• Basic Research •

Astragalin attenuates diabetic cataracts *via* inhibiting aldose reductase activity in rats

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Received: 2022-08-30 Accepted: 2022-12-28

Abstract

● **AIM:** To investigate the aldose reductase (AR) inhibition capacity of astragalin (AST) against streptozoticin-induced diabetic cataracts (DCs) in rats.

● **METHODS:** *Ex vivo* investigations were conducted by treating the lens of a goat placed for 72h in artificial aqueous humor (AAH) of pH 7.8 at room temperature with cataract-causing substance (55 mmol/L of galactose) and *in vivo* studies were performed on rats *via* induction with streptozotocin. AST was administered at different dose levels and scrutinize for DC activity.

● **RESULTS:** In diabetic rats, AST improved the body weight, blood insulin, and glucose as well as the levels of galactitol in a dose-dependent way, other biochemical parameters *i.e.* inflammatory mediators and cytokines, and also suppress AR activity. The level of the antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) activity were also altered on a diabetic lens after the administration of the AST.

● **CONCLUSION:** AST protects against lens opacification to avoid cataracts and polyols formation, indicating that it could be used as a potential therapeutic agent for diabetes.

● **KEYWORDS:** astragalin; diabetic cataract; lens; opacification; aldose reductase rats

DOI:10.18240/ijo.2023.08.02

Citation: Wang N, Singh D, Wu Q. Astragalin attenuates diabetic cataracts *via* inhibiting aldose reductase activity in rats. *Int J Ophthalmol* 2023;16(8):1186-1195

INTRODUCTION

Diabetic mellitus is a key risk factor for cataracts, which is a major leading reason for blindness and vision impairment globally. Diabetic cataract (DC) has become more common as the prevalence of diabetes has increased[1]. Approximately 25%-35% of surgical procedures are assessed solely on diabetic patients. The lens is a crucial component of the eye, and alterations to its physiological makeup significantly affect the eye's biological parameters[2-3]. Dysfunction in the optical or opacification of a lens that is crystalline caused by the breakdown of the micro-architecture of the eye lens and averts light from reaching the retina[4-5]. The glucose cellular content rises significantly in diabetes mellitus in tissues, and entry of glucose is not dependent on insulin, such as the retina, peripheral nerves, kidneys, and lenses. The lens becomes rigid as a result of the increased pressure, which destroys the production of cataract point cells. Hyperglycemia, or a prolonged rise in blood glucose, in a three-way, causes the formation of cataracts: oxidative stress, lens protein non-enzymatic glycation of an eye, and activation of the polyol pathway in the disposal of glucose[6-8].

Aldose reductase (AR) is a key enzyme during the polyol pathway that catalyzes the conversion from sorbitol to glucose, which accumulates intracellularly and causes osmotic stress, leading to lens fiber degeneration and the formation of cataracts. Association of polyol accumulation to lens liquefaction leads to lens opacities formation[9-11]. Therefore, it is believed that the AR enzyme's activation is a crucial step in the development of underlying diabetic problems, including cataracts. Inhibition of the AR enzyme has been shown in many studies to be useful for DCs treatment. Although numerous chemo-synthetic AR inhibitors (ARIs) are developed to treat problems of diabetes, they have little permeability for targeting particular sites and a long list of negative effects[12]. As a result, researchers are now focusing on identifying prospective drugs derived from natural sources useful in diabetes problem therapy while having minimal or no adverse effects.

Cuscuta chinensis Lam belongs to the *Convolvulaceae* family and has been found to contain astragalin (AST; $C_{21}H_{20}O_{11}$), one of the active flavonoids as shown in Figure 1. Cuscuta seeds

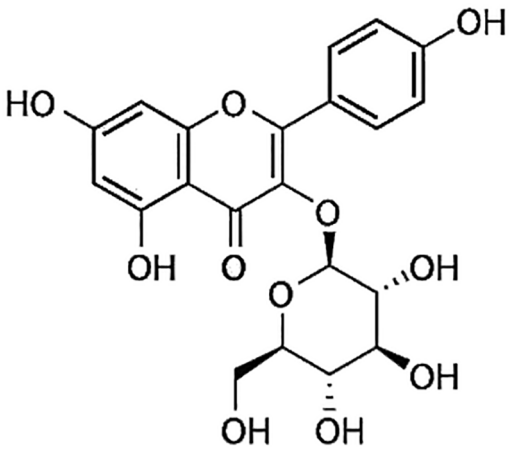

Figure 1 Astragalin.

are high in AST and are used as a conventional remedy for the treatment of osteoporosis in several Asian countries[13-14]. In comparison to other species, *C. chinensis* has a great amount of AST, accounting for 29–34 percent of total phenolics. *In vivo*, AST can be made by glycosylating kaempferol at the 3C-O location[15]. The biologically active and beneficial chemical AST possesses a wide range of pharmacological properties, including neuroprotective, antifibrotic cardioprotective, antioxidant, anti-inflammatory, antiulcer, anticancer, and antidiabetic, properties. In the interim, several investigations related to AST (*in vivo* and *in vitro*) have clarified its therapeutic properties and processes[16]. It was reported that AST functions as an antioxidant, promoting mitochondrial permeability, lipid peroxide inhibition, and free radical scavenging. It also acts as an inhibitor, lowering inflammatory factor levels and preventing inflammatory signal pathways. AST has the best antioxidant defenses and anti-inflammatory benefits, according to all of this research[17]. Because these flavonoids have comparable chemical structures and biological roles, we anticipated that AST could help DC dysfunction. As a result, the current study suggested that AST had protective potential on cataracts *in vitro* experimental models, including antioxidant enzyme activity and inflammation inhibition.

MATERIALS AND METHODS

Ethical Approval The present study involves the usage of animals that complies with all applicable national rules and policies of an institution for the use and care of animals (Research Ethics Committee, Madurai Kamaraj Univerity, Approval No.20226113AA).

Ex Vivo Anti-Cataract Property

Experimental procedure All the lenses (n=30) were separated from the goat eye and divided into various groups as follows (each including six lenses).

Group I consider normal and treated with artificial aqueous humor (AAH) only; Group II was placed in AAH and 55 mmol/L galactose and denoted as a diabetic lense; Group III was placed in AAH+55 mmol/L galactose+standard drug vitamin E (100 g/mL); Group IV was placed in AAH+55 mmol/L galactose+AST (10 mg/kg); Group V was placed in AAH+55 mmol/L galactose +AST (20 mg/kg).

Lens media Fresh Lens was taken out from the goat and kept at a temperature of 0°C–4°C. Extracapsular extraction was used to remove the lenses, which were then incubated for 72h in AAH which is composed of 5.5 mmol/L glucose, 140 mmol/L NaCl, 0.4 mmol/L $CaCl_2$, 2 mmol/L $MgCl_2$, KCl 5 mmol/L, 0.5 mmol/L NaH_2PO_4, and 0.5 mmol/L $NaHCO_3$ at room temperature and pH 7.8. In culture media, an addition of 32 mg percent of penicillin and 250 mg percent of streptomycin was done to prevent bacterial contamination. To induce cataracts, galactose at a dose of 55 mmol/L was utilized[18].

Morphology of lens Graph sheets was used to assess the morphology of the lens by placing them on it. The opacity of the lens was counted as several squares visible within the lens. There were also morphological alterations for example swelling, haziness, and new morphological disorders. The cataract alterations were graded which are as follows: Grade 0 refers to the shape and outline of the lens not affected; Grade 1 refers to the lens became swelled and outline not affected; Grade 2 refers to moderate change in the lens (swelling and faintly visible grid lines), Grade 3 refers to moderate to a severe change in the lens (shape and outline distorted and obstructed grid lines), Grade 4 refers to a severe change in the lens (shape and outline destructed, rupture, matured cataract and grid lines invisible)[19].

Lens homogenate preparation for biochemical parameter Separation of lenses after 72h of incubation from each group, and 50 mmol/L (pH 7.4) phosphate buffer was used for the preparation of 10% (w/v) homogenate. With the help of a cooling centrifuge, the homogenate of the lense was allowed to centrifuge for 20min at 10 000 g (4°C). Lens biochemical analysis such as malondialdehyde (MDA) was used to evaluate lipid peroxidation[20], catalase (CAT), superoxide dismutase (SOD), glutathione (GSH)[21], total protein, lactate dehydrogenase (LDH)[22], sorbitol dehydrogenase (SDH), electrolytes, sorbitol, calcium, which were estimated from the supernatant as per the reported method[23].

In Vivo Experimental Design

Animals Male Wistar rats weighing between 160 to 210 g were from the department for experimental purposes and were given unlimited food and drink access. Polypropylene cages were used to house the rats with a 12-hour day/night cycle following sanitary situations.

Induction of diabetes and dose regimen of 60 mg/kg body weight of streptozotocin (STZ) was administered to induce

diabetes and 2 percent of carboxyl methyl cellulose (CMC) was used to suspend AST and was given to rats by oral gavage to treat experimental diabetes. All the rats were divided into the following categories.

Group I was considered as normal control rats and received only vehicle; Group II was treated as DC rats without treatment; Group III rats had diabetes cataract+AST (10 mg/kg); Group IV rats had DC+AST (20 mg/kg), and Group V rats had DC+AST (30 mg/kg).

Homogenate preparation from lens tissue of diabetic rats All the animals were euthanized *via* cervical dislocation after the experiment's completion and lenses were collected from the eyeballs. After removing the lens, it was cleaned and centrifuged for 5min at 10 000 g in phosphate buffer solution (PBS; 10%). The resultant supernatant was collected for further biochemical analysis[24].

Assessment of progression of cataracts of experimental rats We used tropicamide (0.25%) for the dilation of pupils and assess the cataract in diabetic rats with the help of the slit lamp technique. After 5min, all group rats were treated with an *i.p.* injection of chloral hydrate (10%). The reported method used for the classification of cataract scores (scale I-V) which are as follows: Grade I, normal clear lens; Grade II, subcapsular opacity; Grade III, nuclear cataract; Grade IV, strong nuclear cataract; and Grade V, dense opacity[25].

Aldose reductase preparation from isolated rat lenses The lenses of rats were dissolved in three times the volume of cold water and homogenized for 20min. The homogenate of the lens was subjected to centrifuge at 4℃ for 10 000 rpm for 15min and collection of supernatant after being treated with 70% ammonium sulfate. Again it was dialyzed for 24h. Bradford method was used to estimate the content of protein and crude AR was kept at a temperature of -80℃[26].

Assessment of antioxidant parameter The homogenate was utilized for the measurement of the level of oxidative stress marker. The activity of antioxidant parameters such as antioxidant capacity (MAK334, Sigma Aldrich, Merck, Beijing, China), MDA levels (MAK085, Sigma Aldrich, Merck, Beijing, China), glutathione peroxidase (GPx) activity (A005-1-2, Nanjing Jiancheng Bioengineering Institute), GSH assay kit (CS0260, Sigma Aldrich, Merck, Beijing, China) as per specification defined in the assay kit.

Assay of inflammatory markers Quantification of inflammatory markers [interleukin (IL)-1β and vascular endothelial growth factor (VEGF)] in the retina of rats. The assay was done using IL-1β (583311, Cayman Chemical, Michigan, USA) and VEGF (EK0539, VEGF ELISA Kit PicoKine®, Boster Biological Technology, 3942 Valley, CA, USA) enzyme linked-immunosorbent assay (ELISA) kit as per instruction of the manufacturer. It was transferred to a 96-well plate and incubated at 37℃ for 2h. The solution was assessed on the microplate reader at the wavelength of 450 nm.

Estimation of mRNA and aldose reductase activity in rat lenses The lense of each rat was subjected to homogenization as per the method stated above and a collected supernatant was used to assess the activity of enzyme AR. The number of enzymes that involve as catalysts for the 1 μmol NADPH/h/100 mg protein oxidation. TRIzol reagent was used to extract total RNA from the lens, and the real-time reverse transcription polymerase chain reaction (RT-PCR) technique was used to measure the AR mRNA expression[27].

Estimation of galactitol in rat lens The level of lenticular galactitol was determined with 20% of trichloroacetic acid (TCA) about 20 μL was added to the homogenate of the lens (80 μL) and subjected to centrifuge for 10min at 5000 rpm (4℃) and the level of galactitol was assessed using collected supernatant. Galactitol level was measured at 570 nm spectrophotometrically[28].

Western Blot Analysis The tissue of the lens was lysed with the help of a phosphorylase inhibitor and a bicinchoninic acid (BCA) protein quantitation kit was used to measure the amount of protein. The enhanced chemiluminescence image analyzer was used to see the bands, and image-pro plus software was used to measure the protein levels (Media Cybernetics, Inc., MD, USA)[29].

Statistical Analysis The findings of parametric data were examined by the Tukey-Kramer multiple comparison tests and one-way ANOVA which are shown as the mean standard error of the mean (SEM) by using Graph Pad Prism software.

RESULTS

Morphology of Lens The lenses of the normal rat group were found to be clear and translucent, and graph paper grid lines were seen as very clear. In comparison to the normal group's lenses, the diabetic group's lenses had completely lost their transparency, and shown opacity, as evidenced by swelled, matured cataracts on the verge and the disappearance of grid lines and indicated Grade 4 alterations. At 20 mg, AST reduced the development of cataracts, as demonstrated through the presence of grid lines, limited swelling on the lens, and complete shape of the lens, resulting in Grade 1 alterations in both cases. The lower dose level of AST on lenses, on the other hand, displayed minor swelling.

Biochemical Analysis of Lens Homogenate Lens homogenate was used to observe the effect of AST on biochemical parameter, and the outcomes are described below.

Estimation of enzymatic antioxidant parameter Levels of MDA in the diabetic lens were significantly higher (P<0.001) than in normal rats which suggests more lipid peroxidation (LPO) in lenses treated with galactose. When compared to the diabetic groups, AST treatment on lenses

Table 1 Effect of AST on the enzymatic antioxidant parameter of the lens mean±SEM, *n*=6

Parameters	Normal control	Diabetic control	Vitamin E acetate, 100 μg/mL	AST-10	AST-20
MDA (nmol/100 mg)	0.8480±0.086	4.210±0.891[a]	1.928±0.163[b]	2.988±0.062[b]	2.287±0.063[b]
CAT (U/mg of tissue)	8.315±0.325	0.4168±0.123[a]	6.935±0.301[b]	2.945±0.428[b]	6.467±0.446[b]
SOD activity (U/mg of tissue)	2.073±0.137	0.1783±0.031[a]	1.399±0.048[b]	0.9224±0.045[b]	1.068±0.046[b]
GSH (nmol/100 mg)	23.05±2.918	11.01±0.5017[a]	16.95±0.8280[b]	14.04±1.502[b]	18.15±0.8728[b]

[a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathione; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg; CAT: Catalase.

Table 2 Effect of AST on the sorbitol, SDH, and LDH of lens mean±SEM, *n*=6

Parameters	Normal control	Diabetic control	Vitamin E acetate, 100 μg/mL	AST-10	AST-20
Sorbitol (μg)	3.488±0.1697	7.754±0.2672[a]	4.642±0.2105[b]	6.382±0.2301[b]	5.748±0.2287[b]
SDH	0.2561±0.145	0.849±0.005[a]	0.3834±0.004[b]	0.4561±0.003[b]	0.3722±0.004[b]
LDH (U/mg of solid)	14.58±0.711	23.48±0.815[a]	15.23±0.782[b]	13.71±0.614[b]	11.95±0.975[b]

[a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. SDH: Sorbitol dehydrogenase; LDH: Lactate dehydrogenase; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg.

Table 3 Effect of AST on the electrolytes and proteins of the lens mean±SEM, *n*=6

Parameters	Normal control	Diabetic control	Vitamin E acetate, 100 μg/mL	AST-10	AST-20
Calcium (mg/dL)	8.436±0.4762	15.31±0.4183[a]	12.83±0.783[b]	14.378±0.4682[b]	11.73±0.5579[b]
Sodium (%)	0.0234±0.00017	0.0351±0.00056[a]	0.02335±0.000357[b]	0.02879±0.000378[b]	0.02583±0.00025[b]
Potassium (%)	0.004159±0.0003875	0.01234±0.000465[a]	0.008878±0.00021[b]	0.01007±0.000638[b]	0.002052±0.00044[b]
Protein (mg/dL)	11.27±0.3517	3.247±0.1358[a]	9.462±0.3740[b]	5.688±0.4293[b]	8.274±0.1435[b]

[a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg.

showed a considerably lower content of MDA at two different concentrations (*P*<0.001).

Level of catalase, superoxide dismutase, and glutathione In comparison with normal group rats, diabetic groups shown considerably lower levels of SOD, CAT, and GSH. The diabetic group when treated with different doses of AST showed a significant increase in the activity of CAT and SOD. AST at a dose level of 20 mg significantly enhanced the activity of GSH (*P*<0.001), whereas treatment with 10 mg of AST did not affect these levels (Table 1).

Estimation of the level of sorbitol Lenses treated with galactose show an increased content of sorbitol than the normal control lenses. Sorbitol content was found to be considerably reduced at a dose level of 10 mg (*P*<0.01) and 20 mg (*P*<0.001), of AST, respectively (Table 2).

Estimation of levels of sorbitol dehydrogenase and lactate dehydrogenase The diabetic group's lenses showed a substantial improvement (*P*<0.001) in the level of LDH and SDH, whereas two different concentrations of AST significantly (*P*<0.001) decrease the lens's SDH and LDH (Table 2).

Estimation of content of electrolytes (Ca^{2+}, Na^{+}, K^{+}) In comparison with the control group lens, diabetic lens groups had higher electrolyte levels. The contents of electrolytes were significantly lowered (*P*<0.001) in the diabetic lens after supplementation of AST in a dose-dependent way (Table 3).

Estimation of protein Protein levels in the diabetic lens was found to be 3.247±0.1358 (*P*<0.001) and were substantially lower than those in the normal control (11.27±0.3517; Table 3). The levels of lens protein were dramatically enhanced (*P*<0.001) when treated with AST at a higher dose.

***In Vivo* Experimental Activity**

Therapeutic property of astragalin (blood glucose, insulin, and body weight) in rats Figure 2 portrayed the therapeutic properties of AST (blood glucose, insulin, and body weight) in rats. It has been found that in rats treated with AST, their body weight increased and the insulin amount in their blood increased. Also, the glucose level in the blood of the AST-treated group was found to be much lower depending on the dose (Figure 2).

Estimation of astragalin on the lens opacity in experimental animals The rat eye was inspected with the help of a slit lamp biomicroscope. The opacity of lens progression was scrutiny as per the reported method[30]. It was observed that no trace of turbidity was found in normal control rats. On the other hand, diabetic rats had the presence of cloudiness in a lens that has nuclear opacity and comes under the grade of III. When DC rats treated with a low dose of AST showed a reduction in cloudiness which means an average cataract score. A high dose of AST reveals a beneficial activity in delaying the maturation and progression of DC (Table 4).

Effect of astragalin on oxidative stress markers in experimental rats STZ-induced DC group animals showed

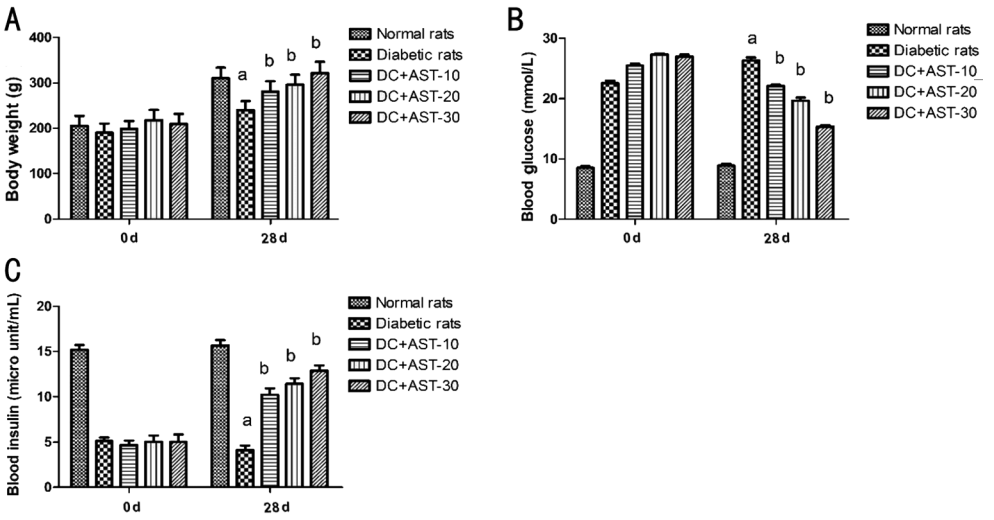

Figure 2 Effect of AST on the biochemical profile of experimental rats A: Body weight; B: Blood glucose; C: Blood insulin. [a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. DC: Diabetic cataract; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg; AST-30: Astragalin at a dose level of 30 mg.

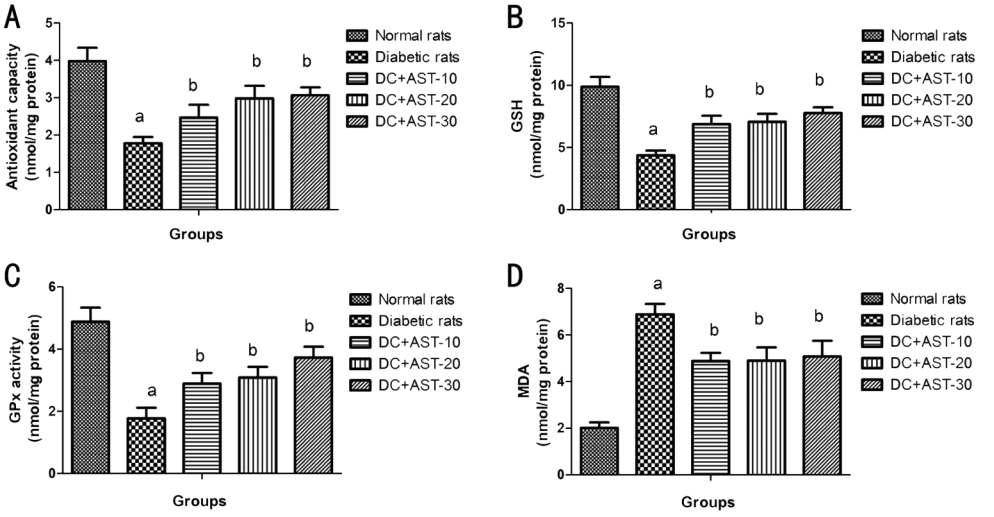

Figure 3 Effect of AST on oxidative stress marker expression in experimental rats A: AOC; B: GSH; C: GPx; D: MDA. [a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. DC: Diabetic cataract; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg; AST-30: Astragalin at a dose level of 30 mg; AOC: Antioxidant capacity; GSH: Glutathione; GPx: Glutathione peroxidase; MDA: Malondialdehyde.

Table 4 Estimation of AST on the lens opacity in experimental animals

Groups	Score of cataract				
	0	1	II	III	IV
Normal	12	0	0	0	0
DC	0	0	3	1	8
DC+AST-10	0	1	4	3	4
DC+AST-20	0	3	4	3	2
DC+AST-30	0	4	5	2	2

DC: Diabetic cataract; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg; AST-30: Astragalin at a dose level of 30 mg.

an unpredicted value of an antioxidant parameter such as GSH, MDA, total antioxidant capacity (AOC), and GPx than the normal control rats. However, the rats that received different doses of AST showed a significant elevation in the level of GSH and AOC than the DC group. Although a significant increase was also observed in the GPx levels when treated with AST in a dose-dependent manner in that DC group. Last, a significant reduction in the level of MDA after administration of AST in a dose-dependent manner to the DC group as shown in Figure 3.

Effects of astragalin on VEGF and IL-1β levels in experimental rats The retina was isolated from all group rats after the completion of the study to measure the VEGF and IL-1β protein levels with the help of ELISA. The result indicates that STZ-induced DCs in the rat group showed a significant elevation in the levels of IL-1β and VEGF than a normal control group. Moreover, there was a significant reduction in the protein level of VEGF and IL-1β after treatment AST

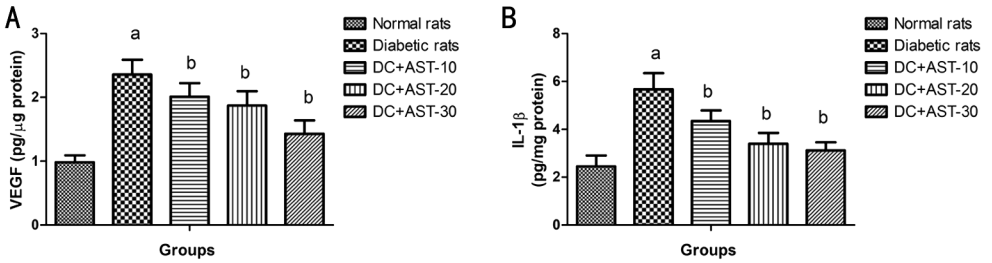

Figure 4 Effect of AST on VEGF (A) and IL-1β (B) levels in experimental rats [a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. DC: Diabetic cataract; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg; AST-30: Astragalin at a dose level of 30 mg; VEGF: Vascular endothelial growth factor; IL: Interleukin.

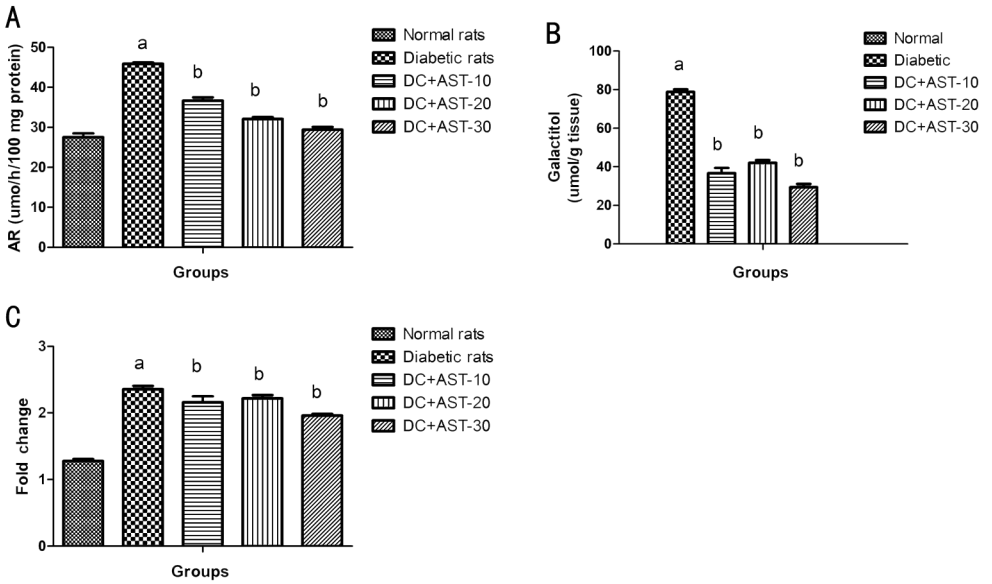

Figure 5 Effect of AST on AR (A), galactitol (B) and fold change (C) in rat lenses [a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. DC: Diabetic cataract; AR: Aldose reductase; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg; AST-30: Astragalin at a dose level of 30 mg.

in a dose-dependent manner than STZ-induced DC in rats as illustrated in Figure 4.

Estimation of mRNA, aldose reductase activity and amount of galactitol in rat lenses As portrayed in Figure 5, the activity of aldose eductase was found to be 27.56 nmol/h/100 mg protein and content of galactitol was undetectable in the normal animals. STZ also induced AR of the lens in diabetic rats and was found to be 45.89 nmol/h/100 mg protein), resulting in galactitol accumulation *i.e.* 78.89 nmol/g lens weight) and significantly increased levels of mRNA AR by 2.38-fold. The activity of AR, level of galactitol, and AR mRNA expression were all partially inhibited by AST in a dose-dependent manner *i.e.* 29.45 nmol/h/100 mg protein, and 29.45 nmol/g lens weight, respectively and with AST (30 mg/kg) producing the highest inhibition (1.96-fold).

Effect of astragalin on aldose reductase 2 expression level To find out more about how AST works, its ability to stop the AR2 expression of homogenate in the animals was tested. Figure 6 shows that the AST lowers the amount of AR2 expression in a dose-dependent manner.

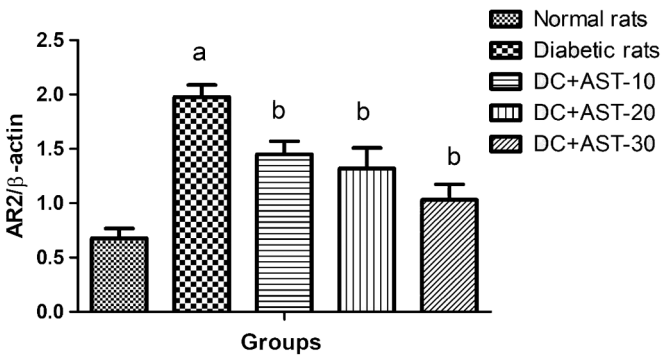

Figure 6 Effect of AST on AR2 expression in experimental rats [a]*P*<0.001 in comparison with normal control; [b]*P*<0.001 in comparison with diabetic control. DC: Diabetic cataract; AST-10: Astragalin at a dose level of 10 mg; AST-20: Astragalin at a dose level of 20 mg; AST-30: Astragalin at a dose level of 30 mg; AR: Aldose reductase.

DISCUSSION

Cataract induced by diabetes is the utmost prevalent and common problem in patients with diabetic mellitus. The significance of the polyol pathway generated fundamental and

metabolic alterations in the diabetic lens is well regarded as one of many processes implicated in DC pathogenesis. The crucial enzyme, AR catalyzes the polyol pathway's initial and most essential rate-determining reaction in cataracts[31-32]. Hyperglycemia is thought to activate the AR and subsequently, the polyol pathway, resulting in an increase in sorbitol build-up in the cell, oxidative stress, and protein loss, developing DCs[33]. The present investigation shows that AST has an AR inhibitory effect as well as anti-cataract activity.

The anti-cataract activity is studied using a variety of experimental models, the most popular of which is galactose-induced cataract. Galactose at a dose of 55 mmol/L was employed to induce cataracts in this investigation. Galactitol which is a reduced form of galactose within the lens creates a substantial quantity and causes osmotic stress. When a large concentration of polyols builds up in the lens, it causes an upsurge in intracellular ionic strength, which causes leakage of myoinositol, GSH, and free amino acids, eventual membrane failure, and extreme hydration. Conversion of galactose, xylose, and glucose to their sugar alcohol counterparts, sorbitol is catalyzed by AR. Organic exploration of lenses treated with galactose revealed increased activity of AR[34-36], on the other hand, significantly reduced after the treatment of AST at different dose levels, demonstrating that it can suppress the potential of AR enzyme.

Significant lens fiber swelling and sorbitol buildup created osmotic stress, which in turn caused stress in the endoplasmic reticulum and the generation of free radicals. The damaging effects of reactive oxygen species produced in the lens are reduced by a variety of antioxidant enzymes, including CAT and SOD. In cataracts, both enzyme activities are decreased, making the lens more vulnerable to the damaging effects of free radicals. These alterations are consistent with the findings of our research[37]. A significant upsurge was observed in sorbitol levels and a corresponding drop in the level of the enzymatic antioxidant parameter in diabetic rats. Administration of AST significantly reduced sorbitol levels while increasing levels of CAT and SOD, indicating that AST has antioxidant properties. Furthermore, a lower amount of sorbitol endorses that the AST repressed AR and disallowed polyol production.

GSH is a significant antioxidant in the lens that helps to retain proteins in a reduced state. In practically all types of cataracts, such as cataracts induced by diabetes, the lens shows a reduction in the content of GSH. In the same way, lipid peroxidation which is autocatalytic progression is also responsible for cell death[38]. The hazardous chemicals lipid hydroperoxides (LH) and MDA produced by lipid peroxidation have been implicated in the pathophysiology of cataracts, owing to their crosslinking capability[39]. All of the alterations indicated above were observed in the current investigation and were normalized using AST in a dose-dependent manner. Moreover, there was a substantial suppression in the activity of LDH and SDH, indicating that it is effective in preventing cataract formation and lens opacity.

Free radicals and oxidative stress are recognized as important contributors to cataract formation. Oxidative stress specifically causes lipid peroxidation and protein. Cataracts can develop in the lens as a result of insoluble aggregates made of proteins and peroxidized lipids. Lipid peroxidation has also been linked to the emergence of cataracts by altering the cell membrane and cytoplasm[40].

AST is a flavonoid with many medicinal benefits, including antioxidant action. A sign of oxidative stress is MDA. In the current study, the levels of MDA were lower in the groups treated with AST than in the diabetic group that did not get any treatment. Moreover, the groups receiving AST treatment showed a considerably higher level of antioxidant capacity than the DC group. GSH plays a comparable role to the antioxidant vitamins A, E, and C as well as selenium in the retina's defense against cataract development. This study reveals that AST treated group showed higher levels of GSH than diabetic rats who weren't given any treatment. The development of cataracts has previously been linked to retinal inflammatory factors such as VEGF and IL-1β. The ELISA data revealed that AST caused a decrease in the protein expression levels of VEGF and IL-1β[40].

Totally 55 mmol/L of high galactose serves as media for lenses that promote electrolytes such as Na^+ and K^+ accumulation resulting in lens fibers' thickening and hydration and responsible for the development of cataracts[41]. Because of the change in the levels of Na^+ and K^+, the lens protein content also changes, resulting in a water-soluble protein reduction and the reason for lens opacification. Total proteins were increased in the AST-treated lenses, although the ions of Na^+ and K^+ had decreased. Reduction in lens opalescence can be attributed to the reduction of protein loss from the lens.

Lens inactivation Ca^{2+} ATPase is caused by the generation of free radicals in the lens of the eye because excess galactose promotes Ca^{2+} buildup[42]. Proteolysis induced by calpain in the lens can be activated by this increased calcium, after opacification of the lens. The diabetic rat group's lenses had a higher calcium concentration, whereas the lenses treated with AST had a lower level of calcium, showing that AST has the capacity against lens cataractogenesis.

One of the bad effects is the oxidation of the sulfhydryl component which leads to disulfide cross-linking, protein precipitation, and then clouding of the lens[37-43]. It was also seen that the Group II lenses became cloudy after 72h, but this didn't happen to the AST-treated lenses, showing that AST can

stop cataracts from forming. The DC induced by STZ-model was used to test how well AST stopped cataracts from forming, diabetic animals given STZ started to get cataracts, and after some weeks, diabetic animals in the control group had fully formed cataracts[44]. After the completion of the experiment, treating diabetic rats with AST slowed the cataract progression in a way that depended on the dose. Rats with diabetes had substantial growth in eye lens AR activity, but when they were given AST, the AR activity went down in a dose-dependent way. At the time of the experiment, the rat's eyes seemed to be clear and healthy in a dose-dependent way with AST. Blood glucose levels were tracked to see if AST slowed the progression of cataracts by lowering the hyperglycemia caused by STZ. Even though blood glucose levels dropped to a small degree after treatment with AST, the delay in the progression of cataracts in diabetic rats was not due to AST's ability to lower glucose. Instead, it could be due to its ability to change other pathways linked to DC development. So, a lot of research needs to be done to find the underlying mechanism of AST and figure out if it can be used to treat DCs.

The outcomes of the present study suggested the potential of AST by reducing the cataract incidence at the start and deferred the progression in lens throughout the study, although there was no clear dose-response association among the AST at three different concentrations. Although the galactitol hyperosmotic action, galactitol resulted in a unique occurrence in which body weight rise lagged far behind the fast growth in the weight of the lens, the lens weight/body weight became the major indicator of osmotic expansion in the lens[45]. Our findings revealed that the diabetic group's ratio was much greater than the normal value and AST had the ratio increase at either dosage. On the other hand, the content of galactitol, activity of AR level, and mRNA of AR in the lens were also used as indicators of the degree of osmotic stress in the lens. All of these findings nearly matched the effects of AST.

The current research suggests that in the lens epithelium and superficial cortical fibers in response to hyperglycemia, AR catalyses the intracellular buildup of sorbitol. Although sorbitol buildup can lead to a hyperosmotic imbalance inside the cells that accumulate it, it does not directly lower GSH levels, indicating that glucose-linked oxidation may not take place. VEGF and IL-1β protein synthesis start when the sorbitol-accumulating lens cells eventually react to the osmotic stress. In addition, the presence of auch protein levels in the lens suggests that ER stress-related ROS production had a place. The observed activation of signaling pathway leads one to believe that these may initially have protective properties; nevertheless, signaling disruption may be a cause of the eventual production of cataracts[46].

AST is a flavonoid, so its potential was discovered with several different parameters and outcomes. Flavonoids have been shown to have antioxidant activity and AR-inhibiting action as reported by various researchers. Improved AR expression and accompanying oxidative stress have been linked to cataract formation[47]. AST contained elements that inhibited AR *in vivo*, which can be explained by its ability to bind AR. As a result, the anti-cataract potential of AST against DCs induced by galactose *in vitro* could be attributed to the AR inhibitory activity.

In conclusion, it was concluded from the current study that AST is found to be a new AR inhibitor *in vitro* and *in vivo*. In the case of *in vitro*, it significantly interrupts the opacification of the lens in a dose-dependent manner and improved the various biochemical parameters as well as *in vivo*. Simply, we can say the antioxidant activity and AR inhibitory action of the flavonoid nature of AST are primarily responsible for this anti-cataract potential. However, using the AST in clinical trials might be conducted to back up the assertion against DCs.

ACKNOWLEDGEMENTS

Conflicts of Interest: Wang N, None; **Singh D,** None; **Wu Q,** None.

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