Anti-osmotic and antioxidant activities of gigantol from *Dendrobium aurantiacum var. denneanum* against cataractogenesis in galactosemic rats

Hua Fang a, Xiaohong Hu a, Meiling Wang a, Wencheng Wan a, Qiaohong Yang a, Xiaosheng Sun a, Qiong Gu b, Xinxin Gao a, Zhengtao Wang a, Lianquan Gu b, C.-Y. Oliver Chen d, Xiaoyong Wei a,d,*

a School of Basic Medical Sciences, Guangzhou University of Chinese Medicine, Guangzhou 510006, PR China
b Institute of Drug Synthesis and Pharmaceutical Processing, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510275, PR China
c Shanghai R&D Center for Standardization of Chinese Medicines, Institute of Chinese Materia Medica of Shanghai University of Traditional Chinese Medicine, Shanghai 201203, PR China
d Antioxidants Research Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111, USA

ARTICLE INFO

Article history:
Received 19 December 2014
Received in revised form 7 May 2015
Accepted 11 June 2015
Available online 25 June 2015

Keywords:
Dendrobii
Gigantol
Catarract
Aldose reductase (AR)
Inducible nitric oxide synthase (iNOS)

Mechanisms

Chemical compounds studied in this article:
Gigantol (PubChem CID: 10221779)
NADPH (PubChem CID: 52945042)
D-galactose (PubChem CID: 86278404)
Dimethyl sulfoxide (PubChem CID: 679)
L-arginine (PubChem CID: 6222)
Pencillin G (PubChem CID: 5904)
Streptomycin (PubChem CID: 19649)
Ethanol (PubChem CID: 702)
Ethyl acetate (PubChem CID: 8857)
Amonium acetate (PubChem CID: 517165)
Butyl alcohol (PubChem CID: 263)
Methanol (PubChem CID: 887)
Dichloromethane (PubChem CID: 6344)
Tetrahydrobiopterin (PubChem CID: 1125)
Piroperoxine sodium (PubChem CID: 23695982)

ABSTRACT

Ethnopharmacological relevance: *Dendrobium aurantiacum var. denneanum* widespread in southern China, locally known as “Shihu,” “Huangcao” or “Fengdou,” has long been used in traditional Chinese medicine for antipyretic, immunomodulatory, anti-aging effects and eye benefiting.

Aim of this study: To investigate the effects of gigantol extracted from the stem of *D. aurantiacum var. denneanum* on the formation of galactose-induced cataractogenesis and the potential mechanisms underlying these effects.

Materials and methods: Cataract lens models were induced by D-galactose both in vitro and in vivo. The transparency of the rat lenses in vitro and in vivo was observed with an anatomical microscope and a slit lamp microscope. The differential protein and action targets of gigantol were determined and compared among the control group, model group, and gigantol group using two-dimensional electrophoresis and mass spectrometry (MS). Enzyme kinetics was used to show the ability of gigantol to repress aldose reductase (AR) and inducible nitric oxide synthase (iNOS). Quantitative real-time PCR (RT-qPCR) was used to detect repression of the expression of AR and iNOS genes. Molecular docking and dynamic simulation were used to predict the interaction points and combination patterns between gigantol, AR, and iNOS.

Results: Gigantol was found to prevent galactose-induced damage to the rat lenses both in vitro and in vivo, to delay lens turbidity, and to keep the lenses transparent. Differential proteomes, MS, and RT-qPCR showed AR and iNOS to be the target proteins of gigantol. Gigantol reduced the galactose-induced AR and iNOS mRNA expression by 51.2% and 60.9%, respectively. The IC50 of gigantol for inhibition of AR and iNOS activities were 65.72 μg/mL and 8.768 μg/mL, respectively. Gigantol–AR binding sites were Trp111, His110, Tyr48, and Trp20, and gigantol–iNOS binding sites were Ile195 and Gin257. The main forms of interaction were hydrophobic forces, hydrogen bonds, and van der Waals forces.

Conclusion: Gigantol extracted from *D. aurantiacum var. denneanum* was found to inhibit galactose-induced formation of cataracts through repression of the gene expression and activity of AR and iNOS.

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1. Introduction

Cataract is the leading cause for impaired vision and blindness in patients with diabetes worldwide (Harding et al., 1993). Although the pathogenesis of diabetic cataracts (DC) is not fully understood, hyperglycemia-associated increases in osmotic pressure and oxidative damages definitely contribute to their development and progression (Hashim and Zarina, 2012). In a hyperglycemic condition, aldose reductase (AR) in the polyol pathway is upregulated and overproduces sorbitol from glucose (Costantino et al., 2002; Suzen and Buyukbingol, 2003). As sorbitol is unable to cross the cell membrane freely via diffusion, its overproduction augments intracellular osmotic pressure and lens swelling due to excess fluid infusion against the osmotic gradient. Free radicals generated in endoplasmic reticulum under enhanced osmotic pressure can also damage lens fibers (Pollreisz and Schmidt, 2010). Thus, chronic sorbital accumulation in lens degenerates hydropic lens fibers and induces damage in ocular cells and apoptosis in lens epithelial cells, eventually leading to the formation of cataracts (Varma et al., 1977; Lightman, 1993; Kinoshita, 1974; Takamura et al., 2001). In addition to AR induced harms on lens, continuous formation of nitric oxide (NO) from the inducible nitric oxide synthase (iNOS) during hyperglycemia contributes to promotion of DC development (Nathan, 1995; Nathan and Xie, 1994; Förstermann et al., 1994; Lechner et al., 2005). Furthermore the formation of peroxynitrite from NO and superoxides exacerbates oxidative damages on lens.

Current available treatments for DC include cataract surgery using the phacoemulsification technique and anti-cataract medications, e.g., aldose reductase inhibitors (ARI) and antioxidants. Cataract surgery is effective to improve vision but the outcomes tend to be poor in patients with diabetes as compared to patients without diabetes (Matsumoto et al., 2008). Preclinical evidence has shown a variety of compounds including plant extracts, animal tissues, and small molecules, can act as an ARI to delay, prevent, or even reverse the development of cataracts (Sadiq et al., 1995; Kador and Sharpless, 1978).

Dendrobium aurantiacum var. denneanum (D. aurantiacum) locally known as “Shihu”, “Huangcao” or “Fengdou” in China is widely distributed in southern China, Burma, Laos, Thailand and other parts of South Asia. It has long been used in traditional Chinese medicine for treatment of multiple symptoms or indications, e.g., nourishing yin and clearing heat, nourishing the stomach, moistening the lungs to stop cough, improving vision, and anti-aging (Commission of Chinese Pharmacopoeia, 2010; Yang et al., 2006, 2007; Ng et al., 2012). “Mi Chuan Yan Ke Qi Shi Er Zheng Quan Shu” (Seventy-two Esoteric Ophthalmology Book, written by Xue-yuan Yuan, Ming-China) described Herba Dendrobii as pungent in the nature, making the blind see. “Shennong’s Herbal” also indicated that D. aurantiacum has the actions of tonifying deficiency of five zang-organs, nourishing yin, and improving vision. There are a few herbal recipes containing D. aurantiacum, such as "Shihu-ye-guang-wan" (Shihu yeguang pills, from Volume 14 of “Tai Ping Shen Hui Fang”, Holy Prescriptions for Universal Relief, Song-China), Shi-hu-san (from Volume 110 of “Shen Ji Zong Lu”, General Records of Holy Universal Relief, Song-China). These D. aurantiacum-derived Chinese herbal medications are now extensively administered to treat cataracts in clinical ophthalmology (Commission of Chinese Pharmacopoeia, 2010; West et al., 2006).

Nevertheless, D. aurantiacum has been used for multiple indications or symptoms, the bioactives exerting the mechanism of actions remain uncharacterized (Li et al., 2008; Lo et al., 2004a; Lo et al., 2004b).

Gigantol (3′,4-dihydroxy-3,5′-dimethoxy-benzyl) is a benzyl-type phenolic compound extracted from the stem of D. aurantiacum (Yang et al., 2006). Gigantol displays a wide range of pharmacological activities and does not cause adverse effects (Miyazawa et al., 1997; One et al., 1995). In this study, we aimed to examine the effect of gigantol on the development and progression of galactose-induced cataract in rats. We also examined whether its protective effect was mediated through regulation of AR and iNOS.

2. Materials and methods

2.1. Reagents

DL-glyceraldehyde (purity ≥ 90%), trifluoroacetic acid (TFA) and β-actin were purchased from Sigma-Aldrich (St. Louis, MO, USA), NADPH from Roth Co. (Italy), β-galactose (purity ≥ 99%) and dimethyl sulfoxide (DMSO, purity ≥ 99.9%) from Amresco (Solon, OH, USA), Pirenoxine sodium (purity ≥ 98%) from Langchem Inc. (Shanghai, P.R. China), Pirenoxine sodium eye drops (Batch no. 070102, 0.8 mg pirenoxine sodium in 15 mL vehicle solution) from Wuhan Tianianting Pharmaceutical Group Co., Ltd. (Wuhan, P.R. China), PCR kit, restriction enzyme, T4 ligase, and low molecular mass standard protein from TaKaRa Corp (Japan), plasmid extraction kit, gel retrieval kit, and PCR product retrieval kit from Qiagen Co. Ltd (Germany), Quik Change XL site-directed mutagenesis kit from Stratagene (La Jolla, CA, USA), DpnI enzyme from Promega (Madison, WI, USA), and the pET28b-AR plasmid from Novagen (Germany). Dulbecco’s modified Eagle’s Medium (DMEM), fetal bovine serum (FBS), penicillin /streptomycin were obtained from Gibco BRL (Grand Island, NY, USA), Reverse Transcription System Kit from Promega (Madison, WI, USA), Nitric Oxide Synthase Assay Kit from Nanjing Jiacheng Bioengineering Institute (Nanjing, P.R. China). The other reagents were in analytical grade and obtained from vendors in China.

2.2. Plant authentication and extraction

The stems of D. aurantiacum var. denneanum (kerr) Z.H. Tsi were collected from Lesan County, Sichuan Province, P.R. China, December 2012, and authenticated by Professor Min Li and Tingmo Zhang of the Chengdu University of Chinese Medicine, where a voucher specimen (No. 2012122001) has been deposited in Wan’an Dendrobium Industry and Development Co., Ltd. (Sichuan Province, P.R. China). Gigantol was extracted from Dendrobium according to the protocol of Yang et al. (2006).

2.3. Evaluation of lens opacification in vitro

Lens opacification was assessed according to Lentini et al. (2011) with slight modifications. Briefly, the lenses were carefully enucleated from Wistar rat eyes using a posterior approach. After rinsing twice with phosphate buffer saline (PBS) and once with Dulbecco’s modified eagle medium (DMEM), the lenses were incubated in DMEM containing 20% fetal bovine serum (FBS),...
above a black background and then observed under an anatomical microscope (Olympus ST-PT type, Japan). The degree of opacity was graded according to the line clarity through the lens: grade 0 (0): absence; grade 1 (+): slight opacity; grade II (++): presence of diffuse opacity; grade III (+++): presence of extensive, thick opacity. The incidence of cataract is expressed as the percentage of different grades of lens in each group at different times (Neal et al., 2010).

2.4. Evaluation of lens opacification in vivo

Eighty Wistar rats (6 weeks old, 80–120 g body weight) were acquired from the Research Animal Laboratory of Guangzhou University of Chinese Medicine. They were housed and cared according to the National Institute of Health (NIH) animal care guidelines. The animal protocol was approved by the Animal Care Committee of Guangzhou University of Chinese Medicine prior to the initiation of the experiment.

Rats with transparent lenses were subject to slit-lamp examination and used for evaluation of lens opacification. During one week of acclimation, both eyes of each rat were treated with tropicamide eye drops daily. The rats were then randomly assigned to one of 4 groups (n=20). Control group received 10 mL/kg saline daily via ip injection and had free access to standard drinking water throughout the experiments. To develop DC, rats in the other 3 groups were ip injected with 10 mL/kg 50% (w/v) α-galactose twice a day for 30 days and provided with water containing 10% (w/v) α-galactose (Suryanarayana et al., 2005). Initiation and progression of lenticular opacity was ranked in 5 grades: stage 0, clear lenses and no vacuoles present; stage 1, vacuoles cover approximately one half of the surface of the anterior pole, forming a subcapsular cataract; stage 2, some vacuoles have disappeared and the cortex exhibits a hazy opacity; stage 3, a hazy cortex remains and dense nuclear opacity is present; and stage 4, a mature cataract with dense opacity develops in both cortex and nucleus (Suryanarayana et al., 2005). On day 30, the rats with the stage 1 and 2 grade were randomly assigned to one of 3 groups: Model group (cataractous rats treated with 50 μL saline), Gigantol group (cataractous rats treated with 50 μL of 4 mg/mL gigantol), and Pirenoxine group (cataractous rats treated with 50 μL of 0.053 mg/mL pirenoxine sodium eye drops as the manufacturer suggested). They received the eye drops 3 times per day and one drop (50 μL/eye drop) each time to each eye. Lens opacity was monitored for 60 days using continuous slit lamp biomicroscopy (Phoenix Optical Co., Ltd. China). At the end of experiment, lenses were collected and treated with Radio-Immunoprecipitation Assay (RIPA) lysis buffer (1:10, w/v) for 1 h at room temperature. After centrifugation for 30 min at 2000 rpm and 4 °C (Eppendorf, Germany), the supernatant was collected of proteomics and mass spectrometry analyses.

2.5. Differential proteomics and MS analyses

Protein content in the supernatant of the rat lens in vivo lysate was determined using Bradford assay kit. Proteins were separated according to their isoelectric point (pI) by isoelectric focusing (IEF) using a Bio-Rad IEF Cell. Proteins (350 μg) separated as they migrated on a 20 cm Immobilized pH gradient (IPG) Bio-Rad strip through the pH gradient from pH 5 to 8 in response to the applied voltage. The gel images were analyzed using Bio-Rad PD Quest image analysis software (Version 7.1.1).

Proteins expressed between groups in the IEF analysis were dissolved in 0.1% trifluoroacetic acid (TFA) and then analyzed using JMS-T100LP LC-Tandem TOFMS (Jasco, Japan). Sample (0.4 μL) was loaded onto a 384-well stainless steel plate, followed by addition of 0.4 μL of 10 mg/mL α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution. After the sample and matrix solutions were air-dried, proteins were characterized by the LC–TOF–MS equipped with GPS Explorer V. 3.6 software (AB SCIEX, USA).

2.6. Assessment of AR and iNOS activity in rat lens

AR was extracted and purified from rat lens using Chakrabarti et al. (1987) method with slight modifications. Briefly, the whole procedure was carried out in the presence of 5 mmol/L β-mercaptoethanol at 4 °C. Fresh rat lens was homogenized, and after centrifugation at 10,000 rpm for 30 min at 4 °C, the resulting supernatant was collected and mixed with 17.5 g/100 mL ammonium sulfate until the solution reached saturation.

The ability of gigantol to modulate AR activity was assessed using the spectrophotometric method of Sahoo and Behera (2010). Briefly, AR was incubated with gigantol at selected concentrations. Gigantol stock solution was prepared in DMSO. The experiment was performed in triplicate. The percentage of gigantol-mediated inhibition on AR activity was calculated as described by Halder et al. (2003). The data is expressed as IC50 obtained from the regression line plotted with gigantol concentrations and % inhibition.

The ability of gigantol to inhibit iNOS activity was assessed using a colorimetric assay. iNOS activity was assessed by monitoring NO production using Nitric Oxide Synthase Assay Kit, which was developed according to the method of Frohlich et al. (1999). The iNOS inhibition is expressed as % inhibition rate calculated using the equation: (% inhibition)=[(B0–B1)]/B0]×100%, where B0 and B1 represent the absorbance values obtained for the blank (control solution) and standard, respectively, and B2 represents the absorbance with the addition of gigantol. The experiment was performed in duplicate.

2.7. Quantitative real-time PCR

Total RNA in homogenized rat lenses in vivo was extracted using TRizol reagent. RNA purity was assessed using an ultraviolet spectrophotometer and its integrity was assessed on 1.2% formaldehyde denaturing agarose gel. cDNA was synthesized using an Improm-II TM Reverse Transcription System Kit. The abundance of AR (951 bp), iNOS (262 bp), and β-actin (420 bp) mRNA in the samples were quantified using SYBR Green-based Prism 7000 real-time PCR system (ABI, Grand Island, NY, USA). The amplification was performed for 40 cycles (denaturing at 95 °C for 10 s, annealing at 90 °C for 5 s, and extension at 60 °C for 31 s). The primers were synthesized by Boya Biotechnology Co. Ltd. (Shanghai, China). The forward and reverse primer sequences were as follows: 5′-AGGGTTTATTCACTACGGT TT-3′ and 5′-AGGGCAGTCGTTCACTTCCAGCA CACGC C-3′ for iNOS, and 5′-GAGGCTCAGCAACACCAGCC-3′ and 5′-GCGGGGCATCGGAACGC-3′ for β-actin.

2.8. Molecular docking

A flexible docking module, Flex X in Sybyl v.17.3, was used to
study the molecular docking (MD) of gigantol onto AR. A core site in the ligand molecule was first selected and then docked into the active site of AR. Subsequently, the rest of the fragments were connected using a tree-search method. Binding between the ligand and the active site was evaluated based on the semi-empirical free energy formula similar to that proposed by Böhm (1992a, 1992b, 1994). The crystal structure of AR was obtained from the Protein Data Bank (PDB) database (PDB No: 1USO, Koukoulitsa et al., 2010). The range within a 6.5 Å distance around the ligand in the crystal structure of AR was defined as the active pocket for gigantol docking.

To investigate the molecular docking between gigantol and iNOS, the crystal structure of iNOS–ligand complex (ID No: 2NOS, ligand amino guanidine) was obtained from PDB database (http://www.rcsb.org/pdb/home/home.do). The resolution of this crystal structure was 2.30 Å, satisfying the precision requirement of docking. Water molecules were detected using Molecular Operating Environment (MOE) docking software, and a Merck molecular force field 94x (MMFF94X) force field was selected for protonation. The MMFF94 X force field was also used to calculate partial charges and to optimize the minimum energy conformation. The optimized conformation was used for analysis of docking events. In this way, an original model was constructed and the interactions between the ligand and the receptor were evaluated. Amino guanidine was selected as a reference compound (Wang et al., 2009; Kyselova et al., 2004).

2.9. Statistical analysis

All data are expressed as mean ± SD. All analyses were performed using the SPSS 16.0 for Windows. P < 0.05 was considered statistically significant.

3. Results

3.1. Effect of gigantol on rat lens opacification in vitro

All the lenses in control group remained transparent up to 24 h (Figs. 1 and 2). In parallel with treatment duration, galactose alone induced opacification and resulted in 100% grade III opacification with a milky-white color clearly visible to the naked eyes at 24 h. Both gigantol and pirenoxine displayed different degrees of protection against galactose-induced opacification at 12, 18, and 24 h. Among 3 gigantol dosages, the 4 mg/mL dose appeared to be the most effective to protect against galactose induced opacification. The Hi-gigantol displayed a comparable protection to pirenoxine. Lo-gigantol kept 80% of lenses in grade II opacification at 24 h when all lenses treated with galactose had grade III opacification.

3.2. Effects of gigantol on the opacification of rat lenses in vivo

α-galactose induced cataracts were first noted on day 23 and reached stage 2–3 grades on day 30. On day 90, 50% of rat lenses developed stage 4 cataracts. Gigantol treatment significantly attenuated severity of cataracts in a time-dependent manner. After 10 days of the gigantol treatment, lens opacification was significantly improved, and the majority of rats had the opacification regressed to stage 0 after 60 days of the treatment (Figs. 3 and 4). Pirenoxine was effective to treat α-galactose induced cataracts by delaying the progression or even curing opacification, but gigantol was found to be more effective than pirenoxine (P < 0.05, Fig. 3).

3.3. Protein expression in rat lens and the target protein

There were 340 ± 6 protein spots in the control group, 427 ± 5 in the α-galactose, and 300 ± 8 in the gigantol (Fig. 5). Most proteins were mainly distributed in relative molecular mass (Mr) 14,400–43,000, and 6–8 regions. Analysis of electrophoretic repeatability showed the matching ratios of two-dimensional electrophoresis (2-DE) maps at 90 ± 4%, 89 ± 8%, and 91 ± 2% in the control, galactose, and gigantol, respectively. As compared to the control, galactose enhanced expression of 32 protein spots by > 2 fold with a 99% confidence level. As compared to galactose, gigantol intensified 4 protein spots by 2 fold with a 99% confidence level and weakened 7 protein spots. Eleven protein spots were identified as α-, β-, and γ-proteins of the lens and related active enzymes using mass spectrometry. The expression of lens structural proteins βA3, βB1, and γN were increased by galactose and significantly decreased by gigantol. The expression of cell-metabolism-related cytoplasmic proteins in lens epithelial cells were significantly elevated by galactose and significantly decreased by gigantol. The lens proteins αA and βA4 showed up-regulation in

![Fig. 1. Gigantol protected galactose treated lenses from development of opacification in vitro. The results are expressed as mean ± SD (n = 10). *P < 0.05 vs all other groups; †P < 0.01 vs. Hi-gigantol and Lo-gigantol.]()
AR (5205R) and iNOS (3505R) were identified in the 2-DE map, and their expressions were increased by galactose and ameliorated by gigantol (Fig. 5).

### 3.4. Effect of gigantol on AR and iNOS activities

Gigantol at concentrations of 5, 10, 100, and 200 μg/mL inhibited AR activity in a dose dependent manner with the IC50 of 65.67 μg/mL. Gigantol at concentrations of 0.0596, 0.164, 0.493, and 0.74 μg/mL inhibited iNOS activity in a dose dependent manner with the IC50 of 8.768 μg/mL. The double reciprocal plot shows that gigantol was an uncompetitive inhibitor in modulation of AR and iNOS (Figs. 6 and 7).

### 3.5. AR and iNOS gene expression in rat lens

AR and iNOS mRNA expression was increased by galactose by 4.3 and 5.1 fold, respectively, compared to the control (Fig. 8).
Gigantol reduced the galactose-induced AR and iNOS mRNA expression by 51.2% \((p < 0.01)\) and 60.9% \((p < 0.05)\), respectively.

### 3.6. Gigantol binding sites on AR and iNOS

The docking analysis showed that AR has 10 docking conformations using the rule of the smallest root-mean-square deviation (RMSD) value and the highest score (Fig. 9). The literature has shown that the hotspot residues for AR inhibitors include Trp111, His110, and Tyr48 (Steuber et al., 2006; Maccari et al., 2008, 2010; Koukoulitsa et al., 2010). In addition to binding to the above 3 hotspot residues, gigantol could bind to Trp20. We predicted that the 1′-hydroxy moiety of gigantol formed hydrogen bonds with His110 and Tyr 48, the left benzene ring formed a π–π conjugation with Trp111 through van der Waals forces, and the 5′-methoxy moiety formed a hydrogen bond with Trp20.

Molecular docking simulation between gigantol and iNOS showed the 4-OH and 3-methoxy moieties of gigantol formed hydrogen bonds with Ile195 and the 5′-methoxy moiety formed a hydrogen bond with Gln257 (Fig. 10). These 3 hydrogen bonds contribute to gigantol-mediated iNOS inhibition.

Hydrophobic forces also existed between gigantol and AR. We anticipated that all of these bonds between gigantol and AR active site contribute to its inhibitory effect. Molecular docking simulation between gigantol and iNOS showed the 4-OH and 3-methoxy moieties of gigantol formed hydrogen bonds with Ile195 and the 5′-methoxy moiety formed a hydrogen bond with Gln257 (Fig. 10). These 3 hydrogen bonds contribute to gigantol mediated iNOS inhibition.

### 4. Discussion and conclusions

Although the pathogenesis of DC is not yet elucidated, its development and progression could be attributed to a few theories, e.g., osmotic pressure, oxidative stress, and protein glycosylation...
Both osmotic pressure and oxidative stress induced by hyperglycemia up-regulate the expression and activity of AR, in turn resulting in the conversion of glucose into sorbitol and its ultimate accumulation in lenses (Hong et al., 2000). Such sorbitol accumulation enables a vicious cycle of osmotic pressure and sorbitol production. The osmotic pressure itself causes ischemia, anoxia, and iNOS induction, which all contribute to generations of free radicals and consequent oxidative stress, damages in lens proteins and membranes of lens epithelium, lens opacification (Kisić et al., 2009; Alexiou et al., 2009). Thus, approaches to inhibiting AR expression and/or expression may hold a great potential for prevention and treatment of diabetic cataracts (Srivastava et al., 2005; Reddy et al., 2011; Sadiq et al., 1995; Kojima et al., 2007).

*D. aurantiacum* has been used in Chinese medicine to improve/maintain vision and treat certain eye diseases, including cataracts, for many years (Commission of Chinese Pharmacopoeia, 2010). Nevertheless, bioactive constituents in *D. aurantiacum*, as well as mechanism of actions, have not been characterized (Ding et al., 2008). Of identified constituents, gigantol contains active phenolic hydroxyl groups, displays aromatic and hydrophobic characteristics, and exerts a wide range of pharmacological activities (Fan et al., 2014; Sadiq et al., 1995; Kojima et al., 2007; Reyes-Ramírez et al., 2011). Our results demonstrate that gigantol was effective to prevent and delay the development of lens opacification in vitro and the development of cataracts in vivo.

AR and iNOS play an important role in development and progression of DC (Lee et al., 2014). We found that gigantol could be administered to prevent/treat DC through its inhibitory effect on these 2 critical enzymes at the level of enzymatic activity as well as the gene expression. First, based on enzyme kinetics, gigantol can inhibit directly the enzymatic activities of the AR and iNOS. By suppressing the AR, gigantol decreased the osmotic pressure within the lens, reducing the fluid infusion and the eventual damage caused by the osmotic pressure stress. The inhibitory effect of gigantol on the iNOS also led to decreased production of the free radical NO, alleviating the damage caused by oxidation was also alleviated. Second, based on proteomics and mRNA levels, gigantol can also significantly inhibit expression of the AR and iNOS genes and reduced the amount of AR and iNOS in lens epithelial cells. Those also indicate that gigantol is a multi-target therapeutic drug. Its molecular targets were AR, iNOS, AR gene and iNOS gene, respectively. Gigantol targeted the pathogenesis of cataracts through these two mechanisms simultaneously, both decreasing osmotic...
pressure stress and reducing oxidative damage. This explains why gigantol was able to prevent and treat the cataracts effectively and quickly.

Simulations of molecular docking and dynamics were performed to uncover more details regarding the molecular mechanisms underlying the interactions between gigantol, the AR, and iNOS. Gigantol was found to interact with the AR at sites Trp111, His110, Tyr48, and Trp20, mainly via hydrophobic forces, hydrogen bonding, and van der Waals forces. Especially, gigantol and AR form a π-π conjugation by the binding site Trp111. Furthermore, the gigantol has a new binding site, Trp20, not commonly found in most AR inhibitors. In this way, the binding between the AR and gigantol is more stable and stronger. In the direct inhibitory activity of gigantol against the iNOS, 4-OH and 3′-methoxy moieties of gigantol formed hydrogen bonds with Ile195, the 5′-methoxy moiety formed a hydrogen bond with Gln257. These interactions inhibit iNOS activity. Given the dual-enzyme inhibition activity, the gigantol may prevent and treat cataracts very effectively.

In summary, D-galactose was found to induce opacification in rat lenses both in vitro and in vivo, possibly through up-regulation of the expression and activity of AR and iNOS. It can in this way be used to establish diabetic rat models (Abdul et al., 2014). Gigantol, an active component of D. aurantiacum, can significantly attenuate this increase in the AR and iNOS expression and activity and the opacification of rat lenses induced by galactose. These experiments demonstrate for the first time the key role played by gigantol in the therapeutic effects of dendrobe against cataracts. We have, also for the first time, shown the putative docking sites of the gigantol, specifically Trp111, His110, Tyr48 and Trp20 on the AR molecule and Ile195 and Gln257 on the iNOS molecule. The results of in this study will facilitate a better understanding of the bioactive components of D. aurantiacum and the molecular mechanisms underlying its anti-cataract activity.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

This study was supported by Grants from the National Natural Science Foundation of China (81274157, 81102674, and 30850012), Guangdong Natural Science Foundation (S20-11010005661), Guangdong Science & Technology Planning Project (2011B031700076 and 2009B090300335), Guangzhou Science & Technology Planning Project (2014J100082) and the U.S. Department of Agriculture (USDA)/Agricultural Research Service (Cooperative Agreement no. 1950-5100-087).

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