

INFLUENCE OF GRAPE SEED EXTRACT AND ZINCOVIT TABLETS (NUTRITIONAL FOOD SUPPLEMENT) ON GLUCOSE LEVEL IN NORMAL AND STREPTOZOCIN INDUCED DIABETIC RATS

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ABSTRACT

Objective: To evaluate the influence of combination of Grape seed extract and Zincovit tablets (nutritional food supplement) on blood glucose level in normal and streptozocin induced diabetic rats.

Method: A total of 48 adult male Wistar rats (24 normal rats, 24 diabetic surviving rats) were divided into 8 groups of 6 rats each. Experimental hyperglycemia was produced in rats by single dose of Streptozocin (35 mg/kg; *i.p.*). Assessment of glucose in blood and urine of rats was used as marker of hyperglycemia.

Results: Oral administration of combination of Grape seed extract and Zincovit tablets (nutritional food supplement) for 45 days significantly reduced the levels of blood glucose in a dose dependent manner among diabetic treated rats when compared to diabetic control rats ($P < 0.0001$, at 40 mg/kg, 80 mg/kg and 160 mg/kg) whereas it had significantly increased the blood glucose level in normal treated rats in comparison with normal control rats ($P = 0.011, 0.003$ and 0.029 at 40 mg/kg, 80 mg/kg and 160 mg/kg respectively). There was significant increase in the weight of isolated dry liver among diabetic treatment group when compared to diabetic control in a dose dependent manner. There was no significant body weight change in diabetic and normal rats as compared to diabetic control and normal control rats respectively.

Conclusion: The hypoglycemic effect of combination of Grape seed extract and Zincovit tablets may be attributed to enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation.

Keywords: Zincovit, Grape seed extract, Streptozocin, Hyperglycemia, Diabetes

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by derangements in carbohydrate, protein and fat metabolism, caused by complete or relative insufficiency of insulin secretion and insulin action [1, 2]. The global prevalence of diabetes is predicted to rise to 300 million by 2025 [1, 3, 4]. India has today, become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 [5]. In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications. Two mechanisms have been proposed that may explain how hyperglycemia causes increased ROS formation. One mechanism involves the transition metal-catalyzed auto-oxidation of protein-bound Amadori products which yields superoxide and hydroxyl radicals and highly reactive di-carbonyl compounds [6]. The other mechanism involves the transition metal-catalyzed auto-oxidation of free sugars, which also yields di-carbonyl compounds and superoxide and hydroxyl radicals [6]. The management of diabetes is intimately linked to food therefore; knowledge about food and nutrition and the scientific base of biochemistry, physiology, and pathogenesis go a long way towards the understanding and dealing with the disorder [7]. Though different therapies are available for the treatment of diabetes mellitus but there is a growing interest in herbal remedies, due to less or no side effects associated with these therapeutic agents [8].

Zincovit is an advanced formulation of high concentration of vitamins and minerals. Zincovit releases a stream of anti-oxidant benefits. In diabetes mellitus, oxygen free radicals (OFRs) are generated by stimulating H_2O_2 in-vitro, as well as in-vivo, in pancreatic β -cells [3, 6]. OFR-scavenging enzymes can respond to conditions of oxidative stress with a compensatory mechanism that increases the enzyme activity in diabetic rats [3, 6]. The antioxidant effect of flavonoids present in the combination of Grape seed extract and Zincovit tablets acts as strong superoxide radicals and singlet oxygen quencher. Therefore, combination of Grape seed extract and Zincovit tablets may be beneficial in controlling the blood glucose level and improving the lipid metabolism and thus in prevention of diabetic complications from lipid per oxidation and antioxidant

systems in Streptozocin induced diabetic rats. This could be useful for prevention or early treatment of diabetic disorders.

Hence a study was planned to estimate the effect of combination of Grape seed extract and Zincovit tablets on fasting blood glucose level in normal and streptozocin induced diabetic rats and to determine the clinical significance of the detected glycemic influence of combination of Grape seed extract and Zincovit tablets administered orally in Wistar rats.

MATERIALS AND METHODS

Drugs and Reagents

A single combined formulation of Zincovit tablets and grape seed extract was obtained as gift sample from Apex Laboratories, Chennai, India. Streptozocin (STZ) was procured from Sigma Aldrich, Mumbai, India). One touch glucometer (Accu-Chek Active) with glucose oxidase-peroxidase reactive strips was purchased from Roche Diagnostics, Germany. The other chemical reagents used in the study were obtained from Merck Chemicals, Bangalore, India.

Animals

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/34/2012) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines on the use and care of experimental animals.

Experimental procedure

In the experiment a total of 48 adult male Wistar rats (24 normal rats, 24 diabetic surviving rats) was used. The rats were divided into 8 groups of 6 rats each. Treatment was done for 45 days as follows-

Group I: Normal control rats were given 1ml/kg of 2% gum acacia orally for 45 days.

Group II: Streptozocin (35mg/kg, i.p.) induced diabetic rats were given 1ml/kg of 2% gum acacia orally for 45 days.

Group III: Non-diabetic rats were given combination of Grape seed extract and Zincovit tablets at the dose of 40 mg/kg orally for 45 days.

Group IV: Non-diabetic rats were given combination of Grape seed extract and Zincovit tablets at the dose of 80 mg/kg orally for 45 days.

Group V: Non-diabetic rats were given combination of Grape seed extract and Zincovit tablets at the dose of 160 mg/kg orally for 45 days.

Group VI: Streptozocin (35mg/kg, i.p.) induced diabetic rats were given combination of Grape seed extract and Zincovit tablets at the dose of 40 mg/kg orally for 45 days.

Group VII: Streptozocin (35mg/kg, i.p.) induced diabetic rats were given combination of Grape seed extract and Zincovit tablets at the dose of 80 mg/kg orally for 45 days.

Group VIII: Streptozocin (35mg/kg, i.p.) induced diabetic rats were given combination of Grape seed extract and Zincovit tablets at the dose of 160 mg/kg orally for 45 days.

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 250 mg/dl) were considered as diabetic rats and used for the experiment.

Collection of blood sample and glucose determination

Fasting blood samples were drawn from tail tip of all the experimental rats before administration of gum acacia or combination of Grape seed extract and Zincovit tablets for the estimation of their baseline fasting blood glucose level. Each group

were given their respective dose as per above chart. Day of drug administered was counted as 0th day. Fasting blood samples of group I after administration of gum acacia and of rest other groups after single administration of combination of Grape seed extract and Zincovit tablets on 1st day and on 7th, 15th, 30th and 45th day before administration of next dose of gum acacia for group I, II and combination of Grape seed extract and Zincovit tablets for group III, IV, V, VI, VII and VIII. All the fasting blood samples were drawn on from tail vein of rats for the estimation of blood glucose by glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). Urine sugar was detected by O-Toluidine method.

Body weight and isolated liver weight

Body weight of each animal was checked on 0th and 45th day among normal and diabetic rats. At the end of 45 days, the rats were sacrificed by anesthetizing with overdose of ketamine (i.p). Liver was dissected out, washed in ice cold saline, patted dry and weighed.

Statistical analysis

Using SPSS 16.0, blood glucose, body weight and dry liver weight data was analyzed by general linear model repeated measures test, analysis of covariance (ANCOVA) and one way analysis of variance (ANOVA) followed by post hoc test Tukey test respectively. P value less than 0.05 was considered as statistically significant.

RESULTS

Blood glucose level

Oral administration of Zincovit tablet with grape seed extract to normal and diabetic rats for 45 days significantly reduced the levels of blood glucose in a dose dependent manner among diabetic treated rats when compared to diabetic control rats whereas it has significantly increased the blood glucose level in normal treated rats in comparison with normal control rats (Table 1).

Body weight

A significant decrease in body weight was observed in diabetic rats when compared to normal control rats but there was no significant body weight change in diabetic and normal rats when compared to diabetic control and normal control rats respectively (Table 2).

Isolated Liver weight

There was no significant difference in dry liver weight of normal treated rats as compared to normal control rats. But, there was significant increase in the weight of dry liver in a dose dependent manner among diabetic treatment group when compared to diabetic control rats (Table 3).

Table 1: Effect of different doses of combination of Grape seed extract and Zincovit tablets on fasting blood glucose levels (in mg/dl) of normal and diabetic rats

Groups	0 th day	7 th day	15 th day	30 th day	45 th day
I-Normal control	84.5 ±2.18	75.33 ±2.27	82.16 ±2.13	68.66 ±3.01	84.16 ±2.49
II-Diabetic control	525.66 ±22.79	521.16 ±16.03	524.00 ±12.16	419.50 ±17.30	407.50 [#] ±14.44
III-Normal+Zincovit (40 mg/kg/day, p.o)	69.66 ±3.40	68.50 ±2.34	75.16 ±4.71	62.33 ±3.98	84.16* ±3.08
IV-Normal+Zincovit (80 mg/kg/day, p.o)	73.16 ±3.66	60.33 ±4.03	72.16 ±2.08	66.50 ±1.23	81.50* ±4.58
V-Normal+Zincovit (160 mg/kg/day, p.o)	77.16 ±2.24	60.50 ±2.12	72.16 ±1.83	77.33 ±6.17	77.33* ±1.78
VI-STZ+Zincovit (40 mg/kg/day, p.o)	401.50 ±41.88	209.16 ±27.61	176.16 ±39.19	229.50 ±12.30	133.83** ±12.83
VII-STZ+Zincovit (80 mg/kg/day, p.o)	461.00 ±46.32	189.66 ±43.02	209.83 ±55.35	191.00 ±48.33	207.00** ±56.55
VIII-STZ+Zincovit (160 mg/kg/day, p.o)	487.00 ±36.70	191.66 ±22.62	198.83 ±45.78	198.83 ±42.72	202.00** ±38.49

Values are given as mean ± SEM for groups of six animals each.

[#]P < 0.0001 compared with the normal control group rats.; ^{**}P < 0.0001 compared with the streptozocin induced diabetic control group rats.

* P ≤ 0.05 compared with the streptozocin induced diabetic control group rats.

Table 2: Effect of different doses of combination of Grape seed extract and Zincovit tablets on body weight (in gram) of normal and diabetic rats

Group differences	Body weight differences (in gram)
(Normal control) – (Normal: Zincovit;40 mg/kg/day, p.o)	5.24±10.70
(Normal control) – (Normal: Zincovit;80 mg/kg/day, p.o)	-1.56±10.78
(Normal control) – (Normal: Zincovit;160 mg/kg/day, p.o)	3.72±10.66
(Diabetic control) – (Normal control)	-23.64±10.65 ^s
(Diabetic control) – (Diabetic: Zincovit;40 mg/kg/day, p.o)	6.14±10.66
(Diabetic control) – (Diabetic: Zincovit;80 mg/kg/day, p.o)	-10.13±10.85
(Diabetic control) – (Diabetic: Zincovit;160 mg/kg/day, p.o)	5.61±10.73

Values are given as mean ± SEM for groups of six animals each.

^sP < 0.05 compared with the normal control group rats

Table 3: Effect of different doses of combination of Grape seed extract and Zincovit tablets on isolated liver weight (in gram) of normal and diabetic rats

Groups	Isolated liver weight (in gram) after the treatment (45 th day)
I-Normal control	4.50±0.34
II-Diabetic control	3.73±0.24
III-Normal+Zincovit (40 mg/kg/day, p.o)	4.50±0.22
IV-Normal+Zincovit (80 mg/kg/day, p.o)	4.33±0.33
V-Normal+Zincovit (160 mg/kg/day, p.o)	4.83±0.16
VI-STZ+Zincovit (40 mg/kg/day, p.o)	5.00±0.44*
VII-STZ+Zincovit (80 mg/kg/day, p.o)	5.33±0.21*
VIII-STZ+Zincovit (160 mg/kg/day, p.o)	5.33±0.21*

Values are given as mean ± SEM for groups of six animals each.

* P ≤ 0.05 compared with the streptozocin induced diabetic control group rats.

DISCUSSION

The results of present study reveals glycemic influence of combined formulation of Grape seed extract and Zincovit tablets in normal and streptozocin-induced diabetic rats in dose and duration dependent fashion. Streptozocin injection results diabetes mellitus, which may be due to destruction of β cells of Islets of Langerhans [9]. After 7 days supplementation of combined formulation of Grape seed extract and Zincovit tablets resulted significant decrease in fasting blood glucose level with respect to diabetic control rats. There was significant increase in fasting blood glucose level in all the three normal rats treatment group which were administered combination of Grape seed extract and Zincovit tablets in comparison to normal control group rats but the increase in blood glucose level was not beyond 84.16 mg/dl in test drug treatment group rats. Supplementation of the combined formulation of Grape seed extract and Zincovit tablets for 45 days resulted in significant reduction in fasting blood glucose level and restoration of liver weight in diabetic treated rats as compared to diabetic control rats. Restoration of liver weight in diabetic treatment group gives one possible way of anti-diabetic action of this supplementation by improving glycogenesis process in muscle and liver [10, 11]. Streptozocin induced type-I diabetes mellitus is also developed due to reduction of glucose-6-phosphate-dehydrogenase activity in liver that obstruct glucose utilization through pentose phosphate pathway as this enzyme activity is under insulin [12, 13]. In our previous unpublished study on effect of this supplementation in diabetic cataract, we found that the combined formulation of Grape seed extract and Zincovit tablets at all the three doses 40 mg/kg, 80 mg/kg and 160 mg/kg had significantly increased the activity of glucose-6-phosphate-dehydrogenase level in eye lens homogenate of diabetic cataract treated rats as compared to diabetic cataract control rats. This suggests the active role of this supplementation in pentose phosphate pathway. The combined formulation of Grape seed extract and Zincovit tablets might have significantly improved this enzyme activity in hepatic tissue which enlighten it's another possible way of anti-diabetic activity. The loss in body weights observed in diabetic control group rats may be due to muscle wasting and loss of tissue proteins upon induction of diabetes with streptozocin [10]. Symptoms like loss of body weight, weakness, polyuria and polyphagia that accompany type-I diabetes mellitus were significantly absent in this test drug

supplemented group. The gain in body weight was observed in both normal and diabetic treated groups but it was not significant as compared to their respective control groups. Long-term daily administration of grape seed extract especially proanthocyanidins present in this offers enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation, and could therefore be associated with reduced glucose levels in diabetic rats [2]. 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 [3]. A synergistic effect of vitamins C and E and Zinc, which are present in Zincovit tablets, could be expected based on the different environments in which they act. Vitamin C acts in the hydrophilic milieu, scavenging reactive oxygen species; Zinc, located in the interphase of the bilayer, will prevent iron or copper binding to the membrane and alpha-tocopherol in the hydrophobic domains of the bilayer, will inhibit the lipid peroxidation free-radical chain reaction [3]. Effects of magnesium and zinc on lipid peroxidation have previously been reported. Magnesium inhibits malondialdehyde (MDA) formation in endothelial cells and low magnesium oxide induced lipid peroxidation [3]. From such information it may be stated primarily that the combined formulation of Grape seed extract and Zincovit tablets may sensitize the insulin receptor to insulin or stimulate the β stem cell of Islets of Langerhans in pancreas by decreasing the oxidative stress in streptozocin-induced diabetic rat that may restore plasma level of insulin or it may results the improvement of carbohydrate metabolic enzymes towards the reestablishment of normal blood glucose level.

CONCLUSIONS

It can be concluded that the anti-hyperglycemic effect of Grape seed extract and Zincovit tablets may be attributed to enhanced antioxidant potential and protection against tissue lipid peroxidation and protein oxidation due to synergistic antioxidant effect of proanthocyanidins present in grape seed extract, vitamins C, E, biotin and minerals like zinc, copper, selenium, magnesium, manganese, chromium and molybdenum mainly. This could be useful for prevention or early treatment of diabetes mellitus and its associated disorders. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of its anti-diabetic effect.

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