated with several risk factors affecting the body and diabetes is one of the main culprits that is associated with it[1,2]. Diabetic cataract is a late stage complication in diabetic patient and one which dramatically affects the quality of life. Aldose reductase (AR) which is the first and rate-limiting enzyme, plays an important role in conversion of glucose to sorbitol through the polyol pathway. In diabetes, persistent hyperglycemia results in the formation of sorbitol through the polyol pathway in insulin-insensitive tissues such lens, retina, nerve and kidney[3]. Accumulation of sorbitol, an osmolyte, in the cells due to its reduced penetration through cellular membranes and slow metabolism by sorbitol dehydrogenase, leads to various biological alteration like osmotic swelling, change in membrane permeability, leakage of glutathione and myo-inositol, in addition the generation of free radicals and hydrogen peroxide leading to generation of oxidative offense[4]. The resulting hyperosmotic stress to cells is postulated to be the primary cause for the development of diabetic complications such as cataract, retinopathy, neuropathy and nephropathy[5]. The accumulation of polyol in eye lens fibers, influx of water and generation of osmotic stress has been implicated as some of the early events in diabetic cataracts which are mainly due to the increase in AR activity[6]. Thus, the inhibition of AR enzyme is therefore one of the potential pharmacological approaches that have been proposed to treat or ameliorate the secondary complications of diabetes causing cataract complication[7]. However, there are very few drugs that are available for the treatment of diabetic cataract. Till date, most of the aldose reductase inhibitor (ARI) has shown inadequate success, and some of the synthetic ARIs were associated with deleterious side effects and poor penetration of target tissues such as nerve and retina. Two main classes of ARI have been tested in phase III trials. While carboxylic acid inhibitors (zopolrestat, ponalrestat and tolerestat) have shown poor tissue permeability and are not very potent *in vivo*, spiroimide (spirohydantoin) inhibitors (sorbinil) penetrate tissues more efficiently but many have been associated with skin reactions and liver toxicity[8]. Hence the use of traditional medicines, mainly derived from plant sources, has been a major part in the management of many chronic ailments including diabetes, particularly in countries like India. Moreover, there is an increased interest in recent times to identify natural sources for their therapeutic properties. It is because most of the plant and plant products are mostly free from adverse effects and are being used as a source of diet and traditional medicine. A single plant may have many pharmacological activities (anti-diabetic, anti-oxidant and anti-stress activity), and they can be effectively utilized to delay or counter diabetic

*Corresponding author: Hemalatha Siva, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, India.
Tel: +91 9415256481
E-mail: shemalatha.phe@itbhu.ac.in

complications, such as cataract. These results together with recent clinical, experimental and pharmacological data provide powerful new support for the rationale for research and development of ARI. Plants like *Buddleja officinalisx* flower, *Corydalis turtschaninovii* rhizome and compound of plants like curcumin have shown delaying effect on experimental cataract. Therefore, it is important to evaluate new plant-derived agents scientifically[9,10]. Roots of *Ceasalpinia digyna* (*C. digyna*) and leaves of *Alangium lamarckii* (*A. lamarckii*), in Indian system of medicine are used traditionally for the treatment of diabetes[11,12]. Hence, in present study these two Indian medicinal plants were selected to evaluate inhibitory effect on AR of lens *in vitro*.

2. Materials and methods

2.1. Chemicals and instrumentation

DL-Glyceraldehyde and quercetin used for AR activity determination were obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA). NADPH was purchased from HiMedia laboratory Pvt. Limited, India. Other reagents and solvents were of analytical grade. Double beam UV spectrophotometer (Shimadzu, Pharmaspec 1700) was used for determining the absorbance of the sample.

2.2. Plant material & extraction

Dried leaves of *A. lamarckii* were collected from Panakudi, Tirunelveli District, India in the month of July 2009, whereas the root of *C. digyna* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India. Both two specimens were identified and authenticated by Prof. Chelladurai V, Research Botanist (Retd.), Palayamkottai, Tamilnadu, India. For future reference the herbarium of the voucher specimen: COG/CD/08 for *C. dignya* and COG/AL/09 for *A. lamarckii* were deposited at the Pharmacognosy Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi, India.

The root of *C. digyna* was chopped manually to small pieces and dried in shade. The dried root was powdered and passed through sieve No. 20 and extracted in a soxhlet extractor for 3 days using 95% ethanol as the menustrum. Extracts were concentrated to dryness using rotary evaporator under reduced pressure. Alcoholic extracts yielded a dark brown solid residue weighing 15% w/w.

Leaves of *A. lamarckii* were freed from foreign matter, dried under shade. They were crushed to coarse powder and subjected to extraction by cold maceration method using alcohol (95%) as an extraction menustrum for 7 days. The alcoholic extract of *A. lamarckii* was concentrated under reduced pressure to dryness. The final yield of the alcoholic extracts was 11.2% w/w. Both extract was preserved in a desiccator till further use.

2.3. Preparation of lens homogenate

Healthy albino rats of Charles foster strain (150-200 g) were obtained from the Central Animal House, (Reg. No. 542/02/ab/CPCSEA) Banaras Hindu University, Varanasi, India. They were kept in the departmental animal house at 22-28 ℃ and relative humidity 60%-70%, light and dark cycles 12-h dark: 12-h light. Animals were provided with standard rodent pellet diet and water was allowed *ad libitum*. Crude AR was prepared from rat lens. Eyeballs from normal rats were immediately taken out after sacrifice by cervical dislocation. All experiments on animals were conducted in accordance with and after approval by Institutional Animal Ethics Committee (No. Dean/2009-10/579). Lenses were dissected through posterior approach, washed with saline and their fresh weights were recorded. Non-cataractous transparent lenses were pooled and a 10% homogenate was prepared in 0.1M phosphate buffer saline (pH 7.4). After centrifugation at 5 000 × *g* for 10 min in a refrigerated centrifuge, the supernatant was collected and kept in ice for the determination of both AR activity and protein content. The determination of protein content in the supernatant of the lens homogenate was done as per the method of Lowry *et al*[13].

2.4. Determination of AR activity

AR activity was assayed according to the method described by Hayman and Kinoshita (1965). The assay mixtured in 1 mL contained 0.7 mL of phosphate buffer (0.067 M), 0.1 mL of NADPH (25×10^{-5} M), 0.1 mL of lens supernatant, 0.1 mL of DL-glyceraldehyde (substrate) (5×10^{-4}M). Appropriate reference blanks were employed for corrections containing all components except the substrate, DL-glyceraldehyde. The enzymatic reaction was started by the addition of the substrate and the absorbance or optical density (OD) was recorded in a double beam UV-spectrophotometer at 340 nm for at least 3 min at 30-s interval. AR activity was expressed as Δ OD/min/mg protein[13].

2.5. Lens AR activity along with plant extract

Dried extracts were reconstituted in phosphate buffer saline to prepare stock solutions. To determine their AR inhibiting activity, various concentration of two plant extract ranging from 25 μg/mL to 300 μg/mL were prepared. The reaction was initiated by the addition of 0.1 mL DL-glyceraldehyde with 0.1 mL plant extract and the rate of reaction was measured as described above. For standard quercetin the concentration was prepared ranging from 1.25 to 10 μg/mL. Δ OD/min/mg protein was calculated for each sample. Percent inhibition of AR activity was then calculated considering the activity of normal rat lens as 100%. IC_{50} value for each extract and standard quercetin were obtained from a dose-response curve calculated by plotting concentration versus percent inhibition.

2.6. Kinetic studies of inhibitory activity against AR

Kinetic studies of inhibitory activity against AR among the substrate, *A. lamarckii* and *C. digyna* extract were analyzed using the Lineweaver-Burk plot with varying concentrations of glyceraldehydes (5×10^{-4} to 30×10^{-4}) in the absence and presence of extracts. K_m and V_{max} were also estimated. K_i value in presence of extracts was determined by applying Cheng-Prusoff equation[14].

2.7. Statistical analysis

All values were expressed as mean±standard deviation (SD) of three independent readings. One-way ANOVA followed by Newman-Keuls multiple comparison tests was applied for

the statistical analysis of data. GraphPad Prism (version 5) software was used for all statistical analysis. A difference in the mean values of $P<0.05$ was considered to be statistically significant.

3. Results

Rat lens AR was utilized for the present study. The AR activity in normal rat lens was 0.0143±0.0001. Alcoholic extracts of both two plants were found to inhibit lens AR to various extent. The AR inhibitory activity of *C. digyna*, *A. lamarckii* and standard quercetin at different concentrations is presented in Figure 1. Quercetin, which is well known for its potent ARI, was used as a positive control and showed excellent activity with IC_{50} of (2.95±1.53) μg/mL. *C. digyna* had a considerable ARI activity with an IC_{50} value of (46.29 ±11.17) μg/mL as compared to that of *A. lamarckii* [(106.00 ±5.11) μg/mL]. One-way ANOVA showed significant differences among quercetin, *C. digyna* and *A. lamarckii* ($P<0.001$). From the IC_{50} value of *C. digyna* and *A. lamarckii*, it was observed that alcoholic extract of *C. digyna* offer more inhibition for the enzyme as compare to that of *A. lamarckii*.

Effects of alcoholic extract of *C. digyna* and *A. lamarckii* in the Lineweaver-Burk plot of RLAR-inhibitory activity with DL-glyceraldehyde as a substrate are shown in Figure 2. Kinetic studies of AR enzyme alone and with inhibitors were studied and their findings are presented in Table 1. Statistical analysis of the data obtained from the kinetic studies has showed significant differences between kinetic data obtained when only DL-glyceraldehyde substrate and DL-glyceraldehyde along with alcoholic extract of *C. digyna* and *A. lamarckii* ($P<0.001$). K_m value (0.599×10^{-3} mM) of enzyme was same in all the cases when glyceraldehydes was only substrate or in presence of extracts. Hence binding efficiency of enzyme towards substrate was same. Low value of K_m suggests that AR has higher affinity towards substrate (DL-glyceraldehyde). It was seen that there is a significant difference between the V_{max} and K_i value which showed that *C. digyna* offer more inhibition towards enzyme.

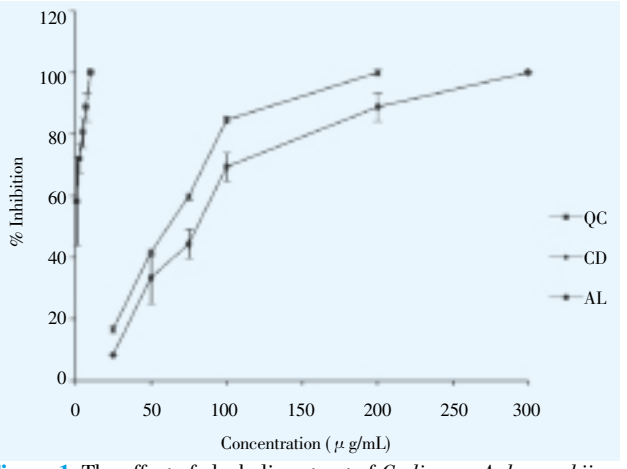

Figure 1. The effect of alcoholic extract of *C. digyna*, *A. lamarckii* and quercetin on AR activity.

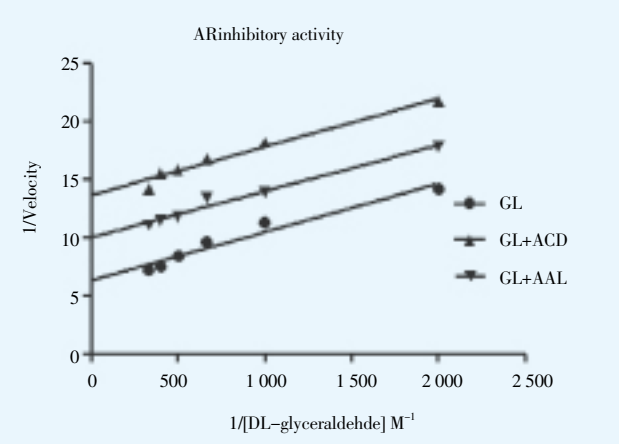

Figure 2. Effects of *C. digyna* and *A. lamarckii* extract on the Lineweaver-Burk plot of AR activity with DL-glyceraldehyde as substrate .

(●) absence of extract. (▲) and (▼) both *C. dignya* and *A. lamarckii* extract at concentration of 100 μg/mL.

Table 1

Kinetic properties of rat lens aldose reductase.

AR Activity	K_m	V_{max}	K_i
DL-glyceraldehyde	0.599×10^{-3} mM	0.1615 ± 0.0045	0.0000 ± 0.0000
DL-glyceraldehyde + *C. digyna*	0.599×10^{-3} mM	0.0750 ± 0.0025[a]	0.0905 ± 0.0211[a]
DL-glyceraldehyde + *A. lamarckii*	0.599×10^{-3} mM	0.1004 ± 0.0006[ab]	0.207 ± 0.0084[ab]

a: $P<0.001$ when compared to DL-glyceraldehyde and b: $P<0.001$ when compared to DL-glyceraldehyde + *C. digyna*.

4. Discussion

'Cataract' is regarded as a multifactorial eye disease which is medically associated with several risk factors and it is responsible for the cause of around 50% blindness worldwide. The only remedy for the treatment of such kind of disease is surgery. However, the incidence is so large that the available surgical facilities are unable to cope up with problems because of the occurrence of postoperative complications such as posterior capsular opacification, endopthalmitis and uncorrected residual refractive error[15]. AR which is the key enzyme in the polyol pathway, is a NADPH-dependent oxidoreductase, and plays a central role in the reduction of aldose to polyol. Under diabetic conditions excessive influx of glucose in tissues takes place, resulting in an increased rate of the AR related polyol pathway[16]. Furthermore, growing evidence suggests that the enzyme AR may provide a common biochemical link in the pathogenesis of many of these diabetic complications, including sugar cataract. Several animal studies and preliminary clinical trials indicate that inhibition of AR may prevent the onset of other diabetic complications such as neuropathy, corneal epitheliopathy, retinopathy and microangiopaty[17,18]. The present study on alcoholic extract of both medicinal plants has showed remarkable inhibitory activities on rat lens AR, and inhibitory action of *C. digyna* was significantly higher than *A. lamarckii*. The Lineweaver-Burk plot of 1/velocity *vs.* 1/(DL-glyceraldehyde) in the

presence of each extract was parallel to that in the absence of each compound. Thus, *C. digyna* and *A. lamarckii* extract inhibited rat lens AR activity in a noncompetitive manner. Thus, it is speculated that both the extracts combined neither with the free enzyme nor binded with the normal substrate (DL-glyceraldehyde), but rather with the enzyme-substrate complex to produce the inactive enzyme-substrate-inhibitor complex. Chemicals obtained from plants constitute a rich source of bioactive molecule against AR. Additionally, some flavonoids and polyphenols as well as sugar derivatives are found to be effective inhibitors of AR[19]. Preliminary phytochemical screenings of alcoholic extract of *A. lamarckii* have showed the presence of phenol and tannin[20]. Further phenolic and tannin also reported to present in *C. digyna* and possess significant antioxidant activity both *in vitro* and *in vivo*[21]. In our previous study the alcoholic extract and sub-fractions of *A. lamarckii* have already been reported to possess significant antioxidant activity different *in vitro* models[22]. AR activity might be due to presence of phenolic and tannaoid principals present in the extract of *C. digyna* and *A. lamarckii*. However, it was subjected to further investigation. Hyperglycemia can induce oxidative stress via several mechanisms. These include glucose autoxidation, formation of advanced glycation end-products, and activation of the polyol pathway. The latter induces intracellular overload of sorbitol and have been proposed as initial event of various types of ocular lesions. Additionally, this polyol was involved in generation of peripheral neuropathy[23]. Both the extracts *ie. C. digyna* and *A. lamarckii* have displayed significant antioxidant (*in vitro* and *in vivo*) and can cause significant inhibitory effect on activation of polyol pathway which can ameliorate the secondary complications of diabetes due to long term glucose toxicity[21,22].

In conclusion, these results indicate that both the extracts have inhibitory effects against rat lens AR *in vitro*. Further, activity guided fractionation may leads to isolation of molecule(s) responsible for AR inhibitory activity and their use *in vivo* and clinical efficacies remain to be evaluated.

Conflict of interest statement

The authors report no conflict of interest. The authors themselves are responsible for writing and content of paper.

References

[1] Agrawal SS, Gupta SK, Srivastava KS, Saxena R. Potential of food supplement, soya bean as a novel anti cataract agent. *Int J Integr Biol* 2008; **3**(1): 9-17.

[2] Lee AYW, Chung SSM. Contributions of polyol pathway to oxidative stress in diabetic cataract. *Fed Am Soc Exp Biol* 1999; **13**(1): 23-30.

[3] Ueda H, Tachibana Y, Moriyasu M, Kawanishi K, Alves SM. Aldose reductase inhibitors from the fruits of *Caesalpinia ferrea* Mart. *Phytomedicine* 2001; **8**(5): 377-381.

[4] Moghaddam MS, Kumar PA, Reddy GB, Ghole VS. Effect of Diabecon on sugar-induced lens opacity in organ culture: mechanism of action. *J Ethnopharmacol* 2005; **97**(2): 397-403.

[5] Lee HS. Rat lens aldose reductase inhibitory activities of *Coptis japonica* root-derived isoquinoline alkaloids. *J Agric Food Chem* 2002; **50**(24): 7013-7016.

[6] Jung HA, Islam MDN, Kwonc YS, Jin SE, Son YK, Park JJ, et al. Extraction and identification of three major aldose reductase inhibitors from Artemisia Montana. *Food Chem Toxicol* 2011; **49**(2): 376-384.

[7] Sivakumari K, Rathinabai AFMC, Kaleena PK, Jayaprakash P, Srikanth R. Molecular docking study of bark-derived components of *Cinnamomum cassia* on aldose reductase. *Indian J Sci Technol* 2010; **3**(8): 1081-1088.

[8] Muthenna P, Suryanarayana P, Gunda SK, Petrash J M, Reddy GB. Inhibition of aldose reductase by dietary antioxidant curcumin: Mechanism of inhibition, specificity and significance. *FEBS Letters* 2009; **583**(22): 3637-3642.

[9] Suryanarayana P, Kumar PA, Saraswat M, Petrash JM, Reddy GB. Inhibition of aldose reductase by tannoid principles of Emblica officinalis: Implications for the prevention of sugar cataract. *Mol Vis* 2004; **10**: 148-154.

[10] Vats V, Yadav SP, Biswas NR, Grover JK, Anti-cataract activity of *Pterocarpus marsupium* bark and *Trigonella foenum*-graecum seeds extract in alloxan diabetic rats. *J Ethnopharmacol* 2004; **93**(2,3): 289-294.

[11] Kiritikar KR, Basu BD. *Indian medicinal plants*. Dehradun: International Booksellers and Publishers; 1999.

[12] Pullaiah T, Chandrasekhar NK. *Antidiabetic plants in India and herbal based antidiabetic research*. New Delhiw: Regency Publications; 2003.

[13] Patel MB, Mishra SM, Aldose reductase inhibitory activity of a C-glycosidic flavonoid Derived from Enicostemma hyssopifolium. *J Altern Complement Med* 2009; **6**(1): 1-17.

[14] Daniellou R, Zheng H, Palmer DRJ. Kinetics of the reaction catalyzed by inositol dehydrogenase from *Bacillus subtilis* and inhibition by fluorinated substrate analogs. *Can J Chem* 2006; **84**(4): 522-527.

[15] Verma SD, Hegde KR. Effect of α-kitoglutarate against selenite *Cataract formation*. *Exp Eye Res* 2004; **79**(6): 913-918.

[16] Jung HA, Kim YS, Choi JS. Quantitative HPLC analysis of two key flavonoids and inhibitory activities against aldose reductase from different parts of the Korean thistle, *Cirsium maackii*. *Food Chem Toxicol* 2009; **47**(11): 2790-2797.

[17] Reddy GB, Satyanarayana A, Balakrishna N, Ayyagari R, Padma M, Viswanath K, et al. Erythrocyte aldose reductase activity and sorbitol levels in diabetic retinopathy. *Mol Vis* 2008; **14**: 593-601.

[18] Scotti L, Fernandes MB, Muramatsu E, Pasqualoto KFM, Emereciano VP, Tavares LC, et al. Self-organizing maps and volsurf approach to predict aldose reductase inhibition by flavonoid compounds. *Rev Bras Phcog* 2011; **21**(1): 170-180.

[19] Lee H. Inhibitory activity of *Cinnamomum cassia* bark-derived component against rat lens aldose reductase. *J Pharm Pharmaceut Sci* 2002; **5**(3): 226-230.

[20] Kumar R, Hemalatha S. Pharmacognostical standardization of leaves of *Alangium lamarckii* Thwaites. *Phcog J* 2011; **2**(18): 19-25.

[21] Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B, Antioxidant activity of *Caesalpinia digyna* root. *J Ethnopharmacol* 2007; **113**(2): 284-291.

[22] Kumar R, Hemalatha S. In vitro antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. *J Chem Pharm Res* 2011; **3**(1): 259-267.

[23] Pastene E, Avello M, Letelier MM, Sanzana E, Vega M, González M. Preliminary studies on antioxidant and anti-cataract activities of *Cheilanthes glauca* (Cav.) Mett. through various in vitro models. *Electronic J Food Plants Chem* 2007; **2**(1): 1-8.