J Food Sci Technol (May 2015) 52(5):2824–2832
DOI 10.1007/s13197-014-1305-y

ORIGINAL ARTICLE

Grape seed extract and Zinc containing nutritional food supplement delays onset and progression of Streptozocin-induced diabetic cataract in Wistar rats

Shakta Mani Satyam · Laxminaryana Kurady Bairy · Rajadurai Pirasanthan · Rajdip Lalit Vaishnav

Revised: 20 December 2013 / Accepted: 23 February 2014 / Published online: 23 March 2014

Abstract Prevention of hyperglycemia and enhancement of antioxidant defense mechanisms remain major goals in the treatment of diabetic cataract. Earlier, we reported strong anti-hyperglycemic and in vitro antioxidant potential of the combined formulation of grape seed extract and Zincovit tablets. Therefore, the current study was designed to investigate effects of combined formulation of grape seed extract and Zincovit tablets against streptozocin-induced diabetic cataract in Wistar rats. Adult Wistar rats were selected and diabetes was induced by streptozocin (35 mg/kg, *i.p*) and divided into four groups (group II-V). The normal control (group I) and streptozocin-induced diabetic cataract control rats received only vehicle. Groups III, IV and V animals received orally 40, 80 and 160 mg/kg of combined formulation of Zincovit tablets with grape seed extract respectively for a period of 150 days. The biochemical pathways involved in the pathogenesis of cataract such as oxidative stress, polyol pathway and alterations in adenosine triphosphate, glucose-6-phosphate dehydrogenase and blood glucose were investigated, to understand the possible mechanism of action of combined formulation of grape seed extract and Zincovit tablets. Rats treated with combined formulation of grape seed extract and Zincovit tablets delayed the progression of diabetic cataract as well as it showed significant alterations in oxidative stress markers along with blood glucose, aldose reductase, glucose-6-phosphate dehydrogenase and adenosine triphosphate level in lens. Over all, the results suggest that single combined formulation of grape seed extract and Zincovit tablets may be of great value in delaying diabetic cataract of human subjects as nutritional food supplement.

Keywords Diabetic cataract · Oxidative stress · Polyol pathway · Adenosine triphosphate · Grape seed extract · Zincovit tablets

S. M. Satyam
Department of Pharmacology, Melaka Manipal Medical College, Manipal University, Manipal 576104, Karnataka, India

L. K. Bairy (✉) · R. Pirasanthan · R. L. Vaishnav
Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal 576104, Karnataka, India
e-mail: klbairy@gmail.com

Introduction

Cataract, a visual impairment characterized by cloudiness or opacification of the crystalline lens, is the leading cause of blindness all over the world. The total number of persons with cataracts is estimated to rise to 30.1 million by 2020 and it can vary from country to country (Gupta et al. 2009). World Health Organization launched Vision 2020, to eliminate cataract as priority diseases. In view of the widespread prevalence of diabetes in developing countries such as India, diabetic cataract may pose a major problem in the management of blindness (Mohan et al. 2007; King et al. 1998; Zimmet 1999).

Chronic hyperglycemia is a major determinant in the development of secondary complications of diabetes, including diabetic cataract (Suryanarayana et al. 2005). The etiology of diabetic cataract is not fully understood, oxidative damage to the constituents of the eye lens is considered to be a major mechanism in the initiation and progression of various types of cataracts, including diabetic cataract (Spector 2000). Diabetes causes increased oxidative stress in various tissues, as evidenced by increased levels of oxidized DNA, proteins, and lipids, which are thought to play an important role in the pathogenesis of various diabetic complications (Chung et al. 2003).

Currently, surgical removal of the opacified lens is the mainstay of the management of cataracts, but there continues to be a backlog in the services provided in many parts of the world. It is estimated that a delay of 10 years in the development of the cataract would reduce the necessity for surgery in

45 %, saving millions (Cariello et al. 2006). Therefore, there is a search for pharmacological intervention that will maintain the transparency of the lens. Zincovit tablet is an advanced combined formulation of vitamins, minerals and grape seed extract (Table 1). Long-term daily administration of grape seed extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation (Chis et al. 2009). The biologically active constituents of grape seed extracts are proanthocyanidins, which represent a variety of polymers of flavan-3-ol, such as catechin and epicatechin and have a strong antioxidative effect in aqueous systems (Nakamura et al. 2003). One of the studies suggests the anticataract activity of grape seed extract (GSE, which contains 38.5 % procyanidins) in hereditary cataractous rats (ICR/f rats) (Yamakoshi et al. 2002). Studies suggest that Zinc prevents diabetes-induced GSH loss in the retina (Moustafa 2004), vitamin C and E prevents inhibition of glutathione peroxidase, glutathione reductase and superoxide dismutase activities in retina (Mustata et al. 2005; Kowluru et al. 2001; Penn et al. 2008). Increased oxygen free-radical production lowers the intracellular magnesium concentration and in light of such evidence, vitamin E administration might also regulate the intracellular magnesium concentration (Farvid et al. 2005). A synergistic effect of vitamins C and E along with Zinc could be expected based on the different environments in which they act (Farvid et al. 2005). Vitamin C acts in the hydrophilic milieu, scavenging reactive oxygen species, zinc located in the interphase of the bilayer prevents iron or copper binding to the membrane and alpha-tocopherol in the hydrophobic domains of the bilayer inhibits the lipid oxidation free-radical chain reaction (Farvid et al. 2005). Magnesium inhibits Malondialdehyde (MDA) formation in endothelial cells and low Magnesium oxide induced lipid peroxidation (Farvid et al. 2005).

Prevention of hyperglycemia and enhancement of antioxidant defense mechanisms remain major goals in the treatment of diabetic cataract. In previous studies, we found strong anti-hyperglycemic (Satyam et al. 2013) and in vitro antioxidant potential of the combined formulation of grape seed extract and Zincovit tablets (Satyam and Bairy 2013). Therefore, the present study was undertaken to investigate effects of combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) against Streptozocin-induced diabetic cataract in Wistar rats.

Table 1 Composition of Zincovit tablet

Ingredients	per tablet contains
Vitamin C	75 mg
Vitamin B_3	50 mg
Vitamin E	15 mg
Vitamin B_1	10 mg
Vitamin B_2	10 mg
Vitamin B_5	10 mg
Vitamin B_6	2 mg
Folic acid	1 mg
Vitamin A	5,000 IU
Vitamin D_3	400 IU
Biotin	150 mcg
Vitamin B_{12}	7.5 mcg
Zinc	22 mg
Magnesium	18 mg
Silica	1 mg
Manganese	0.9 mg
Copper	0.5 mg
Iodine	150 mcg
Boron	150 mcg
Selenium	50 mcg
Chromium	25 mcg
Molybdenum	25 mcg
Grape Seed Extract	50 mg

Materials and methods

Drugs and reagents

Single combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) was obtained as kind gift sample from Apex Laboratories Private Limited., Chennai (India). Streptozocin, Glutathione reductase, Reduced glutathione (GSH), NADPH, Thiobarbituric acid (TBA), Trichloroacetic acid (TCA) and 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB) were procured from Sigma Aldrich, Mumbai (India). One touch glucometer (Accu-Chek Active) with glucose oxidase-peroxidase reactive strips was purchased from Roche Diagnostics, (USA). Aldose reductase, Sorbitol dehydrogenase and Catalase assay kits were purchased from Cusabio (USA), Uscn Life Science Inc. (USA) and Bioassay Systems (USA) respectively. Both ATP and Glucose-6-phosphate dehydrogenase assay kits were procured from Abcam Inc. (USA). Sodium azide, Potassium chloride, Sodium chloride, Sodium hydroxide, Ethylene-di-amine-tetra-acetic acid (EDTA) and all other chemicals were obtained from Merck Chemicals, Mumbai (India). All reagents were analytical grade. All reagents except for the phosphate buffers were prepared every day and stored in a refrigerator at 4 °C. The reagents were equilibrated at room temperature for 30 min before use, either at the start of analysis or when reagent containers were refilled. Phosphate buffers were stable at +4 °C for one month.

Preparation of aqueous solution of Zincovit tablets for oral administration

Zincovit tablet is a single combined formulation of vitamins, minerals and grape seed extract (Table 1). Each tablet of Zincovit weighs 850 mg. Ten tablets of Zincovit were crushed and fine powder form was dissolved in 100 ml of distilled water containing 2 g gum acacia (2 % gum acacia). The aqueous solution of Zincovit tablets was stored in an amber colored bottle at 4 °C in refrigerator. The gum acacia was used for the proper binding of drug particles during oral administration of drug. Every week, fresh aqueous solution of Zincovit tablets was prepared. Only 2 % gum acacia (1 ml/kg) was given to control group animals to rule out any additional effect of gum acacia other than test drug in experimental animals.

Animals

Adult male Wistar albino rats weighing 150–300 g were housed in separate polypropylene cages, maintained under standard conditions with temperature (22–24 °C), 12-h light/12-h dark cycle and relative air humidity 40–60 %. Rats had continuous access to normal calorie standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and to tap water. After randomization into various groups, the rats were acclimatized to the laboratory conditions for one week before the start of the experiment. Animals described as fasted were deprived of food for 16-h but had allowed free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/06/2012) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment (Government of India), Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines and Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Research.

Induction of diabetes mellitus in experimental animals

After fasting, diabetes was induced by intraperitoneal (*ip*) injection of Streptozocin dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 35 mg/kg and maintained on ice prior to use. The animals were allowed to drink 5 % glucose solution overnight to overcome the drug induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (fasting blood glucose range of above 200 mg/dl) were considered as diabetic rats and used for the experiment.

Experimental design

In the experiment, 30 adult male Wistar rats (150–300 g) were used. The rats were divided into five groups ($n=6$). Treatment was done for 150 days as follow- *Group I:* Normal control rats were given 2 % gum acacia (1 ml/kg/day; *p.o*). *Group II:* Streptozocin (35 mg/kg, i.p.) induced diabetic cataract control rats were given 2 % gum acacia (1 ml/kg/day; *p.o*) *Group III:* Streptozocin (35 mg/kg, i.p.) induced diabetic cataract rats were given Zincovit tablets with grape seed extract (40 mg/kg/day; *p.o*) *Group IV:* Streptozocin (35 mg/kg, i.p.) induced diabetic cataract rats were given Zincovit tablets with grape seed extract (80 mg/kg/day; *p.o*) *Group V:* Streptozocin (35 mg/kg, i.p.) induced diabetic cataract rats were given Zincovit tablets with grape seed extract (160 mg/kg/day; *p.o*).

Blood, lens collection and processing

Cataract formation and its prevention were monitored every day during the treatment period, by examining both eyes with pen light illumination and subsequent photography of the isolated lenses was done on 151st day. Body weight of each rat was checked before start and at the end of the experiment. On 151th day, all the rats were sacrificed by administering overdose of ketamine, *i.p.* according to the annexure-6 of euthanasia of laboratory animals in the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility. The eye lenses were dissected by the posterior approach and stored at -70 °C until further analysis. Lens wet weight of each rat was taken. Fasting blood samples were drawn on from retro-orbital plexus of all the experimental animals using capillary tube for the estimation of blood glucose with the help of glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). Lens homogenates (10 % w/v) were prepared from three to five pooled lenses in 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10,000 rpm for 20 min using a Remi C-24 refrigerated centrifuge. The resulting supernatant was stored at −80 °C. All biochemical parameters were analyzed in the soluble fraction of the lens homogenate except for malondialdehyde (MDA) which was determined in the total homogenate. The following biochemical analysis was done in triplicate manner and optical density was also read for reagent and sample blank.

Determination of Malondialdehyde (MDA) level

To 20 μl lens homogenate sample, 200 μl 0.67 % thiobarbituric acid and 100 μl 20 % trichloroacetic acid were added and incubated at 100 °C for 20 min. Then, it was centrifuged at 12,000 rpm for 5 min and 100 μl of supernatant was

transferred to 96- wells of micro test plate. Optical density of supernatant was read at 540 nm by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Reduced glutathione (GSH) level

Mixture of 100 μl of lens tissue homogenate and 100 μl of 5 % trichloro acetic acid (TCA) solution was centrifuged at 5,000 rpm for 5 min. Then, 25 μl of tissue supernatant, 150 μl sodium phosphate buffer (PBS 0.2 M, pH 8.0) and 25 μl DTNB (0.6 mM) was added together in 96-wells of micro test plate and incubated for 10 min at room temperature and absorbance was read at 412 nm by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Glutathione peroxidase (GPx) level

Five hundred fifty microliter phosphate buffer (100 mM) containing 0.1 mM EDTA (pH 6.5), 50 μl Sodium azide (2 mM), 50 μl lens tissue homogenate, 100 μl glutathione reductase (2.5 U/ml) and reduced glutathione (GSH, 100 mM) were added together and incubated at 37ºC for 10 min. Then, 100 μl NADPH (2.5 mM) was added in the above mixture. Reaction was started by adding 100 μl hydrogen peroxide (1.5 mM) and optical density was read at 340 nm at one minute interval for five minutes by using UV-2450 spectrophotometer, Shimadzu Corporation, Tokyo (Japan).

Determination of Protein thiol (PT) level

Twenty microliter of lens tissue homogenate sample was added in the mixture of 180 μl disodium edetate (2 mM disodium edetate in 0.2 M disodium hydrogen phosphate) buffer solution and 4 μl DTNB solution (10 mM DTNB in 0.2 M disodium hydrogen phosphate) in 96-wells of micro test plate. Then, optical density was read at 412 nm by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Superoxide dismutase (SOD) activity

To 25 μl of lens tissue homogenate sample, 925 μl sodium carbonate buffer (0.1 M, pH 10–11) and 50 μl of adrenaline bitartarate (1 mM) was added and absorbance (A_{0s}-A_{60s}) was read at 480 nm by using UV-2450 spectrophotometer, Shimadzu Corporation, Tokyo (Japan).

Determination of Catalase (CAT) activity

Catalase activity in lens homogenate was measured according to the standard protocol given along with the Catalase assay kit of Bioassay Systems, Hayward (USA) by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Glucose-6-phosphate dehydrogenase (G6PD) activity

Glucose-6-phosphate dehydrogenase activity in lens homogenate was measured according to the standard protocol given along with the Glucose-6-phosphate dehydrogenase assay kit of Abcam Inc., (USA) by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Adenosine triphosphate (ATP) level

Adenosine triphosphate level in lens homogenate was measured according to the standard protocol given along with the Adenosine triphosphate assay kit of Abcam Inc., (USA) by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Aldose reductase (AR) level

Aldose reductase concentration in lens homogenate was measured according to the standard protocol given along with the Aldose reductase assay kit of Cusabio Inc., (USA) by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Determination of Sorbitol dehydrogenase (SDH) level

Sorbitol dehydrogenase level in lens homogenate was measured according to the standard protocol given along with the Sorbitol dehydrogenase assay kit of Uscn Life Science, (USA) by using an ELISA reader Bio Tek Instruments ELx800- MS, (USA).

Statistical analysis

Using Statistical Package for the Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, USA), normally distributed data were expressed as mean ± standard error of mean and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. Data with non-uniform distribution were expressed as median, Quartile (Q_1, Q_3) and analyzed by non-parametric K Independent samples test followed by Kruskal-Wallis H test. A level for $P \leq 0.05$ was considered to be statistically significant (two-sided).

Results

Effect on lens morphology

Morphological examination of both eyes of each rat was done during the study period. All six rats in normal control group (which received 2 % gum acacia; 1 ml/kg, *p.o*) exhibited complete transparency of the lens (Figs. 1 and 2a). Out of 12 eye lenses of six rats in streptozocin-induced diabetic cataract

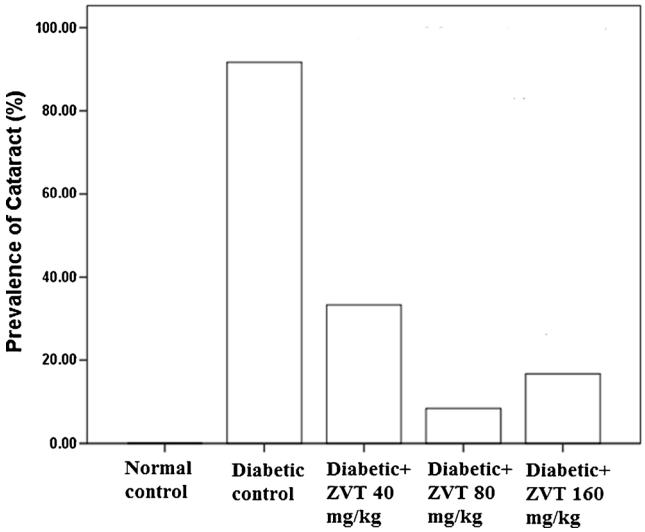

Fig. 1 Effect of different doses of combined formulation of grape seed extract and Zincovit tablets on prevalence of streptozocin induced diabetic cataract among experimental groups

control group (which received 2 % gum acacia; 1 ml/kg, *p.o*), 11 eye lenses exhibited dense opacification of the lenses (Figs. 1 and 2b). In contrast, only 4, 1 and 2 among 12 lenses which received combined formulation of grape seed extract and Zincovit tablets 40 mg/kg, 80 mg/kg and 160 mg/kg; *p.o*, respectively exhibited mild lenticular opacification (Figs. 1 and 2c–e).

Effect on body weight and isolated lens wet weight

There was significant decrease in body weight of diabetic cataract control rats in comparison with normal control group of rats ($p<0.001$). Body weight was restored significantly in the rats treated with combined formulation of grape seed extract and Zincovit tablets in a dose dependent fashion ($p<0.05$; 40 mg/kg, $p<0.001$; 80 and 160 mg/kg treatment groups) when compared with streptozocin-induced diabetic cataract control rats (Fig. 3c). Isolated lens wet weight was significantly lower in group of rats treated with combined formulation of grape seed extract and Zincovit tablets ($p<0.01$; 80 and 160 mg/kg treatment groups) in comparison with streptozocin-induced diabetic cataract control group (Fig. 3d).

Effect on biochemical parameters

There was significant increase in reduced glutathione ($p=0.010$), glutathione peroxidase ($p=0.014$), glucose-6-phosphate dehydrogenase ($p=0.003$), protein thiol ($p=0.002$), adenosine triphosphate ($p=0.021$) and catalase ($p=0.021$) in the lens of rats treated with combined formulation of grape seed extract and Zincovit tablets in dose dependent manner when compared with streptozocin-induced diabetic cataract control rats (Tables 2 and 3). Fasting blood glucose and lens aldose reductase level was significantly high in streptozocin-induced diabetic cataract control rats in comparison with normal control rats (for fasting blood glucose and lens aldose reductase; $p<0.01$, 0.001 respectively). In contrast, there was significant decrease in blood glucose ($p<0.001$; in 40 mg/kg, 80 mg/kg and 160 mg/kg treatment groups, Fig. 3a) and lens aldose reductase level ($p<0.05$; 40 mg/kg, $p<0.001$; 80 mg/kg and $p<0.01$; 160 mg/kg treatment groups, Fig. 3b) in the rats treated with combined

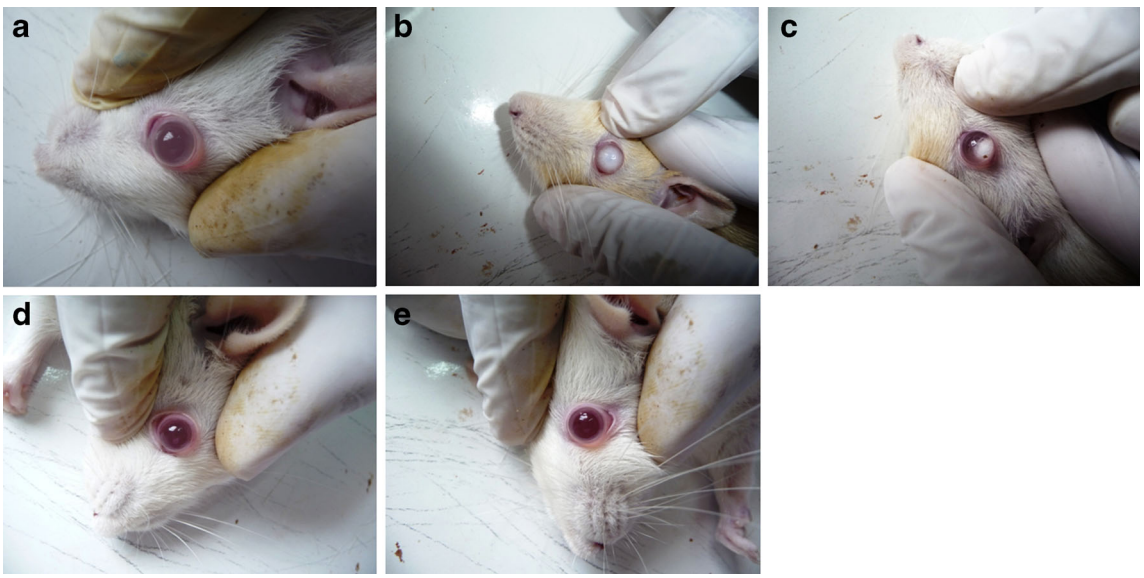

Fig. 2 Photographs of experimental rats on 151th day: (**a**) Normal control rat (**b**). Streptozocin-induced diabetic cataract control rat (**c**). Streptozocin-induced diabetic cataract rat treated with combined formulation of grape seed extract and Zincovit tablets 40 mg/kg (**d**). Streptozocin-induced diabetic cataract rat treated with combined formulation of grape seed extract and Zincovit tablets 80 mg/kg (**e**). Streptozocin-induced diabetic cataract rat treated with combined formulation of grape seed extract and Zincovit tablets 160 mg/kg

formulation of grape seed extract and Zincovit tablets in a dose dependent fashion when compared with streptozocin induced diabetic cataract control rats. There were no significant changes in lens malondialdehyde, superoxide dismutase and sorbitol dehydrogenase levels among experimental groups.

Discussion

At present, surgical removal of the opacified lens is the mainstay of the management of cataracts. Any strategy that prevents or slows the progression of cataract has a significant health impact. Since, the mechanism of galactose-induced cataract is different from typical diabetic cataract (Creighton et al. 1985; Kinoshita 1974). In contrast to sorbitol, galactitol is not further metabolized by sorbitol dehydrogenase in galactosemia and may represent osmotic changes of sugar cataract than oxidative stress (Tausz et al. 2004). Hence, we set out to investigate the role of combined formulation of grape seed extract and Zincovit tablets in the prevention or delay of Streptozocin-induced diabetic cataract.

The significant increase in the activity of lens glucose-6-phosphate dehydrogenase in diabetic cataractous rats treated with combined formulation of grape seed extract and Zincovit tablets in comparison with diabetic cataract control rats may enhance formation of ribose for nucleic acids synthesis and also increase the renewal of lens protein (Table 2). The pentose phosphate pathway supplies NADPH which is the main component of the glutathione system.

The increased level of lens aldose reductase in diabetic cataractous rats treated with combined formulation of grape seed extract and Zincovit tablets in comparison with diabetic cataract control rats may be owing to an increased availability

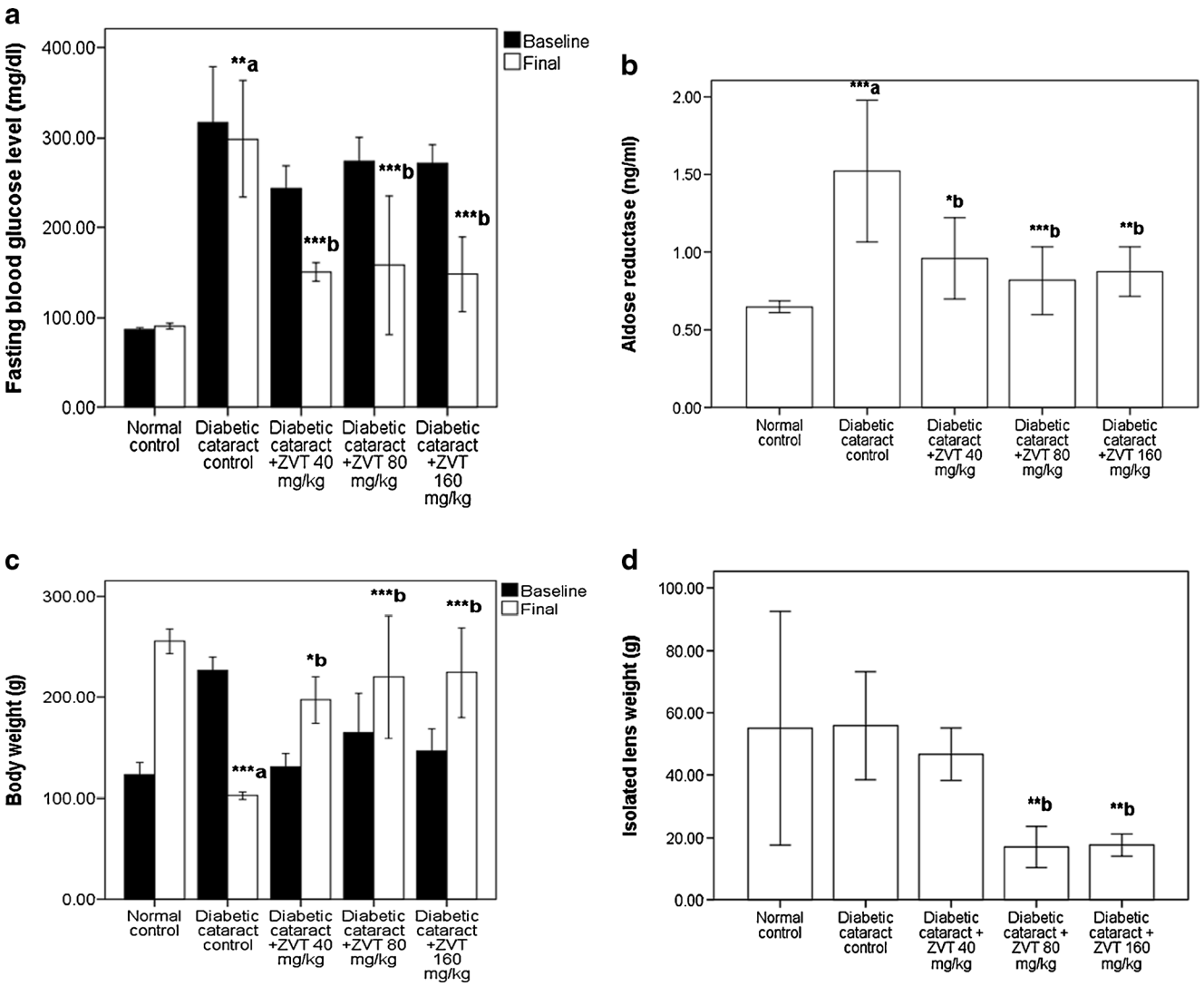

Fig. 3 Effect of different doses of combined formulation of grape seed extract and Zincovit tablets (ZVT) on: (**a**). Fasting blood glucose (**b**). Aldose reductase in lens homogenate (**c**). Body weight (**d**). Isolated lens wet weight. n=6; number of rats in each group. Data are expressed as mean ± standard error of mean and analyzed by one way analysis of variance (ANOVA) followed by post-hoc Tukey test

Table 2 Effect of combined formulation of grape seed extract and Zincovit tablets on reduced glutathione (μmoles/mg), glutathione peroxidase (μmoles/ml) and Glucose-6-phosphate dehydrogenase (μmoles/min/ml) in lens tissue homogenate

Groups (n=6)	GSH (Q_1, Q_3)	GPx (Q_1, Q_3)	G6PD (Q_1, Q_3)
I- Normal control (2 % gum acacia)	10.41 (2.63, 22.14)	53.56 (8.29, 120.10)	9.59 (3.23, 19.47)
II- Diabetic control (2 % gum acacia)	4.47 (2.98, 6.31)	20.76 (19.33, 26.55)	3.28 (2.28, 4.78)
III- Diabetic+ZVT (40 mg/kg/day)	4.77 (3.83, 5.66)	32.14 (17.56, 53.84)	3.91 (3.24, 5.26)
IV- Diabetic+ZVT (80 mg/kg/day)	18.61 (9.25, 25.32)	96.68 (81.24, 222.36)	11.84 (7.69, 16.67)
V- Diabetic+ZVT (160 mg/kg/day)	12.68 (11.90, 21.14)	61.56 (46.28, 99.11)	8.44 (8.07, 14.36)

Data are expressed as the median (quartiles- Q_1, Q_3) and different treatments were analyzed by non-parametric test K Independent sample test followed by Kruskal-Wallis H test

n Number of rats in each group, *GSH* Reduced glutathione (p=0.010), *GPx* Glutathione peroxidase (p=0.014) and *G6PD* Glucose-6-phosphate dehydrogenase (p=0.003)

of NADPH from the enhanced pentose phosphate pathway activity in the treatment groups (Fig. 3b).

It has been reported that when the intensity of a stress increases, GSH concentrations usually decline and redox state becomes more oxidized, leading to deterioration of the system (Tausz et al. 2004). In the present study, there was decrease in reduced form glutathione (GSH) and protein thiol level in the diabetic cataract control group whereas Zincovit tablets with grape seed extract treated group at the dose of 40 and 80 mg/kg restored the level of reduced glutathione and protein thiol might be due to zinc as one of its constituent which is known to prevent diabetes-induced GSH loss but further decrease in the GSH and protein thiol level in higher dose (160 mg/kg) treatment group of Zincovit tablets with grape seed extract might be due to presence of copper as one of the constituent of Zincovit tablets which may resulted into its more binding to membrane and finally decrease in GSH and protein thiol level (Tables 2 and 3).

Decrease in the activity of glutathione peroxidase, catalase in lenses of diabetic cataract control rats may be due to either direct inactivation of these enzymes by reactive oxygen species or by decreased aerobic metabolism in lens. In this study, the significant increase in the activity of glutathione peroxidase following the administration of the combined formulation of grape seed extract and Zincovit tablets may be adduced to the presence of elements such as zinc that might have enhanced the synthesis of this enzyme (Tables 2 and 3).

A consistent finding in the present study was significant increase of lens ATP levels in the rats treated with combined formulation of grape seed extract and Zincovit tablets in a dose dependent fashion in comparison with streptozocin-induced diabetic cataract control rats (Table 3). The decrease in ATP level in lens of diabetic cataract control rats could be due to irreversible damage of the lens. ATP is needed as a cofactor for the conversion of glucose to phosphorylated glucose by hexokinase. Decrease in ATP might have affected glucose 6-phosphate generation and thereby diverting glucose through the polyol pathway. ATP is also known to affect enzymes involved in the glutathione synthesis.

There was no significant change in lens malondialdehyde level among experimental groups might be due to very low concentration of polyunsaturated fatty acids in normal and cataractous lenses, which indicates that lens is less prone to lipid peroxidation than other tissues. The retina, rich in polyunsaturated fatty acids than other ocular tissues is the effective site of lipid peroxidation and from this; membrane peroxidation products may probably diffuses and causes damage to other ocular structures.

The significant decrease in wet weight of lens in test drug treated animals at the dose of 80 and 160 mg/kg might be

Table 3 Effect of combined formulation of grape seed extract and Zincovit tablets on protein thiol (μmoles/mg), adenosine triphosphate (mmoles/ml) and catalase (units of hydrogen peroxide oxidized/min/mg) in lens tissue homogenate

Groups (n=6)	PT (Q_1, Q_3)	ATP (Q_1, Q_3)	Catalase (Q_1, Q_3)
I- Normal control (2 % gum acacia)	77.07 (29.05, 158.84)	0.72 (0.10, 3.47)	6.03 (1.27, 10.77)
II- Diabetic control (2 % gum acacia)	58.97 (39.90, 99.80)	0.20 (0.11, 0.30)	0.80 (0.51, 1.58)
III- Diabetic+ZVT (40 mg/kg/day)	91.97 (79.02, 99.69)	0.14 (0.07, 0.18)	3.60 (2.45, 4.85)
IV- Diabetic+ZVT (80 mg/kg/day)	232.44 (120.96, 309.01)	0.78 (0.45, 1.04)	6.26 (1.83, 16.62)
V- Diabetic+ZVT (160 mg/kg/day)	175.76 (163.74, 319.28)	0.42 0.29, 0.66)	6.27 (3.66, 11.90)

Data are expressed as the median (quartiles- Q_1, Q_3) and different treatments were analyzed by non-parametric test K Independent sample test followed by Kruskal-Wallis H test

n Number of rats in each group, *PT* Protein thiol (p=0.002), *ATP* Adenosine triphosphate (p=0.021) and *CAT* Catalase (p=0.046)

attributed to increased level of reduced glutathione (GSH) which lead to prevention of disruption, disintegration of lenticular fibers and thus inhibited leakage and loss of metabolites resulting into increased water content. This finding is consistent with some of the other pre-clinical and clinical studies where lens wet weight significantly increased in cataract groups (Aleo et al. 2005; Matsuoka et al. 1997).

The ability of the combined formulation of grape seed extract and Zincovit tablets (Nutritional food supplement) to delay the progression and maturation of streptozocin-induced diabetic cataract might be attributed to synergistic interplay of constituents of Zincovit tablets, such as- grape seed extract proanthocyanidins which comprise only procyanidins [subunits constituted of (+)-catechin (C) and (−)-epicatechin (EC)], Vitamins A, B, C, D, E, folic acid, biotin and minerals like zinc, copper, selenium, magnesium, manganese, chromium and molybdenum mainly, which are promoters of antioxidant activity (Table 1). Vitamin C, E (mainly tocopherols) and a variety of carotenoids are present in lens tissue and in the fluid that surrounds it and have an important part to play in lenticular antioxidant status. Riboflavin is a precursor to flavin-adenine dinucleotide (FAD), which is a coenzyme for the biosynthesis of glutathione reductase. Selenium is an integral part of the enzyme, glutathione peroxidase. Both the lenses of the eye and the aqueous humor contain protective enzymes that breakdown the damaged proteins that clump together and cause cataracts. This combined formulation due to its strong antioxidant potential may keep these enzymes from being destroyed.

Conclusions

Thus, the present study demonstrates that the single combined formulation of grape seed extract and Zincovit tablet is the potential functional nutritional food supplement that can prevent the onset, progression and maturation of streptozocin-induced diabetic cataract in Wistar rats. The therapeutic effect seen in animal studies cannot always be entirely extrapolated to humans. Hence, clinical evaluation should be performed to precisely define the anti-cataractogenesis role of Zincovit tablets with grape seed extract in humans. Our study opens the perspective to clinical studies specifically designed to evaluate the impact of the single combined formulation of grape seed extract and Zincovit tablets on the onset and progression of diabetic cataract of human subjects as nutritional food supplement. “This information would eventually complement our findings, opening the way to sustain diabetic cataract development in human population”.

Acknowledgments All the authors declare that they do not have any conflict of interest. This work was financially supported by Apex Laboratories Private Ltd., Chennai (India) for which authors are grateful.

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