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Research Paper

# KIOM-79 inhibits aldose reductase activity and cataractogenesis in Zucker diabetic fatty rats

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## Abstract

**Objectives** KIOM-79, a combination of four plant extracts, has a preventive effect on diabetic nephropathy and retinopathy in diabetic animal models. In this study, we have investigated the inhibitory effects of KIOM-79 on diabetic cataractogenesis.

**Methods** We evaluated aldose reductase activity during cataractogenesis using Zucker diabetic fatty (ZDF) rat, an animal model of type 2 diabetes. ZDF rats were treated orally with KIOM-79 (50 mg/kg body weight) once a day for 13 weeks.

**Key findings** In vehicle-treated ZDF rats, lens opacity was increased, and lens fibre swelling and membrane rupture were observed. In addition, aldose reductase activity and aldose reductase protein expression in diabetic lens were markedly enhanced. However, the administration of KIOM-79 inhibited the development of diabetic cataract through the inhibition of aldose reductase activity and protein expression in diabetic lenses.

**Conclusions** These observations suggested that KIOM-79 was useful against the treatment of diabetic cataractogenesis.

**Keywords** aldose reductase; diabetic cataract; KIOM-79; Zucker diabetic fatty rat

## Introduction

Aldose reductase is the first and rate-limiting enzyme of the polyol pathway.[1] Under euglycaemic conditions, aldose reductase plays a minor role in glucose metabolism; however, during diabetes, its contribution is significantly enhanced, leading to a conversion of excess glucose to sorbitol in insulin independent tissues such as the lens.[2] Aldose reductase-dependent synthesis of excess polyols has been implicated as one of the mechanisms leading to diabetic cataracts.[3]

Diabetic cataracts are a major complication of diabetes mellitus. Some biochemical pathways, such as the polyol pathway, advanced glycation end products (AGEs) and oxidative stress, have been implicated in the pathogenesis of diabetic cataractogenesis.[4] Blood sugar can enter into lens by both facilitated and passive diffusion mechanisms and is then converted by aldose reductase to polyols, which then accumulate because they cannot diffuse passively out of the lens. The accumulation of polyols causes an osmotic change, which leads to lens hydration and swelling.[5]

KIOM-79 is a mixture of an 80% ethanol extract of parched Puerariae radix, gingered Magnoliae cortex, Glycyrrhizae radix and Euphorbiae radix. In previous work, KIOM-79 prevented the development of diabetic nephropathy in streptozocin-induced diabetic rats and type 2 diabetic Goto-Kakizaki rats.[6,7] In addition, KIOM-79 was shown to prevent apoptosis of retinal ganglion cells in diabetic db/db mice.[8] Recently, KIOM-79 inhibited xylose-induced lens opacity and protected HLE-B3 cells from the toxic effects of high glucose.[9] Although various preventive effects of KIOM-79 on diabetic complications have been reported, the in-vivo effect on diabetic cataracts is still unknown. The pharmacological effect of KIOM-79 as an aldose reductase inhibitor is not yet well defined. Therefore in this study, we have evaluated the inhibitory effect of KIOM-79 on diabetic cataractogenesis in Zucker diabetic fatty (ZDF) rat, an animal model of type 2 diabetes.

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## Materials and Methods

### Preparation of KIOM-79

The cortex of *Magnolia officinalis*, radix of *Pueraria lobata*, radix of *Glycyrrhiza uralensis* and radix of *Euphorbia pekinensis* were collected from the Gamsuk Province (China) and identified by botanist Professor J. H. Kim (Division of Life Science, Kyungwon University, Korea). All voucher specimens have been deposited at the herbarium of the Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine (nos 1240, 2, 7, and 207, respectively). KIOM-79 was prepared as described previously.[8] Briefly, Magnoliae cortex (100 g) was simmered with 3 g Zingiberis rhizoma for 60 min. Puerariae radix (100 g) was stir-roasted at 75°C for 45 min. Equal amounts of gingered Magnoliae cortex, parched Puerariae radix, Glycyrrhizae radix, and Euphorbiae radix were mixed, pulverized, extracted in 80% ethanol for one week at room temperature, concentrated with a rotary evaporator and lyophilized. The entire procedure was repeated four times. The quality of KIOM-79 was controlled by HPLC.[6]

### Determination of aldose reductase activity in-vitro

To determine the effect of KIOM-79 on aldose reductase activity, KIOM-79 and a positive control inhibitor (3, 3-tetramethyleneglutaric acid) were assayed according to methods described previously.[10] Briefly, the incubation mixture contained 135 mmol/l Na, K-phosphate buffer (pH 7.0), 100 mmol/l lithium sulfate, 0.03 mmol/l NADPH, 0.04 mmol/l DL-glyceraldehyde and 100 µl of an enzyme preparation, with or without 50 µl KIOM-79 or positive inhibitor, in a total volume of 1.0 ml. The reaction was initiated by adding NADPH at 37°C and stopped by adding 0.3 ml 0.5 M hydrochloric acid. Then 1 ml 6 M NaOH containing 10 mmol/l imidaxole was added, and the mixture was incubated at 60°C for 10 min to convert NADP to a fluorescent product. The fluorescence was measured at room temperature with a spectrofluorophotometer (Ex/Em = 360 nm/460 nm; Synergy HT, Bio-Tek, VT, USA). All measurements were performed in triplicate. The concentration of KIOM-79 that led to 50% inhibition of enzyme activity (IC50) was estimated from the least square regression line of the logarithmic concentration plotted against the remaining activity.

### Animals and experimental design

Male 6-week-old ZDF rats (ZDF/Gmi-fa/fa) and Zucker lean (ZL) counterparts (ZDF/Gmi-lean) were purchased from Charles River Laboratory (Waltham, MA, USA) and acclimated for one week before the study. Rats were individually housed in plastic cages and maintained at 24 ± 2°C with a 12 h light : dark cycle and received a diet of Purina 5008 (Ralston Purina, St Louis, MO, USA) and tap water, which were freely available. Rats were randomly divided into three groups of eight rats as follows: group 1, normal ZL rats; group 2, vehicle-treated ZDF rats; and group 3, ZDF rats treated with KIOM-79 (50 mg/kg body weight). The dosage of freeze-dried powder was calculated based on the minimum human equivalent dosage of raw herbs. KIOM-79 was dissolved in distilled water and administered daily by oral gavage for 13 weeks. Rats were examined weekly in a dark room using an ophthalmoscope after papillary dilatation with tropicamide solution. When the rats reached 21-weeks-of-age, blood glucose, glycosylated haemoglobin (HbA1c), total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and free fatty acid were measured using an automated analyser (Wako, Japan). Blood samples were collected from the tail vein after a 16-h fast. All procedures involving rats were approved by the Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee.

### Analysis of cataract formation

At necropsy, eyes were enucleated under deep anaesthesia following an intraperitoneal injection of pentobarbital sodium (30 mg/kg). The lenses were excised from the eyeball under an optical microscope, transferred onto 24-well plates each containing 2 ml saline solution, and then photographed under an optical microscope with a charged-coupled device camera. The opaque area of the lens was analysed using an imaging system program (Image analysis 42D 3D measuring software, TDI Scope Eyemedia 3.0, Olysia, Japan). Data are expressed as the percentage of opaque area compared with the entire area of the lens.

### Biochemical estimations

A 10% lens homogenate was prepared from two to three pooled lenses in a 50 mM phosphate buffer, pH 7.4. The activity of aldose reductase was assayed according to the methods described above. Sorbitol level in the lens was estimated using an enzymatic method.[11] Briefly, the lens homogenate was centrifuged at 3000 rev/min for 10 min. The supernatant was neutralized with 2 M potassium carbonate, centrifuged again, and the resulting supernatant was diluted with distilled water and used for the sorbitol assay. A total of 1 ml extract from each tissue was mixed with 2 ml glycine buffer (0.05 M, pH 9.4) containing 2 mM NAD and 0.05 ml sorbitol dehydrogenase (25.6 U/ml) and incubated at room temperature for 60 min. After incubation, fluorescence of the generated NADH was measured at an excitation wavelength of 366 nm and emission wavelength of 452 nm using a spectrofluorometer (Synergy HT, Bio-Tek, VT, USA). Based on the difference in fluorescence intensity with or without addition of sorbitol dehydrogenase, sorbitol levels in each extract were calculated from a calibration curve of D-sorbitol. Sorbitol content in the lens was expressed as µmol/g wet weight.

### Western blot analysis

Proteins were extracted from enucleated eyes and then separated by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Bio-Rad, Richmond, CA, USA). Membranes were probed with anti-aldose reductase antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and then the immune complexes were visualized with an enhanced chemiluminescence detection system (ECL; Amersham Bioscience, NJ, USA). Protein expression levels were determined by analysing the signals

captured on the nitrocellulose membranes using an image analyser (Las-3000, Fuji photo, Tokyo, Japan).

### Analysis of lens fibre degeneration

The isolated lenses were fixed in 10% neutralized formalin for 24 h and embedded in paraffin. To analyse lens fibre degeneration, fibre cells were visualized by labelling their membranes with wheat germ agglutinin. The lens sections were deparaffinized in xylene and rehydrated. The sections were then incubated with 2.5 mg/ml rhodamine-conjugated wheat germ agglutinin (Vector Laboratories, Burlingame, CA, USA) for 60 min. All specimens were examined with a fluorescence microscope (BX51, Olympus, Tokyo, Japan).

### Immunofluorescence staining

Immunofluorescence staining was performed on the lens sections. The lens sections were deparaffinized, hydrated with water, and stained with mouse anti-aldose reductase (Santa Cruz Biotechnology, Santa Cruz, CA, USA). For detection of aldose reductase, the sections were incubated with rhodamine-conjugated goat anti-mouse antibody (Santa Cruz, CA, USA). The intensity of the fluorescence was analysed in five randomly selected $mm^2$ areas using Image J software (NIH, Bethesda, MD, USA).

### Statistical analysis

The statistical evaluation of the results was performed using a one-way analysis of variance followed by Tukey's multiple comparison test using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA).

## Results

### KIOM-79 inhibited rat lens aldose reductase activity *in vitro*

KIOM-79 was tested for its effect on an in-vitro aldose reductase activity assay. The IC50 values of KIOM-79 in this assay were compared with a known aldose reductase inhibitor, 3,3-tetramethyleneglutaric acid (Table 1). KIOM-79 had an apparent inhibitory effect on aldose reductase activity (IC50 value 10.09 μg/ml).

### Metabolic parameters

At 21-weeks-old blood glucose, LDL, HDL, triglycerides, total cholesterol, and HbA1c levels were significantly increased in vehicle-treated ZDF rats ($P < 0.01$ vs normal ZL rats). The body weights of vehicle-treated ZDF rats were elevated approximately 130% compared with normal ZL rats. KIOM-79 induced a minor decrease of blood glucose levels, but had no effect on HbA1c levels. ZDF rats treated with KIOM-79 showed significantly reduced LDL, triglyceride and free fatty acid compared with vehicle-treated ZDF rats. However, no differences in HDL levels or total cholesterol were noted between KIOM-79-treated and vehicle-treated ZDF rats (Table 2).

### Cataract formation analysis

Vehicle-treated ZDF rats started to show lens opacity beginning at 15-weeks-old. At the end of the study, cataracts were observed in the eyes of all vehicle-treated ZDF rats. In KIOM-79-treated ZDF rats, cataracts were first observed at 17-weeks-

**Table 1** Inhibition of rat lens aldose reductase in-vitro activity by KIOM-79

| Treatment | Concentration (μg/ml) | Inhibition (%) | IC50 value[a] (μg/ml) |
|---|---|---|---|
| KIOM-79 | 5 | 29.67 ± 1.10 | 10.09 ± 0.573 |
| | 10 | 47.99 ± 3.53 | |
| | 20 | 92.31 ± 3.81 | |
| 3, 3-Tetramethyleneglutaric acid[b] | 3.7 | 31.42 ± 5.71 | 5.34 ± 0.891 |
| | 5.5 | 56.42 ± 9.60 | |
| | 7.4 | 69.69 ± 8.15 | |

[a]The inhibitory effect is expressed as the mean of four experiments. IC50 values were calculated from dose inhibition curves. [b]3, 3-Tetramethyleneglutaric acid was used as a positive control.

**Table 2** Metabolic and physical parameters

| | ZL | ZDF-vehicle | ZDF-KIOM-79 |
|---|---|---|---|
| Body weight (g) | 338.5 ± 40.5 | 433.2 ± 69.4* | 414.6 ± 45.0 |
| Blood glucose (mg/dl) | 92.9 ± 10.8 | 489.8 ± 38* | 391.7 ± 113.2 |
| HbA1c (%) | 3.7 ± 0.1 | 7.88 ± 1.3* | 7.1 ± 0.6 |
| Total cholesterol (mg/dl) | 100.6 ± 7.3 | 254.7 ± 18.1* | 228.3 ± 21.7 |
| HDL (mg/dl) | 34.69 ± 2.1 | 72.7 ± 6.9* | 68.2 ± 10.3 |
| LDL (mg/dl) | 8.6 ± 0.8 | 34.6 ± 6.5* | 26.3 ± 7.9# |
| Triglyceride (mg/dl) | 93.1 ± 24.1 | 1112.6 ± 330.7* | 881.0 ± 335.5# |
| Free fatty acid (μEq/l) | 896.3 ± 185.3 | 1775.8 ± 331.6* | 1224.1 ± 172.4# |

ZL, Zucker normal lean rat; ZDF-vehicle, vehicle-treated Zucker diabetic fatty rat; ZDF-KIOM-79, ZDF rat treated with KIOM-79 (50 mg/kg); HbA1c, glycosylated haemoglobin; HDL, high-density lipoproteins; LDL, low-density lipoproteins. All data were expressed as the mean ± SE. *$P < 0.01$ vs ZL group; #$P < 0.01$ vs ZDF-vehicle group.

of-age, and three rats developed cataracts at 20-weeks-of-age (Figure 1a). In the analysis of lens opacification, the mean opaque area of the lens was 6.03 ± 2.88% for normal ZL rats and 49.83 ± 28.48% for vehicle-treated ZDF rats, indicating severe lens opacity. In KIOM-79-treated ZDF rats, 19.15 ± 11.52% of the lenses were opaque (Figure 1b). Thus, the administration of KIOM-79 decreased the percentage of eye opacity by 60%. This result indicated that KIOM-79 retarded the development of cataract in ZDF rats.

## Lens fibre cell degeneration

The membrane-labelled lens section demonstrated that normal lenses consistently showed a perfect crystalline packing of fibre cells. In cataractous lenses, large areas of liquefaction, fibre cell swelling and multiple membrane rupture were observed. However, this histological change in lens fibres in ZDF rats was prevented after KIOM-79 treatment (Figure 2).

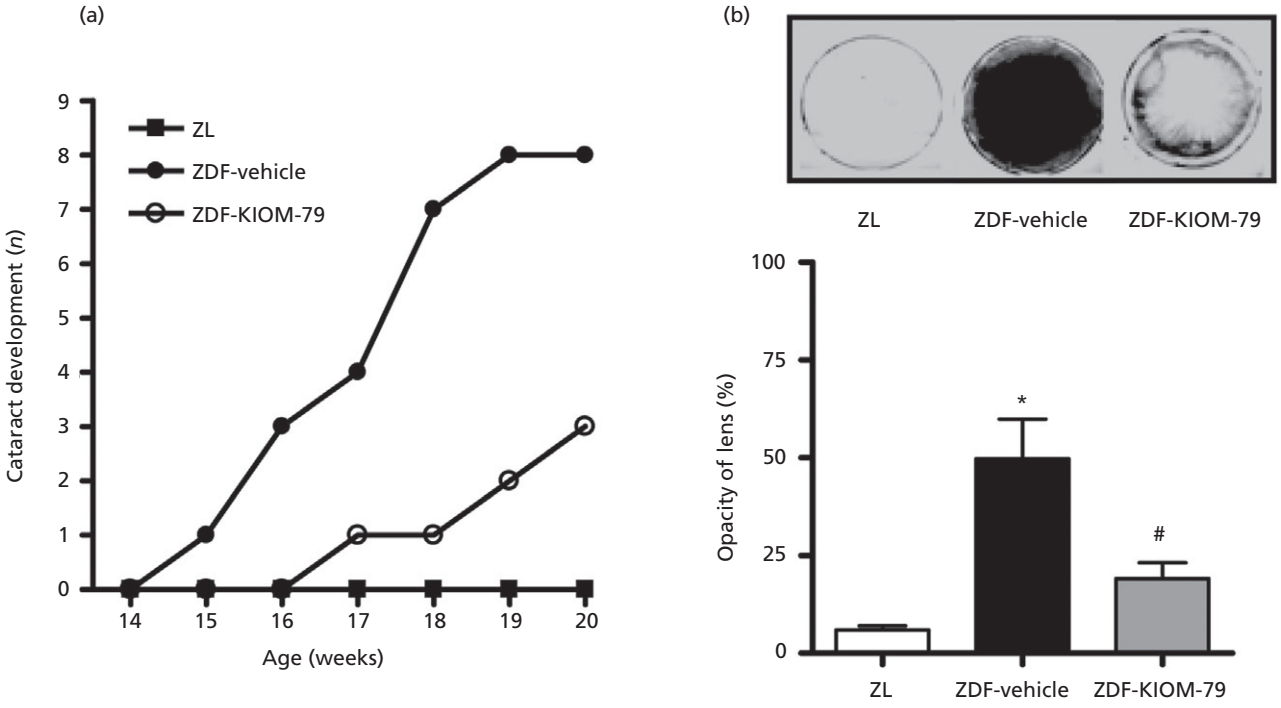


**Figure 1** Lens opacity in Zucker lean rats and Zucker diabetic fatty rats treated with and without KIOM-79. (a) Cataract development in the Zucker lean rat (ZL), vehicle-treated Zucker diabetic fatty rat (ZDF-vehicle) and ZDF rat treated with KIOM-79 (ZDF-KIOM-79). (b) Representative image of lenses in each group and analysis of lens opacity. All opacities were analysed from each lens. All data are expressed as the means ± SE, $n = 8$. *$P < 0.01$ vs normal ZL rats, #$P < 0.01$ vs vehicle-treated ZDF rats.

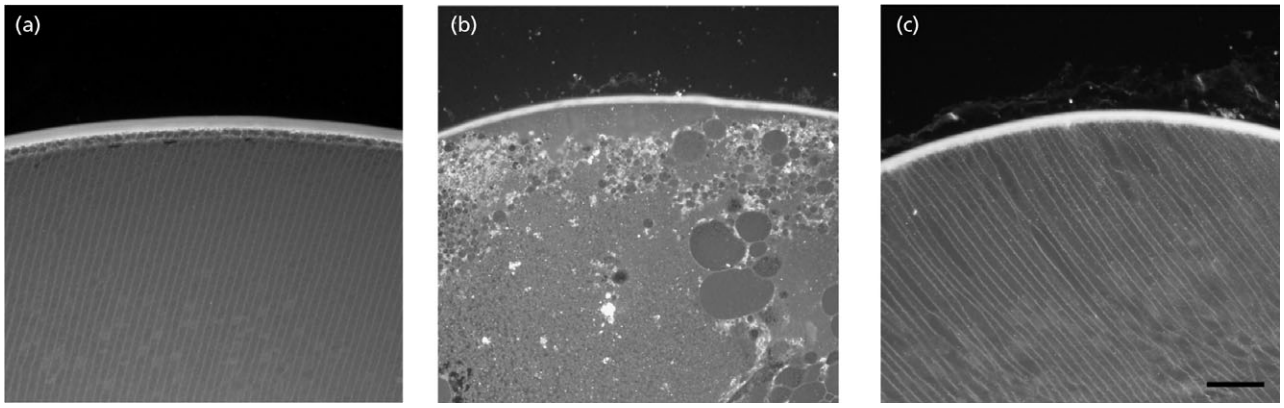


**Figure 2** Lens fibre degeneration in Zucker lean rats and Zucker diabetic fatty rats treated with and without KIOM-79. Lens sections from a normal Zucker lean rat (a; $n = 8$), a vehicle-treated Zucker diabetic fatty (ZDF) rat (b; $n = 8$) and a ZDF rat treated with KIOM-79 (c; $n = 8$) were labelled with rhodamine-conjugated wheat germ agglutinin. Fibre cell liquefaction and membrane rupture were observed in diabetic cataractous lens. Scale bar = 50 μm.

### Polyol pathway

The activity of aldose reductase, a key enzyme of the polyol pathway, was significantly elevated in vehicle-treated ZDF rats (Figure 3a). Aldose reductase activity in lenses from animals treated with KIOM-79 was decreased, consistent with our observations on the in-vitro inhibition of rat lens aldose reductase by KIOM-79. Using Western blotting, the expression of aldose reductase protein was shown to be 2.7-fold higher in vehicle-treated ZDF rats than in normal ZL rats (Figure 3b). However, treatment with KIOM-79 reduced the expression of the aldose reductase protein in lens tissues of ZDF rats. Furthermore, there was an increase of sorbitol levels in vehicle-treated ZDF rats when compared with normal ZL rats (Figure 3c), which was expected following activation of the polyol pathway in diabetic lens. However, administration of KIOM-79 resulted in a lower level of diabetes-induced sorbitol accumulation. Similarly, in vehicle-treated ZDF rat, an increase in immunoreactive staining for aldose reductase was observed in the cytoplasm of lens epithelial cells and extended into the deeper cortical fibres. However, treatment with KIOM-79 prevented aldose reductase expression in the lens epithelial cells and inhibited the extension of aldose reductase beneath the epithelial region (Figure 4a). Using morphometric analysis, the intensity of aldose reductase immunolabelling was shown to be significantly increased 2-fold in the vehicle-treated ZDF rats compared with the normal ZL rats, and it was suppressed by treatment with KIOM-79 (Figure 4b, $P < 0.01$).

## Discussion

Plant medicines and herbal drugs have traditionally been used to treat diabetes. Some medicinal herbs are investigated for their potential against diabetic complications such as

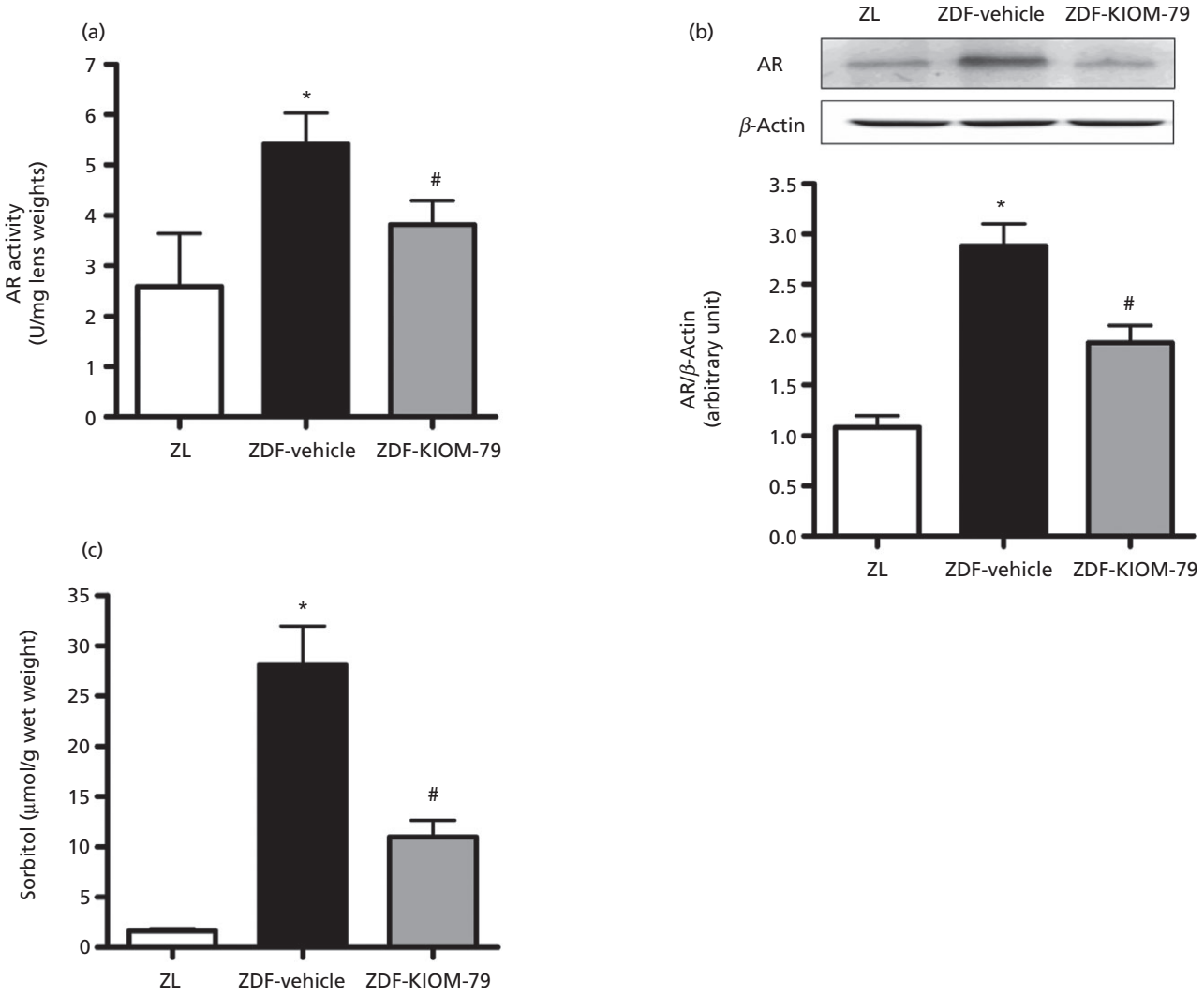


**Figure 3** Polyol pathway in Zucker rats. (a) Aldose reductase (AR) activity, (b) Western blot analysis of AR and (c) sorbitol levels in lenses from normal Zucker lean rats (ZL), vehicle-treated Zucker diabetic fatty rats (ZDF-vehicle) and ZDF rats treated with KIOM-79 (ZDF-KIOM-79). Values in the bar graphs represent means ± SE, $n = 8$. *$P < 0.01$ vs normal ZL rats, #$P < 0.01$ vs vehicle-treated ZDF rats.

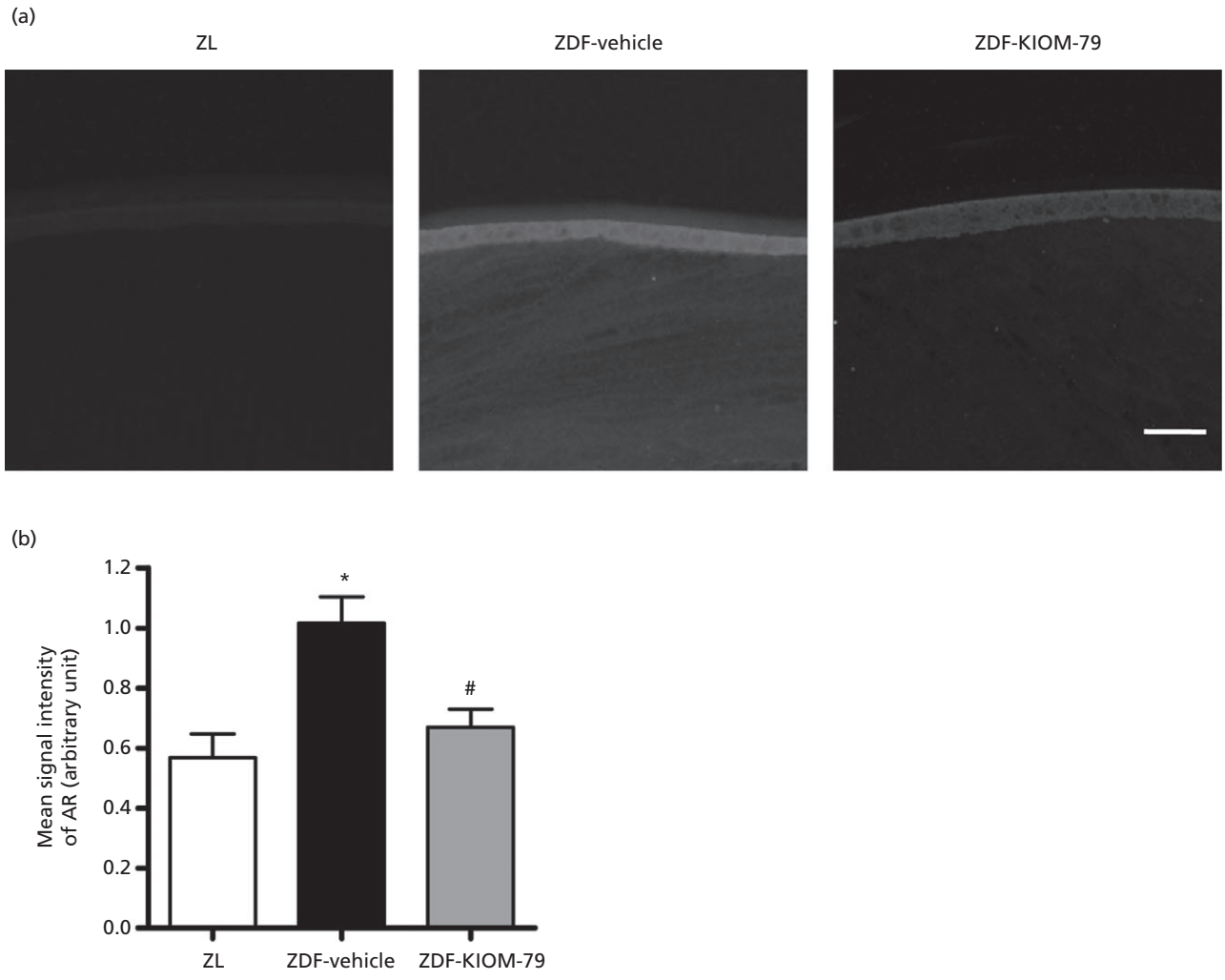


**Figure 4** Immunofluorescence staining of aldose reductase in Zucker lean rats and Zucker diabetic fatty rats. (a) Representative immunostaining of aldose reductase (AR) in lenses from a normal Zucker lean rat (ZL), a vehicle-treated Zucker diabetic fatty rat (ZDF-vehicle) and a ZDF rat treated with KIOM-79 (ZDF-KIOM-79). A strong immunoreactivity of AR was observed in the cytoplasm of lens epithelial cells and lens cortical fibres. Scale bar = 50 μm. (b) Quantitative analysis of AR immunoreactive intensity. Values in the bar graphs represent means ± SE, $n = 8$. *$P < 0.01$ vs normal ZL rats, #$P < 0.01$ vs vehicle-treated ZDF rats.

cataracts.[12,13] Sugar cataract formation associated with diabetes has been linked to the aldose reductase-catalysed production of sugar alcohols or AGEs accumulation.[14,15] In this study, KIOM-79, a mixture of an 80% ethanol extract of parched Puerariae radix, gingered Magnoliae cortex, Glycyrrhizae radix and Euphorbiae radix, was tested for potential inhibitory effects on aldose reductase activity and diabetic cataractogenesis. KIOM-79 significantly inhibited aldose reductase activity *in vitro* and prevented lens opacity as well as lens fibre degeneration in ZDF rats. In addition, aldose reductase activity and sorbitol level were also reduced by KIOM-79 treatment in ZDF rats.

Aldose reductase, the key enzyme in the polyol pathway, plays important roles in cataract formation in diabetic lens.[16] However, few drugs are available for the treatment of diabetic cataract, and they target aldose reductase. A number of studies have been undertaken to identify natural and synthetic compounds that inhibit aldose reductase.[17] In this study, aldose reductase activity was significantly increased in diabetic lenses, whereas in lenses treated with KIOM-79, aldose reductase activity was decreased, substantiating the in-vitro inhibitory effect of KIOM-79 against rat lens aldose reductase. Moreover, the expression of aldose reductase protein in the lens of ZDF rats was suppressed by treatment with KIOM-79. Lenticular aldose reductase activity and immunoreactivity are significantly increased in diabetic animals compared with age-matched nondiabetic controls, and the increased aldose reductase activity can be explained in part by enhanced aldose reductase protein expression.[18–20] Thus, these results suggested that aldose reductase activity was directly inhibited by KIOM-79 and indirectly decreased via suppression of the aldose reductase protein expression in the lens tissues. Furthermore, KIOM-79 prevented the accumulation of sorbitol in the lens tissue. In the lens, osmotic stress imposed by sorbitol accumulation has long been believed to be the major factor in the progression of

diabetic cataracts.[21] Based on these results, the prevention of diabetic cataracts by KIOM-79 is related partly to both the inhibition of aldose reductase activity and the inhibition of sorbitol accumulation in the lens.

Most of the time herbs are not used alone; herbal mixtures are known to have various advantages of synergy and interactions among the various phytocompounds present in the different herbs. Four major compounds, magnolol, honokiol, glycyrrhizine and puerarin, were recently identified in KIOM-79.[22] Magnolol and honokiol are the potent aldose reductase inhibitors, with IC50 values of 28 and 36 μM, respectively.[23] Glycyrrhizine inhibits aldose reductase activity and sorbitol formation in rat lenses with high concentrations of glucose.[24] Puerarin from Puerariae radix is an active inhibitor of aldose reductase.[25] Therefore, the inhibitory effect of KIOM-79 on aldose reductase may have been a result of the activity of these compounds. Moreover, in our previous study, KIOM-79 exhibited a stronger anti-AGE effect compared with its four herb components.[6] Similarly, KIOM-79 has a stronger inhibitory effect against aldose reductase activity compared with its four herb components (data not shown). These results suggested that the four herbs of KIOM-79 acted synergistically. Interestingly, it was previously reported that the effect of aldose reductase inhibitors (ARI) such as epalrestat, tolrestat, zenarestat, SG-210 and SNK-860 on cataract formation was inadequate in spite of their potent ameliorating effect on aldose reductase *in vitro*.[26–30] This suggested that a single compound may not be as effective as an anti-cataract drug in animal models *in vivo*. Thus, it was assumed that the anti-cataract effect of KIOM-79 may have been due to the synergistic activity among the four herbs. It remains to be seen whether KIOM-79 was responsible for a stronger prevention of diabetic cataractogenesis than the four herb components, which would provide evidence for the synergistic effect of KIOM-79.

## Conclusions

We propose that KIOM-79 significantly inhibited diabetic cataractogenesis via inhibition of aldose reductase. We suggest that KIOM-79 is useful particularly in the treatment of diabetic cataracts.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

### Funding

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