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ORIGINAL ARTICLE

Anticataract Activity of Forskolin by Inhibiting Polyol Pathway for the Prevention of Diabetic Complication

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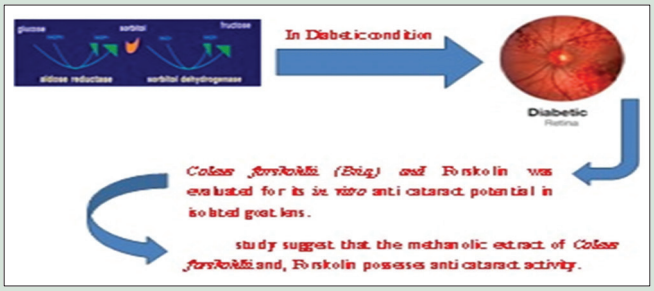
ABSTRACT

Background: Cataract is the opacification or optical dysfunction of the crystalline lens. Diabetes has been considered to be one of the major risk factors of cataract. **Objective:** The present study was designed to evaluate the anticataract activity of antioxidants such as *Coleus forskohlii* (CF) Briq and its phytoconstituent forskolin (FS) which were subjected to prevent cataract formation *in vitro* on glucose-induced cataract model. **Materials and Methods:** Goat lenses were incubated in Krebs-Ringer bicarbonate buffer pH 7.5 (supplemented with Taxim and streptomycin) containing 55 mM glucose (cataractogenesis) with fidarestat; CF methanolic extract; and FS at a concentration of 1 μg/mL, 100 μg/mL, and 10 μg/mL for 24 h at 37°C with 5% CO_2 and 95% air. Glucose-induced opacification of goat lens began 8–10 h after incubation and was complete in 24 h. Polyol (galactitol) levels in incubated lenses were estimated spectrophotometrically. **Results:** Cataractous lenses showed higher content of galactitol. However, lens treated with fidarestat, CF methanolic extract, and its phytoconstituent FS showed lower content of galactitol. **Conclusion:** CF and FS prevented the formation and progress of cataract by glucose, as evidenced by lens transparencies with photographic evaluation and lens galactitol levels.

Key words: Aldose reductase, cataract, *Coleus forskohlii*, forskolin, polyol pathway

SUMMARY

- Cataract was induced in goat lens by incubating the lens in high glucose medium (55 mM). The methanolic extract of *Coleus forskohlii* and its active constituent forskolin were evaluated for their *in vitro* effect on inhibition of aldose reductase enzyme, a key enzyme in polyol pathway and prevention of decrease in lens transparency in cataract lens. The results of the preset study have shown that the *Coleus forskohlii* and forskolin possesses *in vitro* anti-cataract activity via inhibition of the aldose reductase enzyme and decrease in lens transparency.



Abbreviations Used: CF: *Coleus forskohlii*, FS: Forskolin, FDST: Fidarestat ARI: Aldose reductase inhibitory, GLUT: Glucose transport, NADPH: Nicotinamide adenine dinucleotide phosphate, NIH: National Institutes of Health, LOCI: Laboratory for Optical and Computational Instrumentation, KOH: Potassium hydroxide, RLAR: Rat lens aldose reductase, DMSO: Dimethyl sulphoxide, UV: Ultra violet, SEM: Standard error to mean, ANOVA: Analysis of variance, AR: Aldose reductase

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INTRODUCTION

Cataract is the opacification or optical dysfunction of the crystalline lens, associated with the breakdown of the lens microarchitecture, which interferes with transmission of light onto the retina.[1] A major complication of diabetes is the early development of cataract in lens. Studies conducted over the past 30 years have established a clear link between the excess accumulation of intracellular sorbitol levels and the onset of diabetic complications such as cataract.[2] The lens is one of the body parts most affected in diabetes; during hyperglycemia, extracellular glucose diffuses into the lens uncontrolled by the hormone insulin. The proteins of the lens are long, and ingestion of excessive glucose leads to the accumulation of sorbitol (polyols) in the lens fibers, and consequently, due to osmotic stress, the sorbitol thus accumulated does not easily cross the cell membrane leading to damage by disturbing the osmotic homeostasis.[3]

This mechanism has been confirmed by numerous studies in which administration of aldose reductase (AR) inhibitors at the onset of diabetes significantly prevents this progression of cataract.[4] Literature has revealed that cataract progression can be slowed or prevented by the use of natural therapies with plants showing considerable hypoglycemic and AR inhibitory (ARI) activity.[5] Halder *et al.* 2003 reported the anticataract activity of *Curcuma longa L., Withania somnifera L., and Caesalpinia digyana.*[6]

Coleus forskohlii (CF) Briq. is an important medicinal plant of family Lamiaceae, growing wild in the subtropical climate of India, Nepal, Bhutan, Thailand, Burma, and Sri Lanka. India is considered to be native place of the plant.[7] The plant has been described in Ayurveda by the name "Mayani" or "Makandi."[8] The tuberous roots are found to be rich source of the labdane diterpenoid forskolin (FS). In Central India, the plant roots are used as condiments and for making pickles also.[9] CF has been used to treat hypertension, congestive heart failure, eczema, colic, respiratory disorders, painful urination, insomnia and convulsions, asthma, bronchitis, intestinal disorders, burning sensation, constipation, epilepsy, and angina.[10]

FS plays a role in treating conditions associated with diabetes which improves cellular insulin response. It also decreases blood sugar levels

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in rats. Although there are no data that directly show that FS helps in diabetic conditions, it was proposed as a drug to help treat one of the complications of diabetes, i.e., diabetic retinopathy. FS blocks two proteins responsible for glucose transport – GLUT1 and GLUT4, thus helping to decrease blood sugar levels. It is also good for suppressing inflammation, which often worsens diabetes.[11]

Thus, in view of the potent ARI activity of CF (Briq.) and its phytoconstituent, FS was further evaluated for its *in vitro* anticataract potential in isolated goat lens. Further, since the plant was well known for its antidiabetic activity, the methanolic extract of CF along with its phytoconstituent FS was studied for its effect on cataract development in cultured goat lens with respect to opacification and accumulation polyol levels in the lens.[12]

MATERIALS AND METHODS

Krebs-Ringer buffer medium and dulcitol, Nicotinamide adenine dinucleotide phosphate (NADPH), were obtained from HiMedia Laboratories (Mumbai, India).

Instruments

Double-beam ultraviolet (UV)-visible spectrophotometer (Elico-SL-159), tissue homogenizer (ART Micra D-1 N-300063), cooling centrifuge (Remi R-8C), millipore water filtration system (Direct-Q-UV-3), CO_2 incubator (Innova-42), refrigerator, and freezer were used in this study.

Preparation of lens culture

Fresh bovine eyes were obtained from a local slaughterhouse, and the lenses were isolated from the eyes by extracapsular extraction and were washed free from any exogenous tissue with sterile normal saline; the experiment was conducted on the same day. The isolated lenses were incubated in Krebs-Ringer bicarbonate buffer pH 7.5 (supplemented with Taxim and streptomycin) for 24 h at 37°C with 5% CO_2 and 95% air. Cataract was induced by glucose at a concentration of 55 mM.[13]

Lenses were divided into the following groups (n = 3): Group I (naïve): Krebs-Ringer bicarbonate buffer with 10 mM glucose, Group II (control): Krebs-Ringer bicarbonate buffer with 55 mM glucose, Group III (CF): Krebs-Ringer bicarbonate buffer with 55 mM glucose and CF 100 µg/ml, Group IV (FS): Krebs-Ringer bicarbonate buffer with 55 mM glucose and FS 10 µg/ml, and Group V Fidarestat (FDST): Krebs-Ringer bicarbonate buffer with 55 mM glucose and fidarestat 1 µg/ml. After the incubation period, the photographs of lenses were taken. The extent of transparency of the lens was measured by analyzing the images of the lenses using software ImageJ 152-win-java8 (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, LOCI, University of Wisconsin), NIH followed by the estimation of polyol levels in the lens on the same day.

Estimation of lens polyols

The lens polyols were estimated by the previously reported method.[14] The lenses were homogenized in 0.6N perchloric acid followed by centrifugation at 5000 rpm for 30 min. The supernatant was separated and neutralized with 2N KOH and again centrifuged. The supernatant was treated with 0.2 mL of 0.03 M periodic acid, 0.2 mL of stannous chloride and 2 mL of 0.2% chromotropic acid for 30 min on a water bath to obtain a purple colored complex. Absorbance was measured at 570 nm using UV spectrophotometer (SL-159, Elico). The polyol concentration was measured using a calibration curve.

Rat lens aldose reductase inhibitory activity of *Coleus forskohlii* and forskolin *in vitro*

Rats were sacrificed by cervical dislocation followed by removal of eyes. The lenses were enucleated through posterior approach. Both lenses homogenized separately with three volumes of 0.1 M phosphate buffer, pH 6.2, and centrifuged for 30 min at 16,000 rpm at 4°C, and the supernatant collected was used as crude rat lens AR (RLAR) preparation.[15]

In vitro AR inhibitory activity of the plant extract and its phytoconstituent was assayed as previously described method of Jung *et al.*[16] FS at concentration of 1, 5, and 10 µg/ml; fidarestat at a concentration of 0.1, 0.5, and 1 µg/ml; and methanolic extract of CF at a concentration of 10, 50, and 100 µg/ml were prepared separately in 10% dimethyl sulfoxide (DMSO). The reaction mixture consists of 300 µL of crude enzyme preparation and 300 µL of test or standard drug solution (test drug solution was replaced by 10% DMSO in blank). The reaction was initiated by addition of 300 µL of 10 mM DL-glyceraldehyde as substrate (double-distilled water in blank). The absorbance of the resultant solution was measured at 340 nm using double-beam UV spectrophotometer (SL210, Elico, India) for 1 min at 5-s interval. Absorbance was recorded for all the concentrations in triplicate. The AR inhibitory activity of each sample was calculated using a previously reported formula.

Statistical analysis

All the values were expressed as mean ± standard error of mean. The data were analyzed using analysis of variance followed by Dunnett's test, and statistical significance was considered to be $P < 0.05$.

RESULTS

Effect on lens transparency

Lens transparencies are shown in Figure 1 and 2. Photographic evaluation of lens revealed that all the lenses incubated in Krebs-Ringer bicarbonate buffer with 10 mM glucose remained transparent, while incubation of lenses in the presence of high glucose (55 mM) led to a decrease in lens transparency when compared to negative control.

The presence of CF and FS in high glucose medium prevented the decrease in lens transparency. The transparency in lens of test groups was found to be higher when compared to standard group.

Effect on polyol levels

Effect on alterations in polyol levels of the lens is shown in Figure 3. Polyol levels were significantly increased in lenses incubated in high

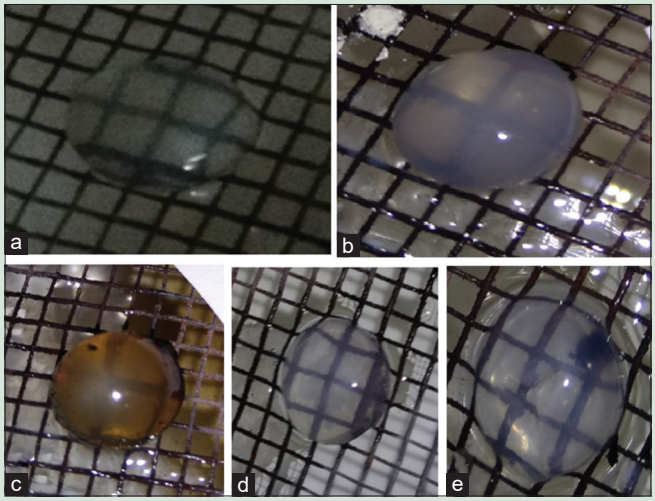

Figure 1: Effect of plant extracts and their phytoconstituents on lens transparencies in sugar-induced opacity model in goat lens. (a) Negative control, (b) positive control, (c) *Coleus forskohlii* (100 µg/ml), (d) forskolin (10 µg/ml), and (e) fidarestat (1 µg/ml)

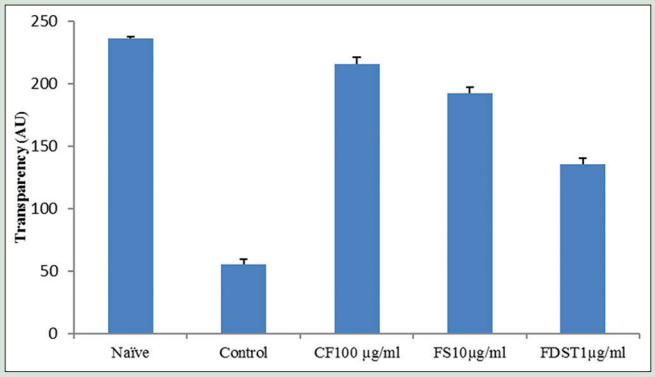

Figure 2: Effect of *Coleus forskohlii* and forskolin on lens transparency in goat lens in sugar-induced opacity model. Data were analyzed by one-way analysis of variance followed by Dunnett's test. (n = 3); $P < 0.05$ as compared to control group

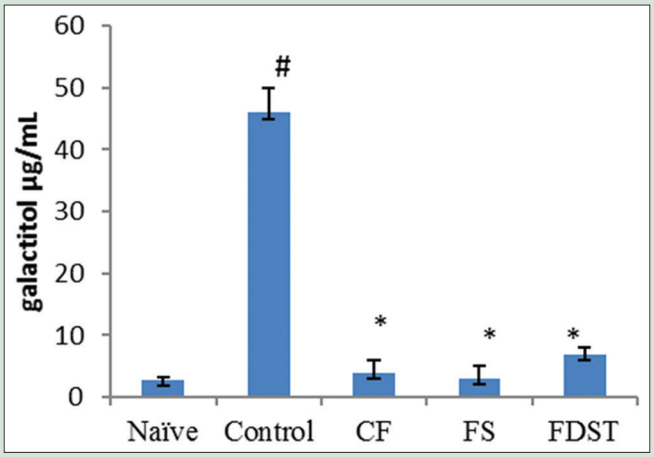

Figure 3: Effect of *Coleus forskohlii* and forskolin on polyol levels in goat lens in sugar-induced opacity model. Data were analyzed by one-way analysis of variance followed by Dunnett's test. (n = 3); $P < 0.05$ as compared to control group

glucose medium in comparison to negative control. The presence of CF, FS, and standard fidarestat in the high glucose medium significantly reduces osmotic stress induced by increase in polyol levels indicating a protective effect against the formation of cataract.

In vitro aldose reductase activity against rat lens aldose reductase

In vitro AR inhibitory activity of the plant extract and its phytoconstituent on rat lens is shown in Table 1. RLAR inhibitory activity of the plant extract and phytoconstituent was compared with that of fidarestat. The plant extract showed RLAR inhibitory activity in a concentration-dependent manner with maximum activity obtained at the highest concentration (100 µg/ml). The plant extract showed inhibitory activity against RLAR with an IC_{50} value of 27.454 ± 3.25 µg/mL. The phytoconstituent FS was found to exhibit potent inhibitory activity against RLAR with an IC_{50} value of 0.372 ± 2.64 µM when compared to that of fidarestat with an IC_{50} value of 15.080 µM.

Table 1: Effect of *Coleus forskohlii* and forskolin on rat lens aldose reductase inhibitory activity *in vitro*

	Concentration µg/ml	Percentage inhibition	IC_{50} (µg/ml)	IC_{50} (µM)
CF	10	48.006±3.546	27.454±3.25	
	50	52.866±1.2		
	100	75.272±8.453		
FS	1	65.504±6.387	0.153±2.64	0.372
	5	67.123±2.3		
	10	84.630±5.242		
Fidarestat	0.1	3.09±0.951	4.211±0.048	15.080
	0.5	61.52±2.013		
	1	73.459±2.087		

All values are expressed as mean±SD, n=3. CF: *Coleus forskohlii*; FS: Forskolin; SD: Standard deviation; IC_{50}: Half-maximal inhibitory concentration

DISCUSSION

The key event in the glucose-induced cataract is the activation of the polyol pathway, with the conversion of glucose into sorbitol by AR. Sorbitol accumulates in lens as the cellular membranes of the lens are impermeable to sorbitol, and this leads to hyperosmotic cell swelling, which produces scattering of light and diminished lens transparency.[17]

Cataract during diabetes is mainly caused by hyperglycemia-induced AR-mediated increase in polyol levels in crystalline lens leading to increase osmotic stress, thereby causing excessive hydration with loss of membrane integrity.[18] Besides this, increased polyols in the lens react with lens proteins leading to the formation of high-molecular-weight insoluble proteins which are responsible for loss of transparency.[19] Oxidative stress may also be implicated in the cataract induced by glucose, due to the formation of superoxide (O–2) radicals and H_2O_2. High glucose (55 mM) has shown to induce antioxidant enzymes, suggesting oxidative stress in the cells.[20]

Diabetic cataractogenesis in experimental animals might be due to increased formation of polyols from reducing sugars by AR enzyme which is an important rate-limiting enzyme that contributes to cataract induction in a diabetic patient. It converts galactose to galactitol and glucose to sorbitol.[21] AR inhibitors could be an effective strategy in the prevention or delay of cataract. Oxidative stress is a common underlying mechanism of cataractogenesis, and antioxidant defenses have been shown to prevent or delay cataract.[22] Previous studies on experimental models have demonstrated a correlation between AR inhibitors and prevention of diabetic cataract.[23]

In the present study, CF and FS were evaluated for the AR inhibitory activity on RLAR *in vitro* and showed potent AR inhibitory activity with low IC_{50} values than fidarestat.

Studies were carried out for the antioxidant properties of CF, and it was observed that the tubers possessed a significant potential of both enzymatic and nonenzymatic antioxidants that could protect against oxidative and free radical injuries, in addition to having various other medicinal properties. *In vitro* study showed FS stimulation of glucose-induced insulin secretion. This appears to reflect a general stimulatory influence of FS on adenylate cyclase activity, obviating its specific suitability as an antidiabetic treatment.[10]

FS significantly decreases lipid peroxidation. The antioxidant activity of it was comparable to the effects of Vitamin E. *In vitro* FS protected against the intracellular effects of H_2O_2 and increased the levels of the antioxidant, glutathione, by 1.6–2-fold.[24,25]

In the present study, CF and FS were evaluated for their ARI activity-mediated anticataract potential against high glucose-induced opacity and biochemical changes in bovine lenses maintained in organ culture medium. In consistent with the above theory, incubation of lenses in the high glucose medium led to the opacity of the lens when compared to that incubated in low glucose medium. The presence of CF

and FS or fidarestat in the medium prevented the induction of opacity which can be attributed to the inhibition of lens AR by the compounds. This can be supported by the fact that the accumulation of polyols was significantly prevented by the presence of CF and FS or fidarestat in the high glucose medium.

CONCLUSION

In the present study, CF and FS were evaluated for the AR inhibitory activity on RLAR, and subsequently, their anticataract activity was evaluated against glucose-induced increase in the polyol levels in goat lens maintained in culture medium in the presence or absence of the CF and FS, which were reported for their antidiabetic activity. The presence of CF and FS prevented the accumulation of polyols caused by high glucose concentration. Thus, the results of the present study suggest that the methanolic extract of CF and its respective phytoconstituent, FS possesses anticataract activity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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