Antidiabetic cataract effects of GbE, rutin and quercetin are mediated by the inhibition of oxidative stress and polyol pathway

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One of the earliest critical secondary complications of diabetes is the opacification of the eye lens – a condition strictly associated with diabetic cataract. The study presented here was designed to investigate the effect of *Ginkgo biloba* extract (GbE), rutin and quercetin on streptozotocin (STZ) induced diabetic cataract (DC) rats. Ten weeks after administration of GbE, rutin and quercetin, the opacity of diabetic rats' lenses was graded under a slit lamp. Then, the levels of malondialdehyde (MDA), reduced glutathione (GSH), advanced glycosylation end products (AGEs), and the activities of aldose reductase (AR) were estimated. The DC-induced rats produced less GSH, higher levels of MDA and AGEs as well as elevated AR activity when compared to the normal group. Administration of GbE, rutin and quercetin remarkably inhibited the AR activity, stimulated the production of glutathione, and decreased the levels of MDA and AGEs in the lenses of DC-induced rats, which eventually delayed the progression of lens opacification in diabetic rats to various degrees. Our results revealed that quercetin had the highest significant ($P<0.05$) potential to delay the progression of STZ-induced diabetic cataract when compared with rutin and GbE. The mechanism dictating this interesting prowess of quercetin might be attributed to its AR inhibitory strength, anti-lipid peroxidation potential and anti-AGEs activity.

Key words: *Ginkgo biloba* extract, rutin, quercetin, diabetic cataract

Received: 07 July, 2016; revised: 20 April, 2017; accepted: 11 July, 2017; available on-line: 28 December, 2017

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INTRODUCTION

One of the most common diabetic complications contributing menace to the diabetic populace is diabetic cataract (Wong *et al.*, 2006). Diabetic cataract (DC) is characterized by cloudiness or opacification of the lens that is responsible for focusing light and producing clear and sharp images (Bahmani *et al.*, 2012). However, the etiology of diabetic cataract remains incompletely understood. Multiple factors are considered to be involved in the progression of DC, such as activation of the aldose reductase (AR), up-regulation of the advanced glycation end products (AGEs) and accumulation of reactive oxy-gen species (ROS) that ultimately induce oxidative stress injury (Obrosova *et al.*, 2010). Currently, surgical extraction is the only way available to cure cataract, which unfortunately often causes considerable financial burden and serious postoperative complications (Bockelbrink *et al.*, 2008). Therefore any inexpensiveand effective non-surgical therapeutic remedy with little or no side effect will be definitely preferred (Olson *et al.*, 2003).

Gingko biloba extract (GbE) is derived from the leaves of *Gingko biloba*, which contains 22–27% flavone glycosides (e.g. kaempferol, quercetin, and isorhamnetin) and 5–7% terpenes (Chan *et al.*, 2007). Recent studies emphasized the pharmacological effects of GbE which include prevention of DC. It has been reported that GbE inhibits lens epithelial oxidative stress-induced apoptosis which is critical to the management/treatment of DC (Wu *et al.*, 2008). GbEalso inhibitsthe AR activity, increases antioxidant ability and reduces the levels of AGEs to suppress the opacification of rat lens *in vivo* and *in vitro* (Liu *et al.*, 2013; Lu *et al.*, 2014). Therefore, knowing the active ingredients and mechanism of action of these compounds might present a potent therapeutic strategy towards the management/treatment of diabetic cataract.

Gingko biloba extract contains rutin and quercetin flavonoids with 13.95% and 3.87% content, respectively (Wang *et al.*, 2005). Recent studies revealed that rutin, a derivative of quercetin, has been shown to possess a wide range of pharmacological activities with scientists demonstrating its capacity to put the lens' AGE at bay (Muthenna *et al.*, 2012), suppress sorbitol accumulation and AR activity (Reddy *et al.*, 2011), prevent selenite-induced cataract in rats by preventing depletion of the reduced glutathione (GSH) and inhibiting lipid peroxidation (Isai *et al.*, 2009). Furthermore, quercetin, a hydrolyzate of rutin, exerts potent reactivity against oxidation and AR (Stefek 2011; Shetty *et al* 2004). It is reported that quercetin plays a beneficial role in relieving diabetic complications by inhibiting AR (Shetty *et al.*, 2004). Our previous studies also established that quercetin could delay occurrence of cataract in rat lens *in vitro* and *in vivo*. These findings present rutin and quercetin as promising functional agents to manage/treat DC.

The focus of this study was to investigate the protective prowess of GbE, rutin and quercetin on streptozotocin (STZ)-induced DC rats by measuring their effect on lens opacity, antioxidant activities, AGEs inhibitory capacities and AR inhibitory strengths. This research elucidates the mechanisms exploited by these three compounds in the management/treatment of DC and may also create some insights for the development of func-

tional foods and nutraceuticals for the management/treatment of DC.

MATERIALS AND METHODS

Reagents. STZ (cat.no.0130), DL-glyceraldehyde (Lot#BCBF4113V) and collagenase I (CAS9001-12-1) were purchased from Sigma Chemicals Company (St. Louis, MO, USA). *Ginkgo biloba* extract, rutin and quercetin ($\geq 98\%$ purity) were supplied by Haoyang Biological Technology Co., Ltd. (Shanxi, China). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) (041939) and nicotinamide adenine dinucleotide phosphate (NADP) (004669) were purchased from Roche Co., Ltd. (Basel, Switzerland). The details of other reagents used are specified accordingly.

Animals. Adult male Sprague-Dawley rats (weighting about 200 g) were provided by the Laboratory Animal Center of Xuzhou Medical College (Xuzhou, China). The rats were maintained in a controlled environment (12 h/12 h light/dark cycles) at 21°C±2°C. All animals were fed with a standard pellet diet and acclimatized to laboratory conditions before experiments. All procedures strictly followed the Guiding Principles for Care and Use of Laboratory Animals of Xuzhou Medical College.

Experimental Designs. Slit lamp microscopy was used to examine the lens of 30 rats while those without any lens defect were induced for diabetic cataract. Twenty four overnight fasted rats were intraperitoneally injected with 65 mg/kg STZ, which was freshly dissolved in 100 mmol/L sodium citrate buffer (pH 4.5) before injection. Sodium citrate buffer: 2.1 g of citric acid are dissolved in 100 ml double distilled water (solution A); 2.94 g of trisodium citrate are dissolved in 100 ml of double distilled water (solution B); 1:1 mixture of solution A and B is made and pH adjusted to 4.2–4.5 with acid. 1% STZ-sodium citrate buffer: before the injection, the corresponding STZ powder was dissolved in a 1% sodium citrate buffer solution. Six normal rats (NC group) received the same amount of buffer only. Blood glucose measurement was performed three days after STZ injection, in which those with ≥13.88 mmol/L fasting blood glucose were considered diabetic rats (Lu et al., 2015a). These rats were then randomly divided into four groups according to their various treatments of 1% sodium carboxymethyl cellulose (CMC-Na) solution (DC group, n=6), 200 mg/kg of *Ginkgo biloba* extract (GbE group, n=6) (Lu et al., 2015b; Lu et al., 2007), 90 mg/kg of rutin (RU group, n=6) (Hao et al., 2008), and 90 mg/kg of quercetin (QU group, n=6) (Lu et al., 2015a). Meanwhile, the rats in the NC group were administered 1% CMC-Na solution of the same volume. The animals were allowed free access to food and water *ad libitum* and were sacrificed 10 weeks after which their body weight and blood glucose were measured before and after treatment. The blood glucose levels were measured through the bleeding of the tail tip after which Qiangsheng glucometer was used to measure blood glucose level while the weighing balance was used to measure the weight of the experimental rats.

Evaluation of cataract progression. Tropicamide, 0.25%, was used to examine the development of rat cataract by dilating the pupils using slit lamp technique. Five minutes later, the rats were anaesthetized by intraperitoneal injection of 10% chloral hydrate at doses of 0.35 mL/100 g body weight. The cataract was scored on a scale of I–V according to the description of Arnal and coworkers (Arnal et al., 2009). Grade I was a normal clear lens, Grade II was a subcapsular opacity, Grade III was nuclear cataract, Grade IV was strong nuclear cataract, and Grade V was dense opacity involving entire lens. Relevant images were taken by a 66VisionTech YZ5T900 imaging system.

Lens preparation and protein determination. Rats were sacrificed at the end of week 10. Lenses were dissected and homogenized in pre-chilled 0.9% neutral normal saline. The resultant homogenate was centrifuged at 4°C at $10\,000 \times g$ for 15 min, storing the supernatant and pellets at −80°C until further analysis. The supernatant was decanted and used to determine protein concentrations, AR activity, GSH, and MDA, while the pellets were harvested for measurement of the AGEs levels. Protein concentration was determined by a protein assay kit (Thermo Scientific, Rockford, IL, USA) according to the bicinchoninic acid method.

Measurement of reduced glutathione in the lens. Glutathione is a tripeptide, which exists in reduced (GSH) and oxidized (GSSG) states. Under physiological conditions, most of glutathione is present in a reduced state, which can prevent unnecessary oxidation of proteins and enzymes by free radicals. The levels of GSH in the lenses were measured by spectrophotometer using kits from Jiancheng Bioengineering Institute (Nanjing, China). Operating procedure: firstly, the tissue has to be accurately weighed and then homogenized, using the ratio of 1:9 with the normal saline buffer; next, the samples are centrifuged at 2500 r/min for 10 minutes. The supernatant isdecanted. Secondly, 0.5 ml of the supernatant is added to 2 mls of the reagent (Precipitant, from the kit) and mixed homogenously using a vortex shaker, centrifuged at 3500–4000 r/min for 10 minutes after which 1 ml of the supernatant istaken for color reaction. The color reaction istaken after the samples and reagent (A coloring agent for catalytic reaction from the kit, DTNB) are added, mixed homogenously and incubated at room temperature for 5 minutes, after which the colorimetric determination is carriedat 420 nm wavelength and 1 cm optical path (Ellman, 1959).

Determination of malondialdehyde (MDA) levels in the lens by HPLC. Lens tissues preparation and MDA determination method were according to Tukozkan and coworkers (Tukozkan et al., 2006). For alkaline hydrolysis of protein bound MDA, 40 μL 6 mol/L sodium hydroxide was added to 200 μL lens homogenate, and the sample was incubated in a 60°C water bath for 30 min. The hydrolyzed sample was acidified with 100 μL 35% perchloric acid. The resulting suspension was then vortex for 30 s and centrifuged at $12\,000 \times g$ for 10 min. The upper clear supernatant was mixed with 25 μL DNPH solution (5 mmol/L DNPH solution in 2 mol/L HCl) and incubated for 30 min at room temperature. After derivatisation, the samples were filtered through a 0.2 μm filter. Aliquots of 50 μL were injected into the HPLC system. MDA levels were measured by RP-HPLC with pre-column derivatization on an Agilent Zorbax SB-C18 column (Agilent United States, 4.6×150 mm, 5 μm). The mobile phase consisted of acetonitrile-distilled water (38:62, v/v) containing 0.2% acetic acid at a flow rate of 1 mL/min. The RP column was set at 35°C and a wavelength of 310 nm was used for UV detection. The concentration of MDA was calculated based on a standard curve and expressed as nmol/g protein. The MDA standard was prepared by dissolving 1,1,3,3-tetraethoxypropane (TEP) in water to give a stock solution of 1 mmol/L. The working standard was prepared by serial dilution of the TEP stock solution with 1% sulfuric acid to 20,10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 μmol/L, yielding a standard curve for total MDA content determination.

Table 1. Effects of *Ginkgo biloba* extract, rutin and quercetin on the body weight and blood glucose of STZ-induced diabetic rats before and after treatment.

Groups	Body weight (g)		Blood glucose (mmol/L)	
	Initial	Final	Initial	Final
NC	210.3±8.5	397.8±15.6	4.6±2.1	5.2±0.8
DC	210.5±7.9	177.0±10.5##	17.1±4.3##	22.8±3.0##
GbE	193.3±7.5	178.0±13.3	18.6±3.9	22.5±5.8
RU	192.5±6.9	184.3±14.3	17.1±4.9	21.8±4.5
QU	188.3±8.2	187.0.3±13.3	18.0±5.5	21.3±8.1

Data are expressed as mean ± S.D., 6 rats in each group, ##$P<0.01$ as compared to the normal control group.

Determination of advanced glycation end products (AGE) levels in the lenses by fluorospectrophotometry. The levels of AGEs in the lens tissues were determined by fluorospectrophotometer as previously reported (Lu *et al.*, 2014; Liu *et al.*, 2013) and expressed as the enzymatic activity of type I collagenase (U) per milligram of protein (mgprot).

Measurement of aldose reductase (AR) activity in lenses by fluorospectrophotometry. AR activity in the rat lenses was detected by spectrophotometry according to Liu and coworkers (Liu *et al.*, 2013; Brownlee, 2001). The enzymatic reaction system adopted was 200 µL consisting of 135 mmol/L phosphate buffers, 100 mmol/l of lithium sulfate, 150 µmol/L NADPH, 5 µL lens homogenate, and 1.0 mmol/L DL-glyceraldehyde. The fluorescence was measured with a spectrofluorophotometer (Ex/Em – 360 nm/460 nm; GloMax-Multijr, Promega Co., Ltd., USA), and expressed as the unit of AR enzyme activity (U) per gram of protein (g prot).

Statistical analysis. Data are presented as the mean ± standard deviation (S.D.). Statistical analysis was performed using SPSS software, version 13.0 (SPSS, Inc., Chicago, IL, USA). Intergroup differences were compared using one-way analysis of variance (ANOVA) followed by the Dunnett's test. Cataracts were graded using the Wilcoxon signed-rank test. Statistical significance was defined as $P<0.05$.

RESULTS

Effects of *Ginkgo biloba* extract, rutin, and quercetin on the blood glucose and body weight of STZ-Induced diabetic rats

The blood glucose and body weight of the experimental rats were measured before and 10 weeks after treatment with *Ginkgo biloba* extract, rutin, orquercetin.After the administration of STZ, rats in the DC group presented obvious increase in blood glucose ($P<0.01$), which was in contrast with their declined body weights ($P<0.01$), as compared with their baseline weights. However, no remarkable changes were observed as to blood glucose and body weight after administration of *Ginkgo biloba* extract, rutin, and quercetin (Table 1).

Effects of *Ginkgo biloba* extract, rutin, and quercetin on the lens opacity of STZ-induced diabetic rats

Eyes were examined using a slit lamp biomicroscope. The progression of lenticular opacity was assessed according to the description of Arnal and coworkers (Liu *et al.*, 2013). The lens of normal control rats showed no trace of turbidity with confirmed clearance and transparency according to the slit lamp findings after treatment. In contrast, the rats in the DC group presented cloudy lenses, with nuclear opacity within a range of grades III-V. The QU group reported an average cataract score that was significantly lower than those in the GbE and RU groups ($P<0.05$), indicating a superior effect of quercetin over *Ginkgo biloba* extract and rutinindelaying the progression and maturation of diabetic cataract (Table 2 and Fig. 1).
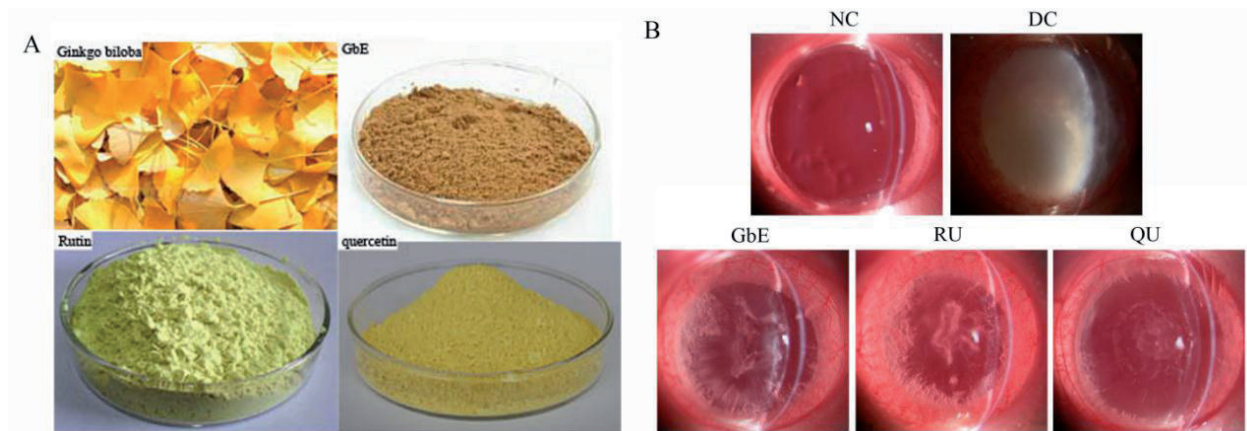
Effects of *Ginkgo biloba* extract, rutin, and quercetin on reduced glutathione in the lens of STZ-induced diabetic rats

When the normal control group was compared with the DC group, a dramatic decline in the level of GSH was detected in the DC group ($P<0.01$), which represented a striking decrease in the antioxidant capacity of DC rats. However, such a decrease was reversed by treatment with GbE, rutin,and especially quercetin ($P<0.01$) (Fig. 2).

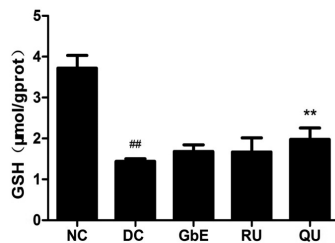**Table 2.** Effects of *Ginkgo biloba* extract, rutin and quercetin on the lens opacity of STZ-induced diabetic rats.

Groups	Cataract grades				
	0	I	II	III	IV
NC	12	0	0	0	0
DC##	0	0	2	1	9
GbE	0	0	3	4	5
RU**	0	2	5	2	3
QU**ΔΔ	0	5	4	1	2

6 rats in each group; ##$P<0.01$ compared with the NC group; **$P<0.01$ compared with the DC group; ΔΔ$P<0.01$ compared with the GbE group.

Figure 1. Effects of *Ginkgo biloba* extract, rutin and quercetin on the lens opacity of STZ-induced diabetic rats.
NC: normal control group; DC: diabetic cataract rats treated with 1% CMC-Na solution; GbE: diabetic cataract rats treated with 200 mg/kg of *Ginkgo biloba* extract; RU: diabetic cataract rats treated with 90 mg/kg of rutin; QU: diabetic cataract rats treated with 90 mg/kg of quercetin. A: *Ginkgo biloba*, *Ginkgo biloba* extract, quercetin and rutin powder; B: the lens opacity of STZ-induced diabetic rats.

Figure 2. Effects of *Ginkgo biloba* extract, rutin and quercetin on GSH levelsin the lens of STZ-induced diabetic rats.
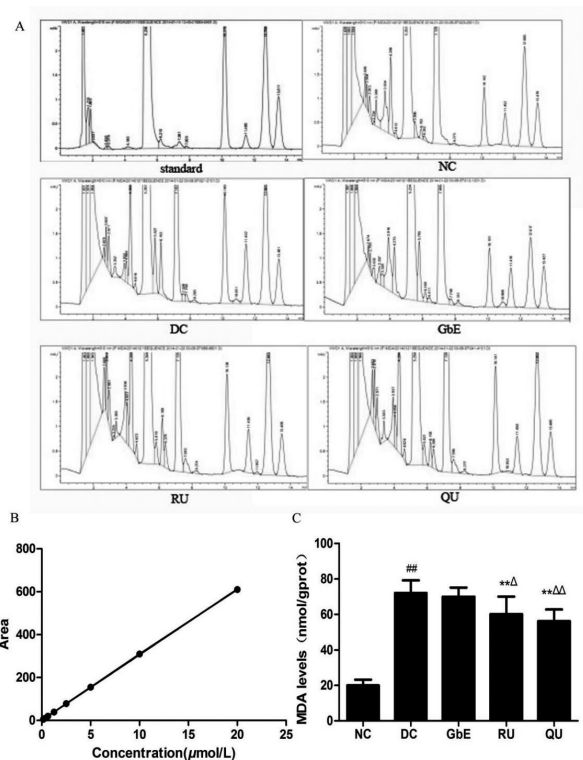The levels of reduced GSH in rats are presented as mean ±S.D., n=6,##$P<0.01$ compared with the NC group;**$P<0.01$ compared with the DC group; NC: normal control group; DC: diabetic cataract rats treated with 1% CMC-Na solution; GbE: diabetic cataract rats treated with 200mg/kg of *Ginkgo biloba* extract; RU: diabetic cataract rats treated with 90 mg/kg of rutin; QU: diabetic cataract rats treated with 90 mg/kg of quercetin.

Effects of *Ginkgo biloba* extract, rutin, and quercetin on malondialdehyde (MDA) levels in the lens of STZ-induced diabetic rats

The retention time of MDA in samples averaged at 10.0 min, which was consistent with the standard forqualitative analysis (Fig. 3A, B). HPLC separation provided a standard curve with a linear dose-response for total MDA content and HPLC peak areas within a range of 0.15625 to 20 μmol/L (y=1.1167+30.536x; r²=1) (Fig. 3C). Compared to baseline, the quantity of the MDA lipid oxidation product rose significantly in the DC group ($P<0.01$). Treatments with GbE, rutin and quercetin could prevent this increase. Moreover, both the RU and QU groups presented obviously reduced levels of MDA in comparison to the DC group ($P<0.01$ for both) and the GbE group ($P<0.05$ for RU, and $P<0.01$ for QU) (Fig. 3C).

Effects of *Ginkgo biloba* extract, rutin, and quercetin on the levels of advanced glycation end products (AGEs) in the lens of STZ-induced diabetic rats

AGEs levels were enhanced in the lenses of the DC group relative to the normal controls ($P<0.01$), which dramatically declined after administration of rutin and quercetin ($P<0.01$) but not with GbE. Meanwhile, quercetin demonstrated a stronger decrease in AGEs in the lens than rutin did ($P<0.01$) (Fig. 4).
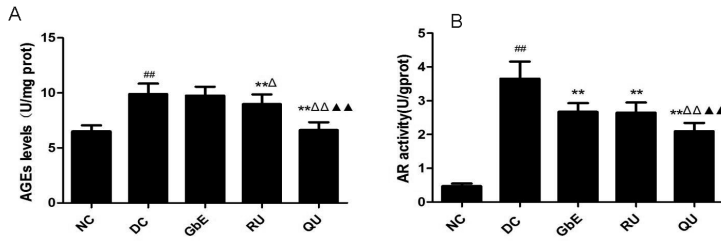

Figure 3. Effects of *Ginkgo biloba* extract, rutin and quercetin on MDA levels in the lens of STZ-induced diabetic rats.
(**A**) Peak position of MDA in standard and in samples. (**B**) MDA standard curve ranged from 0 to 20 μmol/L. (**C**) Statistical analysis of MDA in samples. The levels of MDA in rats are presented as mean ±S.D., n=6, ##$P<0.01$ compared with the NC group;**$P<0.01$ compared with the DC group; ΔΔ$P<0.01$, Δ$P<0.05$ compared with the GbE group; NC: normal control group; DC: diabetic cataract rats treated with 1% CMC-Na solution; GbE: diabetic cataract rats treated with 200 mg/kg of *Ginkgo biloba* extract; RU: diabetic cataract rats treated with 90 mg/kg of rutin; QU: diabetic cataract rats treated with 90 mg/kg of quercetin

Effects of *Ginkgo biloba* extract, rutin, and quercetin on aldose reductase (AR) activity in the lens of STZ-induced diabetic rats

The AR activities in the DC group significantly increased when compared with those of the normal controls ($P<0.01$). The diabetic rats receiving *Ginkgo biloba*

Figure 4. Effects of *Ginkgo biloba* extract, rutin, quercetin on the AGEs levels (A) and AR activity (B) in the lens of diabetic rats.
Mean ±S.E.M., n=6, ##$P<0.01$ compared with the control (NC) group; **$P<0.01$ compared with the DC group; △△$P<0.01$, △$P<0.05$ compared with the GbE group; ▲▲$P<0.01$compared with the RU group; NC, normal controls; DC, diabetic cataract group; GbE, *Ginkgo biloba* extract treatment group; RU, rutin treatment group; QU, quercetin treatment group.
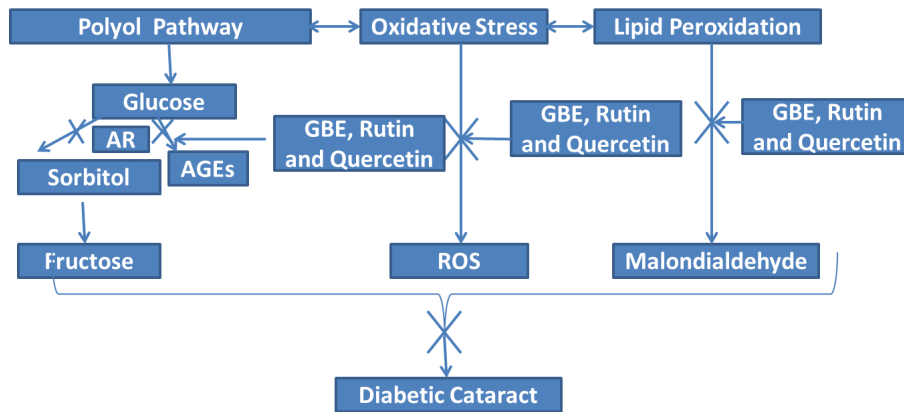
extract, rutin orquercetin showed weaker AR activity than those in the DC group ($P<0.01$), where the inhibitory effect of quercetin seemed stronger than the effect of GbE ($P<0.01$) and rutin ($P<0.01$) (Fig. 4).

DISCUSSION

The molecular complications surrounding the progression of diabetic cataract include the activation of polyol pathway, non-enzymatic glycation of eye lens proteins and oxidative stress. AR, the rate limiting enzyme of the polyol pathway, reduces glucose to sorbitol underhyperglycemic conditions, while sorbitol dehydrogenase (SDH) oxidizes sorbitol to fructose. Because the hydrophilic nature of sorbitol makes its diffusion across the membrane impossible, it accumulates intracellularly, thereby imposing osmotic damage to the cell's cortex and lens, inducing opacification (Arnal *et al.*, 2009; Giacco & Brownlee, 2010; Brownlee, 2001; Aronson, 2008). In this study, the significant ($P<0.05$ and $P<0.01$) AR activity in the DC group could be attributed to the abnormal blood glucose level in the diabetic rats. This increase was arrested by the administration of *Gingko biloba* extract, rutin and quercetin. *Gingko biloba* extract, quercetin and its derivative rutin reduced the activity if this rate limiting enzyme, while quercetin proved itself to be the most potent flavonoid by having the most significant inhibitory

strength. Our result was in agreement with a previous study where Aldose Reductase Inhibitors (ARIs) were administered topically in diabetic rats over the duration of 3 months (Stefek, 2011). Decrease cataract formation associated with decreased lens sorbitol was observed in the treated rats when compared to controls. This is also in line with another study, where ARI-809 (a recently discovered aldose reductase inhibitor) had high selectivity for aldose reductase. It was observed that ARI-809 inhibited the polyol pathway in the retina of insulinizedstreptozotocin-induced diabetic rats after four months of administration (Sun & Oates, 2006). Another group reported that fidarestat counteracted the cataract formation in diabetic cataractogenesis and early retinopathy and therefore presented it as a therapeutic measure for the management/treatment of this disease (Drel *et al.*, 2008).

Underthe high glucose conditions, glucose stands another chance of forming covalent adducts with the plasma proteins through a non-enzymatic process called glycation. Thelens' AGEs are formidable enough to induce irreversible alterations in proteins which cause high molecular lens protein aggregates to scatter light and impede vision (Nagaraj *et al.*, 2012). It has been proven beyond scientific controversy that AGEs alter the cell surface protein charges, thereby imposing a conformational change that might interfere with protein-protein and protein-water interactions which ultimately reduces the eye lens transparency – a critical hallmark of diabetic cataract (Kumar *et al.*, 2004). Glycation affects some biomolecules in nature, like proteins, and therefore changes their structures and functions; it also affects enzymes by altering their activities. AGEs could also bind to their plasma membrane receptors AGERs to effect a molecular conformational change that ultimately interferes with intracellular signal transduction cascade process and downstream gene expression which might release some pro-inflammatory molecules in association with free radicals. Therefore, denaturation, functional deterioration of target proteins and lipids that ultimately provokes organ-

Figure 5. *Gingko biloba* extract, rutin and quercetin atenuated streptozotocin-induced diabetic cataract in rats.
In diabetic cataract, chronic hyperglycemia could provoke oxidative insults, osmotic damage and lipid peroxidation in optical lens, which lead the decreased activity of antioxidant enzymes, excessive activation of aldose reductase (AR) and the accumulation of advanced glycation end products (AGEs). In this study, *Gingko biloba* extract, rutin and quercetin improved the aldose reductase activity, arrested lipid peroxidation process through MDA reduction and ameliorates ameliorate oxidative stress by increasing glutathione activity, scientifically proves their effectiveness towards the management/treatment of diabetic cataract. The figure summarizes the essence of the whole project where *Gingko biloba* extract, Rutin and quercetin attenuated streptozotocin-induced diabetic cataract in rats.

opathy (due to AGEs accumulation in tissues), activation of receptor-mediated signal pathways in cells together with the generation of oxidative stress could connive together to erupt an arsenal of mechanism through which glycation alters cell functions (Yonekura et al., 2005). Our results indicated a significant increase in AGEs when compared with control, which might indicate the onset or progression of DC. Reduced level of AGEs was observed in the diabetic rats after administration of rutin and quercetin, while GbE had a weaker effect. The strongest inhibitory effect was observed in rats treated with quercetin.

NADH oxidase uses the product of sorbitol dehydrogenase (NADH) for its activity thereby, generating intracellular ROS which could provoke oxidative stress. It has been reported that a decrease in NADH/NADP is strong enough to provoke oxidative insults by decreasing cellular antioxidants that use NADPH as cofactor (glutathione peroxidase) thereby increasing polyol pathway susceptibility to intracellular oxidative stress which ultimately weakens retina's integrity (Giacco & Brownlee, 2010; Baynes, 2010; Gabbay, 1973). The oxidative stress, that serves as the converging point for many pathways that lead to the onset and progression of diabetes and its downstream complications, is characterized by a decreased level of antioxidants, reduced glutathione and lipid peroxidation (Obrosova et al., 2010). GSH attenuates DC by exploiting three different mechanisms which include the maintenance of protein thiols in reduced state to prevent light scattering and lens opacification, protection of membrane -SH groups that are important in cation transport and permeability, detoxification of hydrogen peroxide and other organic peroxides (Srinivas, 2014). MDA is the toxic byproduct of lipid peroxidation, resulting in the polymerization and cross-linking of nucleic acids and other macromolecules. It has been explored several times as a potential biomarker to evaluate the extent of cell damage caused by oxidative stress (Januszewski et al., 2003; Libondi et al., 1994). In light of this, we unravel the effect of Gingko biloba extract, rutin and quercetin against the oxidative stress generated under high glucose conditions. Our result show an obvious decrease in the GSH levels while an increase in the MDA level was seen in the lenses of hyperglycemic rats. This significant reduction in GSH and increment of MDA actually signifies the actually marks the onset and progression of oxidative stress. After the administration of Gingko biloba extract, rutin and quercetin, the GSH level was increased in the lens by all extracts, while quercetin showed the most potent activity when compared to rutin and Gingko biloba. All extracts inhibited lipid peroxidation by reducing malondiadehyde (MDA) content in the lens, thereby putting cataractogenesis on hold. Our results are consistent with those of others, where when the mesangial cells were incubated with quercetin, the antioxidant enzymes' activities (T-SOD), Catalase and (GSH-Px) increased, while malondialdehyde content decreased significantly, suggesting that quercetin possesses a strong antioxidant prowess. Functional foods containing flavonoids have been found to possess antioxidant activity against cataract (Durukan et al., 2006; Patel et al., 2012). Isoflavone genistin has been noted for its ability to delay galactose-induced cataract progression. Therefore, our studies fall in perspective with most antioxidant literature on how flavonoids could eliminate diabetes and its complications early on.

Recent studies confirmed the relationship between the abnormal glucose-induced polyol metabolic pathway, non-enzymatic protein glycation and oxidative stress.

Persistent oxidative stress can influence non-enzymatic and polyol metabolic pathway by promoting the continuous generation of AGEs, enhancing AR activities and over expressing AR mRNA (Stitte et al., 2002). Meanwhile, the activation of the polyol pathway caused consumption of NADPH, resulting in a decrease of GSH, and an increase in MDA (Tang et al., 2012). AGEs generate a large amount of hydroxyl groups and superoxide anion by autoxidation. Therefore, it makes sense to say multiple factors are responsible for the progression of diabetic cataract and any therapeutic measure that could tackle it in more than a single perspective might present a better efficacy.

CONCLUSION

In summary, we established the effective role of Gingko biloba extract, rutin and quercetin in the prevention/treatment of STZ-induced diabetic cataract through inhibition of the polyol pathway, lipid peroxidation and oxidative stress. The ability of these compounds to decrease the aldose reductase activity, arrest lipid peroxidation process through MDA reduction and ameliorate oxidative stress by increasing glutathione activity, scientifically proves their effectiveness towards the management/treatment of diabetic cataract. Our result had verified quercetin as the most potent flavonoid that could put this disease at bay, and therefore it might be a useful nutraceutical for the treatment/management of this condition.

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