International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(3): May-June 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 05-05-2016; Revised: 10-05-2016; Accepted: 11-05-2016

IN-VITRO ANTICATARACT ACTIVITY OF ETHANOLIC EXTRACT OF SHOOTS OF *BAMBUSA BALCOOA ROXB.* ON GOAT LENS

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Keywords:

Cataract, Antioxidant activities, Anticataract activity

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ABSTRACT

To study the in-vitro anticataract activity of ethanolic extract of shoots of Bambusa balcooa (EESBB) on goat lens. Ethanolic extract of shoots of Bambusa balcooa (EESBB) was prepared by Percolation method. Goat lenses were made in 6 groups with 5 lenses in each group.Artificial aqueous humor was prepared and 32% Penicillin G and 250% Streptomycin were added to it.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 EESBB (1 mg/kg, 1.5 mg/kg, 2 mg/kg) 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. Glucose induced opacification of lens was started 10-12 hours post incubation & was completely opacified in 72 hrs. Lens treated with the EESBB at concentrations of 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. Hence the study suggested that EESBB possesses significant anticataract activity which can be attributed to its antioxidant property.

INTRODUCTION

- Cataract is the opacity of the lens that produces painless gradual loss of vision. Cataract formation is mainly an age-related phenomenon, although socioeconomic and lifestyle factors such as nutritional deficiency, sunlight, smoking, environmental factors, lack of consumption of antioxidants may also influence its occurrence. Apart from ageing, diabetes has been considered to be one of the major risk factors of cataract.[1]
- 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.[2]
- 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.[3,4]

- Under physiological conditions, glucose is metabolized through the glycolytic pathway. An excess amount of glucose is converted to sorbitol by enzyme aldose reductase via polyol pathway. The glucose converted into sorbitol by utilizing NADPH results in the reduction of NADPH/NADP+. Sorbitol does not easily cross cell membrane.
- Intra lenticular accumulation of sorbitol, leads to lens damage. As, the lens starts to swell in response to the hyper osmotic effects of polyol, membrane permeability changes resulting in an increase in lenticular sodium and decrease in the levels of lenticular potassium, reduced glutathione, ATP and free amino acids.[5]

- Also the lens Na^+- K^+-ATPase activity plays an important role in maintaining lens transparency, and its impairment causes accumulation of Na+ and loss of K+ with hydration and swelling of the lens fibers leading to cataractogenesis.[6]
- Due to the presence of high fibre and phytosterols in bamboo shoot it reduces fat and cholesterol levels of blood making them one of the most sought after health foods among patients with life style related disorders. The dietary fibre contains a number of health benefits as it controls blood pressure, hypertension, and obesity and also protects from coronary diseases and potential carcinogens

- Though surgery is the preferred treatment of cataract,there are various complication of surgery and its being more significant in case of diabetes. Diabetes and cataract combining pose an enormous health and economic burden, particularly in case of developing countries where diabetes treatment insufficient and cataract surgery often inaccessible.[7,8]

MATERIALS AND METHODS

- Plant: Fresh shoots of bambusa balcooa were collected and was being authenticated in Dibrugarh University by Professor Dr. L. R.Saikia Department of Life Sciences (No. DU L.Sc 437)
- Preparation of plant extract:The seeds were dried in shade and then grounded to fine powder. It was then soaked in 90% ethanol and allowed to stand for 15 min in tightly covered container. The entire solution was transferred to a percolator and sufficient quantity of 90% ethanol was added. It was then allowed to macerate for 72 hours after that it was allowed to percolate slowly. and the extract was collected. The extract was then dried with rotator flask evaporator and stored below ambient temperature. [9]
- Phytochemical screening: The extract were subjected to qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, diterpenes, triterpenes and phenols as per the standard methods.
- Drugs like Enalapril, Penicillin and streptomycin were obtained from Abbott Healthcare Pvt Ltd (AHPL), Alembic Chemical Works Co Ltd and Cadila Pharmaceuticals Ltd. Respectively.
- **Eye Balls**: Goat eye balls were used for the study were obtained from local slaughterhouse immediately after slaughter and transported to laboratory at 0-4 degree Celsius.
- **Preparation of Lens Culture**:The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl - 140 mM, KCl - 5 mM, MgCl2 - 2 mM, NaHCO3 - 0.5 mM, NaH(PO4)2 - 0.5 mM, CaCl2 - 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Strict aseptic measures were followed and any possible contamination of the culture media was prevented by adding penicillin 32mg%, streptomycin 250mg% and replacing the aqueous humor with fresh solution every 24hrs. Glucose in a concentration of 55 mM was used to induce cataract.[10]

- **Experimental design**: Groups: A total of 60 lenses were divided into following categories (n=10 in each category). The lens in group A were incubated in glucose at 5.5 mM and were taken as a negative control and group B incubated in glucose 55mM serve as positive control. Three concentrations 1mg, 1.5mg and 2mg of the plant extract were chosen. The standard drug Enalapril (group C) was taken at a concentration of 5ng/ml.

Groups	GROUP STRUCTURE
Group A	Lens culture + Glucose 5.5mM (negative control)
Group B	Lens culture + Glucose 55mM (positive control)
Group C	Lens culture + Glucose 55mM + Enalapril 5ng/ml
Group D	Lens culture + Glucose 55mM + 1mg/kg EEBBS
Group E	Lens culture + Glucose 55mM + 1.5mg/kg EEBBS
Group F	Lens culture + Glucose 55mM + 2mg/kg EEBBS

- After 72 hours of incubation, visual evaluation was done by placing the lens on a graph paper and counting the number of squares visible through the lenses as a measure of lens opacity. Then 10% W/V homogenate of 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.
- supernatant was used for the determination of lipid peroxidation[11] enzymatic antioxidant like superoxide dismutase[12] and catalase[13] The total proteins were estimated by Lowery et al. method.[14]
- 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 6.

Results and observations

- After 72 hours of incubation, transparency was maintained in the normal control group A (fig. 1) but there was complete loss of transparency in the positive control group B (fig. 2) indicating complete cataractogenesis.
- Goat lenses of groups containing escalated doses of the EEBBS (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 EEBBS) was more effective in suppressing cataract formation (fig. 6) than Group D (fig. 4) and Group E (fig. 5).

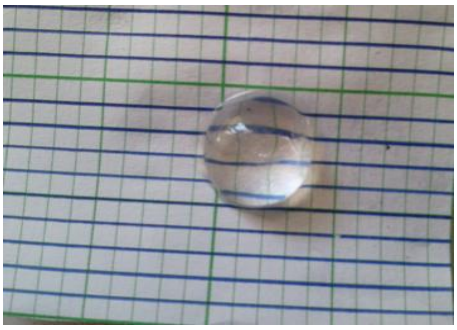


Fig 1:Normal control(Group A)

Fig 2:Positive Control(Group B)

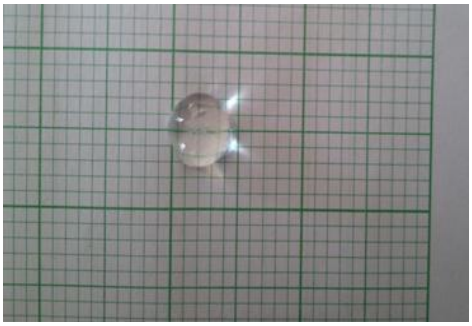


Fig 3:Standard drug (Group C)

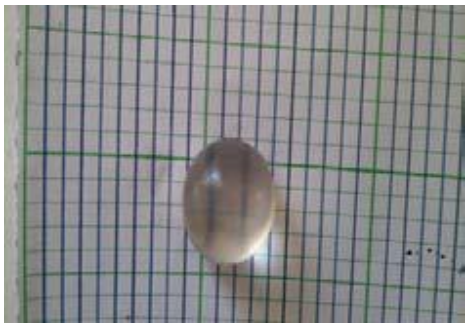


Fig 4:Group D(EEBBS 1mg)

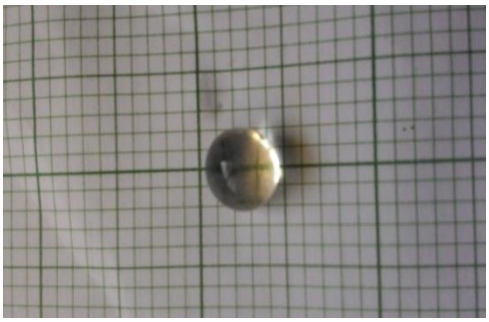


Fig 5: Group E(EEBBS 1.5mg)

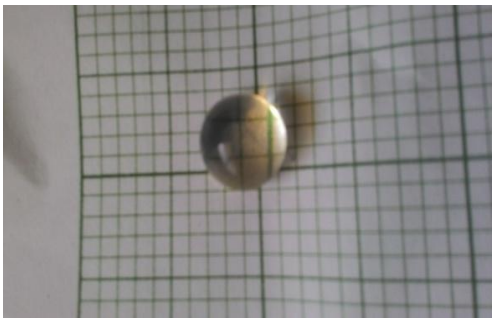


Fig 6:Group F(EEBBS 2mg)

- Lens homogenate of Group B lenses showed significantly lower levels of catalase and SOD activities and higher levels of MDA 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 .
- Group D, E, F containing 1 mg, 1.5 mg and 2 mg EEBBS respectively showed significant 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 . Group F was more efficient in suppressing the oxidative stress as the catalase activity was significantly higher than Group D and E. Group F also showed significantly higher total protein , water soluble protein and lower MDA level 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).

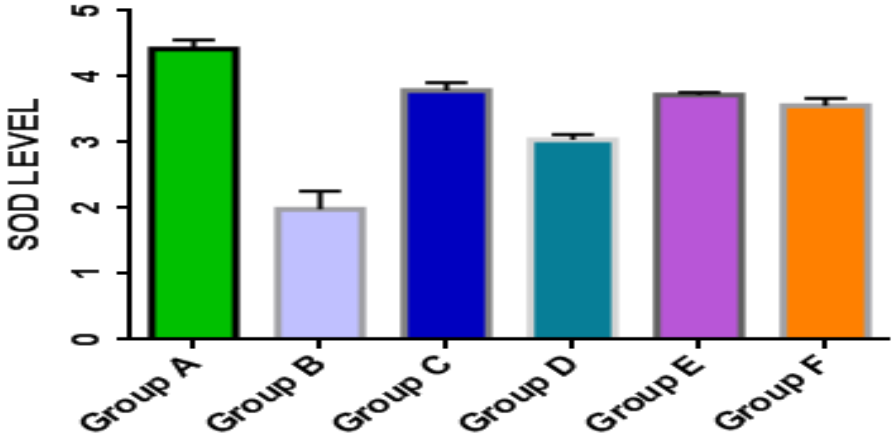
Table 1: Shows the effects of EESBB on biochemical and oxidative parameters in the lens homogenate after 72 hours of incubation

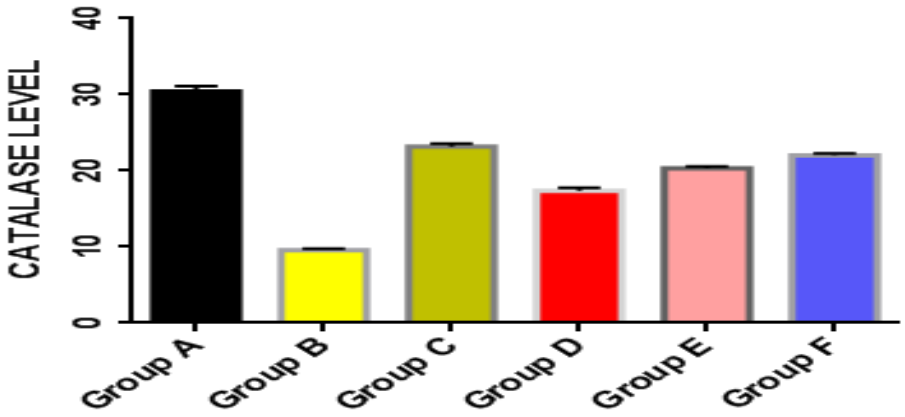
Groups	SOD activity (unit/mg of protein	Catalase activity(µmol/min/g)	MDA level (nmol/gm)	Total protein(mg/gm)	Water soluble protein(mg/gm)
Group A	4.42±0.14	30.42±0.68	7.32±0.34	179.7±2.14	78.28±2.53
Group B	1.98±0.28[a]	9.54±0.22[a]	16.53±0.41[a]	133.2±2.83[a]	45.14±1.51a
Group C	3.79±0.12 [b]	23.16±0.38 [b]	11.12±0.52[b]	170.25±0.91[b]	72.13±1.33 [b]
Group D	3.04±0.08[b]	17.38±0.40[b]	13.38±0.33[b]	153.2±0.82[b]	64.24±1.51[b]
Group E	3.72±0.04[b]	20.32±0.22[b]	13.08±0.42[b]	160.7±0.41[b]	65.55±0.83[b]
Group F	3.56±0.11[b]	22.02±0.24[b]	11.38±0.41[b]	164.2±0.28[b]	72.24±1.71[b]

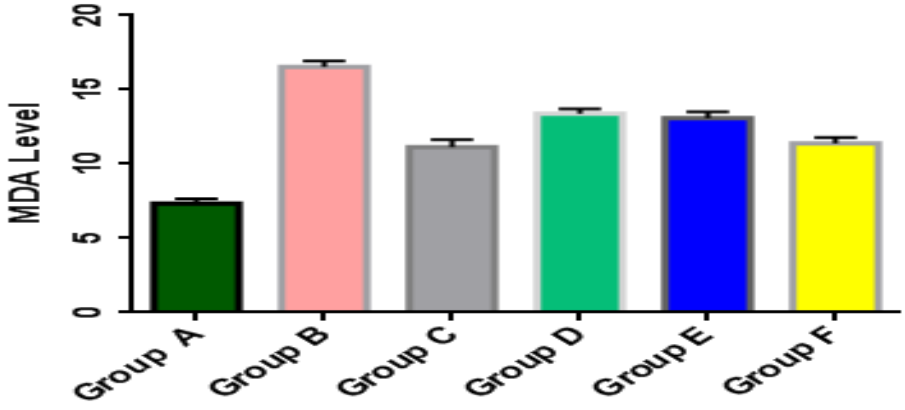
- Mg=milligram,µmol/min/ml=micromoles/minute/milliliter,nmol/gm=nanomoles/gram,mg/gm=milligrams/ gram. The values are expressed as means±SD.; n=10.

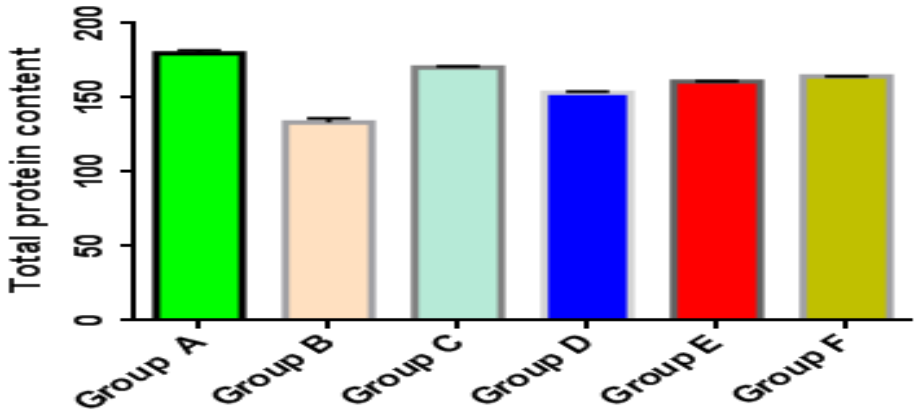
a:$p<0.05$ as compared with their corresponding values in glucose 5.5mmol group(-ve control)

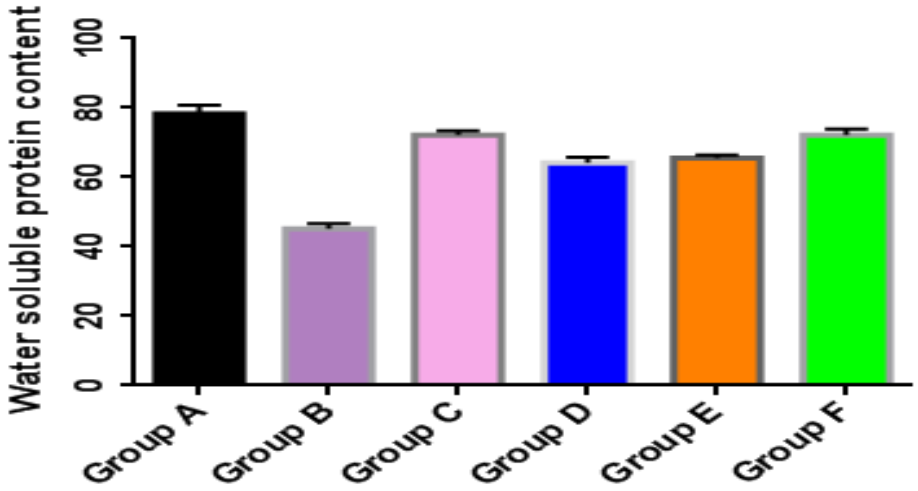
b:$p<0.05$ as compared with their corresponding values in Glucose 55mmol group(+ve control)


SOD LEVEL
0
1
2
3
4
5
Group A
Group B
Group C
Group D
Group E
Group F


CATALASE LEVEL
0
10
20
30
40
Group A
Group B
Group C
Group D
Group E
Group F


MDA Level
0
5
10
15
20
Group A
Group B
Group C
Group D
Group E
Group F





DISCUSSION

- Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defence systems. Numerous scientific investigations have confirmed the presence of oxidative stress in ocular diseases, and ROS may play a significant role for the pathophysiology in cataracts.
- ROS have physiological functions at low levels but are toxic to the cell at high levels. To protect against toxic effects of ROS and to modulate physiological effects of ROS, the cell has developed antioxidant defence systems. The systems are very complex, being composed of antioxidative enzymes (such as SOD and CAT) and antioxidant compounds (vitamins A, C, E, and so on). SOD decomposes superoxide into hydrogen peroxide. CAT reduces H_2O_2 to water. They protect cells against ROS produced during normal metabolism and after an oxidative insult.[15]

- In the present study the cataract is generated by the incubation of lens culture in the media containing high glucose (55mM) concentration. There is strong evidence to show that diabetes is associated with increased oxidative stress.[16] The toxic effects of the reactive oxygen species so produced are neutralized in the lens by various antioxidants out of which SOD and catalase is one of them.
- The MDA levels were significantly less in the test drug treated groups at all concentrations. The test Group F have also been shown to increase the content of water-soluble proteins, retarding the process of cataractogenesis initiated by high glucose concentration.[17]
- However with standard drug Enalapril and as well with the plant extracts the process of cataract formation is slowed down to a significant level. The measured parameters used in the present study reflects the antioxidant activities of the plant extracts in possessing free radical scavenging property which may help in prevention of cataract.

CONCLUSION

- To conclude, the study suggested that the shoots of bambusa balcooa roxb. possess anticataract and antioxidant activities, which might be helpful in preventing or slowing the progress of cataract. Further *in vivo* studies and investigations on the isolation and identification of active components in the shoots may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

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