International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-3, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Evaluation of Anti-Cataract Activity of *Asparagus Racemosus* Root Extract Using *In-Vitro* Model of Goat Lens

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Manuscript No: IJPRS/V2/I3/00125, Received On: 15/07/2013, Accepted On: 22/07/2013

ABSTRACT

The aim of the present work to evaluate the anticataract activity of aqueous extract of *Asparagus racemosus* root using in vitro model of goat lens. In the in vitro study, goat lenses were incubated in artificial aqueous humor containing 55 mM glucose (cataractogenesis) with aqueous extract of *Asparagus racemosus* root (AEAR) at different concentrations of 250 µg/ml and 500µg/ml at room temperature for 72 hours. Biochemical parameters studied in the lens were electrolytes (Na^+, Ca^+, and K^+), total proteins, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and Catalase. Photographic evaluation was also done. AEAR significantly prevented the glucose induced changes in biochemical parameters like sodium, calcium, and potassium, total proteins, MDA, SOD, GSH and Catalase. Photographic evaluation also indicated that AEAR prevented the opacity of the lens compared to model control group in vitro. These results suggest that prevention of cataract by aqueous extract of *Asparagus racemosus* root may be through a mechanism involving free radical scavenging, preventing lipid peroxidation and direct antioxidative capacity.

KEYWORDS

Cataract, glucose, *Asparagus racemosus*, Anti oxidant.

INTRODUCTION

Cataract (lens opacification) is a major contributing factor of blindness. It is defined as a clouding of the natural lens, a part of the eye responsible for focusing and producing a clear sharp image. It is called as a “peril of sight” because cataracts have blinded more people throughout the ages than any other affliction of the eye. It is also called as “Senile cataract”.

Cataract is derived from the Latin word “cataracta” meaning waterfall. ARN (Age-Related Nuclear Cataract) is the most common form of cataract which is found in ages more than 45 year and opacity forms in the centre of the lens. [1]

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Cataract is nothing but visual impairment as a result of a disturbance of lens transparency. It is one of the leading cause of blindness worldwide, it accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 28000 new cases are reported daily worldwide. Approximately 25% of the populations over 65 and about 50% over 80 have serious loss of vision because of cataract. [2, 3]

Cataractogenesis is influenced by multiple risk factors, such as aging, diabetes mellitus, drugs, trauma, toxins, genetics, smoking and other ocular diseases. Multiple mechanisms such as osmotic graduation, protein aggregates, oxidative stress, post translational protein changes, phase separation are proposed for cataract formation. Combined factors of

heritage, UV light exposure, diet, some metabolic disorders, quality of life, cationic pump malfunction and lens metabolism disorder are believed to have a role in cataract formation. The increased incidence of cataracts, in diabetic patients is also well known. [4]

Presently, surgery is the only approach for the treatment of cataract, and while favorable outcomes are quite predictable, the limited number of surgeons is underdeveloped countries and the high cost of surgery have made cataract a major health problem. Drugs developed to delay or prevent lens opacification have failed to give convincing positive results in clinical trials. This stimulates the research towards the experimental work on cataract to understand the all possible pathway and mechanism which is responsible for the generation of cataract. While the main treatment for cataract is surgical intervention, it is associated with certain risks and subsequent suboptimal outcomes. [5]

The prophylactic and therapeutic effect of many herbal extract had been reported. Such as *Adhatoda vasica, Allium cepa, Cassia fistula, Citrus aurantium, Cochlospermum religiosum, Curcuma longa, Ginkgo biloba, Momordica charantia, Ocimum sanctum, Vitex negundo* having anticataract activity. [6]

Asparagus racemosus root extract having pharmacological action like Antimicrobial, Hepatoprotective, and Antioxidant. Antiulcer, Antidepressant, Antineoplastic, activity is also reported.[7, 8]

With this background the objective of current study was to evaluate the Anticataract activity of aqueous extract of *Asparagus racemosus* root.

MATERIALS AND METHODS

Plant Extract

Aqueous extract of *Asparagus racemosus* root was purchased from the Konark herbals & health Care, Mumbai (Mfg. Date.July-2011 & Batch NO. KH/AR/001/11).% yield of extract from certificate of analysis was found to be 25% w/w.

In Vitro Evaluation of Anti-Cataract Activity

In this study, goat lens was used as they were easily available. Fresh goat eyes were obtained from slaughterhouse from bardoli. Other chemicals and reagents were under analytical grade.

Lens Culture

Fresh goat eyeballs were obtained from slaughterhouse was immediately transported to the laboratory at 0-40 ºC. The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (Nacl: 140 mM, HCl: 5 mM, $MgCl_2$: 2 mM, $NaHCO_3$: 0.5 mM, NaH $(PO4)_2$: 0.5 mM, $CaCl_2$: 0.4 mM and Glucose: 5.5 mM) at room temperature and pH-7.8 for 72 hours. Penicillin 32 mg and streptomycin 250 mg were added to the culture media to prevent bacterial contamination.[9]

Induction of *In Vitro* Cataract

Glucose in a concentration of 55 mM was used to induce cataract. Glucose in the lens metabolizes through sorbitol pathway and accumulation of polyols (sugar alcohols) causing over hydration and oxidative stress. This led to cataractogenesis. A total of 24 lenses were used for the study. These lenses were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM served as normal control and 55 mM served as toxic control) for 72 hours. [9]

Study Groups

A total of 24 lenses were divided into following groups (n = 6 in each group)

Group 1: aqueous humor (5.5mM Glucose) (Normal control)

Group 2: aqueous humor + Glucose 55 mM (Model control)

Group 3: aqueous humor + Glucose 55 mM + AEAR 250µg/mL

Group 4: aqueous humor + Glucose 55 mM + AEAR 500µg/ml

Photographic Evaluation

After 72 hours of incubation, lenses were placed on a wired mesh with posterior surface touching the mesh and the pattern of mesh (number of squares clearly visible through the lens) was observed through the lens as a measure of opacity.

The degree of opacity was graded as follows:

'0' - Absence of opacity

'1' - Slight degree of opacity

'2' - Presence of diffuse opacity

'3' - Presence of extensive thick opacity

Preparation of Lens Homogenate

After 72 hours of incubation, homogenate of lenses was prepared in tris buffer (0.23 M, pH-7.8) containing 0.25 × 10^{-3} M EDTA and homogenate was adjusted to 10% w/v which was centrifuged at 10,000 G at 4ºC for 1hour and the supernatant was used for the estimation of biochemical parameters. [9]

Biochemical Parameters

Electrolyte (Na^+) estimation was done by flame photometry method and protein estimation was done by Modified Biuret End Point Assay method. The degree of oxidative stress was assessed by measuring malondialdehyde (MDA) levels by TBARS-method. Estimation of Potassium (K^+) was done in lens homogenate by Potassium (MONOTEST) Colorimetric Method. Estimation of Calcium (Ca^+) was done in lens homogenate by O Cresolphthalein Complexone End Point Assay Method. Estimation of Catalase in lens homogenate was done by Aeibe et al. Estimation of superoxide dismutase (SOD) by Misra et al. Estimation of glutathione (GSH) was done in lens homogenate by Sedlak and Lindsay.[10-17]

Statistical Analysis

Results were expressed as mean ± S.E.M. The statistical significance of the difference between groups for the various treatments were determined by one way analysis of variance (ANOVA) followed by Tukey's test. $P<0.05$ was considered statistically significant.

RESULTS

Photographic Evaluation

Incubation of lenses with glucose 55 Mm showed opacification starting after 8 hours at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 72 hours as compared to lenses incubated in 5.5 mM glucose where transparency maintained and squares were clearly visible. Incubation of lenses with AEAR at (250 µg/ml, 500 µg/ml) concentrations seems to retard the progression of lens opacification.

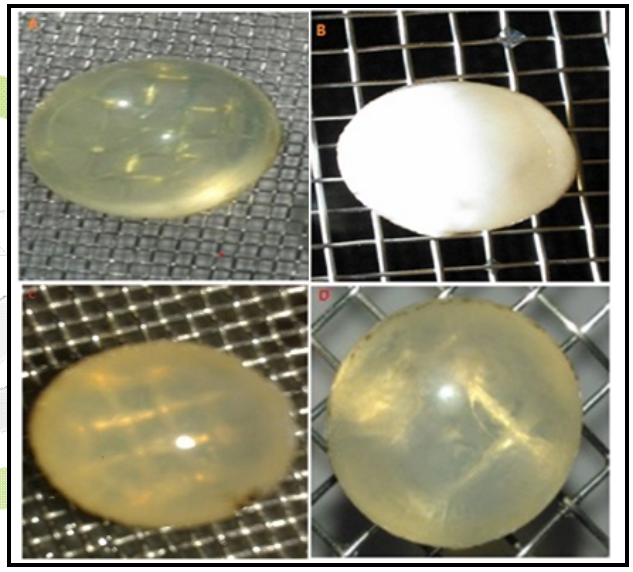

Figure 1: Photographic Evaluation of Goat Eye Lens

Table 1: Effect of AEAR on Opacity of Goat Eye Lens

Study Groups	Grade
(A) Normal control	'0'
(B) Model control	'3'
(C) AEAR 250µg/ml	'1'
(D) AEAR 500µg/ml	'1'

Biochemical Estimation

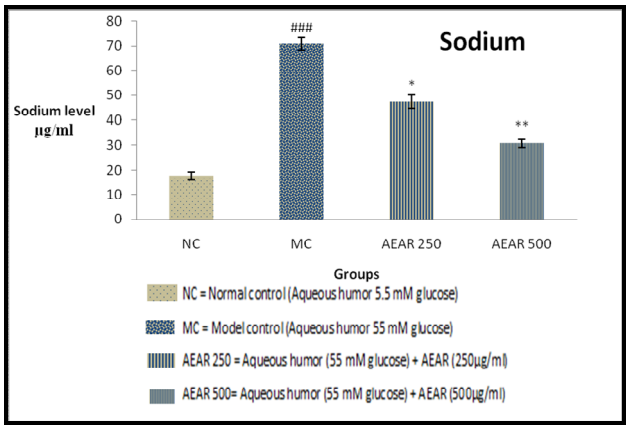

Figure2: Effect of AEAR on Sodium Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

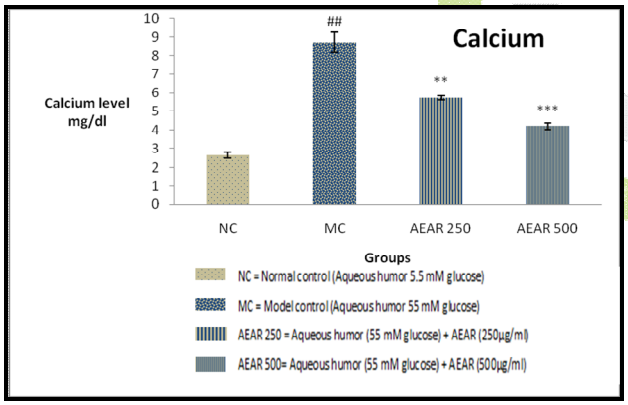

Figure 3: Effect of AEAR on Calcium Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

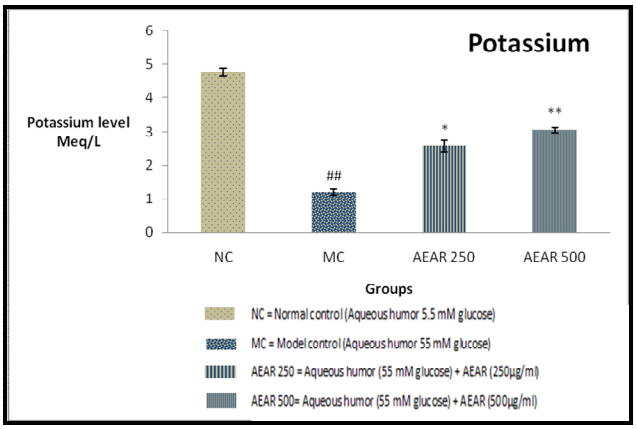

Figure 4: Effect of AEAR on Potassium Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

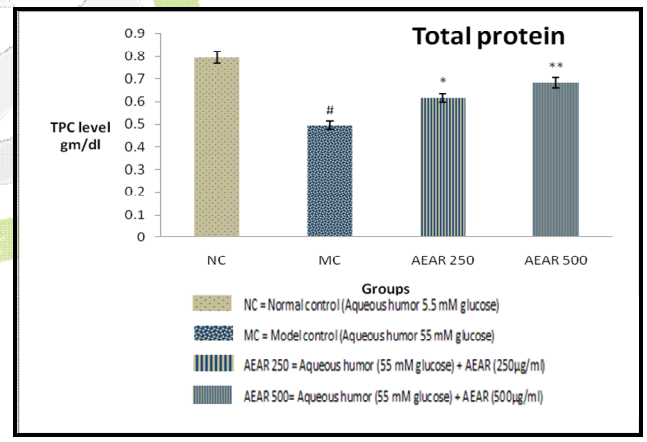

Figure 5: Effect of AEAR on Total Protein Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

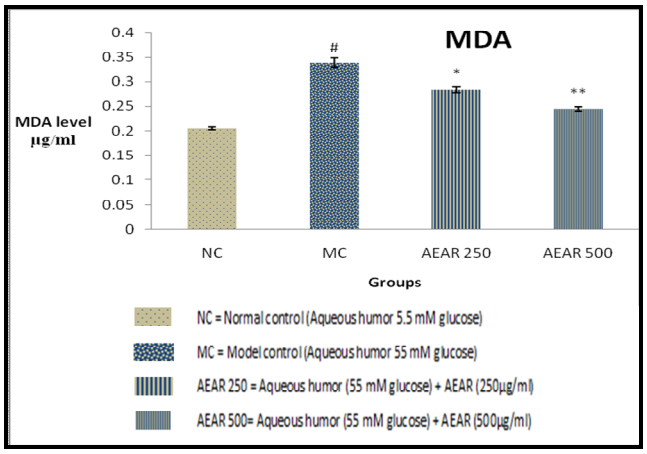

Figure 6: Effect of AEAR on MDA Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

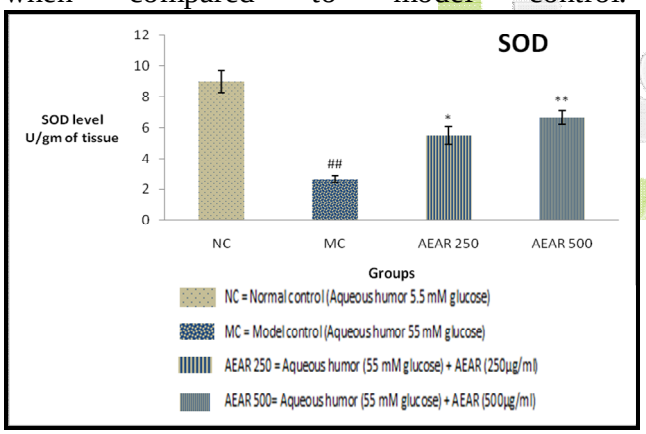

Figure 7: Effect of AEAR on SOD Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

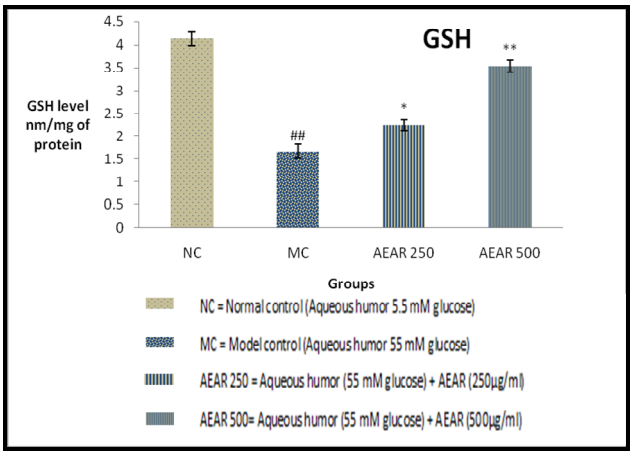

Figure 8: Effect of AEAR on GSH Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

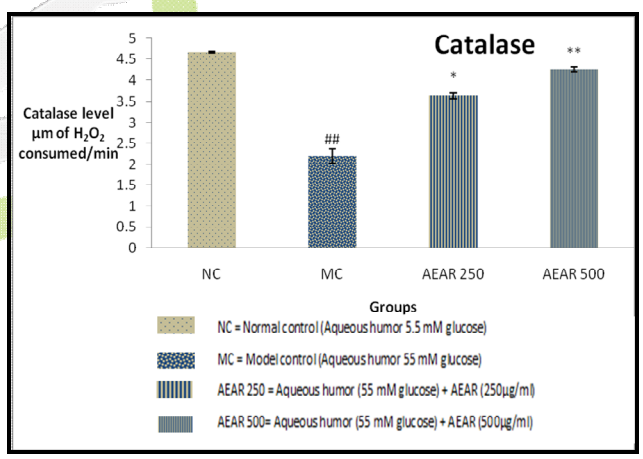

Figure 9: Effect of AEAR on Catalase Level in Goat Lens Homogenate after 72 Hours of Incubation

Data presented as mean ± S.E.M. (n=6). Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ Significant when compared to normal control.

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ Significant when compared to model control.

DISCUSSION

Cataract is a major cause of blindness all over the world. It is an age related phenomenon, over and above oxidative stress also plays its role. Surgical treatment has remained the only remedy till now. Hence, if a drug is sought which can either reverse or prevent lenticular opacity, it will be a great advance in the treatment of this disorder. A number of drugs have been shown to interfere with the process of cataract formation like aldose reductase inhibitors, restatin, sulindac, aspirin, etc.[18]

Cataract is one of the universal processes of ageing and is consequence of cumulative effect of various insults to the lens. The oxidation of lens proteins by free radicals and reactive oxygen species play an important role in the process leading to lens opacification. This oxidative crisis is one of the reasons for generation of cataract.

In vitro model for inducing cataract using glucose concentration 55 mM provides an effective model on isolated lenses of goat. Incubation of goat lenses in the media containing high glucose (55 mM) concentration induce cataract has shown to cause considerable drop in Na^+/K^+-ATPase activity, with progression of opacity. The impairment of Na^+/K^+-ATPase causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis. This alteration in the Na^+, K^+ ratio change the protein content of the lens, leading to a decrease in total proteins causing lens opacification. In this study showed higher total proteins ($P < 0.05$ at all concentration) and K^+ ions ($P<0.05$ at all concentration) whereas lower concentrations of Na^+ ions ($P<0.05$ at all concentration) with AEAR treated groups. The imbalance of Na^+ and K^+ is prevent due to an action of AEAR which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentrations, and intracellular glucose.[19]

In the present study, the mean level of calcium was found to be significantly higher in lenses of model control groups than that in the lenses of normal groups. However, administration of test drug to goat lenses significantly reduced the mean calcium level in treated groups. The increased calcium levels in the lenses of glucose induce cataract may have been caused by oxidation of sulfhydryls and other changes to the membranes caused by glucose leading to inhibition of Ca-ATPase pump leading to influx of calcium. The influx of calcium causes activation of the calcium-dependent proteases calpain II and Lp82, which partially degrade α and β crystallins, ultimately causing insolubilization of protein and scattering of light.[20]

Catalase is an important part of the innate enzymatic defense system of the lens which is responsible for the detoxification of H_2O_2. Decrease in the activities of this enzyme in tissue has been linked with the build up of highly reactive free radicals leading to injurious effect such as loss of integrity and the function of the cell membranes. The catalase keeps the level of free radicals below toxic levels. In cataractous lenses its concentration is decreased. Hence, with the use of antioxidants cataract formation can be prevented. In this study the level of Catalase was found to be less in to experimentally induced cataract lenses as compared to normal control group ($P<0.05$). The lenses treated with AEAR showed significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity.[21]

The amount of reduced glutathione in the lens decreases in almost in any type of cataract. The role of reduced glutathione in the preservation of lens clarity is of substantial interest; it serves as the major antioxidant in the lens and prevents protein oxidation. In this study the level of GSH was found to be less in to experimentally induced cataract lenses as compared to normal control group ($P<0.05$). The restoration of reduced glutathione levels by *AEAR* extract also demonstrated its anticataract potential.[22]

Oxidative stress may also be implicated in the cataract induced by glucose due to the formation of H_2O_2. In this study MDA levels were significantly higher in Positive control groups. The MDA levels were significantly less in the AEAR treated groups at 250 µg/ml and 500 µg/ml concentrations ($P<0.05$) respectively.[23]

With an increase in the severity of cataract, there is a leakage of hydrolyzed crystallins from the lens into the aqueous humor. The total protein content in lenses in this study indicated that protein is significantly ($p<0.05$) decreased in experimentally induced cataract groups, when compared to normal control group. The total protein levels were significantly high in the AEAR treated groups at 250 µg/ml and 500 µg/ml concentrations ($P<0.05$) respectively. Under stress full condition the protein of the lens denatures and creates the disulfide cross linking causing disulfide and mixed disulfide bond formation causing a protein aggregation and this precipitation leading to lens opalescence.

SOD, a chain-breaking antioxidant, was first described by McCord and Fridovich in red blood cells. SOD converts superoxide to H_2O_2. The enzyme exists in two forms, one containing $Mn^{+2,}$ restricted to the mitochondria, and a cytosolic form containing Mn^{+2} and Cu^{+2}. The SOD content was significantly reduced in experimentally induced cataract group as compared to normal control group. The SOD levels were significantly high in the AEAR treated groups at 250 µg/ml and 500 µg/ml concentrations ($P<0.05$) respectively.[24]

CONCLUSION

The Present investigation suggests that aqueous extract of *Asparagus racemosus* root effectively prevent the cataractogenic condition which was indicated by increase in the total protein content, potassium level and decrease in the sodium and calcium level. However, antioxidant property of aqueous extract of *Asparagus racemosus* root was confirmed by increase in lens glutathione content, SOD, Catalase and decrease in lens MDA level. In conclusion all the above finding lends credence to roots of *Asparagus racemosus* in the treatment of cataract.

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