DOI: 10.21276/ijabpt.2016.7.3.2

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-3, July-Sept-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 26th Apr-2016 *Revised: 24th May -2016* *Accepted: 25th May 2016*

Research article

ANTI-CATARACT POTENTIAL OF *CYANADON DACTYLON* IN CATARACTOUS GOAT LENS *IN VITRO*

Nishmitha S*, Brindha.S, Elango.N, Gokila.V, Nithya.k, Rosheni.N and Kokila.S

School of Biotechnology, Dr G R Damodaran College of Science
(Autonomous, affiliated to **Bharathiar university)** Coimbatore 641014, Tamil Nadu, India.

ABSTRACT: Cataract is a disease characterized by vision impairment due to opacification of the lens. Several factors causing cataract include diet, oxidative stress, deficiency of nutrients antioxidants, smoking constant exposure to bright light etc., Of all the factors causing cataract, diabetes is found to be a common risk factor and cataract is one of the secondary complications of diabetes mellitus. Diabetes can cause cataract by either of the following three mechanisms: polyol pathway, oxidative stress and non-enzymatic glycation. Medicinal plants are a rich source of phytoconstituents that are capable of curing various human diseases including diabetes mellitus and cataract. Of all the medicinal plants having anti- diabetic potential a few have been studied to be anti-cataractic also. Based on data collected from various research works on diabetes mellitus and the anti-diabetic potential of *Cyanadon dactylon*, the current study was performed to investigate the anti-cataract potential of *C. dactylon in vitro*, using goat lens culture. The experimental design included four groups of lenses including a positive, negative and normal control along with a group of lenses exposed to an optimal concentration of hydro-ethanolic plant extract of *C. dactylon* . Photographic evaluation of lens opacity was done to compare and check the effectiveness and potential of *C. dactylon* . Further anti-cataractic potential was studied with the lens homogenate by estimating the following biochemical parameters: total protein content, malondialdehyde assay, super oxide dismutase assay and determination of aldose reductase activity. The results showed significant anti-cataractic activity.

Key words: Cataract, Diabetes mellitus, anti- cataractic potential, *Cyanadon dactylon*, goat lens culture, hydro-ethanolic extract, biochemical parameters.

*Corresponding author: Nishmitha S, Department of Biochemistry, Patna University, Patna-800 005, Bihar, India, E-mail: nishy511@gmail.com

INTRODUCTION

Cataract is a condition of the human lens which causes opacification or optical dysfunction of human lens leading to deterioration in vision. Various factors cause cataract formation including daylight, diet, diabetes, dehydration, oxidation of lens protein due to oxidative stress and peroxidation of lipids. The risk factors of cataract include smoking, deficiency of nutrients and antioxidants etc., (Patel *et al.,* 2011). Studies have related cataract as a secondary complication of diabetes mellitus and it is a major risk factor. Cataractogenesis or the formation of cataract is mainly due to excessive sorbitol accumulation in the lens fibers as a result of "Polyol Pathway" wherein glucose is reduced to sorbitol by the enzyme aldose reductase (AR) utilizing NADPH as a cofactor. Sorbitol is trapped within the cell membranes and this accumulation causes cell damage due to imbalance in osmotic homeostasis.

Oxidative stress is another major risk factor of cataract. This causes an excessive free radical generation disturbing the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase. These enzymes are responsible for defending the lens against cell damage and injury (kalekar *et al*., 2014). *Cyanadon dactylon* (L).Pers. is a perennial grass belonging to family Poaceae that has a variety of medicinal properties. It is cultivated throughout the tropics and subtropics. Whole herb and its root stalk are used for medicinal use. It is native to north and east Africa, Asia and Australia and southern Europe (Abhishek *et al*., 2012). It is noted to have many medicinal properties. Research has concluded that it has Antihelmintic, Antidiuretic, Antiinflamatory, Hepatoprotective activity, treatment of urinary tract infections, Prostatitis and Dysentry. Traditional medicines derived from C. dactylon were used to treat diabetes, jaundice, kidney problems, urinary disease, gastrointestinal disorder, constipation and abdominal pain (Kanimozhi *et al*., 2012). Freshly extracted juice of *C. dactylon* is useful in hematuresis, vomiting, as an application in catarrhal ophthalmia and can also be applied to cuts and wounds as it checks bleeding and in chronic diarrhea. Root decoctions are used to treat vesical calculus, secondary syphilis, stoppage of bleeding from piles and irritation of urinary organs. It was also found that an alkaloid isolated from the plant has hypoglycemic effect, slows down the flow of blood in mesenteric capillaries, reduce bleeding and clotting time and hypotension (Chidrawar *et al*., 2011). Recent studies showed that the aqueous and ethanolic extracts of *Cyanadon dactylon* have hypoglycaemic and antidiabetic potential (Saroja *et al*., 2012). The principal constituents of *C. dactylon* crude proteins, carbohydrates, mineral constituents, oxides of magnesium, phosphorous, calcium, sodium, potassium, β-sitosterol, flavonoids, alkaloids, glycosides, triterpenoids, vitamin C, carotene, fats, palmitic acid, etc., were reported. The green grass was analysed and found to have 10.47 % crude protein, 28.17%, fiber and 11.75% of total ash (Badri, Renu, 2011). All of these medicinal properties were employed by traditional medicine systems including Sidhha and Ayurveda. According to Ayurveda and traditional pharmacopoeia, *C. dactylon* plant is a pungent, bitter, fragrant, heating, appetizer, vulnerary, anthelmintic, antipyretic and alexiteric which destroys foulness of breath, useful in leukoderma, bronchitis, asthma, tumours and enlargement of the spleen and also used as a purifying agent (Kondaveeti *et al.*, 2012).

Based on data collected from various research works on diabetes mellitus and the anti-diabetic potential of *Cyanadon dactylon*, the current study was performed to investigate the anti-cataract potential of *Cyanadon dactylon in vitro*, using goat lens culture.

MATERIALS AND METHODS

A. Collection of plant material: Fresh samples of *Cyanadon dactylon* were collected from Coimbatore district, Tamil Nadu, India

B. Preparation of Plant Extract: The plant sample was shade dried and subjected to extraction with Hydroethanol (1:1) solvent (20g/200ml).

C. Anticataract activity: The *in vitro* anticataract activity was carried out using the modified protocol from Shabeer *et al*. (2011).

1) Collection of Goat eye balls

The anticataract potential of the plants extracts was studied *in vitro* in glucose induced cataractogenesis using goat eye lens. Goat eye balls were used in the present study. They were obtained from a slaughter house in Coimbatore, immediately after slaughter and transported to the laboratory at 0 - 4˚C.

2) Preparation of lens culture

The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl: 140 mM, KCl: 5 mM, $MgCl_2$: 2 mM, $NaHCO_3$: 0.5 mM, $NaH(PO_4)_2$: 0.5 mM, $CaCl_2$: 0.4 mM, and Glucose: 5.5 mM) at room temperature and pH 7.8 for 72 hours. Penicillin G 32 mg% and Streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose at the concentration of 55 mM was used to induce cataract.

3) Experimental design

Group I: Normal lens glucose 5.5 mM (Normal control)

Group II: Lens + Glucose 55 mM (Negative control)

Group III: Lens + Glucose 55 mM + *Cynodon dactylon* (500 µg/ml)

Group IV: Lens + Glucose 55 mM + Standard drug Enalapril (10 ng/ml)

4) Photographic evaluation of Lens opacity

After 72 hours of incubation, lenses were observed for opacity and photographs were taken by placing the lenses on the wire meshes with posterior surface touching the mesh, and the pattern of mesh was observed through the lens as a measure of lens opacity.

5) Preparation of lens Homogenate

Lenses were homogenized in Tris buffer (0.23 M pH 7.8) and 0.25 x 10^{-3} M EDTA. The homogenate was adjusted to 10% w/v. The homogenate was centrifuged at 10,000 rpm at 4°C for 1 hour. The supernatant was used for studying various biochemical parameters.

6) Study of anticataract potential of *Cyanadon dacylon*

The anticataract potential of the plant extracts was determined. The following biochemical parameters were analyzed using the modified protocol of Umamaheswari *et al*. (2012).

a) 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 at room temperature for 30 mins for color 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 μg/mg lens tissue.

b) Estimmation of Malondialdehyde (MDA)

Lenses were homogenized in10% (w/v) 0.1 M Tris–HCl buffer (pH 7.5). One milliliter 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 1000 rpm for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The values are expressed as MDA/ min/ mg lens protein.

c) Assay of Superoxide dismutase (SOD)

The assay mixture contained 1.2 ml sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml of 186 μM phenazonium methosulphate (PMS), 0.3 ml of 300 μM NBT, 0.2 ml of 780 μM NADH, 1.0 ml homogenate and distilled water to a final volume of 3.0 ml. Reaction was started by the addition of NADH and incubated at 30ºC for 1 min. The reaction was stopped by the addition of 1.0 ml glacial acetic acid and the mixture was stirred vigorously. Precisely 4.0 ml of n-butanol was added to the mixture and shaken well. The mixture was allowed to stand for 10 min, centrifuged, the butanol layer was taken out and the absorbance was measured at 560 nm against a butanol blank. A system devoid of enzyme served as the control.

d) Determination of Aldose Reductase (AR) Activity

AR activity was assayed according to the modified protocol described by Rajesh et al. (2011). The assay mixture in 1 ml contained 0.7 ml phosphate buffer (0.067 M), 0.1 ml of NADPH (25×10^{-5}), 0.1 ml of lens supernatant, 0.1 ml of D L-glyceraldehydes (substrate) (5×10^{-4} M). Appropriate reference blanks were employed for corrections containing except the substrate, D L-glyceraldehydes. The enzymatic reaction was started by the addition of substrate and the absorbance was recorded in UV-Spectrophotometer at 340 nm for at least 3 min at 30 sec interval. AR activity was expressed as Δ OD /min/mg protein and the % inhibition activity was found using the following formula:

$$\text{AR Inhibition Activity (\%)} = \frac{\text{A340nm (Control) - A340nm (Sample)}}{\text{A340m (Control)}} \times 100$$

Where,

A_{340nm} (Control) is the Absorbance of the control at 340nm;

A_{340nm} (Sample) is the Absorbance of the plant samples at 340nm

The anticataract effect of hydroethanolic extract of *Cyanadon dactylon* were studied by photographic evaluation and by determining the Total protein content, malondialdehyde, aldose reductase activity and superoxide dismutase assays. Enalapril was taken as the standard drug as it shows to have potent anticataract activity *in vitro* due to antioxidant and free radical scavenging activity (Langade *et al*., 2006).

RESULTS AND DISCUSSIONS

On performing the above experiments according to the parameters chosen, the following results were evaluated and discussed.

1) Photographic evaluation of lens opacity

Group I : The photograph showed the normal lens incubated in artificial aqueous humour solution and glucose (5.5 mM) and complete transparency is seen. (Figure 1).

Group II : The lens was incubated with 55mM concentration of glucose and was taken as the negative control. The photograph showed complete opacification (Figure 2).

Group III : The lens incubated with hydro-ethanolic extract of *Cyanadon dactylon* (500 µg/ml) showed significant reduction in lens opacity (Figure 3).

Group IV : This lens was incubated with the standard anti-cataract drug enalapril and this showed almost normal transparency when compared to cataractous lenses (Figure 4).

The result indicates a positive effect of the hydro-ethanolic extract of *Cyanadon dactylon* by exhibiting reduction in the opacity of cataractous lens.

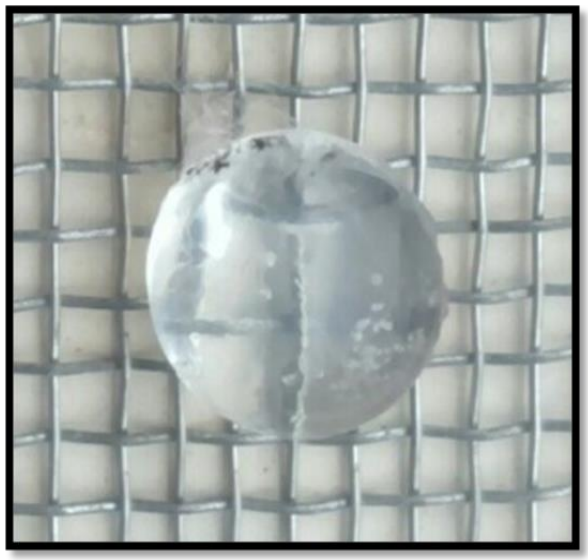

Figure 1. Normal control incubated with nominal glucose (5.5mM) showing complete transparency

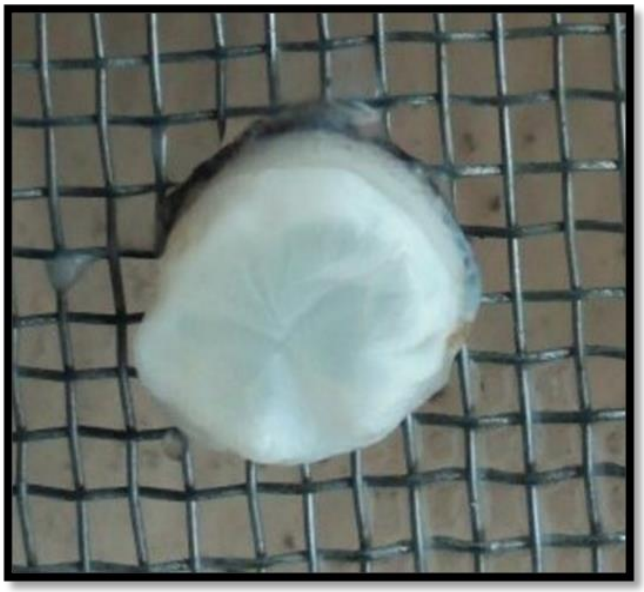

Figure 2. Negative control incubated with glucose (55mM) showing complete opacification

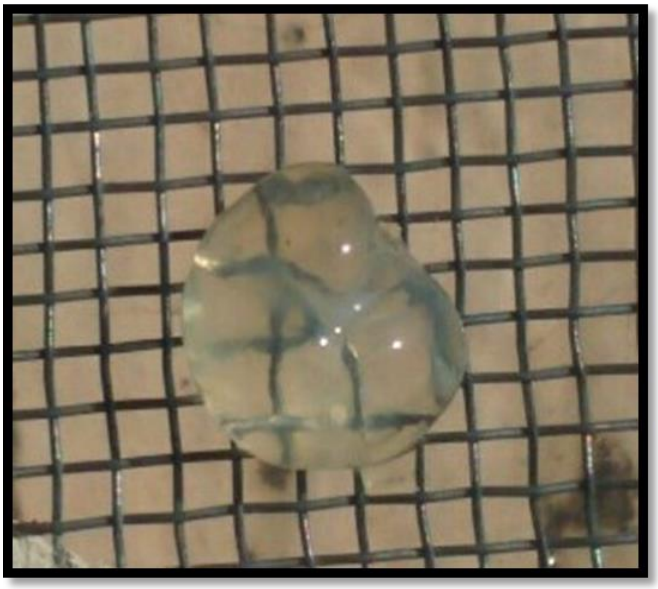

Figure 3. Lens incubated with plant extract (500µg) Showing significant transparency.

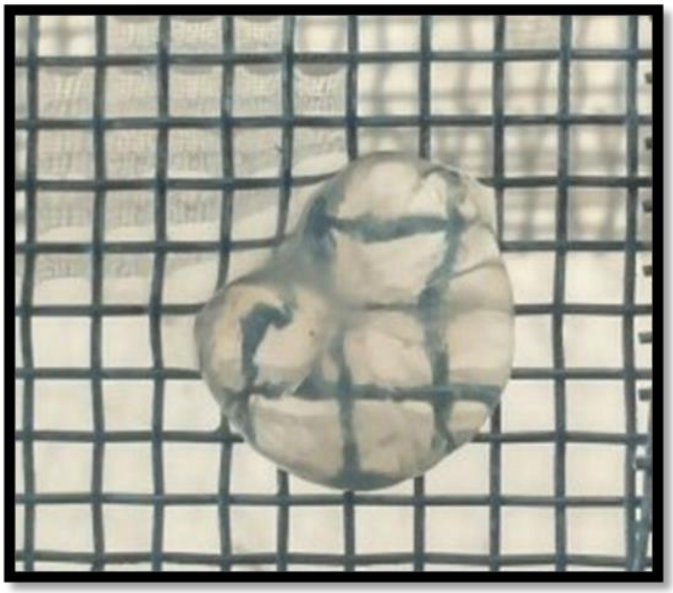

Figure 4. Positive control incubated with enalapril showing complete transparency

2) Anti-cataract potential based on biochemical parameters

The Effect of the hydro-ethanolic leaf extracts on lens protein and Malondialdehyde (MDA) was evaluated (Table 1). It was evident that there was significant decrease in total protein and a simultaneous increase in MDA in Group II, cataractous lens (negative control) when compared to the normal control. Group III and IV, including plant extract induced and enalapril induced lenses respectively, showed positive results with an increase in total protein and decrease in the level of MDA. Similar results were achieved from the study carried out by Umamaheshwari *et al*, 2012, where the biochemical parameters (Total protein content and MDA) were checked for all groups of lenses.

The superoxide dismutase (SOD) assay and aldose reductase (AR) activity were evaluated (Table 2). Group I with normal lens showed the highest amount of SOD activity whereas the negative control showed very less activity. Group III containing the plant extract and group IV containing the drug enalapril showed significantly restored SOD activity. The results of SOD activity of lens homogenate in the study performed by Hajarnavis and Bulakh, 2013, were found to be similar.

On evaluating the percentage inhibition activity by aldose reductase (AR), the highest activity was found in the normal control and the least in the negative control (Table 2). Group III lenses significantly increased the percentage inhibition activity. Group IV with enalapril, similarly, had significant increase in percentage inhibition activity. The aldose reductase activity studied by kalekar *et al*, 2014, in lens homogenate revealed a congruous result as well.

Table 1. Effect of the hydro-ethanolic leaf extracts on lens protein and MDA (Malondialdehyde)

Groups	Protein (mg/ml)	MDA (MDA/ min/ mg lens protein)
Group 1	16.6 ± (0.316)	0.0003 ± (0.00158)
Group 2	2.2 ± (0.158)	0.0026 ± (0.000354)
Group 3	13.4 ± (0.1)	0.0013 ± (0.000158)
Group 4	12.8 ± (0.224)	0.0005 ± (0.000316)

The values are given as mean ± (standard deviation)

Table 2. Effect of the hydro-ethanolic leaf extracts on lens ARA (Aldose Reductase Activity) and SOD (Superoxide Dismutase)

Groups	AR – Inhibition Activity (%)	SOD (units/mg tissue)
Group 1	98.98 ± (0.0509)	9.21 ± (0.01)
Group 2	69.22 ± (0.0316)	3.11 ± (0.0223)
Group 3	90.10 ± (0.158)	8.36 ± (0.0254)
Group 4	91.40 ± (0.0509)	8.50 ± (0.10024)

The values are given as mean ± (standard deviation)

Angiotensin Converting Enzyme (ACE) inhibitors have been found to afford protection from free radicals induced damage in many experimental conditions. Oxidative stress being an important factor in cataractogenesis, the increase in the level of SOD in the plant extract helps treat cataract. Aldose reductase is one of the key enzyme in polyol pathway and this enzyme induced/ mediated changes are major insults in the development of diabetic complications such as cataract and retinopathy (Williamson et al., 1992). The inhibition of AR is one of the potential pharmacological approach to treat secondary complications of diabetes including cataract (Moghaddam et al., 2005). The results observed from this study clearly implicit the potential role of *Cyanadon dactylon* in decreasing the oxidative stress and hence the opacity of lens.

CONCLUSION

Cyanadon dactylon contains antioxidants that can prevent oxidative stress, glycation and polyol pathway. This study confirms the anticataract potential of *Cyanadon dactylon* and further studies can be done *in vivo*, on diabetic animals to isolate the potential compound responsible for its anticataract activity.

ACKKNOWLEDGEMENT

We wish to place on record our sincere gratitude to our institution, respected Correspondent, Dr D Padmanaban, Secretary, Mrs Geetha Padmanaban and our Principal Dr T Santha, Dr G R Damodaran College of Science (Autonomous), Coimbatore for their kind patronage.

We acknowledge our sincere thanks to Dr R Suganthi, Associate Professor & Head, School of Biotechnology, Dr G R Damodaran College of Science (Autonomous), Coimbatore, for her guidance and help throughout our project. We wish to place on record our deep sense of gratitude and profound thanks to our respected guide, Mrs S Kokila, Assistant Professor & Coordinator, Department of Biochemistry, Dr G R Damodaran College of Science (Autonomous), Coimbatore, for her complete guidance and constant support during the course of our project.

REFERNECES

Abhishek B and Anita Thakur (2012). Anthelmintic Activity of *Cynodon Dactylon*, Journal Of Pharmacognosy And Phytochemistry, 2278- 4136, Ic Journal No: 8192, Volume 1 Issue 3.

Badri Prakash Nagori and Renu Solanki (2011). *Cyanadon Dactylon* (L.) Pers.: A Valuable Medicinal Plant, Research Jornal of Medicinal Plant, Volume: 5(5), P508-514.

Chidrawar Vr, Chitme Hr, Patel Kn, Patel Nj, Racharla Vr, Dhoraji Nc And Vadalia Kr (2011). Effects of *Cynodon Dactylon* on Stress-Induced Infertility In Male Rats, Journal Of Young Pharmacists, Volume: 3(1), P26-35.

Hajarnavis A M and Bulakh P M (2013). "Antioxidant Potential of *Emblica Officinalis* (Amla) Aqueous Extract Delays Cataractogenesis in Hyperglycemic Goat Lenses", Iosr Journal of Pharmacy and Biological Sciences, Volume: 6(1), P32-35.

Kalekar Sa, Munshi Rp And Kulkarni Dk (2014). Evaluation of The Anti-Cataract Potential Of *Tinospora Cordifollia* & *Phyllanthus Emblica* In An *In Vitro* Sugar Induced Cataracteric Lens Organ Culture Model, Internation Journal of Pharma And Bio Sciences, January 2014, 5(1): (P) 120-130.

Kanimozhi D. And Ratha Bai V. (2012). Evaluation of Antimicrobial Activity of *Cyanadon Dactylon*, International Journal of Research In Pharmacy And Science, Volume: 2(2), P34-43.

Kondaveeti Suresh Babu, Ivvala Anand Shaker, Dabburu Kumaraswamy, S. Saleem Basha and Inampudi Sailaja (2012). Indigenous Effect of *Cyanadon Dactylon* In Experimental Induced Ulcers And Gastric Secretions, International Research Journal Of Pharmacy, Volume: 3(5), P301-304.

Langade D G, Rao G, Girme R C, Patki P S and Bulakh P M (2006). "*In Vitro* Prevention By Ace Inhibitors Of Cataract Induced By Glucose", Indian Journal Of Pharmacology, Volume: 38(2), P107-110.

Moghaddam, M.S, P. A. Kumar, B. G. Reddy and V. S. Ghole (2005). "Effect of Diabecon on Sugar Induced Lens Opacity In Organ Culture: Mechanism Of Action", Journal of Ethnopharmocology, Volume: 97, P397 – 403.

M.Saroja, R.Santhi and S.Annapooran (2012). Antioxidant Potential of Ethylacetate Fraction Of *Cynodon* Dactylon Against Ela Implanted Swiss Albino Mice, International Journal Of Pharma And Bio Sciences, Volume: 3(2), P415-419.

Patel Dk, Prasad Sk, Kumar R And Hemalatha S (2011). Cataract: A Major Secondary Complication of Diabetes, Its Epidemiology And An Overview on Major Medicinal Plants Screened For Anticataract Activity. Asian Pacific Journal of Tropical Disease: P1-7.

Shabeer Ahmed N, Niyas Ahmed I, Ashar Waheed M P And Abdul Jaffar Ali H (2011). Anticataract Activity of Ethanolic Extract of *Nigella Sativa* on Glucose Induced Cataract in Goat Eye Lens, International Journal of Applied Biology and Pharmaceutical Technology, IJABPT-Volume: 2(4), P0976-4550.

Umamaheswari M, Dhinesh S, Sivashanmugam T, Subhadradevi V, Puliyath J, Madeswaran M (2012). "Anticataract and Antioxidant Activities of *Achyranthes Aspera* Linn. Against Glucose-Induced Cataractogenesis Using Goat Lenses, Journal of Natural Products and Plant Resources, Volume: 2 (1): P153-161.

Williamson, J., C. Kilo and R. G Tilton (1992). "Mechanism Of Glucose And Diabetes Induced Vascular Dysfunction. In: Hyperglycemia, Diabetes And Vascular Disease, Ruderman, N., J. Williamson And M. Brownlee (Eds.)." , American Physiology Society, New York, Usa., P107-132.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com