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

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NADPH (PubChem CID: 52945042)
D-galactose (PubChem CID: 86278404)
Dimethyl sulfoxide (PubChem CID: 679)
L-arginine (PubChem CID: 6322)
Pencillin G (PubChem CID: 5904)
Streptomycin (PubChem CID: 19649)
Ethanol (PubChem CID: 702)
Ethyl acetate (PubChem CID: 8857)
Ammonium acetate (PubChem CID: 517165)
Butyl alcohol (PubChem CID: 263)
Methanol (PubChem CID: 887)
Dichloromethane (PubChem CID: 6344)
Tetrahydrobiopterin (PubChem CID: 1125)
Pirenoxine sodium (PubChem CID: 23695982)

ABSTRACT

Ethnopharmacological relevance: *Dendrobium aurantiacum var. denneanumis* 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 respress 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 65.67 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 Gln257. 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.

Abbreviations: DC, diabetic cataracts; AR, aldose reductase; NO, nitric oxide; iNOS, inducible nitric oxide synthase; ARI, aldose reductase inhibitors; PBS, phosphate buffer saline; FBS, fetal bovine serum; NIH, National Institute of Health; MD, molecular docking; PDB, Protein Data Bank; Mr, relative molecular mass; pI, isoelectric point; IEF, isoelectric focusing; IPG, Immobilized pH gradient; TFA, trifluoroacetic acid; MS, mass spectrometry; 2-DE, two-dimensional electrophoresis; CHCA, α-cyano-4-hydroxycinnamic acid; RIPA, Radio-Immunoprecipitation Assay; DMEM, Dulbecco's modified eagle medium; MOE, Molecular Operating Environment; MMFF94x, Merck molecular force field 94x; IC50, half-maximal inhibitory concentration; RMSD, smallest root-mean-square deviation; *D.aurantiacum*, *Dendrobium aurantiacum var. denneanum*; RT-qPCR, quantitative real-time reverse transcription polymerase chain reaction

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Trifluoroacetic acid (PubChem CID: 6422)
dl-glyceraldehyde (PubChem CID: 24895192).

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 et al., 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 contribute to promotion of DC development (Lechner et al., 2005). Furthermore the formation of peroxynitrite from NO and superoxides exacerbate 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).

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, antipyretic, immunomodulatory, and anti-aging (Commission of Chinese Pharmacopoeia, 2010; Li 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 flavor, cold in the nature, making the blind see. “Shennong's Herbal” also indicated that *D. aurantiacum* had 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 (Yan et al., 2008).

Gigantol (3′,4-dihydroxy-3,5′-dimethoxy-bibenzyl) is a bibenzyl-type phenolic compound extracted from the stem of *D. aurantiacum* (Li 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), D-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 Tiantianming 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 /streptomyci 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 Jiancheng 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), 100 U/mL penicillin, and 100 μg/mL streptomycin in a pre-warmed 24-well culture plate for 5 h at 37 °C and 5% CO_2. Sixty lenses were randomly divided into 6 groups ($n=10$) and treated for 24 h with

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