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RESEARCH ARTICLE

Targeting the Polyol Pathway with *Pithecellobium dulce* for Diabetic Cataract Prevention

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Abstract

Background: Diabetic cataracts are a prevalent complication in diabetes, primarily resulting from the accumulation of sorbitol in the lens via the polyol pathway. Targeting the polyol pathway to prevent diabetic cataracts has garnered significant interest. *Pithecellobium dulce,* a traditional medicinal plant, has shown potential in inhibiting aldose reductase activity, thereby reducing sorbitol accumulation. **Aim:** The present study investigates the efficacy of *P. dulce* in preventing diabetic cataract formation by modulating the polyol pathway. **Methods:** In this study, goat lenses were used for anti-cataract activity. A total of 16 lenses were divided into 4 groups group I normal control, group II Dexamethasone, group III and IV methanolic extract of *P. dulce* 100mg/ml, 200mg/ml respectively. At the end of the study, goat lenses were examined for opalescence score and estimate the Lens polyol, biochemical parameters like sodium, potassium, total proteins and catalase levels. **Results:** Dexamethasone treated lens showed more opalescence and increased levels of polyols, electrolytes and catalase levels when compared to the normal group. Whereas methanolic extract of *P. dulce* 200mg/ml treated lens significantly decreased opalescence, decreased levels of polyols, electrolytes and catalase levels when compared to dexamethasone-treated groups. **Conclusion:** Targeting the polyol pathway with *P. dulce* holds significant promise for the prevention of diabetic cataracts. Through its inhibitory effects on the polyol pathway, *P. dulce* can potentially mitigate the excessive accumulation of sorbitol within the lens, thus preventing the emergence and progression of cataracts in diabetic individuals.

Keywords: Cataract, Dexamethasone, Diabetes, Polyol Pathway, Target

1. Introduction

Diabetic cataract is a prevalent complication of diabetes mellitus, significantly contributing to visual impairment and blindness among diabetic patients. One of the critical metabolic pathways implicated in the development of diabetic cataracts is the polyol pathway. This pathway becomes overly active due to high levels of glucose, leading deposition of sorbitol in the eye lens. The enzyme, aldose reductase is produced by sorbitol and is subsequently converted to fructose. However, the rate of sorbitol accumulation often exceeds its conversion to

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fructose, causing osmotic stress, lens fibre swelling, and oxidative damage, which ultimately results in cataract formation[1]. Targeting the polyol pathway has evolved as a magnificent therapeutic strategy for preventing diabetic cataracts. Aldose Reductase Inhibitors (ARIs) have been explored extensively to halt or slow down the progression of lens opacification by preventing sorbitol accumulation. However, many synthetic ARIs have shown limited success in clinical trials due to adverse side effects and insufficient efficacy. This has sparked interest in exploring natural compounds with potential ARI activity and minimal side effects[2]. Manila tamarind is the common name for *P. dulce,* a tropical plant known for its diverse medicinal properties. Traditionally used in various folk medicines, *P. dulce* possesses anti-inflammatory, antimicrobial, and antioxidant activities, making it a potential candidate for addressing complications of chronic diseases, including diabetes[3]. Recent studies have suggested that extracts from *P. dulce* exhibit inhibitory effects on the key enzyme aldose reductase in the polyol pathway[4]. The bioactive compounds in *P. dulce*, such as flavonoids and phenolic acids, are believed to contribute to its aldose reductase inhibitory activity[5]. These natural inhibitors may offer a safer and more effective alternative to synthetic ARIs. By preventing the sorbitol accumulation in the lens, *P. dulce* could reduce osmotic stress and oxidative damage, thereby mitigating the cataract formation risk in diabetic patients[6].

Also, *Pithecellobium dulce's* antioxidant properties can provide additional protective effects against oxidative stress, an important complicated factor in diabetes. Oxidative stress is caused by the disparity of the Reactive Oxygen Species production and defence of antioxidants in the human body that leads to cell damage. By increasing the antioxidant capacity of the body and scavenging free radicals, *P. dulce* may help preserve lens transparency and function[7].

2. Materials and Methods

2.1 Collection and Identification

Leaves of *P. dulce* from the medicinal herbarium of the School of Pharmaceutical Sciences, Sandip University, Nashik – India. This is approved by the Department of Botany, Sandip University and voucher specimen is preserved for future testimonial. All chemicals employed in the current work are of analytical-grade fetched from Himedia laboratories, Mumbai.

2.2 Drying and Grinding

The leaves were isolated by removing extraneous matter and then subjected to drying at room temperature for two weeks under shade. Then these are finely pulverised into a fine powder and stored in an airtight jar.

2.3 Extraction

500g of leaves that are finely powdered and extracted using petroleum ether at 60-80 °C for one week to wipe out the fatty material. The remaining free fatty substance is mixed with 75% methanol for Soxhlet extraction. This extract is evaporated to dryness at a low temperature of 30°C under reduced pressure and a brownish-yellow colour was obtained[8].

2.4 Phytochemical Preliminary Screening

Various constituents were detected by subjecting MEPD to preliminary phytochemical screening[9].

2.5 *Ex Vivo* Examination: Anti-cataract Activity

Goat lenses were used due to their wide availability. The fresh lens of the goat was acquired from a slaughterhouse in Guntur.

2.6 Lens Culture

The obtained goat eye lens was transported immediately to the laboratory at a temperature of 0-40 °C. Extracapsular extraction was employed to remove the lens and then incubate it in artificial aqueous humour. This aqueous humour comprises a mixture of Hydrochloric acid – 5mM, Sodium chloride – 140mM, Magnesium Chloride - 2mM, Sodium dihydrogen phosphate – 0.5mM, Sodium bicarbonate – 0.5mM, Glucose – 5.5mM, and Calcium Chloride – 0.4mM stored at room temperature maintained at a pH of 7.8. To the culture media, 500mg of Cefixime was added to prevent contamination from bacteria[10].

2.7 *Ex Vivo* Extracts Induction

The drug Dexamethasone 10 mg was employed to induce posterior subcapsular cataracts. The mechanism of induction is via osmotic change, oxidative stress, conformational protein change, and hydration.

A sum of 16 lenses was used for this research and all these lenses were incubated. Incubation was carried out in artificial aqueous humour containing Dexamethasone of 10mg per kg that was served for 5 days as a toxic control[11].

The four groups in the study comprise lenses 16 in number and each group contains 4 lenses i.e. n=4 as below:

- Group – I: Aqueous humour i.e. normal control
- Group II: Aqueous humour + 10mg* (Model/ Toxic control)
- Group III: Aqueous humour + 10mg* + 100mg/ml MEPD
- Group-IV = Aqueous humour + 10mg* + 200mg/ml MEPD

10mg* = 10mg of Dexamethasone

2.8 Photographic Evaluation

Lenses after five incubation days, were kept on wire meshing posteriorly adhering to the lens. A mesh pattern of visible squares through the lens was observed which a measure of its opacity is. Grading was given as per degree of opacity as:

- 0 - No opacity
- 1 - Slight opacity
- 2 - Diffuse opacity
- 3 - Extensive thick opacity

2.9 Lens Polypols - Estimation

By the previously reported method[12], the lens polypols were estimated. Lenses were homogenized using 0.6N Per chloric acid and then by centrifugation for 30 min for 5000rpm. The supernatant liquid was isolated and neutralized using 2N of KOH and later centrifuged. Then the supernatant was treated by employing 0.2ml stannous chloride, 2ml of 0.2% chromo tropic acid and 0.2ml of 0.03M periodic acid in a water bath for 30 min. A purple complex was obtained, and its absorbance was measured by a calibration curve.

2.10 Lens Homogenate Preparation

The lens homogenate was prepared in 0.23M Tri's buffer of pH = 7.8 in 0.25 X 10^{-3} EDTA solution. The homogenate mixture was made to 10% W/V and further centrifuged at 10000 rpm for one hr at 4°C. The supernatant that is obtained was utilised in the determination of biochemical parameters[13] like Na^+ and K^+ electrolytes. These were estimated using the Flame photometric method and modified biuret method for the estimation of proteins using the endpoint assay technique. Catalase estimation in the lens homogenate is demonstrated in the literature, by Aeibe *et al*[14-19].

2.11 Statistical Interpretation

The obtained values are depicted in terms of mean ± standard mean error. Interpretation of results among groups for multiple therapies was evaluated using one-way ANOVA (Analysis of Variance), further using Dennett's test. The value of $P < 0.05$ is found to be significant.

3. Results

Determination of various phytochemical substances in MEDP was performed by phytochemical evaluation and the results were summarized in Table 1.

3.1 Photographic Examination

The lenses were treated with 10mg dexamethasone and later subjected to incubation. Moderate opacification on the lens is seen at the peripheral sides after 2 days posteriorly. The said activity gradually started increasing towards the centre with complete opacification after five days when compared to the incubated lenses in the normal aqueous humour and, the indication of squares was visible with transparency. Progress of lens opacification is retarded when the lenses were incubated with MEDP at (100µg/ml and 200 µg/ml) as

Table 1. Results of MEDP phytochemical constituents

S. No.	Phytochemical analyses	Results
1	Alkaloids	X
2	Glycosides	X
3	Tannins	X
4	Phenols	✓
5	Flavonoids	✓
6	Saponins	X
7	Steroids	✓

Here, ✓ denotes present X denotes absent

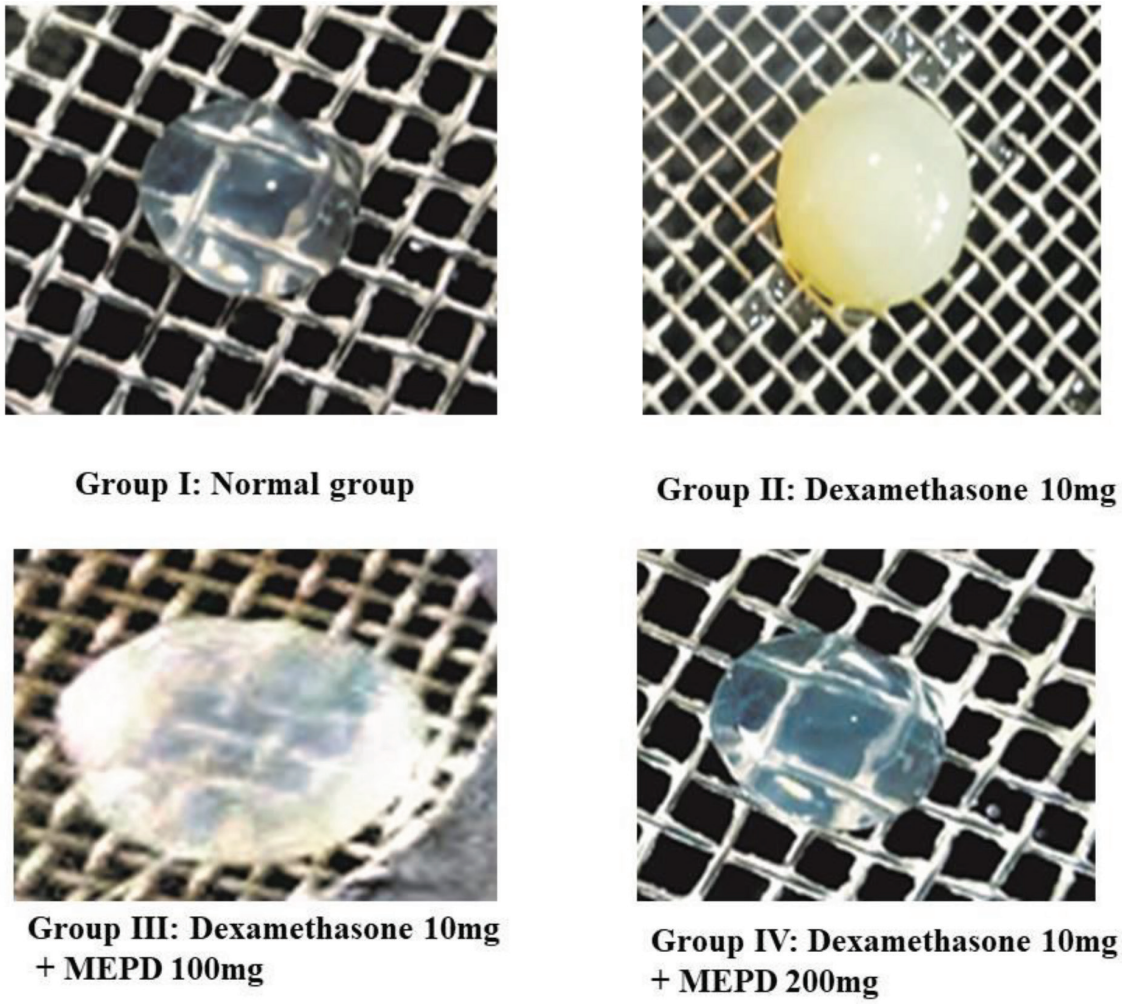


Figure 1. Opacification of lenses.

Table 2. Grades of opacity for lens

Sl. No.	Name of group	Grade
1	Group I - Normal group	0
2	Group II - Dexamethasone 10mg	3
3	Group III - Dexamethasone 10mg + MEPD 100mg	2
4	Group IV - Dexamethasone 10mg + MEPD 200mg	1

in (Figures 1 and 2). The opacity grades for the lenses and their results are depicted in Table 2.

3.2 Effect on Levels of Polyol

The effect of fluctuations in polyol levels in the lens is depicted in Figure 3. The levels of polyol increased significantly in lenses when incubated in high content of Dexamethasone medium by comparing to negative control. MEPD presence at a high level of Dexamethasone medium markedly lowers the osmotic stress that is induced by a reduction in polyol levels (Figure 3). This feature indicated the protective action against cataract formation.

3.3 Biochemical Parameters

The present research implied a higher content of total proteins and K^+ ions and a lesser concentration of Na^+ ions when the groups were treated with MPED. The catalase level was less found in cataract lenses that are induced experimentally when compared to the normal group. Lenses that are treated with MEPD have a remarkable elevation in enzyme levels

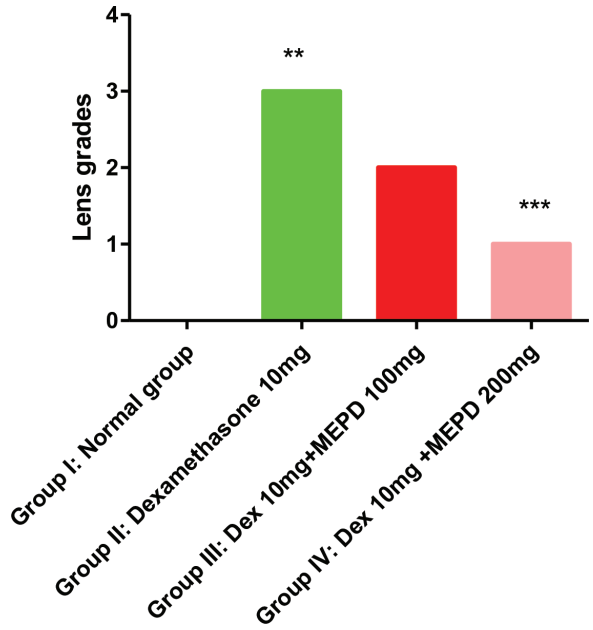


Figure 2. Represents the effect of MEPD on opacification of lens in Dexamethasone-induced cataract. Compared to normal control, Dexamethasone control shows increased opacification of the lens (**p<0.001) whereas MEPD 200 µg/ml treated control significantly decreases (***p<0.001) opacification of the lens when compared to the negative control.

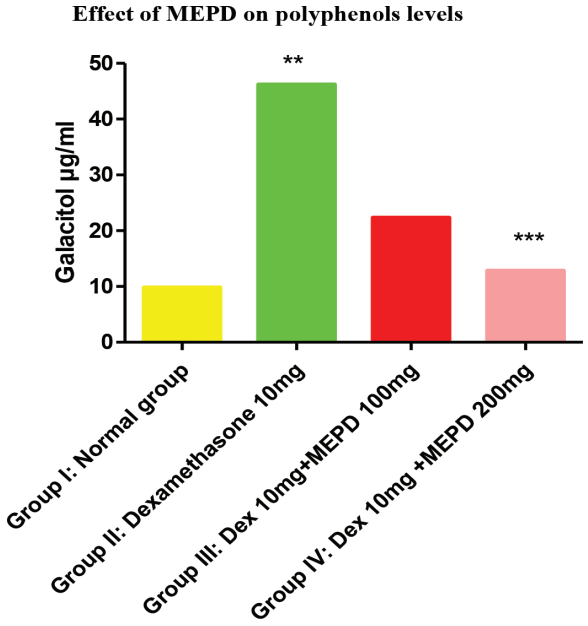


Figure 3. Represents the MEPD effect on levels of Galacitrol in cataracts induced by Dexamethasone. Dexamethasone control shows increased galacitrol levels (**p<0.001) in comparison to normal control whereas, when compared to negative control, MEPD 200 µg/ml treated control significantly decreases (***p<0.001) galacitrol levels.

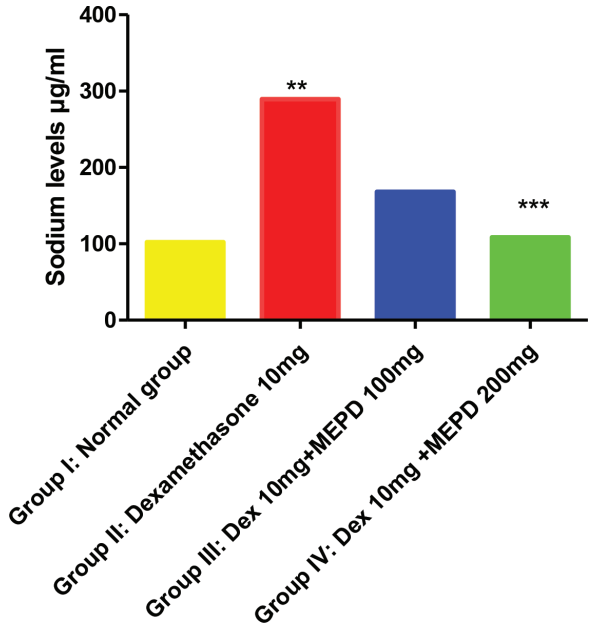


Figure 4. Represents the effect of MEPD on levels of sodium in high glucose that induced cataracts. Dexamethasone control shows an increase in sodium levels (**p< 0.001) to that of normal control whereas MEPD 200 µg/ml treated control significantly decreases (***p<0.001) sodium to that of negative control.

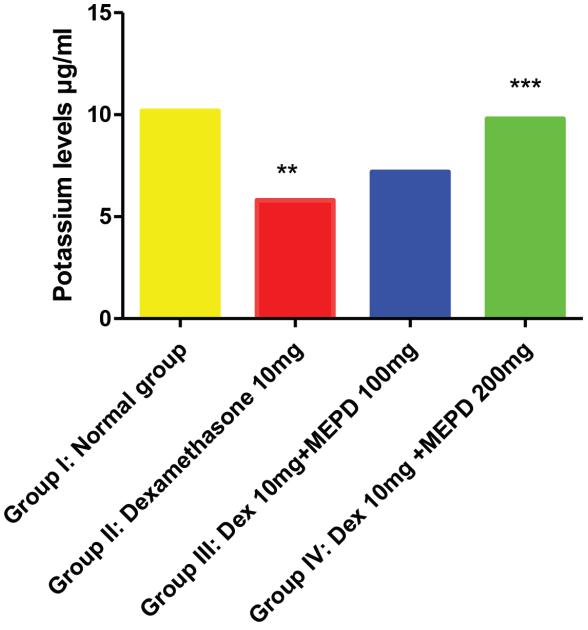


Figure 5. Represents the action of MEPD on the levels of potassium in high glucose content-induced cataracts. Dexamethasone control shows decreased potassium levels (**p<0.001) than the normal control whereas MEPD 200 µg/ml treated control significantly increases (***p<0.001) potassium levels than the negative control.

Table 3. Effect of MEPD on biochemical parameters

Sl. No.	Name of group	Levels of sodium µg/ml	Levels of potassium µg/ml	TPC value gm/dl	Catalase level µmol H_20_2/mint
1	Group I: Normal group	102.2±0.12	10.2±0.01	3.1±0.27	208±0.56
2	Group II: Dexamethasone 10mg/kg	289.4 ±0.25	5.8 ±0.52	1.02±0.68	128±0.89
3	Group III: Dexamethasone 10mg/kg+100 mg MEPD	168.0 ±0.68	7.2 ±0.36	1.98±0.82	196±0.01
4	Group IV: Dexamethasone 10mg/kg +200 mg MEPD	108.4 ±0.78	9.8 ±0.54	2.75±0.26	202.2±0.24

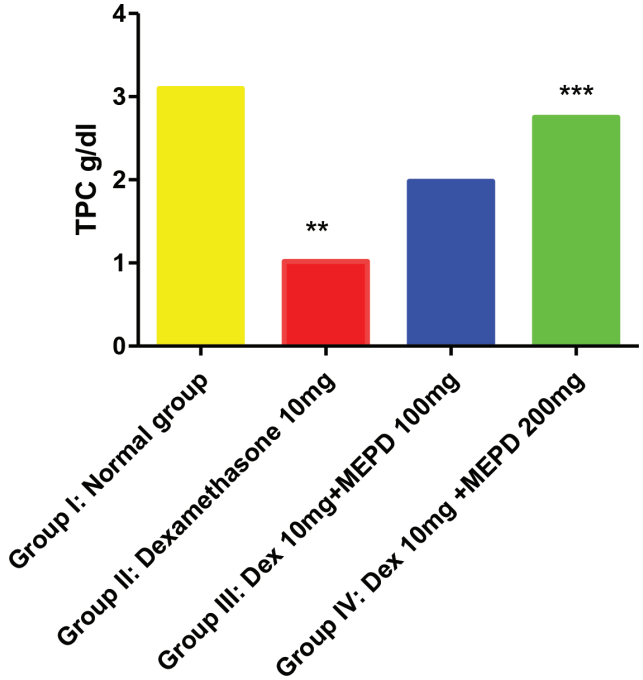


Figure 6. Represents the impact of MEPD on the content of total protein in cataracts induced by high glucose. Compared to normal control, negative control shows decreased levels of total protein (**p<0.001) whereas compared to negative control, MEPD 200 µg/ml treated control prominently increased (***p < 0.001) total protein levels.

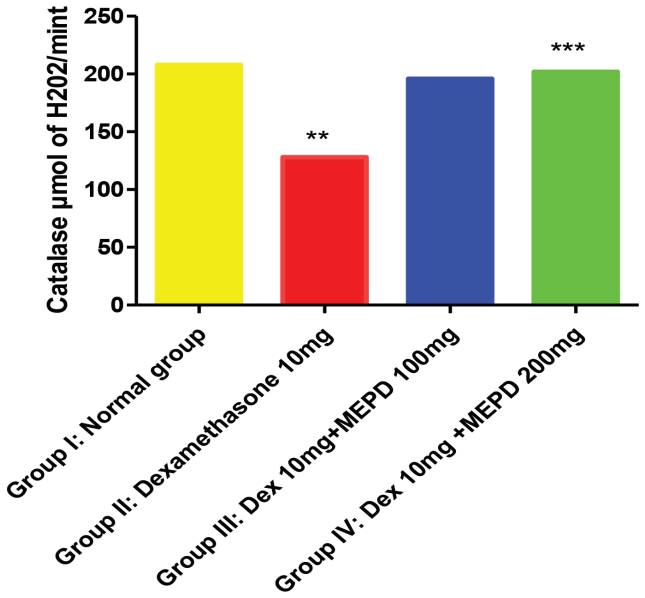


Figure 7. Represents the effect of MEPD on levels of catalase in cataracts that is induced with high glucose content. Negative control shows a decrease in catalase levels (**p<1.001), compared to normal control whereas MEPD 200 µg/ml treated control significantly elevated (***p<0.001) catalase levels, as that of negative control.

and thereby, propose maintaining the integrity of antioxidant enzymes. The results were tabulated in Table 3.

3.4 Effect of MEPD on Sodium Levels in Goat Lens

In this study, the Dexamethasone control lens increases the levels of proteins 289.4 ±0.25, whereas the MEPD 200ug/ml treated lens significantly decreases the protein levels 108.4 ±0.78. This indicates that MEPD maintains normal lens transparency by decreasing sodium levels (Figure 4).

3.5 MEPD Effect on Levels of Potassium in Goat Lens

In this study, the Dexamethasone control lens increases the levels of potassium 5.8 ±0.52 whereas the MEPD 200ug/ml treated lens significantly increases the proteins levels 9.8 ±0.54. This indicates that MEPD maintains normal lens transparency by decreasing sodium levels (Figure 5).

3.6 MEPD Effect on Total Protein Content in Goat Lens

Transparency in the eye's lens is dependent on the maintenance of its native tertiary structure and the solubility of crystalline proteins in the lens. So, proteins are used for lens transparency. In this study,

the Dexamethasone control lens decreases the levels of proteins by 1.02±0.68, whereas the MEPD 200ug/ml treated lens significantly increases the protein levels by 2.75±0.26. This indicates that MEPD maintains normal lens transparency by increasing protein levels (Figure 6).

3.7 Effect of MEPD on Catalase in Goat Lens

Catalase is one of the antioxidants present in goat lenses for the prevention of oxidative stress. Negative control lenses show decreased levels of catalase 128±0.89 and the MEPD 200µg/ml treated lens exhibits increased catalase levels 202.2±0.24 when compared to negative control lens. It indicates that MEPD increases catalase levels and prevents oxidative stress-induced cataracts (Table 3 and Figure 7).

4. Discussion

The study highlights the promising role of *Pithecellobium dulce* methanolic extract (MEPD) in preventing diabetic cataracts by targeting the polyol pathway. The results show that MEPD effectively reduced lens opacification and normalized biochemical parameters in dexamethasone-induced cataract models. Specifically, the extract significantly decreased polyol levels and sodium ion concentration while increasing potassium ion levels, total proteins, and catalase activity in treated lenses. These outcomes indicate MEPD's dual action: reducing osmotic stress caused by sorbitol accumulation and enhancing antioxidant defences to counter oxidative damage.

The untreated dexamethasone-induced cataract lenses exhibited extensive opacification, elevated polyol levels, and significant biochemical imbalances, closely resembling the pathological features of diabetic cataracts. However, MEPD, particularly at a concentration of 200 mg/ml, demonstrated substantial efficacy in restoring lens transparency and maintaining biochemical homeostasis. This suggests that MEPD's inhibitory effect on aldose reductase, a key enzyme in the polyol pathway, plays a critical role in reducing sorbitol accumulation and preventing osmotic stress. Additionally, its antioxidant properties, attributed to its phytochemical constituents such as flavonoids and phenols, provide further protection against oxidative stress a major factor in cataract genesis.

These findings align with the growing interest in natural alternatives for managing diabetic complications. Synthetic Aldose Reductase Inhibitors (ARIs) have faced limitations due to side effects and inconsistent efficacy, whereas *P. dulce* offers a potentially safer and more effective approach. The study also highlights MEPD's ability to maintain lens protein stability, essential for preserving lens transparency.

Overall, the results validate the therapeutic potential of *P. dulce* in diabetic cataract prevention. Future research, including preclinical and clinical studies, is needed to confirm its efficacy, determine optimal dosages, and evaluate safety profiles. This could pave the way for its development into natural health products or adjunct therapies for diabetic eye complications.

5. Conclusion

In conclusion, targeting the polyol pathway with *P. dulce* holds significant promise for the prevention of diabetic cataracts. Through its inhibitory effects on the polyol pathway, *P. dulce* can potentially mitigate the excessive accumulation of sorbitol within the lens, thus preventing the emergence and progression of cataracts in diabetic individuals. The rich phytochemical composition of *P. dulce* further enhances its therapeutic potential, offering a natural and potentially safer alternative to conventional diabetic management strategies. However, further preclinical and clinical studies are warranted to fully elucidate its efficacy, safety profile, and optimal dosage regimen.

The study on *P. dulce* suggests its potential as a natural, safer alternative for preventing diabetic cataracts by targeting the polyol pathway. It could inspire new treatments, supplements, and ethnopharmacological research. Clinical validation may establish its broader applications in managing diabetic complications and age-related cataracts, improving global eye health outcomes.

6. Acknowledgement

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7. Author Contribution

Conceptualization: NB, NVID, YDM; Methodology: MGB, APG, NB, PKT; Data curation: NB, PS, PKT: Formal analysis: NB, MGB; Writing - original draft: NB, DTN, PS; Supervision: NB, MSG; Writing - review and editing: NB, MGB, MSG.

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