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ANTICATARACT ACTIVITY OF ETHANOLIC EXTRACT OF *NIGELLA SATIVA* ON GLUCOSE INDUCED CATARACT IN GOAT EYE LENS

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ABSTRACT : The present investigation was aimed to evaluate efficacy of ethanolic extract seeds of *Nigella sativa* against glucose induced cataract in goat eye lens. In the in vitro study, goat lenses were subjected to photographic evaluation and biochemical parameters such as protein, GSH, MDA, NA^+/K^+ ATPase and sodium and potassium were also analyzed. Photographic examination of the eyes showed that treatment with ethanolic extracts of seeds of *N. sativa* retarded the progression of lens opacification. Cataract lens treated with *N. sativa* elevated the activity of Na^+ K^+ ATPase, total and water soluble proteins and K^+ ions to the level of normal level whereas reduced concentrations of Na^+ ions. The MDA levels were significantly less in the *N. sativa* treated groups whereas, the level of GSH, in high glucose (55mM), compared to the normal control group was significantly low but *N. sativa* treated groups showed higher level of GSH. These results support the view that ethanolic extract of seeds of *N. sativa* as seen in this in vitro model may, counteracts the effects of glucose in inducing cataract to some extent.

Key words: Anticataract, *Nigella sativa*, goat lens, in vitro

INTRODUCTION

Cataract is one of the complications that diabetic patients are at higher risk of developing. Osmotic stress imposed by sorbitol accumulation in the ocular lens has long been suggested to be the major cause of this complication (Harding, 1991 and Nirmalan *et al,* 2004) since sorbitol was found to be accumulated to a substantially high level incataractous lenses in diabetic animals like rats, rabbits, and dogs (Ughade *et al,* 1998). Under hyperglycemic conditions, sorbitol is formed from the reduction of glucose by the enzyme aldose reductase (AR) of the polyol pathway (Congdon *et al,* 2003). There is accumulating evidence, however, showing the contribution of oxidative stress to the development of diabetic cataract (Taylor *et al*, 1995; Kyselova *et al,* 2004; Mares, 2004). These findings led tothe conclusion that the major culprit of diabetic cataract is sorbitol accumulation.

Recently, the conversion of sorbitol to fructosevia sorbitol dehydrogenase (SD) has also been suggested to contribute to redox imbalance in diabetic tissues (Elena *et al* 2000 & Chandorkar *et al,* 1981).Na^+K^+ATPase plays an important role in maintaining the lens transparency and its alteration is one of the major event leading to cataract formation. Impairment of Na^+ - K^+ ATPase activity causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to catactogenesis (Chylack and Kinashita, 1969). Reduced glutathione (GSH) was found to be depleted in diabetic lenses (Gillis *et al*, 1992; Gupta *et al,* 1997) which was accompanied by an increase in the level of lipid peroxidation products (LPO)

Dietary intervention, particularly the use of traditional food and medicines derived from natural sources, is the mainstay in the management of diabetes. In this context, there has been a growing interest in recent times in identifying as many dietary/spice sources as possible for their ability to control diabetes (Swanston*et al,* 1991; Chang, 2000; Suryanarayana *et al*, 2003). Nevertheless, exhaust review of literature showed that studies of natural sources focusing on their ability to maintain blood glucose levels are copious but investigations for their beneficial effects on secondary complications of diabetes such as cataract; retinopathy, nephropathy and neuropathy are scanty. Therefore, we have been interested in investigating various dietary sources for their potential to prevent the secondary complications of diabetes.

The surgery has its own limitations; pronounced post-operative inflammation, loss of vitreous humor, posterior capsule opacification and expensive (Kyselova *et al,* 2004). So there is a need to look at the impact of treating cataract and relate it not just to surgery but also to scholastic achievements and development. In recent times, herbal drugs and natural products are targeted to develop more safe, effective and economical treatment for prevention or delay the cataract (Gupta and Halder, 2002 & Gupta and Srinivasa, 2005).

The seed of *Nigella sativa* is known by different names like black seeds or black cumin. In Latin, it is called as 'Panacea' meaning 'cure all', in Arabic it is termed as 'Habbah Sawda' or 'Habbat el Baraka' translated as 'seeds of blessing'. In China it is referred as Hak Jung Chou while in India it is called as Kalonji and in Persian, it is called as Shoneez. This plant belongs to the Ranunculaceae family of flowering plants and genus Nigella consists of about 14 species including therapeutically used seven species such as, *Nigella arvensis, Nigella ciliaris, Nigella damascene, Nigella hispanica, Nigella integrifolia, Nigella nigellastrum Nigella orientalis and Nigella sativa.* Among these, *Nigella sativa* is the species most exhaustively investigated for therapeutic purposes. The historical references to use of seeds of *Nigella sativa* also found in some of the oldest religious and medical texts. The seeds of *Nigella sativa* Linn. (Ranunculaceae) are used in herbal medicine all over the world for the treatment and prevention of a number of diseases.

Several studies have been carried out on the effect of herbal extracts against cataract formation in-vitro and in-vivo in various animals by various authors (Ho *et al,* 2000; Rosen *et al,* 2001; Wolff and Dean, 1987; Mullarkey *et al,* 1990; Schmidt *et al,* 1994). However, there is a paucity of information about the effects of extracts of seeds of *Nigella sativa* against cataract lens in goat. With this in context, the present investigation was aimed to evaluate efficacy of seeds of *Nigella sativa* against glucose induced cataract in goat eye lens. Since biochemical parameters such as protein, GSH, MDA, NA^+/K^+ ATPase and sodium and potassium are important for reflecting the healthy state of lens, these parameters were analyzed in the present study.

MATERIALS AND METHODS

Plant material

Plant material consists of dried powdered seeds of *Nigella sativa* belonging to the family *Ranunculaceae*. The seeds were purchased from local market, Vaniyambadi, India, during the month of November 2009.

Preparation of Ethanolic extract of *Nigella sativa*

The standard method (Bhargava *et al* 1998)was followed for the extraction of material. Seeds of *Nigella sativa* were dried in shade under room temperature and pulverized to a coarse powder. They were extracted by percolation at room temperature with 70% ethyl alcohol. The extract was concentrated under pressure (bath temperature 50°C) and finally dried in vacuum desiccator.

Collection of Eye Balls

Fresh goat eye balls of young and healthy goats were collected from the slaughter house, Vaniyambadi immediately after the slaughter. These eye balls were immediately transferred to the laboratory at 0-4°C. Sliced the Cornea from the front of the eye to gain access to the lens.

Lens culture

The lenses were incubated in artificial aqueous humor (NaCl 140mM, KCl 5mM, $MgCl_2$ 2mM, $NaHCO_3$ 0.5mM, Na_2HPO_4 0.5mM, $CaCl_2$ 0.4mM, Glucose 5.5mM) for 72 hours at room temperature at a pH of about 7.8 is maintained. In addition to this 32mg of penicillin and 250mg of streptomycin were added to prevent bacterial contamination. Glucose 55mM served as cataract inducer(Chandrokar *et al,* 1981).

Drug study

Group I : Normal lens glucose 5.5mM (control)
Group II : Glucose 55mM (induced)
Group III :
A. Glucose 55mM+*Nigella sativa* (100µg/ml) (Treated)
B. Glucose 55mM+*Nigella sativa* (300µg/ml) (Treated)
C. Glucose 55mM+*Nigella sativa* (500µg/ml) (Treated)

Photographic Evaluation

After 72 hours of incubation, lenses were observed for opacity and photographs were taken by placing the lenses on the wire meshes with posterior surface touching the mesh, and the pattern of mesh was observed through the lens as a lens as a measure of lens opacity.

Homogenate preparation

Lenses were homogenized in Tris buffer (0.23M pH 7.8) and $0.25X10^{-3}$ M EDTA. The homogenate was adjusted to 10% W/V. The homogenate was centrifuged at 10000 g at 4°C for 1 hour. The supernatant was used for estimation of protein (Lowry et al., 1951), GSH(Moron *et al*., 1979), MDA(Satohwith modification, 1978), NA^+/K^+ ATPase(Bonting, 1970) and sodium and potassium (flame photometry).

Statistical Analysis

All data were expressed as mean ± SD. All data were analyzed with SPSS/10 student software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by LSD. The values are expressed as mean ± S.D. and results were considered significantly different if $P<0.05$. Statistical variations are compared as follows: Normal Goat lens vs Goat lens + Glucose 55mM, Goat lens + Glucose55mM vs Goat lens + Glucose55mM + *N. sativa* extract.

RESULTS

Photographic examination of the eyes showed that the lenses of control were in normal stage throughout the duration of experimental period. Lenses treated with glucose (55mM) showed varying degree of cataractogenic changes as evidenced by opacification starting at the periphery after 8 hrs(Fig 1). Treatment with various concentrations of ethanolic extracts of seeds of *Nigella sativa* retarded the progression of lens opacification and this could be evidenced with clear visibility of gridlines through the lens.

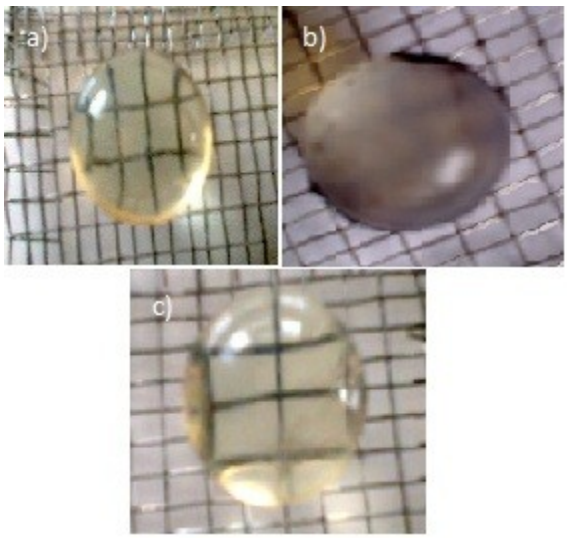

Figure 1. Effect of the treatment on lens opacification
a : Normal group (Glucose 5.5 mM); **b :** Control group (Glucose 55 mM);
c : Test group (Glucose 55 mM) + (Ethanolic extract of seeds of *N. sativa*500 µg /mL

Glucose (55mM) treated lenses showed significantly higher Na^+ ($P<0.05$) and lower K^+ & Na^+ K^+ ATPase activity ($P<0.001$) compared with normal lenses. *Nigella sativa* treated lenses showed significantly increased level of K^+ and Na^+ K^+ ATPase activity ($P<0.001$) with increasing concentration and the maximum activity of 10.7±1.8 meq/g and 42.6± 3.8 µg / g lens was registered at concentration of 500 µg/ml respectively (Table 1).

Table 1. Na^+, K^+ and Na^+ K^+ ATPase activity in lens homogenate after 72 hours of incubation.

Description	Na^+(meq/g m)	K^+(meq/gm)	Na^+K^+ATPase activity (µg / g lens)
Normal Goat lens	153±57.1	11.6±1.2	46.2±4.5
Goat lens + Glucose55mM	218.4±22.6	6.4±0.3	19.3±2.1
Goat lens + Glucose55mM + extract of seeds of *N. sativa*			
a) 100µ/ml	171.8±17.3	8.6±0.94	28.3±3.1
b) 300µ/ml	167.5±19.8	9.8±0.9	33.8±4.1
c) 500µ/ml	160.5±29.4	10.7±1.8	42.6±3.8

Values are mean ±S.D. n=5 for each group.

Lower concentration of protein and GSH was observed in the homogenate of glucose induced lenses whereas, very high MDA ($P<0.001$) was recorded against control group having normal lenses (Table 2). Homogenate of lens treated with extract increased total lens protein and GSH at 500 µg/ml concentration and decreased MDA content ($P<0.05$).

Table 2. Total Protein, Malondialdehyde (MDA) and Glutathione (GSH)in lens homogenate after 72 hours of incubation.

Description	Total protein (mg/gm)	MDA (µmole/gm)	Glutathione GSH (µmole/gm)
Normal Goat lens	226.8±24.9	2.7±1.8	2.8±0.19
Goat lens + Glucose55mM	160.8±17.68	56.4±6.3	1.2±0.05
Goat lens + Glucose55mM + extract of seeds of *N. sativa*			
a) 100µ/ml	187.4±20.6	53.6±5.4	1.5±0.07
b) 300µ/ml	201.6±18.14	42.8±12.2	2.0±0.25
c) 500µ/ml	220±19.8	34.5±3.9	2.4±0.3

Values are mean ±S.D. n=5 for each group.

DISCUSSION

In cataractogenesis, the parameters commonly considered are electrolytes (Na^+ and K^+), malondialdehyde (MDA), GSH and proteins (total proteins and water soluble proteins). Incubation of lens in the media containing high glucose (55mM) concentration has shown to cause considerable drop in Na^+ K^+ ATPase activity, with the progression of opacity. This study is in agreement with the findings of Lokesh*et al* (2010). Na^+ K^+ ATPase is important in maintaining the ionic equilibrium in the lens, and its impairment causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis (Wilbur, 1949). This alteration in the Na^+ K^+ 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 (Chylack and Kinashita, 1969). In the present study, cataract lens treated with *N. sativa* elevated the activity of Na^+ K^+ ATPase, total and water soluble proteins and K^+ ions to the level of normal level whereas reduced concentrations of Na^+ ions. This clearly evidenced that *N. sativa* seems to prevent the alteration of Na^+ and K^+ imbalance, which may be due to a direct effect on lens membrane Na^+ K^+ - ATPase or indirect through their free radical scavenging activity.

In this study, MDA levels were significantly higher in high glucose (55mM) groups, compared with normal control group. The MDA levels were significantly less in the *N. sativa* treated groups. The level of GSH, in high glucose (55mM), compared to the normal control group was significantly low but *N. sativa* treated groups showed higher level of GSH.

Incubation in the presence of high glucose (55mM) concentration stimulates a state of clinical diabetes. A prevention role of *Nigella sativa* as seen in this in vitro model may, to some extent suggest in preventing and/or retarding the progression of diabetic cataracts.This in vitro study may not directly correlate with the in vivo conditions. Therefore in vivo studies in different animal models are required for further elucidation of the role of *Nigella sativa* in preventing cataract formation.

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