

# In Vitro Anti-Cataract Activity of Hydro-Alcoholic Extract of *Pennisetum purpureum* (Cenchrus purpureus) Against Sodium Selenite-Induced Cataractogenesis in Isolated Goat Lens

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## ABSTRACT

### 1. Objective

The in vitro anti-cataract activity of the hydro-alcoholic extract of *Pennisetum purpureum* (HAEPP) was assessed in sodium selenite induced isolated goat lenses.

### 2. Methods

The water: Ethanol (1:1 v/v) extracted shade dried whole plant material of *Cenchrus purpureus* (synonym *Pennisetum purpureum*) was subjected to physicochemical evaluation (loss on drying, total ash, acid-insoluble ash and water-soluble ash) and the extraction was carried out by continuous hot extraction in a Soxhlet apparatus for 48 hours. The resultant hydro-alcoholic extract (HAEPP) was put to qualitative preliminary phytochemical screening. The anti-cataract activity was determined by using isolated goat lenses kept in artificial aqueous humor and randomly divided into five groups (n = 5) as follows: normal control; cataract control (sodium selenite 100  $\mu$ M); reference control (sodium selenite 100  $\mu$ M + ascorbic acid 10  $\mu$ g/mL); sodium selenite 100  $\mu$ M + HAEPP 100 mg/mL; and sodium selenite 100  $\mu$ M + HAEPP 200 mg/mL. Lenses were graded after 72 hours of incubation for opacity on a semi-quantitative scale by photographic means and then total soluble lens protein was estimated by Lowry's method. One-way ANOVA with Dunnett's t-test was used to analyse the data and a value of  $p < 0.05$  was adopted as statistically significant.

### 3. Results

The loss on drying, total ash, acid insoluble ash and water soluble ash of the powdered drug was 1% w/w, 4.2% w/w, 13.5% w/w and 2% w/w respectively and the extractive yield was 14.8% w/w. The phytochemical screening revealed the presence of tannins, saponins, flavonoids, carbohydrate, alkaloids and triterpenoids. Sodium selenite showed dense lenticular opacification (cataract score  $3.64 \pm 0.24$ ) and significantly reduced the total protein of the lens ( $1.05 \pm 0.01$  g/dL) compared with the normal control (cataract score 0;  $3.25 \pm 0.11$  g/dL). Co-incubation with HAEPP produced a dose-dependent reduction in opacity, with cataract scores of  $2.62 \pm 0.14$  ( $p < 0.05$ ) at 100 mg/mL and  $1.50 \pm 0.07$  ( $p < 0.01$ ) at 200 mg/mL, the higher dose approaching the reference standard ( $1.23 \pm 0.08$ ,  $p < 0.001$ ). The total protein content was similarly restored at the two doses,  $2.54 \pm 0.14$  and  $3.08 \pm 0.16$  g/dL, respectively, which were comparable with that of the ascorbic acid reference group ( $3.21 \pm 0.20$  g/dL).

### 4. Conclusion

The protection against selenite-induced cataractogenesis seen by HAEPP is dose dependent, likely due to the actions of its individual active components, the alkaloid, flavonoids and saponins, which are thought to inhibit oxidative protein precipitation within the lens. The results suggest that *Pennisetum purpureum* can be studied for anti-cataract activity using further pharmacological and mechanistic studies.

The article provides a detailed report of the anti-cataract activity of the hydro-alcoholic extract of *Pennisetum purpureum* and *Cenchrus purpureus* on isolated goat lens with the analysis of phytochemical screening, estimation of total lens protein, oxidative stress, and hydro-alcoholic extract.

**Keywords:** *Pennisetum purpureum*; *Cenchrus purpureus*; Elephant grass; Anti-cataract activity; Sodium selenite; Isolated goat lens; Oxidative stress; Phytochemical screening; Hydro-alcoholic extract; Lowry's method; Total lens protein.

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## 1. INTRODUCTION

In the botanical sense, a herb is a seed-producing plant that lacks persistent woody tissues and has green succulent stems, herbs can be annual, biennial or perennial, and have different uses such as aromatic, culinary and medicinal uses [1]. Several factors have contributed to this resurgence: Firstly, the preference for natural, minimally processed therapeutic options over synthetic drugs; secondly, a greater cultural attitude towards preventive and holistic health and wellbeing over the treatment of illness alone; thirdly, easier access to health information that is empowering patients to conduct their own research and make therapeutic choices; fourthly, the perception, although not always accurate, that herbal therapies have a less serious adverse-effect profile than conventional pharmaceuticals; and fifthly, the expansion of pharmacologic research that is increasingly supporting traditional health uses for plants. The significance of herbal medicine is equally profound as it is used as a primary healthcare system, a traditional knowledge system that is passed down from generation to generation, and a source of novel bioactive scaffolds for novel drugs development [3]. Loss of transparency and progressive, often irreversible, opacification of the lens may result from a variety of degenerative processes, congenital disturbances of lens growth, fibrous metaplasia of the lens epithelium, cortical hydration between lens fibres, and pigment deposition, and in the end denature and precipitate the structural lens crystallin proteins. The degenerative mechanisms can be generally characterized as occurring during lens development (congenital cataract), as a metaplasia of the lens epithelium (subcapsular cataract), as a hydration of the cortical area between the lens fibres (cortical cataract) and as a deposition of pigments like urochrome in the nucleus of the lens (nuclear cataract), all leading to an opacification of the lens preventing light passage to the retina. There is a remarkable amount of evidence pointing to oxidative stress as a key, if not dominant, mechanism of cataractogenesis. Oxidative stress is the result of a shift in the balance between the production of ROS and the ability of antioxidant defenses to neutralize the ROS. The non-vascularized, metabolic, biochemically unique lens is especially sensitive to this imbalance, and its transparency depends critically on the maintenance of a proper intracellular redox state, mostly maintained by reduced glutathione (GSH). When the GSH level of the lens falls, the lens accumulates reactive oxygen species, which result in a parallel decrease of antioxidant enzyme activity, favouring the oxidative modification of the membrane of the fibre cells and, therefore, the aggregation and precipitation of the soluble lens proteins (crystallins) which are responsible for lens transparency. The sequence of

oxidative insult, GSH depletion, crystallin denaturation and crystallin precipitation has now been shown to be a clear unifying pathway in several experimental cataract models including the sodium selenite model employed in the current study, and is an appropriate pharmacologic target for the antioxidants found in natural products. The understanding of this oxidative mechanism has inspired a body of literature in the field of pharmacology, where natural antioxidants are being investigated as anti-cataract drugs. N-acetylcysteine amide has been shown to be a useful preventive and even restorative for selenite-induced cataract in experimental animals, and its advocates have proposed that agents of this type might alter the treatment of cataract from a surgical correction to a relatively inexpensive pharmacological prevention, particularly in high-risk populations [10]. Curcumin also has been reported to have a protective effect against selenite cataract by reducing the generation of reactive oxygen species inside the cells and the level of oxidative damage to the lens epithelial cells [11]; similarly, the aqueous whole plant extract of *Heliotropium indicum* has been proved to have an effective effect on cataract in rats by several partially characterized mechanisms [12]. Traditional plant preparations have exhibited similar promise, with ethanolic extract of *Cineraria maritima* being anticataract in an ex vivo study and preventing selenite-induced cataractogenesis in an in vivo study [29, 32] and the polyherbal Ayurvedic formulation *Triphala* exhibiting anticataract activity in both selenite-induced in vitro and in vivo studies [30]. *Cenchrus purpureus* belongs to kingdom Plantae, to the clade Tracheophyta, to the clade Angiosperms, to the clade Monocots, to the clade Commelinids, to the order Poales, to the family Poaceae, to the subfamily Panicoideae, to the genus *Cenchrus*, and to the accepted binomial *Cenchrus purpureus* (Schumacher). Morrone [26]. A tall, robust, bamboo like C4 perennial grass, which mainly reproduces vegetatively by stolons, with a low requirement for both water and nutrients, and which can grow to 7-8 metres in 4 months under favourable conditions. The species is currently most valued for the plants' nutritional value as a fodder for dairy cattle and buffalo in East Africa, especially in the hairless cultivar forms, with reported dry-biomass yields of 15-40 dry tonnes per hectare per year under drier conditions to 60-100 under optimal management; these can be converted to bioenergy through thermal pyrolysis to give biogas, bio-oil and charcoal [13,14,18,19,20,21,24]. The species also provides windbreak and firebreak, protects dry and marginal lands from soil erosion, and has been developed as the major "pull" element of the push-pull intercropping system implemented throughout sub-

Saharan Africa for trapping cereal stemborers away from maize and sorghum, which also has been found to enhance soil fertility and reduce the incidence of parasitic Striga weed in smallholder farming systems [15,16,17,22,23]. This extensive agronomic characterisation contrasts with the relatively short pharmaceutical literature for the species and highlights the novelty of the evaluation of the species for a particular pharmaceutical indication such as the eye, like cataract. The aim of the present study was accordingly to evaluate the in vitro anti-cataract activity of the hydro-alcoholic extract of Pennisetum purpureum (HAEPP). This was achieved by five progressive steps: (i) collection and shade-drying of plant materials; (ii) physicochemical (ash-value) analysis of powdered drug; (iii) extraction of powdered drug by hot continuous-extraction technique; (iv) preliminary phytochemical screening of the extract; and (v) testing the in vitro anti-cataract activity of the extract in a validated isolated goat lens model using sodium selenite-induced cataract, estimating total lens protein content as a biochemical correlate of lens transparency.

## **2. MATERIALS AND METHODS**

### **2.1 .The collection and authentication of plant materials.**

The whole plant material of *Cenchrus purpureus* (synonym *Pennisetum purpureum*) commonly referred as elephant grass or Yannai Pull was collected from Veravadampalayam village, Tiruppur District, Tamilnadu, India. The plant material was identified by the Botanical Survey of India, Coimbatore (BRI). Vouchers were kept for future reference.

### **2.2 Drying and Powdering**

The aerial parts (Leaves and Stems) of the plant were collected carefully and thoroughly washed to remove soil and other debris and then shade dried for 7-15 days with frequent turning to ensure uniform drying and to avoid microbial spoilage. The dried plant material was then mechanically blended to get uniform and free flowing coarse powder using laboratory blender which was passed through suitable mesh, stored in an airtight container away from light and moisture and used for all the subsequent physicochemical, extraction and phytochemical work.

### **2.3 Discuss about the Physicochemical Evaluation of the Powdered Drug.**

Ash values are the pharmacognostic parameters which are used to evaluate the quality, purity and inorganic or other extraneous contamination in powdered form of a crude drug [25]. The mineral content of the crude drug, plus the care given in collecting and processing it, determines the total ash value, which is determined by incineration, since the ash consists chiefly of the carbonates, phosphates and silicates of sodium, potassium, calcium, and magnesium; and an unusually

high acid-insoluble ash content is especially significant, when surface contamination with siliceous earthy material or a high native calcium oxalate content is suspected.

The loss on drying was measured gravimetrically. Five grams of the accurately weighed, air-dried powdered material was transferred into a flat-bottomed, previously dried and tared weighing bottle and dried in a hot-air oven at 100-105 °C until the weights of 2 successive weighings did not differ by more than 5 mg. The percentage loss on drying was determined by calculating the loss of weight as a percentage of the sample taken.

To determine the total ash content, 4 g of the finely ground sample was transferred to a silica crucible that was previously heated to 500-600 °C and tared, spread in a thin layer, and burned to obtain a white or greyish-white ash which means that the carbonaceous matter is completely burned. The crucible was cooled in a desiccator and weighed, and the percentage total ash (per gram of air-dry material) calculated as:

$$\% \text{ Total ash} = \% \text{ ash/ weight of sample taken} \times 100$$

After total ash was placed in the crucible, 25 mL of dilute hydrochloric acid was added to the crucible, covered with a watch glass and boiled gently for 5 minutes, and then the contents of the watch glass were washed off and added to the crucible for acid-insoluble ash determination. The insoluble residue was filtered on ash-less filter paper, washed free from excess acid, returned to the original crucible and dried on a hot plate and then fired to constant weight. The residue was weighed immediately after cooling in a desiccator and the percentage of acid-insoluble ash calculated on the basis of one gram of the air-dried material.

The total ash was boiled for 5 minutes with 25 mL of distilled water and the insoluble material was collected on the ash-less filter paper and washed with hot water and dried to constant weight at a low temperature for the water soluble ash. The weight loss of the total ash minus that of the ash insoluble in water was termed water soluble ash per g of air dry material.

### **2.4 Extraction of the Powdered Drug**

The plant material was dried and powdered and extracted using a Soxhlet apparatus using a hydro-alcoholic solvent with water:ethanol (1:1 v/v) ratio [26]. Extraction by Soxhlet is an old extraction method that is well known for exhaustively extracting thermally stable analytes; the solvent in the distillation flask below is condensed and percolates through the powdered sample contained in a cellulose thimble, which is placed in the extraction chamber. The solvent level is raised in the thimble chamber and is periodically siphoned back into the distillation flask which carries dissolved constituents with it; the non-volatile constituents extracted remain in the distillation flask while the volatile solvent is returned to the

thimble chamber and the process of siphoning is repeated by refluxing for an extended period of time to progressively concentrate the extractable constituents of the drug in the distillation flask.

Extraction was continued for 48 hours to insure complete extraction of extractable constituents. The hydro-alcoholic extract was subjected to controlled evaporation of the solvent, and then the concentrated extract was transferred to a tared china dish, dried to constant weight and stored in a desiccator away from light and moisture until further use. The extractive yield, in percentage, was defined as the percentage of the weight of the product obtained by extraction to the total weight of the raw material used, on a weight for weight basis.

**% Yield = (Weight of dried extract/ Weight of powdered drug subjected to extraction) × 100**

### **2.5 Preliminary Phytochemical Screening**

Standard qualitative chemical screening test was carried out on the hydro-alcoholic extract of *Pennisetum purpureum* (HAEPP) to determine the general classes of secondary metabolites present in the extract, according to standard pharmacognostic procedures [27,28].

Alkaloids were tested by Mayer's reagent (formation of white precipitate), Dragendorff's reagent (reddish-brown precipitate), Wagner's reagent (brown precipitate), Hager's reagent (creamy-white precipitate) and picric acid solution (yellow colouration) and a positive reaction was considered as a sign of the presence of alkaloidal constituents.

The following tests were carried out for the presence of tannins: the gelatin test in which the extract dissolved in distilled water was treated with 1% gelatin solution and a white precipitate was formed indicating tannins; the ferric chloride (Braymer) test in which the filtrate was treated with dilute ferric chloride solution, a blue-green colouration was formed, indicating the presence of tannins; and treatment with 10% sodium hydroxide, formation of an emulsion again indicating the presence of tannins.

The froth test was used to assess saponins, where vigorous shaking of water extract with water followed by boiling results in a persistent froth with small bubbles, whereas the sodium bicarbonate test involved vigorous shaking of extract with sodium bicarbonate solution which led to a stable froth in the shape of a honeycomb, both tests being held to be indicative of saponin glycosides; the haemolysis test involved dropping fresh blood on a glass slide and treating it with the extract which resulted in a visible zone of haemolysis, both observations regarded as indicative of the presence of saponin glycosides.

The following tests were conducted: alkaline reagent test, in which the extract treated with 2% sodium hydroxide solution turned intense yellow and then

became colourless when treated with dilute hydrochloric acid; second alkaline reagent test (using 10% ammonium hydroxide solution), in which the extract in alcohol turned an intense yellow colour and then became colourless when treated with dilute hydrochloric acid; Shinoda test (Magnesium hydrochloride reduction) showed extract turning a pink to crimson colour on adding magnesium turnings to the extract and concentrated hydrochloric acid; Shibata test (Cyanidin) showed extract turning red (flavone) or orange (flavonol) colour on adding magnesium metal to it and concentrated hydrochloric acid; Lead acetate test showed a yellow precipitate on treating with a 10% lead acetate solution.

The following tests were used to screen for carbohydrates: Bradford's test (A red precipitate is formed when carbohydrates are heated with Bradford's reagent, indicating monosaccharides); Molisch's test (A violet ring is formed when concentrated sulphuric acid is poured over an alcoholic extract and the solvent mixture is then heated, indicating ketoses); a test for pentoses (A red colour is obtained on heating the extract with an alcoholic extract of phloroglucinol and concentrated hydrochloric acid, indicating pentoses); a test for starch (A canary-yellow colour is obtained after treating the aqueous extract with 5% potassium hydroxide solution, indicating starch).

Standard Liebermann-Burchard type colour reactions were used to evaluate the triterpenoids, a positive test being indicated by a colour change typical of the steroidal/triterpenoidal nucleus.

The anti-cataract activity in vitro was evaluated. In Vitro Anti-Cataract Activity was evaluated.

#### **2.6.1 Collection of Goat Eyeballs and Lens Isolation**

25 fresh goat eyeballs were collected from the local slaughterhouse immediately after slaughter in ice bag and kept under 0-4 °C for maintaining the viability of the lens. Aseptically the lenses were carefully dissected away from the surrounding vitreous and other ocular tissue and immediately placed in freshly prepared artificial aqueous humor of the following composition: sodium chloride 140 mM, potassium chloride 5 mM, magnesium chloride 2 mM, sodium bicarbonate 0.5 mM, sodium dihydrogen phosphate 0.5 mM, calcium chloride 0.4 mM and glucose 5.5 mM, adjusted at and maintained at pH 7.8. To prevent microbial contamination during the incubation period, streptomycin (100 µg/mL) and penicillin (100 IU/mL) were added to the medium [29].

Students will develop and conduct experimental designs, and they will establish study groups.

Five groups each containing 5 lenses were set up and incubated in the artificial aqueous humor described above with the additives described below: Group I: Normal control (incubated in plain artificial aqueous humor); Group II: Cataract control (incubated in

artificial aqueous humor containing 100  $\mu$ M sodium selenite); Group III: Reference control (incubated in artificial aqueous humor containing 100  $\mu$ M sodium selenite and ascorbic acid 10  $\mu$ g/mL); Group IV: Incubated in artificial aqueous humor containing 100  $\mu$ M sodium selenite and HAEPP (100 mg/mL); Group V: Incubated in the artificial aqueous humor containing 100  $\mu$ M sodium selenite and HAEPP (200 mg/mL). Experimental cataractogenesis was induced in this study by sodium selenite, which is known to induce oxidative modification and precipitation of lens crystallins and is a common feature of age-related nuclear and cortical cataract.

The morphological examination and cataract grading is provided. The morphological examination and cataract grading are done.

The lenses in each of the 5 groups were cultured for 72 h, removed and then visually examined under standardized light and background conditions for opacity and remaining transparency and photographed. There was no quantitative measurement of opacity; instead a semi-quantitative grading scale was used: 0 indicates no opacity (grid visible); 1 indicates slight opacity (grid barely visible); 2 indicates diffuse opacity (grid can still be seen); and 4 indicates extensive, dense opacity (grid not visible) [30].

The Total Lens Protein was estimated using the method of Lowry et al. (1981).

After 72 hours, lenses from each group were homogenised to make 10 % w/v homogenates in 0.1 M sodium phosphate buffer pH 7.4. Homogenates were centrifuged at 10,000 rpm for 30 minutes at 4 °C in a refrigerated centrifuge and the clear supernatant of each homogenate was collected for estimation of total soluble protein by Lowry's method. The protein in the supernatant was precipitated by adding 0.1 mL of 90% ethanol to 1 mL of the supernatant and then the alcoholic precipitate was redissolved in 1 mL of 0.1 N sodium hydroxide, and 0.05 mL was transferred to a fresh test tube. Four millilitres of 2 g sodium carbonate in 100 mL of 0.1 N sodium hydroxide containing 1 mL of 4% aqueous copper sulphate was added and left to stand for 10 minutes followed by the addition of 0.4 mL of phenol reagent of Folin-Ciocalteu and left to stand for another 10 minutes. The amount of absorbance for each sample was measured at 610 nm using a UV-visible spectrophotometer and the protein content of each sample was interpolated from a calibration curve using bovine serum albumin and expressed as g/dL protein [31].

## 2.7 Statistical Analysis

All data are expressed as mean  $\pm$  standard error of the mean (SEM). Differences among groups were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons

against the cataract control group, using GraphPad Prism software. A probability value of  $p < 0.05$  was considered statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Plant Collection and Authentication

The botanical identity of the plant used in the present study was confirmed by the Botanical Survey of India, Coimbatore from which the whole plant of *Cenchrus purpureus* (synonym *Pennisetum purpureum*) collected from the village Veruvadampalayam, Tiruppur District, Tamilnadu was authenticated. Before use the authenticated material has been shade dried, ground and kept in conditions to prevent up-take of moisture, microbial or enzymatic degradation.

### 3.2 Physicochemical Evaluation

The physicochemical parameters of the powdered drug determined as ash value and loss on drying are summarized as follows: Loss on drying is 1% w/w whereas the total ash value is 4.2% w/w, acid-insoluble ash value is 13.5% w/w, and water-soluble ash value is 2% w/w. The low loss-on-drying value suggests that the shade-drying procedure used was appropriate to ensure low moisture content that is not likely to favour microbial growth or hydrolytic breakdown of phytoconstituents during storage. The total ash value gives an overall information about the physiological (intrinsic) and non-physiological (extraneous or adulterant) mineral content of the drug and the acid-insoluble ash is the residue remaining after the acid treatment of the total ash which represents the siliceous contaminant.

### 3.3 Extractive Yield

The powdered drug was extracted with water:ethanol (1:1) using a Soxhlet for 48 hours to give a hydro-alcoholic extract of 14.8% w/w of the drug. This yield corresponds to the high proportion of moderately polar-to-polar extractable constituents, such as the phenolic fraction, the flavonoid fraction and the saponin fraction, which were later confirmed by phytochemical screening, and is typical for the choice of hydro-alcoholic solvent system, which is well suited for the co-extraction of both polar glycosidic and moderately lipophilic phenolics from plant matrices.

### 3.4 Preliminary Phytochemical Screening

The qualitative phytochemical analysis of *Pennisetum purpureum* hydro-alcoholic extract showed the presence of tannins, saponins, flavonoids, carbohydrates, alkaloids and triterpenoids (Table 1).

**Table 1. Preliminary phytochemical screening of the hydro-alcoholic extract of *Pennisetum purpureum* (HAEPP)**

**In Vitro Anti-Cataract Activity of Hydro-Alcoholic Extract of Pennisetum purpureum (Cenchrus purpureus) Against Sodium Selenite-Induced Cataractogenesis in Isolated Goat Lens**

| S. No. | Phytoconstituent | Remark  |
|--------|------------------|---------|
| 1      | Tannins          | Present |
| 2      | Saponins         | Present |
| 3      | Flavonoids       | Present |
| 4      | Carbohydrates    | Present |
| 5      | Alkaloids        | Present |
| 6      | Triterpenoids    | Present |

The antioxidant, anti-microbial, anti-cytotoxic, anti-hypertensive and anti-inflammatory effects of Pennisetum extracts are attributed to this broad phytochemical profile, which extends the results of earlier reports on the genus Pennisetum that documented at least 35 different types of secondary metabolites from the various species of the genus [9, 32]. The identified flavonoids, alkaloids and saponins are of particular pharmacological relevance as each of these classes has been independently reported for free-radical scavenging and antioxidant activity, properties which are mechanistically plausible, and consistent with previous studies demonstrating that *P. purpureum* extracts exhibited significant antioxidant activity and antimicrobial activity related to their phytochemical constituents [7] which is essentially the observed effect in the subsequent bioassay.

The anti-cataract activity was investigated in vitro. In vitro anti-cataract activity was investigated.

The anti-cataractic activity of HAEPP was assessed in isolated goat lens model on the basis of opacity evaluation by morphological examination and quantification of total soluble lens protein by biochemical method. The findings are presented in a summary table (Table 2) and in photographic and graphical presentations.

**Table 2. In vitro anti-cataract activity of the hydro-alcoholic extract of Pennisetum purpureum (HAEPP) in the sodium selenite-induced isolated goat lens model**

| Group and Treatment      | Cataract Score | Total Protein (g/dL) |
|--------------------------|----------------|----------------------|
| Group I – Normal control | 0              | 3.25 ± 0.11          |

| Group and Treatment                                                                | Cataract Score | Total Protein (g/dL) |
|------------------------------------------------------------------------------------|----------------|----------------------|
| Group II – Cataract control, sodium selenite (100 µM)                              | 3.64 ± 0.24    | 1.05 ± 0.01          |
| Group III – Reference control, sodium selenite (100 µM) + ascorbic acid (10 µg/mL) | 1.23 ± 0.08*** | 3.21 ± 0.20          |
| Group IV – Sodium selenite (100 µM) + HAEPP (100 mg/mL)                            | 2.62 ± 0.14*   | 2.54 ± 0.14          |
| Group V – Sodium selenite (100 µM) + HAEPP (200 mg/mL)                             | 1.50 ± 0.07**  | 3.08 ± 0.16          |

*Values are expressed as mean ± SEM (n = 5). \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 compared with the cataract control group (Group II), one-way ANOVA followed by Dunnett's t-test.*

The 72 hours of incubation did not cause any cloudiness in the lenses stored in plain artificial aqueous humor (Group I), and cataract score remained at 0, whereas the level of total soluble protein remained at 3.25 ± 0.11 g/dL, indicating structural and biochemical stability of the isolated organ-culture system for the entire experimental period. Incubation with 100 µM sodium selenite alone (Group II) resulted in thick and extensive opacification in the shape of lens lenticules, accompanied by significant reduction in total soluble protein to 1.05 ± 0.01 g/dL, which is almost two-thirds of the normal control. This simultaneous visual opacification with a similarly marked loss of soluble protein is the expected biochemical signature of selenite-induced cataractogenesis, in which the previous soluble lens crystallins are converted to insoluble, light-scattering aggregates, which are lost from the soluble protein fraction on homogenisation and centrifugation, and that macroscopically manifest as visual opacification of the lens.

The reference antioxidant ascorbic acid (10 µg/mL; Group III) showed a highly significant decrease in cataract score to 1.23 ± 0.08 (p<0.001 vs cataract

control) and maintained the normal level of total soluble protein ( $3.21 \pm 0.20$  g/dL) which was statistically similar to the normal control, thus validating and demonstrating the sensitivity of the experimental model to a known antioxidant therapy. The protective effect was clearly seen and dose-dependent when HAEPP was co-incubated. Cataract score was significantly decreased to  $2.62 \pm 0.14$  ( $p < 0.05$  versus cataract control), and a corresponding partial recovery of TSP to  $2.54 \pm 0.14$  g/dL in the lower dose group (Group IV, 100 mg/mL). However, the protective effect was significantly more pronounced at the higher dose of 200 mg/mL (Group V) when the cataract score was reduced to  $1.50 \pm 0.07$  ( $p < 0.01$  vs. cataract control), which was close to, but not the same as, the ascorbic acid reference group, and total soluble protein was restored to  $3.08 \pm 0.16$  g/dL, which was close, but not the same as, both the reference control ( $3.21 \pm 0.20$  g/dL) and the normal control ( $3.25 \pm 0.11$  g/dL). Photographic comparison of representative lenses from each group confirmed the quantitative grading with the lenses from the high-dose HAEPP group demonstrating marked preservation of transparency compared with the densely opacified cataract control lenses, and similar characteristics to the appearance of the reference control lenses.

**Figure 1. *In-vitro* anti-cataract activity of hydro-alcoholic extract of *pennisetumpurpureum***

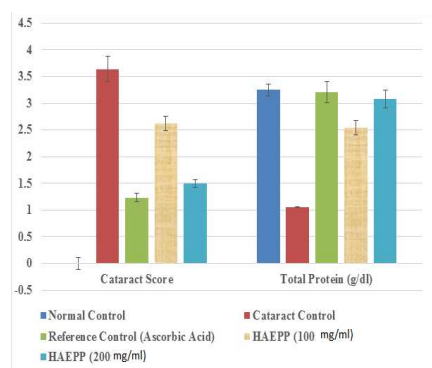


**Figure 2. Photograph of different groups of goat lens**

Normal Induced  
Standard

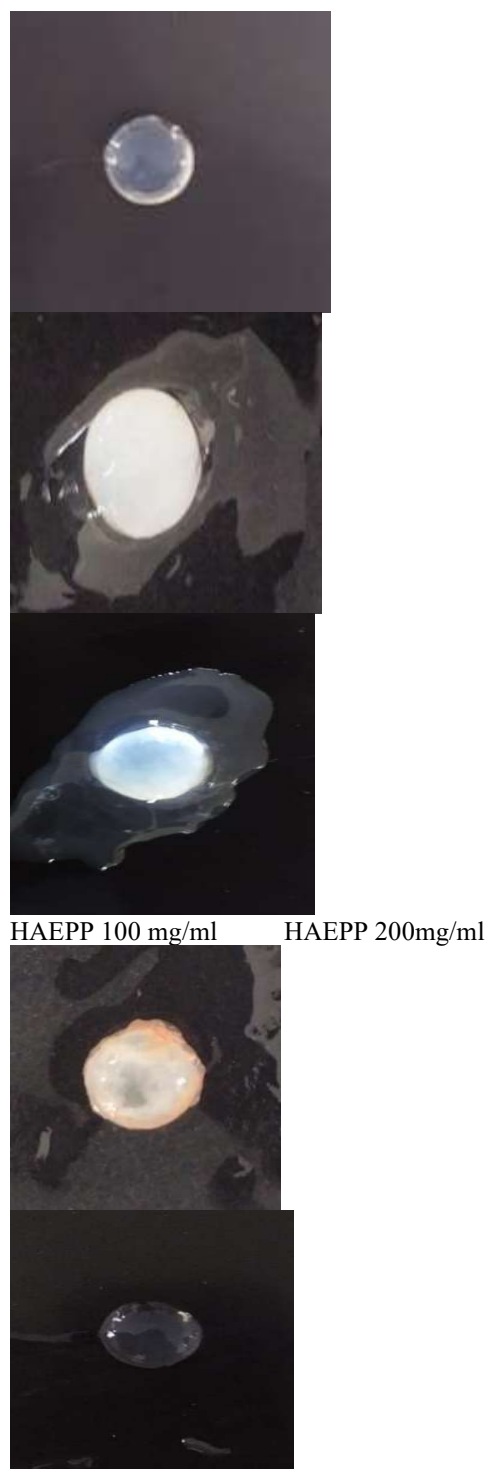


**Figure 2. Photograph of different groups of goat lens**

### 3.6. Mechanistic Basic of The observed anti-cataract activity attribute to the mechanism.

Oxidative stress is one of the major causes of biochemically defined cataract, which is defined as opacification of the lens caused by precipitation of lens natural crystalline proteins that are essential for lens

transparency [32]. Oxidative stress is basically defined as the imbalance between the generation of reactive oxidant species and their enzymatic and non-enzymatic degradation [33] and there is considerable experimental evidence that links ROS and oxidative damage directly to the development of cataract [34,35]. The multifactorial aetiology of cataract formation is well established, and in most experimental and clinical studies, the loss of the antioxidant defence system of the lens is believed to be one of the most important factors in cataract formation. Maintenance of a favourable intracellular redox balance is critical to lens transparency, and is largely dependent on the high native content of reduced glutathione in the lens; loss of this high native content makes it easier for lens glutathione to fail in its role as a radical scavenger, allowing lipid peroxidation of the fibre-cell membranes and subsequent reduction in antioxidant enzyme activity both of which have been directly linked as proximate causes of cataract formation [39,40].

It is known that sodium selenite causes cataract in this way, leading to an oxidative modification of the lipids of the lens membrane and the crystallin proteins, which ultimately leads to the above-mentioned biochemical cascade. As a potent small molecule antioxidant, ascorbic acid is known to block the selenite-induced cataract formation by its antioxidant action, which is likely its primary role in protecting against cataract formation as detected in the present study. It can be inferred by analogy and from the confirmed phytochemical profile of this extract that antioxidants such as the alkaloids, flavonoids and saponins contained in HAEPP could be the most plausible antioxidants responsible for its anti-cataract activity, most probably by direct free-radical scavenging and by subsequent maintenance of the status of the antioxidant enzymes and glutathione in the lens, reducing the amount of crystallin precipitated due to oxidative stress caused by sodium selenite. This proposed mechanism is consistent with and extends to a new botanical source an antioxidant mechanism that has already been established for other antioxidant-rich phytomedicines that have been evaluated in similar selenite-induced cataract models, such as N-acetylcysteine amide [10] and curcumin [11] *Heliotropium indicum* [12] *Cineraria maritima* [29,32] and the polyherbal formulation *Triphala* [30] each of which have been shown to attenuate selenite-induced lens opacification through primarily antioxidant mechanisms.

The protective effect of the higher extract dose (200 mg/mL) was significantly more than that of the lower dose (100 mg/mL) again for both the morphological opacity score and the biochemical protein-recovery endpoint, suggesting that there is a real, dose-

dependent pharmacological effect rather than a non-specific or artefactual effect, and thus further evidence that the antioxidant phytoconstituents present in the extract may be responsible for this activity. The close approximation of the high dose HAEPP group to the ascorbic acid reference group in both endpoints further suggests that, at the appropriate dose, the antioxidant activity of the crude extract may be comparable to that of ascorbic acid, a well characterised reference antioxidant in this ex vivo model, but this should be interpreted with appropriate caution as the crude extract is a multi-constituent product and no data on isolated-constituent or dose-response was found across a broader range of concentrations.

### 3.7 Study Limitations

The present study has several drawbacks to be noted. In addition, the anti-cataract evaluation was limited to an ex vivo isolated goat lens organ-culture model, which is well established for anti-cataract screening and can serve as a model for the core oxidative biochemistry of selenite induced cataract, but cannot fully represent the systemic pharmacokinetic, penetration to the eye, and chronic exposure factors relevant to in vivo or clinical anti-cataract efficacy; therefore, the present findings should be considered as screening-level evidence, and in vivo confirmation is required. Second, the physicochemical (ash value, loss on drying) and extractive yield determination reported here were single determinations on one batch of authenticated plant material, and therefore should not be considered as validated mean values with associated variability, but as indicative. Future work is needed to report the determinations on other independent batches. Third, the phytochemical screening carried out was of qualitative nature, not quantitative and no attempt to isolate marker phytoconstituents or chromatographic fingerprinting was made, which means that the responsible phytoconstituent(s) for the anti-cataract activity cannot be identified. Lastly, only two concentrations of the extract were used (100 and 200 mg/mL), and it is recommended to carry out a broader dose-response curve, together with the formal antioxidant assays such as DPPH or FRAP, that could more accurately correlate the antioxidant activity of the extract with its observed anti-cataract activity.

### 4. CONCLUSION

The current study has, for the first time, shown that the hydro-alcoholic extract of *Pennisetum purpureum* (*Cenchrus purpureus*) has appreciable dose-dependent anti-cataract activity in both the endpoints of the validated sodium selenite induced isolated goat lens model in vitro, and the higher dose concentration (200 mg/mL) showed a significant decrease in the lens opacity score and restoration of the total soluble protein of the lens, and approached the efficacy of the

ascorbic acid reference standard in both of the examined endpoints. Preliminary phytochemical screening confirmed the extract to contain high levels of tannins, saponins, flavonoids, alkaloids, carbohydrates and triterpenoids, known phytochemical classes with antioxidant activity and the protective effect was attributed, most plausibly, to the ability of these constituents to interrupt the oxidative cascade, production of Reactive Oxygen Species, glutathione depletion, lipid peroxidation and crystallin precipitation in the selenite-induced cataract model. The results indicate that *Pennisetum purpureum* is a new and promising grass species with pharmacological potential against cataract development and is among the species that have not been explored for anti-cataract phytoconstituents, thus suggesting a rationale for in-depth bio-activity guided fractionation, quantitative antioxidant profile and in vivo pharmacological investigation to isolate and characterise the responsible phytoconstituent(s) for this activity.

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