Available online at www.scholarsresearchlibrary.com

Scholars Research Library

J. Nat. Prod. Plant Resour., 2012, 2 (1): 153-161
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Anticataract and antioxidant activities of *Achyranthes aspera* Linn. against glucose-induced cataractogenesis using goat lenses

Muthuswamy Umamaheswari*, Sundaram Dhinesh, Thirumalaiswamy Sivashanmugam, Varadharajan Subhadradevi, Jagannath Puliyath, Arumugam Madeswaran

Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, Tamilnadu, India

---

ABSTRACT

The present study evaluated the in vitro anticataract and antioxidant activities of aqueous leaves extract of Achyranthes aspera Linn. against glucose-induced cataractogenesis using goat lenses. Transparent isolated goat lenes were incubated in artificial aqueous humor and divided into five experimental groups. The extracts at a dose of 100 µg/ml and 200 µg/ml were incubated simultaneously with glucose (55 mM) for a period of 72 h. Vitamin E (100 µg/ml) was used as the standard drug. At the end of the incubation, levels of various biochemical parameters such as protein content, malondialdehyde (MDA), lipid hydroperoxides (LH), Cu^{2+}-induced lipoprotein diene formation, Ca^{2+}ATPase and enzymatic antioxidants like catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GSSH) and non-enzymatic antioxidant like reduced glutathione (GSH) were measured in the lens homogenate. Incubation with glucose produced a mature cataract and there was a significant increase in LH and MDA and a decrease in protein content, Ca^{2+}ATPase, Cu^{2+}-induced lipoprotein diene formation and enzymatic and non-enzymatic antioxidants when compared to normal control. Our results indicated that simultaneous incubation of the plant extracts prevented the preoxidative damage caused by glucose, which is evidenced from the improved antioxidant potential. The aqueous leaves extract of Achyranthes aspera protected the lens against glucose-induced oxidative damage which might be helpful in delaying the progression of cataract.

Key words: antioxidant; cataract; glucose; *Achyranthes aspera.*

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. It is the most important cause of blindness worldwide. Several biochemical processes

such as oxidative stress, altered epithelial metabolism, calcium accumulation, calpain-induced proteolysis, crystalline precipitation, phase transition, and cytoskeletal loss occur during the development of cataract [1].

Cataract is a major cause of blindness worldwide and more so in developing countries. Surgery is the only effective treatment for cataract and the exact mechanism is not clear. Although the surgery is recognized as being one of the safest procedure, there are significant rate of complications, leading to irreversibly blind eyes. Pharmacological interventions to inhibit or delay lens opacification is yet at experimental stage. Studies are being conducted to explore the mechanism of cataractogenesis using various models of cataract and to target crucial steps to stop this process. Limitations in acceptability, accessibility and affordability of cataract surgical services make it more relevant and important to look into alternative pharmacological measures for treatment of this disorder Thus, much eagerness is being laid on identification of natural compounds that will help to prevent cataractogenesis [2].

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. The overall antioxidant status of the lens decreases because of depletion of glutathione (GSH) [3].

Oxidative stress may also be implicated in the cataract induced by glucose and age related process due to the formation of superoxide ($O_2^{\cdot-}$) radicals and H_2O_2 because of these free radicles are readily react with the biomolecules [3]. The toxic effects of the reactive oxygen species are neutralized in the lens by antioxidants such as ascorbic acid, vitamin E, the glutathione system (GSH peroxidase, GSH reductase), superoxide dismutase and catalase. The enzymatic (superoxide dismutase,glutathione peroxidase, catalase) and non-enzymatic (ascorbate, glutathione, cysteine) antioxidant system activities are decreased in the lens and aqueous humor during aging and in the development of cataract [4].

Acyranthus aspera Linn. belonging to the family Amaranthaceae is a shrub widely distributed in southern region of India. The leaves are reported to possess alkaloids, flavonoids, saponins, tannins and phenolic compounds. The leaves of this species are used in Indian traditional medicine for the treatment of ophthalmic and other eye infections, it also has antifertility, nephroprotective, post-coital antifertility, antioxidant, reduce inflammatory swellings, nephroprotective and immunomodulatory activities [5]. Various studies have been reported for its hypoglycaemic effect [6] anti cancer [7] anti fungal [8] and increase the thyroid hormone levels [9].

The main objective of the study was to evaluate the aqueous extract of leaves of *Acyranthus aspera* Linn. for its *in vitro* anticataract and antioxidant activities against glucose-induced cataractogenesis using goat lenses.

MATERIALS AND METHODS

Drugs and chemicals

Glucose and vitamin E were obtained from SD fine chemicals, Mumbai. 1-amino 2-naphthol-4-sulfonic acid, 5, 5-dithiobis-(-2-nitrobenzoic acid) (DTNB), Nitro blue tetrazolium chloride (NBT), Nicotinamide adenine dinucleotide reduced salt (NADH), Nicotinamide adenine dinucleotide phosphate reduced tetra sodium salt (NADPH), Oxidised glutathione, Reduced glutathione, Adenosine-5'-triphophate (ATP) were obtained from Himedia Laboratories Ltd., Mumbai. Fresh goat lenses were obtained from the slaughterhouse, Coimbatore. All other chemicals used in the study were obtained commercially and were of analytical grade.

Plant collection and authentication

The leaves of *Acyranthus aspera* Linn. were collected from Pachai hills, Salem, Tamilnadu, India during the month of June 2010. The plant was identified and authenticated by Mr. G.V.S. Murthy, Joint Director, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference number BSI/SRC/5/23/10-11/Tech-595.

Plant extract

Achyranthes aspera aqueous leaf extract

The fresh leaves were shade dried, powdered and 25 g of the powder was soaked separately with 250 ml of sterile distilled water and kept at room temperature for 24 hrs and macerated using a mechanical shaker for 4 h. The extract was filtered through muslin cloth and the marc was again soaked with the same volume of water for 12 h and then further extracted using a mechanical shaker for 4h and filtered. The filtrates were then combined concentrated at 40°C [9]. The *Achyranthes aspera* Linn. leaves extract was abbreviated as ALE.

Glucose-induced cataract

Fresh goat eyeballs were obtained from a local slaughterhouse within two hours after killing of the animals and the lenses were isolated. The isolated lens were incubated in artificial aqueous humor at 37ºC and pH 7.8 for 72 h. Glucose at a concentration of 55 mM was used to induce cataract [10, 11].

A total of 30 goat lenses were used and divided into five experimental groups consisting of 6 in each group.

Group I : Artificial aqueous humor alone (solvent control)
Group II: Glucose 55 mM alone (negative control)
Group III: ALE (100 µg/ml) + Glucose 55 mM
Group IV: ALE (200 µg/ml) + Glucose 55 mM
Group V: Vitamin E (100 µg/ml) + Glucose 55 mM

Examination of lens opacity

To study the opacity of the lens, the lenses from the control and experimental groups were placed on a wire mesh and photographed [12].

Preparation of lens homogenate

After incubation, lenses were homogenized in 10 volumes of 0.1M potassium phosphate buffer, pH 7.0. The homogenate was centrifuged at 10,000 rpm for 1 h and the supernatant was used for estimation of biochemical parameters [13].

Biochemical parameters

Estimation of total protein content

To 0.1 ml of lens homogenate, 4.0ml of alkaline copper solution was added and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for colour 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 bovin serum albumin and expressed as µg/mg lens tissue [14].

Estimation of lipid hydroperoxides (LH)

About 0.1ml of lens homogenate was treated with 0.9 ml of Fox reagent (188 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 ammonium ion sulphate were added to 90 ml of methanol and 10ml 250 mM sulphuric acid) and incubated for 3 min. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/ mg lens protein [15].

Estimation 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 1000g for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The values are expressed as nmoles of MDA/ min/ mg lens protein [16].

Inhibition of Cu^{2+} induced lipoprotein diene formation

Lens homogenate was diluted to 0.67% in phosphate buffered saline. Control experiments consisted of identical assay conditions but without the sample. Oxidation was initiated immediately after addition of sample by the addition of 12 µM final concentration of Cu^{2+} added as CuSO4_5H2O dissolved in deionized distilled water. Oxidation was determined by measuring the absorbance at 234 nm using a UV–Visible Spectrophotometer. Absorbance was taken after 120 mins at 37ºC.The lipoprotein diene formation was measured from the absorbance at a time. The absorbance provides an indication of protection of tissue lipoprotein against oxidation [16].

Assay of Ca^{2+}-ATPase activity

To added 0.1 ml of the lens homogenate (10% (w/ v) in 0.25 M sucrose) and add 0.2 ml of the substrate, ATP. The tubes were incubated for 30 min in a water bath at 37ºC. The enzyme activity is stopped by adding 2 ml of 10% TCA. Then 0.2 ml of ATP is added and the same kept in ice for 20 min. All the tubes were then centrifuged at 2500 rpm for 10 min and the supernatant collected. The protein free supernatant was analyzed for inorganic phosphate. For that 3 ml of the supernatant was treated with 1 ml of ammonium molybdate and 0.4 ml 1-amino-2-naphthol-

4-sulfonic acid (ANSA). The colour developed was read at 680 nm after 20 min and the inorganic phosphate value expressed as nm three parallel experiments were conducted [17].

Determination of enzymatic antioxidants

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 (lens were homogenized in10% (w/v) 0.25 M sucrose buffer) 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 stirred vigorously. 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 taken out and the absorbance was measured at 560 nm against a butanol blank. A system devoid of enzyme served as the control and three parallel experiments were conducted [18].

Assay of catalase (CAT)

The reaction mixture contained 2.0 ml of homogenate (lens were homogenized in 10% (w/v) 50 mM phosphate buffer, pH 7.0) and 1.0 ml of 30 mM hydrogen peroxide (in 50 mM phosphate buffer, pH 7.0). A system devoid of the substrate (hydrogen peroxide) served as the control. Reaction was started by the addition of the substrate and decrease in absorbance monitored at 240 nm for 30 s at 25ºC. The difference in absorbance per unit time was expressed as the activity and three parallel experiments were conducted. One unit is defined as the amount of enzyme required to decompose 1.0 M of hydrogen peroxide per minute at pH 7.0 and 25ºC [19].

Estimation of glutatione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidised glutathione and 0.1 ml (10 mg/ml) of bovine serum albumin (BSA). The reaction was started by the addition of 0.02 ml of lens homogenate with mixing and the decrease in absorbance at 340 nm was measured for 3 min against a blank. Glutathione activity was expressed as nmoles NADPH oxidized/min/ mg lens protein at 30ºC [20].

Estimation of glutatione peroxidise (GPx)

The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2ml of lens homogenate supernatant incubated at 37ºC for 10 min. The reaction was arrested by the addition of 10% TCA and the absorbance was taken at 340 nm. Activity was expressed as nmoles/min/mg lens protein [21].

Determination of non enzymatic antioxidant:

Estimation of glutathione (GSH)

Lenses were homogenized in10% (w/v) cold 20 mM EDTA solution on ice. After deproteinization with 5% TCA, an aliquot of the supernatant was allowed to react with 150 µM DTNB [5, 5-dithiobis-(-2-nitrobenzoic acid)]. The product was detected and quantified spectrophotometrically at 416 nm. Pure GSH was used as standard for establishing the calibration curve and three parallel experiments were conducted [22].

Statistical analysis

Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM of six lenses in each group. P values < 0.05 were considered significant.

RESULTS

Phytochemical screening

The aqueous leaf extract of *Achyranthes aspera* Linn. was subjected to the phytochemical screening and for the presence of various phytochemical constituents.

Table 1. Phytochemical screening

Phytochemicals	*Achyranthes aspera* L.
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins and phenolics	+
Terpenoids	+

Photographs of lenses in normal and experimental groups incubated with glucose are shown in Fig 1(a-g).

Fig 1a shows the normal lens incubated with artificial aqueous humor showing complete transparency compared with the experimental groups. **Fig 1b** is the lens incubated with glucose (55 mM) for a period of 72 hrs showing complete opacification of the lens fibres compared to normal control. **Fig 1c & d** are the lenses incubated simultaneously with glucose (55 mM) and ALE at a concentration of 100 and 200 µg/ml respectively, showing a decrease in opacity compared to cataractous lenses. **Fig 1e** is the lens incubated with glucose (55 mM) and vitamin E (100 µg/ml) showing almost normal transparency when compared to catatactous lenses.

Table 2. Effect of the aqueous leaf extract of *Achyranthes aspera* Linn. on lens protein, MDA, LH, Ca^{2+}ATPase and Cu^{2+} induced lipoprotein diene in control and experimental groups

GROUP	**Protein** (mmoles/min/ mg lens tissue)	**MDA** (µmoles/min/mg protein)	**LH** (nmoles/min/mg protein)	**Ca^{2+}ATPase** (µmoles/min/mg inorganic phosphate)	**Cu^{2+}induced Lipoprotein diene** absorbance
Normal control	97.84±2.9	0.198±0.04	6.29±0.32	5.11±0.39	0.6862±0.05
Glucose control (55 mM)	54.35±2.71[a]	0.690±0.15[a]	13.87±1.31[a]	1.61±0.23[a]	0.1565±0.02[a]
ALE (100 µg/ml)	70.73±4.28[b]	0.311±0.07[b]	9.28±1.58[b]	4.39±0.42[c]	0.5041±0.03[c]
ALE (200 µg/ml)	75.77±4.21[c]	0.299±0.05[c]	8.79±0.73[c]	4.73±0.26[c]	0.5424±0.06[c]
Vitamin-E (100 µg/ml)	84.41±3.18[c]	0.243±0.05[c]	7.82±1.03[c]	4.98±0.42[c]	0.6244±0.06[c]

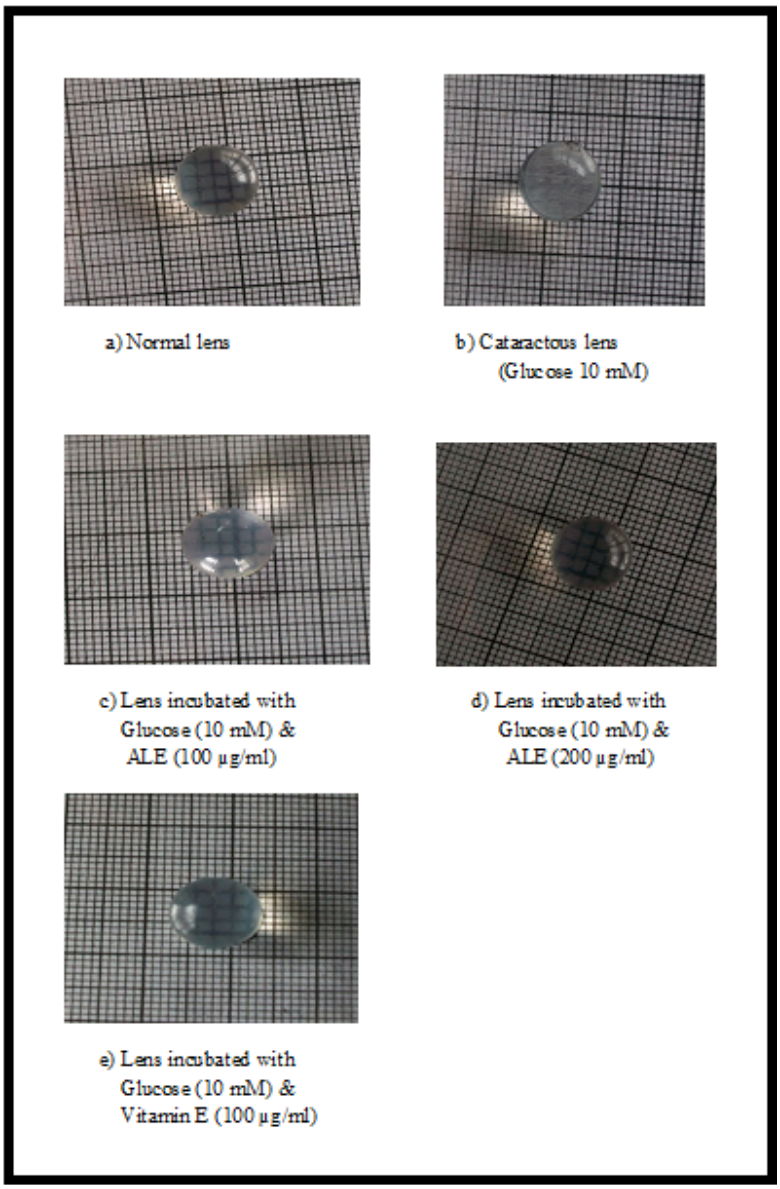
Values are mean ± SEM; n=6 in each; [a]P <0.01 when compared to normal control; [b]P<0.05, [c]p<0.01 when compared to glucose control (one way ANOVA followed by Dunnett's test).

Effect of the aqueous leaf extract of *Achyranthes aspera* Linn. on lens protein and lipid peroxidation in control and experimental groups

There was a significant (P<0.01) decrease in the level of total protein, Ca^{2+}ATPase, Cu^{2+}induced lipoprotein diene formation and an increase in the level of malondialdehyde and lipid hydroperoxides in glucose-induced cataractous lenses when compared to normal control.

Incubation with the aqueous leaf extract of *Achyranthes aspera* at dose of 100 & 200 µg/ml and Vitamin E (100 µg/ml) simultaneously with glucose for 72 h caused a significant ($P<0.05$) increase in the total protein, Ca^{2+}ATPase, Cu^{2+}induced lipoprotein diene formation and a decrease in the level of malondialdehyde and lipid hydroperoxides (Table 2).

Fig 1. Photographs of lenses in normal and experimental groups incubated with glucose



3.4. Effect of the aqueous leaf extract of *Achyranthes aspera* Linn. *on* lens enzymatic and non enzymatic antioxidants in control and experimental groups

Incubation with glucose 55 mM for 72 h produced a significant ($P<0.01$) decrease in the enzymatic antioxidants like catalase, superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the lens

homogenate when compared to normal control. Incubation with the aqueous leaf extract of *Achyranthes aspera* at doses of (100 & 200 µg/ml) and Vitamin E simultaneously with glucose significantly (P<0.01) restored the levels of both enzymatic and non enzymatic antioxidant enzymes which is almost similar to the control group (Table 3).

Table 3. Effect of the aqueous leaf extract of *Achyranthes aspera* Linn. on lens enzymatic and non enzymatic antioxidants in control and experimental groups

GROUP	Catalase (mmoles/min/ mg protein)	GPx (µmoles/min/ mg protein)	SOD (µmoles/min/ mg protein)	GSSH (µmoles/min/ mg protein)	GSH (µmoles/min/ mg protein)
Normal control	2.34±0.16	4.06±0.19	4.96±0.15	3.03±0.09	3.77±0.38
Glucose control (55 mM)	0.768±0.07[a]	1.78±0.05[a]	2.08±0.24[a]	1.22±0.07[a]	1.38±0.16[a]
ALE (100 µg/ml)	1.65±0.18[b]	2.89±0.26[b]	3.57±0.33[b]	2.17±0.13[b]	2.97±0.25[b]
ALE (200 µg/ml)	1.87±0.15[b]	3.55±0.34[b]	4.18±0.18[b]	2.69±0.24[b]	3.15±0.24[b]
Vitamin-E (100 µg/ml)	1.97±0.12[b]	3.88±0.25[b]	4.85±0.21[b]	2.93±0.16[b]	3.39±0.12[b]

Values are mean ± SEM; n=6 in each; [a]P <0.01 when compared to normal control; [b]P<0.01 when compared to glucose control (one way ANOVA followed by Dunnett's test).

CONCLUSION

To conclude, the study suggested that the *Achyranthes aspera* L. leaves 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 seeds and leaves may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

REFERENCES

[1] M.S. Moghaddam, A.P. Kumar, G.B. Reddy, V.S. Ghole, *J. Ethnopharmacol*, **2005**, 97, 397.
[2] R. Manikandan, R. Thiagarajan, S. Beulaja, S. Chindhu, K. Mariammal, G. Sudhandiran, M. Arumugam, *Chem. Biol. Inter.* **2009**, 181, 202.
[3] Z. Kyselova, M. Stefek, V. Bauer, *J Diab. & its Compl.*, **2004**, 18, 129.
[4] D. Ozmen, E. Erkin, I. Guner, S. Habil, O. Bayindir, *Turkish J. Endocrin.& Metab.*, **2000**, 1, 1.
[5] M. Umamaheshwari, T. K. Chatterjee, *Inter. J. Biomed. & Pharm. Sci.*, **2008**, 2, 70.
[6] M. S. Akhtar, J. Iqbal, *J. Ethnopharmacol.*, **1991**, 3, 49.
[7] A. Chakraborty, A. Brantner, T. Mukainaka, Y. Nobukuni, H. Tokuda, H. Nishino, *Cancer letters*, **2002**, 177, 1.
[8] E.K. Elumalai, N. Chandrasekaran, T. Thirumalai, C. Sivakumar, V.S. Therasa, E. David, *Int. J. Pharmtech Res.*, **2009**, 1, 1576.
[9] P. Tahiliani, K. Anand, *J. Ethnopharmacol.*, **2000**, 71, 527.
[10] S.K. Gupta, *Drug screening methods*, **2004**, 1, 333.
[11] D.G. Langade, G. Rao, R.C. Grime, P.S. Patki, P.M. Bulakh, *Indian Journal of Pharmacology*, **2006**, 38, 107.
[12] J. Sanderson, J.M. Marcantonio, G.A. Duncan, *Invest. Ophthal. & Vis. Sci.*, **2000**, 41, 2255.
[13] H. Son, H. Kim, Y.H. Kwon, *J. Nut. Sci. Vit.*, **2007**, 53, 324.

[14] O.H. Lowry, N.J. Rosenbourgh, A.L. Farr, R.J. Randall, *J. Biol. Chem.*, **1951**, 193, 265.
[15] W.G. Niehaus, B. Samuelsson, *Eur. J. Biochem.*, **1968**, 6, 126.
[16] J.M. Hodyson, J.M.P. Foot, K.D. Croft, I.B. Puddy, T.A. Mori, L.J. Beilin, *J. Sci. of Food & Agri.*, **1999**, 79, 561.
[17] G. Rorive, A. Kleinzeller, *Methods Enzymol.*, **1974**, 32, 303.
[18] P. Kakkar, B. Das, P.N. Viswanathan, *Ind. J. Biochem.& Biophy.* 1984, 21, 130.
[19] H. Aebi, *Methods Enzymol.*,**1984**, 105, 121.
[20] I. Carlberg, B. Mannervik, *Methods Enzymol.*, **1985**, 113, 484.
[21] A.L. Tappel, *Methods Enzymol.*, **1978**, 52, 506.
[22] G.L. Ellman, *Arch bio-chem biophys* **1959**, 82, 70.