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Effect of Two Different Formulations of Zincovit Tablets on Galactose Induced Cataract in Rats.

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ABSTRACT

Diabetes is one of the major cause for development of early cataract. Control of blood sugar and enhancement of antioxidant defense remain important goals in the prevention of diabetic cataract. Multivitamins may prevent or delay the development of cataract by their antioxidant action. Hence a commercially available nutrient supplement namely Zincovit tablet along with grape seed extract was tested against experimentally induced diabetic cataract. A total of 48 rats were used in this experiment. Diabetic cataract was induced in 42 rats by administration of 0.5 ml of 50% galactose for 45 days. All the animals including normal control were divided into eight groups (n=6). The rats were given two formulations of Zincovit tablets (40, 80 and 160 mg/kg) along with 0.5 ml of 50% galactose orally to their respective groups for 45 days. Eye was examined daily to check cataract formation. On 46th day, the rats were sacrificed and lens was separated for estimation of glutathione peroxidase (GPx) activity, superoxide dismutase (SOD) activity, catalase, glucose-6-phosphate dehydrogenase (G6PD) activity, adenosine triphosphate (ATP) level, aldose reductase (AR) level and sorbitol dehydrogenase (SDH). Data showed that there was significant increase in glutathione peroxidase ($p=0.010$), adenosine triphosphate ($p=0.002$) and significant decrease ($p<0.001$) in aldose reductase level in Zincovit treated animals when compared with galactose induced diabetic cataract control animals. But, there was no significant change in reduced glutathione (GSH), protein thiol, superoxide dismutase, catalase, malondialdehyde, glucose-6-phosphate dehydrogenase and sorbitol dehydrogenase level when compared to control group. Zincovit tablet (vitamin A: 5000 IU with grape seed extract was better in delaying the onset of cataract when compared to other groups.

Keywords: Zincovit tablet, galactose cataract, Zincovit formualtions

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INTRODUCTION

Cataract is the leading cause of blindness all over the world. The risk factors that increase the development of cataract are aging, nutritional deficiencies, smoking, exposure to sunlight, oxidative stress and certain drugs[1]. Uncontrolled blood sugar is important in the development of complications of diabetes mellitus (DM) including early cataract [2]. Since India is a country with high prevalence of DM in developing countries such as India, cataract due to DM may pose a major problem in the management of blindness.

The exact mechanism of development of cataract is not fully understood. The well accepted mechanism is the oxidative damage to the constituents of the eye lens that results in initiation and progression of various types of cataracts, including diabetic cataract. Poorly controlled DM results in hyperglycemia, which is associated in ocular tissues with non-enzymatic protein glycation, osmotic stress, and oxidative stress [3, 4].

Irina et al reported that grape seed extract has antioxidant property and can protect against tissue lipid peroxidation and protein oxidation [5]. Antioxidants such as vitamin C, vitamin E, and carotenoids have been reported to prevent the progression of experimentally induced cataracts. Procyanidins, a powerful antioxidant, which can prevent cataract formation. Yamakoshi et al. reported preventive activity of grape seed extract (GSE, which contains 38.5% procyanidins) in hereditary cataractous rats (ICR/f rats)[6]. Satyam et al reported the effect of Zincovit tablet (vitamin A: 5000 IU with grape seed extract) on delay of onset and progression of streptozocin induced diabetic cataract [7]. We would like to study the effect of different doses of vitamin A on galactose induced diabetic cataract. Two new formulations of Zincovit tablet which contain vitamin A: 2000 IU with grape seed extract and vitamin A: 5000 IU without grape seed extract were prepared by Apex Laboratories Chennai. A study was planned to evaluate the effect of Zincovit tablet which contain vitamin A: 2000 IU with grape seed extract and vitamin A: 5000 IU without grape seed extract on galactose induced diabetic cataract in Wistar rats.

MATERIALS AND METHODS

Twelve week old Wistar rats (150–200 g) of either sex weighing were used in this study. The CPCSEA guidelines on housing and maintenance of laboratory animals were followed during the entire experimental period. The Institutional Animal Ethics Committee (Registration no. 94/1999/CPCSEA/KMC) has approved the study.

Forty eight rats were divided in to 8 groups of 6 each as follows: Group I: Normal control rats were given 0.3 ml of 2% gum acacia, Group II: Diabetic cataract control rats were given 0.5 ml of 50% galactose + 0.3 ml of 2% gum acacia, Group III: Diabetic cataract rats were given 0.5 ml of 50% galactose + Zincovit tablets (vitamin A: 2000 IU with grape seed extract 40 mg/kg/day, Group IV: Diabetic cataract rats were given 0.5 ml of 50% galactose + Zincovit tablets (vitamin A: 2000 IU with grape seed extract 80 mg/kg/day, Group V: Diabetic cataract rats were given 0.5 ml of 50% galactose + Zincovit tablets (vitamin A: 2000 IU with grape seed extract 160 mg/kg/day, Group VI: Diabetic cataract rats were given 0.5 ml of 50% galactose + Zincovit tablets (vitamin A: 5000 IU without grape seed extract 40 mg/kg/day, Group VII: Diabetic cataract rats were given 0.5 ml of 50% galactose + Zincovit tablets (vitamin A: 5000 IU without grape seed extract 80 mg/kg/day and Group VIII: Diabetic cataract rats were given 0.5 ml of 50% galactose + Zincovit tablets (vitamin A: 5000 IU without grape seed extract 160 mg/kg/day. All the drugs were given orally for 45 days.

Eyes of each rat was observed throughout the experiment for any changes including but not limited to cataract formation. On forty sixth day, body weight of each animal was taken and fasting blood samples were drawn for assessing blood glucose. On the same day rats were sacrificed by overdose of urethane (i.p.) and lenses were removed, weighed and stored at -70°C until further analysis. A 10% lens homogenate was prepared in 50 mM phosphate buffer (pH 7.4). All the biochemical parameters such as reduced glutathione (GSH), protein thiol, glutathione peroxidase, superoxide dismutase, catalase, malondialdehyde, glucose-6-phosphate dehydrogenase, adenosine triphosphate (ATP), aldose reductase and sorbitol dehydrogenase were analyzed in the soluble fraction of the lens homogenate (15,000 *rpm* at 4°C) by commercially available assay kits.

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Data was expressed in terms of median (Quartile$_1$, Quartile$_3$) and analyzed by non-parametric K independent sample test followed by Kruskal Wallis test respectively. P value less than 0.05 was considered as statistically significant.

RESULTS

Table 1: Level of Glutathione peroxidase in lens homogenate (nmol/min/ml): Diabetic cataract model

Groups	Dose	Median (Q_1, Q_3)	P value	Significance
I	Normal control-0.3 ml of 2% gum acacia	53.56 (8.29, 120.10)	**0.010***	**S**
II	Galactose induced diabetic cataract (GIDC) control-0.3 ml of gum acacia	18.26 (18.13, 25.42)		
III	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 40 mg/kg/day	42.34 (20.68, 58.34)		
IV	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 80 mg/kg/day	95.48 (70.24, 122.12)		
V	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 160 mg/kg/day	61.56 (46.28, 99.11)		
VI	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 40 mg/kg/day	20.77 (6.44, 40.18)		
VII	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 80 mg/kg/day	32.14 (12.62, 50.23)		
VIII	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 160 mg/kg/day	24.77 (10.82, 40.55)		

*compared among groups, S- Significant

Table 2: Level of Adenosine triphosphate (ATP) in lens homogenate (mol/ml): Diabetic cataract model

Groups	Dose	Median (Q_1, Q_3)	P value	Significance
I	Normal control-0.3 ml of 2% gum acacia	125.0 (52.75, 170.54)	**0.002***	**S**
II	Galactose induced diabetic cataract (GIDC) control-0.3 ml of gum acacia	9.50 (6.0, 48.50)		
III	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 40 mg/kg/day	82.10 (49.5, 107.52)		
IV	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 80 mg/kg/day	24.20 (16.20, 39.25)		
V	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 160 mg/kg/day	108.50 (38.50, 284.10)		
VI	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 40 mg/kg/day	82.32 (39.48, 179.18)		
VII	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 80 mg/kg/day	27.50 (16.24, 45.50)		
VIII	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 160 mg/kg/day	41.50 (38.50, 46.75)		

*compared among groups, S- Significant

In galactose induced diabetic cataract animal model, different significant levels were observed among different doses of both the formulations of Zincovit tablets with respect to their respective toxic control group for glutathione peroxidase, aldose reductase and adenosine triphosphate (Table 1-3). There was no significant change in the fasting blood glucose level, body weight and isolated lens weight of experimental animals in galactose induced cataract animal model. Also, there was no significant change in reduced glutathione (GSH), protein thiol, superoxide dismutase, catalase, malondialdehyde, glucose-6-phosphate dehydrogenase and

sorbitol dehydrogenase level in the lens homogenate of rats who were treated with the respective formulations of Zincovit tablet when compared to the toxic control animals in galactose induced diabetic cataract. In galactose induced cataract model, all lenses in normal control group were clear and normal but among galacotose administered rats, 71%, 68%, 65%, 62%, 48%, 58%, 60% of lenses developed cataract in galactose induced cataractous control, Zincovit tablets (vitamin A: 2000 IU with grape seed extract) and Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 40 mg/kg, 80 mg/kg and 160 mg/kg each treatment groups respectively (Fig. 1-8).

Table 3: Level of Aldose reductase in lens homogenate (ng/ml): Diabetic cataract model

Groups	Dose	Median (Q_1, Q_3)	P value	Significance
I	Normal control-0.3 ml of 2% gum acacia	3.87 (2.97, 10.12)	**<0.001***	**S**
II	Galactose induced diabetic cataract (GIDC) control-0.3 ml of gum acacia	5.54 (4.49, 6.50)		
III	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 40 mg/kg/day	5.25 (5.13, 6.37)		
IV	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 80 mg/kg/day	1.03 (0.63, 1.92)		
V	GIDC- Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 160 mg/kg/day	1.56 (1.32, 3.12)		
VI	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 40 mg/kg/day	1.51 (0.78, 6.96)		
VII	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 80 mg/kg/day	0.91 (0.48, 1.83)		
VIII	GIDC- Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 160 mg/kg/day	1.56 (0.98, 3.56)		

*compared among groups, S- Significant

Figure 1: Normal control rat

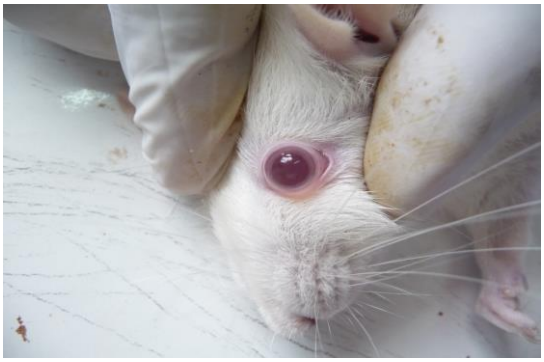

Figure 2: Galactose induced diabetic cataract control rat

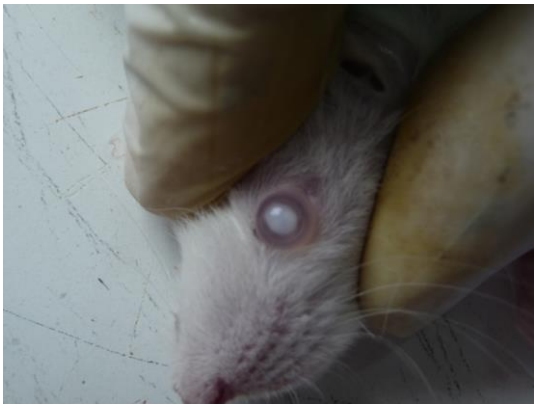

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Figure 3: Galactose induced cataractous rat treated with Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 40 mg/kg

Figure 4: Galactose induced cataractous rat treated with Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 80 mg/kg

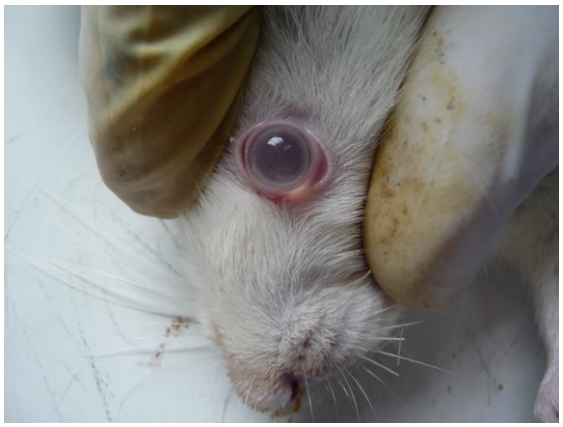

Figure 5: Galactose induced cataractous rat treated with Zincovit tablets (vitamin A: 2000 IU with grape seed extract) 160 mg/kg

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Figure 6: Galactose induced cataractous rat treated with Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 40 mg/kg

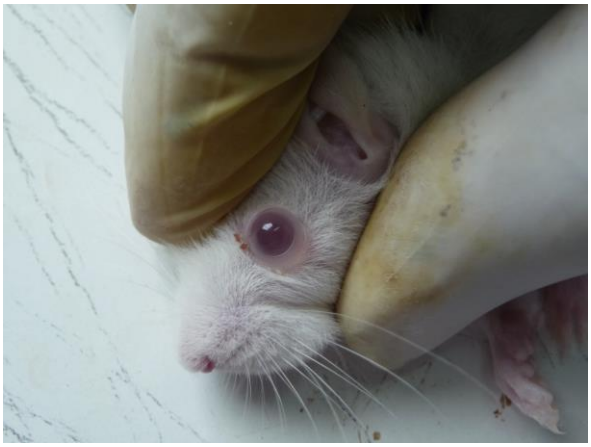
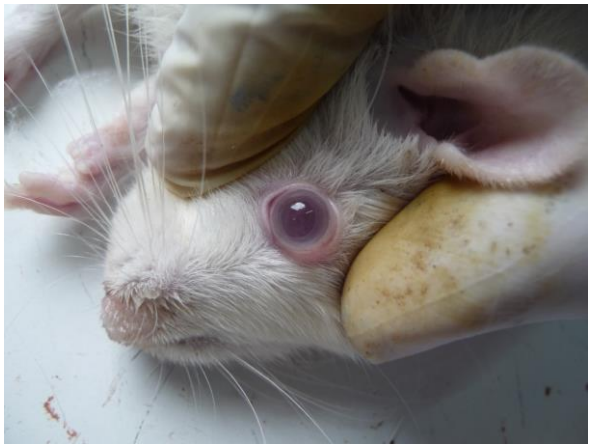


Figure 7: Galactose induced cataractous rat treated with Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 80 mg/kg

Figure 8: Galactose induced cataractous rat treated with Zincovit tablets (vitamin A: 5000 IU without grape seed extract) 160 mg/kg



DISCUSSION

Hyperglycemia-induced oxidative stress is considered as the most likely cause of changes in the sulfhydryl status of lens proteins during diabetic cataractogenesis. A progressive decrease of protein sulfhydryls has been observed generally during the development of diabetic and senile cataracts [8,9].

Sulfhydryl oxidation is thought to be one of the main pathological events leading, through disulfide cross-linking and molecular aggregation, to protein precipitation and lens opacification [10].

Accumulation of high levels of sugar alcohol such as galactose causes hyper tonicity of lens. Increased hypertonicity eventually increases sodium levels and decreases potassium levels leading to cataract formation [11]. Animal studies clearly indicate that the rate and severity of sugar cataract development is proportional to both the lenticular levels of aldose reductase and the severity of the hyperglycemia [12].

In galactose induced diabetic cataract model, the specific activity of aldose reductase, a key enzyme of the polyol pathway was significantly higher in diabetic cataract control group animals than in normal control and both the formulations of Zincovit tablets 40 mg/kg, 80 mg/kg, 160 mg/kg treatment group. Sorbitol dehydrogenase activity was not significantly altered in the treatment groups.

The potential role of vitamins in preventing cataract is well documented, especially vitamin C or ascorbic acid which plays an important part in lens biology, both as an antioxidant and as a UV filter. Dietary deficiency of vitamin C led to reduction in lens concentrations of ascorbate. A research study on guinea pigs shows that ascorbate inhibits galactose cataract [13]. Similarly, another study reveals that intake of ascorbate increases the level of vitamin C in rat lens [14]. Vitamin E also has an important part to play in lenticular antioxidant status. A number of studies have evaluated the anticataract potential of vitamin E and found it to be effective against galactose, steroid and UV radiation-induced cataract. Riboflavin is a precursor to flavin adenine dinucleotide (FAD), which is a coenzyme for the biosynthesis of glutathione reductase [15-18]. Plasma levels of zinc and copper were found to be significantly low in cataract patients [19]. Selenium is an integral part of the enzyme, glutathione peroxidase. A decrease in glutathione peroxidase activity has been found in the lenses of selenium-deficient rats [20].

The present study correlates with our previous study on the effect of Zincovit tablet (vitamin A: 5000 IU with grape seed extract) on delay of onset and progression of streptozocin induced diabetic cataract in Wistar rats. The therapeutic benefit for delay the onset and progression of diabetic cataract was found to be highest with Zincovit tablet (vitamin A: 5000 IU with grape seed extract followed by Zincovit tablet (vitamin A: 5000 IU without grape seed extract) and Zincovit tablet (vitamin A: 2000 IU with grape seed extract) respectively.

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REFERENCES

[1] Greiner J, Chylack L. Posterior subcapsular cataracts: Histopathologic study of steroid-associated cataracts. Arch Ophthalmol 1979; 97:135–44.
[2] Brian G, Taylor H. cataract blindness: Challenges for the 21st century. Bull World Health Organ 2001; 79:249–56.
[3] Derham BK, Harding JJ. Alpha-crystallin as a molecular chaperone. Prog Retin Eye Res 1999;18:463–509
[4] 46. Kador PF, Robison WG, Jr, Kinoshita JH. The pharmacology of aldose reductase inhibitors. Annu Rev Pharmacol Toxicol. 1985; 25:691–714.
[5] Irina CC, Ungureanu MI, Marton A, Simedrea R, Muresan A, Ion -Dan Postescu et al. Antioxidant effects of a grape seed extract in a rat model of diabetes mellitus. Diabetes & Vascular Disease Research 2009; 6(3):200-204.
[6] Yamakoshi J, Makoto S, Shigehiro K, Shoichi T. Procyanidin Rich Extract from Grape Seeds Prevents Cataract Formation in Hereditary Cataractous (ICR/f) Rats. Agric. Food Chem 2002; 50 (17): 4983–4988.
[7] Satyam SM, Bairy KL, Pirasanthan R, Vaishnav RL. Grape seed extract and Zinc containing nutritional food supplement, delays progression and maturation of Streptozocin- induced diabetic cataract in Wistar rats. IFST 2015; 52(5):2824-2832. DOI 10.1007/s13197-014-1305-y.
[8] Hum TP, Augusteyn RC. The nature of disulphide bonds in rat lens proteins. Curr Eye Res 1987; 6:1103–1108.

[9] Duhaiman AS. Glycation of human lens proteins from diabetic and (nondiabetic) senile cataract patients. Glycoconj J 1995; 12:618-621.
[10] Swamy MS, Abraham EC. Lens protein composition, glycation and high molecular weight aggregation in aging rats. Invest Ophthalmol Vis Sci 1987; 28: 1693–1701.
[11] Newell W F. Ophthalmology Principle and Concept, Mosby-Year Group St Louis.1992, pp.358-368.
[12] Lee A Y, Chung S S. Contributions of polyol pathway to oxidative stress in diabetic cataract. FASEB J 1999;13: 23–30.
[13] Yokoyama T, Sasaki H, Giblin FJ, Reddy VN. A physiological level of ascorbate inhibits galactose cataract in guinea pigs by decreasing plyol accumulation in the lens epithelium: A dehydroascorbate-linked mechanism. Exp Eye Res 1994; 58:207–18.
[14] Mody VC , Kakar M, Elfving A, Soderberg PG, Lofgren S. Ascorbate in the rat lens: dependence on dietary intake. Ophthalmic Res 2005; 37(3):142-149.
[15] Ayala MN, Soderberg PG. Vitamin C can protect against ultraviolet radiation induced cataract in albino rats. Ophthal Res 2004; 36:264–269.
[16] Labgle UW, Wolf A, Cordier A. Enhancement of SDZ ICT 322-induced cataracts and skin changes in rats following vitamin E-and selenium deficient diet. Arch Toxicol 1997; 71:283–289.
[17] Kojima M, Shui YB, Murano M, Nagata O, Hockwin K, Sasaki K, et al. Low vitamin E level as a subliminal risk factor in a rat model of predisolone-induced cataract. Investig Ophthalmol Visual Sci 2002; 43: 1116–1120.
[18] Reddy GB, Nayak S, Reddy PY, Seetharam Bhat K. Reduced levels of rat lens antioxidant vitamins upon *in vitro* UVB irradiation. J Nutr Biochem 2001; 12:121–124.
[19] Bhat KS. Nutritional status of thiamine, riboflavin and pyridoxine in cataract patients. Nutr Rep Inter 1987; 36:685–692.
[20] Karakucuk S, Ertugrul Mirza G, Faruk Ekinciler O, Saraymen R, Karakucuk I, Ustdal M. Selenium concentrations in serum, lens and aqueous humor of patients with senile cataract. Acta Ophthalmol Scand 1995; 73:329–332.