Toxicology International, *31(4) : 515-522; 2024. DOI: 10.18311/ti/2024/v31i4/43004*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.co.in/index.php/toxi



DNA Protective, Aldose Reductase Inhibitory and Anti-Cataract Activities of Ferulic Acid

Nagaraju Bandaru[1*], Veera Mani Deepika Pedapati[2], Ms. Matta Sarika[2], Suggu Vangmai Swaroopa[2], M. Aparna[3], Md. Said Ibrahim[3], Makarand Suresh Gambhire[1], Ramu Samineni[1], M. Narender[3] and Prashik B. Dudhe[1]

[1]School of Pharmaceutical Sciences (SOPS), Sandip University, Nasik-422213, Maharashtra , India; bnrajupharma@gmail.com

[2]Department of Pharmacy, Aditya Pharmacy College, Surampalem, Kakinada, India

[3]College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddesweram, Andhra Pradesh, India

Abstract

The current study assessed Ferulic acid's DNA protective, aldose reductase inhibitory and anti- cataract activities. Different concentrations of ferulic acid are examined for DNA protection and incubated with aldose reductase isolated from a goat lens. Ferulic acid demonstrates a strong inhibitory effect against aldose reductase enzyme with IC50 value of 1.43 g/mL and shows potent DNA protective action at the dose of 100 ug/mL against Fenton's reagent induced DNA damage. Ferulic acid also reduces the formation of opacification in goat lens that inhibits the development of cataract and increases antioxidants levels significantly in lenses. This indicated that Ferulic acid inhibits the oxidative stress induced cataract. Based on the findings, Ferulic acid may be an effective option for the treatment of high glucose-induced cataract.

Keywords: Antioxidants, DNA, Docking, Diabetes, Ferulic Acid, Glucose Concentrations

1. Introduction

Diabetes mellitus is a complex and over time carbohydrate metabolism disorder characterized by elevated blood glucose levels, and it is the most prevalent metabolic syndrome in the world.

Diabetes mellitus affects approximately 35 to 40 percent of the global population each year. This population has a significant fatality rate due to micro and macro vascular issues that cause permanent end-organ damage[1]. Tissues and organs that are not regulated by insulin experiences elevated efflux of glucose. Due to the saturation of the glycolysis pathway brought on by the increased glucose intake, many stress signaling pathways are stimulated. Through increased oxidative stress and inflammation, this stress signaling system starts the cascade of problems associated with diabetes mellitus[2]. Stress signaling pathways that is activated by increased glucose outflow is the polyol pathway. In the case of illnesses other than diabetes mellitus, only5% of glucose enters this route.

In diabetes mellitus, approximately 35% of the glucose can reach the polyol system, increasingthe toxicity of the polyol pathway. Aldose Reductase (AR) is the rate-limiting enzyme in the polyol pathway[3]. It is used to detoxify endogenous xenobiotic aldehydes in a physiologically healthy system; however, Aldose Reductase (AR) activity is

**Author for correspondence*

Article Received on: 22.03.2024 Revised on: 20.06.2024 Accepted on: 23.08.2024

increased in diabetic conditions. Aldose Reductase (AR), triggers the transformation of glucose into sorbitolvia polyol pathway, which is associated to the progression of diabetic cataract[4]. As sorbitol continues to be broken down into fructose, NAD+ is changed into NADH by the sorbitol dehydrogenase enzyme[5].

Excessive intracellular levels of sorbitol induce osmotic changes leading to hydropic lens fibersthat tends to degenerate thereby forming sugar cataracts[6,7] and develop a hyper osmotic effect that results in the infusion of fluid reversing the osmotic gradient. Intracellular aggregation of polyols causes lens fibers to break down and liquefy, which finally results in lens opacities. In typical physiological cells, oxidative stress produces Reactive Oxygen Species (ROS) and imbalances in various normal antioxidant enzymes. The ROS can cause severely structural damage of proteins, lipids, and DNA[8,9]. These damage leads to formation of genotoxicity and mutagenicity and leads formation of various disease and disorders like cancers, diabetes mellitus, cataract *etc.*

Cataract is nothing but formation of opacity in clear lens in eye that leads decrease the levels of influx of light, causes decline of vision. Normal lenses are composed of a crystalline substance with precise water and protein structures that aid in the passage of light. Cataract canalso be defined as peering through a cascade of water or waxed paper.

Cataract is more in hyperglycemia patients, and it is a major reason for worldwide blindness. The scope of diabetes and the nature of hyperglycemia are key risk factors in the development of diabetic cataracts[10]. Amplified activation of RA plays a major part of cataract progression in diabetic conditions. Preventing aldose reductase activity is a dynamic strategy for handling diabetic cataracts. Moreover, AR inhibitors have been found to be beneficial in complications of diabetes mellitus. But most of AR inhibitors are withdrawn from the market due to their own toxicity, for instance, Tolrestat administration causes liver toxicity that leads to death. In this context, it is very important to investigate less toxic and more efficient ARI's.

Nowadays more research on diabetes mellitus has been shifted towards herbal sources. Many extracts of plants and isolated phytochemicals inhibit the activity of AR enzyme and are also used for prevention of complications associated with diabetes mellitus[11]. Ferulic acid is a phenolic compound commonly seen in the plant tissues. FA has a minimal toxicity that produces many physiological functions like anti-inflammatory, antioxidant, anti-microbial, anti-cancer and anti-diabetic effects. It is also widely used in Pharma, Food and cosmetic industry and also an inhibitor of enzymes that catalyze free radical generation and increases scavenging activity of enzyme[12,13].

As the positive effects of ferulic acid are encouraging, the authors sought to determine its ability to inhibit aldose reductase against high glucose-induced cataract in goat isolated lenses.

2. Materials and Methods

2.1 Chemicals

The plasmid pBR322, ferulic acid, and fenton's reagent were all procured from sigma chemicalsin india. NADPH and remaining chemicals employed in the current work are of analytical- grade fetched from himedia laboratories, Mumbai.

2.2 Ethical Approval

The protocol of this study was approved by IAEC of Koneru Lakshmaiah College of Pharmacywith CPCSEA Regd No. 439/PO/01/a/CPCSEA. Ethical commission approval has beenwaived as goat eyes were employed that are supplied from a slaughterhouse.

2.3 *In-vitro* DNA Protection Assay

In-vitro DNA protection assay was performed by using Plasmid nicking assay.

2.4. Plasmid nicking assay

The shielding capacity of Ferulic acid against oxidative DNA damage was assessed using the Lee *et al.*, approach in a plasmid nicking assay via supercoiled pBR322 DNA[14]. Plasmid DNA (0.5 µg) was treated with Fenton's reagent (30 mM hydrogen peroxide, 50 µM ascorbic acid, and 80 µM FeCl3) and different amounts of ferulic acid (10-100 µg). The final volume of the reaction is set to 20 µL with tris buffer. The reaction mixture then underwent incubation at 37°C for 30 minutes. The collected samples were combined with 1% tracking dye (0.25% of bromophenol blue in 50% glycerol) and loaded onto a 1% agarose gel (0.5 g of agarose in 50 mL 1× TBE buffer). Electrophoresis was performed, followed by ethidium bromide staining for visualization.

2.5 Assortment of Goat Eyes

Fresh goat eyes were acquired at an abattoir in Vaddeswaram, Guntur, and were transported to a lab within an hour at a temperature of 0-4 °C. Extra capsular extraction had separated the lensat this point.

2.6 Inhibitory Activity of Aldose Reductase *In-Vitro*

Aldose reductase preparation: Following isolation, the lenses are measured and cleaned with normal saline. To produce a 1:3 w/v lens homogenate, the lens was now unified using sodium phosphate buffer, pH 6.2, which comprised 10 mM beta meracaptoethanol. After 10 minutes of centrifuging the mixture at 10,000 rpm and 4 °C, a clear supernatant was drawn out. The supernatant was treated with 10% ammonium sulphate and centrifuged again at 10,000 rpm for 10 minutes. The supernatant was then used to study ARI[15].

2.6.1 The Activity of Inhibiting Aldose Reductase

The aldose reductase inhibitor assay performed with the technique outlined in Somani and Sathaye's paper[16]. Freshly produced enzyme, various ferulic acid concentrations, 0.067 M buffer (pH 6.2), 0.125 mM NADPH, 400 mM Lithium sulphate, and 40 mM xylose are all included in the reaction mixture. The final reaction volume has been set at 200 l. Now check the absorbance at 340 nm; decreasing absorbance values signify the production of NADP. As a standard, quercetin is employed. The results were calculated as follows and expressed as a percentage inhibition [17].

To calculate the percentage of inhibition,

([Absorbance of control/min - absorbance of sample/min]/absorbance of control/min) X 100.

2.6.2 Ferulic Acid Docking Studies on the Enzyme Aldose Reductase

In silico molecular docking analysis was performed using Molegro Virtual Docker v6.0.17.2.8 to understand the interaction and affinity of ferulic acid against the enzyme's active site of the target protein aldose reductase (PDB ID: 1US0 with resolution of 0.66 A).

Goat lens cataract incubated in artificial aqueous humor after lens isolation and high glucose incubation. Cataracts were developed by administering a high dose of glucose (55 mM) while maintaining a pH of 7.8. (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, $NaH(PO_4)_2$ 0.5mM, $CaCl_2$ 0.4 mM, and glucose 5.5 mM) and penicillin 32 mg and streptomycin 250 mg was added to artificial aqueous humor for preventing bacterial contamination[18].

Each of the following groups, which each had four lenses (n=4), was created from the lens. Group I: 5.5 mM glucose and aqueous humour under normal regulation.

Group II: Aqueous humour and 55 mM glucose as a negative control.

Ferulic acid 50 g/ml with 55 mM glucose and aqueous humour is in Group III.

Group IV: Aqueous humour and 100 g/ml ferulic acid with 55 mM glucose.

The lenses were cultured at 37 °C for 72 hours. At the end of the incubation period, the lenses were placed on a wired mesh, with their posterior surfaces touching the mesh, and the mesh pattern (the number of squares readily apparent through the lens) could be seen with the lens to determine opacity.

2.6.3 Levels of Opacity Grading

- 0 - no opacity; 1 - a slight amount of opacity; 2 - diffuse opacity and3 - thick, widespread opacity.

2.6.4 Lens Homogenate Preparation

To make a 10% w/v homogenate, lenses were mashed in Tris buffer (0.23 M; pH 7.8) containing 0.25 103 M EDTA. The supernatant was retrieved after further centrifugation of the blend at 10,000 rpm and 4 °C. Biochemical parameters like catalase, reduced glutathione, and total proteins were estimated using the supernatant[19,20].

3. Statistical Analysis

The statistical procedure used analysis of variance and Dennett's test, producing statistically significant results (***$P < 0.001$) compared to the negative control group.

4. Results

4.1 DNA Protection Activity

The DNA-protective action of ferulic acid against oxidative damage was investigated using the pBR322 plasmid DNA model. When fenton's reagent was exposed to plasmid DNA, the super coiled form was converted to a

linearized and open circular form as a result of the release of hydroxyl free radicals. Adding varying concentrations of ferulic acid (10-100 µg/mL) to the resulting mix increased the formation of supercoiled plasmid DNA. These findings are consistent with phenolic-rich ferulic acid's ability to protect plasmid DNA.

Based on this DNA protective activity of ferulic acid it can be used for treatment of different disorders like cancers and cataract (Figure 1).

4.1.1 Aldose Reductase Inhibition

The AR enzyme in goat lenses was considerably linearly inhibited by ferulic acid in the current investigation at doses ranging from 0.5 to 2 g/mL. Ferulic acid produced the maximum percentage of inhibition, 70%, at 2 g/ml, and IC50 discovered as 1.43 µg/mL.

At 4.5 µg/ml of typical quercetin concentration, the AR enzyme is inhibited 80.11%. Table 1 displays the percentages of AR inhibition caused by ferulic acid and quercetin.

4.2 Docking Studies of Ferulic Acid on Aldose Reductase Enzyme

The result indicated that ferulic acid binds effectively to the target receptor and the MolDock, ReRank and HBond scores were compared with co-crystalized inhibitor. The results show that ferulic acid could bind to aldose reductase at various active sites including Cys303, His110 and Tyr48 with bond lengths 3.36, 3.10 and 3.02 respectively (Figure 2).

4.3 High Glucose Produced Cataract Formation

4.3.1 Visual Investigation and Ranking of Lens

The animals in the negative control group had advanced dense opacities, whereas the control group remained clear at the end of the investigation time frame. The lens opacification commenced at the edge and evolved toward the center. Ferulic acid treatment diminished the

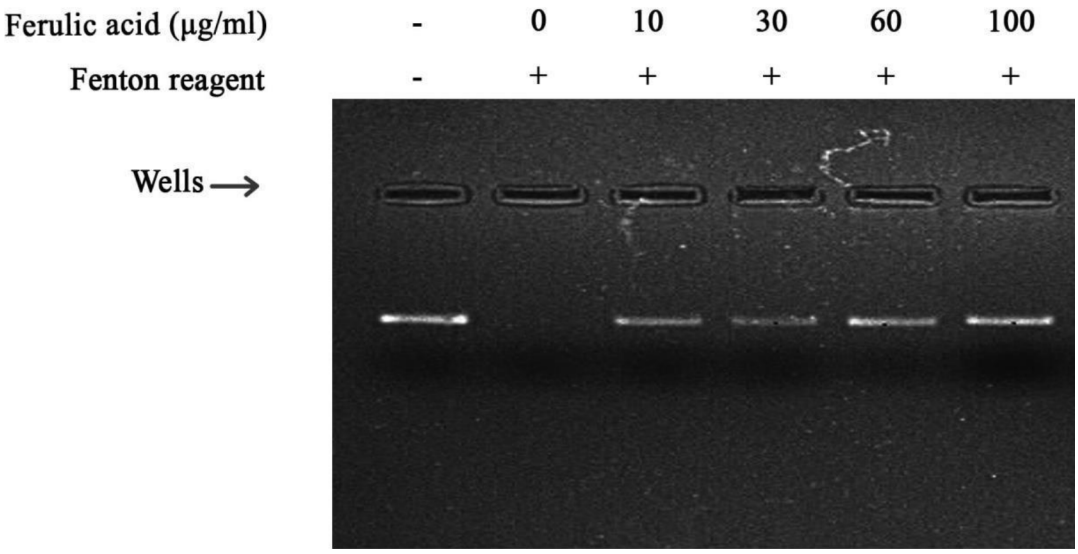


Figure 1. Ferulic acid protective action on pBR322 plasmid DNA against OH radical-induced damage.

Table 1. Aldose reductase percentage inhibition by various doses offerulic acid and common quercetin

Drug concentration (µg/mL)	Percentage inhibition of AR enzyme
0.5 Ferulic acid	36.23 ± 0.12
1 Ferulic acid	46.58 ± 0.12
1.5 Ferulic acid	58.98 ± 0.12
2 Ferulic acid	70.12 ± 0.12
4.5 Quercetin	80.11 ± 0.12

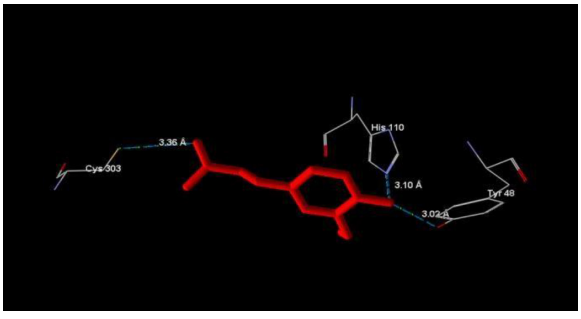


Figure 2. Docking studies of ferulic acid on aldose reductase.

opacification of lens even at highest concentration of 100 µg/mL. The scores of opacities were ranked as 0, 3, 1, and 1 in different Groups such as I, II, III, and IV, respectively. Results were tabulated in Table 2 and Figure 3.

4.4 Effect of Ferulic Acid on Catalase Levels in Goat Lens

Catalase is one of the antioxidants present in goat lens for prevention of oxidative stress. Negative control lenses show decreases levels of catalase 80 ±0.89 and ferulic acid 100ug/ml treated lens exhibits increases catalase levels 192 ±1.25 when compared to negative control lens. It indicates ferulic acid increases catalase levels and prevents the oxidative stress inducedcataract (Figure 4).

4.5 Effect of Ferulic Acid on Reduced Glutathione Levels in Goat Lens

Glutathione is a significant naturally occurring antioxidant found in unusually high amounts in the lens, preventing protein oxidation. It is essential for keeping the lens transparency. Negative control lenses show decreased levels of glutathione levels 8.0 ± 0.78 whereas, ferulic acid 100ug/mL significantly increases glutathione levels 19.1 ± 0.72 when compared to the negative control. So, it indicates that ferulic acid maintainsthe transparency in lens (Figure 5).

Table 2. Effect of ferulic acid on degree of opacity on lens by glucose induced method

S. No	Name of the group	Degree of opacity
1	Group I – Normal control	0
2	Group II – Negative control	3
3	Group III – Ferulic acid 50µg/mL	1
4	Group IV – Ferulic acid 100µg/mL	0

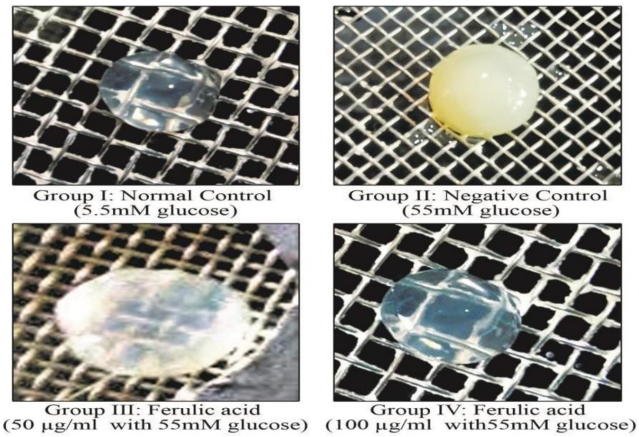


Figure 3. A representative depiction of anti-cataract activity of ferulic acid.

4.6 Effect of Ferulic Acid on Total Proteins Levels in Goat Lens

The transparency of the eye lens is dependent on the retention of the native tertiary structures and the soluble nature of the lens crystalline proteins. So, proteins are used for lens transparency. In this study negative control lens decreases the levels of proteins 90.2 ± 0.37, whereas ferulic acid 100 ug/mL treated lens significantly increases the proteins levels 210.2 ± 0.46. It indicates ferulic acid is used for maintaining normal transparency of lens by increasing protein levels (Figure 6).

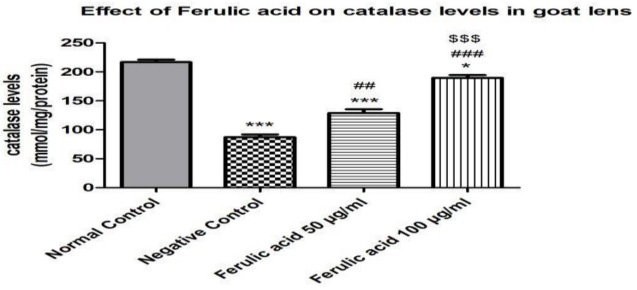


Figure 4. Effect of ferulic acid on catalase levels in high glucose induced cataract.
Result is depicted as mean ± standard error of the mean, n = 4; Negative control shows decreased catalase levels (***p<0.001) than to normal control whereas ferulic acid 100 ug/mL treated control exhibits significantly increased (###p<0.001) catalase levels rather to negative control.

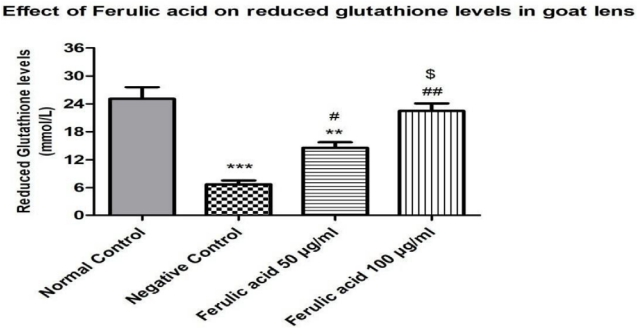


Figure 5. Effect of ferulic acid on reduced glutathione levels in high glucose induced cataract.
Data is expressed as mean ± standard error of the mean, n = 4; Negative control shows decreases levels of reduced glutathione (***p<0.001) than to normal control whereas, ferulic acid 100 ug/mL treated control exhibits significantly increased (###p<0.001)reduced glutathione levels when compared to negative control.

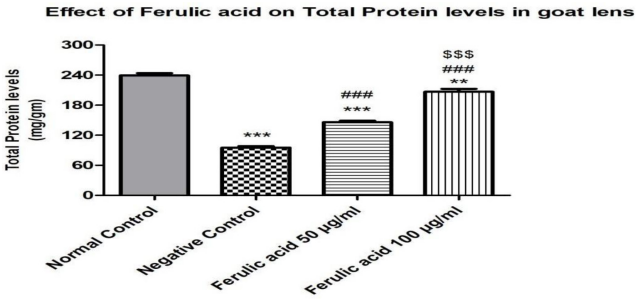


Figure 6. Effect of ferulic acid on total proteins in high glucose induced cataract.
The result is represented as mean ± standard error of the mean, n = 4; Negative control shows decreases levels of total protein (***p<0.001) compared to normal control whereas ferulic acid 100 ug/mL treated control exhibits significantly increased (###p<0.001)total protein levels when compared to negative control.

5. Discussion

It is an antiquated truth that maintaining proper quality diabetic control has a beneficial role in curing and preventing complications of diabetes mellitus. Traditional drugs for diabetes mellitus failed to maintain good glycemic control. As a result, they are also potential to inhibit the diabetic complication progressions. Thus, additional strategies that may inhibit the emergence of adverse effects of diabetes mellitus would offer numerous advantages regardless of controlling glycemic levels[21]. Several natural compounds and plants have shown great results in inhibiting the AR enzyme.To evaluate ferulic acid ability to inhibit this enzyme, the study was investigated with various graded concentrations. Ferulic acid repressed aldose reductase in a dose-proportional manner at an IC50 value of 1.43 µg/mL. Ferulic acid effectively inhibited the enzyme like the quercetin. Ferulic acid is also investigated for the DNA protection assay by plasmid nicking assay method. In this investigation, different concentrations of ferulic acid are used. Among this, 100 µg/mL protects the DNA from the free radicals released by Fenton's reagent. It indicates ferulic acid prevents the oxidative damage of DNA and decreases the incidences of various pathological conditions like cancer and cataract. Molecular docking studies provide the information provides valuable information concerning the binding nature of different compounds (ligands) with targeted proteins or enzymes.

The result indicated that ferulic acid binds effectively to the target receptor and the MolDock, ReRank and HBond scores were compared with co-crystalized inhibitor. The results show that ferulic acid could bind to aldose reductase at various active sites including Cys303, His110 and Tyr48 with bond lengths 3.36, 3.10 and 3.02 respectively. Based on these docking studies ferulic acid binds to aldose reductase enzyme and inhibits action of this enzyme.

Aldose reductase inhibitors have been shown to inhibit or prevent the development of exploratory diabetic cataracts in animals, as well as diabetic retinopathy and nephropathy abnormalities. High glucose-induced cataract studies are conducted in vitro using various models such as goat, chicken, and rat lens cultures. Goat lenses are generally used for the evaluation of test compounds for anti-cataract activity due to their easy availability, economical and convenient handling purpose. Moreover, these lenses are larger size and facilitate the precise visualization of opacity and realization of cataract in lens[22]. Thus goat lenses were employed in the present research to investigate the impact of ferulic acid on overloaded glucose-induced cataract in insulated goat lenses.

Visual inspection of the lens is detected, and grades are categorized based on the opacity of lens after 72 hr of the treatment. Ferulic acid, (100 µg/mL) inhibited the formation of opacification in lens and thus thwart the formation of cataract in high glucose exposed group. Oxidative stress is regarded as one of the major risk factors in the progression of cataracts, due to increased reactive oxygen species lens proteins undergoing oxidation and results in opacification of lens. It is because of alterations in the redox position and declines the glutathione levels. Glutathione is an antioxidant that plays a dynamic role in conserving transparency of lens by inhibiting the oxidation of proteins in lens. Commonly, it is present in high concentration in lens[23].

In high glucose conditions, activation of polyol pathway results in reduced glutathione levels which lead to oxidation of proteins resulting in formation of cataract. Ferulic acid restores the glutathione levels to 19.1 ± 0.72 at dose of 100 µg/mL.

Catalase is also another endogenous antioxidant present in lens. It is also used for inhibiting the degradation of proteins. In high dose glucose lens the levels of catalase is reduced to 80 ± 0.89. Ferulic acid is restore the catalase levels to 192 ± 1.25 at dose of 100 µg/ml. Ferulic acid is also increases proteins levels when compared to glucose lens, results decrease the opacification of lens and prevent the formation of cataract in high glucose induced model.

Based on the above discussion ferulic acid restores the glutathione, catalase, and proteins levels in lens so it indicates ferulic acid inhibits the oxidative stress induced cataract by glucose.

6. Conclusion

Cataract hyperglycemia can be seen due to various swearing to the lens, especially with the activation of the enzyme aldose reductase and oxidative stress. Thus, with ARIs, antioxidants are very essential to mitigate the progression of cataracts. The current study found that ferulic acid's DNA protection from free radicals, aldose reductase inhibitory, and antioxidant activities can stop the development or occurrence of high glucose-induced cataract. Based on these varied functions, ferulic acid is useful for reducing diabetes mellitus problems like cataracts.

7. Acknowledgment

Authors are thankful to management of Sandip University, Nasik for supporting and providing facilities to conducting this work.

8. Reference

1. Imran A, Shehzad MT, Shah SJA. *et al.* Development and exploration of novel substituted thiosemicarbazones as inhibitors of aldose reductase via *in vitro* analysis and computational study. Sci Rep. 2022. https://doi.org/10.1038/s41598-022-09658-z
2. Yu MG, Gordin D, Fu J, Park K, Li Q, King GL. Protective factors and the pathogenesis of complications in diabetes. End Rev. 2024; 45(2):227–252. https://doi.org/10.1210/endrev/bnad030
3. Shahab M, Zheng G, Alshabrmi FM, Bourhia M, Wondmie GF, Salamatullah MA. Exploring potent aldose reductase inhibitors for anti-diabetic (anti-hyperglycemic) therapy: integrating structure-based drug design, and MMGBSA approaches. Front Mol Biosci. 2023; 20(10). https://doi.org/10.3389/fmolb.2023.1271569
4. Mrugacz M, Pony-Uram M, Bryl A, Zorena K. Current approach to the pathogenesis of diabetic cataracts. Int J Mol Sci. 2023; 24(7):6317. https://doi.org/10.3390/ijms24076317
5. Zhu C. Aldose reductase inhibitors as potential therapeutic drugs for diabetic complications. Diabetes Mellitus - Insights and Perspectives. 2013. https://doi.org/10.5772/54642
6. Kador PF, Wyman M, Oates PJ. Aldose reductase, ocular diabetic complications and the development of topical Kinostat(®). Prog Retin Eye Res. 2016; 54:1-29. https://doi.org/10.1016/j.preteyeres.2016.04.006
7. Mrugacz M, Pony-Uram M, Bryl A, Zorena K. Current approach to the pathogenesis of diabetic cataracts. International Journal of Molecular Sciences. 2023; 24(7):6317. https://doi.org/10.3390/ijms24076317
8. Snow A, Shieh B, Chang KC, Pal A, Lenhart P, Ammar D, Ruzycki P, Palla S, Reddy GB, Petrash JM. Aldose reductase expression as a risk factor for cataract. Chem Biol Interact. 2015; 234:247-253. PMID: 25541468. https://doi.org/10.1016/j.cbi.2014.12.017.
9. Robert S, Randall LK, editors. Cataract Overview (Internet). Life Extension. Available from: http://www.garynullforum.com/Articles/pdf/foundarticles1/lef/CATARACTS.pdf
10. Obrosova IG, Chung SS, Kador PF. Diabetic cataract: Mechanisms and management. Diabetes Metab Res Rev 2010; 26:172-180. https://doi.org/10.1002/dmrr.1075
11. Dongare V, Kulkarni C, Kondawar M, Magdum C, Haldavnekar V, Arvindekar A. Inhibition of aldose reductase and anti-cataract action of trans-anethole isolated from *Foeniculum vulgare* Mill. Fruits Food Chem. 2012;132(1):385-390. https://doi.org/10.1016/j.foodchem.2011.11.005
12. Mattila P, Kumpulainen J. Determination of free and total phenolic acids in plant- derived foods by HPLC with diode-array detection. J Agric Food Chem. 2002; 50(13):3660–3667. https://doi.org/10.1021/jf020028p
13. Devi VS, Vellapandian C, Ilavarasan R, Sumithra M. Role of phytochemicals against diabetic nephropathy: An insight into molecular receptors. Toxicol Int. 2023; 30(4):585– 604. https://doi.org/10.18311/ti/2023/v30i4/30998
14. Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica var. Saboten*. J Agric Food Chem. 2002; 50(22):6490– 6496. https://doi.org/10.1021/jf020388c
15. Saraswat M, Muthenna P, Suryanarayana P, Petrash JM, Reddy GB. Dietary sources of aldose reductase inhibitors: Prospects for alleviating diabetic complications. Asia Pac J Clin Nutr. 2008; 17(4):558-565.
16. Somani G, Sathaye S. Bioactive fraction of *Saraca indica* prevents diabetes-induced cataractogenesis: Aldose reductase inhibitory activity. Pharmacogn Mag. 2015; 11(41):102-110. https://doi.org/10.4103/0973-1296.149722
17. Arumugam M, Muthuswamy U, Kuppusamy A, Thirumalaisamy S, Varadharajan S, Puliyath J. Discovery of potential aldose reductase inhibitors using *in silico* docking studies. Orient Pharm Exp Med. 2012; 12:157-1 61. https://doi.org/10.1007/s13596- 012-0065-3
18. Bandaru N, Ramu A, Vijetha P, Vidhyadhara S, Nandini P, Reddy AS. Evaluation of *ex vivo* anti-cataract activity of ethanolic extract of *Alstonia scholaris* leaves on dexamethasone-induced cataract using isolated goat lens.

Asian J Pharm Clin Res. 2017; 10(2):182-185. https://doi.org/10.22159/ajpcr.2017.v10i2.15084

19. Estimation of total protein by the modified biuret endpoint method. Available from: http://www.btprotocolsmaulikblogspot. in/2007/04/estimate-protein-by.html.
20. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 2009; 5(1):51-66. https://doi.org/10.1038/nprot.2009.197
21. Muthenna RP, Akileshwari C, Saraswat M, Petrash JM. Inhibition of aldose reductase and sorbitol accumulation by dietary rutin. Curr Sci. 2011; 101(9):1191-1197.
22. Ganeshpurkar A, Bhadoriya SS, Pardhi P, Jain AP, Rai G. *In vitro* prevention of cataract by Oyster Mushroom *Pleurotus florida* extract on isolated goat eye lens. Indian J Pharmacol. 2011; 43(6):667-670. https://doi.org/10.4103/0253-7613.89823
23. Giblin FJ. Glutathione: A vital lens antioxidant. J Ocul Pharmacol Ther. 2000; 16(2):121-135. https://doi.org/10.1089/jop.2000.16.121