

## ORIGINAL RESEARCH ARTICLE

***In Vitro* Anticataract Activity of *Zingiber officinale* on Goat Lenses****Manish Kumar\*, Talever Singh, Javed Ali and L.K Tyagi***Department of Pharmacy, Institute of Biomedical Education & Research, Mangalayatan University, Aligarh, U. P., India*

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**ABSTRACT**

The antioxidants such as *Zingiber officinale* and Enalapril (ACE Inhibitor) were subjected to prevent cataract formation in vitro on glucose induced cataract model. Goat lenses were incubated in artificial aqueous humor containing 55 mM glucose (cataractogenesis) with enalapril and *Zingiber officinale* extract in different concentrations at room temperature for 72 h. Biochemical parameters studied in the lens were electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ),  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity, malondialdehyde (MDA) and proteins. Glucose induced opacification of goat lens began 8-10 hrs after incubation and was complete in 72-80 hrs. Cataractous lenses showed higher  $\text{Na}^+$ , MDA ( $P < 0.001$ ), lower  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity, and water-soluble protein content. Lenses treated with enalapril 5ng/ml and *Zingiber officinale* extract in concentrations of 100, 300, and 500 ng/ml showed higher protein (total and water soluble proteins) content and prevented formation and progress of cataract by glucose, as evidenced by biochemical parameters.

**Key words:** Antioxidants, *Zingiber officinale*, Free radicals, Anticataract**INTRODUCTION**

Cataract is the opacification of lens often associated with old age and is a major complication of diabetes mellitus because higher glycosylated hemoglobin levels are significantly associated with increased risk of cataract<sup>[1]</sup>. Although many cataractogenic factors have been identified, the biochemical background of cataractogenesis is still unknown. It is a multifactorial disease occurs mainly due to formation of large protein aggregates in the lens. The lens  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity plays an important role in maintaining lens transparency, and its impairment causes accumulation of  $\text{Na}^+$  and loss of  $\text{K}^+$  with hydration and swelling of the lens fibers leading to cataractogenesis<sup>[2]</sup>. Although a number of agents have been tried for prevention and treatment of cataract but non have proved to useful<sup>[3]</sup>. Aldose reductase is a lens enzyme probably involved in the development of this eye problem (cataract)<sup>[4]</sup>. The aldose reductase acts on the sugars like glucose, galactose and xylose and convert them into their respective alcohols. These alcohols, also known as polyols accumulate within the lens there by producing osmotic effects. Since polyols are not

capable of diffusing out easily nor metabolizes rapidly and causes hyper tonicity responsible for formation of cataract<sup>[5]</sup>.

Oxidative mechanism plays an important role in biological phenomena including cataract formation. The formation of superoxide radicals in the aqueous humor and in lens, lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to formation of cataract<sup>[6]</sup>. ACE inhibitors have been found to afford protection from free radical damage in many experimental conditions. Enalapril (ACE Inhibitor) was shown potent anticataract activity in vitro due to antioxidant and free radical scavenging activity<sup>[7]</sup>. Hence we take enalapril as standard and measure various parameters including ( $\text{Na}^+$  &  $\text{K}^+$ ) estimation,  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity, Proteins (total proteins and water soluble proteins) and malondialdehyde (MDA) in vitro on goat lenses.

**MATERIALS AND METHODS**

**Plant:** Fresh *Zingiber officinale* Rhizomes were collected from Medicinal garden Mangalayatan University Aligarh.

**Drugs:** Drugs Enalapril, Penicillin and streptomycin were obtained from Praveen drugs & chemicals Private Ltd. Mathura.

**Extraction of Plant Material (Ginger):**

The ginger rhizomes were washed with distilled water and allowed to dry (air-day) for two days. Extraction was done using the following procedures:

250g fresh ginger was soaked in 100ml of 95% ethanol for 24 hours to obtain the extract which was evaporated to dryness using water bath and stored below ambient temperature.

**Eye Balls:**

Goat eye balls were used in the present study. They were obtained from the slaughterhouse Aligarh immediately after slaughter and transported to laboratory at 0-4 degree Celsius.

**Preparation of Lens Culture:**

The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl - 140 mM, KCl - 5 mM, MgCl<sub>2</sub> - 2 mM, NaHCO<sub>3</sub> - 0.5 mM, NaH(PO<sub>4</sub>)<sub>2</sub> - 0.5 mM, CaCl<sub>2</sub> - 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose in a concentration of 55 mM was used to induce cataract<sup>[8]</sup>.

**Drug Concentration and Groups:**

The standard drug (enalapril) is taken in the concentration of 5ng/ml. And the *Zingiber officinale* extract was taken into three concentrations, 100ng/ml, 300ng/ml and 500ng/ml respectively.

A total of 60 lenses were divided into following categories (n=10 in each category):

**Group-I: Normal lens [Control (Glucose 5.5 mM)]**

**Group II: Glucose 55 mM**

**Group III: Glucose 55 mM + enalapril 5ng/ml**

**Group IV:**

- 1) Glucose 55 mM + *Zingiber officinale* extract 100ng/ml
- 2) Glucose 55 mM + *Zingiber officinale* extract 300ng/ml
- 3) Glucose 55 mM + *Zingiber officinale* extract 500ng/ml

**Homogenate preparation:**

After 72 h of incubation, homogenate of lenses was prepared in Tris buffer (0.23M, pH 7.8) containing 0.25X10<sup>-3</sup>sub M EDTA and homogenate adjusted to 10 % w/v. The homogenate was centrifuged at 10,000 G at 4°C for 1 hour and the supernatant used for estimation of biochemical parameters. For estimation of

water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH 7.4).

**Biochemical estimation:**

Electrolyte (Na<sup>+</sup> & K<sup>+</sup>) estimation was done by flame photometry. Na<sup>+</sup>-K<sup>+</sup>-ATPase activity was assessed by the method of *Unakar & Tsui*<sup>[9]</sup> and protein by *Lowry's method*<sup>[10]</sup>. The degree of oxidative stress was assessed by measuring the MDA levels by *Wilbur's method*<sup>[11]</sup>.

**Photographic Evaluation:**

Lenses were placed 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 lens as a measure of lens opacity.

**Statistical Analysis:**

The data was presented as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc- Dunnett's test using Graph Pad Prism software, version 4.01.

**RESULTS**

Incubation of lenses with glucose 55 mM showed opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 72 hrs.

As shown in (**Table 1**), Glucose 55 mM treated lenses (Group-II) showed significantly higher Na<sup>+</sup> (P<0.05), lower K<sup>+</sup> (P<0.001) and lower Na<sup>+</sup>-K<sup>+</sup>-ATPase activity (P<0.001) compared with normal lenses (Group-I). enalapril treated lenses (Group-III) and Lenses treated with *Zingiber officinale* extract (Group-IV) showed lower Na<sup>+</sup> (P<0.05), higher K<sup>+</sup> (P<0.001) and higher Na<sup>+</sup>-K<sup>+</sup>-ATPase activity (P<0.001) compared with Glucose 55 mM treated lenses (Group-II).

As shown in table-2, Glucose 55 mM treated lenses (Group-II) also showed significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate (P<0.001) and very high concentration of MDA (P<0.001) compared with normal lenses (Group-I). enalapril treated lenses (Group-III) and Lenses treated with *Zingiber officinale* extract (Group-IV) showed higher concentrations of proteins (total and water soluble proteins) (P<0.001) and lower concentration of MDA (P<0.001) compared with Glucose 55 mM treated lenses (Group-II) (**Table-2**).

**DISCUSSION**

The parameters commonly considered in cataractogenesis are electrolytes (Na<sup>+</sup> and K<sup>+</sup>), malondialdehyde (MDA) and proteins (total

proteins and water soluble proteins). Incubation in the media containing high glucose (55 mM) concentration has shown to cause considerable drop in Na<sup>+</sup>-K<sup>+</sup>-ATPase activity, with progression of opacity<sup>[9]</sup>. This study, is in agreement with this finding. Na<sup>+</sup>-K<sup>+</sup>-ATPase is important in maintaining the ionic equilibrium in the lens, and its impairment causes accumulation of Na<sup>+</sup> and loss of K<sup>+</sup> with hydration and swelling of the lens fibers leading to cataractogenesis<sup>[12]</sup>. This alteration in the Na<sup>+</sup>-K<sup>+</sup> ratio alters the protein content of the lens, leading to a decrease in water soluble proteins' content and increase in insoluble proteins. This causes lens opacification<sup>[13]</sup>. This study showed higher Na<sup>+</sup>-K<sup>+</sup>-ATPase activity, total and water-soluble proteins and K<sup>+</sup> ions whereas lower concentrations of Na<sup>+</sup> ions with enalapril and *Zingiber officinale* extract treated groups (Group-III and IV).

Therefore, enalapril and *Zingiber officinale* extract seem to prevent the alteration of Na<sup>+</sup> and K<sup>+</sup> imbalance, which may be due to a direct effect on lens membrane Na<sup>+</sup>-K<sup>+</sup>-ATPase or indirect effect through their free radical scavenging activity.

Oxidative stress may also be implicated in the cataract induced by glucose, due to the formation of superoxide (O<sub>2</sub><sup>-</sup>) radicals and H<sub>2</sub>O<sub>2</sub>. High glucose (55 mM) has shown to induce antioxidant enzymes, suggesting oxidative stress in the cells<sup>[14]</sup>. In this study MDA levels were significantly higher in Glucose 55 mM treated Group, compared with normal lenses Group. The MDA levels were significantly lower in enalapril and *Zingiber officinale* extract treated groups. Enalapril and *Zingiber officinale* extract treated groups have also been shown to increase the content of water-soluble proteins, retarding the process of cataractogenesis initiated by high glucose concentration.

**Table 1: Na<sup>+</sup>, K<sup>+</sup> and Na<sup>+</sup>-K<sup>+</sup>-ATPase activity in lens homogenate after 72 h of incubation**

Study Groups	Na <sup>+</sup> [meq/gm]	K <sup>+</sup> [meq/gm]	Na <sup>+</sup> -K <sup>+</sup> -ATPase activity [μgP/gm]
Normal lens [Control (Glucose 5.5 mM)]	153.7 ± 57.1*	10.5 ± 1.5***	41.8 ± 2.2***
Glucose 55 mM	209.7 ± 29.7	6.4 ± 0.3	17.7 ± 4.9
Glucose 55 mM + enalapril 5ng/ml	180.0 ± 40.4	9.7 ± 2.7**	37.3 ± 5.5***
Glucose 55 mM + <i>Zingiber officinale</i> extract 100ng/ml	187.5 ± 29.4	9.4 ± 1.8*	34.5 ± 7.0 ***
Glucose 55 mM + <i>Zingiber officinale</i> extract 300ng/ml	182.5 ± 28.9	9.5 ± 2.2**	23.8 ± 9.8
Glucose 55 mM + <i>Zingiber officinale</i> extract 500ng/ml	180.0 ± 40.4	9.8 ± 2.3 ***	34.4 ± 9.5 ***

Values are mean + SD. n=10 for each group. \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 as compared with their corresponding value in glucose 55 mM group

**Table 2: Proteins (total proteins and water soluble proteins) and malondialdehyde (MDA) in lens homogenate after 72 h of incubation**

Study Groups	Total proteins [mg/gm]	Water soluble proteins [mg/gm]	MDA [nmole/gm]
Normal lens [Control (Glucose 5.5 mM)]	226.8 ± 33.9***	94.1 ± 18.4**	2.9 ± 1.1***
Glucose 55 mM	160.8 ± 29.1	61.6 ± 29.4	60.7 ± 20.0
Glucose 55 mM + enalapril 5ng/ml	214.9 ± 27.7***	65.7 ± 25.8	27.4 ± 7.4***
Glucose 55 mM + <i>Zingiber officinale</i> extract 100ng/ml	165.7 ± 31.0	69.0 ± 23.8	42.8 ± 12.2**
Glucose 55 mM + <i>Zingiber officinale</i> extract 300ng/ml	203.8 ± 29.0 **	71.3 ± 21.7	32.5 ± 8.7 ***
Glucose 55 mM + <i>Zingiber officinale</i> extract 500ng/ml	202.4 ± 17.1 *	70.7 ± 25.4	24.3 ± 13.1***

Values are mean±SD. n=10 for each group. \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 as compared with their corresponding value in glucose 55 mM group.

**Photographic evaluation:**

All lenses were placed on a wired mesh with posterior surface touching the mesh and observed visually to opacity. (Fig 1) containing the normal lens (Glucose 5.5 mM). (Fig 2) containing the lens having 55 mM Glucose, in which cataract

was induced so it was blurred (not clear). (Fig 3) containing lens treated with 5ng/ml enalapril. And figure 4, 5 and 6 having lenses treated with *Zingiber officinale* extract in concentrations 100ng/ml, 300ng/ml and 500ng/ml respectively.



Fig. 1



Fig. 2



Fig. 3



Fig.4



Fig.5



Fig.6

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