



ANTICATARACT ACTIVITY OF *ACORUS CALAMUS* LINN. AGAINST HYDROGEN PEROXIDE INDUCED CATARACTOGENESIS IN GOAT EYES

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Accepted on: 30-08-2011; Finalized on: 30-11-2011.

ABSTRACT

The anticataract activity of *Acorus calamus* roots extract against induced hydrogen peroxide cataractogenesis was assessed in Goat lenses. The *in vitro* phase of study was performed on lenses from Goat eyes incubated for 52 h at 37°C in Dulbecco Modified Eagle Medium (DMEM) containing H₂O₂ (0.5 mM) (Group I), in DMEM containing H₂O₂ (0.5 mM) and methanolic extract of *Acorus calamus* (MEAC), (Group II), in DMEM containing H₂O₂ (0.5 mM) and isolated compound (β asarone), (Group III), in DMEM containing H₂O₂ (0.5 mM) and standard drug (Catlon and Tobramycin) (Group IV). Gross morphological examination of these lenses revealed dense opacification (cataract formation) in Group I. At the end of the experiment, the biochemical study was performed for estimation of MDA, GSH and proteins. The data suggest that methanolic extract of *Acorus calamus* (MEAC), isolated compound (β asarone) and standard drug (Catlon and Tobramycin) are able to significantly retard experimental hydrogen peroxide induced cataractogenesis.

Keywords: Cataractogenesis, Hydrogen peroxide, Methanolic extract *Acorus calamus*, MDA, GSH and protein estimation.

INTRODUCTION

Cataract is the leading cause of blindness worldwide. Several risk factors have been identified for the development of human cataract which includes: aging, diabetes, diarrhea, malnutrition, poverty, sunlight, smoking, hypertension and renal failure.¹ Although cataract is a multifactorial disease, oxidative stress has been identified as an initiating factor for the development of cataract.²

The oxidizing agent, hydrogen peroxide (H₂O₂) is present in normal aqueous humor at concentrations of approximately 20-30 μ M and is reported to be raised (up to 660 μ M) in patient with cataract.³ In *in vitro* lens culture experiments, Hydrogen peroxide at these higher concentrations causes lens opacification and produces a pattern of oxidative damage similar to that found in human cataract.⁴ Evidence suggests that Hydrogen peroxide is likely to be a major oxidant involved in cataract formation in man. Physiological antioxidants such as pyruvate⁵ and nutritional antioxidants such as ascorbate, flavonoids, vitamin E and carotenoids are reported to delay the development of experimental cataract.⁶

Acorus calamus belonging to the family Araceae is a small genus of herbs of the monocotyledonous; the plant is found in the northern temperate and subtropical region of Asia, North America and Europe. Various therapeutic potentials have been attributed to *Acorus calamus* in the traditional system of medicine as well as in folk fore practices.⁷ The plant is known to be effective as stimulant, emetic, expectorant, aphrodisiac, anthelmintic, epilepsy, memory disorders, homeostatic⁷, antioxidants⁸ and skin diseases⁹ In this study the effect of methanolic extract

and isolated compound of *Acorus calamus* was assessed against H₂O₂ (0.5 mM) induced opacity in goat lenses.

MATERIALS AND METHODS

Animals

Goat lenses were obtained from the freshly frozen eye balls transported immediately from the slaughter house. They were dissected out carefully and placed in a sterile tissue culture dish having the DMEM.

Collection and authentication of plant material

The plant material was collected from Haridwar, Uttarakhand, India. The plant was identified and authenticated by Dr. K. Pradheep, Senior Scientist at National Bureau of Plant Genetic Resources (ICAR) New Delhi. A voucher specimen no. NHCP/NBPGR/2010-36/ is deposited in the herbarium.

Extraction

The powdered *Acorus calamus* roots material was extracted with petroleum ether and methanol by soxhlet extraction method. The filtrate was evaporated using rotary vacuum evaporator under reduced pressure <10 mm Hg.

Preliminary phytochemical screening

Preliminary phytochemical screening were carried out to find the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, sesquiterpenes, fixed oils and fats.

Standard drugs: Catlon, Tobramycin



Effect of extract on H₂O₂ (Hydrogen peroxide) (oxidative stress) 0.5 mM induced cataract in goat lens.

The *in vitro* protective effects of *Acorus calamus* against hydrogen peroxide induced damage was assessed by culturing freshly excised goat lenses in DMEM containing 0.5 mM hydrogen peroxide with or without *Acorus calamus* and by measuring the time taken by lenses to become opaque (Table 1).

Biochemical studies

Reduced glutathione (GSH)

The GSH content was estimated by the method of Moron *et al*¹⁰. Half of the lenses from each group were weighed and homogenized in 1 ml of 5% trichloroacetic acid (TCA), and a clear supernatant was obtained by centrifugation at 5000 rpm for 15 min. To 0.5 ml of this supernatant, 4.0 ml of 0.3 M disodium phosphate (Na₂HPO₄) and 0.5 ml of 0.6 mM 5, 5'- dithiobis-2-nitrobenzoic acid in 1% trisodium citrate were added in succession. The intensity of the resulting yellow color was read spectrophotometrically at 410 nm. Reduced GSH was used as a standard (Table 2).

Estimation of malondialdehyde (MDA)

The extent of lipid peroxidation was determined by the method of Ohkawa *et al*¹¹. Briefly, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.81% thiobarbituric acid aqueous solution were mixed. To this reaction mixture, 0.2 ml of the tissue sample (lens homogenate prepared in 0.15 M Potassium chloride) was added. The mixture was then heated in boiling water for 60 min. After cooling to room temperature, 5 ml of butanol: pyridine (15:1 v/v) solution

was added. The mixture was then centrifuged at 5000 rpm for 15 min. The upper organic layer was separated, and the intensity of the resulting pink colour was read at 532 nm. Tetramethoxypropane was used as an external standard. The level of lipid peroxide was expressed as nmoles of MDA formed in $\mu\text{mol/g}$ wet weight for lenses (Table 2).

Estimation of protein value

For total protein estimation the lens homogenate was prepared in 5% trichloroacetic acid. The precipitated protein was dissolved in sodium hydroxide and used as aliquots for the estimation of total proteins. Soluble and insoluble fractions of the protein were estimated by preparing homogenate in double distilled water. The water soluble supernatant was used for estimation of soluble protein while the residue dissolved in sodium hydroxide was used for the estimation of insoluble protein. The protein content of the samples was determined by the method of Lowry *et al*¹² using bovine serum albumin as the standard (Table 2).

Statistical analysis

Considering standard opacity as base, the ratios of control group were compared with other treatment groups by proportional t-test. Result obtained showed that the time to opacity (in hrs.) in group 2 and 3 (treated methanolic extract and isolated compound) was significantly high ($p < 0.01$) than the control while in other treatments did not differ significantly ($p < 0.05$) from the control group. On the basis of the above analysis it can be deduced that the β asarone (AC 2) and methanolic extract (AC 1) exhibit significant protection against opacity.

Table1: Effect of the extract of *Acorus calamus* and isolated compound on Goat lenses on anticataract activity.

S.No.	Treatment	Treatment H ₂ O ₂ (0.5mM)+ DMEM	Time of Opacity (T _{op}) in Hrs.
1	Control	H ₂ O ₂ (0.5 mM)+ DMEM	17±2
2	Methanolic extract (AC 1)	H ₂ O ₂ (0.5 mM)+ DMEM	45±3**
3	Isolated Compound (0.25 mg) (AC 2)	H ₂ O ₂ (0.5 mM)+ DMEM	42±2
4	Standard drug	H ₂ O ₂ (0.5 mM)+ DMEM	46±4**

Each values represents mean \pm S.E. n=6; **Represents statistical significance vs. control ($p < 0.01$)

Table 2: Effects of methanolic extract of *Acorus calamus* (AC 1), AC 2 (β asarone) and Standard group on GSH, MDA and Protein estimation in hydrogen peroxide induced cataract.

	Control (Group I)	AC1 (Group II)	AC2 (Group III)	Standard (Group IV)
MDA (nmol/mg)	4.75±2.15	1.69±0.01*	1.50±0.02	2.89±0.01*
GSH ($\mu\text{mol/g}$)	0.12±0.05	0.49±0.01*	0.32±0.01	0.55±0.002*
Protein	0.59±0.005	2.159±0.001*	1.571±0.004	2.298±0.001*

All values are expressed as mean \pm SD, Group I: Control, lenses exposed to H₂O₂ only. Group II: lenses exposed to H₂O₂ and MEAC, Group III: lenses exposed to H₂O₂ and β asarone and Group IV: lenses exposed to H₂O₂ and standard drug. Values are mean \pm SD, n=6 statistically significant difference (* $P < 0.01$) when compared with group II values. GSH:glutathione; MDA:malondialdehyde.



RESULTS AND DISCUSSION

Percentage yield of crude extracts

The powdered drug (250 g) was extracted with petroleum ether (60-80°C) and methanol using Soxhlet extraction apparatus. The percentage yields of petroleum ether and methanol were found to be 6.58% and 25.50% w/w respectively.

Phytochemical tests

The phytochemical tests revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, sesquiterpenes, fixed oils and fats in the extract.

Effects on lens morphology

All the lenses in DMEM alone were transparent. However, lenses after 52 h of incubation in the presence of hydrogen peroxide developed dense opacity. *Acorus calamus* (methanolic extract) was found to afford significant, concentration dependent protection against hydrogen peroxide (H₂O₂) damage to goat lenses. *Acorus calamus* extract 0.25 mg/ml prevented opacity from 21 to 44 hrs as compared to control, which become opaque in 17 hrs, where as β asarone prevented opacity for 42 hrs and standard drug (Catlon + Tobramycin) prevented opacity for 46 hrs. (Table 1) (Figure 1)

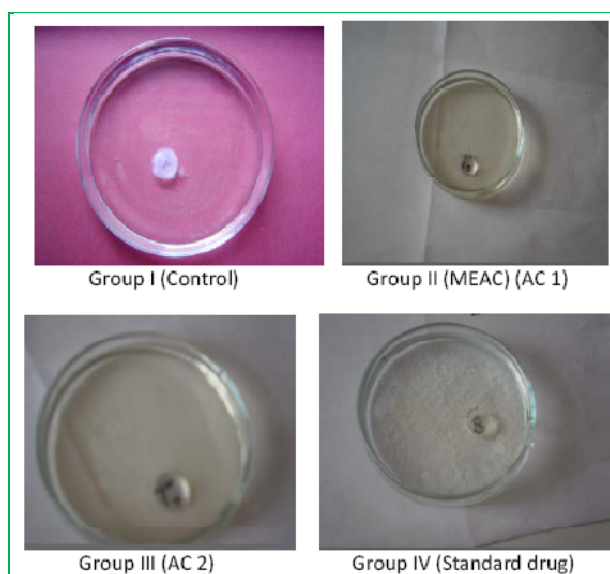


Figure 1: Goat Lenses

Effect on GSH, MDA and Protein estimation in hydrogen peroxide induced oxidative stress

GSH level was observed in control group $0.12 \pm 0.05 \mu\text{mol/g}$ of lenses and the *Acorus calamus* extract and β asarone at the concentration of 0.25 mg/ml significantly restored the GSH level by 0.49 ± 0.01 and 0.32 ± 0.01 of the lens, also standard drug restored the GSH level by 0.55 ± 0.002 (Table 2). MDA level in control group was found to be 4.75 ± 2.15 nmol/mg of lens weight. Incorporation of the *Acorus calamus* extract and β asarone at the concentration of 0.25 mg/ml significantly prevented the rise in MDA level. A decreased level of 1.69 ± 0.01 was observed with 1.50 ± 0.02 nmol/mg. Hydrogen peroxide

0.5mM treated lenses also showed significantly low concentrations of protein in the lens homogenate compared with control group having control lenses. *Acorus calamus*, β asarone and standard drug had significantly higher concentrations of total lens protein, compared with hydrogen peroxide group.

CONCLUSION

Cataract is multifactorial diseases associated with several risk factors and it is responsible for 50% of blindness worldwide. At present, the only remedy for cataract is surgery. However the incidence is so large that the available surgical facilities are unable to cope up with the problem because of postoperative complications such as posterior capsular opacification, endophthalmitis and uncorrected residual refractive error¹³. In the present study, *Acorus calamus* extract and β asarone delayed the progression of cataract in goat eyes and also extract prevented the cataract development in goat lenses. Although, multiple mechanisms may contribute to these effects, the antioxidant effect of *Acorus calamus* and β asarone appears to be the predominant mechanism of action. In conclusion, *Acorus calamus* and β asarone showed anticataract activity against hydrogen peroxide induced cataract in goat eyes. This effect is attributed to the protection of antioxidant defense system.

REFERENCES

1. Harding J. The epidemiology of cataract. In: Harding J, ed. *Cataract — biochemistry, epidemiology and pharmacology*. 1st ed. Madras Chapman & Hall, 1991, 83-124.
2. Spector A. Oxidative stress induced cataract: mechanism of action. *FASEB J*, 9,1995,1173-1182.
3. Spector A, Garner WH, Hydrogen peroxide and human cataract. *Exp. Eye Res*, 33(6), 1981, 673-681.
4. Cui XL, Lou MF, The effect and recovery of long term H₂O₂ exposure on lens morphology and biochemistry. *Exp. Eye Res*. 57(2), 1993, 157-167.
5. Devamanoharan PS, Henein M, Ali AH, Varma SD, Attenuation of sugar cataract by ethyl pyruvate. *Mol Cell Biochem*, 200(1-2), 1999, 103-109.
6. Gerster H, Antioxidant vitamins in cataract prevention, *Z Ernährungswiss*, 28(1), 1989, 56-75.
7. Mukherjee PK, Kumar V, Mal M, Houghton PJ, Acetylcholinesterase inhibitors from plants, *Pharm. Biology*, 45(8), 2007, 651-666.
8. Kim H, Han TH, Lee SG, Antiinflammatory activity of a water extract of *Acorus calamus* L leaves of keratinocyte Ha CaT cells, *Journal of Ethnopharmacology*, 122(1), 2009, 149-156.
9. Devi A, Ganjewala D, *Acta biologica szegediensis*, 53 (1), 2009, 45-49.
10. Moron MS, Depierre JW, Mannervik B, Levels of glutathione, glutathionereductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*, 582(1)(4),1979, 67-78.



11. Ohkawa H, Ohishi N, Yagi K, Assay of lipid peroxide in animal tissue by thiobarbituric acid reaction. Anal Biochem, 95(2), 1979, 351-58.
12. Lowry OH, Rosebrough NJ, Farr AI, Measurement with the folin phenol reagent, J Biol Chem, 193(1), 1951, 265-275.
13. Verma SD, Hedge KR, Effect of alpha ketoglutarate against selenite cataractformation, Experimental eye research, 79, 2004, 913-918.

