



Evaluation of Cataract Preventive Action of Phycocyanin

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ABSTRACT

Phycocyanin is a biliprotein pigment found in blue-green algae *Spirulina platensis*, which have attracted attention because of their nutritional value and medicinal properties. This pigment has antioxidant, anti-inflammatory and hepatoprotective activity in different experimental models. This data supports the view that phycocyanin may prevent cataract progression. Cataract preventive action of phycocyanin was evaluated against naphthalene and galactose induced cataract experimental models in wistar rats at dose 200 mg/kg/day p.o and vitamin E was used as a reference standard. Phycocyanin treated animals showed no opacification in the lens and they also showed significantly increased level of glutathione (GSH), soluble proteins and water content as compared to positive control group in the lens in both the experimental models. Cataract preventive action of phycocyanin may be due to its antioxidant and free radical scavenging activity.

Keywords: Cataract, phycocyanin, antioxidant, glutathione, soluble protein.

INTRODUCTION

Cataract is nothing but visual impairment as a result of a disturbance of lens transparency. 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. [1] Cataract development is usually a very gradual process of normal aging but can occasionally occur rapidly. Many people are in fact unaware that they have cataracts because the changes in their vision have been so gradual. Cataracts commonly affect both eyes, but it is not uncommon for cataracts in one eye to advance more rapidly. Several factors are postulated to be of importance in the generation of lens opacities in the older individual. These have been helpfully summarized by Taylor as the five 'D's: daylight, diet, diabetes, dehydration and doesn't know. [2] The latter catch-all category probably predominantly involves genetic influences in nuclear and cortical opacification, in humans. But the final common pathway by which these different factors exert their influence is predominantly through oxidation of lens proteins and peroxidation of lipids. [3] Presently there are no medications, eye drops, exercise or glasses to cure or prevent cataracts. The symptoms of early cataract may be improved with new eye glasses, brighter

lightening, anti-glare sun glasses, or magnifying lens. If these measures do not help, surgery is the only effective treatment. Apart from knowledge of the pathogenic links between above five D and lens opacity at anti-cataract agent for humans would further require a better understanding of transparency in the normal eye lens systemically or topically to achieve reasonable biological availability, and effective noninvasive methods for monitoring cataract progression in humans. [3] Thus, supplementation with a potential cataract preventive agent is envisaged as an adjunct therapy to help preserve vision and future clinical trials are needed to assess the benefits of pharmacological interventions in lowering the risk of cataract development. [4]

Phycocyanin is one of the major pigment constituents of *Spirulina platensis*, a microalgae used in many countries as dietary supplement whose nutritional and therapeutic values have been very well documented. At present there is a mass of evidence in favor of the antioxidant properties of Phycocyanin. Studies have shown, by different experimental methods that Phycocyanin is an efficient scavenger of oxygen free radicals and also reacts with other oxidants of pathological relevance such as HOCl and ONOO. [5] Antioxidant and free radical scavenging and other beneficial effects are reported for phycocyanin, could justify testing phycocyanin as a potential approach to the prevention of cataract. Hence, *in-vivo* evaluation of cataract preventive activity against naphthalene and galactose induced cataract.

MATERIAL AND METHODS

Drug and chemicals: Phycocyanin was obtained from the parry neutraceuticals, Chennai. And all the other drugs chemicals and drugs was obtained from Himedia chem-

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Mumbai, Loba chem. Wistar Albino rats of either sex were procured from Indian Institute of Sciences, Bangalore for experimental purpose. The Institutional Animal Ethics Committee approved the experimental protocol.

Dose of the phycocyanin was prepared in distill water and vitamin E was prepared in olive

Naphthalene induced cataract

The Wistar rats weighing between 180-200 g were randomly divided into four groups of six each. Normal control group (group I) received liquid paraffin 5ml/kg/day, positive control group (group II) received naphthalene solution 0.5 gm/kg/day p.o. for first three days and 1 g/kg/day p.o. thereafter, group III and IV received phycocyanin 200 mg/kg/day p.o and vit E 50 mg/kg/day p.o respectively. [6]

All the above groups will be treated for 42 days. On 42nd day cataract was examined under slit lamp. On the 43rd day lenses will be removed from the eyes of all the animals for estimation of lens glutathione by Ellman's reagent method [7], lens soluble protein by lowry's method [8] and the lens water content. [9]

Galactose induced cataract

Wistar rats of either sex, weighing 50 to 60 g were randomly divided into four groups of six each. The normal control group (group I) received only distill water, positive control group (group II) received 30% galactose diet, group III and group IV received phycocyanin 200 mg/kg/day p.o and vit E 50 mg/kg/day respectively along with the galactose. [10]

All the above groups will be treated for 40 days. On 40th day cataract was examined under slit lamp. On the 41st day lenses will be removed from the eyes of all the animals for estimation of lens glutathione, lens soluble protein, and the lens water content.

Statistical Analysis

Values are presented as mean $\pm$ SEM. Results were compared by one-way ANOVA followed by Dunnet's t test. A value of $P < 0.001$ was considered significant.

RESULTS

In both naphthalene induced cataract and galactose induced cataract phycocyanin treated animals shown no opacification and lenses was clear. While in positive control group all the animals shown cortical opacity.

The lens glutathione level, lens soluble protein and lens water content of positive control animals showed a significant ($P < 0.001$) decrease as compared to the vehicle control group. Phycocyanin (200 mg/kg) and vitamin E (50 mg/kg) showed a significant increase ($P < 0.001$) in the lens glutathione level, lens soluble protein and lens water content as compared to positive control group in both experimental models (as shown in Table I and II).

DISCUSSION

Naphthalene-induced cataract has been extensively used to test potential anti-cataract drugs. Because the morphology as well as the toxic manifestations of naphthalene-induced cataract is reported to be similar to that of age-related cataract, naphthalene cataractogenesis in rats has been used as a valuable animal model to study the etiology of age-related cataract in humans. [11] Ingested naphthalene is metabolized in the liver to the stable compound naphthalene-1, 2-dihydrodiol and it is further metabolized to NQ by an enzyme dihydrodiol dehydrogenase. Which has ability quickly react with glutathione or protein sulfhydryl groups

and causes its alkylation. This lead to the formation of disulphide bridges causing precipitation of high molecular weight protein, hence opalescence in the lens. The formation of NQ is considered to be the underlying mechanism of cataract development in naphthalene fed animals. [12] Aldose reductase is the key enzyme for the metabolism of naphthalene-1, 2-dihydrodiol in the process of naphthalene cataract development.

Table I: Effect of phycocyanin and vit E on lens Glutathione, soluble protein and water content in naphthalene induced cataract in rats

Group	Treatment	Glutathione ($\times 10^{-5}$ moles)	Soluble protein (mg)	Water content (%)
I	Vehicle control	19.05 $\pm$ 0.69	10.99 $\pm$ 0.32	62.05 $\pm$ 1.84
II	Positive control	10.07 $\pm$ 0.26*	5.22 $\pm$ 0.23*	45.29 $\pm$ 1.48*
III	Phycocyanin treated	13.13 $\pm$ 0.46 [#]	9.43 $\pm$ 0.49 [#]	56.23 $\pm$ 1.51 [#]
IV	Vitamin-E treated	17.42 $\pm$ 0.30 [#]	10.39 $\pm$ 0.30 [#]	56.84 $\pm$ 1.55 [#]

Values are mean $\pm$ SEM. n = 6 for each group. *Significantly different from normal control (* $P < 0.001$), [#]significantly different from positive control (* $P < 0.001$) (One-Way ANOVA followed by Dunnet's t-test)

Table II: Effect of phycocyanin and vit E on lens Glutathione, soluble protein and water content in galactose induced cataract in rats

Group	Treatment	Glutathione ($\times 10^{-5}$ moles)	Soluble protein (mg)	Water content (%)
I	Vehicle control	11.87 $\pm$ 0.35	8.53 $\pm$ 0.34	56.25 $\pm$ 1.73
II	Positive control	5.38 $\pm$ 0.32*	5.41 $\pm$ 0.33*	38.58 $\pm$ 1.55*
III	Phycocyanin treated	7.52 $\pm$ 0.30 [#]	7.54 $\pm$ 0.21 [#]	49.07 $\pm$ 1.31 [#]
IV	Vitamin-E treated	9.77 $\pm$ 0.31 [#]	7.89 $\pm$ 0.26 [#]	51.68 $\pm$ 1.55 [#]

Values are mean $\pm$ SEM. n = 6 for each group. *Significantly different from normal control (* $P < 0.001$), [#]significantly different from positive control (* $P < 0.001$) (One-Way ANOVA followed by Dunnet's t-test)

Galactose induced cataract in rats was used to study the biochemical basis for the protective effect of Phycocyanin; a galactose cataract is an accepted model for diabetic cataract. Three possible mechanisms that may be involved in cataract formation as a result of hyperglycemia or hypergalactosemia are the polyol pathway, oxidation and non enzymatic glycation. The relative contributions to galactosemic cataract by the polyol pathway resulting in osmotic balance versus oxidative stress remains under debate. [13] With an increase in the severity of cataract, there will be a leakage of hydrolyzed crystallins from the lens into the aqueous humor.

The result of the present study indicates phycocyanin treated rats prevents the cataract progression in naphthalene and galactose induced cataract models. Biochemical estimation of the lens showed that the phycocyanin treated animals increased the level of glutathione, soluble proteins of the lens and water content of the lens compared to the positive control group in both experimental models. This indicates phycocyanin provides favorable effect on anti-oxidative defense system such as increase in soluble protein level and water content level. It may be suggest that the anti-oxidative property of phycocyanin contributes to cataract preventive effect observed in present study. Furthermore the distinct diminution in GSH content of lens in diabetic and naphthalene treated rats and its subsequent attainment of near normalcy in Phycocyanin administered group reveal the

protection offered by Phycocyanin in combating oxidative insult due to diabetes and by formation of Naphthoquinone.

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