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Research Article

A COMPARATIVE STUDY OF ANTI-CATARACT ACTIVITY OF TRIPHALA AND ITS CONSTITUENTS

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ABSTRACT
Cataract is referred as clouding of lens of the eyes, a major cause of blindness all over the world. There is a wide spread belief that the herbal medicines have fewer side effects compared to allopathic medicines. Hence the present study comprises the comparison of anti-cataract activity of Triphala, an herbal formulation and its constituents *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis in-vitro*.
Goat lenses were incubated in artificial aqueous humor containing 55mM glucose with Triphala formulation and its individual constituents separately at different dose levels (400µg/ml, 800µg/ml and 1200µg/ml) at room temperature for 72h. Evaluation was done by using biochemical parameters like superoxide dismutase (SOD), malondialdehyde (MDA), total protein, electrolytes (Na^+, K^+) and Na^+/ K^+- ATPase activity. The glucose induced opacification of goat lens was also studied as a part of visual evaluation.
Present study reveals that lenses treated with Triphala and its individual constituents shows significant decrease in, loss of transparency in the lens, MDA and Na^+ levels. There was also significant increase in SOD, total protein and Na^+/K^+- ATPase activity, thereby Triphala and its individual constituents prevents the formation and progress of cataract induced by glucose. The Triphala formulation at higher dose level (1200 µg/ml) shows much more significant preventive effect on cataract formation than its individual components.
From the present we conclude that Triphala formulation effectively shows anti-cataract activity comparatively to its individual components. Hence the finding of the present study provides the evidence of beneficial effect of Triphala in the patients suffering from cataract.
KEYWORDS: Cataract, Triphala, *Terminalia bellerica, Terminalia chebula, Emblica officinalis*.

INTRODUCTION

Cataract is a visual impairment as a result of a disturbance of lens transparency. It is one of the leading cause for blindness worldwide and accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 2800 new cases are reported daily worldwide. Approximately 25% of the populations over 65 and about 50% over 80 have serious loss of vision which is caused because of the cataract formation.[1,2]

Cataract formation is an ageing process. In adult, the nucleus constitutes a large portion of the lens. As the lens fibers of the nucleus are the oldest fibers, they are devoid of DNA and RNA in their nuclei, therefore the lens has got to depend mainly on the cortical fibers for nutrition. Biochemical analysis shows that in cataractous lens there is preferential loss of gamma crystalline as compared to alpha and beta fractions. This gives rise to formation of bigger sized protein aggregate which results in light scattering. This phenomenon occurs in nucleus of the lens.[3]

There are several causes for cataract such as aging, long term exposure to sunlight, cigarette smoking, high cholesterol / triglycerides, diabetes, certain eye-conditions associated with myopia, retinopathy. Hypertension, kidney disease and direct trauma to the eye can also cause cataract.[4] Surgical treatment is the only remedy for cataract till now.[2] Hence, if a drug is sought which can either reverse or prevent lenticular opacity, it will be a great advance in the treatment of cataract. A number of drugs have been shown to interfere with the process of cataract formation like aldose reductase inhibitors, restatin, sulindac, aspirin, quercetin.[5]

There is a widespread belief that the natural products are less toxic when compared to pure chemicals. Recent data suggests that 80% drug molecules are natural products or natural compound inspired.[6] Hence in the present study we have undertaken an herbal formulation Triphala and its individual components for the anti-cataract activity. Triphala is an herbal formulation consisting of the dried and powdered fruits of three plants, *Terminalia chebula*, *Emblica officinalis* and *Terminalia bellerica* in equal proportions. Triphala is listed in Ayurveda system of medicine and also referenced in traditional Indian texts Charaka Samhita and Sushruta samhita. It is an important medicine of the 'rasayana' group and is believed to promote health, immunity and longevity. This formulation, rich in antioxidants, is frequently used traditionally in Ayurvedic medicine to treat many diseases such as anemia, jaundice, asthma, fever, chronic ulcers, gout and arthritis. Triphala has been scientifically proved for cardio protective, radio protective abilities, antiarthritic effect, anticancer activity and immunomodulatory activity.[7-10]

So far, no single drug has proved effective in preventing cataract. To best of knowledge no scientific data regarding the anti-cataract activity of Triphala, and its constituents *Terminalia chebula*, *Terminalia bellerica*, and *Emblica officinalis* is available. Hence, on the basis of the claims made in ancient literature the present study was carried out to establish the efficacy of Triphala formulation and its individual constituents in the treatment of cataract.

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazoleum, Thiobarbituric acid and all other chemicals were of analytical grade were procured from Highmedia, Mumbai, India.

Eye balls

Goat eye balls used in the present study were obtained from the slaughterhouse immediately after slaughter and transported to laboratory at 0-4 ^oC. The study was conducted after approval by Institutional Animal Ethics Committee (KSHEMA/AEC/05/2011).

Preparation of Lens Culture

The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl: 140 mM, KCl: 5 mM, $MgCl_2$: 2 mM, $NaHCO_3$: 0.5 mM, $NaH(PO_4)_2$: 0.5 mM,

$CaCl_2$: 0.4 mM and Glucose: 5.5 mM) at room temperature and pH - 7.8 for 72 hours. Penicillin G 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose in concentration of 55 mM was used to induce cataract.[11]

Experimental design

Anti-cataract study was carried out with a commercial formulation of Triphala at three different dose levels (400µg/ml, 800µg/ml and 1200µg/ml). Study was also carried out with individual component of Triphala formulation namely *Terminalia bellerica, Terminalia chebula* and *Emblica officinalis* at three different dose levels (400µg/ml, 800µg/ml and 1200µg/ml).

Study comprised following groups with 6 lenses in each group.

Group 1: Lens culture (Negative control)

Group 2: Lens culture + Glucose 55 mM (Positive control)

Group 3: Lens culture + Glucose 55 mM + Triphala 400 µg/ml

: Lens culture + Glucose 55 mM + Triphala 800 µg/ml

: Lens culture + Glucose 55 mM + Triphala 1200µg/ml

Group 4: Lens culture + Glucose 55 mM + *Terminalia bellerica* 400µg/ml

: Lens culture + Glucose 55 mM + *Terminalia bellerica* 800µg/ml

: Lens culture + Glucose 55 mM + *Terminalia bellerica* 1200µg/ml

Group5: Lens culture + Glucose 55 mM + *Terminalia chebula* 400µg/ml

: Lens culture + Glucose 55 mM + *Terminalia chebula* 800µg/ml

: Lens culture + Glucose 55 mM + *Terminalia chebula* 1200µg/ml

Group 6: Lens culture + Glucose 55 mM + *Emblica officinalis* 400µg/ml

: Lens culture + Glucose 55 mM + *Emblica officinalis* 800µg/ml

: Lens culture + Glucose 55 mM + *Emblica officinalis* 1200µg/ml

After 72 hours of incubation, the visual evaluation was done by placing lenses on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of hexagons clearly visible through the lens) was observed through the lens as a measure of lens opacity. Then 10% w/v homogenate of lenses were prepared in tris buffer (0.23 M, pH-7.8) containing 0.25×10^{-3} M EDTA and centrifuged at 10,000 *g* at 4°C for 1hour and the supernatant was used for the estimation of biochemical parameters like SOD, MDA, total protein, electrolytes (Na^+, K^+) and Na^+/ K^+- ATPase activity.

Electrolyte (Na^+ & K^+) estimation was done in automatic analyzer by colorimetric method and protein by Lowry's method.[12] Na^+/K^+-ATPase activity was assessed by the method of Unakar & Tsui.[13] The degree of oxidative stress was assessed by measuring malondialdehyde (MDA) levels by TBARS-method[14,15] The superoxide anion radical scavenging activity was assessed by estimation of superoxide dismutase.[13]

Statistical analysis

All data were expressed as mean ± standard error of the mean (S.E.M.) of 6 lenses per experimental group. Parametric one way analysis of variance (ANOVA) followed by Tukey's post test. Statistical analysis was performed using Graph pad prism 5.0. The minimal level of significance was identified at $P < 0.05$.

RESULTS

Visual Evaluation

Transparency was maintained in normal lens (negative control) after 72 hours of incubation in aqueous humor, in positive control complete cataractogenesis was observed after 72 hours of incubation in aqueous humor + glucose 55 mM with complete loss of transparency. The lens which were incubated in aqueous humor + 55mM of glucose with higher doses (1200 µg/ml) of Triphala, *Terminalia bellerica, Terminalia chebula* and *Emblica officinalis* appeared less hazy and grids were visible more clearly indicating the suppression of cataract formation. Triphala formulation (1200 µg/ml) was more effective in the suppression of cataract than its individual components. (Figure 1)

Biochemical parameters

The Positive control group (Lens culture + Glucose 55 mM) showed significant ($P<0.01$) increase in Na^+ and MDA levels compared with normal lenses group indicating development of cataract. At higher doses (1200 µg/ml) Triphala ($P<0.01$) and its individual constituents ($P<0.05$) shows significant decrease in the Na^+ and MDA levels. (Table 1)

The Positive control group (Lens culture + Glucose 55 mM) showed significant decrease in total protein ($P<0.001$), SOD ($P<0.001$), K^+ levels ($P<0.05$) and Na^+/K^+-ATPase activity ($P<0.01$) when compared with normal lenses group further supporting the cataract formation. Significantly Triphala at higher dose level (1200 µg/ml) increases total protein ($P<0.01$), SOD ($P<0.001$), K^+ levels ($P<0.05$) and Na^+/K^+-ATPase activity ($P<0.01$) gives an evidence for suppression of cataract formation. The individual components of Triphala at higher dose significantly increases total protein ($P<0.05$) and SOD ($P<0.01$) levels only. (Table 2) All these biochemical estimation activities were efficient at higher doses (1200 µg/ml) and Triphala formulation was more significant than its individual components in suppression of cataract.

DISCUSSION

Cataract is an age related phenomenon in which oxidative stress, by the formation of free radicals in lens *milieu* (includes superoxide anion, lipid hydroperoxides, -OH radicals and hydrogen peroxide) plays its important role in the cause of cataract. Other risk factors for cataract formation include diabetes, galactosemia, electromagnetic radiation, life-threatening diarrhea, renal failure, and many drugs. Naturally the young lens has substantial reserves of antioxidants (e.g., vitamins C and E, carotenoids, and glutathione) and antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione reductase/peroxidase) that may prevent damage.[16] But in cataractous lenses antioxidants concentration is decreased. Hence, with the use of antioxidants cataract formation can be prevented which is proved in the anti-cataract activity of lisinopril and enalapril (ACE inhibitors) on glucose induced cataract in goat lenses may be because of the antioxidant and free radical scavenging activity of the drugs.[17]

In the present study the cataract is generated by the incubation of lens culture in the media containing high glucose (55 mM) concentration leading to oxidative stress due to the formation of superoxide (O_2^-) radicals and H_2O_2. High glucose (55 mM) has also shown to induce antioxidant enzymes, suggesting oxidative stress in the cells.

In cataractogenesis, the parameters commonly considered are electrolytes (Na^+ and K^+), malondialdehyde (MDA), total proteins and Na^+/K^+-ATPase activity.[17] Na^+/K^+-ATPase is important in maintaining the ionic equilibrium in the lens,

this enzyme which is embedded in the lens plasma membrane, uses the energy from one molecule of ATP to pump three molecules of sodium outward and two molecules of potassium inward. The impairment accumulation of Na^+/K^+-ATPase causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis. This alteration in the Na^+, K^+ ratio alters the protein content of the lens, leading to a decrease in total proteins, this causes lens opacification leading to cataract.[15]

From the present study the higher doses (1200 µg/ml) of Triphala and its individual components decreases the opacification of goat lens may be by increasing the Na^+/K^+-ATPase activity. The study reports of biochemical parameters reveals the prevention of cataract by Triphala at 1200 µg/ml may be through a mechanism involving anti-oxidant and free radical scavenging by preventing lipid peroxidation i.e. decreasing MDA level and increasing SOD levels which further supports the anti-cataract activity.

CONCLUSION

Triphala formulation at higher dose level 1200µg/ml shows better anti-cataract activity compare to its individual constituents namely *Terminalia bellerica, Terminalia chebula* and *Emblica officinalis.* Hence the present investigation opened avenues for the treatment of cataract from the Triphala formulation.

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Table 1: Na^+ and MDA (lipid peroxidation) levels in lens homogenate after 72 hours of incubation

Group No.	Treatment		Na^+[mEq/g]	MDA (µmol/g)
1	Lens culture (Negative control)		182.7 ± 14.55	0.362 ± 0.10
2	Lens culture + glucose 55 mM (Positive control)		293.7 ± 24.15**	1.467 ± 0.25**
3	Lens culture + glucose 55 mM + Triphala	400µg/ml	245.1 ± 17.42	1.182 ± 0.19
		800µg/ml	232.5 ± 18.33	1.013 ± 0.21
		1200µg/ml	206.6 ± 18.44##	0.458 ± 0.12##
4	Lens culture + glucose 55 mM +*Terminalia Bellerica*	400µg/ml	278.4 ± 19.88	1.292 ± 0.29
		800µg/ml	256.7 ± 20.06	1.082 ± 0.28
		1200µg/ml	226.1 ± 20.71#	0.668 ± 0.19#
5	Lens culture + glucose 55 mM +*Terminalia Chebula*	400µg/ml	271.2 ±23.29	1.380 ± 0.15
		800µg/ml	254.0 ± 23.94	1.085 ± 0.14
		1200µg/ml	228.7 ± 23.00#	0.683 ± 0.13#
6	Lens culture + glucose 55 mM +*Emblica Officinalis*	400µg/ml	277.1 ± 22.46	1.375 ± 0.14
		800µg/ml	250.3 ± 21.74	1.083 ± 0.11
		1200µg/ml	228.4 ± 22.10#	0.686 ± 0.12#

mEq/g = milliequivalents/gram, µmol/g = micromoles/gram, mM = milimoles

The values are expressed as mean ± SEM, n=6 lens. ** represents statistical significance of P< 0.01 when compared with Lens culture (negative control) treated group. # and ## represents statistical significance of P<0.05 and P< 0.01 respectively when compared with positive control group.

Table.2. Protein estimation, K+, Na+/K+-ATPase activity and superoxide dismutase in lens homogenate after 72 hours of incubation.

Group No.	Treatment		Total Proteins (mg/g)	Superoxide dismutase (unit / mg of protein)	Na^+/K^+-ATPase activity[µgP/g]	K^+ [mEq/g]
1	Lens culture (Negative control)		281.5 ± 4.5	1.531 ± 0.18	47.38 ± 2.58	5.390 ± 0.55
2	Lens culture + glucose 55 mM (Positive control)		205 ± 3.9***	0.269 ± 0.02***	27.60 ± 3.49**	3.388 ± 0.49*
3	Lens culture + glucose 55 mM + Triphala	400µg/ml	218.3 ± 5.7	0.436 ± 0.03	33.17 ± 3.29	3.661 ± 0.56
		800µg/ml	224.7 ± 5.7	0.670 ± 0.06##	39.85 ± 3.28	4.614 ± 0.54
		1200µg/ml	234.2 ± 5.7##	1.05 1± 0.14###	44.42 ± 3.58##	5.130 ± 0.51#
4	Lens culture + glucose 55 mM +*Terminalia Bellerica*	400µg/ml	209.1 ± 7.3	0.373 ± 0.02	28.12 ± 2.95	3.468 ± 0.49
		800µg/ml	215.3 ± 5.2	0.598 ± 0.03#	33.34 ± 2.72	4.215 ± 0.46
		1200µg/ml	229.4 ± 4.2#	0.679 ± 0.03##	38.57 ± 3.58	4.720 ± 0.55
5	Lens culture + glucose 55 mM +*Terminalia Chebula*	400µg/ml	208.2 ± 4.1	0.307 ± 0.01	25.19 ± 3.33	3.415 ± 0.31
		800µg/ml	214.5 ± 4.7	0.603 ± 0.03#	29.59 ± 3.73	4.220 ± 0.31
		1200µg/ml	227.2 ± 3.5#	0.674± 0.03##	33.42 ± 3.65	4.712 ± 0.29
6	Lens culture + glucose 55 mM +*Emblica Officinalis*	400µg/ml	208.9 ± 5.7	0.324 ± 0.02	27.57 ± 3.33	3.405 ± 0.19
		800µg/ml	215.0 ± 5.4	0.599 ± 0.02#	32.56 ± 3.63	3.937 ± 0.16
		1200µg/ml	228.6 ± 3.7#	0.669 ± 0.02##	36.55 ± 4.20	4.417 ± 0.16

mEq/g = milliequivalents/gram, µmol/g = micromoles/gram, mM = milimoles

The values are expressed as mean ± SEM, n=6 lens. *, ** and *** represents statistical significance of P< 0.05, P< 0.01 and P<0.001 respectively when compared with Lens culture (negative control) treated group. #, ## and ### represents statistical significance of P<0.05, P< 0.01 and P<0.001 respectively when compared with positive control group.

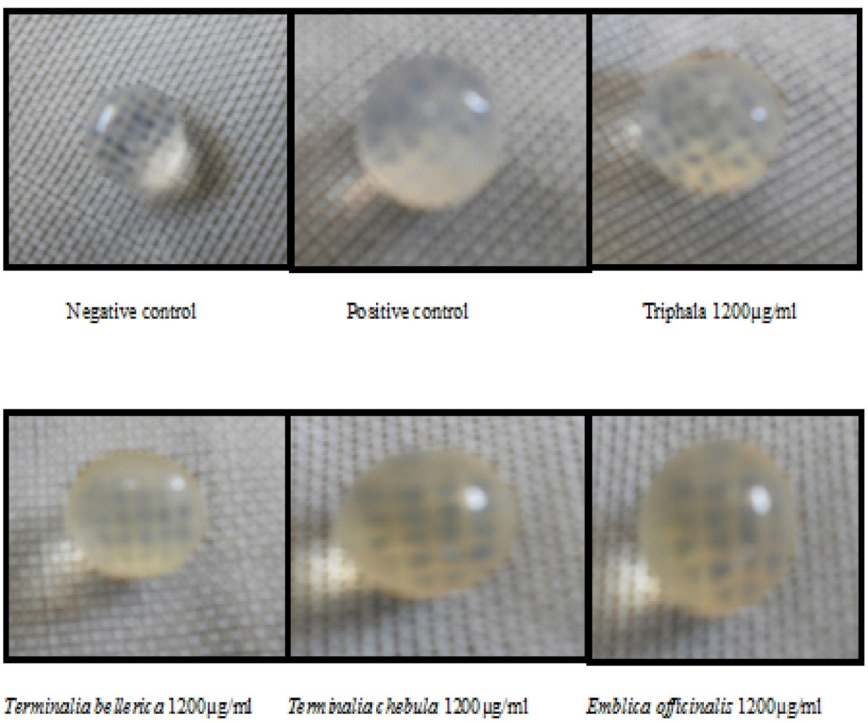

Figure 1: Visual Evaluation

Negative control transparency is maintained, Positive control loss of transparency with complete cataractogenesis, Triphala, *Terminalia bellerica, Terminalia chebula* and *Emblica officinalis* appeared more clear transparency than positive control which indicates the suppression of cataract formation.

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