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Research Article

A 45-DAY RANDOMIZED, OPEN-LABEL, COMPARATOR STUDY TO EVALUATE THE SAFETY AND EFFICACY OF ZINCOVIT TABLETS WITH GRAPE SEED EXTRACT (NUTRITIONAL FOOD SUPPLEMENT) IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: To evaluate the efficacy of Zincovit (ZVT) tablets with grape seed extract (GSE) in patients with Type 2 diabetes mellitus by testing the hypothesis of a greater reduction in plasma glucose levels (fasting blood sugar [FBS] and post-prandial blood sugar [PPBS]) from baseline and after 45 days of therapy as compared to standard comparator.

Methods: This was a randomized, open-label, comparative (2-arm), prospective 45 days study. Treatment consisted of 2 arms: Antidiabetic drug plus non-pharmacological measure alone or ZVT tablets with GSE plus non-pharmacological measures. A total of 30 patients (15 in each arm) were included in the study.

Results: ZVT tablet did not alter the FBS, PPBS, and HbA1c level in diabetic patients compared to diabetic patients treated with placebo. No changes were seen in any of the safety parameters when given for 45 days.

Conclusion: ZVT tablets do not possess antidiabetic activity in spite of good safety profile in our study design. This could be due to several limitations of the study such as inadequate sample size, short duration of the study, and wrong selection of the patients. A long-term, double-blind, placebo controlled study in a large sample of population measuring glycemic parameters, and cardiovascular outcomes could give a clear picture of the anti-diabetic effect of ZVT with GSE tablets.

Keywords: Diabetes mellitus, Zincovit tablets, Grape seed extract, Antioxidant, Safety parameters.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by derangements in carbohydrate, protein, and fat metabolism caused by complete or relative insufficiency of insulin secretion and insulin action. The pharmaceutical drugs are either too expensive or have undesirable side effects. Treatment with sulfonylureas and biguanides are also associated with side effects [1]. 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. Free radicals are continuously produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems that have increased free radical production or reduced activity of antioxidant defenses or both. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free-radical-generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes, and formation of lipid peroxides [1-4]. In addition to reduced glutathione (GSH), there are other defense mechanisms against free radicals such as the enzymes superoxide dismutase (SOD), GSH peroxidase, and catalase, whose activities contribute to eliminate superoxide, hydrogen peroxide, and hydroxyl radicals. Diabetes is associated with lower levels of endogenous antioxidants such as Vitamins A, C, E, lycopine, and lipoic acid. Total serum antioxidant capacity is reduced, and antioxidant effect of serum albumin and high density lipoprotein is impaired in people with diabetes. It is uncertain, however, whether depleted levels of these endogenous antioxidants and free radical scavengers are a cause or an effect of oxidative stress [5,6]. Conflicting results are available about the activity of various antioxidant enzymes. Cytosolic Cu^{2+}/Zn^{2+} SOD (SOD 1) and mitochondrial Mn^{2+} SOD (SOD 2) are lower in human diabetic neutrophils. Renal SOD 1 and glutathione peroxidase is found to be increased in diabetic rat kidneys. SOD 2 shows no change. Glutathione oxidase activity is higher in the renal cortex of diabetic mice than non-diabetic controls. The exact contribution of antioxidant enzymes to oxidative stress in diabetes is not fully understood. Many of the complications of diabetes mellitus, including retinopathy and atherosclerotic vascular disease, the leading cause of mortality in diabetes mellitus, have been linked to oxidative stress, and antioxidants have been considered as treatments. Oxidative stress is increased in experimental models of streptozotocin (STZ)-induced diabetes mellitus [7,8].

Zincovit tablet (ZVT) is an advanced formula of high concentration of vitamins, minerals, and grape seed extract (GSE). ZVT releases a stream of antioxidant benefits. In diabetes mellitus, oxygen free radicals (OFRs) are generated by stimulating H_2O_2 *in-vitro*, as well as *in-vivo*, in pancreatic ß-cells. OFR-scavenging enzymes can respond to conditions of oxidative stress with a compensatory mechanism that increases the enzyme activity in diabetic rats [1]. The antioxidant of flavonoids present in the ZVT with GSE acts as strong superoxide radicals and singlet oxygen quencher STZ-induced experimental diabetes is a valuable model for induction of Type 1 diabetes. Few studies such as the one done by Irina *et al.* regarding antioxidant effects of GSE suggest that the treatment of STZ-induced diabetic rats with GSE lowered the protein's oxidant damage in rat's plasma and liver tissues [1,9]. The antioxidant activity of phenols is due to their redox properties that allow them to act as reducing agents by donating hydrogen, quenching singlet oxygen, or acting as metal chelators. It has been reported that various proteins, including hemoglobin, albumin, collagen, low-density lipoprotein (LDL), or crystalline proteins undergo non-enzymatic glycation in diabetes. The rate of glycation is proportional

to the concentration of blood glucose [10,11]. Therefore, ZVT with GSE supplementation may be beneficial in controlling the blood glucose level. Hence, this study.

METHODS

Trial design

This was a randomized, open-label, comparative (2-arms), prospective 45 days study. Treatment consisted of 2 arms: Antidiabetic drug plus non-pharmacological measure alone or ZVT with GSE plus non-pharmacological measures. Patients who met entry criteria were randomized after screening to one of the two treatment arms in a 1:1 ratio. The study was approved by the Institutional Ethics Committee (IEC), and all included patients gave their written informed consent prior to entry into the study, and the study was performed in accordance with the ethical principles consistent with good clinical practice and international harmonization guidelines. Patients were evaluated at day 0 and day 45.

Endpoints

The primary endpoint of the study was comparative assessment percentage change from baseline (day 0) in fasting blood sugar (FBS) and post-prandial blood sugar (PPBS) to 45 days in both the groups. Secondary endpoints included safety assessment by doing routine hematological and biochemical investigations. Patients were also observed for any adverse signs and symptoms.

Inclusion criteria

Outpatients and inpatients (both males and females) with Type 2 diabetes mellitus between the age of 18 and 75 years. Patients with fasting glucose levels above 125 mg/dl and below 250 mg/dl, and or post-prandial glucose levels above 200 mg/dl and below 350 mg/dl will be included. Patients who are eligible and able to participate in the study must consent to do so after the details of the study are explained to them (written informed consent).

Exclusion criteria

Patients with Type 1 diabetes mellitus, with known allergy to zinc, with severe comorbid conditions such as renal impairment, hepatic impairment, Stage IV congestive cardiac failure, carcinoma, immunosuppression, refusal to give written informed consent voluntarily, alcohol or drug abuse, pregnancy or lactation or pediatric age group, and any other medical condition that in the opinion of the investigator, may be an unacceptable additional risk to the patient.

Safety assessments

Safety assessments included recording of treatment-emergent adverse events (adverse events that started or worsened during randomized treatment), hematological and biochemical measurements, and physical examination.

Patient characteristics

A total of 30 patients (15 in each arm) were included in the study and all the 30 patients completed the study. Both males and females were included in the study. Patients were selected carefully as per the inclusion/exclusion criteria, and the comorbid conditions were minimal/clinically not significant/well under control.

RESULTS

Efficacy

Table 1 shows the pre-treatment and post-treatment values of FBS, PPBS, and HbA1c values. ZVT tablet did not alter the FBS, PPBS, and HbA1c level in diabetic patients compared to diabetic patients treated with placebo. Results were analyzed using the non-parametric unpaired t-test (Mann–Whitney test).

Safety

Overall ZVT tablets were very well-tolerated over 45 days. Occasional side effects observed in a couple of patients were nausea, abdominal pain, headache, diarrhea, and constipation (Table 2). The intensity of these adverse effects was mild in nature. There were no other significant changes in the laboratory parameters at the end of the treatment in both the groups. Table 2 shows the pre-treatment and post-treatment values of liver function test, renal function test lipid profile, blood glucose, electrolyte balance, and hematological parameters. There is no change in any of the safety parameters when given for 45 days.

DISCUSSION

There are several published reports indicating the beneficial effects of complementary and ayurvedic medicine in the treatment of Type 2 diabetes mellitus. Chromium and possibly Gymnema appears to improve glycemic control. In the indigenous Indian system of medicine (Ayurveda), a mention was made on a good number of plants for the cure of diabetes or "madhumeha" and some of them have been experimentally evaluated and the active principles were isolated [12-15].

In the present study, ZVT tablets at a dose of 1-2 tablets per day half an hour before food as an add-on therapy (with the standard background antidiabetic medications) over a 45-day period was found to statistically non-inferior ($p>0.05$) to the existing standard antidiabetic medications in lowering the FBS, PPBS, and HbA1c levels. As per the current literature, oral administration of ZVT tablet with GSE 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 [16]. In our study, there is no beneficial effect in diabetes patients. This could be due to several limitations of the study such as inadequate sample size, short duration of the study, and wrong selection of the patients. Studies investigating diabetes management should be of at least 4 month's duration. Furthermore, diabetic studies must focus on cardiovascular outcomes also apart from glycemic control. This is also one of the serious limitations of our study. This is important because better glycemic control might not always lead to real-world clinical benefits [17]. However, in spite of all these negative results literature clearly confirms the antioxidant effect of ZVT tablets. The antioxidative effect of flavonoids present in the ZVT with GSE acts as strong superoxide radicals and singlet oxygen quencher [16]. Therefore, ZVT with GSE supplementation may be beneficial in controlling the blood glucose level as an add-on drug in Type 2 diabetic patients.

Table 1: Comparison of diabetic parameters before and after the administration of study drug/placebo in the diabetic patients

Variable	[#]Pre-treatment values (mean±SEM) (n=15)		95% CI		[‡]Post-treatment values (mean±SEM) (n=15)		95% CI	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug
FBS (mg/dl)	94.37±3.04	104.4±4.9	87.19-101.6	93.39-115.4	99.38±2.43	103.9±3.2	93.64-105	196.74-111
PPBS (mg/dl)	110±8.3	114.2±5.3	90.35-129.7	102.3-126.1	114.75±7.5	106.5±4.6	96.9-132.5	96-116.92
HbA1c	5.43±0.14	5.7±0.12	5.09-5.76	5.49-6.04	5.59±0.03	5.62±0.03	5.52-5.66	5.44-5.8

[#]$p>0.05$ between the pre-treatment values of the control and drug groups indicating that both groups are similar to each other. Statistical analysis carried out by Mann–Whitney test (non-parametric unpaired t-test), [‡]$p>0.05$ between the post-treatment values of the control and drug groups indicating that there is no difference between the drug and the placebo on the measured parameters. Statistical analysis carried out by Mann–Whitney test (non-parametric unpaired t-test), SEM: Standard error of mean, FBS: Fasting blood sugar, PPBS: Post-prandial blood sugar, CI: Confidence of interval

Table 2: Comparison of safety parameters (hematological/biochemical) before and after the administration of study drug/placebo in diabetic patients

Variable	#Pre-treatment values (mean±SEM) (n=15)		95% CI		‡Post-treatment values (mean±SEM) (n=15)		95% CI	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug
ALT (IU/l)	27.7±4	22.6±4	14.41-41.11	15.7-29.5	26.5±3.7	23.9±3.32	16.84-36.16	17.39-29.4
AST (IU/l)	24.5±2.72	22.4±1.6	15.5-33.9	18.4-25.5	23.8±2.5	21.8±1.62	16.8-30.9	18.8-24.7
TC (mg/dl)	181±15.12	171.6±14.79	156.9-206.1	143.1-199	195.25±20.8	163.7±10.6	166.9-225.6	144.8-181.6
TG (mg/dl)	126±32.9	129.2±16.2	81.5-172.5	99.5-159.9	138.75±32.2	115.7±15.42	80.2-196.28	87.8-144.6
HDL (mg/dl)	51.25±12	48.9±4.67	32.7-97.7	39.3-56.4	50.71±11.3	50.23±5.09	31.29-69.21	41.29-58.3
LDL (mg/dl)	104±12.2	97.5±13.29	96.2-122.8	73.4-121.6	117.75±20.5	92.7±9.25	85.62-149.9	76.79-108.6
TC/HC	3.84±0.89	3.63±0.29	2.6-5.4	3.1-4.2	4.4±0.78	3.3±0.32	2.4-6.3	2.7-3.9
Urea (mg/dl)	23.25±2.59	25±4.79	11.9-35.5	17.17-33.8	22.5±2.72	23.5±2.8	13.83-31.17	18.16-27.8
Creat (mg/dl)	0.87±0.05	0.83±0.09	0.54-1.19	0.62-1.03	0.87±0.16	0.82±0.12	0.37-1.38	0.55-1.09
Na (mmol/l)	136.14±1.7	136.92±0.39	132.2-139.78	135.9-138.7	141.25±1.32	37.1±0.92	139-143.43	135.32-138.4
K (mmol/l)	4.12±0.35	4.4±0.14	3.6-4.6	4.16-4.71	4.38±0.24	4.31±0.13	3.9-4.7	4.1-4.53
Hb (g/dl)	12.25±0.59	12.6±0.3	10.7-13.7	12.1-13.2	12.5±0.15	12.6±0.41	11.01-14	11.9-13.3
HCT (%)	36.9±1.36	38.5±0.86	33.9-40.4	36.7-40.2	38.13±1.66	38.23±1.11	34.44-42	36.3-40.24
PLC (10^3/µl)	248.8±8.9	250.6±18.7	209.8-288.6	217.5-283.9	259.5±14.72	252.2±17	212.65-306.3	213.7-290.7
TWBC (10^3/µl)	8.25±0.54	6.9±0.58	6.1-10.4	5.9-7.9	7.9±1.02	7.5±0.51	5.9-9.9	6.6-8.36
N (%)	51.9±3.25	51.12±2.2	44.8-59.2	45.3-56.6	49.85±3.84	51.63±2.18	37.64-62.06	46.69-56.57
L (%)	36.13±3.45	35.87±2.7	30.5-41.6	29.8-41.8	39.37±4.3	36.12±2.36	25.65-53.09	30.78-41.5
M (%)	6.4±1.3	87.5±0.3	4.38-8.67	6.75-8.3	6.4±0.4	6.6±0.34	5.1-7.8	6.4-7.3
E (%)	6.13±1.72	4.8±0.86	1.8-10.4	3.3-6.3	6.1±1.72	5.2±0.84	3.03-9.3	3.64-6.87
B (%)	0.4±0.03	0.67±0.09*	0.23-0.56	0.51-0.84	0.5±0.04	0.62±0.12	0.34-0.65	0.39-0.85
ESR	31.7±7.98	24.3±3.23	17.35-46.07	16.9-31.1	40.25±7.04	25±4.17	17.85-62.65	15.57-34.43

#p>0.05 between the pre-treatment values of the control and drug groups indicating that both groups are similar to each other. Statistical analysis carried out by Mann–Whitney test (non-parametric unpaired t-test), ‡p>0.05 between the post-treatment values of the control and drug groups indicating that there is no difference between the drug and the placebo on the measured parameters. Statistical analysis carried out by Mann–Whitney test (non-parametric unpaired t-test), ESR: Erythrocyte sedimentation rate, TWBC: Total white blood cell, Hb: Hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate transaminase, TC: Total cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, SEM: Standard error of mean, CI: Confidence of interval

CONCLUSIONS

From the present study, we need to conclude that ZVT tablets do not possess antidiabetic activity in spite of good safety profile. However, the long-term, double-blind, placebo controlled study in a large sample of population measuring glycemic parameters, and cardiovascular outcomes could give a clear picture of the antidiabetic effect of ZVT with GSE tablets.

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