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# Protective effect of *Tephrosia purpurea* in diabetic cataract through aldose reductase inhibitory activity

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ABSTRACT

*Purpose: Tephrosia purpurea (T. purpurea)* has been reported to prevent cataract formation in senile cataract model as well as proven effective in STZ induced type 1 diabetes. Aldose reductase (AR) is a key enzyme in the intracellular polyol pathway responsible for the development of diabetic cataract.
*Objective:* To investigate the effects of *T. purpurea* in the light of inhibition of aldose reductase enzyme in polyol pathway.
*Methods:* We studied the effects of alcoholic extract and flavonoid fraction of *T. purpurea* in streptozotocin (STZ, 45 mg/kg, i.v.)-induced type I diabetic cataract in rats. The animals were divided into five groups as control, control treated with alcoholic and flavonoid fraction, diabetic control and diabetic treated with alcoholic and flavonoid fraction. *In-vitro* aldose reductase inhibitory activity was also evaluated. Further, molecular docking study was performed with crystal structure of aldose reductase and its known chemical constituents of the plant.
*Results:* The $IC_{50}$ value of alcoholic extract for aldose reductase inhibition was found to be 209.13 μg/ml, and that of flavonoid fraction was found to be 46.73 μg/ml. Administration of STZ produced significantly abnormal levels of serum glucose, serum insulin, soluble protein and antioxidants in the lens homogenate. Treatment with alcoholic extract and flavonoid fraction of *T. purpurea* were able to normalize these levels. Some of the active constituents of *T. purpurea* showed significant interactions with aldose reductase enzyme in molecular docking studies.
*Conclusions:* Our data suggested that both the extracts might be helpful in delaying the development of diabetic cataract due to the presence of rutin and quercetin. This beneficial effect may be due to its significant inhibition of aldose reductase enzyme and anti-oxidant activity.


## 1. Introduction

Cataract is one of the major causes of blindness in diabetic patients in developing countries. Aldose reductase (AR) is a key enzyme in the intracellular polyol pathway, responsible for the development of diabetic cataract. The contribution of AR during chronic hyperglycemia is reported to be enhanced as compared to euglycemic conditions where it play a minor role [1,2]. AR catalyzes the reduction of glucose to sorbitol, which causes an accumulation of sorbitol in lens which results in increase in osmotic pressure, and thereby swelling of the tissues [3]. Thus, by inhibiting aldose reductase localized in lens epithelial cells, it may be possible to prevent cataract progression especially in diabetic conditions. A number of aldose reductase inhibitors have been studied experimentally in animal studies for their efficacy in diabetic complications like epalrestat, fidarestat, ranirestat, zenarestat, sorbinil and tolrestat [4]. Despite numerous efforts, only a few drugs have succeeded to reach into clinical trials, but some of them are still associated with unacceptable side effects, as well as poor pharmacokinetics [5]. Thus, there is a need to develop aldose reductase inhibitors with potent efficacy and fewer side effects. Currently natural products are in demand for the treatment of diabetes and its complications [6].

*Tephrosia purpurea* (Linn.) pers, (Fabaceae), a widely growing herbaceous perennial is reported to exhibit significant anti-hyperglycemic activity in animal models [7–9] as well as able to delay the development of diabetic complications [10,11]. Recently, we have reported *T. purpurea* for its beneficial effects in senile

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cataract, and the activity was correlated with the presence of rutin and quercetin [12]. Both rutin and its aglycone quercetin have been reported previously to possess aldose reductase inhibition [13,14]. Thus, it could be hypothesized that the effects of *T. purpurea* extracts on cataract and diabetes might be due to inhibition of AR and reduction of oxidative stress by rutin. Thus, the objective of the present the study was to evaluate the effects of alcoholic extract and flavonoid fraction of *T. purpurea* in diabetic cataract model and to determine its mechanism of action.

## 2. Materials and methods

### 2.1. Materials

Quercetin was purchased from MP Biomedicals Ltd., Inc, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and streptozotocin from SRL Pvt., Ltd., Mumbai, NADPH and D-xylose from Sigma Aldrich Ltd., Mumbai.

### 2.2. Collection of plant material and preparation of extracts

The whole plant of *T. purpurea* was collected from the medicinal plant garden of Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India and botanically authenticated. Voucher specimen (No. PL08SVBRKGtp001) was deposited in herbarium of the Institute of Pharmacy, Nirma University. It was shade-dried and powdered. Dried plant of *T. purpurea* (200 g) was soxhlet-extracted with 95% alcohol. After concentration under vacuum, the alcoholic extract (AcTp) (9.28%w/w) was stored for further use. The same extract was suspended in distilled water and partitioned into ethyl acetate. Residue obtained after evaporation of the ethyl acetate fraction was dissolved in alcohol, and treated with neutral lead acetate solution. Precipitate obtained was centrifuged, resuspended in alcohol, treated with hydrogen sulfide and filtered. Filtrate was evaporated under vacuum to yield the flavonoid rich fraction (FFTp) (3.07%w/w) [11,12,15], which gave positive Shinoda test.

### 2.3. Phytochemical analysis

Total phenolic content of the alcoholic extract and flavonoid rich fraction was determined through the method previously described by Singleton and Rossi [16] and calculated from the calibration curve of gallic acid standard solution. Results were expressed as %w/w of gallic acid equivalent in dry extract. Total flavonoid content was measured using a previously described method [17]. Quercetin was used as the standard for the calibration curve. Results were expressed as %w/w of quercetin equivalent in dried extract.

The FFTp and AcTp were analyzed by thin layer chromatography for the presence of rutin using ethyl acetate: n-butanol: formic acid: water (5:3:1:1 v/v) and quercetin using toluene: ethyl acetate: formic acid (5:4:1 v/v) as solvent systems. The resulting chromatograms of the alcoholic extract and flavonoid fraction along with the standards i.e. rutin and quercetin were scanned and quantified using CAMAG TLC scanner III at 254 nm for rutin and 374 nm for quercetin.

The protocol of the experiment was approved by our institutional animal ethics committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India (Protocol no. IPS/PCOL/FAC 10-11/2001 dated 12th January, 2011).

### 2.4. In-vitro *DPPH free radical assay*

Anti-oxidant activity of the plant extracts were measured in terms of electron transfer/hydrogen donating ability using a stable radical DPPH by the method described by Molyneux [18]. Briefly, 1.0 ml of 0.1 mM solution of DPPH in methanol was added in 3.0 ml of test solution of AcTp (10 μg, 20 μg, 50 μg, 100 μg, 200 μg 300 μg, 400 μg and 500 μg/ml) and FFTp (10 μg, 20 μg, 50 μg, 80 μg and 100 μg/ml). These solutions were kept in dark for 30 min to protect from the light. The absorbance was measured spectrophotometrically (Shimadzu, Japan) at 517 nm. Absorbance was recorded and the% inhibition was calculated using the following formula:

$$\%\text{Reduction in DPPH radical} = (A_{control} - A_{test})/A_{control} \times 100$$

where $A_{control}$ – Absorbance of control and $A_{test}$ – Absorbance of test sample.

### 2.5. In-vitro *lens aldose reductase inhibitory activity*

#### 2.5.1. Animals

Healthy adult Sprague-Dawley rats (200–250 g) were taken for the study. They were housed in polypropylene cages in rooms maintained at 25 ± 1 °C and 12 h/12 h light-dark cycle. The animals were maintained on a standard laboratory animal diet (Pranav Agro Ltd., India) and provided water *ad libitum* throughout the experimental period.

#### 2.5.2. Preparation of lens homogenate/enzyme preparation

Eyes of normal Sprague-Dawley rats were removed immediately after sacrifice. The lenses were enucleated through posterior approach, washed with saline and their fresh weights were recorded. Twelve non-cataractous lenses were pooled, and a 10% homogenate was prepared in 0.1 M phosphate buffer saline (pH 7.4). After centrifugation at 5000g for 10 min in a refrigerated centrifuge, supernatant was collected and kept at −20 °C for the determination of AR activity and protein content.

#### 2.5.3. Protein estimation

Protein content in supernatant of the lens homogenate was determined by the method of Lowry et al. [19].

#### 2.5.4. Determination of AR activity

Lens AR activity was measured according to the method of Hayman and Kinoshita [20]. A sample cuvette containing 0.7 ml of phosphate buffer (0.067 M), 0.1 ml of NADPH ($25 \times 10^{-5}$ M), 0.1 ml of lens supernatant, 0.1 ml of D-xylose (substrate) (10 mM) to a final volume of 1.0 ml was read against a reference cuvette containing all the components but not the substrate, D-xylose. Final pH of the reaction mixture was 6.2. The enzymatic reaction was started by addition of substrate and the absorbance (OD) was recorded spectrophotometrically at 340 nm for 3 min at 30 second interval. AR activity was expressed as ΔOD/min/mg protein.

#### 2.5.5. Lens AR activity and plant extracts

The alcoholic extract and flavonoid fraction were suspended in 5% DMSO in phosphate buffer saline for stock solutions. To determine their AR inhibitory activity, 0.1 ml of the each plant extract from various stock solutions (final concentrations: 100–700 μg/ml for AcTp and 10–80 μg/ml for FFTp) was added to both the reference and standard cuvettes. The reaction was initiated by the addition of 0.1 ml D-xylose. ΔOD/min/mg protein was calculated for each sample. 5% DMSO in phosphate buffer saline was used as negative control. The assays were run in triplicate and average inhibitory activities were calculated using the following

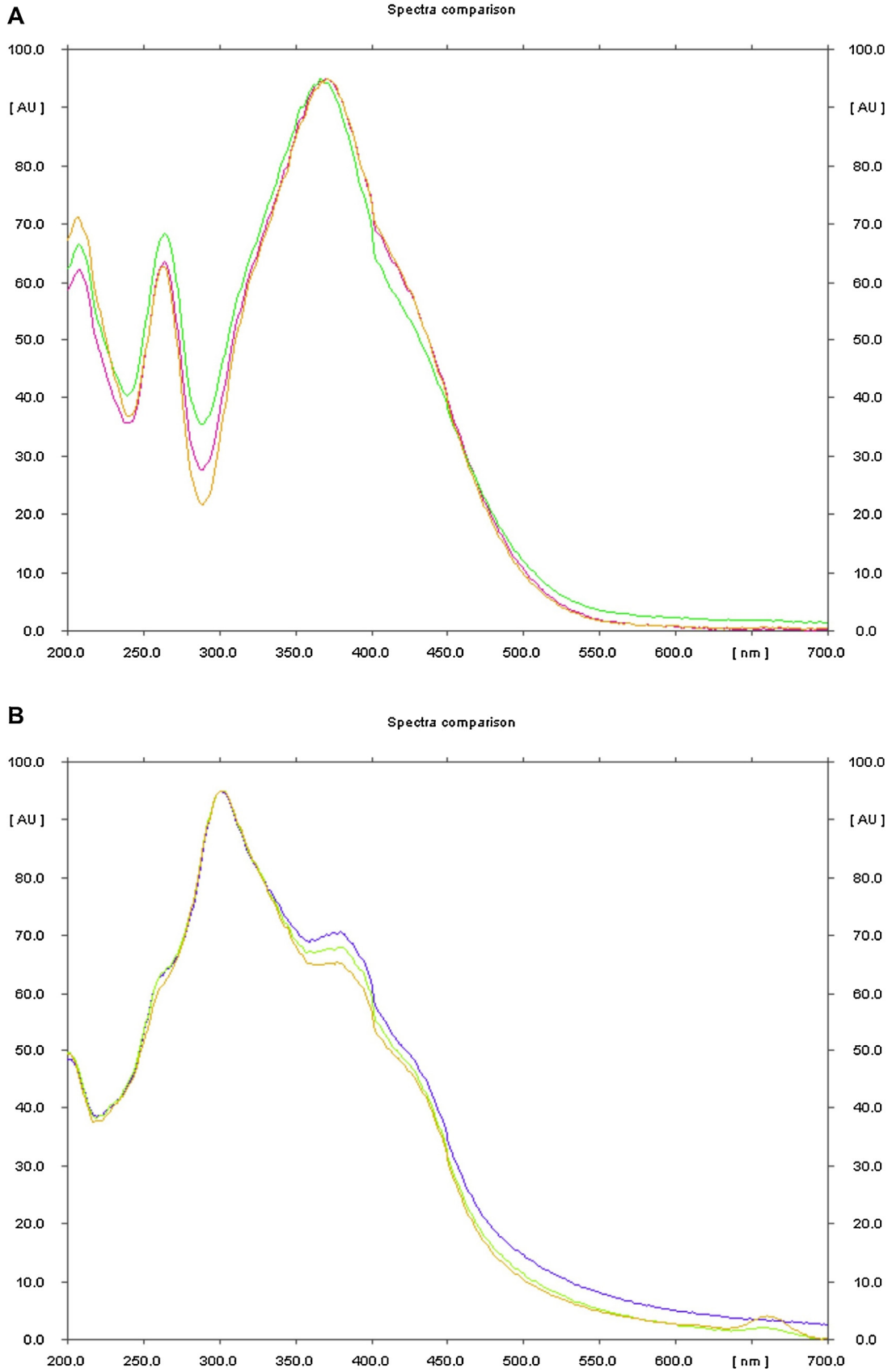


**Fig. 1.** Spectral overlay of alcohol extract (Yellow) and flavonoid rich fraction of *T. purpurea* (Green) with A. Standard rutin (Red) B. Standard quercetin (Purple)[12].

formula:

$$\%\text{AR inhibition} = (\Delta OD_{(\text{Negative control})} - \Delta OD_{(\text{Extract})}) / \Delta OD_{(\text{Negative control})}$$

Percent inhibition of AR activity was then calculated and $IC_{50}$ value for both the extracts were calculated from the dose response curve (DRC) obtained by plotting concentration in μg/ml versus percent inhibition.

### 2.6. Evaluation of T. purpurea effect in type I diabetic cataract in rats

#### 2.6.1. Animals

Healthy Sprague Dawley rats of either sex (200–250 g) were made diabetic by single tail vein injection of streptozotocin (STZ) (45 mg/kg) dissolved in 0.1 M (pH 4.5) of citrate buffer. Control rats were injected with 0.1 M (pH 4.5) of citrate buffer alone. The induction of diabetes was checked 48 h after the STZ injection by measuring the extent of glycosuria with Diastix (Bayer Diagnostics, Ltd). Rats displaying glycosuria more than 2% were considered as diabetic. Rats were then randomly divided into 6 groups consisting of 6 animals each: control (CON), control treated with AcTp (COA), control treated with FFTp (COF), diabetic control (DIC), diabetic treated with AcTp (DIA), and diabetic treated with FFTp (DIF). AcTp and FFTp were suspended in distilled water and administered at a dose of 300 mg/kg/day and 40 mg/kg/day, p.o. respectively for eight weeks. The food (Pranav Agro Ltd., India) and water were given *ad libitum*.

#### 2.6.2. Blood sample collection and serum analysis

At the end of 8 weeks of treatment, animals were fasted for 12 h and blood samples were collected from the retro-orbital plexuses of each rat under light ether anesthesia and serum was separated. The serum samples were analyzed for glucose spectrophotometrically (Shimadzu UV, Japan) using available biochemical diagnostic kit (Accucare Diagnostics, Ltd., India). Serum insulin was estimated by radioimmunoassay technique using kit obtained from Board of Radiation and Isotope Technology, Mumbai, India in gamma counter (Packard, USA).

#### 2.6.3. Oxidative stress in lens

Animals were sacrificed after the blood collection and eye lenses were dissected by posterior approach. Lenses of both the eyes were rinsed with ice-cold distilled water, blotted, weighed and stored at −20 °C for further analysis. Known weights of lens were homogenized in phosphate buffer saline using a glass motor-pestle to prepare 10% homogenate and centrifuged at 4000*g* in the refrigerated centrifuge for 30 min. Supernatant were taken as aliquots for the estimation of antioxidant parameters. The oxidative stress inflicted to lens was assessed by measurement of glutathione (GSH) depletion [21], superoxide dismutase [22], as well as the level of lipid peroxidation in form of malondialdehyde (MDA) [23]. Moreover, soluble protein levels were assessed in lens homogenate by the method described by Lowry et al. [19].

### 2.7. Molecular docking

The Surflex–Dock module of SYBYL was used for molecular docking. The ultrahigh resolved (0.66 Å) crystal structure of human aldose reductase (PDB ID: 1US0) [24] was retrieved from the protein data bank (PDB) at http://www.rcsb.org/pdb/explore/explore.do?structureId=1US0 (accessed March 2015), and modified for docking calculations. Co-crystallized inhibitors IDD594 was removed from the structure, water molecules were removed, -H atoms were added and side chains were fixed during protein preparation. Protein structure minimization was performed by applying Tripos force field, and partial atomic charges were calculated by Gasteiger-Huckel method.

### 2.8. Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). Statistical differences between means of the various groups were evaluated using 1-way analysis of variance followed by Tukey's test. Data were considered statistically significant at a p value $< 0.05$.

## 3. Results

### 3.1. Phytochemical studies

Preliminary studies showed the presence of flavonoids and phenolics. The level of total phenolic content and flavonoids in alcohol extract was found to be 22.67%w/w and 18.09%w/w,

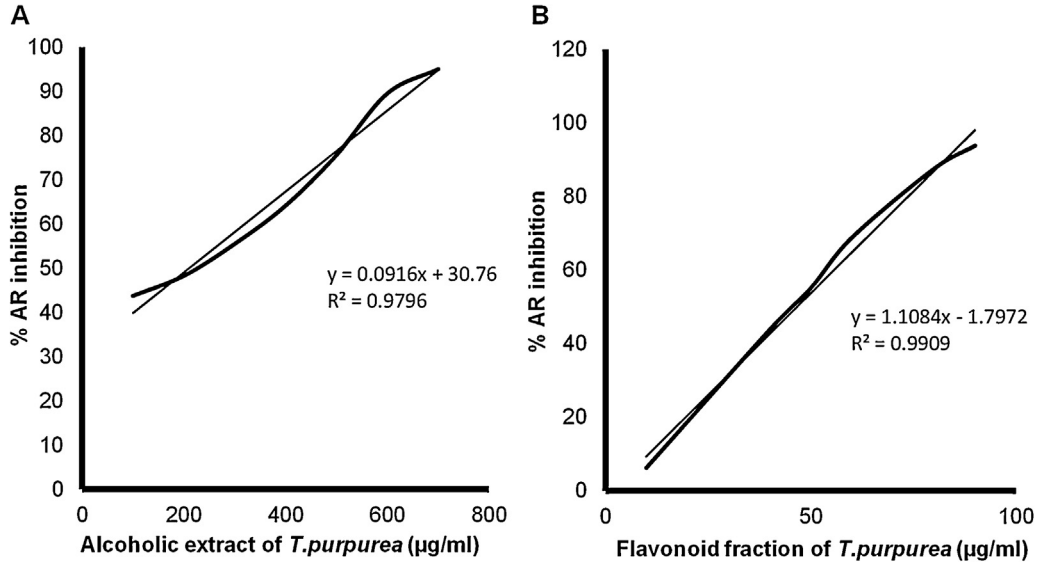


**Fig. 2.** Aldose reductase inhibitory activity of (A) Alcoholic extract and (B) Flavonoid rich fraction of *T. purpurea*.

respectively, whereas in flavonoid rich fraction was found to be 3.83%w/w and 26.94%w/w respectively [12].

On performing HPTLC analysis of the alcohol extract and flavonoid rich fraction of *T. purpurea*, rutin (5.37%w/w in alcohol extract and 2.37%w/w in flavonoid fraction of *T. purpurea*) and quercetin (1.05%w/w in alcohol extract and 1.75%w/w in flavonoid fraction of *T. purpurea*) were found to be present in both the extracts (Fig. 1) [12].

### 3.2. In-vitro *DPPH free radical scavenging assay*

AcTp and FFTp were found to produce reduction in DPPH free radical concentration. The $IC_{50}$ value of AcTp and FFTp was found to be 345.29 μg/ml and 48.51 μg/ml respectively. At the concentration of 400 μg/ml, AcTp showed 54% reduction whereas FFTp at the concentration of 100 μg/ml showed 70.65% reduction in DPPH free radical concentration.

### 3.3. In-vitro *lens aldose reductase inhibitory activity:*

The AcTp and FFTp were found to inhibit lens AR activity to various extent. At the concentration of 700 μg/ml, the alcoholic extract showed 95% AR inhibition, whereas flavonoid fraction showed 93.75% AR inhibition at a concentration of 90 μg/ml. The $IC_{50}$ value of alcoholic extract was found to be 209.13 μg/ml and that of flavonoid fraction was found to be 46.73 μg/ml. The AR inhibitory activity of both the extracts at different concentrations is presented in Fig. 2.

### 3.4. In-vivo *evaluation of* T. purpurea *in type I diabetic cataract in rats*

Eight weeks diabetic rats were examined for visual presence of cataract with the help of opthalmoscope. But visual cataract was not found to be present in type 1 diabetic rats.

#### 3.4.1. Serum glucose and insulin

STZ-diabetic rats were found to exhibit significant hyperglycemia and hypoinsulinemia as compared to control rats. Chronic treatment with AcTp (300 mg/kg/p.o./day) and FFTp (40 mg/kg/p.o./day) produced significant decrease in the elevated serum glucose levels (Table 1) accompanied by significant increase in serum insulin levels (Table 1).

#### 3.4.2. Soluble protein levels in lens

Soluble protein levels were found to be significantly reduced in eye lens due to STZ administration as compared to control rats. Chronic treatment with AcTp (300 mg/kg/p.o./day) and FFTp (40 mg/kg/p.o./day) significantly prevented this reduction in soluble protein levels (Table 2).

### 3.5. Antioxidant levels in lens

STZ administration exhibited significantly decreased reduced glutathione and superoxide dismutase (SOD) levels, and significantly elevated lipid peroxidation levels in lens of diabetic animals as compared to those of control animals. Treatment with AcTp (300 mg/kg/p.o./day) and FFTp (40 mg/kg/day) significantly prevented the loss of reduced glutathione and SOD levels in lens of diabetic rats (Table 2). Also, chronic treatment with AcTp (300 mg/kg/p.o./day) and FFTp (40 mg/kg/day) showed a significant reduction in the lipid peroxidation levels (Table 2).

### 3.6. Analysis of molecular docking

To study the binding modes of known natural constituents in the active site of aldose reductase, we performed molecular docking study. The Surflex–Dock uses an empirically derived scoring function that is based on the binding affinities of enzyme-ligand complexes, and on their X-ray structures. The protomol is a unique and important factor of the docking algorithm and is a computational representation of assumed ligands that interact with the binding site. The ultrahigh resolved structure of aldose reductase-inhibitor (IDD594) complex was retrieved from the PDB. After running Surflex–Dock, the scores of the active docked conformers were ranked in a molecular spread sheet. We selected the best total score conformers and speculated regarding the detailed binding patterns in the cavity. A total score represent Surflex–Dock score (docking score), which include hydrophobic, polar, repulsive, entropic, and salvation is given in Table 3.

## 4. Discussion

Cataract is considered to be a major cause of visual impairment in diabetic patients. Chronic elevation of blood glucose in diabetes plays a critical role in the development and progression of major diabetic complications. Prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism, and long-term irreversible changes in stable macromolecules. The injurious effects of hyperglycemia are characteristically observed in tissues, which are not dependent on insulin for glucose entry into the cell (e.g., eye lens, kidneys) and, hence, they are not capable of down-regulating glucose transport along with the increase of extracellular sugar concentrations [25]. Moreover, the role of increased oxidative stress is also widely accepted for the development and progression of diabetes and its complications [26]. Reports indicated that diabetic complications are associated with overproduction of free radicals and accumulation of lipid peroxidation by-products [27]. However, many non-enzymatic antioxidants like glutathione (GSH) and enzymatic antioxidants like superoxide dismutase (SOD) are involved in the protection of free radicals induced oxidative damage. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins [28]. Three important mechanisms have been implicated in the development of diabetic cataract i.e. the polyol pathway [29], oxidative stress [30] and formation of advanced glycosylation end products [31,32]. Although, there is cross talk between these mechanisms, results

**Table 1**
Effect of alcoholic extract and flavonoid rich fraction of *T. purpurea* on Blood glucose and insulin levels.

| Parameter | CON | COA | COF | DIC | DIA | DIF |
|---|---|---|---|---|---|---|
| Glucose | 75.10 ± 3.90 | 83.10 ± 4.05 | 85.30 ± 4.85 | 317 ± 8.98[a] | 141.78 ± 11.83[b] | 135 ± 12.80[b] |
| Insulin | 35 ± 0.71 | 31.12 ± 0.99 | 33.8 ± 0.15 | 18 ± 1.56[a] | 27.52 ± 1.52[b] | 32 ± 1.49[b] |

Values are expressed as mean ± standard error of the mean, n = 6 in each group. CON—Control animals, COA—Control animals treated with alcoholic extract of *T. purpurea* (300 mg/kg/p.o/day), COF—Control animals treated with flavonoid rich fraction of *T.purpurea* (40 mg/kg/p.o/day), DIA—Diabetic control animals, DIA—Diabetic animals treated with alcoholic extract of *T.purpurea* (300 mg/kg/p.o/day), DIF—Diabetic animals treated with flavonoid rich fraction of *T. purpurea* (40 mg/kg/p.o/day).

[a] Significantly different from control ($P < 0.05$).

[b] Significantly different from diabetic control ($P < 0.05$).

**Table 2**
Effect of alcoholic extract and flavonoid rich fraction of *T. purpurea* on antioxidant parameters in lens.

| Parameter | CON | COA | COF | DIC | DIA | DIF |
|---|---|---|---|---|---|---|
| Soluble Protein (μg/mg wet tissue) | 57.11 ± 4.03 | 47.19 ± 4.47 | 56.12 ± 4.13 | 23.62 ± 1.84[a] | 60.69 ± 6.56[b] | 59.87 ± 5.23[b] |
| Reduced Glutathione (μg/mg protein) | 23.13 ± 2.07 | 25.48 ± 3.84 | 21.71 ± 2.54 | 10.57 ± 0.88[a] | 22.77 ± 2.32[b] | 21.28 ± 1.96[b] |
| Superoxide dismutase (Units/min/mg protein) | 4.21 ± 0.10 | 4.02 ± 0.88 | 4.05 ± 0.05 | 2.56 ± 0.07[a] | 3.87 ± 0.09[b] | 4.17 ± 0.28[b] |
| Lipid Peroxidation (nmoles MDA/mg protein | 2.56 ± 0.36 | 2.69 ± 0.43 | 2.92 ± 0.09 | 5.65 ± 0.56[a] | 2.41 ± 0.40[b] | 2.27 ± 0.31[b] |

Values are expressed as mean ± standard error of the mean, n = 6 in each group. CON—Control animals, COA—Control animals treated with alcoholic extract of *T. purpurea* (300 mg/kg/p.o/day), COF—Control animals treated with flavonoid rich fraction of *T. purpurea* (40 mg/kg/p.o/day), DIA—Diabetic control animals, DIA—Diabetic animals treated with alcoholic extract of *T.purpurea* (300 mg/kg/p.o/day), DIF—Diabetic animals treated with flavonoid rich fraction of *T. purpurea* (40 mg/kg/p.o/day).

[a] Significantly different from control ($P < 0.05$).

[b] Significantly different from diabetic control ($P < 0.05$).

**Table 3**
Docking score of natural constituents with interacting binding site amino acids.

| Compound | [a]Total Score | [b]Crash | [c]Polar | Interacting amino acid residue of binding site |
|---|---|---|---|---|
| Rutin | 5.549 | −3.711 | 4.636 | Trp20, Lys21, Tyr48, Gln49, Phe122, Pro123, Ser302, |
| Quercetin | 4.435 | −0.368 | 4.432 | Trp20, Val47, Tyr48, Gln49, Trp111, Phe122, |
| Fidarestat | 3.427 | −0.634 | 1.962 | Lys21,Tyr48, Gln49, Trp219, Phe122 |
| Lupeol | 3.142 | −1.022 | 0.000 | Gln49, Phe122, Trp219, |
| Tephrosin | 2.993 | −1.153 | 0.007 | Trp20, Tyr48, Phe122, His110 |
| Degurelin | 2.971 | −0.826 | 0.999 | Trp20, Tyr48, Phe122, Pro218 |
| Rotenone | 2.790 | −1.330 | 0.903 | Trp20, Phe122 |
| Elliptone | 2.602 | −0.856 | 0.998 | Trp20, Phe122, Ser302 |

[a] Total Score indicated the total Surflex-Dock score expressed as -log(Kd).

[b] Crash value indicated the degree of inappropriate penetration by the ligand into the protein and of interpenetration (self-clash) between ligand atoms that are separated by rotatable bonds.

[c] Polar value showed the contribution of the polar non-hydrogen bonding interactions to the total score.

in several studies suggested that oxidative stress is a major determinant in diabetic complications [33–35]. Cataract requires not just a surgical solution, but a chemical and pharmacological complement as well. Since, oxidative stress is a common initiator of many diabetic complications, including cataract, chemical approach to delay the onset or retard the progression of cataract is valuable. Therefore, agents or compounds that exert multiple actions, such as antioxidant, hypoglycemic and aldose reductase (AR) inhibitory activity could be more effective therapeutics.

Oxidative stress may be a predominant mechanism in STZ-induced hyperglycemia. Oxidative stress may cause direct modification of the inner lens proteins, such as cross-linking, aggregation, and precipitation [36]. The increased TBARS (MDA levels) along with the decreased GSH and altered activities of antioxidant enzymes like SOD in the present study suggested increased oxidative stress in diabetic conditions. Chronic treatment with AcTp and FFTp decreased lipid peroxidation as well as was found effective in restoring the levels of GSH and antioxidant enzymes like SOD. As *T. purpurea* has been proven to possess potent anti-oxidant activity [37–39], it is possible that delay in the development of STZ-induced diabetic complications may be predominantly due to its antioxidant activity.

Decrease in soluble protein content in diabetic lenses compared with those in control lenses in present study could be due to leakage of proteins and insolubilization. *T. purpurea* treatment could increase the levels of soluble proteins in diabetic lenses may be by preventing cross-linking/aggregation and distribution of soluble proteins. Under conditions of severe oxidative stress, free radical generation leads to protein modification. *T. purpurea* treatment possessing potent anti-oxidant potential may prevent protein modification, and hence, may be helpful in preventing insolubilization of proteins. This may prevent or delay the development of opacity of lens.

Aldose reductase (AR) is a small monomeric protein belonging to aldo-keto reductase superfamily. AR-derived polyols like sorbitol accumulate in the diabetic ocular lens [40], which causes osmotic swelling resulting in ionic imbalance, and protein insolubilization leading to cataractogenesis. Osmotic swelling of diabetic lens may render the cells leaky [41], enhancing loss of GSH accumulated in the lens [42]. Disrupted cell membrane by osmotic stress may also interfere with amino acid transport into the lens [43], and hence biosynthesis of GSH [42]. Moreover, AR reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its cofactor NADPH, which is also required for the regeneration of GSH [44]. Studies suggested that persistent high intracellular glucose concentration-induced superoxide generation inhibits GAPDH activity [45] which increases the levels of all the glycolytic intermediates located in upstream of GAPDH, finally increasing the first glycolytic metabolite, glucose [46]. Moreover, inhibition of GAPDH is responsible for an increased formation of the AGE-forming compound methylglyoxal [47]. As shown by Chang et al. [48], methylglyoxal is also responsible for substrate-induced upregulation of AR, which may further facilitate development of diabetic cataract. AR inhibiting activity of investigated plant *T. purpurea* was evaluated *in-vitro* using rat lens homogenate. The alcoholic extract as well as flavonoid fraction of the plant have shown significant AR inhibiting activity, which may propose probable mechanism of plant in delaying development of diabetic complications like cataract. Also, docking results suggested that most of the known constituents have a common binding mode in the vicinity of active site of subunit A of aldose reductase, lying between the catalytic amino acid residues, Trp20, Tyr48, Trp111, Phe122, and His110. These compounds showed interactions with several residues near the active site, including Trp20, Lys21, Tyr48, Trp111, Phe122, Pro218 and Ser302, which seems to play a key role in the activity of the enzyme (Table 3). The overall binding of rutin, quercetin and fidarestat (reference standard for docking) in human aldose reductase is illustrated in Fig. 2. Docking results showed that rutin possessed highest docking score of 5.549 and formed five hydrogen bonds (Fig. 3A)

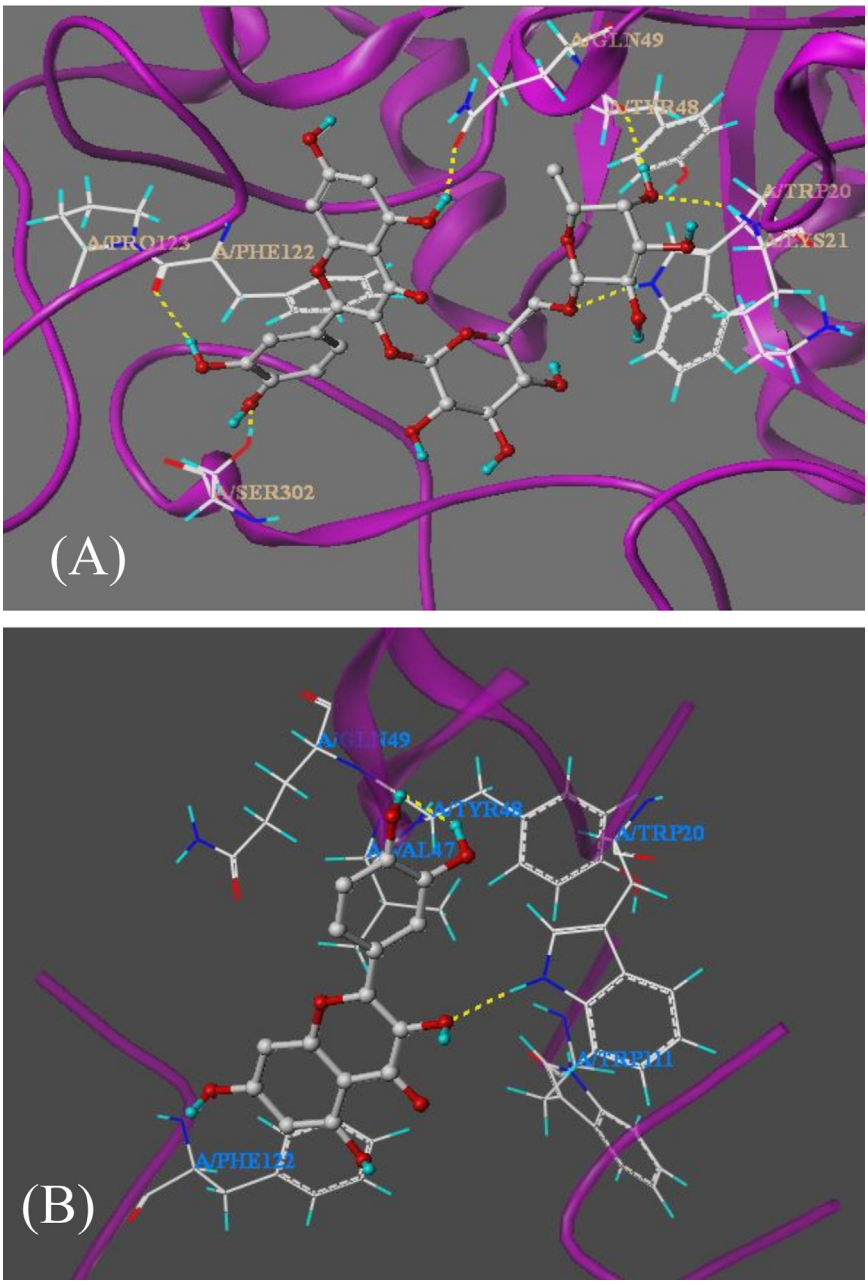


**Fig. 3.** Binding mode of rutin (A) and quercetin (B) in aldose reductase enzyme proposed by docking studies using Surflex-Dock. Rutin and quercetin is in ball-and-stick model with color by atoms, H-bond interaction were highlighted using yellow line. The labeled protein residues are in capped stick model with color by atom.

with Trp20, Tyr48, Gln49, Lys121 and Pro123 which are important residues for binding of inhibitors. The hydrophobic phenyl ring was found in contact with Phe122, which is an important residues for binding of inhibitors. Quercetin has second highest docking score of 4.43, and formed three hydrogen bonds (Fig. 3B) with Tyr48, Gln49 and Trp111, which are an important residues for binding of inhibitors. Fidarestat as reference molecules formed three hydrogen bonds with Lys21, Tyr48 and Gln49 with a docking score of 3.42. Docking study explored the interaction mechanism, and reasonable binding mode of these natural constituents in the active site of aldose reductase.

## 5. Conclusions

The present study indicated that *T. purpurea* possessed significant anti-hyperglycemic activity, as well as anti-oxidant activity in diabetic rats and, also possessed significant *in-vitro* AR inhibiting activity. Together with the ability to reduce oxidative stress and inhibition of AR, *T. purpurea* might be beneficial not only in preventing hyperglycemia but also in delaying the development of diabetes induced complications, due to hyperglycemia induced oxidative and osmotic stress.

## Conflict of interest

The authors declare no conflict of interest.

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