Research Journal of Pharmacology 10 (1): 1-5, 2016
ISSN: 1815-9362

Pharmacological Exploration of *Vernonia cinerea* Flower Extract on Experimental Cataract Using Isolated Goat Lenses

Iwin Thomas, K.R. Resmy and S.K. Kanthlal
Department of Pharmacology, Amrita School of Pharmacy,
Amrita Vishwa Vidyapeetham University, AIMS Health Science Campus, Kochi, Kerala, India

Abstract: The aim of the present study was to evaluate the methanol extract of *Vernonia cinerea* flowers for its *in vitro* anticataract activity on isolated goat lenses. Transparent isolated goat lenses were incubated in artificial aqueous humor for 72 h. Experimental catatact was induced by adding glucose at 55 mM and the methanol extracts *Vernonia cinerea* at the doses of 200 and 400 μg mL^{-1} were added simultaneously into the aqueous humor. After the incubation period photographic evaluation of lens was performed and biochemical estimation of electrolytes (Na^+ and K^+), total protein, malondialdehyde and lipid peroxidase were done in the lens homogenate. Photographic evaluation clearly indicates the suppression of cataract at 400 μg mL^{-1} of extract in which the lenses appears hazy and less cloudy. Cataractous lenses showed higher Na^+, MDA and LPH ($p<0.001$) and lowers the K^+ and total protein content. Lenses treated with *Vernonia cinerea* extract prevented the formation of cataract in a dose dependent manner as evidenced by the correcting the altered biochemical parameters. The anticataract activity of *Vernonia cinerea* may because of balancing antioxidant defense system either by direct antioxidant action or by free radical scavenging mechanism as evidenced by correcting MDA and LPH levels. Further, *in vivo* studies are required for confirmation and elucidation of molecular the role of *Vernonia cineria* in preventing cataract formation.

Key words: Anticataract, *Vernonia cinerea*, aqueous humor, antioxidant, India

INTRODUCTION

Cataract is the opacification or optical dysfunction of the crystalline lens, associated with the breakdown of the eye lens micro-architecture which interferes with transmission of light onto the retina (Moghaddam *et al*., 2005). It is often associated with old age and is a major complication of diabetes mellitus because higher glycosylated hemoglobin (Klein *et al*., 1997). There are many causative factors for cataract formation other than age-like nutritional deficiencies, environmental changes, radiation, metabolic diseases like diabetes and oxidative stress (Harding and Heyningen, 1987). It is a multifactorial disease occurs mainly due to formation of a large protein aggregates in the lens (Unakar and Tsui, 1983). It is leading cause of blindness worldwide. There are about 12 million blind people due to cataract in India alone (Minassian and Mehra, 1990). Aldose reductase the key enzyme of polyol pathway probably involved in the development of this eye problem. The impairment of lens Na^+/K^+-ATPase causes accumulation of Na^+ and loss K^+ with hydration and swelling of the lens fibers leading to cataractogenesis (Jung *et al*., 2011).

Vernonia cinerea (Linn) of family Astraceae a flowering common weed grown in roadsides, open waste places, dry grassy sites and habitat to almost all Asian countries. The herb has been traditionally used to treat a number of disorders including inflammation, malaria, insomnia, fever, pain, diuresis, cancer, abortion and various GI disorders (Sathyanathan *et al*., 2012). Various studies showed the presence of alkaloids, steroidal glycosides, triterpenoids and esters in a methanolic extract of stem bark roots and flowers (Rizvi *et al*., 2011).

Although, many people have used *Vernonia cinerea* in the traditional medicine for a long time, there are no scientific data regarding the anti cataract studies. Hence, the present study was carried out to establish the *in vitro* anti-cataract evaluation of methanol extract of *Vernonia cinerea* flowers on isolated goat lenses.

MATERIALS AND METHODS

Plant material: The flowers of *Vernonia cineria* were collected from Thrissur, Kerala during the month of

Corresponding Author: S.K. Kanthlal, Department of Pharmacology, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Science Campus, AIMS, Kochin, 682041 Kerala, India

August 2013. The plant was identified and authenticated by Dr. Elsamma Joseph Arackal, Associate Professor and HOD, Department of Botany, Maharajas College, Ernakulum and certified as *Vernonia cinerea* (Linn) of family Astraceae from available literature.

Preparation of plant extract: The shade dried powdered plant material was successfully extracted with methanol for 48 h at 55-65°C. The extract was filtered and concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness at room temperature. Then the extract was stored in the desiccators and used for subsequent experiment.

Capsular extraction of goat lens: Fresh eye balls were obtained from the slaughter house and lenses were removed with cataract knife by intercapsular lens extraction method. Lens were weighed immediately and placed in sterile tissue culture dish and maintained in isotonic saline solution and pH was carefully maintained at 7.2. Saline was changed at fine intervals. Lenses were observed for development of generalized opacification and cell disruption (Chandorkar *et al*., 1983).

Preparation of lens culture: The extracted lenses were incubated in artificial aqueous humor (NaCl: 140 mM, KCl: 5 mM, $MgCl_2$: 2 mM, NaHCO3: 0.5 mM, $NaH(PO_4)_2$: 0.5 mM, $CaCl_2$:0.4 mM and Glucose 5.5 mM) at 37°C and pH 7.8 for 72 h. Penicillin 32% mg and streptomycin 250% mg were added to the culture media to prevent bacterial contamination. Glucose at a concentration of 55 mM was used to induce experimental cataract (Chandorkar *et al*., 1981).

Experimental design: Anticataract study was carried out with Methanol Extract of *Vernonia Cineria* flower (MEVC) at two different dose levels (200, 400 μg mL^{-1}). A total of 20 goat lenses were used and divided into four experimental groups consisting of 5 in each group:

- Group I: lens in artificial aqueous humor (normal control)
- Group II: normal goat Lens+ Glucose 55mM (cataract control)
- Group III: MEVC (200 μg mL^{-1})+Glucose (55 mM)
- Group IV: MEVC (400 μg mL^{-1})+Glucose (55mM)

The lenses were incubated at 37°C and pH 7.8 for 72 h and the following parameters were evaluated.

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

Biochemical estimation: Lenses were homogenized in Tris buffer (0.23M pH 7.8) and 0.25X10-3 M EDTA. The homogenate was adjusted to 10% W/V. The homogenate was centrifuged at 10000 g at 4°C for 1 h. The supernatant was used for biochemical estimations.

Electrolytes (Na^+ and K^+) estimation was done by flame photometry, total protein by Lowry's method and the degree of oxidative stress was assessed by the evaluation of Malondialdehyde (MDA) and Lipid Peroxidase (LPH) (Lowry *et al*., 1951; Uchiyama and Mihara, 1978; Ohkawa *et al*., 1979).

Statistical analysis: All data expressed as Mean±SEM. The statistical significance between groups compared using one way ANOVA followed by Tukey's test. The $p<0.05$ was considered as significant. Statistical variations are compared as follows: normal control with cataract control and cataract control with treated groups.

RESULTS

Photographic visualization: Transparency was maintained in normal group lenses after 72 h of incubation in aqueous humor. Incubation of lenses with glucose 55mM showed a complete loss of transparency after 72 h indicating the cataractogenisis. The lenses which incubated in glucose 55mM aqueous humor with MEVC 400 μg mL^{-1} appeared less hazy and grids were visible which clearly indicates the suppression of cataract formation (Fig. 1).

Biochemical parameters: Lens incubated aqueous humor with glucose (55 mM) showed a significant increase ($p<0.001$) in Na^+ level and lowers the K^+ level ($p<0.001$) when compared with control group. MEVC treated groups showed a mild significant reversal ($p<0.05$) at 200 μg mL^{-1} and a highly significant ($p<0.001$) action at 400 μg mL^{-1} dose level (Table 1).

Glucose (55 mM) treated lenses showed significantly, low concentrations of total proteins and LPH in the

Table 1: Effect of MEVC on Na^+ and K^+ levels in lens homogenate after 72 h of incubation

Groups	Treatment	Na^+(mEq g^{-1})	K^+(mEq g^{-1})
I	Normal control	150.15±2.53	10.45±0.82
II	Cataract control	219.53±8.0***	6.08±0.29***
III	MEVC (200 μg)	198.51±4.16*	8.97±0.42*
IV	MEVC (400 μg)	186.17±3.33**	9.89±0.57**

Values are Mean±SEM (n = 5) statistically significant at *, **, ***$p<0.05$, 0.01, 0.001. Group II compared with group I and Groups III and IV were compared with group II

Table 2: Effect of MEVC on total protein, MDA and LPH levels in lens homogenate after 72 h of incubation

Groups	Treatment	Total protein (mg gm of lens)	MDA (nmoles/mg of lens)	LPH (nm/gm of lens)
I	Normal control	229.92± 4.32	7.27±0.38	3.86±0.57
II	Cataract control	159.42±3.69***	42.52±2.54***	8.63±0.53***
III	MEVC (200 μg)	181.93±3.06**	32.42±2.16**	7.11±0.34
IV	MEVC (400 μg)	201.45±4.70***	22.98±1.31***	5.27±0.43***

Values are Mean±SEM (n = 5) statistically significant at *, ** ,**p<0.05, 0.01, 0.001. Group II compared with group I and Groups III and IV were compared with group II

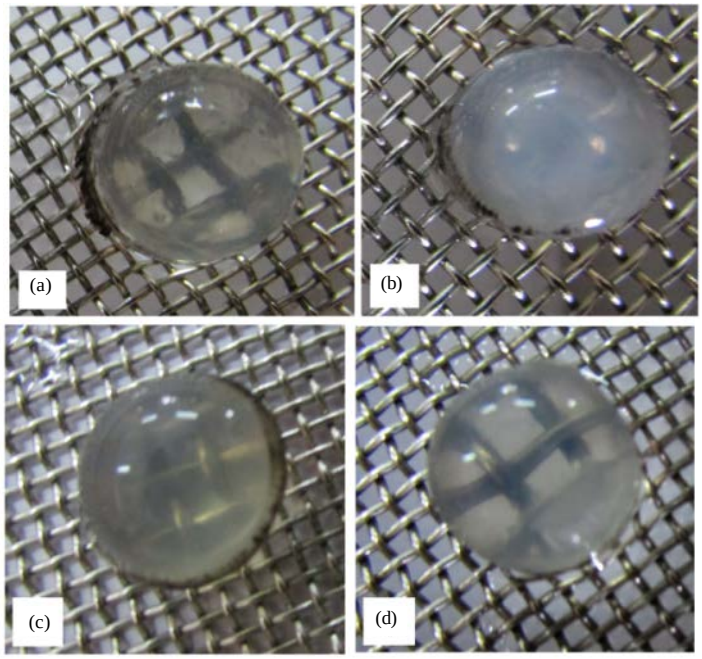

Fig. 1: Photograph showing clear transparent normal lens: a) After 72 h incubation. A complete loss of transparency in cataract control; b) Showing the induction of cataract. Lens appears slightly hazy in MEVC 200 μg groups; c) Very less hazy and wires were clearly visible in MEVC 400 μg treated lenses; d) Indicating the suppression of cataract 11

homogenate ($p<0.001$) compared with control groups in normal aqueous humor. MEVC treated group had significantly higher concentrations of total proteins and LPH at 400 μg mL^{-1} concentration ($p<0.001$), compared with cataract control group. MDA levels also found to be very high in glucose 55 mM treated lenses. There was a dose dependent significant reduction ($p<0.01$, 0.001) of MDA content in MEVC treated groups at 200 and 400 μg mL^{-1}, respectively (Table 2).

DISCUSSION

Cataract is one of the universal processes of ageing and is consequence of cumulative effect of various insults to the lens. 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 (Minassian and Mehra, 1990).

In vitro model for inducing cataract using glucose concentration 55 mM provides an effective model on isolated lenses of goat. Incubation of lenses in the artificial aqueous homor containing high glucose (55 mM) concentration has shown to cause considerable drop in Na^+/K^+-ATPase activity (Unakar and Tsui, 1980). Impairment of Na^+/K^+-ATPase causes an imbalance in ionic equilibrium in the lens which causes accumulation of Na^+ and a considerable K^+ loss with hydration causes swelling of lens fibers leading to cataractogenisis (Chylack and Kinoshita, 1969). The alteration in these ionic levels (both Na^+ and K^+) is the major cause for the reduction of soluble proteins content in the lens which leads to the opacification (Shinohara and Piatigorsky, 1977; Kumar, 2011). This study showed that MEVC seem to prevent the alteration of ionic imbalance which may be due to the direct effect on lens membrane Na^+/K^+-ATPase or indirect effect through other mechanisms.

Apart from the above ionic imbalance, oxidative stress may also be implicated in the cataract induced by glucose, due to the formation of superoxide radicals and H_2O_2. High glucose (55 mM) has shown to induce antioxidant enzymes, suggesting oxidative stress in the cells (Rizvi *et al*., 2011). Hence, estimation of Malondialdehyde (MDA) and Lipid Peroxidase (LPO) will reveals the antioxidative effects of *Vernonia cineria* on induce cataract. Lipid Peroxidation (LPO) is considered a pathogenetic factor of cataractogenesis. Since, the cell membranes have lipoprotein structure, these are the most common substrates of oxidative attacks. Structural changes of the cell membrane and its increased permeability change the cell volume and the configuration of the lens, leading to refractory changes that are associated with the early cataract (Stark, 2005; Halliwell and Chirico, 1993). The elevated MDA seen in cataract can react with amino-groups of proteins and thus affect the structural and functional properties of lens proteins and inhibits the thiol dependent enzymes such as glucose-6-phosphatase and Na^+/K^+-ATPase, etc. (Choudhary *et al*., 2003; Berlett and Stadtman, 1997). In the present study, MEVC corrected the elevated levels of MDA and LPH significantly reveals that the antioxidant defense system may balanced either by direct antioxidant action or by free radical scavenging mechanism. From the present study, it had been reported that methanol extract of *Vernonia cineria* at the dose of 400 μg mL^{-1} decreases the lens opacification along with the correction of altered biochemical parameters reveals the prevention of cataract.

CONCLUSION

Incubation of lenses in high glucose (55 mM) concentration stimulates a state of clinical model of cataract. A prevention role of *Vernonia cineria* as seen in this *in vitro* model may to some extent suggest in preventing and/or retarding the progression of diabetic and other diseases related cataracts. This study may not directly correlate with the *in vivo* conditions. Therefore, *in vivo* studies in different animal models are required for further confirmation and elucidation of molecular the role of *Vernonia cineria* in preventing cataract formation.

ACKNOWLEDGEMENTS

Researchers are grateful to Dr. Elsamma Joseph Arackal for authentication of plant species and Dr. Umadevi P, Mr. Anilkumar B and Mr. Jipnomon Joseph for their wise advice and encouragements.

REFERENCES

Berlett, B.S. and E.R. Stadtman, 1997. Protein oxidation in aging, disease and oxidative stress. J. Biol. Chem., 272: 20313-20316.

Chandorkar, A.G., M.V. Albal, P.M. Bulakh and M.P. Muley, 1981. Lens Organ Culture. Indian J. Opthamol., 29: 151-152.

Chandorkar, A.G., M.V. Albal, P.M. Bulakh and V.V. Gumaste, 1983. Electrolyte pattern in normal goat lenses and its alteration in lens culture during cataractogenesis. Indian J. Ophthalmol., 31: 262-264.

Choudhary, S., S. Srivastava, T. Xiao, U.P. Andley, S.K. Srivastava and N.H. Ansari, 2003. Metabolism of lipid derived aldehyde, 4-hydroxynonenal in human lens epithelial cells and rat lens. Invest. Ophthalmol. Visual. Sci., 44: 2675-2682.

Chylack, L.T. and J.H. Kinoshita, 1969. A biochemical evaluation of a cataract induced in a high-glucose medium. Invest. Ophthalmol. Visual Sci., 8: 401-412.

Halliwell, B. and S. Chirico, 1993. Lipid peroxidation: Its mechanism, measurement and significance. Am. J. Clin. Nutr., 57: 715S-724S.

Harding, J.J. and R.V. Heyningen, 1987. Epidemiology and risk factors for cataract. Eye, 1: 537-541.

Jung, H.A., M.N. Islam, Y.S. Kwon, S.E. Jin and Y.K. Son *et al*., 2011. Extraction and identification of three major aldose reductase inhibitors from *Artemisia montana*. Food Chem. Toxicol., 49: 376-384.

Klein, B.E., R. Klein and K.E. Lee, 1997. Cardiovascular disease, selected cardiovascular disease risk factors and age-related cataracts: The beaver dam eye study. Am. J. Ophthalmol., 123: 338-346.

Kumar, M., 2011. *In vitro* anticataract activity of *Zingiber officinale* on goat lenses. Int. J. Pharm. Biol. Arch., 2: 1430-1433.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein easurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Minassian, D.C. and V. Mehra, 1990. 3.8 million blinded by cataract each year: Projections from the first epidemiological study of incidence of cataract blindness in India. Br. J. Ophthalmol., 74: 341-343.

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. J. Ethnopharmacol., 97: 397-403.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Rizvi, S.M.D., D. Biswas, J.M. Arif and M. Zeeshan, 2011. *In-vitro* antibacterial and antioxidant potential of leaf and flower extracts of *Vernonia cinerea* and their phytochemical constituents. Int. J. Pharm. Sci. Rev. Res., 9: 164-169.

Sathyanathan, V., A.K. Eswar, M.S. Babu, I. Rizwana and F. Fatima, 2012. Sedative-hypnotic activity on whole plant extract of *Vernonia cinerea* (Linn.) less. Int. J. Res. Pharm. Pharmacotherapeutics, 1: 169-171.

Stark, G., 2005. Functional consequences of oxidative membrane damage. J. Membr. Biol., 205: 1-16.

Uchiyama, M. and M. Mihara, 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 86: 271-278.

Unakar, N.J. and J.Y. Tsui, 1980. Measurment of Na^+ K^+ ATPase activity. Invest. Opth. Vis. Sci., 19: 378-385.

Unakar, N.J. and J.Y. Tsui, 1983. Inhibition of galactose induced alteration in ocular lens with sorbinil. Exp. Eye Res., 36: 685-694.