Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(1); 31-39

ISSN: 0975-4873

Research Article

Aldose Reductase Inhibitiory Effect of Gymnemic Acid, Trigonelline and Ferulic Acid- An *In Silico* Approach

Roshana Devi Vellai*, Subramanian Sorimuthu Pillai

Department of Biochemistry, University of Madras, Guindy Campus, Chennai 600 025, India

Received: 6th Dec, 16; Revised: 15th Dec, 16; Accepted: 18th Dec,16; Available Online: 15th January, 2017

ABSTRACT

Diabetic retinopathy (DR) is one of the earliest complications of chronic hyperglycemia and is a major cause of vision loss. Nearly all patients with type 1 diabetes mellitus and more than 60 % with chronic type 2 diabetes develop some degree of retinopathy after 10 years. Aldose reductase, the first enzyme in polyol pathway chiefly contributes to the development of diabetic retinopathy and other secondary complications. None of the currently available drugs used to inhibit the activity of aldose reductase is found to be ideal due to their adverse effects. Hence, the screening and identification of more effective and safer aldose reductase inhibitors from natural products have been critical requirement in the management and treatment of T2DM. Recently, we have reported the antidiabetic properties of phytochemicals such as Gymnemic acid, Trigonelline and Ferulic acid in high fat diet fed- low dose STZ induced experimental type 2 diabetes in rats. In the present study, the structure based computational method was employed to identify the *in silico* inhibitory effect of the above phytoingredients on aldose reductase activity. Auto Dock 4.2 is used to study the molecular interactions between the ligands and the receptor. The data obtained evidenced that the docking efficacy of the antidiabetic ligands which are comparable with fidarestat, the standard used in the present study. Thus, the antidiabetic properties of the above lead molecules may attribute to its aldose reductase inhibitory effect.

Keywords: Diabetic Retinopathy, Polyol pathyway, Aldose reductase, Gymnemic acid, Trigonelline, Ferulic acid, Autodock

INTRODUCTION

Diabetes mellitus is a multifactorial, multisystemic metabolic disorder characterized by persistent elevation in fasting as well as post prandial blood glucose levels. It arises due to absolute lack of insulin secretion (T1DM) from the pancreatic β-cells or its action on peripheral tissues (T2DM) or both. More than 90% of the diabetics belong to T2DM. According to International Diabetic Federation (IDF), more than 415 million people worldwide had diabetes mellitus in 2015 and are expected to rise to 642 million in 2040 unless otherwise immediate preventive measures are initiated[1]. This is a minimum number because, for each diagnosed case, there will be one undiagnosed case in first world and eight in the third world countries.

Diabetes mellitus can go undiagnosed until one of the life threatening complications develops. According to a report by the US National Health and Nutrition Examination Survey, nearly 60 % of patients with diabetes have more than one secondary complication caused by chronic diabetes[2]. About 85% of all diabetics develop retinopathy, 60-70% has mild to severe form of nerve damage and 25-50% develops kidney disease[3]. Diabetic retinopathy (DR) is the most severe secondary complication arises due to chronic hyperglycemia because it can result in irreversible blindness[4]. Nearly all patients with type 1 diabetes mellitus and more than 60 % with type 2 diabetes have some degree of retinopathy after 10 years[5]. Despite of DR causing eventual blindness, only a few visual or ophthalmic symptoms are developed until visual loss develops[6]. It is generally accepted that early metabolic abnormalities in the eyes resulting from chronic hyperglycemia strongly influence the development of DR[7]. Ocular diabetic complications include retinopathy, cataract and corneal epitheliopathy and loss of vision[8]. DR leads to increased retinal vasopermeability and breakdown of blood retinal barrier resulting in retinal haemorrhages, swelling, exudates and retinal detachment which are characterized by microaneurysms, inter-retinal oedema, haemorrhages, exudates and intraocular pathological neovasculization[6]. Several studies have shown that retinal pigment epithelial cells, glial cells and retinal pericytes undergo chronic hyperglycemia induced apoptosis through the activation of several proteins involved in apoptotic cell death including the members of the caspase and Bcl-2 family[9]. Under normoglycemic conditions approximately 3% of the glucose metabolized is routed through the polyol pathway[10]. However, under chronic hyperglycemic conditions, this pathway accounts for more than 30% of the glucose utilized[11]. Aldose reductase (alditol/NAD^+ oxidoreductase, E.C.1.1.1.21, ALR2) is the first enzyme of the polyol pathway that stereospecifically transfer the hydride from the excess D-glucose into D-sorbitol with concomitant conversion of NADPH into $NADP^{+}$[12].

*Author for Correspondence:subbus2020@yahoo.co.in

Sorbitol dehydrogenase in turn, utilizing NAD^+ oxidizes this intermediate polyol to fructose. AR is of prime importance in the etiology of diabetic complications, not only in lenses but also in peripheral nerves and kidneys[13].

AR is cytosolic, monomeric oxidase having a low affinity (High Km) for glucose. Its crystal structure has a single domain folded into an eight-stranded parallel α/β barrel motif, with the substrate binding site located in a cleft at the carboxy – terminal end of the β-barrel[14]. This sulfhydryl containing enzyme has the molecular weight ranging from 28 and 45 K contains 284-315 amino acid residues depending upon the species and the tissue from which the enzyme is isolated and that apparent isoenzymes of AR may exist[15,16]. The enzyme molecule is nearly spherical as indicated by a frictional ratio f/fo of 1.14[17]. The human AR gene has been mapped to chromosome 7 region q35[18]. The gene extends over 18Kb and consists of 10 exons[19].

AR has been purified from several tissues including lens, placenta, brain, kidney, muscle and seminal vesicles from such sources as cow, rat, rabbit, pig, dog and human[20]. Retinal ganglion cells, muller glia, vascular pericytes and endothelial cells are endowed with aldose reductase in all species studied including humans[21]. However, antibodies raised against the purified enzymes reveal significant differences in cross-reactivity between species and perhaps even tissues[8]. Retinal capillary pericytes contain the enzyme AR and the accumulation of excess sorbitol catalysed by AR in pericytes has been implicated in their degeneration and selective apoptosis[22]. Pericyte degeneration is considered as the hall mark of early DR.

Aldose reductase and the sorbitol pathway were first described in 1956 by Hers in the seminal vesicles where it generates fructose for sperms[23]. Its discovery in the lens by Van Heyningen indicated that this pathway was not merely restricted to reproductive tissues. Van Heyningen was the first to found polyols in sugar cataracts in 1959[24]. Adverse effects of the aldose reductase associated polyol pathway were first described by Kinoshita in the lens[25-27]. These studies formed the basis for the osmotic hypothesis of sugar cataract formation (polyol concept) indicating that the aldose reductase initiated intracellular accumulation of excess sorbitol that eventually leads to diabetic cataract formation.

Though the affinity of hexokinase for glucose is greater than that of AR, when its activity is saturated by elevated levels of glucose[28], sorbitol is formed more rapidly than it is converted to fructose, resulting in a net accumulation of sorbitol. However, galactose is much better substrate than glucose for AR and hence galactosemic animal model is often preferred to induce DR[29]. The harmful polyol pathway of glucose metabolism becomes active when the intracellular glucose levels are persistently elevated[30].

Sorbitol being an alcohol is polyhydroxylated and strongly hydrophilic[31]. Accumulation of sorbitol in the cells due to its poor penetration across membrane and inefficient metabolism results in the development of diabetic complications including DR[32]. The fructose produced by the polyol pathway gets phosphorylated to form fructose-3-phosphate[33] which is broken down to 3-deoxyglucosone; both compounds are powerful glycosylating agents that enter in the formation of AGEs[34]. Further, the usage of NADPH by aldose reductase may result in fewer co-factors available for glutathione reductase which is essential for the maintenance of the intracellular pool of non-enzymatic antioxidant, reduced glutathione (GSH)[35]. Similarly, the usage of NAD by sorbitol dehydrogenase leads to an increased ratio of $NADH/NAD^+$, which has been termed as "pseudohypoxia" and linked to a multitude of metabolic and signaling changes known to alter major cellular functions[36].

It has been proposed that oxidation of sorbitol by NAD+ increases the cytosolic NADH: NAD^+ ratio, thereby inhibiting the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and increasing the concentration of triose phosphate[35]. Raised triose phosphate levels could increase the formation of both methylglyoxal, a precursor of AGEs and Diacylglycerol (DAG) thus activating PKC. Further, the excess NADH may become a substrate for NADH oxidase and this would be a mechanism for the generation of intracellular ROS[37]. Thus, activation of the polyol pathway by altering intracellular tonicity, generating AGEs precursors and exposing cells to oxidative stress perhaps through decreased antioxidant defenses and generation of ROS can initiate and multiply several mechanisms of cellular damage.

Polyols are osmolytes, compounds that poorly penetrate biological membranes, and thus, when they are formed and retained in the lens cells, they could cause an osmotic imbalance. The induction of osmotic stress from the excess intracellular accumulation of sugar alcohol (polyol) can lead to altered membrane permeability and subsequently to biochemical changes that result in the inhibition of cellular lesions, commonly known as the "polyol osmotic theory of sugar cataracts"[25,26]. Because the polyol level is not sufficiently high to exert osmotic effects in other tissues, the polyol osmotic theory for sugar cataracts is thought not to apply to other diabetic complications. The contribution of polyol pathway to diabetic complications may be very much species, site and tissue dependent. However, several studies implicate a role for AR in the detoxification of aldehydes, the major bioactive products of lipid peroxidative[38-40].

While visual impairment in diabetic retinopathy may often be improved by photocoagulation or vitreous surgery, blindness cannot be prevented in all cases. Therefore, elucidating the mechanism for initiating AR is important so that specific methods for the prevention and/or treatment of diabetic retinopathy can be developed. AR inhibitors offer the possibility of preventing or arresting the progression of long term diabetic complications, despite the high glucose levels and hence with no risk of hypoglycemia, since they have no effect on blood glucose levels[41]. The first compound capable of modifying the cataractous process through the inhibition of AR was tetramethylene glutaric acid. However, the inability to penetrate membranes hinders its chemical use[26]. The inhibition of AR along with tight control of blood glucose levels at the early stages of diabetes may be beneficial in preventing pericyte degeneration in the retinal capillaries

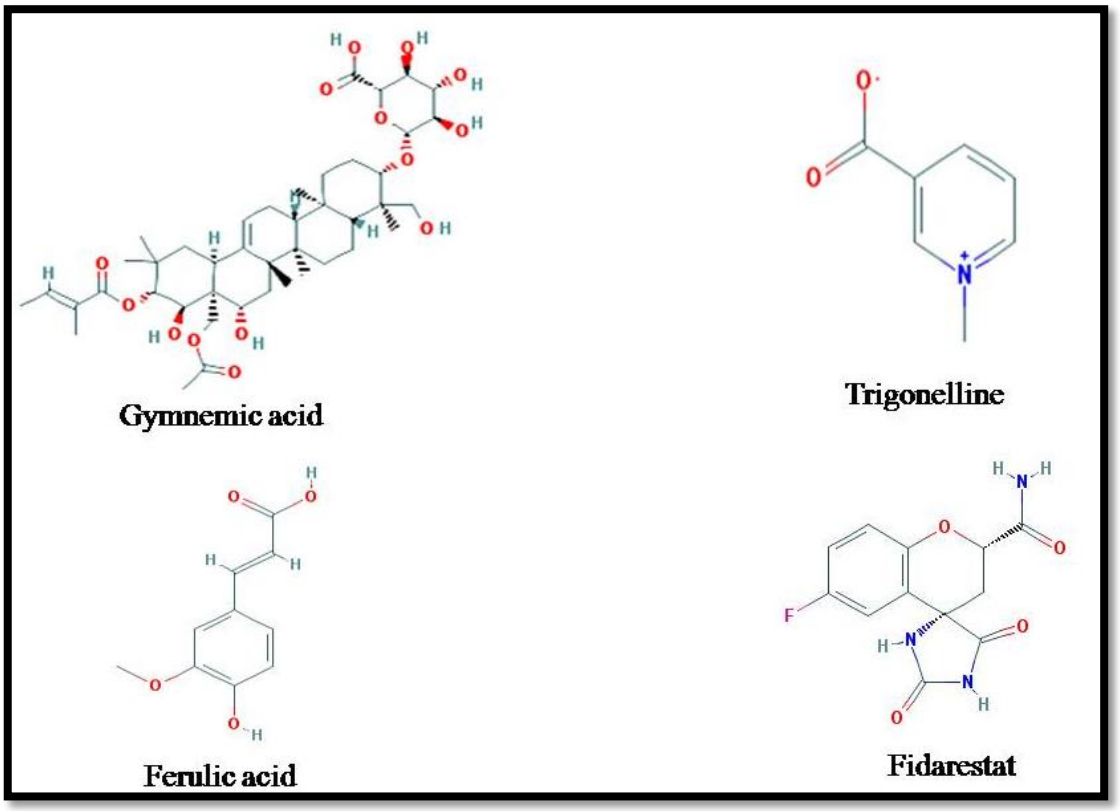


Figure 1: The chemical structure of ligands and fidarestat

Table 1: Docking energy for gymnemic acid with aldose reductase

Aldose Reductase		Gymnemic Acid	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
LEU301	N	O	3.17	
ALA299	N	O	2.71	–5.72
VAL297	O	H	2.204	
LYS221	NZ	O	2.94	

Table 2: Docking energy for trigonelline with aldose reductase

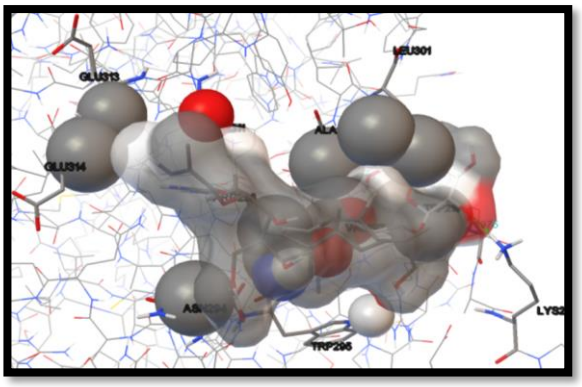
Aldose Reductase		Trigonelline	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
ARG250	NH1	O	2.69	
ARG250	NH2	O	2.92	–5.59
MET1	N	O	2.68	
MET1	N	O	2.90	

that can lead to the development and progression of diabetic retinopathy[4].

Several studies are aimed at dietary sources with AR inhibitory potential as a food based adjuvant therapy for improving the effectiveness of chemotherapeutic drugs. Currently known ARIs can be divided into four main classes according to their structure: (i) acetic acid derivatives (ii) cyclic amides (iii) phenolic derivatives and phenylsulfonyl nitromethane derivatives. Even though numerous clinical trials for the treatment of diabetic neuropathy have been conducted with the above derivates, they have resulted in the lack of observed efficacy[42].

Nature is a very important source of novel ligands of high chemical diversity, many of them possessing interesting biological activities and medicinal properties. In the context of the worldwide spread of diabetes, an intensive search for new lead molecules for the development of novel pharmacological therapeutics is extremely important[43]. Traditional Indian Medicines (TIMs) have been used for more than 2000 years in India and other Asian countries with the fundamental aim of targeting not only the treatment of diabetes but also the prevention of the secondary complications such as DR. A growing body of evidence has shown the benefit of combining TIMs with western treatment strategies[44]. Traditionally, "blood-vitalizing herbs" are used to treat microvascular related DR.

Thus, the study of active compounds from the extract of traditional medicinal plants and the possible applicable mechanisms are warranted for better understanding the effect of phytochemicals in the treatment of DR[45]. Medicinal plants acts as a "stepping stone" for the discovery of novel pharmacologically active ligands[46] and remain an important source of material for maintaining good health with unparallel diversity which have improved the quality of life[47]. Medicinal plants are "gold mine" for novel ligands to be discovered[48,49]. However, the mechanisms of action for most of the herbal medicines associated with modulating the harmful pathways have not been fully elucidated. Nevertheless, the experience



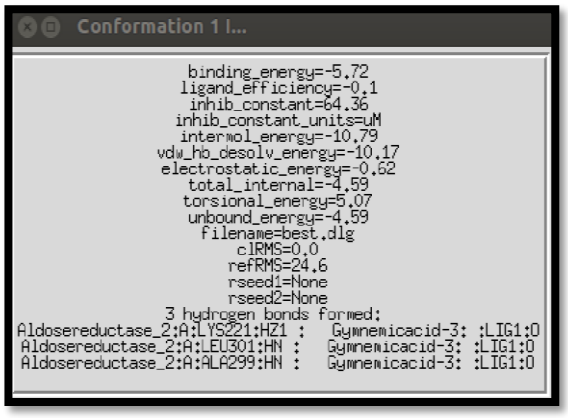


Figure 2 (a): Docking conformation of aldose reductase with gymnemic acid.

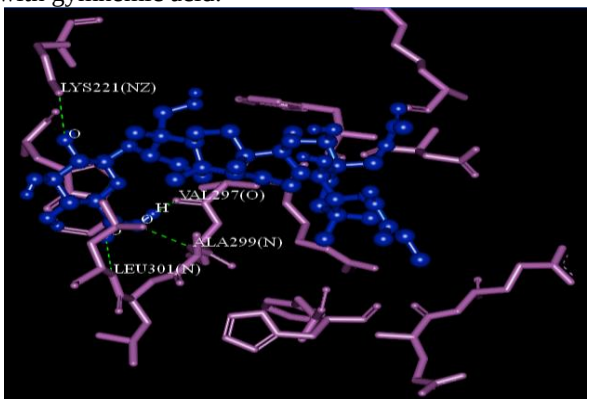


Figure 2(b): Molecular interactions showing the binding sites of aldose reductase with gymnemic acid.

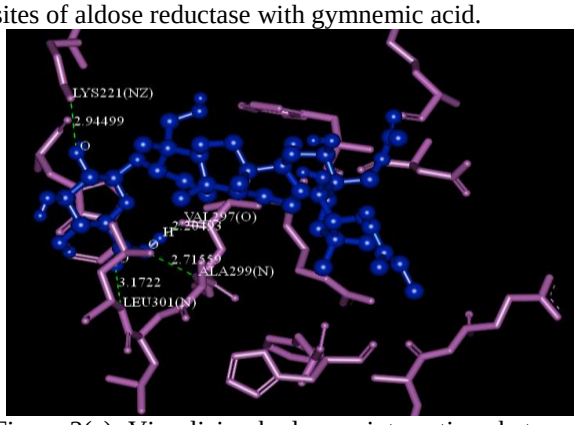


Figure 2(c): Visualizing hydrogen interactions between aldose reductase and gymnemic acid using acceryls discovery studio visualize.

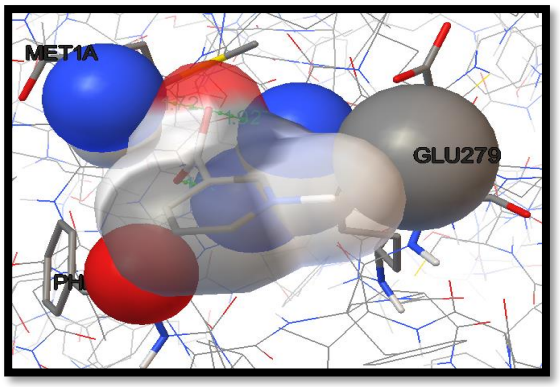


Figure 3 (a): Docking conformation for aldose reductase with trigonelline.

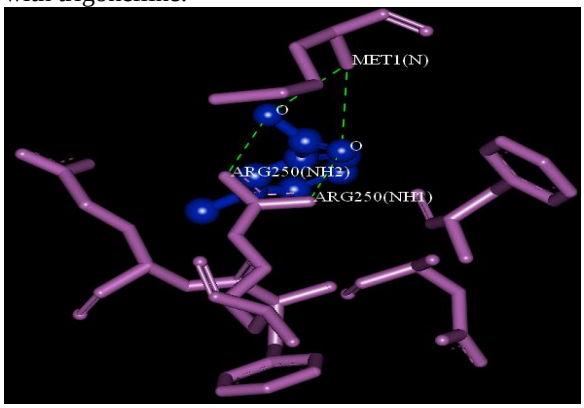


Figure 3(b): Molecular interactions showing the binding sites of aldose reductase with trigonelline.

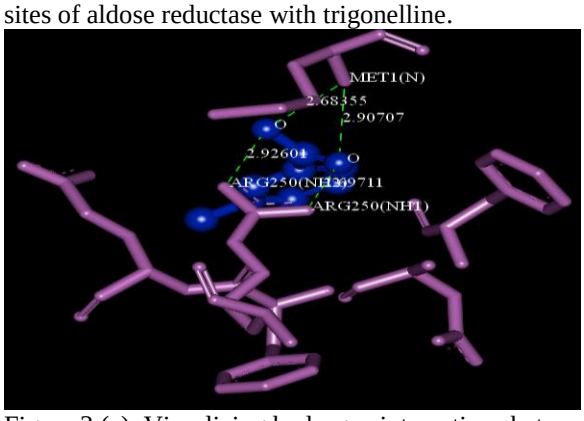
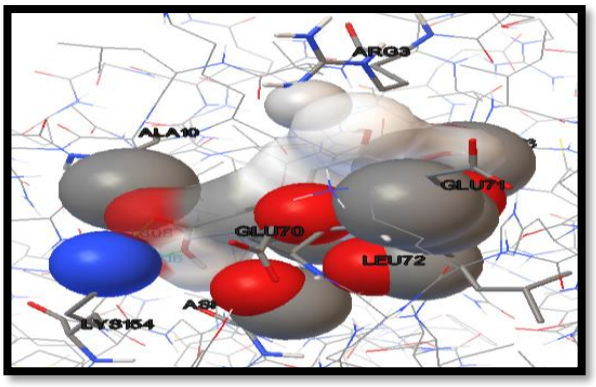


Figure 3 (c): Visualizing hydrogen interactions between aldose reductase and trigonelline using acceryls discovery studio visualize.

obtained from their traditional use over many years should



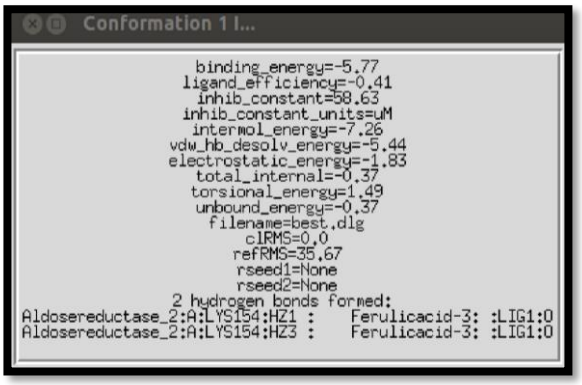


Figure 4(a): Docking conformation for aldose reductase with ferulic acid.

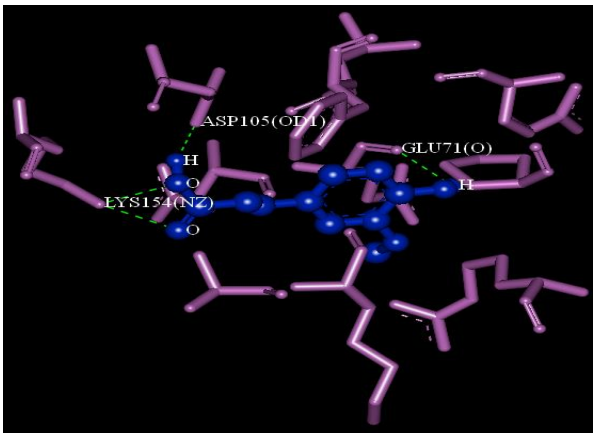


Figure 4(b): Molecular interactions showing the binding sites of aldose reductase with ferulic acid.

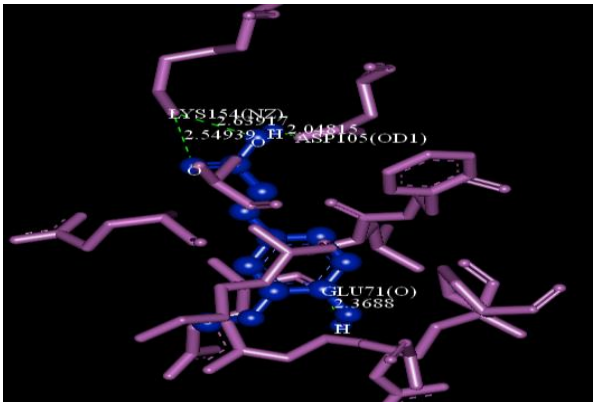
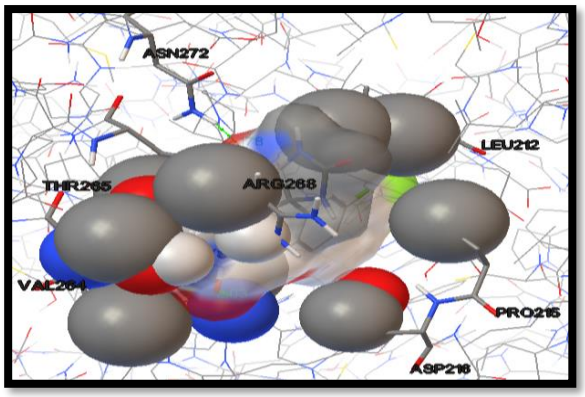


Figure 4(c): Visualizing hydrogen interactions between aldose reductase and ferulic acid using acceryls discovery studio visualize.



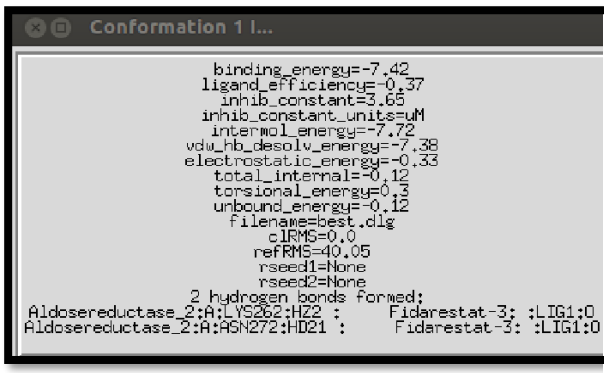


Figure 5 (a): Docking conformation for aldose reductase with fidarestat.

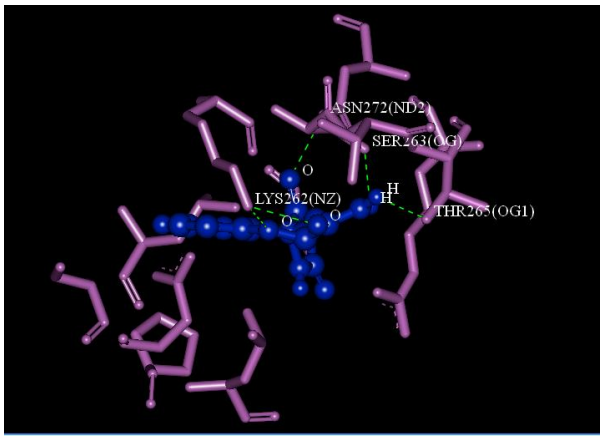


Figure 5 (b): Docking conformation for aldose reductase with fidarestat.

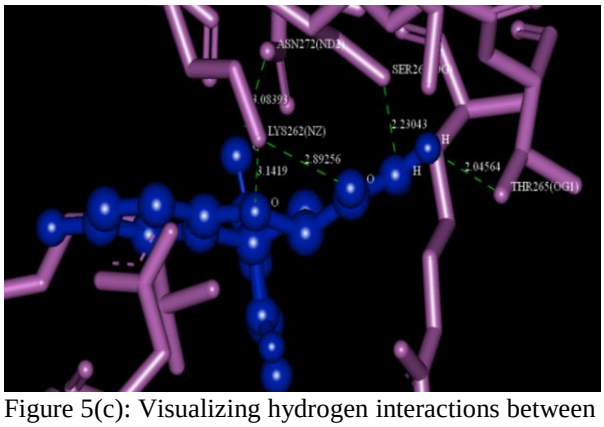


Figure 5(c): Visualizing hydrogen interactions between aldose reductase and fidarestat using acceryls discovery studio visualize.

Table 3: Docking energy for ferulic acid with aldose reductase.

Aldose Reductase		Ferulic Acid	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
GLU71	O	H	2.36	
ASP105	OD1	H	2.04	–5.77
LYS154	NZ	O	2.63	
LYS154	NZ	O	2.54	

Table 4: Docking energy for fidarestat with aldose reductase.

Aldose Reductase		Fidarestat	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
THR265	OG1	H	2.01	
SER263	OG1	H	2.23	
LYS262	NZ	O	2.89	–7.42
LYS262	NZ	O	3.14	
ASN272	ND2	O	3.08	

Table 5: The binding energies ranked according to docking scores.

Ligands	Receptor	Binding energy
Fidarestat	Aldose reductase	–7.42
Ferulic acid	Aldose reductase	–5.77
Gymnemic acid	Aldose reductase	–5.72
Trigonelline	Aldose reductase	–5.59

not be ignored[50]. Current treatments of DR rarely improve visual function and are limited to surgical options in an advanced stage with excessive side effects and huge financial burden. Hence, emerging treatments possibly in combination with standard therapy may provide superior efficacy and safety profile for the prevention or treatment of DR[51].

Several experimental and clinical studies evidenced that the inhibition of aldose reductase activity can lead to the amelioration of diabetic retinopathy[52]. In this study, we have presented an *in silico* analysis on the inhibition of aldose reductase activity by the pharmacologically known phytochemicals such as gymnemic acid, trigonelline and ferulic acid. Recently, we have reported the hypoglycemic properties of the above phytochemicals. For the *in silico* analysis we have used Auto Dock[53] for the binding of ligands (Gymnemic acid, Trigonelline and Ferulic acid) and receptor (aldose reductase).

Computational Methods

Preparation of receptor

Crystal structure of aldose reductase was obtained from the RSCB protein data bank. Preparation of aldose reductase with the Auto Dock Tools involved in the addition of hydrogen atoms to the target enzyme, which is a necessary step for the computation of partial atomic charges. Kollman united atom charges, salvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein docking simulation.

Preparation of ligands

The phytochemicals such as Gymnemic acid, Trigonelline and Ferulic acid were considered as ligand molecules. The ligands were constructed using Chemsketch and then converted into PDB file format by adding the hydrogen bonds.

Auto Dock

The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run and analyze the docking simulations. Auto Dock 4.2 is used to study the molecular interactions between the phytochemical ligands such as Gymnemic acid, Trigonelline and Ferulic acid and the enzyme receptor, aldose reductase.

Auto Dock requires pre-calculated grid maps, one for each type of atom present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. This grid must surround the region of interest in the rigid macromolecule. The grid box size was set at 126, 126 and 126 Å (*x, y* and *z*) to include all the amino acid residues which are present in rigid macromolecules. Auto Grid 4.2 Program, supplied with Auto Dock 4.2 was used to produce grid maps. The spacing between grid points was 0.375 angstroms.

Auto Dock offers a variety of search algorithms to explore a given docking problem. In the present study, Lamarckian Genetic Algorithm (LGA) was chosen search for the best conformers. During the docking process, a maximum of 10 conformers was considered. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 500000, maximum number of generations 1000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, Step sizes were 0.2 Å for translations, 5.0° for quaternions and 5.0° for torsions. Cluster tolerance 0.5Aº, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed.

Auto Dock results were analyzed to study the interactions and the binding energy of the docked structure. It was run several times to get various docked conformations and to analyze predicted docking energy. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were visualized using the Acceryls Visualizer discovery studio tool.

RESULTS AND DISCUSSION

Activation of polyol pathway due to chronic hyperglycemia in diabetes has been implicated in the initiation and onset of secondary complications such as retinopathy, cataract, neuropathy, nephropathy, cardiovascular diseases and oxidative stress. Over expression of aldose reductase, the major rate limiting enzyme in the polyol pathway chiefly contribute to the development of diabetic retinopathy and hence the inhibition of aldose reductase activity is considered as a potential target for the prevention of most of the diabetic complications. A large number of molecules have been synthesized to inhibit aldose reductase activity. However,

none is found to be ideal due to several drawbacks and others are still in clinical trials. Hence, the search for novel aldose reductase inhibitors preferably from plant origin with improved efficacy and safety continues.

Recently, we have reported the antidiabetic properties of pharmacologically important phytochemicals such as gymnemic acid, trigonelline and ferulic acid in high fat diet fed –low dose STZ induced experimental type 2 diabetes in rats[54]. We have also found that the polyherbal preparation of the above three phytochemicals in the ratio of 2:3:1 exerts significant antidiabetic properties at a relatively less concentration[55]. Based on the results obtained through the *in vivo* experiments, the present study was aimed to identify the inhibitory potential of the above lead molecules against the activity of aldose reductase using *in silico* molecular docking studies. The efficacy of the phytoingredients was compared with fidarestat, an aldose reductase inhibitor.

The chemical structures of the phytochemical ligands and the standard were presented in figure 1. The *in silico* interaction between gymnemic acid and aldose reductase was presented as Figure 2(a), 2(b), 2(c) and the data obtained for binding energy is presented as table 1. The amino acids present in the active site of aldose reductase capable of interaction with gymnemic acid are found to be LEU301, ALA299, VAL297 and LYS221. The data obtained for trigonelline and ferulic acid were depicted as figure 3(a), 3(b), 3(c), table 2 and figure 4a, 4b, 4c and table 3 respectively. The amino acids responsible for the binding of aldose reductase with the ligands trigonelline and ferulic acid were identified as ARG250, ARG250, MET1, MET1 and GLU71, ASP105, LYS154, LYS154 respectively. The data obtained for the binding energy of the standard fidarestat is represented as 5a, 5b, 5c and table 4 depicts the amino acids present in the active site of aldose reductase having the potential to bind with fidarestat as THR265, SER263, LYS262, LYS262 and ASN272.

In the docking studies, if a compound shows lesser binding energy compare to the standard, it implies that the test compounds have higher activity. The docking poses were ranked according to their docking scores and both the ranked list of ligands and their corresponding binding poses are presented as table 5. The binding energy of the ligands was found to be –7.42, –5.77, –5.72, –5.59 for fidarestat, ferulic acid, gymnemic acid and trigonelline respectively. It is evidenced from the data obtained that the binding energy of all the three antidiabetic ligands possesses significant aldose reductase inhibitory activities which are comparable with the standard, fidarestat. Several reports are available in the literature regarding the aldose reductase inhibitory activity of various phytochemicals derived from traditionally important medicinal plants[56-58].

Further studies are in progress to evaluate through both *in silico* and *in vitro* studies to assess the modulatory effects of the above phytochemicals in the maintenance of normoglycemia during experimental diabetes. Further, the results of the *in silico* findings evidenced that lead molecules are capable of significantly reduce the intracellular sorbitol accumulation, which has been implicated in the pathogenesis of late-onset diabetic complications like retinopathy, neuropathy and nephropathy. Further studies are in progress to evaluate the effect of these phytochemicals on the activities of other relevant receptors involved in the secondary complications of diabetes mellitus.

CONCLUSION

In the present study, we have demonstrated the binding interactions between the phytochemicals such as Gymnemic acid, Trigonelline and Ferulic acid with Aldose reductase using Autodock. Based on the binding energies obtained, it can be concluded that the phytochemicals have enhanced binding sites, interactions and potent inhibitory effect on aldose reductase activity. The results also indicate that the significant antidiabetic properties of these phytochemicals may attribute to its aldose reductase inhibitory effects.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Chan RSM, Woo J. Prevention of Overweight and Obesity: How Effective is the Current Public Health Approach. Int. J. Environ. Res. Public. Health 2010; 7(3): 765–783.
2. Veeresham C, Rama Rao A, Asres K. Aldose reductase inhibitors of plant origin. Phytother. Res. 2014; 28(3): 317-33.
3. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin Epidemiologic Study of Diabetic retinopathy: II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch. Ophthalmol 1984;102 (4): 520–526.
4. Oishi N, Kubo E, Takamura Y, Maekawa K, Tanimoto T, Akagi Y. Correlation between erythrocyte aldose reductase level and human diabetic retinopathy. Br. J. Ophthalmol. 2002; 86(12): 1363–1366.
5. Williams R, Airey M, Baxter H, Forrester J, Kennedy-Martin T, Girach A. Epidemiology of diabetic retinopathy and macular oedema: a systematic review. Eye (Lond). 2004; 18: 963-83.
6. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL, Klein R. American Diabetes Association, Retinopathy in diabetes. Diabetes Care. 2004; 27: S84-7.
7. Kinoshita JH, Fukushi S, Kador P, Merola LO. Aldose reductase in diabetic complications of the eye. Metabolism. 1979; 28; 462-469.
8. Kador PF, Kinoshita JH, Sharpless NE. Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. J. Med. Chem. 1985; 28: 841–849.
9. Allen DA, Yaqoob MM, Harwood SM. Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications. J. Nutr. Biochem. 2005; 16: 705-13.
10. Morrison AD, Clements RS, Travis SB et al. Glucose utilization by the polyol pathway in human

erythrocytes. Biochem. Biophys. Res. Commun. 1970; 40:199–205.
11. Gonzalez RG, Barnet P, Aguayo J et al. Direct measurement of polyol pathway activity in the ocular lens. Diabetes. 1984; 33: 196–199.
12. Kinoshita JH, Kador PF, Datiles M. Aldose reductase in diabetic cataracts. J. Am. Med. Assoc. 1981; 246: 257-61.
13. Kadar PF, Kinoshita H. Role of aldose reductase in the development of diabetes-associated complications. Am. J. Med. 1985; 79: 8-12.
14. Wilson DK, Bohren KM, Gabbay KH, Quiocho FA. An unlikely sugar substrate site in the 1.65 A structure of the human aldose reductase holoenzyme implicated in diabetic complications. Science.1992; 257: 81–84.
15. Gabbay KH, Cathcart ES. Purification and immunologic identification of aldose reductases. Diabetes. 1974; 23: 460-8.
16. Tanimoto T, Fukuda H, Kawamura J. Characterization of aldose reductases Ia and Ib from rabbit lens. Chem. Pharm. Bull. 1984; 32: 1025-31.
17. Sheaff CM, Doughty CC. Physical and kinetic properties of homogeneous bovine lens aldose reductase. J Biol. Chem. 1976; 251: 2696-2702.
18. Graham A, Heath P, Morten JEN, Markham AF. The human aldose reductase gene maps to chromosome region 7q35, Hum Genet. 1991a; 86: 509-514.
19. Graham A, Brown L, Hedge PJ, Gammack AJ, Markham AF. Structure of the human aldose reductase gene. J Biol. Chem. 1991b; 266; 6872-6877.
20. Bohren KM, Bullock B, Wermuth B, Gabbay KH. The aldo-keto reductase superfamily. J Biol. Chem. 1989; 264: 9547-9551.
21. Lorenzi M, Gerhardinger C. Early cellular and molecular changes induced by diabetes in the retina. Diabetologia. 2001; 44: 791–804.
22. Akagi Y, Kador PF, Kuwabara T et al. Aldose reductase localization in human retinal mural cells. Invest. Ophthalmol. Vis. Sci. 1983; 24:1516–19.
23. Hers HG. The mechanism of the transformation of glucose in fructose in the seminal vesicles. Biochim. Biophys. Acta. 1956; 22: 202-3.
24. van Heyningen R. Formation of polyols by the lens of rats with sugar cataract, Nature (London). 1959; 184: 194-196.
25. Kinoshita JH. Cataracts in galactosemia. Invest. Ophthalmol. 1965; 4: 786-799.
26. Kinoshita JH. Mechanisms initiating cataract formation. Proctor Lecture. Invest. Ophthalmol. 1974; 13: 713-24.
27. Obazawa H, Merola L, Kinoshita JH. The effects of xylose on the isolated lens. Invest. Ophthalmol. 1974; 13: 204-9.
28. Lou MF, Kinoshita JH. Control of lens glycolysis. Biochim. Biophys. Acta, 1967; 141: 547-559.
29. Kinoshita JH. A thirty year journey in the polyol pathway. Exp. Eye Res. 1990; 50: 567-73.
30. Szwergold S, Kappler F, Brown TR. Identification of fructose 3-phosphate in the lens of diabetic rats. Science.1990; 247: 451–454.
31. The Diabetes Control and Complications Trial Research Group, "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus," The New England Journal of Medicine, Vol. 329, no. 14, p 977–986, 1993.
32. Kador PF, Kinoshita JH, Tung WH et al. Differences in the susceptibility of various aldose reductases to inhibition. II. Invest. Opthalmol. Vis. Sci. 1980; 19: 980–982.
33. Barnett PA, Gonz´alez RG, Chylack Jr LT. Cheng H.-M. The effect of oxidation on sorbitol pathway kinetics. Diabetes.1986; 35:426–432.
34. Gonz´alez RG, Miglior S, Saltza IV, Buckley L, Neuringer LJ, Cheng H.-M. 31P NMR studies of the diabetic lens. Magn. Reson. Med. 1988; 6: 435– 444.
35. Williamson JR, Chang K, Frangos M et al., Hyperglycemic pseudohypoxia and diabetic complications. Diabetes. 1993; 42: 801–813.
36. Lass`egue, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2003; 285: 277– 297.
37. Dagher Z, Park YS, Asnaghi V, Hoehn T, Gerhardinger C, Lorenzi M. Studies of rat and human retinas predict a role for the polyol pathway in human diabetic retinopathy. Diabetes 2004; 53: 2404–2411.
38. He Q, Khanna P, Srivastava S et al. Reduction of 4-hydroxynonenal and 4-hydroxyhexenal by retinal aldose reductase. Biochem. Biophys. Res. Commun. 1998; 247: 719–22.
39. Rittner HL, Hafner V, Klimiuk PA et al. Aldose reductase functions as a detoxification system for lipid peroxidation products in vasculitis. J. Clin. Invest. 1999; 103: 1007–13.
40. Vander Jagt L, Hunsaker LA. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. Chem. Biol. Interact. 2003; 143-144: 341–51.
41. Costantino L, Rastelli G, Vianello P et al. Diabetes complications and their pote;ntial prevention: aldose reductase inhibition and other approaches. Med. Res. Rev. 1999; 19: 3–23.
42. Pfeifer MA, Schumer MP, Gelber DA. Aldose reductase inhibitors: the end of an era or the need for different trial designs? Diabetes. 1997; 46: S82.
43. Fuente JA, Manzanaro S. Aldose reductase inhibitors from natural sources. Nat. Prod. Rep. 2003; 20: 243–251.
44. Donnelly R, Wang B, Qu X. Type 2 diabetes in China: partnerships in education and research to evaluate new antidiabetic treatments. Br. J. Clin. Pharmacol. 2006; 61:702–705.
45. Su W, Dai DZ, Liu HR, Na T, Dai Y. Upregulated endothelin system in diabetic vascular dysfunction and early retinopathy is reversed by CPU0213 and total triterpene acids from *Fructus Corni*. Clin. Exp. Pharmacol. Physiol. 2007; 34:1228–1233.

46. Barnett AH, Grant PJ, Hitman GA, Mather H, Pawa M, Robertson L et al, Rosiglitazone in Type 2 diabetes mellitus: an evaluation in British Indo-Asian patients. Diabet. Med. 2003; 20: 387–393.
47. Li D, Chen JH, Chen Q, Li GW, Chen J, Yue JM et al. *Swietenia mahagony* extract shows agonistic activity to PPAR (gamma) and gives ameliorative effects on diabetic db/db mice. Acta. Pharmacol. Sin. 2005; 26: 220–222.
48. Egan AD. Addressing use of herbal medicine in the primary care setting. J. Am. Acad. Nurse. Pract. 2002; 14:166–171.
49. Shen J, Xu X, Cheng F, Liu H, Luo X, Chen K et al. Virtual screening on natural products for discovering active compounds and target information. Curr. Med. Chem. 2003; 10: 2327–2342.
50. Elvin-Lewis M. Should we be concerned about herbal remedies. J. Ethnopharmacol. 2001; 75: 141–164.
51. Song MK, Roufogalis BD, Huang THW. Modulation of diabetic retinopathy pathophysiology by natural medicines through PPAR-γ-related pharmacology. Br. J. Pharmacol. 2012; 165: 4–19.
52. Obrosova AIG, Pacher P, Szabo C, Zsengeller Z, Hirooka H, Stevens MJ, Yorek MA. Aldose Reductase Inhibition Counteracts Oxidative-Nitrosative Stress and Poly(ADP- Ribose) Polymerase Activation in Tissue Sites for Diabetes Complications. Diabetes 2005; 54: 234-242.
53. Gabbay KH. The sorbitol pathway and the complications of diabetes. N. Engl. J. Med. 1973; 288: 831–836.
54. Roshana Devi V, Subramanian S. Biochemical Evaluation of Hypoglycemic Effect of GTF-231, a Polyherbal Preparation in High Fat Diet Fed- Low Dose STZ Induced Experimental Type 2 Diabetes in Rats. Der Pharmacia Lettre. 2016; 8: 121-132.
55. Roshana Devi V, Sriram Prasath G, Subramanian S. Antidiabetic properties of GTF – 231, an ayurvedic formulation studied in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats. Der Pharmacia Lettre, 2015; 7: 113-123.
56. Vyshali P, Thara Saraswathi KJ, Sanakal RD, Kaliwal BB. Inhibition of Aldose Activity by Essential Phytochemicals of *Cymbopogon citratus* (DC.) Stapf. 2011; 5: 257 – 267.
57. Umamaheswari M, Aji CS, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P, Madeswaran A. *In Silico* Docking studies of Aldose Reductase Inhibitory activity of selected Flavonoids, Int. J. Drug Dev. & Res. 2012; 4: 328-334.
58. Sathyadevi M, Subramanian S. Aldose reductase inhibitors from the fruits of *Physalis peruviana* Linn.- An *In silico* Approach. J. Pharm. Res. 2014; 8:743-1750.