

EFFECT OF AMBROXOL, SPIRULINA AND VITAMIN-E IN NAPHTHALENE INDUCED CATARACT IN FEMALE RATS

S. E. HAQUE* AND K. M. A. GILANI

*Department of Pharmacology,
Faculty of Pharmacy,
Jamia Hamdard, New Delhi - 110 062*

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Abstract : Anticataract activity of Ambroxol, Spirulina and Vitamin E was examined using the naphthalene cataract model. Adult female albino rats of Wistar strain weighing between 180 and 220 grams were taken and divided into eight groups. Group I received light liquid paraffin 5 ml/kg/day p. o. for 6 weeks. Group II received naphthalene solution 0.5 gm/kg/day p. o. for first three days and 1 gm/kg/day p. o. thereafter for six weeks. Group III received Ambroxol suspension in 0.5% carboxy methyl cellulose (CMC) at the dose of 100 mg/kg/day p. o. alongwith naphthalene. Group IV received Spirulina in distilled water at the dose of 1500 mg/kg/day p. o. alongwith naphthalene. Group V received Vitamin E emulsion at the dose of 50 mg/kg/day p. o. alongwith naphthalene. Group VI received Ambroxol alone at the dose of 100 mg/kg/day p.o. Group VII received Spirulina alone at the dose of 1500 mg/kg/day p. o. Group VIII received vitamin E alone at the dose of 50 mg/kg/day p. o. Lens glutathione, soluble protein and water content profiles revealed the preventive role of Ambroxol, Spirulina and Vitamin E in naphthalene-induced cataract in female rats.

Key words : cataract antioxidant glutathione
soluble protein lens water content naphthalene.

INTRODUCTION

Cataract is opacification or optical dysfunction of crystallin lens (1). It is the most significant current problem leading to blindness and visual disability. Number of blind people due to cataract was estimated 25 million in the year 2000 (2). In India out of 12 million cases of blindness, cataract alone accounts for 81% (3). The only present remedy of cataract is surgery (4). Surgery although an effective means of reversing cataract has not however eliminated the problem because of many reasons.

- (i) Long waiting list : In India about 2.7 million operations are performed annually instead of required 5 to 6 million (5). Most of the patient will spend more than a tenth of their remaining life expectancy visually impaired on the list; some die with their cataract (6).
- (ii) Cost of surgery : It is a worry to some authorities, accounting for 12% of medicare budget in US (6).
- (iii) Risk of complications : Posterior

*Corresponding Author :

capsular opacification occurs in 50% patient but in young patient the effect is 100% (7).

- (iv) Lack of technical hand and equipments : In many of the countries with the greatest cataract problems the equipment to deal with this complication is a rarity, a point which is ignored by those advocating greater use of the latest western surgical techniques in developing countries (8). Though surgery may be an effective mean to reverse cataract blindness, visual outcome will be poor where experienced surgeons and appropriate post operative care, including refraction, are not available (9).

Though a new long term initiative to expand the capabilities of cataract surgery and service levels with financial assistance from the world bank has been undertaken, alternative means to slow down the advancement of the disease by prophylactic means is a desirable goal (6, 10). Thus finding a means to delay cataract may curb the growing number of these operations. For example it has been hypothesized that delaying cataract by 10 years could reduce the number of extractions by 50% (11). So, it is appropriate to search for a drug which can prevent or delay cataract formation.

Cataract develops later in life and is most likely the consequence of decades of accumulated damage to the long-lived lens proteins. In vivo and in vitro studies have suggested that much of this damage is a result of oxidation due to the generation of oxygen radicals, and that antioxidant might protect the lens against formation of cataract (12, 13). A number of

epidemiological studies have demonstrated a reduce risk of developing cataract in person who consume a diet with high content of nutritional antioxidants. These foodstuffs contain essential vitamins, micro nutrients, carotenoids and flavonoids which either alone or in combination attribute to their anticataract activity (14).

In this study we therefore, intended to evaluate some of the antioxidants, which can prevent or delay the cataract formation. Since ambroxol, Spirulina and Vitamin - E have good antioxidant effect (15-17), we have chosen them to assess their anticataract role.

Vitamin E is a lipid soluble antioxidant (18). It can prevent oxidant injury to polyunsaturated fatty acids and thiol-rich protein constituents of cellular membranes and the cytoskeleton and can enhance glutathione recycling in the lens and aqueous humour (19).

The benzylamine ambroxol is a semisynthetic derivative of vasicine, an alkaloid obtained from Indian shrub *Adhatoda vasica*. Ambroxol has been shown to have hydroxyl radical (OH), hypochlorous acid (HOCl) and superoxide anion (O_2^-) scavenging property (20). The exact mechanism by which ambroxol scavenges oxidants is unknown. However, it has been hypothesized that the ambroxol molecule can be oxidized through a transfer of electron to the amino-benzyl structure of the aromatic moiety (20).

Spirulina is a multicellular, filamentous cyanobacterium, belonging to a blue-green algae of cyanophyta (21). It is a good source of carotenoids, micronutrients,

tocopherols and beta-carotene. It is a potent peroxy radical scavenger (22). Spirulina increases the level of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and reduced glutathione (21).

METHODS

Ninety-six adult female albino rats of Wistar strain weighing between 180 and 220 grams, 4–5 weeks old were obtained from Central Animal House, Jamia Hamdard, New Delhi. Spirulina (*Spirulina platensis*) was purchased from Burman Lab. Pvt. Ltd., Maksi (M.P.), Ambroxol (Ambroxol hydrochloride) from Aristo Pharma. Ltd., Raisen (M.P.), and Vitamin E (tocopheryl acetate) from Merck Ltd., Goa. All the other reagents used were of the analytical grade.

Rats were acclimated to the new facility for at least three days. They were housed in clean polypropylene cages under a 12-hour light/12-hour dark cycle and were allowed food and water ad libitum. Animals were randomly distributed into 8 groups of 12 animals each. All the groups were kept in same condition in cages. Before starting the experiment, permission from the Animal Ethical Committee was obtained.

Treatment schedule

Group I: Normal Control group, received light liquid paraffin 5 ml/kg/day p.o. for 6 weeks.

Group II: Toxic control group, received naphthalene solution 0.5 gm/kg/day p.o. for first three days and 1 gm/kg/day p.o. thereafter.

Group III: It received Ambroxol suspension in 0.5% carboxy methyl cellulose (CMC) at the dose of 100 mg/kg/day p.o. alongwith naphthalene.

Group IV: It received Spirulina in distilled water at the dose of 1500 mg/kg/day p.o. alongwith naphthalene.

Group V: It received Vitamin E emulsion at the dose of 50 mg/kg/day p.o. alongwith naphthalene.

Group VI: It received Ambroxol alone at the dose of 100 mg/kg/day p.o.

Group VII: It received Spirulina alone at the dose of 1500 mg/kg/day p.o.

Group VIII: It received vitamin E alone at the dose of 50 mg/kg/day p.o.

All the above groups were treated for 42 days (23). Cataract was examined by torchlight and ophthalmoscope. Cataract was developed in all animals. On the 43rd day lenses were removed from the eyes of all groups of rat for estimation of lens glutathione by the modified method of Ellman, 1959 (24), lens soluble protein by Lowry et. al., 1951 (25) and the lens water content (26).

Glutathione estimation

For the estimation of glutathione in lens, the lens was taken on filter paper, dried and homogenized in 1 ml of 10% trichloroacetic acid solution (TCA) using

tissue homogenizer. The homogenate was centrifuged ($g = 12473.5 \times 10^4$) for 15 minutes and 0.6 ml of supernatant was taken and mixed with 1.77 ml of Tris HCl buffer containing 0.2 M EDTA (pH-8.2) and 30 μ l of Ellman's reagent. The final volume was brought to 3 ml with 0.05 M EDTA and the absorbance of the solution was measured immediately at 415 nm.

Calculation :

GSH (Lens) was calculated from following expression and expressed as moles :

$$\text{GSH (Co)} = A/E \times D$$

Where E = Extinction coefficient
(13,600/M/cm)

A = Absorbance

D = Dilution factor (5.25)

Co = Concentration of glutathione

Protein estimation using Folin's reagent

For the estimation of soluble protein in lens, the lens was dried on filter paper and weighed. A 20% W/V homogenate was prepared in distilled water, using tissue homogenizer. The homogenate was centrifuged ($g = 171105 \times 10^4$) for 15 minutes and the supernatant was used for the estimation of lens soluble protein. 5 ml of alkaline solution was added to 1 ml of the supernatant obtained after centrifugation of 20% homogenate and allowed to stand for 10 minutes. 0.5 ml of diluted Folin's reagent was then added and the tube was shaken to mix the solution: After 30 minutes the absorbance against appropriate blank at 750 nm was recorded.

Preparation of calibration standard curve of protein

100 ml of bovine serum albumin (100 μ g/ml) was prepared and different volumes were taken in 10 test tubes. To all the tubes distilled water was added to make up the volume in each tube to 1 ml. The protein concentrations in these tubes were estimated in the same way as that of the sample. A graph was plotted between the concentration of protein and the optical density. The calibration standard plot thus obtained was used to measure the concentration of protein in each ml of the sample.

Lens water content

For the estimation of lens water content, the lens was taken out and fresh weight was estimated. Lens was then dried in an oven at 110°C till constant dry weight was obtained. The differences in the weights were used as an index of the percentage water content in that lens.

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 $P < 0.05$ was considered significant.

RESULTS

Lens soluble protein level

Table-I shows the protein level in various groups. The lens protein level of naphthalene treated animals (Group II) showed a significant ($P < 0.001$) decrease as

compared to the normal control group (Group I). Ambroxol at the dose of 100 mg/kg/day p.o. (Group III), Spirulina at the dose of 1500 mg/kg/day p.o. (Group IV) and Vitamin E at the dose of 50 mg/kg/day p.o. (Group V) showed a significant increase ($P < 0.001$) in lens protein as compared to the toxic control (Group II). Groups VI, VII, and VIII i.e., only Ambroxol, Spirulina and Vitamin E treated, groups showed the same levels of protein as that of control.

TABLE I: Effect of Ambroxol, Spirulina and Vitamin E on the level of lens soluble protein in the rats treated with naphthalene.

Groups	Treatment	Protein (mg)
I	Normal Control-Paraffin	7.35±0.19
II	Toxic Control-Naphthalene lg/kg/day p.o.	5.63±0.40*
III	Ambroxol 100 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	6.68±0.37*
IV	Spirulina 1500 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	6.71±0.22*
V	Vitamin E 50 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	6.88±0.27*
VI	Ambroxol 100 mg/kg/day p.o.	7.10±0.21*
VII	Spirulina 1500 mg/kg/day p.o.	7.31±0.47*
VIII	Vitamin E 50 mg/kg/day p.o.	7.05±0.60*
Significance difference between various groups at $P < 0.01$ (ANOVA)		* $P < 0.001$

Each group comprises of 6 samples. Data shown in mean ± SEM.

Lens glutathione level

Table II shows the glutathione level in various groups. The lens glutathione level of naphthalene treated animals (Group II) showed a significant ($P < 0.001$) decrease as compared to the normal control group (Group I). Ambroxol at the dose of 100 mg/

TABLE II: Effect of Ambroxol, Spirulina and Vitamin E on the level of lens glutathione in the rats treated with naphthalene.

Groups	Treatment	Glutathione ($\times 10^{-5}$ moles)
I	Normal Control-Paraffin	16.31±3.9
II	Toxic Control-Naphthalene lg/kg/day p.o.	8.83±0.33*
III	Ambroxol 100 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	12.56±0.50*
IV	Spirulina 1500 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	11.77±1.10**
V	Vitamin E 50 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	15.39±1.15*
VI	Ambroxol 100 mg/kg/day p.o.	16.97±1.32*
VII	Spirulina 1500 mg/kg/day p.o.	16.65±2.17*
VIII	Vitamin E 50 mg/kg/day p.o.	18.24±0.87*
Significance difference between various groups at $P < 0.01$ (ANOVA)		* $P < 0.001$ ** $P < 0.01$

Each group comprises of 6 samples. Data shown in mean ± SEM.

kg/day p.o. (Group III), Spirulina at the dose of 1500 mg/kg/day p.o. (Group IV) and Vitamin E at the dose of 50 mg/kg/day p.o. (Group V) showed a significant increase ($P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively) in lens glutathione as compared to the toxic control (Group II). Groups VI, VII and VIII i.e. test agents only treated groups showed almost the same levels of glutathione as that of control.

Lens water content

Table III shows the lens water content in various groups. The lens water content of naphthalene treated animals (Group II) showed a significant ($P < 0.001$) decrease as compared to the normal control group (Group I). Ambroxol at the dose of 100 mg/

TABLE III: Effect of Ambroxol, Spirulina and Vitamin E on the level of lens water content in the rats treated with naphthalene.

Groups	Treatment	Water Content (%)
I	Normal Control-Paraffin	52.54±2.67
II	Toxic Control-Naphthalene lg/kg/day p.o.	42.97±3.46*
III	Ambroxol 100 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	45.46±0.98***
IV	Spirulina 1500 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	45.96±1.65**
V	Vitamin E 50 mg/kg/day p.o. + Naphthalene lg/kg/day p.o.	50.90±1.41*
VI	Ambroxol 100 mg/kg/day p.o.	53.12±1.54*
VII	Spirulina 1500 mg/kg/day p.o.	53.70±1.58*
VIII	Vitamin E 50 mg/kg/day p.o.	52.67±1.22*
Significance difference between various groups at		*P<0.001
P<0.01 (ANOVA)		**P<0.02
		***P<0.05

Each group comprises of 6 samples. Data shown in mean ± SEM.

kg/day p.o. (Group III), spirulina at the dose of 1500 mg/kg/day p.o. (Group IV), and Vitamin E at the dose of 50 mg/kg/day p.o. (Group V) showed a significant increase ($P<0.05$, $P<0.02$ and $P<0.001$, respectively) in lens water content as compared to the toxic control (Group II). Groups VI, VII and VIII showed almost the same levels of water content.

DISCUSSION

Naphthalene has been known to be cataractogenic agent with powerful oxidizing effect for many years (27). Cataract induced by it develops and progress more rapidly in pigmented strains than in albino animals, presumably due to the presence of the melanin synthesizing enzyme phenol oxidase (28).

Naphthalene cataract can be induced by feeding animals with naphthalene and also by culturing lenses in vitro in a medium containing naphthalene-1,2-dihydrodiol or 1,2-naphthoquinone (NQ) (29, 30). It has been demonstrated that direct injection of NQ into the anterior chamber of mouse eye forms anterior cataract with pathological features similar to that of naphthalene cataract (31).

Ingested naphthalene is metabolized in the liver to the stable compound naphthalene-1,2-dihydrodiol (32). Naphthalene-1,2-dihydrodiol is further metabolized to NQ by dihydrodiol dehydrogenase (33), an enzyme also referred to as catachol reductase in early publications. Because of its ability to quickly react with glutathione or protein sulfhydryl groups (34), the formation of NQ is considered to be the underlying mechanism of cataract development in naphthalene fed animals (27). Aldose reductase is the key enzyme for the metabolism of naphthalene-1,2-dihydrodiol in the process of naphthalene cataract development (35). Although lens epithelial mitochondria are the target of NQ toxicity, cataract begins to develop before mitochondria and other sub cellular organelles become totally dysfunctional (36).

Oxidative mechanisms and reactive oxygen species are believed to play a major role in development of age related or senile cataract, a leading cause of impaired vision and blindness in elderly people (37). A characteristic feature of the aging human lens is a 14-fold loss of the key cellular antioxidant, glutathione.

Water-soluble protein and glutathione

decreases in naphthalene-induced cataract (38, 39). Naphthalene induced cataract has been utilized as an experimental model for investigating oxidative insults that are linked to human senile cataracts (27).

The results of the present study show the anticataract effect of Ambroxol, Spirulina and Vitamin E. There is a clear indication of the prevention of cataract by these drugs. The underlying mechanism is attributed to the decrease of soluble-protein level in toxic control group under oxidative stress created by 1,2-naphthaquinone (38). Under this condition the protein gets denatured and forms disulfide cross linkages leading to disulfide and mixed disulfide bond formation, hence protein aggregation, precipitation and lens opalescence. The situation is however improved along with an increase in the protein level in rats treated with Ambroxol, Spirulina and Vitamin E. The induction of cataract and its prevention by Ambroxol, Spirulina and Vitamin E is also indicated by the lens glutathione (GSH) level. As the oxidative stress increases following 1,2-naphthaquinone treatment, the glutathione level decreases in order to combat with the situation to

prevent oxidative damages (39). The situation here again is indicated improved with the administration of Ambroxol, Spirulina and Vitamin E, which have shown the antioxidant effect. The preventive role of these drugs has also been substantiated by the estimates of lens water content.

In the lens 1,2-naphthaquinone, a highly reactive compound (33), causes alkylation of protein, glutathione and amino acid. This lead to the formation of disulphide bridges causing precipitation of high molecular weight protein, hence opalescence in the lens. The test agents used possesses high antioxidant property. They neutralize the oxidative stress; prevent alkylation of protein, glutathione and amino acid. The test agents only treated groups show a slight increase in biochemical parameters as compared to the normal control indicating their strong antioxidant property playing role in day-to-day oxidative stress.

Thus, the lens soluble protein, glutathione and water content profiles show the preventive role of Ambroxol, Spirulina and Vitamin E in naphthalene-induced cataract in rats.

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