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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Aldose Reductase inhibitory activity of baicalin for the management of Diabetic complication through *In Vitro* and *In Silico* approach

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ABSTRACT
Aldose reductase is a cytosolic NADPH- dependent oxidoreductase and a principal enzyme of polyol pathway which plays an important role in diabetes and its complications. The present study evaluates the aldose reductase inhibitory activity of baicalin by molecular docking studies and in vitro evaluation. The software which was used for in silico evaluation was Molegro Virtual Docker software (MVD). PDB ID: 1PWM was selected for aldose reductase enzyme. Aldose reductase was isolated from goat eye lens. The in vitro assay was performed by using DL- glyceraldehyde as a substrate and NADPH as a starting material. The MolDock Score of baicalin and the standard drug (ranirestat) was found to be -190.593, -151.57, Rerank score -165.68, -63.72 and H bond-14.47, -8.61respectively. The IC_{50} value of baicalin was found to be 02.914±0.133µg/ml and ranirestat was 09.261±0.107. From the result of in silico approach and in vitro evaluation it was concluded that baicalin fits perfectly at the active site of the enzyme and considered as a potential inhibitor of aldose reductase then the standard drug, and in the future this flavone glycoside, baicalin which is a bioactive compound will be helpful in the management of diabetes and its complication.
Keywords: *Molecular docking, Aldose reductase, baicalin, Diabetes mellitus, in vitro evaluation*

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INTRODUCTION

Diabetes mellitus (DM) is a serious, chronic, and complicated metabolic disorder with numerous etiologies that has severe acute and chronic consequences. [1]. Diabetes is primarily caused by a lack of insulin production, but it can also be caused by a decrease in the cell's capacity to utilise insulin [2]. Diabetes is regarded as the crucial risk factor for the development of a variety of clinical problems, including ischemic heart disease, peripheral neuropathies, ulcerations, and delayed wound healing, and ultimately reducing the patient's life expectancy. [3]. Chronic consequences of diabetes are broadly classified as microvascular (Neuropathy, nephropathy, and retinopathy) and macrovascular (cardiovascular disease, stroke, and peripheral artery disease) [4]. Diabetic foot syndrome is a leading cause of lower limb amputation and is described by the development of a foot ulcer along with neuropathy, peripheral artery disease, and infection. [5]. Several enzymes are involved in the diabetic pathway which plays a specific role in the progression of the disease [6].

The primary cause of blindness worldwide is "cataract." which is linked to a number of risk factors that affect the body, with diabetes being among the primary reason [7]. The first and rate-limiting enzyme, aldose reductase (AR), is involved in the reduction of glucose to sorbitol via the polyol pathway and this step oxidizes the NADPH to NADP+. In the second step this sorbitol is oxidised to fructose by sorbitol dehydrogenase with the production of NADH from NAD+. In the normal condition aldose reductase has a relatively less substrate affinity for the tissue which results in little catalysis of the conversion of glucose to sorbitol. But in diabetes mellitus when the concentration of glucose in the blood increases so the AR activity also increases and this enhances the formation of sorbitol via polyol pathway in tissues such as the lens,

retina, nerve, and kidney [8]. Due to its sluggish degradation by sorbitol dehydrogenase and limited penetration across cellular membranes, sorbitol accumulates in the cells. This results in osmotic swelling, alterations to membrane permeability, as well as other physiological changes which are the major causes of diabetes complication such as cataractogenesis[9,10].

Baicalin has received increased scientific attention because of its numerous pharmacological properties, like antioxidant, anticancer, anti-inflammatory, and hepatoprotective [11]. The current study aims to evaluate the inhibitory potential of baicalin against the enzyme aldose reductase which is involved in diabetic complications like cataractogenesis.

MATERIALS AND METHODS

The chemicals and enzymes utilised in this study were purchased from Loba Chemie and Sigma-Aldrich (India). Commercial suppliers provided the baicalin and standard drug.

Molecular docking:

Preparation of Ligand

The PubChem chemical database was used to identify the 3D structure of baicalin and standard drug. Chem3D was used to create the structures, and the MM2 force field was used to minimise energy (Figure 1). The structures were stored in .mol format. Baicalin and standard drug was prepared for docking analysis by importing to the workspace and assigning the missing bond orders, charges, bonds, and hybridization states in MVD Software [12].

Preparation of Enzyme

The 3D Structure of Aldose Reductase (PDB ID: 1PWM), was retrieved from Protein Data Bank. The protein was prepared by removing all water molecules, ligands, and cofactors and assigned bonds, bond order, hybridization, and charges in MVD software [12].

Docking search algorithms and scoring functions

The Piecewise Linear Potential (PLP) scoring functions are the source of the scoring function in MolDock. The active binding site region was described as a spherical zone including protein within 15.0 Å with specified coordinates of the X, Y, and Z axes. Docking was done using a grid resolution of 0.30 Å and a maximum of 1500 iterations on a single population of 50 individuals for each of the 10 independent runs [13]. Three cavities were restricted for docking analysis and the large volume cavity was selected as the origin for the binding site [14]. The baicalin is docked with the specified protein and based on the MVD docking scores; best-generated poses were selected.

Re rank score

Although the re-ranking score function required more processing resources than the docking score function, it is frequently more effective than the docking score approach at determining the best pose among several poses originating from the same ligand [15]. The energy parameters like E-Inter total, E-Inter, Steric, Van der Waal's, hydrogen bonding energy, E-Intra E-Solvation, E-Total, etc. were employed in the reranking coefficients [16].

Biological Evaluation:

Aldose reductase (ARE) inhibitory activity

Preparation of Enzyme

The lenses were quickly removed from goat eyeballs obtained from a nearby butcher soon after slaughtering. To remove the insoluble material from lenses, 100 gm lenses were placed in a pestle mortar, homogenised in 3 litres of cold distilled water, and then centrifuged at 10,400 RPM for 15 minutes at 0-4°C. To achieve 40% saturation, saturated ammonium sulphate was added to the supernatant fluid. The thick suspension was obtained and centrifuged after standing for 15 minutes to achieve full precipitation. By raising the ammonium sulphate concentration to 50% saturation and centrifuging the liquid, additional inert protein was removed. After this powdered ammonium sulphate solution was added to 75% saturation, and then centrifuged. This results in a more complete separation of aldose reductase from contaminated protein. The precipitate obtained was used for the enzymatic assay [17].

Aldose reductase inhibition assay

A sample cuvette was prepared which containing mixture of 0.75 mL of phosphate buffer (pH 6.2,0.1 M), 0.5 ml of NADPH (0.104mM), 0.3 ml of lens supernatant, 0.1 ml of baicalin (2-10 µg/mL). After incubating the above mixture at 30°C for 10 minutes, 0.75 ml of DL-glyceraldehyde (substrate) (10 mM) was added and the absorbance at 340 nm was measured. The assay was performed in triplicate and ranirestat was used as a standard drug. A dose-response curve was used to obtain the IC_{50} value and % inhibitions [18].

Statistical Analysis:

All the results were expressed as mean ± SEM for triplicate determinations.

RESULTS

Molecular Docking results:

In this work, the molecular docking approach was employed to determine the 'best fit' of baicalin against the enzyme aldose reductase. The lowest binding energy of baicalin against target protein was considered acceptable.

Table 1 and Figure 2A clearly indicate that baicalin fits perfectly in the active site of aldose reductase and it forms 11 hydrogen bonds with aldose reductase. Cys298, His110, Lys77, Tyr48, Gln183, Asp43, Ser210, Pro261, lle260, Asp216, Lys21 were the amino acid residues involved in hydrogen bond formation in the baicalin-aldose reductase complex. Ranirestat formed nine hydrogen bonds with aldose reductase. The amino acid residue involved in hydrogen bond formation were Cys298, Tyr48, Thr19, Asp43, Trp20 and Ser210 (Figure 2B). According to docking studies, Baicalin has a higher affinity for the enzyme than the standard drug.

Biological Evaluation results:

Blood glucose control is essential in the early diagnosis of diabetes mellitus as well as in the macro and microvascular complications. Cataract is a multifactorial eye disease that has been scientifically linked to several risk factors and is responsible for almost half of all blindness globally. Many studies show that inhibiting the aldose reductase enzyme helps to reduce the development of cataract genesis, a diabetic condition.

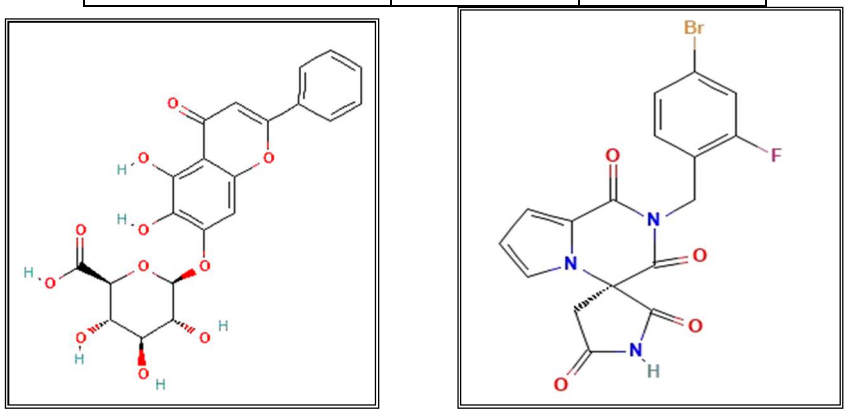
The inhibitory ability of baicalin (2-10 µg/mL) on aldose reductase, was investigated in this research (Table 2, Figure 3). It has a strong inhibitory effect against aldose reductase with IC_{50} value of 02.914±0.133 µg/mL, for standard drug, ranirestat the IC_{50} value was found to be IC_{50}:09.261±0.107 µg/mL respectively. The result indicated that baicalin inhibited the enzyme efficiently in vitro, with a dose-dependent increase in percentage inhibitory activity against the enzyme. As a result, the plant isolate exhibits a higher percentage of inhibition than the standard drug.

Table 1: Result of Molecular docking study of baicalin and standard drug with aldose reductase enzyme.

MVD Result	Baicalin	Ranirestat
Moldock score	-190.59	-151.57
Rerank score	-165.68	-63.72
H bond	-14.47	-8.61

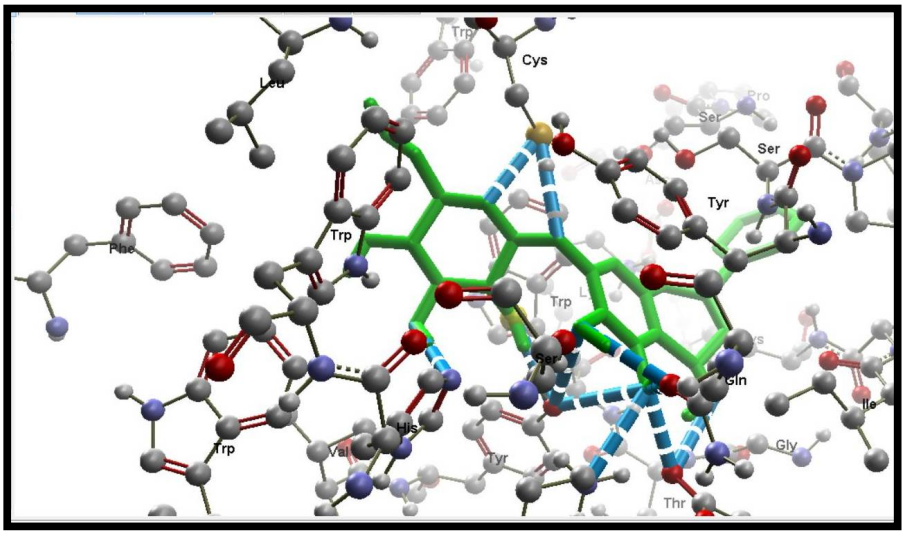
Table 2: Percent inhibitory activity of baicalin and ranirestat on aldose reductase enzyme.

Concentration (µg/ml)	Percent Inhibitory Activity	
	Baicalin	Ranirestat
2	45.74 ±0.795	21.48 ±1.05
4	56.36 ±0.665	32.06 ±1.12
6	62.36 ±1.309	39.48±0.712
8	78.49 ±0.57	44.2 ±0.777
10	85.56 ±0.751	52.57±0.692
IC50	02.914±0.133	09.261±0.107

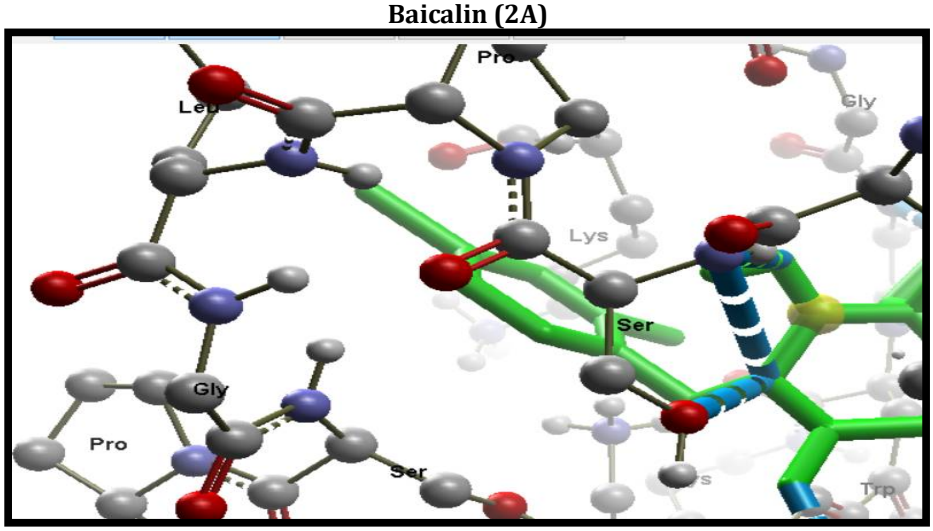


Baicalin **Ranirestat**

Figure 1: Chemical structure of bioactive compound and standard drug



Baicalin (2A)



Ranirestat (2B)

Figure 2: Ligand Map of Reverse Molecular docking study of baicalin and ranirestat with aldose reductase

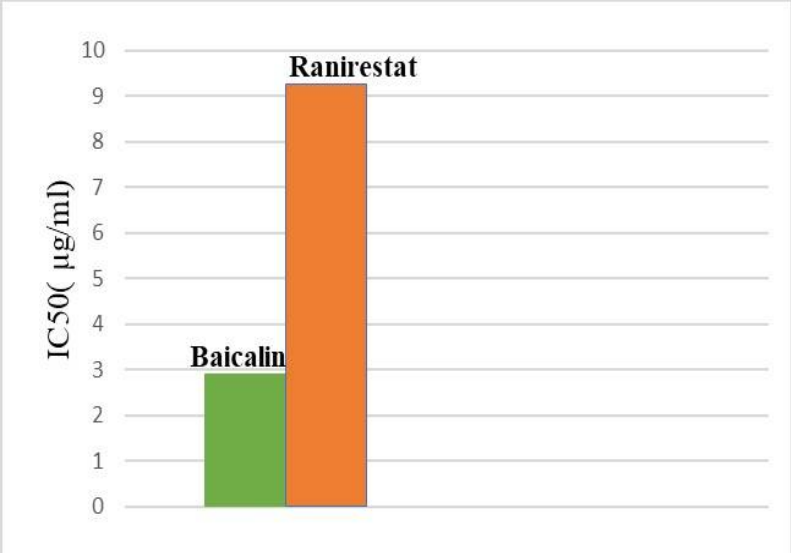


Figure 3: IC_{50} value of baicalin and standard drug on aldose reductase enzyme

DISCUSSION

Diabetes mellitus also called DM, is a severe, long-lasting, and complicated metabolic condition that affect about 25% of the world population [19]. It is a metabolic disorder associated with complications like microvascular and macrovascular that leads to the tissue damage. Retinopathy or cataract genesis is the microvascular complication of diabetes [3]

Aldose reductase is a cytosolic NADPH- dependent enzyme catalyze the reduction of glucose to sorbitol, involved in polyol pathway and plays an important role in cataractogenesis [20]

A cataract is a complicated condition that has a number of risk factors. An important element in the development and progression of cataracts is oxidative stress [21,22].

An effective antioxidant defence system protects the eye lens from damaging from ROS or free radical [23] Free radicals in the body causes gene mutations which results in the formation of cataract [24]. Baicalin which is a flavonoid and bioactive compound present in many species of scutellaria, it is a potent antioxidant compound work through the removal of ROS and act as free radical scavenger, and has health benefits including antiviral, antiinflammation, antiallergic, anticancer, antibacterial, antitumor Properties,[25] but no information has been yet published on the aldose reductase inhibition of the plant.

In the present study aldose reductase inhibitory potential of baicalin was performed by using molegro virtual docker software and through biological assay. From the result it was found that baicalin have high affinity for the enzyme and fits perfectly at the active site of aldose reductase, it was also shown through biological assay that baicalin inhibit the enzyme more potently than the standard drug, ranirestat.

CONCLUSION

The potential of baicalin to inhibit target enzyme was investigated in this research using the ligand-protein molecular docking simulation approach and in vitro evaluation.

According to the results, baicalin has more stable bonding and a higher docking score with aldose reductase, than the standard drug ranirestat. It was found using molecular docking and in vitro study that it can control cataract genesis by blocking the polyol pathway. More study may be done to find pharmacokinetic properties and establish safety and efficacy parameters at both the preclinical and clinical phases, as well as to produce an effective formulation employing baicalin for the treatment of diabetic complication.

Conflict of Interest:

The authors have no conflicts of interest.

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