

Phytochemical Analysis And Effects Of Trevo Supplement On Glucose, Lipid Metabolism And Some Micronutrients In Alloxan-Induced Diabetic Wistar Rats.

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Abstract: Existing treatment regimens have not provided adequate solution to the metabolic dysfunctions associated with diabetes mellitus. We determined the effect of Trevo supplement on blood glucose, lipid profile and some micronutrients in alloxan-induced diabetic rats.

Phytochemical analysis of Trevo was carried out. Eighteen Wistar rats weighing 150-250 g were allocated to three groups of six rats: Group 1, Wistar rats fed on feed only; Group 2, diabetic Wistar rats without Trevo; Group 3, diabetic rats on Trevo. Blood glucose, lipid profile and essential trace metals were determined using colorimetric method and atomic absorption spectrometry.

Trevo showed varying concentrations of different phytochemical constituents. There is significant decrease ($p < 0.05$) in the blood glucose of treated diabetic Wistar rats when compared with untreated diabetic Wistar rats. There was a significant ($p < 0.05$) increase in the HDL-cholesterol and a significant ($p < 0.05$) decrease in LDL-cholesterol of the treated diabetic Wistar rats when compared with the untreated diabetic Wistar rats. Plasma levels of Zn, Cr and Mn were significantly ($p < 0.05$) higher in untreated diabetic, while Se increased significantly in the treated diabetic Wistar rats.

This study suggests Trevo supplement could assist in regulating blood sugar, low density Lipoprotein cholesterol, high density lipoprotein cholesterol and Se levels in diabetic Wistar rats.

Key words: Trevo, Reactive oxygen species, Micronutrients, Phytochemical, Wistar rat.

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I. Introduction

Diabetes mellitus (DM) is a syndrome characterized by insulin deficiency or tissue resistance to insulin [1], elevated blood glucose level over a prolonged period, frequent urination, increased thirst and hunger. If untreated, complications such as diabetic ketoacidosis, non-ketotic hyperosmolar coma [2], dyslipidaemia, heart disease, stroke, kidney failure, foot ulcers and blindness [3] are imminent. This insulin dysfunction or deregulation usually leads to metabolic derangements of macro and micro-molecules [1]. Previous reports show that typical lipoprotein pattern in diabetes mellitus include moderate elevated triglyceride levels, low HDL cholesterol values, and increased level of LDL particles [4]. There are speculations that certain trace metals could play critical roles in preventing type 2 diabetes mellitus by regulating blood sugar and alleviating insulin insensitivity [5]. While the roles of chromium have been established in enhancing the effects of insulin [6], the exact roles of other trace metals have not been proved. For example, Garg *et al.* [7] reported that there is loss of zinc into the extracellular fluid from the cells causing low serum levels and hyperzincuria in type 2 diabetes mellitus, while Hashemipour *et al.* [8] reported that supplementation with zinc lower oxidative stress-related by-products and attenuate the synthesis of inflammatory mediator proteins. Studies have suggested that selenium could enhance insulin sensitivity by mediating insulin-like actions [9]. In another study, plasma manganese and cobalt decreased significantly in type 2 diabetic patients.

Alloxan is a toxic glucose analogue that selectively destroys beta cells of the pancreas when administered to rodents and certain animal species. It accumulates in the beta cells through uptake via the GLUT2 glucose transporter and in the presence of intracellular thios, generates reactive oxygen species in a

cyclic reaction with its reduction product, dialuric acid. Toxic effect of reactive oxygen species generated by alloxan therefore induces insulin dependent DM [10]. Trevo is a natural product claimed to containing about 174 micronutrients and phytonutrients including vitamins and minerals, vital trace minerals, amino acids, essential fatty acids, antioxidants, digestive enzymes and Co-Enzyme Q10 from fruits, green superfoods, garden vegetables, sea vegetables, herbs, and coral calcium complex [11]. Despite its very high cost, 11,000 – 12,000 Nigerian naira which is very expensive for an average Nigerian, the popularity of Trévo is on the increase vis-à-vis its use for treatment or prevention of varying diseases particularly the metabolic ones there is little or no data to support these claims. Therefore, the present study was designed to determine the effect of Trevo supplement on blood sugar level, and lipid profiles in alloxan-induced diabetic rats.

II. Materials and Methods

2.1 Experimental Animals

Eighteen Wistar rats weighing between 150 and 250g were obtained from the Animal Care Unit, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo. Each group of six Wistar rats was housed separately in a wire mesh cage, under standard conditions (25-29 °C, 12 hours light and 12 hours darkness cycle). They were fed with free standard pellet diet and tap water, allowed to acclimatise for 2 weeks before the diabetes induction. The Wistar rats were divided into three groups, Group 1: non-diabetic rats fed on rat feed only; Group 2: diabetic rats fed on rat feed without Trevo; Group 3: diabetic rats treated with 0.9 ml of Trevo per kg body weight (Table 1).

2.2 Induction of Diabetes

The Wistar rats were allowed to fast for 12 hours. Then, 180 mg/kg body weight of alloxan monohydrate in 0.9% w/v NaCl was injected intraperitoneally. After 24 hours of alloxan administration, the blood glucose level was measured by using glucose oxidase/peroxidase enzymatic techniques as described by Sada *et al.* [12]. Rats having blood glucose level of ≥ 20 mmol/L were selected for this study

2.3 Sample Collection and Preparation

The animals were sacrificed by cervical dislocation and 4 ml of blood was collected from each rat directly by cardiac puncture into fluoride oxalate (2 ml) and lithium heparin (2 ml) bottles for plasma preparation and biochemical analysis. Blood was centrifuged and the plasma separated at 4000 rpm for 5 minutes.

2.4 Analytical Methods

2.4.1 Phytochemical Screening

Phytochemical tests were carried out on Trevo using standard procedure to identify the constituents as described by Trease and Evans [13] and Sofowora [14].

2.4.2 Determination of total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein (LDL-C)

Plasma concentrations of glucose, total cholesterol, triglyceride, HDL-C and LDL-C were determined using standard spectrophotometric techniques as described by Allain *et al.*, [15]; Bucolo and David, [16]; Albers *et al.*, [17]; Friedwald *et al.*, [18] respectively.

2.4.3 Determination of Trace Metals

Trace metals' levels were determined by Atomic Absorption Spectrophotometer (AAS), as described by Kaneko [19].

2.5 Statistical Analysis

Data were expressed as Mean \pm SD. Student's (t) test was used for comparison of analytes between groups. Changes were considered significant when p-values were less than 0.05.

III. Results

3.1 Phytochemical constituents of Trevo

Qualitative assessment of the phytochemical contents of the Trevo supplement showed cardenolides and chalcones were not present in the Trevo food supplement (-), while phytochemicals such as alkaloids (+++), tannin (+++), phlobatannin (++), saponin (+++), flavonoids (+++), anthraquinones (+), steroids (++), phenol (+++) cardiac glycosides (++), terpenes (++) were present at different levels of concentrations.

3.2 Effects of Trevo on plasma glucose level and lipids of alloxan-induced diabetic Wistar rats

Plasma glucose level of the treated Wistar rats decreased significantly when compared with untreated diabetic Wistar rats. Total cholesterol and triglyceride levels of the treated diabetic Wistar rats were insignificantly lower ($p > 0.05$) when compared with untreated diabetic Wistar rats. But there was a significant ($p < 0.05$) increase in the HDL-cholesterol and a significant ($p < 0.05$) decrease in LDL- cholesterol of the treated diabetic Wistar rats when compared with the untreated diabetic Wistar rats (Table 1).

3.3 Effects of Trevo on micronutrients of alloxan-induced diabetic Wistar rats

Plasma levels of Zn, Cr and Mn were significantly ($p < 0.05$) higher in untreated diabetic Wistar rats when compared with the treated diabetic Wistar rats when the two groups were compared. Plasma level of Se increased significantly in the treated diabetic Wistar rats when compared with the untreated diabetic Wistar rats (Table 2).

IV. Discussion

Despite the understanding of the aetiology and pathogenesis of diabetes mellitus, the management strategies are still insufficient. This study was conducted in response to the suggestion of Triptych *et al.* [20] that there is need for a more effective treatment strategy. Blood glucose levels of those diabetic Wistar rats treated with Trevo showed significant decrease when compared with the untreated. It could be hypothesized that the antioxidants in the Trevo counter the effect of ROS generated by cyclic reaction with dialuric acid (a metabolite of alloxan) in the beta cells of pancreas, thereby reversing the actions of alloxan on beta cells of the pancreas. Although, Dembinska-Kiec *et al.* [21] earlier reported that phytochemicals especially polyphenols in fruits, vegetables and herbal medicines may modify imbalance glucose metabolism. The presence of a significant concentration of phenols (+++) in Trevo is a confirmation of the report of Dembinska-Kiec *et al.* [21] that phenols ameliorate glucose intolerance. It was also demonstrated that plasma LDL cholesterol level decreased significantly in diabetic Wistar rats treated with Trevo. This study agrees with Belobrajdic and Bird [22] who reported that diets high in whole grains are associated with 30% reduction risk of developing diabetic dyslipidemia. The significant decrease in plasma LDL cholesterol of diabetic Wistar rats treated with Trevo could accompany decreased plasma glucose observed in treated diabetic Wistar rats.

Tissue breakdown and plasma hyperosmolarity might account for significant increase in plasma levels of zinc and chromium of the untreated diabetic Wistar rats in this study. This finding is similar to the previous study that prolonged hyperglycemia increases chromium urinary excretion [23]. Our findings suggest that Trevo treatment of diabetic Wistar rats increased tissue uptake of zinc and chromium for metabolic purposes. Selenium is a micronutrient that is required as an essential co-factor for the antioxidant enzyme, glutathione peroxidase that counteracts the damaging effect of hydrogen peroxide in the tissues [24]. A significantly increased plasma level of selenium observed in Trevo treated diabetic Wistar rats could indicate that Trevo has effects on selenium metabolism in the diabetic Wistar rats. Cobalt also showed a significant increase in the treated diabetic Wistar rats. The increase in cobalt level is proposed to have the potential beneficial effect in the glucose-insulin metabolism [25]. Forte *et al.* [26] reported manganese deficiency in type 2 diabetic patients, while in contrary; this study shows a significantly increased manganese level in untreated diabetic Wistar rats. Studies examining the Mn status of diabetic humans have generated contradictory results [27]. Leonhardt *et al.* [28] had posited that diabetes mellitus with high level of blood manganese are better protected from oxidation of LDL which contributes to intra-arterial plaque causing heart attack and stroke. In conclusion, Trevo appeared to have regulatory effects on blood glucose level, lipid and micronutrients metabolisms. However the present study strongly recommends clinical trials with Trevo to establish its role as adjuvant therapy in the management of diabetes and its complications.

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Competing interests

The authors declare that they have no competing interests.

References

- [1]. Shoback A. *Greenspan's basic & clinical endocrinology* (9th ed.). Edited by David G: (Gardner & Dolores New York: *McGraw-Hill Medical*, Chapter 17), 2011.
- [2]. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hyperglycemic crises in adult patients with diabetes. *Diabetes Care*, 32(7): 2009, 1335–1343.
- [3]. [World Health Organization, Department of non-communicable disease, definition, diagnosis and classification of diabetes mellitus and its complications. *Diabetic Medicine* 14: 2014, 31-57.
- [4]. Jayawardena R, Ranasinghe P, Galappaththy P, Malcanthi RLDK, Constantine GR and Katulanda P, Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. *Diabetol. Metab. Syndr.* 4: 2012, p13.
- [5]. Maggini S, Wintergerst ES, Beveridge S and Hornig H, Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral responses. *Br. J. Nutr.*, 98(1): 2007, S29-35
- [6]. Cefalu WT and Hu FB, Role of chromium in human health and in diabetes. *Diabetes Care*, 27(11): 2004, 2741-2751.
- [7]. Garg VK, Gupta R, Goyal RK, Hypozincemia in diabetes mellitus. *J Assoc. Physicians India*, 42: 1994, 720-721.
- [8]. Hashemipour E, Dabiri N, Hashemipour H, Effect of diets containing different levels of stream treated pith bagasse on performance and carcass characteristics of fattening Arabi lambs. *J. Anim. Vet. Adv.*, 8(11):2009, 2139-214.
- [9]. Mueller AS and Pallauf J, Compendium of the antidiabetic effects of supranutritional selenate doses. In vivo and in vitro investigations with type II diabetic db/db mice. *J. of Nutr. Biochem.*, 17(8): 2006, 548–560.

- [10]. Lenzen S, The mechanisms of alloxan and streptozotocin induced diabetes. *Diabetologia*, 51: 2008, 216-226.
- [11]. Stevens MA: Trévo – The power of wellness. www.trevocorporate.com. 2014.
- [12]. Sada NM, Tanko Y and Mabrouk MA, Modulatory role of Soyabeans supplement on lipid profiles and liver enzymes on Alloxan-induced diabetic wistar rats. *European Journal of Experimental Biology*. 3(2): 2013, 62-67.
- [13]. Trease GE and Evans WC, *A Text-book of Pharmacognosy*. 14th Edn. (W.B Saunders, London 1989), pg. 13-53.
- [14]. Sofowora AO, Medicinal plants and traditional medicines in Africa. (2nd Edn. University of Ife Press 1993), Pg 320.
- [15]. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC, Enzymatic determination of total serum cholesterol. *Clin. Chem.*, 20(4): 1974, 470-5
- [16]. Bucolo G and David H, Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.*, 19: 1973, 476-82.
- [17]. Albers JJ, Warnick GR and Cheung MC, Quantification of high density lipoproteins. *Lipids*, 13: 1978, 926-932.
- [18]. Friedewald W, Levy R, Fredrickson D, Estimation of concentration of low-density lipoprotein in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, 18: 1972, 499-502.
- [19]. Kaneko JJ, *Clinical Biochemistry of Animal*. (4thEdn.) (Academic press Inc.,1999) pp: 932.
- [20]. Triptych S, Sumathi S and Raj GB, Minerals nutritional status of type II diabetic subjects. *International Journal of Diabetes in Developing Countries*, 24: 2004, 27–28.
- [21]. [Dembinska-Kiec A, Mykkanen O, Kiec-Wilk B and Mykkanen H, Antioxidant phytochemicals against type 2 diabetes. *British Journal of Nutrition* 99: 2008, 109-117.
- [22]. Belobrajdic DP and Bird AR, The potential role of Phytochemicals in whole grain cereals for prevention of type-2 diabetes. *Nutr. J.* 2013, 12:62.
- [23]. Kazi TG, Afridi HI, Kazi N, Jamali MK, Arain MB, Jalbani N and Kandhro GA, Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients *Biological Trace Element Research*, 122(1): 2008, 1-18.
- [24]. Akbaraly TN, Arnaud J, Rayman MP, Hininger-Favier I, Roussel A, Berr C and Fontbonne A, Plasma selenium and risk of dysglycemia in an elderly French population: results from the prospective epidemiology of vascular ageing study. *Nutr. Metab.*, 7: 2010, p21.
- [25]. Pandey G, Jain GC, Mathur N, Therapeutic potential of metals in managing diabetes mellitus: a review. *J. Mol. Pathophysiol.* 1(1): 2012, 63-76.
- [26]. [Forte G, Bocca B, Peruzzi A, Tolu F, Asara Y, Farace C et al., Blood metals concentration in type 1 and type 2 diabetics. *Biological Trace Element Research*, 156(1–3): 2013, 79-90.
- [27]. Nicoloff G, Mutaftchiev K, Strashimirov D, Petrova C, Serum manganese in children with diabetes mellitus type 1. *Diabetologia Croatica*, 33(2): 2004, 47-51.
- [28]. Leonhardt W, Hanefeld M, Muller G, Impact of concentrations of glycated haemoglobin, alpha-tocopherol, copper and manganese in oxidation of low-density lipoproteins in patients with type 1 diabetes, type 2 diabetes and control subjects. *Clin Chim Acta*, 254: 1996, 173-186.

Table 1. Lipid Profile and Blood Glucose Levels in Diabetics and Controls.

Group	N	Total cholesterol (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	Triglyceride (mmol/l)	Glucose (mmol/l)
Diabetics+Trevo	6	3.27±0.35	1.61±0.19	1.29±0.11	1.62±0.16	14.3±3.98
Diabetics-Trevo	6	3.71±0.19	0.98±0.14	2.93±0.56	1.78±0.06	27.6±2.28
Controls	6	3.26±0.84	1.50±0.29	1.43±0.33	1.32±0.10	5.35±0.66
p values ^a		>0.05	<0.05*	<0.05*	>0.05	<0.05*
p values ^b		>0.05	>0.05	>0.05	<0.05*	<0.05*

^a = level of significance of difference between Diabetics+Trevo and Diabetics-Trevo

^b = level of significance of difference between Diabetics+Trevo and Controls

* = significantly different

Table 2. Mean ± SD of Trace elements in Diabetics and controls

Group	N	Zn (mg/L)	Cr (mg/L)	Se (mg/L)	Co (mg/L)	Mn (mg/L)
Diabetics+Trevo	6	0.350±0.005	0.330±0.013	0.007±0.002	0.024±0.004	0.018±0.003
Diabetics-Trevo	6	0.75±0.005	0.51±0.011	0.005±0.001	0.022±0.001	0.026±0.001
Controls	6	0.390±0.005	0.310±0.012	0.009±0.001	0.015±0.002	0.011±0.002
p values ^a		<0.05*	<0.05*	<0.05*	>0.05	<0.05*
p values ^b		<0.05*	>0.05	<0.05*	<0.05*	<0.05*

^a = level of significance of difference between Diabetics+Trevo and Diabetics-Trevo

^b = significance of difference between Diabetics+Trevo and Controls

* = significantly different

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