

**Short Communication**

**IN-VITRO ANTICATARACT ACTIVITY OF *HIBISCUS ROSA-SINENSIS* LINN ON GOAT LENS**

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**ABSTRACT**

**Objectives:** To study the effects of ethanolic extract of leaves of *Hibiscus rosa-sinensis* Linn (EEHRS) on glucose induced cataract in an *in vitro* model of goat lens.

**Methods:** Ethanolic extracts of leaves of *Hibiscus rosa-sinensis* Linn (EEHRS) were prepared by Percolation method. Goat lenses, obtained from a local slaughterhouse were made in 6 groups with 5 lenses in each group. They were incubated in artificial aqueous humor for 72 hrs at room temperature with 5.5 mM glucose (negative control group), 55 mM glucose (cataractogenesis group), enalapril (standard drug group) and 3 dosage of EEHRS (1 mg, 1.5 mg, 2 mg) with 55 mM glucose (test drug groups). Opacification of lens was assessed by counting the number of clear squares when placed over a graph paper. Parameters studied were catalase and superoxide dismutase (SOD) activities, tissue Malondialdehyde (MDA) and total and water soluble protein in the lens homogenate. All the data relating to biochemical parameters were compared using one way ANOVA followed by Bonferroni multiple comparison tests.

**Results:** Glucose induced opacification of lens was started 10-12 hours post incubation & was completely opacified in 72 hrs. Lens treated with the EEHRS at concentrations of 1 mg, 1.5 mg and 2 mg showed significantly ( $p < 0.05$ ) decreased opacity and decreased tissue MDA level, increased catalase and SOD activities and increased total protein and water soluble protein levels respectively compared to the positive control.

**Conclusion:** Hence the study suggested that EEHRS possesses significant anticataract activity which can be attributed to its antioxidant property.

**Keywords:** Cataract, Antioxidant activities, Anticataract activity.

Any opacity of the lens or its capsule whether developmental or acquired is called a cataract. Cataract is caused by degeneration & opacification of the lens fibre already formed, the formation of aberrant lens fibre or deposition of other material in their place [1]. WHO estimates that around 285 million people in the world are visually impaired out of which 90.5 million of them are from the South-East Asian Region. Of the estimated 39 million blind people in the world, 90% are in developing countries; 22% in India alone. Therefore, the burden of blindness is largely in developing countries where 9 out of 10 of the world's blind live. Cataract alone is the cause of approximately 50% of the world's blindness [2].

Though the exact cause of cataract is not known, it is often associated with old age. It is also a major complication of diabetes mellitus. Other risk factors associated are loss of antioxidant mechanisms of lens, smoking, corticosteroids etc. Though the biochemical background of cataractogenesis is still unknown, three biochemical factors are evident in the process of cataract formation; *hydration* may be due to osmotic changes within the lens or due to changes in the semi permeability of the capsule. Second is *denaturation* of the lens protein with an increase in insoluble protein and the third is *slow sclerosis*. Normal lens contains sulphydryl containing reduced glutathione and ascorbic acid as antioxidants. With increase in age this antioxidative mechanism becomes less effective and there is increase in inactive insoluble proteins and semi permeability of the lens capsule which may lead to cataract formation [1].

Surgical remedy remains the only effective treatment of cataract till date. Although a number of agents have been tried for prevention and treatment of cataract but none have proved to be useful [3]. Hence if a drug is sought which can effectively prevent lenticular opacification, it will be of great advance in the treatment of cataract.

There is a widespread belief that natural products are less toxic when compared to pure chemicals. Recent data suggest that 80% of drug molecules are natural products or natural compound inspired [4]. *Hibiscus rosa sinensis* Linn. (commonly known as china rose) is an evergreen shrub native to the tropical and subtropical regions of the world including India [5]. Hibiscus is traditionally used for the treatment

of hypertension, fever, menstrual irregularities (stimulates menstruation), headache, arthritis, cough and as analgesic [6, 7]. Hibiscus tea is rich in vitamin C [6] and contains several flavonoids which have antioxidant properties [8]. Hence in the present study we had undertaken *H. rosa sinensis* Linn. Leaves extracts. Angiotensin Converting Enzyme (ACE) inhibitors have been found to afford protection from free radicals induced damage in many experimental conditions. Enalapril (ACE Inhibitor) was shown to have potent anticataract activity *in vitro* due to antioxidant and free radical scavenging activity [9]. Hence we had taken enalapril as the standard drug.

Fresh *Hibiscus rosa sinensis* Linn. leaves were collected from nearby localities of Assam Medical College campus, Dibrugarh, Assam and was authenticated by Dr. L. R. Saikia, Prof. of dept of Life Sciences, Dibrugarh University bearing V No. DUL. Sc. 462. Enalapril, Penicillin and Streptomycin; chemicals like thiobarbituric acid (TBA), EDTA, triss buffer and other chemicals of analytical grade were bought from Surgical Mart, Dibrugarh. Ethanolic extract of leaves of *H. rosa-sinensis* Linn. was prepared by percolation method [10].

Goat eyeballs used in the study were obtained from a local slaughter house immediately after slaughter and transported to the laboratory at 0-4°C. Lenses were extracted by extra capsular lens extraction. After extraction of the goat lens it was 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<sub>2</sub>PO<sub>4</sub>-0.5 mM, CaCl<sub>2</sub>-0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 and cultured for 72 hours. To prevent bacterial contamination, Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media [11]. Glucose in a concentration of 55 mM was used to induce cataract [12].

The standard drug (enalapril) was taken in the concentration of 5ng/ml. And the EEHRS was taken into three doses, 1 mg, 1.5 mg and 2 mg respectively. A total of 60 lenses were divided into following categories (n=10 in each category):

GROUP A: Lens Culture+Glucose 5.5 mM (negative control)

GROUP B: Lens Culture+Glucose 55 mM (positive control/cataractogenesis).

GROUP C: Lens Culture+Glucose 55 mM+Enalapril 5ng/ml (standard drug group)

GROUP D: Lens Culture+Glucose 55 mM+1 mg EEHRS

GROUP E: Lens Culture+Glucose 55 mM+1.5 mg EEHRS

GROUP F: Lens Culture+Glucose 55 mM+2 mg EEHRS

After 72 hours of incubation, visual evaluation was done by placing the lens on a graph paper and counting the no. of squares visible through the lenses as a measure of lens opacity. 10% W/V homogenate of the lenses were prepared in Tris buffer (0.23 mm, pH 7.4) containing 0.25 mm EDTA and centrifuged at 10,000 g at 4°C for 60 minutes and supernatant was used for estimating tissue catalase and superoxide dismutase (SOD) activities, malondialdehyde (MDA) and total protein. For estimation of water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH 7.4) [13]. Catalase [14] and SOD activities [15] were estimated by using spectrophotometry. MDA by TBARS method [16] and total protein and water soluble protein was estimated by Lowry's method [17]. The data were expressed as mean±SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison tests using Graph Pad Prism software, version 5.

After 72 hours of incubation, transparency was maintained in the normal control group (fig. 1) but there was complete loss of transparency in the positive control group (fig. 2) indicating complete cataractogenesis. Goat lenses of groups containing escalated doses of the EEHRS (Group D, E, F) were less hazy and the squares of the graph paper were visible through the lenses indicating suppression of cataract formation (fig. 4, 5, 6). Group F (containing 2 mg EEHRS) was more effective in suppressing cataract formation (fig. 6) than Group D (fig. 4) and Group E (fig. 5). Lens homogenate of Group B lenses showed significantly lower ( $p<0.0001$ ) levels of catalase and SOD activities and higher levels of MDA ( $p<0.0001$ ) than that of Group A lenses. Total and water soluble protein was also significantly lower in Group B lens homogenate than the Group A lenses ( $p<0.0001$ ). Group D, E, F containing 1 mg, 1.5 mg and 2 mg EEHRS respectively showed significant ( $p<0.001$ ) increase in catalase and SOD activities and total and water soluble protein in the lens homogenate than Group B. There were also significant reduction in the MDA levels in the test groups (Group D, E, F) compared to Group B ( $p<0.001$ ). Group F was more efficient in suppressing the oxidative stress as the catalase activity was significantly higher ( $p<0.05$ ) than Group D and E. Group F also showed significantly higher total protein ( $p<0.001$ ), water soluble protein ( $p<0.05$ ) and lower MDA level ( $p<0.05$ ) than Group D. However, there was no significant difference in SOD activities in groups D, E and F. All the biochemical parameters of Group F were comparable to the standard drug (enalapril) group (Group C).

**Table 1: Shows the effects of EEHRS on biochemical and oxidative parameters in the lens homogenate after 72 hours of incubation**

| GROUP   | SOD activity (unit/mg of protein) | Catalase activity ( $\mu\text{mol}/\text{min}/\text{mg}$ ) | MDA level (nmol/gm)       | Total protein (mg/gm)      | Water soluble protein (mg/gm) |
|---------|-----------------------------------|--|---------------------------|----------------------------|-------------------------------|
| Group A | 4.44±0.21                         | 31.32±0.58   | 7.37±0.32                 | 183.7±2.14                 | 79.56±2.56                    |
| Group B | 1.98±0.28 <sup>a</sup>            | 8.56±0.27 <sup>a</sup>                                     | 17.59±0.44 <sup>a</sup>   | 139.2±2.83 <sup>a</sup>    | 49.14±1.53 <sup>a</sup>       |
| Group C | 3.83±0.13                         | 25.18±0.42   | 11.16±0.50                | 170.5±0.91                 | 72.16±1.33                    |
| Group D | 3.09±0.04 <sup>b</sup>            | 17.32±0.44 <sup>b</sup>                                    | 13.44±0.36 <sup>b</sup>   | 156.2±0.88 <sup>b</sup>    | 64.04±1.53 <sup>b</sup>       |
| Group E | 3.76±0.06 <sup>b</sup>            | 20.36±0.29 <sup>b</sup>                                    | 13.15±0.46 <sup>b</sup>   | 162.7±0.43 <sup>b</sup>    | 65.76±0.91 <sup>b</sup>       |
| Group F | 3.62±0.15 <sup>b</sup>            | 24.02±0.25 <sup>b,*</sup>                                  | 11.34±0.33 <sup>b,*</sup> | 165.9±0.31 <sup>b,**</sup> | 72.30±1.75 <sup>b,*</sup>     |

Mg, milligram;  $\mu\text{mol}/\text{min}/\text{mg}$ , micromoles/minute/milligram; nmol/gm, nanomoles/gram; mg/gm, milligrams/gram. The values were stated as mean±SEM. <sup>a</sup> represents  $p<0.0001$  when compared to the negative control group (Group A). <sup>b</sup> represents  $p<0.001$  when compared with the positive control group (Group B). \*and \*\* represents  $p<0.05$  and  $p<0.001$  when compared to Group D.

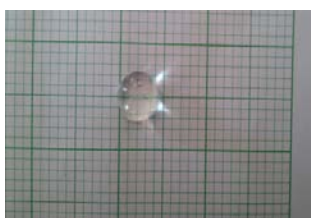
#### Photographic evaluation



**Fig 1: Normal control (group A) showed transparent lens.**



**Fig 2: Positive control (Group B) showed complete cataractogenesis**



**Fig. 3: Standard drug group (Group C)**



**Fig. 4: Group D**



**Fig 5: Group E**



**Fig 6: Group F**

Group C, D, E, F showed suppression of cataract formation after 72 hours of incubation in high concentration glucose solution.

The parameters considered in this study were total protein, water soluble protein and oxidative stress markers such as tissue MDA level, catalase activity and SOD activity. Incubation of goat lens in media containing 55 mm glucose concentration had shown a decrease in total protein in general and water soluble protein in particular in the lens homogenate. This finding is in agreement with previous studies [11, 13]. Alteration of Na<sup>+</sup>-K<sup>+</sup> ratio due to reduction in Na<sup>+</sup>-K<sup>+</sup>ATPase activity in the lens causes hydration and swelling of the lens [18] and alteration of the protein content [13]. Both of these contribute to the formation of cataract<sup>(1)</sup>. This study showed higher total and water soluble protein in Enalapril (Group C) and *H. rosa sinensis* extract treated groups (Group D, E, F). Therefore Enalapril and *H. rosa sinensis* extract seem to prevent pathogenesis of cataract formation. This may be due to their free radical scavenging activity.

Loss of lens's antioxidant mechanisms is one of the important risk factors for cataractogenesis induced by high glucose concentration due to the formation of free radicals like superoxide ion (O<sub>2</sub><sup>-</sup>) and H<sub>2</sub>O<sub>2</sub> [1, 13]. High glucose had shown to induce antioxidant enzyme suggesting oxidative stress in the cell [19]. In the present study MDA level was significantly raised and catalase and SOD activities were significantly reduced in the lens culture containing 55 mm glucose compared to the normal lens (5.5 mM). Lens culture treated with Enalapril and different doses of *H. rosa sinensis* extract had shown a significant increase in catalase and SOD activities and reduction in MDA level compared to the Group B lens culture. This shows a significant increase in antioxidant activities in the treated groups. This also helps in retarding the process of cataractogenesis induced by high glucose concentration. The present study shows EEHRS possesses anticataract activity due to the presence of antioxidant activities which might be helpful in preventing and/or slowing cataract formation and hence the present study opened avenues for the treatment of cataract from *Hibiscus rosa-sinensis* Linn. However, further study is needed for identification of the lead compound.

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