



Research article

## Study of anticataract activity of *Sesbania grandiflora* Linn & *Mentha arvensis* Linn.

Singh Amarjeet<sup>1\*</sup>, Sharma Pankaj<sup>2</sup>, Lamba H.S<sup>3</sup>., Lamba Avneet Kaur<sup>4</sup>

<sup>1</sup>Innovative College of Pharmacy, Gr. Noida U.P.

<sup>2</sup>Jaipur National University, Jaipur.

<sup>3</sup>H.R. Institute of Pharmacy, Ghaziabad.

<sup>4</sup>B.S. Anangpuria Institute of Pharmacy, Faridabad.

### Abstract

Cataract - opacification of the lens - is closely related to Diabetes, Unhealthy diet, Aging as one of its major late complications. This study deals with three molecular mechanisms that may be involved in the development of cataract, non-enzymatic glycation of eye lens proteins, oxidative stress, and activated polyol pathway in glucose disposition. The natural nutrients and antioxidants such as *Sesbania grandiflora* Linn & *Mentha arvensis* Linn. was subjected to prevent cataract formation in vitro on glucose induced cataract model. The goat lenses were incubated in artificial aqueous humor containing 55 mM glucose with *Sesbania grandiflora* Linn & *Mentha arvensis* Linn extracts separately at different dose levels (75 mg/ml, 150 mg/ml and 250 mg/ml) and (250 mg/ml, 500 mg/ml and 750 mg/ml) at room temperature for 72 hrs. Evaluation was done by using biochemical parameters like Estimation of Total Protein Content, Estimation of Malondialdehyde (MDA) Level, Estimation of Glutathione (GSH) Level, Estimation of Lipid Peroxidase Level. The glucose induced opacification of goat lens was also studied as a part of visual evaluation. The data suggest that ethanolic extract of *Sesbania grandiflora* Linn (EESG), *Mentha arvensis* Linn (EEMA) and standard drug (Catlon) are able to significantly retard experimental glucose induced cataractogenesis.

**Key words:** Cataractogenesis, MDA, GSH and Protein estimation, EESG, EEMA, Polyol pathway.

**\*Corresponding Author:** Singh Amarjeet, Innovative College of Pharmacy, Gr. Noida U.P.

### Introduction

Cataract is a disease causing clouding of the eye lens that reduces the amount of incoming light and resulting in deteriorating vision. Cataract is often described as being similar to looking through a waterfall or waxed paper. Cataract is one of the most ancient diseases known being an age- related disorder. It is for

this reason that there is no medical solution or cure for this affliction [1]. Mamtigate the problem have thus been revolving around preventive measures and towards delaying its onset or progression. Considerable research has been done on the complex aspects of this disease. Important leads have come from

studies on the epidemiological, nutritional, photochemical and genetic aspects of cataract. In addition, the role of behavioral habits such as smoking, alcohol intake, or drug addiction has also been investigated. During the past fifteen years, a number of cross sectional surveys have provided data on the prevalence of cataract, which indicate that cataract is by far the most common cause of blindness and visual disability worldwide[7]. Cataract can include cases of family/genetic origin, or secondary to trauma, systemic diseases, drugs and age-related factors. Senile or age-related cataract is responsible for more than 80% of the total number of cataract cases[20]. The proportion of cataract patients with diabetes has been found to range from 8.7 to 21%, which shows a high risk of cataract associated with diabetes[13].

Although cataracts can be surgically removed, in many countries surgical services are inadequate or do not produce equal outcomes, and cataract remains the leading cause of blindness. Preventive interventions must be identified, perfected and delivered, through research. The challenges are to prevent or delay cataract formation, and cure that which does occur. There are many herbal preparations, vitamin supplementations and drugs used to prevent cataractogenesis and reverse cataract [6]. Oxidative mechanism plays an important role in biological phenomena including cataract formation. The formation of superoxide radicals in the aqueous humor and in lens, lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to formation of cataract [2].

In the present study, investigation of lens opacity was carried out to differentiate the control and experimental lenses. Incubation with glucose in high concentration results in various biochemical changes leading to formation of cataract. However the groups incubated with (EESG & AESG) and (EEMA &

AEMA) have reversed the lens opacity which is almost similar to the standard drug, Catlon. The prevention of glucose-induced cataractogenesis by the extract of *Sesbania grandiflora*( L.) leaves and the extract of *Mentha arvensis* (L.) leaves . This effect is associated with increased GSH, decreased MDA levels, LH Levels and maintaining of protein level, mineral homeostasis and increased activities.

## **Materials and methods**

### **Collection and Authentication of plant**

The plant *Mentha arvensis* L. was collected from botanical garden of Innovative college of pharmacy, Greater Noida and authenticated by Dr. K.C. Bhatt, Principal Scientist at National Bureau of Plant Genetic Resources (ICAR) New Delhi. A voucher specimen no. NHCP/NBPGR /2014-14 is deposited in the herbarium.

The second plant of my research *Sesbania grandiflora* L. was collected from the field of Dadari, Gautham Budh Nagar and authenticated by Dr. K. Pradheep, Senior Scientist at National Bureau of Plant Genetic Resources (ICAR) New Delhi. A voucher specimen no. NHCP/NBPGR /2014-24 is deposited in the herbarium.

### **Extractions of Plant Material**

#### **Extraction of *Sesbania grandiflora*( L.) leaves with various solvent**

About 500 g of dried powder leaves of *Sesbania grandiflora* plant was subjected to Soxhlet apparatus. It was first defatted with petroleum ether then exhaustively extracted with ethanol and aqueous in a Soxhlet apparatus for 36 hours. The temperature was maintained at (40-500 C). The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract [10].

#### **Extraction of *Mentha arvensis* (L.) leaves with various solvent**

The leaves of *Mentha arvensis* were collected and shade dried. The dried leaves were coarse powdered and the powder was packed in to soxhlet column and extracted successively with petroleum ether (60 – 80°C), ethanol (64.5 – 65.5°C) and distilled water. The extracts were concentrated under reduced pressure (bath tem. 50°C). The dried extracts were stored in airtight container in refrigerator.

**Eye Balls:** Goat eye balls were used in the present study. They were obtained from the local slaughter house, immediately after slaughter and transported to laboratory at 0-4 degree Celsius.

### Pharmacological screening

All the experimental procedures and protocols were approved by the Institutional Animal Ethics Committee (IAEC), reference no. 1346/c/10/CPCSEA provided to Innovative college of Pharmacy and were in accordance with the guidelines of the IAEC.

### Preliminary Phytochemical Screening of *Sesbania grandiflora* L.

The phyto-chemical screening of three extracts (petroleum ether, ethanol and aqueous) of leaves confirmed the presence of various constituents such as alkaloids, steroids, volatile oils, tannins, saponins, flavonoids, etc. However, flavonoids and phenolic compounds were found in almost all the extracts.

### Preliminary Phytochemical Screening *M.arvensis* L.

It was found that petroleum ether extract contained steroids, fat and fixed oils, aqueous extract contained carbohydrates, amino acids, steroids, flavonoid, alkaloids, glycosides and tannins, ethanolic extract also showed tannins, steroids and flavanoids.

### Removal of tannins

The crude ethanolic and aqueous extract (2 g) of *Sesbania grandiflora* and *Mentha arvensis*

separately dissolved in 20 mL of ethanol was applied on a column (5 × 40 cm) packed with Sephadex LH-20 gel. Ethanol (1L), used as first eluent, allowed removing low molecular weight phenolic compounds. Then 600 mL of 50% acetone (v/v) was used to elute tannins. Solvent from tannin fractions was removed using rotary evaporator, and water was removed during lyophilisation. After the removal of tannins from both the ethanolic and aqueous extract of *Sesbania grandiflora*, and *Mentha arvensis*, it is used for the preparation of dosage form which was used for pharmacological screening [16].

**Preparation of Lens Culture:** The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl - 140 mM, KCl - 5 mM, MgCl<sub>2</sub> - 2 mM, NaHCO<sub>3</sub> - 0.5 mM, NaH (PO<sub>4</sub>)<sub>2</sub> - 0.5 mM, CaCl<sub>2</sub> - 0.4 mM and Glucose 5.5mM) at room temperature and pH 7.8 for 72 hrs. Gentamycin or Streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Galactose in a concentration of 55 mM is used to induce cataract.

### Inducer and Treating Groups:

#### Group of *Sesbania grandiflora* extract

Group I - Artificial aqueous humor alone (Normal control)

Group II - Glucose 55 mM alone (Negative control)

Group III- Glucose 55 mM +PESG (75 mg/ml)

Group IV- Glucose 55 mM +EESG (150 mg/ml)

Group V- Glucose 55 mM +AESG (250 mg/ml)

Group VI: Glucose 55 mM + Catlon, Gentamycin (Standard Drug)

#### Group of *Mentha arvensis* extract

Group I- Artificial aqueous humor alone (Normal control)

Group II- Glucose 55 mM alone (Negative control)

Group III-Glucose 55 mM + PEMA (250 mg/ml)

Group IV- Glucose 55 mM + EEMA (500 mg/ml)

Group V- Glucose 55 mM + AEMA (750 mg/ml)

Group VI : Glucose 55 mM + Catlon, Gentamycin

**Homogenate preparation:** After 72 hrs of incubation, homogenate of lenses were prepared in Tris buffer (0.23M, pH 7.8) containing  $0.25 \times 10^{-3}$  sub MEDTA and homogenate adjusted to 10 % w/v. The homogenate was centrifuged at 10,000 G at 4°C for 1 hour and the supernatant used for estimation of biochemical parameters. For estimation of water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH 7.4).

### Photographic Evaluation

Lenses were placed on a graph paper with posterior surface touching the graph paper, and the pattern of graph square (number of square clearly visible through the lens) was observed through the lens that show the opacity of the lens. (shown in Figure 1 & 2)



Figure 1. Lenses showing the transparency of visibility



Figure 2. Lens become opaque after adding the inducer

**Statistical Analysis:** The data was presented as mean  $\pm$  SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc- using Graph Pad Prism software.

### Biochemical estimation:

#### Estimation of total protein content

To 0.1 ml of lens homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 min for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as  $\mu\text{g}/\text{mg}$  lens tissue [16].

#### Estimation of malondialdehyde (MDA)

Lenses were homogenized in 10% (w/v) 0.1 M Tris-HCl buffer (pH 7.5). One ml of the homogenate was combined with 2 ml of TCA-TBA-HCl reagent (15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl) and boiled for 15 min. Precipitate was removed after cooling by centrifugation at 1000g for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The levels of MDA were calculated using extinction coefficient calculation. The values are expressed as nmoles of MDA/ min/mg lens protein [20].

#### Reduced glutathione (GSH)

The GSH content was estimated by the method of Moron *et al* . Half of the lenses from each group were weighed and homogenized in 1 ml of 5% trichloroacetic acid (TCA), and a clear supernatant was obtained by centrifugation at 5000 rpm for 15 min. To 0.5 ml of this supernatant, 4.0 ml of 0.3 M disodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) and 0.5 ml of 0.6 mM 5, 5'- dithiobis-2-nitrobenzoic acid in 1% tri-sodium citrate were added in succession. The intensity of the resulting yellow color was read

spectrophotometrically at 410 nm. Reduced GSH was used as a standard.

### Estimation of lipid hydroperoxides (LH)

About 0.1 ml of lens homogenate was treated with 0.9 ml of Fox reagent (49 mg of ferrous ammonium sulfate in 50 mL of 250 mM H<sub>2</sub>SO<sub>4</sub>, 0.397 g of butylated hydroxyl toluene, and 38

mg of xylenol orange in 950 mL of methanol) and incubated for 3 min. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/ mg lens protein

### Observation on Goat lenses for anticataract activity

**Table 1. Effect of the extract of *Sesbania grandiflora* L. on Goat lenses for anticataract activity.**

S No.	Treatment	Treatment ( Glucose 55 mM +LCS)	Time of Opacity (Top) in Hrs.
1	Control	( Glucose 55 mM +LCS)	17±2
2	Ethanollic ext.of SG(EESG)	( Glucose 55 mM +LCS)	42±3*
3	Aqueous ext.of SG(AESG)	( Glucose 55 mM +LCS)	45±2
4	Standard drug	( Glucose 55 mM +LCS)	46±3*

**Table 2. Effect of the extract of *Mentha arvensis* L. on Goat lenses for anticataract activity.**

S No.	Treatment	Treatment ( Glucose 55 mM +LCS)	Time of Opacity (Top) in Hrs.
1.	Control	( Glucose 55 mM +LCS)	18±2
2.	Ethanollic ext.of MA(EEMA)	( Glucose 55 mM +LCS)	43±3*
3.	Aqueous ext.of MA(AEMA)	( Glucose 55 mM +LCS)	44±2
4.	Standard drug	( Glucose 55 mM +LCS)	46±3*

Each values represents mean ± S.E. n=6; \*\*Represents statistical significance vs. control (p < 0.01).

**Table 3. Effects of PESG (75 mg/ml) EESG (150 mg/ml),AESG (250 mg/ml) and Standard group on GSH, MDA and Protein estimation in Glucose 55 mM induced cataract.**

Study Group	MDA (nmol/mg)	GSH (µmol/g)	Total Protein	Lipidhydroperoxidase
Control (Gr.I)	4.65±2.13	0.15±0.05	16±1.87	3.44±0.37
Inducer (Gr.II)	5.26±0.54	0.28±0.02	10.04±1.56	8.13±0.85
PESG (Gr. III)	1.65±0.02	0.33±0.01	11.03±1.08	3.84±0.26
EESG (Gr. IV)	1.52±0.02*	0.48±0.01*	14±1.92*	3.50±0.22*
AESG(Gr.V)	1.87±0.02	0.38±0.01	12.06±1.56	3.55±0.73
Standard (Gr. VI)	2.92±0.01	0.53±0.02	13.30±1.23	3.57±0.28

### Effects on lens morphology

All the lenses in artificial aqueous humor alone were transparent. However, lenses after 72 hrs of incubation in the presence of 55 mM concentration of glucose developed dense opacity. *Sesbania grandiflora* (ethanollic extract) was found to afford significant, concentration dependent protection against 55 mM concentration of glucose that damages the goat lenses. *Sesbania grandiflora* extract 150 mg/ml

prevented opacity from 21 to 42 hrs as compared to control, which become opaque in 17 hrs, where as ethanollic extract of *Mentha Arvensis* prevented opacity for 43 hrs and standard drug (Catlon + Gentamycin) prevented opacity for 46 hrs. [Table 1 & 2].

### Observation of *Sesbania grandiflora* (leaves) Graphical represent of GSH, MDA, LH, Total Protein Level of *Sesbania grandiflora*

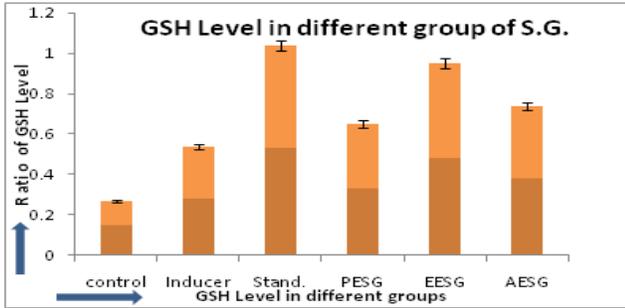


Figure 3. GSH Level in different group of S.G.

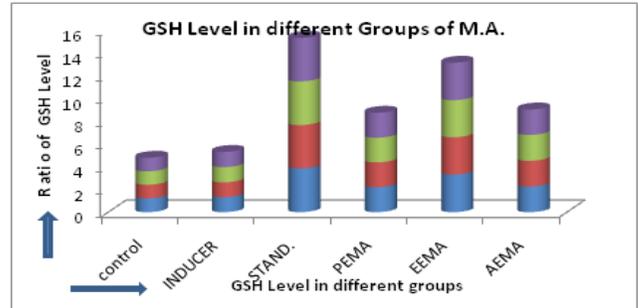


Figure 7. GSH Level in different group of M.A.

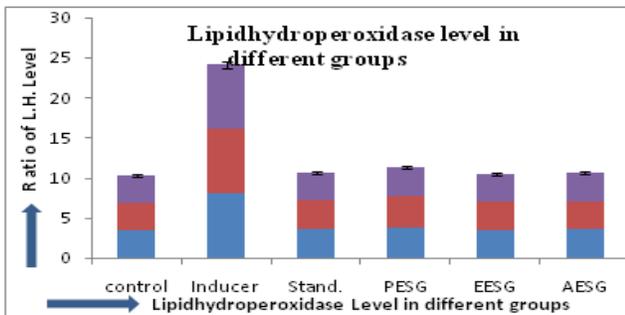


Figure 4. LH Level in different group of S.G.

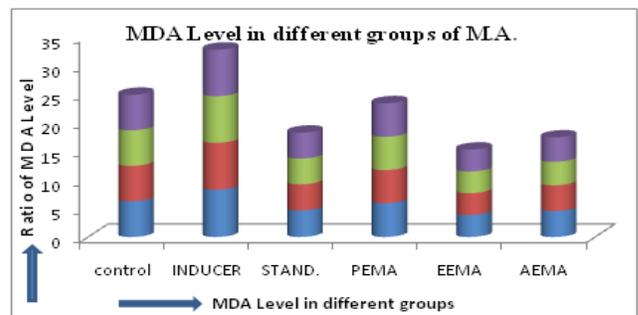


Figure 8. MDA Level in different group of M.A.

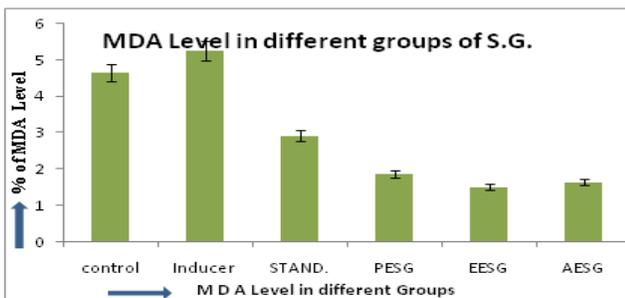


Figure 5. MDA Level in different group of S.G.

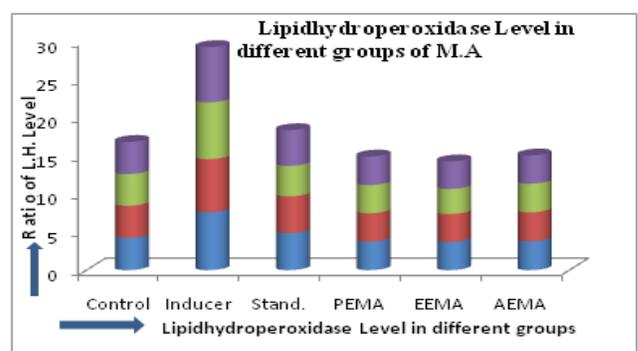


Figure 9. LH Level in different group of M.A.

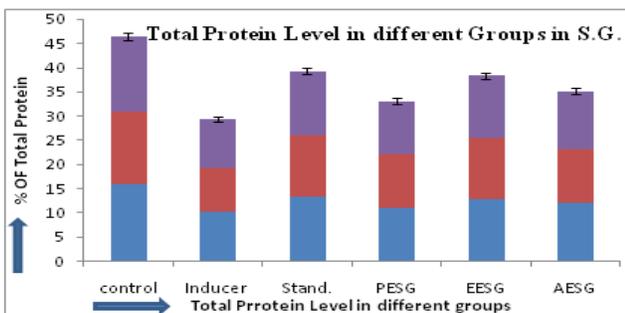


Figure 6. Total Protein Level in different group of S.G.

Observation of *Mentha Arvensis* (leaves)  
Graphical represent of GSH, MDA, LH, Total Protein Level of *Mentha arvensis*

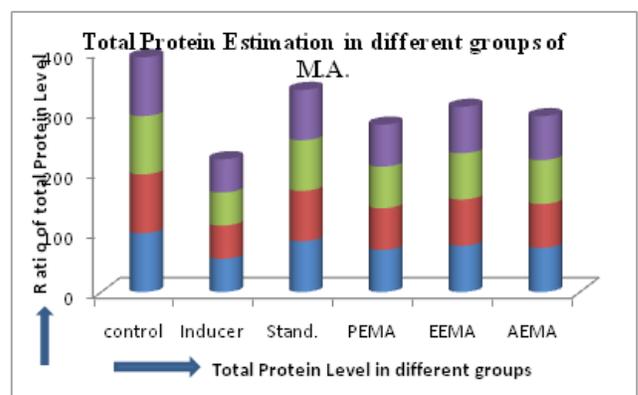


Figure 10. Total Protein Level in different group of M.A.

**Table 4. Effects of PEMA (250 mg/ml) EEMA (500 mg/ml), AEMA (750 mg/ml) and Standard group on GSH, MDA and Protein estimation in Glucose 55 mM induced cataract.**

Study Group	MDA(nmol/mg)	GSH ( $\mu$ mol/g)	Total Protein (mg/g)	Lipidhydroperoxidase (nmol/mg)
Control (Gr.I)	6.23 $\pm$ 0.64	1.21 $\pm$ 0.06	97.82 $\pm$ 2.12	4.24 $\pm$ 0.35
Inducer (Gr.II)	8.25 $\pm$ 0.56	1.32 $\pm$ 0.06	55.32 $\pm$ 2.65	7.56 $\pm$ 0.65
PEMA (Gr. III)	5.89 $\pm$ 0.54	2.20 $\pm$ 0.05	70.12 $\pm$ 2.34	3.74 $\pm$ 0.28
EEMA (Gr. IV)	3.85 $\pm$ 0.14*	3.30 $\pm$ 0.08*	77.00 $\pm$ 3.75*	3.69 $\pm$ 0.73*
AEMA(Gr.V)	4.52 $\pm$ 0.45	2.25 $\pm$ 0.12	73.35 $\pm$ 2.54	3.78 $\pm$ 0.26
Standard (Gr. VI)	4.65 $\pm$ 0.48	3.85 $\pm$ 0.14	84.45 $\pm$ 3.15	3.85 $\pm$ 0.25

## Result and Discussion

### Results

Incubation of lenses with glucose 55 mM showed opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 72 hrs (as shown in Table 1&2). Glucose 55mM treated lenses (Group-II) also showed significantly low concentrations of proteins (total proteins) in the lens homogenate ( $P < 0.01$ ), very high concentration of MDA ( $P < 0.01$ ) and high concentration of lipid hydro peroxidase ( $P < 0.01$ ) compared with normal lenses (Group-I). Catlon treated lenses (Group VI) and Lenses treated with *Sesbania grandiflora* extract [(EESG) & (AESG)] as shown in Table 3 and *Mentha Arvensis* extract [(EEMA)&(AEMA)] Group-IV&V showed higher concentrations of glutathione, proteins (total proteins) ( $P < 0.01$ ), lower concentration of lipid hydro peroxidase ( $P < 0.01$ ) and lower concentration of MDA ( $P < 0.01$ ) compared with Glucose 55 mM treated lenses (Group-II) as shown in Table 4. The comparison has been done in both plants for the observation of significant dose i.e. *Sesbania grandiflora* extract (EESG) 150 mg/ml prevented opacity from 21 to 42 hrs as compared to control and *Mentha arvensis* extract (EEMA) 500 mg/ml prevented opacity from 21 to 43 hrs as compared to control and standard drug (Catlon + Gentamycin) prevented opacity for 46 hrs. The result of the test drugs

(EESG & EEMA) are approximately similar to the result of the standard drug.

### Discussion

Prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism and long-term irreversible changes in stable macromolecules. Non enzymatic glycation, oxidative stress and polyol pathway are the possible mechanisms by which high glucose concentrations induce and accelerate lens opacification leading to cataract formation [2]. High concentrations of glucose contribute to oxidative stress by generating more reactive oxygen species at the mitochondrial level owing to increased intracellular glucose metabolism. Increased reactive oxygen species initiates polyol pathway by stimulating aldose reductase resulting in high levels of sorbitol. Sorbitol does not easily cross cell membranes and accumulates in cells causing damage by disturbing osmotic homeostasis. This intralenticular accumulation of polyols is a major factor in acute models of sugar cataract [17].

The parameters commonly considered in cataractogenesis are Glutathion (GSH), Malondialdehyde (MDA), lipidhydro peroxidase and Proteins (total proteins)[18]. In this study MDA levels, lipidhydro peroxidase levels were significantly higher in Glucose 55 mM treated Group, compared with normal lenses Group. The MDA levels, lipid hydro peroxidase levels were significantly lower in Catlon and *Sesbania grandiflora L.* extract (EESG)& *Mentha arvensis*

L.extract (EEMA) treated groups. Catlon and (EESG) & (EEMA) extract treated groups have also been shown to increase the content of Glutathione level and total proteins, retarding the process of cataractogenesis initiated by high glucose concentration. This proves that *Sesbania grandiflora L.* extract (EESG) & *Mentha arvensis L.* extract (EEMA) delay the cataract formation through its anti-oxidant and natural nutrients properties.

## Conclusion

The present study has depicted that the *Sesbania grandiflora L.* (leaves) and *Mentha arvensis L.* (leaves) extract offers protection from cataract induction by reducing lens protein insolubilization and lens peroxidation and by increasing lens antioxidant status. Consequently, a reduction in lens apoptosis and epithelial proliferation occurred. The study also reports that administration of the extracts after the onset of cataract might be helpful in preventing or slowing the progression of cataract.

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