

Inhibition of aldose reductase and sorbitol accumulation by dietary rutin

G. Bhanuprakash Reddy^{1,*}, P. Muthenna¹, C. Akileshwari¹,
Megha Saraswat¹ and J. Mark Petrash²

¹Biochemistry Division, National Institute of Nutrition, Hyderabad 500 604, India

²Department of Ophthalmology, University of Colorado School of Medicine, Aurora, CO80045, USA

Accumulation of intracellular sorbitol due to increased aldose reductase (ALR2 or AKR1B1) activity has been implicated in the development of various secondary complications of diabetes. Previously we have reported that some common dietary sources such as spices, fruits and vegetables have the potential to inhibit ALR2 under *in vitro* conditions and in animal models. In this study, we describe the inhibition of ALR2 by rutin, a bioflavonoid present in many dietary sources. Rutin inhibited ALR2 with an IC₅₀ value of 13 μM in an uncompetitive manner, but was a poor inhibitor of closely related members of the aldo-keto reductase (AKR) superfamily, particularly aldehyde reductase (ALR1). Results from molecular docking studies were consistent with the pattern of inhibition of ALR2 by rutin and its specificity. Moreover, rutin was able to suppress sorbitol accumulation in human erythrocytes under high glucose conditions, demonstrating an *in vivo* potential of rutin to prevent sorbitol accumulation. These results suggest that rutin holds a promise as an agent to prevent or treat diabetic complications.

Keywords: Aldehyde reductase, aldose reductase, diabetic complications, rutin, sorbitol.

PROLONGED exposure to chronic hyperglycaemia in diabetes can lead to various complications affecting the cardiovascular, renal, neurological and visual systems¹. Although mechanisms leading to diabetic complications are not completely understood, many biochemical pathways associated with hyperglycaemia have been implicated¹. Among these, the polyol pathway has been extensively studied². Aldose reductase (ALR2 or AKR1B1; EC: 1.1.1.21) belongs to the aldo-keto reductase (AKR) superfamily. It is the first and rate-limiting enzyme in the polyol pathway and reduces glucose to sorbitol utilizing NADPH as a cofactor. Sorbitol is then metabolized to fructose by sorbitol dehydrogenase². Accumulation of sorbitol leads to osmotic swelling, changes in membrane permeability and also oxidative stress culminating in tissue injury³.

Many studies with experimental animals suggest that inhibition of ALR2 could be effective in the prevention

of some diabetic complications, including cataract, retinopathy, nephropathy and neuropathy³⁻⁵. A number of ALR2 inhibitors (aldose reductase inhibitor; ARI), both synthetic and natural, have been found to delay or substantially prevent some diabetic complications in animal models and have been evaluated in clinical trials^{5,6}. To date, most ARIs have met with limited success, and some of the synthetic ARIs were associated with deleterious side effects and poor penetration of target tissues such as nerve and retina⁵⁻⁸. Largely, two chemical classes of ARI have been tested in phase-III trials. While carboxylic acid inhibitors (zopolrestat, ponalrestat and tolrestat) have shown poor tissue permeability and are not potent *in vivo*, spiroimide (spiro hydantoin) inhibitors such as sorbinil penetrate tissues more efficiently, but many have been associated with skin reactions and liver toxicity³⁻⁶.

Aldehyde reductase (ALR1; EC: 1.1.1.2) is one of AKR family members that is closely related to ALR2 and known to play a role in the detoxification of reactive aldehydes^{9,10}. Recently, other AKR members have been identified that are similar to ALR2. One such AKR is human small intestine reductase (HSIR or AKR1B10), which has 70–80% sequence similarity^{11,12} with ALR2. Since many ARIs not only inhibit ALR2 but other AKRs, particularly ALR1 (refs 9 and 10), it has been suggested that poor selectivity might have contributed to the poor outcome of ARI clinical trials⁵.

In the course of identifying and testing new and effective ARIs, we have evaluated a number of traditional and common dietary sources and found that some spice principles, fruits and vegetables have the potential to inhibit ALR2 under *in vitro* conditions and in animal models¹³⁻¹⁹. Flavonoids are abundantly found in fruits, vegetables, herbs and spices, and some flavonoids have been shown to inhibit ALR2 (refs 20–24). Rutin is one of the commonly found dietary flavonols. While a previous study demonstrated that rutin may have ARI activity²⁴, much remains to be determined about the potency and specificity of this compound against ALR2 and related AKRs. Therefore, in the present study we have characterized the inhibition of human recombinant ALR2 by rutin and have provided insights into the nature of inhibition. In addition, we have studied the specificity of rutin towards two closely related AKRs, and its effects on intracellular

*For correspondence. (e-mail: geereddy@yahoo.com)

sorbitol accumulation in red blood cells (RBCs) under *ex vivo* high glucose conditions.

Materials and methods

Materials

D-Glucose, DL-glyceraldehyde, lithium sulphate, 2-mercaptoethanol, NADPH, NADP, dimethyl sulphoxide (DMSO), sorbitol, rutin, glycine, methyl orange, perchloric acid, ammonium sulphate, DEAE-cellulose, Tris-HCl, EDTA, sucrose and sorbitol dehydrogenase were purchased from Sigma Chemicals Company (St Louis, MO, USA). All other chemicals were obtained from local companies.

Expression and purification of recombinant human ALR2

Recombinant human ALR2 was over-expressed in *Escherichia coli* and purified from bacterial cultures essentially as described previously²⁵, with a minor modification. Chromatography over AffiGel Blue (Bio-Rad) affinity matrix was used as the final purification step.

Purification of ALR1 from bovine kidney

ALR1 was partially purified from bovine kidney following the previously described methods^{17,26}. Briefly, freshly obtained bovine kidney was homogenized in three volumes of 10 mM sodium phosphate buffer, pH 7.2, containing 0.25 M sucrose, 2.0 mM EDTA and 2.5 mM 2-mercaptoethanol. The homogenate was centrifuged at 16,000 *g* for 20 min and the supernatant was subjected to ammonium sulphate precipitation. Precipitate obtained between 45% and 75% saturation was dissolved in the above buffer and dialysed extensively against the same buffer. DEAE-52 resin was added to the dialysed material and then removed by centrifugation. The supernatant was used as the source of ALR1.

ALR2 assay

The activity of ALR2 was measured as described previously¹³. The change in the absorbance at 340 nm due to NADPH oxidation was followed in a Lambda35 spectrophotometer (Perkin-Elmer, Shelton, USA).

ALR1 assay

The activity of ALR1 was measured spectrophotometrically by monitoring the oxidation of NADPH at 340 nm as a function of time at 37°C using glyceraldehyde as substrate¹⁷. The assay mixture in 1 ml contained 50 mM

sodium phosphate buffer, pH 7.2, 0.2 M ammonium sulphate, 10 mM DL-glyceraldehyde, 5 mM 2-mercaptoethanol and 0.1 mM NADPH.

Inhibition studies

For inhibition studies, concentrated stocks of rutin prepared in DMSO were used and the final concentration of DMSO was not more than 1%. Various concentrations of rutin were added to assay mixtures of ALR2, ALR1 or AKR1B10 and incubated for 5 min before initiating the reaction by NADPH, as described above. The percentage inhibition was calculated considering the activity in the absence of rutin as 100%. The IC₅₀ values were determined by nonlinear regression analysis of the plot of per cent inhibition versus log inhibitor concentration.

Enzyme kinetics

K_m and V_{max} of recombinant ALR2 were determined with varying concentrations of glyceraldehyde as substrate in the absence and presence of different concentrations of rutin by Lineweaver–Burk double reciprocal plots. Inhibitory constant (K_i) was derived by plotting slopes obtained from Lineweaver–Burk plots versus rutin concentration.

In vitro incubation of RBC

Five millilitres blood was collected into heparinized tubes from healthy male volunteers after an overnight fast. RBCs were separated by centrifugation and washed three times with isotonic saline at 4°C. Washed RBCs were suspended in Krebs–ringer bicarbonate buffer, pH 7.4 (pre-equilibrated with 5% CO₂). Duplicate samples were incubated at 37°C in the presence of 5% CO₂ for 3 h under normal (5.5 mM) and high glucose (55 mM) conditions¹⁷. The effect of rutin on sorbitol accumulation was evaluated by incubating the RBC with different concentrations of rutin.

Estimation of sorbitol in RBC

At the end of the incubation period, RBCs were homogenized in nine volumes of 0.8 M perchloric acid. The homogenate was centrifuged at 5000 *g* at 4°C for 10 min and pH of the supernatant was adjusted to 3.5 with 0.5 M potassium carbonate. The sorbitol content of the supernatant was measured by a fluorometric method as described previously²⁷ using a spectrofluorometer (Jasco-FP-6500).

Molecular docking studies

Molecular docking was done using the discovery (Discover 2.7) package from (Biosystems Technologies, San

Diego, CA, USA), on an O2 (R12000) workstation (Silicon Graphics, CA, USA). All ligands were minimized to least energy conformations and taken for docking studies. Crystal structure of aldose reductase (human ALR2 or AKR1B1) was downloaded from Brookhaven databank (PDB: 1PWM) and protein structure minimized using charmM force field. All water molecules were removed. Docking was done by discovery LigandFit module in a protein-created sphere about 12 Å around the active site. After docking, poses were viewed by DS viewer and calculation of binding energy was done using the discovery programmer and figures were prepared by poseview software.

Results and discussion

Rutin is a flavonol glycoside composed of flavonol quercetin and the disaccharide rutinose, i.e. quercetin-3-rutinoside (Figure 1). Lemon, orange, apple and black tea are rich sources of the glycoside and interestingly, crude aqueous extracts of these sources have been shown to inhibit both rat lens and human recombinant ALR2 (ref. 17). Further, rutin possesses anti-inflammatory activity, antioxidant²⁸, anticarcinogenic²⁹, antiplatelet activity³⁰ and these properties have been attributed to some of the ARI. Many flavonoids, including rutin have been shown to inhibit rat lens ALR2 (ref. 24). ARI potential of rutin was reported using rat lens ALR2, but not the mechanism of inhibition, specificity and binding properties. However, lens is known to have the highest AR activity compared to other tissues and more so in rat¹³. Thus the relevance of inhibition of rat lens ALR2 by rutin may have limitations with respect to its extrapolation to

human diabetic complications. This led us to further explore the ARI activity of rutin with human recombinant ALR2 and its specificity of inhibition with a closely related AKR, ALR1. To test for physiological significance, we measured the ability of rutin to block ALR2 activity in freshly harvested human erythrocytes.

Rutin inhibited human recombinant ALR2 with an IC₅₀ value of 13 μM, as evident from Figure 2. Rutin is a flavonol glycoside of quercetin. Hence we compared the IC₅₀ value of rutin with that of quercetin, the aglycoside part of rutin which was also shown to have ARI potential^{24,31}. Interestingly, rutin appears to be more potent than quercetin (13 μM versus 28 μM). The primary structure of ALR2 displays high similarities with ALR1 and AKR1B10. Both ALR1 and ALR2 catalyse the reduction of biogenic aldehydes and NADPH-dependent reduction of a variety of carbonyls such as glyceraldehyde, glucuronate and short-chain alkanals¹⁰⁻¹². Therefore, we have also studied the specificity of rutin with ALR1 and ALR2. Specificity was assessed in terms of selectivity ratio based on the IC₅₀ value of rutin with ALR2 and ALR1 (ref. 17). It was interesting to note that rutin did not inhibit bovine kidney ALR1 up to 200 μM concentration under the conditions employed in the study (Table 1), signifying its marked specificity towards ALR2 over ALR1. In comparison to rutin, quercetin exhibited a low selectivity ratio (Table 1).

Table 1. Specificity of rutin and quercetin against ALR2 and ALR1

Inhibitor	ALR2 (μM)	ALR1 (μM)	Selectivity ratio (ALR1/ALR2)
Rutin	13	200	15.38
Quercetin	28	59	2.11

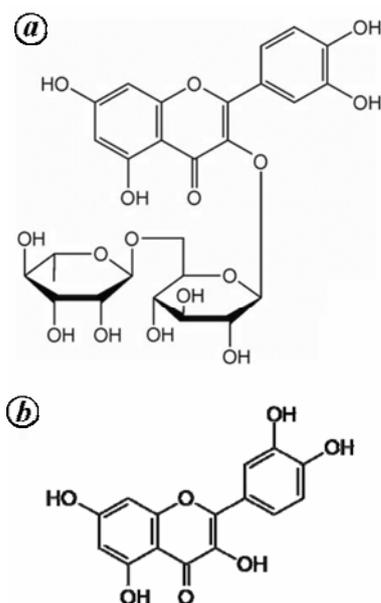


Figure 1. Two-dimensional structure of rutin (a) and quercetin (b).

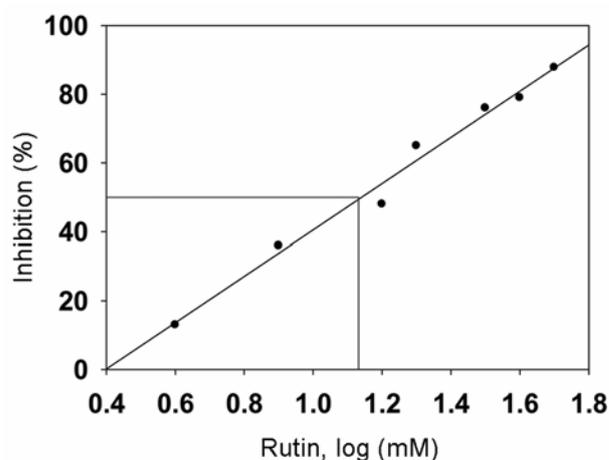


Figure 2. Inhibition of ALR2 by rutin. Representative inhibition curve of human recombinant ALR2 by rutin. ALR2 activity in the absence of rutin was considered as 100%. Data are average of three independent experiments.

Next, we determined some kinetic parameters such as K_m and V_{max} to understand the mechanism of inhibition of ALR2 by rutin. In the presence of different concentrations of rutin, both V_{max} and K_m were found to decrease with glyceraldehyde as substrate (Figure 3 *a* and Table 2). These results suggest that the inhibitor binds only to the enzyme substrate complex and does not compete with the substrate and inhibit ALR2 in an uncompetitive manner. Further, we have determined inhibitory constant (K_i) from the secondary plots of the Lineweaver–Burk plots, and K_i of rutin for ALR2 was found to be 25×10^{-6} M (Figure 3 *b*). As reported by Bohren *et al.*³², although many ionic inhibitors bind to the active site, they still show non-competitive to uncompetitive pattern inhibition because under steady-state conditions most of the enzyme will be present as enzyme–nucleotide binary complex. Hence,

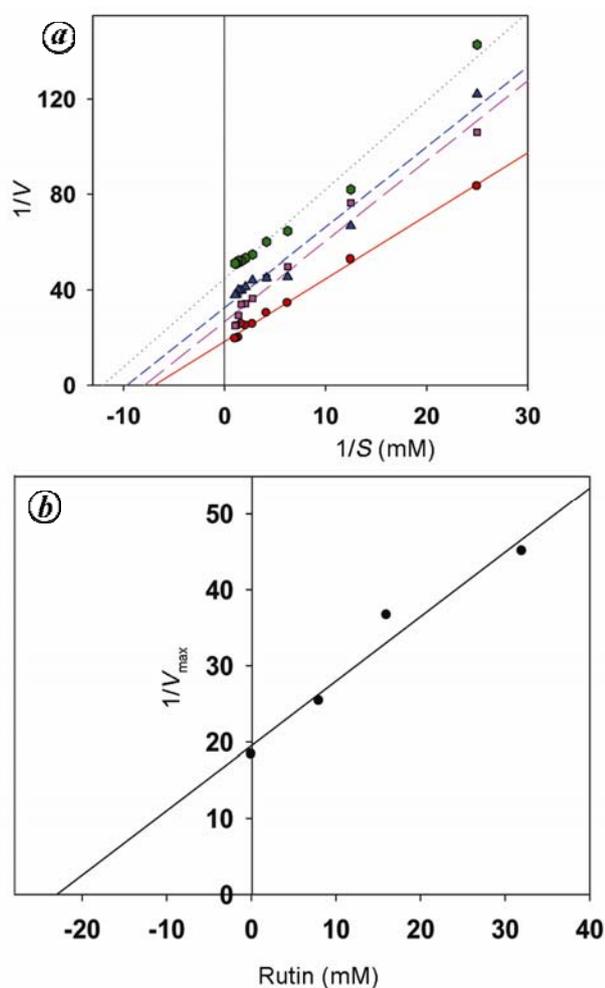


Figure 3. Kinetics of human recombinant aldose reductase inhibition. *a*, Lineweaver–Burk plot of recombinant ALR2 in the absence and presence of various concentration of rutin. Final concentration of rutin used in the assay system: 0 (circle), 8 (triangle), 15 (diamond) and 30 μ M (hexagonal). *b*, Determination of inhibitory constant (K_i). Slopes of the Lineweaver–Burk plot as a function of rutin concentration. X-axis intercept of this plot gives K_i . Data in (*a*) and (*b*) are average of three independent experiments.

compounds that selectively bind to the enzyme–nucleotide complex are more effective than those that bind to the free enzyme.

Table 2. Kinetics of human recombinant ALR2 in the absence and presence of rutin. Data are the mean \pm SE ($n = 6$). V_{max} is reported as μ moles NADPH oxidized/min/mg protein

Rutin (μ M)	K_m (mM)	V_{max}
0	0.182 ± 0.08	0.067 ± 0.019
8	0.143 ± 0.07	0.049 ± 0.011
15	0.100 ± 0.05	0.037 ± 0.014
30	0.084 ± 0.11	0.025 ± 0.013

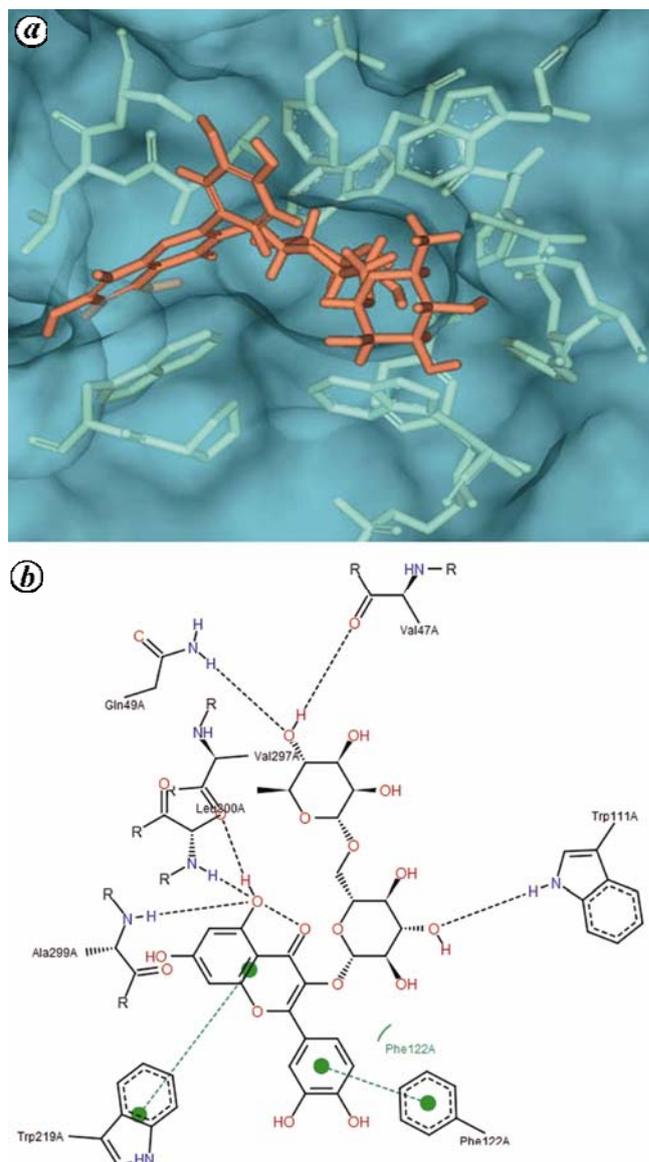


Figure 4. Stereoview of AKR1B1 docked with rutin. *a*, Rutin was docked into the active site of ALR2 and extended towards the hydrophobic pocket. *b*, Rutin docked into the active site of ALR2 and its hydrogen bond interaction with residues Val-47, Gln-49, Trp-111, Leu-300, Leu-301, Val-297 and Ala-299, and hydrophobic interactions with Trp-219 and Phe-122.

Molecular docking studies were conducted to substantiate the binding pattern and selective inhibition of ALR2 by rutin. It was observed that rutin possibly interacts with ALR2 at the active site residues Val-47, Gln-49, Trp-111, Leu-300, Leu-301, Val-297 and Ala-299, and exhibit hydrophobic interactions with Trp-219 and Phe-122. Hence, it appears that rutin might bind to ALR2 in an open type of conformation because of the formation of a hydrogen bond with Leu-300 (Figure 4). However, no contact with Trp-111 was noted. In the case of quercetin, hydrogen bonding was observed with residues Trp-20, His-110, Trp-111, Leu-300 and Leu-301, and hydrophobic interactions with Trp-20 (Figure 5). Compared to the well-

known synthetic inhibitor, fidarestat which occupied the active site of ALR2 with limited contacts, rutin and quercetin extended into the hydrophobic cleft called specificity pocket, suggesting effective inhibition of ALR2. However, compared to rutin, quercetin did not form hydrogen bonds with Val-47, Gln-49, Val-297 and Ala-299, and also no hydrophobic interactions with Trp-219 and Phe-122. This might explain the lower IC_{50} as well lower selectivity for quercetin compared to its glucoside rutin.

Compared to some potent ARIs, the IC_{50} value obtained with rutin (13 μ M) was modest. However, the relative specificity shown by rutin towards human ALR2 over ALR1, underscores its importance in terms of achieving good inhibition of ALR2 without side effects related to off-target inhibition of ALR1. In addition, the data also suggest that rutin might aid in guiding the development or identification of highly specific ARIs. Among human AKRs, ALR2 is unique in its ability to catalyse the NADPH-dependent conversion of glucose to sorbitol¹². In addition to lens, retina, nerve and kidney, activation of ALR2 in RBC leads to the accumulation of sorbitol³³. We have also found a direct correlation between erythrocyte ALR2 and sorbitol levels³⁴. Therefore, we assessed accumulation of sorbitol in RBC under high glucose conditions (*ex vivo*) to understand the significance of *in vitro* inhibition of ALR2 by rutin, particularly its effect on osmotic stress. Incubation of RBC with 55 mM glucose resulted in the accumulation of sorbitol about threefold higher than the control, whereas presence of rutin under high glucose conditions led to a reduction in the accumulation of intracellular sorbitol in a dose-dependent manner (Table 3). The same concentrations of rutin were more effective than quercetin in decreasing sorbitol accumulation (Table 3). These results not only substantiate the inhibition of ALR2 by rutin, but also indicate the significance of rutin in terms of preventing the accumulation of intracellular sorbitol.

Results of the present study showing the inhibition of ALR2 by rutin merit attention in many respects. The data

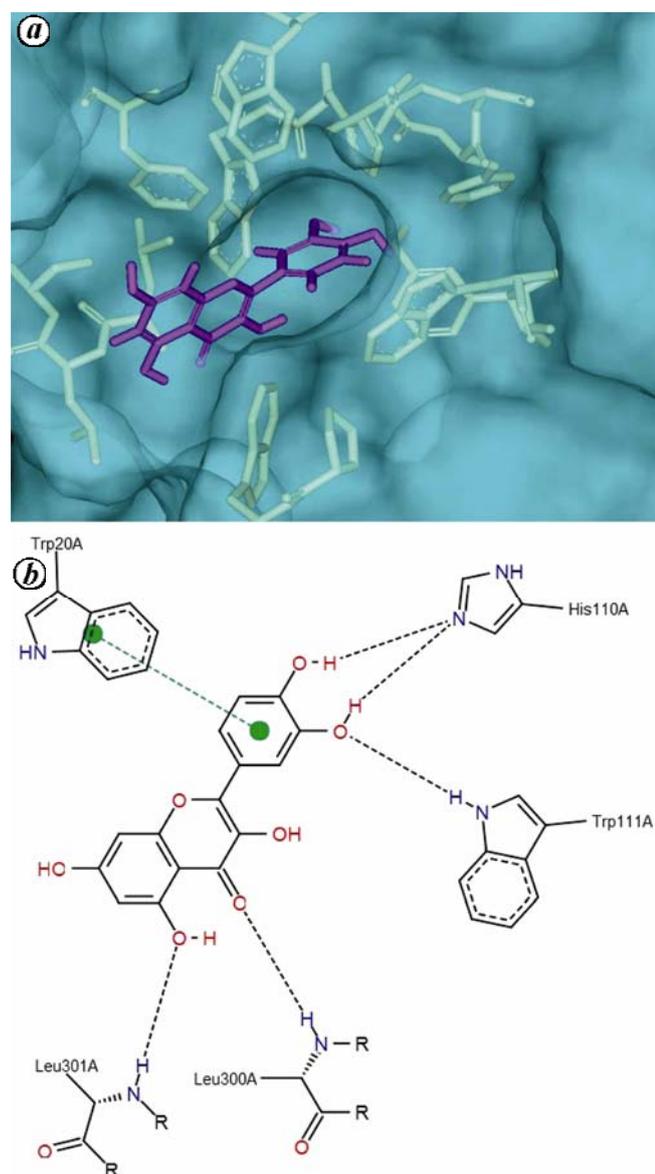


Figure 5. Stereoview of AKR1B1 docked with quercetin. *a*, Quercetin was docked into the active site of ALR2 and extended towards the hydrophobic pocket. *b*, Quercetin docked into the active site of ALR2 and its hydrogen bond interaction with residues Trp-20, His-110, Trp-111, Leu-300 and Leu-301, and hydrophobic interactions with Trp-20 (green dotted line).

Table 3. Effect of rutin and quercetin on intracellular red cell sorbitol level

Group	Rutin	Quercetin
Control	4.5 ± 0.36	4.5 ± 0.45
Glucose 50 mM	11.4* ± 0.53	10.6* ± 0.51
Glucose 50 mM + 50 μ M	6.5 [#] ± 0.63	8.9 [#] ± 0.57
Glucose 50 mM + 100 μ M	4.4 [#] ± 0.20	6.8 [#] ± 0.34

Values are in μ g/ml RBC and are an average of three different experiments. Sorbitol levels were measured in RBC incubated in the presence of normal (5.5 mM) and high (50 mM) glucose for 3 h. Sorbitol is expressed as μ g/ml RBC. *Indicates a statistically significant difference from the control group and [#]indicates a statistically significant difference from the glucose 50 mM group (ANOVA, $P < 0.05$). Values are mean \pm standard deviation of three independent experiments.

indicate that rutin inhibits human recombinant ALR2 in an uncompetitive manner and this inhibition appears to be relatively specific towards ALR2 over ALR1. Suppression of sorbitol accumulation in human erythrocytes under high glucose conditions by rutin is suggestive of translating its impact to *in vivo* conditions. Further, rutin was not only a better ARI than its aglycone quercetin, but also more specific to ALR2. Flavonoids are nonnutritive dietary components that are widely distributed in plants and possess strong antioxidant activity. Since free-radical mediated oxidative modification makes ALR2 insensitive to inhibitors and activation of the polyol pathway also contributes to oxidative stress in target organs³⁵, it may be appropriate to try a combination therapy consisting of ARI and antioxidants. In addition, formation of advanced glycation end-products (AGE) also plays a key role in the several pathophysiologies associated with ageing and diabetes such as retinopathy, nephropathy, neuropathy and cataract. Therefore, inhibition of AGE formation is also one of the approaches to prevent or arrest the progression of diabetic complications. The potential of rutin to prevent and/or inhibit protein glycation has been reported^{36,37}. Thus these multiple properties of rutin support its utility for controlling AGE-mediated diabetic pathological conditions *in vivo*.

Although the beneficial impact of strict glycaemic control on prevention of diabetic complications has been well established, most individuals with diabetes rarely achieve consistent euglycaemia. Hence agents that can substantially delay or prevent the onset and development of diabetic complications, irrespective of glycaemic control, would offer many advantages. In principle, ARI can be included in this category. Thus, intensive research continues to identify and test both synthetic as well as natural products for their therapeutic value to prevent the onset and/or delay progression of diabetic complications.

- Brownlee, M., Biochemistry and molecular cell biology of diabetic complications. *Nature*, 2001, **414**, 813–820.
- Kinoshita, J. H., A thirty-year journey in the polyol pathway. *Exp. Eye Res.*, 1990, **50**, 567–573.
- Bhatnagar, A. and Srivastava, S. K., Aldose reductase: congenial and injurious profiles of an enigmatic enzyme. *Biochem. Med. Metab. Biol.*, 1992, **48**, 91–121.
- Kador, P. F., Robison, W. G. and Kinoshita, J. H., The pharmacology of aldose reductase inhibitors. *Annu. Rev. Pharmacol. Toxicol.*, 1985, **25**, 691–714.
- Pfeifer, M. A., Schumer, M. P. and Gelber, D. A., Aldose reductase inhibitors: the end of an era or the need for different trial designs? *Diabetes*, 1997, **46**, S82–S89.
- Raskin, P. and Rosenstock, J., Aldose reductase inhibitors and diabetic complications. *Am. J. Med.*, 1987, **83**, 298–306.
- Jaspan, J. B., Towle, V. L., Maselli, R. and Herold, K., Clinical studies with an aldose reductase inhibitor in the autonomic and somatic neuropathies of diabetes. *Metabolism*, 1986, **35**, 83–92.
- Foppiano, M. and Lombardo, G., Worldwide pharmacovigilance systems and tolrestat withdrawal. *Lancet*, 1997, **349**, 399–400.
- Srivastava, S. K., Petrash, J. M., Sadana, I. J., Ansari, N. H. and Partridge, C. A., Susceptibility of aldehyde and aldose reductases of human tissues to aldose reductase inhibitors. *Curr. Eye Res.*, 1982, **2**, 407–410.
- Spite, M. *et al.*, Substrate specificity and catalytic efficiency of aldo-keto reductases with phospholipid aldehydes. *Biochem. J.*, 2007, **405**, 95–105.
- Hyndman, D. J. and Flynn, T. G., Sequence and expression levels in human tissues of a new member of the aldo-keto reductase family. *Biochim. Biophys. Acta*, 1998, **1399**, 198–202.
- Crosas, B., Hyndman, D. J., Gallego, O., Martras, S., Pare, S. X., Flynn, T. G. and Farre, S. J., Human aldose reductase and human small intestine aldose reductase are efficient retinal reductases: consequences for retinoid metabolism. *Biochem. J.*, 2003, **373**, 973–979.
- Suryanarayana, P., Kumar, P. A., Saraswat, M., Petrash, J. M. and Reddy, G. B., Inhibition of aldose reductase by tannoid principles of *Emblia officinalis*: implications for the prevention of sugar cataract. *Mol. Vis.*, 2004, **10**, 148–154.
- Suryanarayana, P., Saraswat, M., Mrudula, M., Krishna, T. P., Krishnaswamy, K. and Reddy, G. B., Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. *Invest. Ophthalmol. Vis. Sci.*, 2005, **46**, 2092–2099.
- Suryanarayana, P., Saraswat, M., Petrash, J. M. and Reddy, G. B., *Emblia officinalis* and its enriched tannoids delay streptozotocin-induced diabetic cataract in rats. *Mol. Vis.*, 2007, **24**, 1291–1297.
- Mrudula, T., Suryanarayana, P., Srinivas, P. N. and Reddy, G. B., Effect of curcumin on hyperglycaemia-induced vascular endothelial growth factor expression in streptozotocin-induced diabetic rat retina. *Biochem. Biophys. Res. Commun.*, 2007, **21**, 528–532.
- Saraswat, M., Muthenna, P., Suryanarayana, P., Petrash, J. M. and Reddy, G. B., Dietary sources of aldose reductase inhibitors: prospects for alleviating diabetic complications. *Asia Pac. J. Clin. Nutr.*, 2008, **17**, 58–65.
- Muthenna, P., Suryanarayana, P., Gunda, S., Petrash, J. M. and Reddy, G. B., Inhibition of aldose reductase by dietary antioxidant curcumin: mechanism of inhibition, specificity and significance. *FEBS Lett.*, 2009, **583**, 3637–3642.
- Saraswat, M. *et al.*, Antiglycating potential of *Zingiber officinalis* and delay of diabetic cataract in rats. *Mol. Vis.*, 2010, **16**, 1525–1537.
- Kawanishi, K., Ueda, H. and Moriyasu, M., Aldose reductase inhibitors from the nature. *Curr. Med. Chem.*, 2003, **10**, 1353–1374.
- Zenon 3rd, G. J., Abobo, C. V., Carter, B. L. and Ball, D. W., Potential use of aldose reductase inhibitors to prevent diabetic complications. *Clin. Pharm.*, 1990, **9**, 446–457.
- Fernández, M., Caballero, J., Helguera, A. M., Castro, E. A. and González, M. P., Quantitative structure–activity relationship to predict differential inhibition of aldose reductase by flavonoid compounds. *Bioorg. Med. Chem.*, 2005, **13**, 3269–3277.
- Matsuda, H., Morikawa, T., Toguchida, I. and Yoshikawa, M., Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. *Chem. Pharm. Bull. Tokyo*, 2005, **50**, 788–795.
- Varma, S. D., Mikuni, I. and Kinoshita, J. H., Flavonoids as inhibitors of lens aldose reductase. *Science*, 1975, **188**, 1215–1216.
- Petrash, J. M., Harter, T. M., Devine, C. S., Olins, P. O., Bhatnagar, A., Liu, S. and Srivastava, S. K., Involvement of cysteine residues in catalysis and inhibition of human aldose reductase: site-directed mutagenesis of Cys-80, -298, and -303. *J. Biol. Chem.*, 1992, **267**, 24833–24840.
- Daly, A. K. and Mantle, T. J., Purification and characterization of the multiple forms of aldehyde reductase in ox kidney. *Biochem. J.*, 1982, **205**, 373–380.
- Malon, J. I., Knox, G., Benford, S. and Tedesco, T. A., Red cell sorbitol an indicator of diabetic control. *Diabetes*, 1980, **29**, 861–864.

28. Florek, E., Ignatowicz, E., Wrzosek, J. and Piekoszewski, W., Effect of rutin on total antioxidant status of rats exposed to cigarette smoke. *Pharmacol. Rep.*, 2005, **57**, 84–89.
29. Undeğer, U., Aydin, S., Başaran, A. A. and Başaran, N., The modulating effects of quercetin and rutin on the mitomycin C induced DNA damage. *Toxicol. Lett.*, 2004, **151**, 143–149.
30. Sheu, J. R., Hsiao, G., Chou, P. H., Shen, M. Y. and Chou, D. S., Mechanisms involved in the antiplatelet activity of rutin, a glycoside of the flavonol quercetin, in human platelets. *J. Agric. Food Chem.*, 2004, **52**, 4414–4418.
31. Bhatnagar, A., Liu, S. Q., Das, B., Ansari, N. H. and Srivastava, S. K., Inhibition kinetics of human kidney aldose and aldehyde reductases by aldose reductase inhibitors. *Biochem. Pharmacol.*, 1990, **39**, 1115–1124.
32. Bohren, K. M. and Grimshaw, C. E., The sorbinil trap: A predicted dead-end complex confirms the mechanism of aldose reductase inhibition. *Biochemistry*, 2000, **39**, 9967–9997.
33. Morrison, A. D., Clements, R. S. and Winegard, A. I., Effects of elevated glucose concentration on the metabolism of the aorta wall. *J. Clin. Invest.*, 1972, **51**, 3114–3123.
34. Reddy, G. B., Satyanarayana, A., Balakrishna, N., Ayyagari, R., Padma, M., Viswanath, K. and Petrash, J. M., Erythrocyte aldose reductase activity and sorbitol levels in diabetic retinopathy. *Mol. Vis.*, 2008, **14**, 593–601.
35. Chung, S. S., Ho, E. C., Lam, K. S. and Chung, S. K., Contribution of polyol pathway to diabetes-induced oxidative stress. *J. Am. Soc. Nephrol.*, 2003, **14**, S233–S236.
36. Pashikanti, S., de Alba, D. R., Boissonneault, G. A. and Cervantes-Laurean, D., Rutin metabolites: novel inhibitors of nonoxidative advanced glycation end products. *Free Radical Biol. Med.*, 2010, **48**, 656–663.
37. Muthenna, P., Akileshwari, C., Saraswat, M. and Reddy, G. B., Inhibition of advanced glycation end product formation on eye lens protein by rutin. *Br. J. Nutr.*, 2011, **August 25**, 1–9 [Epub ahead of print].

ACKNOWLEDGEMENTS. This work was supported by grants from the Department of Biotechnology, New Delhi under the 7th FP of Indo-EU collaborative grant on functional foods (Grant agreement # 245030) and Life Sciences Research Board of the Defence Research and Development Organization, Government of India. P.M. was supported by a Senior Research Fellowship from the University Grants Commission, New Delhi. Molecular docking studies were carried out at the Indian Council of Medical Research-Biomedical Informatics Centre, National Institute of Nutrition.

Received 20 June 2011; accepted 30 September 2011