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Effect of Vitamin C and/or Vitamin E on Oxidative Stress and Lipid Profile in Diabetic Rats

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ABSTRACT

Diabetes Mellitus is one of the main threats to human health in the 21st century. The present study was planned to evaluate the effect of vitamin C and/or vitamin E as hypocholesterolemic, antioxidant agent on oxidative stress and serum lipid profile in streptozoticin induced-diabetic rats using glibenclamide as reference drug for treatment of type II diabetes. 120 male adult Sprague Dawley rats were divided into 6 groups, normal control (C), diabetic control (D), diabetic treated with: glibenclamide (D_G), vitamin C (D_C), vitamin E (D_E); vitamin C and E (D_{C+E}). Diabetic group (D) revealed a significant increase in glucose, HbA_{1C} (258.1 vs 88.9 gm/dl; 8.09 vs 5.55 gm/dl respectively), liver and plasma MDA (124.03, 134.24 vs 60.44, 61.27 nmol/gm, nmol/l respectively), lipid profile except HDL-C which showed a significant decrease when compared with normal control group. When treated diabetic groups were compared with diabetic group, significant decrease in serum levels of glucose, HbA_{1C}, total cholesterol, LDL-C, VLDL-C, triacylglycerol, liver and plasma MDA as well as significant increase in serum HDL-C, phospholipids, Vitamin C and liver glycogen were observed. Combined treatment with vitamin C and E gave better results (Cholesterol, LDL-C, VLDL-C and TG decreased by 39.98, 46.88, 56.83, and 45.84 % from diabetic group respectively). Results of our study suggest that it is better to use antioxidant vitamins (C+E) in combination rather than using single one in treatment of type II diabetes.

Keywords: Streptozotocin, Diabetes mellitus, Lipoproteins, Vitamin C, Vitamin E, MDA

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INTRODUCTION

BACKGROUND

Diabetes mellitus is associated with abnormal changes in protein, carbohydrate and fat metabolism[1]. It has been suggested that increased free radicals and decline of antioxidant defense mechanisms are implicated in diabetic complications [2, 3]. Ascorbic acid (vitamin C) is an essential cofactor involved in many biochemical functions and acts as an electron donor or reducing agent. It scavenges singlet oxygen, superoxide, hydroxyl and water soluble peroxyl radical and hypochlorous acid [4]. Among lipid soluble antioxidants, α-tocopherol plays a central role as it controls radical-induced lipoprotein lipid peroxidation [5]. Plasma concentration of ascorbic acid was reported to decrease in both human and experimental diabetes [6]. Similarly, plasma vitamin E levels were found to decrease in Type II diabetic patients [7, 8]. Vitamin C is capable of regenerating α-tocopherol from the tocopheroxyl (chromanoxyl) radical that is formed upon the inhibition of lipid peroxidation by vitamin E [9]. Vitamin C has been reported to contribute up to 24% of the total peroxyl radical-trapping antioxidant activity (TRAP) [10]. Reports in previous literature reported beneficial effect of vitamin E and/or C supplementation in type II diabetes on blood sugar, insulin, serum lipids and levels of HbA1c [11,12,13], although [14] reported no effect.

MATERIALS AND METHODS

Experimental animals:

This study was approved by the high society of scientific ethic committee of NNI (**N**ational **N**utrition **I**nstitute) & GOTHI (**G**eneral **O**rganization for **T**eaching **H**ospitals and **I**nstitutes) in Cairo, Egypt, ARE.

One hundred and twenty male Sprague Dawley rats aged 3 months, weighing 230±20 g were purchased from Helwan Animal farm and maintained under standard conditions of boarding. All rats were housed individually in wire meshed cages to facilitate weighing of rats and their diet. The animals were fed on a standard rat diet for 10 days for acclimatization. The standard control rat diet (AIN-93 M diet formulated for adult rodents) was prepared according to the National Research Council (NRC, 1978) [16] and Reeves et al., (1993) [15]. Diabetes (Type II, NIDDM) was induced in rats by a single intraperitoneal injection of streptozotocin (STZ, Sigma, St. Louis, Missouri, USA) at a dose of 50 mg/ kg body weight. STZ was dissolved immediately before use in 1ml sodium citrate (0.05 mol/L, pH 4.5) to get a final concentration of 50 mg STZ/ml citrate. STZ-injected animals exhibited massive glucosuria and hyperglycemia within 2-3 days. Blood was drawn from the tail vein and blood glucose was measured using Bionime instrument, Rightest, GM 300. Rats were considered diabetic only if their blood glucose levels exceeded 250 mg /dl [17]. Rat diet and body weights were also recorded on a weekly basis. Rat diet and water were given ad libitum

Experimental design: Rats were divided into six groups (20 rat /group) as follows:

1. G1: Control rats: (C): received single i.p injection of sodium citrate buffer in a dose of 1 ml/kg b.w.
2. Groups from 2 to 6 rendered diabetic (Type II) by receiving single intraperitoneal injection of streptozotocin, (50 mg/kg b.w.), then they were divided as follow
3. G2: Diabetic rats: (D): received normal diet
4. G3: Diabetic rats treated with glibenclamide: (D_G): treated with daily i.p. injection of glibenclamide (600 μg /kg b.w. in aqueous solution).
5. G4: Diabetic rats then treated with vitamin C: (D_C): treated with daily i.p. injection of vitamin C (1000 mg/kg b.w. /day).
6. G5: Diabetic rats treated with vitamin E: (D_E): treated with daily i.p. injection of vitamin E (600 mg/kg b.w./day).
7. G6: Diabetic rats treated with vitamin C and E (D_{C+E}): treated with daily i.p. injections of vitamin C and vitamin E as groups D_C and D_E.

The experiment lasted for 6 weeks after induction of diabetes for all groups

Experimental procedure:

After the end of the experiment rats were fasted overnight, then scarified under ether anesthesia (Sigma, USA). Blood samples were taken by cardiac puncture. Fasting blood samples were collected in tubes (plain and coated with anticoagulant). Plain tubes centrifuged for separation of serum at 3,000 rpm for 15 minutes, and sera were stored at –20 °C for determination of the following biochemical measurements; Glucose, vitamin C, total cholesterol, HDL-C, LDL-C, VLDL-C, triacylglycerol, phospholipids and total antioxidant capacity (TAC). One EDTA tubes were centrifuged at 3,000 rpm for 15 minutes to get plasma for the determination of malondialdehyde and insulin, $HbA1_C$ was determined in the other EDTA tubes (whole blood), Rat livers were excised to be used for determination of glycogen and malondialdehyde after being washed with saline, dried and weighed. They were kept at -20 °C till analysis.

Assay of Biochemical Parameters

Glucose was determined using Randox kit **(**Randox Lab, San Francisco, USA**)**. HbA_{1C} was determined using Human kits (Human Gessellschaft Für Biochemica und Diagnostica, mbh, Wiesbaden, Germany). Triacylglycerol (TG) was determined using Bicon kit (Germany). Total cholesterol, TC; Serum HDL-C; and Serum LDL-C; was determined using Bio Mérieux kit (France). VLDL-C was determined by using the following equation: VLDL-C=total cholesterol- (HDL-C+LDL-C). Atherogenic Index (AI) was calculated according to [18] using following equation: AI= (Total cholesterol-HDL-C)/HDL-C. Plasma vitamin C was determined colorimetrically according to the method of [19, 20]**,** malondialdehyde was determined according to the method of [21]**.** Liver glycogen was determined according to the method described by [22]**.** Total antioxidant capacity

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was measured using Bio-diagnostic kits (Cairo, Egypt). The homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance was calculated according to equation (HOMA-IR (mmol/l) = [Fasting plasma insulin (µU/ml) X plasma glucose (mmole/l)]/22.5) according to [23]**.** Insulin was determined using rat insulin ELISA kit EIA 2018 (DRG international Inc, USA)

STATISTICAL ANALYSIS:

Data are expressed as Mean ±SEM. All statistical data and significance tests (T Test for comparison between individual groups and control group; and post hoc Duncan test analysis for comparison between groups) were performed using the Statistical Package for the Social Sciences version 11 (SPSS Inc, Chicago, IL, USA), The correlation coefficients were determined by Pearson's simple linear regression analysis (SPSS, v 11). Statistical significance was accepted at $P < 0.05$.

RESULTS

Changes in body weight:

Initial body weights (IBW) were comparable between all studied groups. However, final body weight (FBW) of all diabetic groups (D, D_G, D_C, D_E and D_{C+E}) became significantly ($P<0.001$) lower than normal controls, but in treated diabetic groups (D_G, D_C, D_E and D_{C+E}), final body weights increased significantly ($P<0.01$, 26.41-29.58%) compared to D group but still significantly lower than normal (C) control group (Table 1). Body weight gain (BWG) in treated groups range was 27-38.5 g.

Table (1): Mean values of initial body weight (IBW), final body weight (FBW), Body weight gain (BWG) of normal controls (C), untreated diabetic (D), diabetic treated with glibenclamide (D_G), diabetic treated with vitamin C (D_C), diabetic treated with vitamin E (D_E) and diabetic treated with vitamin C and E (D_{C+E}).

Rats	IBW (g)	FBW (g)	% Change vs D	BWG (g)
C	229.70±3.59	294.70±6.27		65.00±2.70
D	234.60±2.12	208.60±2.07 [a]		-26.00±0.47
D_G	234.10±1.80	263.70±2.49 [a,b]	26.41	29.60±1.85
D_C	231.80±1.85	270.30±2.25 [a,b]	29.58	38.50±0.19
D_E	232.30±1.68	259.40±2.09 [ab]	24.35	27.10±1.77
D_{C+E}	233.90±2.55	263.80±2.67 [a,b]	26.46	29.90±0.62

Results are expressed as Mean**±**SEM, a: significance with C; b: significance with D; c: significance with D_G; d: significance with DC ; e: significance with DE . by ANOVA and Duncan post hoc test

Changes in serum glucose, HbA_{1c} and liver glycogen:

Serum levels of glucose and $HbA1_C$ of D, D_G, D_C, D_E and D_{C+E} were significantly higher than C group ($P< 0.001$) and decreased significantly in all treated diabetic groups (D_G, D_C, D_E and D_{C+E}) compared to D group ($P< 0.001$). However, Serum glucose remained significantly higher in

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all vitamin treated groups (D_C, D_E and D_{C+E}) compared to D_G group and in D_{C+E} group compared to D_C group but significantly lower than D_E group. Liver glycogen decreased significantly in all diabetic groups (D, D_G, D_C, D_E and D_{C+E}) compared to C group and increased significantly ($P< 0.001$) in all treated diabetic groups (D_G, D_C, D_E and D_{C+E}) compared to D group. However, Liver glycogen was significantly lower in D_C and D_E groups compared to D_G group and significantly higher in D_{C+E} group compared to either D_C or D_E groups (Table 2). Plasma levels of HOMA-IR increased significantly ($P < 0.001$) in G_D rats compared to C rats and decreased significantly ($P < 0.001$) in treated group compared to G_D group (Table 2, figure 1). Data of Table 2 revealed a significant decrease in insulin level and significant increase in HOMA-IR level in diabetic group compared to normal control group. Treatment with vitamin C and/or vitamin E lead to an improvement in insulin and HOMA-IR levels returning to near normal level.

Table (2): Mean values of serum levels of glucose, Homeostasis model assessment of insulin resistance (HOMA-IR), HbA1c, glycogen, vitamin C and MDA in serum and liver. Total Antioxidant Capacity (TAC) of normal controls (C), untreated diabetic (D), diabetic treated with glibenclamide (D_G), diabetic treated with vitamin C (D_C), diabetic treated with vitamin E (D_E) and diabetic treated with vitamin C and E (D_{C+E}).

Rats	Serum Glucose (mg/dl)	Insulin (µU/ml)	HOMA-IR (mmol/l)	Blood HbA_1c (g/dl)	Glycogen (mg/g tissue)	Serum Vitamin C (mg/dl)	Plasma MDA (nmol/l)	MDA (Liver nmol/g)	Total Antioxidant Capacity (TAC, mmol/l)
C	88.90± 2.25	12.30± 0.18	2.70± 0.06	5.55± 0.15	23.29± 0.88	1.78± 0.07	61.27± 1.30	60.44± 1.25	2.21± 0.02
D	258.10± 3.16 ^{a}	8.77± 0.28 ^{a}	5.60± 0.21 ^{a}	8.09± 0.23 ^{a}	12.85± 0.19 ^{a}	1.10± 0.07 ^{a}	134.24± 3.17 ^{a}	124.03± 3.17 ^{a}	1.57± 0.03 ^{a}
D_G	120.50± 3.47 ^{ab}	10.38± 0.30 ^{ab}	3.11±0.17 ^{abfe}	6.24± 0.18 ^{ab}	19.66± 0.83 ^{ab}	1.30± 0.04 ^{ab}	102.87± 2.88 ^{ab}	87.22± 1.79 ^{ab}	2.04± 0.04 ^{abef}
D_C	139.40± 1.69 ^{abc}	9.82± 0.12 ^{abc}	3.38± 0.04 ^{abf}	6.45± 0.12 ^{ab}	17.09± 0.43 ^{abc}	1.60± 0.04 ^{abc}	89.87± 1.18 ^{abc}	70.09± 1.31 ^{abc}	1.96± 0.03 ^{ab}
D_E	152.40± 1.09 ^{abc}	9.96± 0.08 ^{abc}	3.75± 0.03 ^{abcdf}	6.58± 0.13 ^{ab}	15.59± 0.21 ^{abc}	1.33± 0.04 ^{abc}	94.25± 1.11 ^{abc}	76.09± 0.57 ^{abc}	1.91± 0.02 ^{abc}
D_{C+E}	143.70± 0.87 ^{abcde}	10.19± 0.13 ^{ab}	3.62± 0.06 ^{abcde}	6.28± 0.12 ^{ab}	19.19± 0.52 ^{abde}	1.68± 0.05 ^{bce}	69.58± 1.43 ^{abcde}	62.92± 2.83 ^{bcde}	1.91± 0.03 ^{abc}

Results are expressed as Mean±SEM, a: significance with C; b: significance with D; c: significance with D_G; d: significance with DC ; e: significance with DE . by ANOVA and Duncan post hoc test

Changes in serum Vitamin C concentration:

Serum vitamin C levels decreased significantly ($P< 0.001$) in D, D_G, D_C and D_E groups compared to C group ($P < 0.01$) being more pronounced in D group ($P < 0.001$) but treatment with vitamin C and/or vitamin E significantly increase vitamin C level in D_C, D_E and D_{C+E} but still lower than C group (Table 2 , figure 1).

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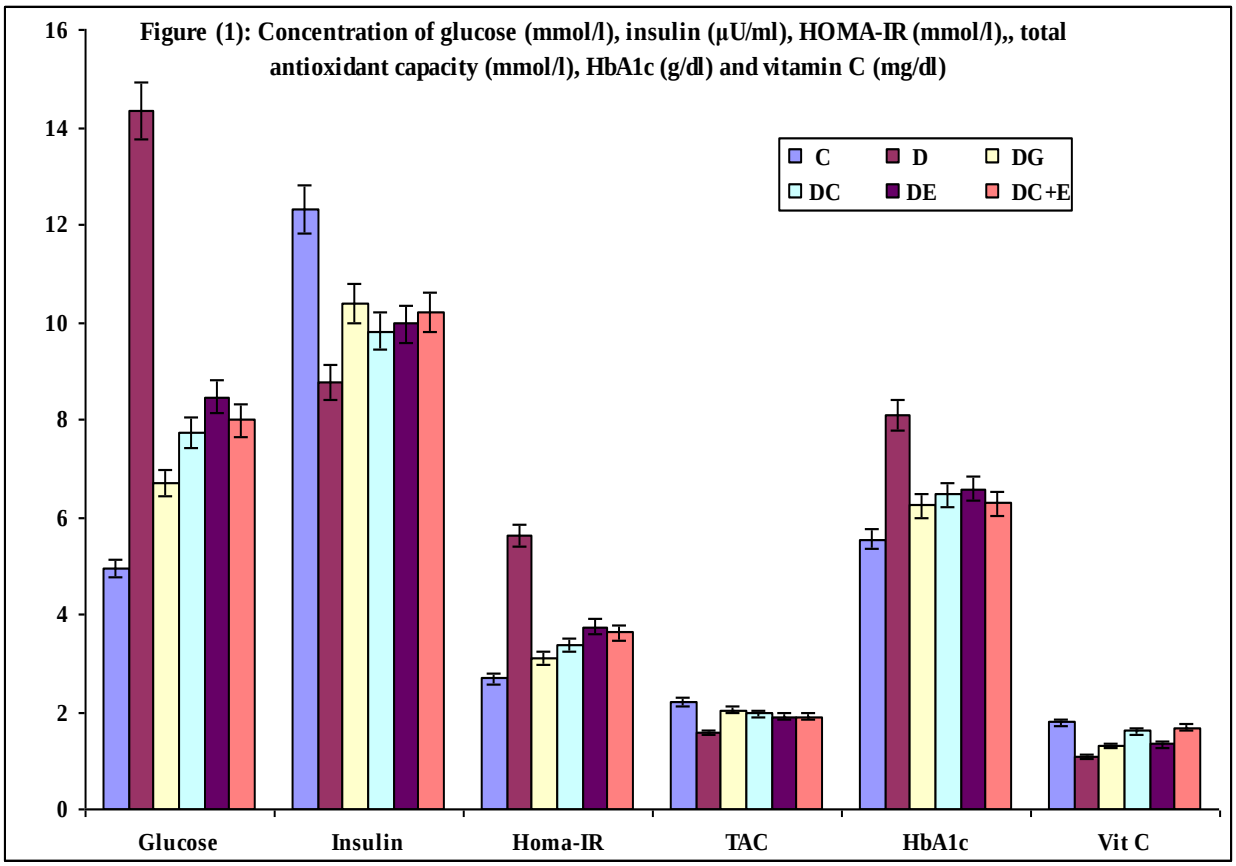

Changes in plasma and liver malondialdehde (MDA), and TAC

Plasma MDA increased significantly (P< 0.001) in all diabetic groups (D, D_G, D_C, D_E and D_{C+E}) compared to C group and decreased significantly (P< 0.001) in all treated diabetic groups (D_G, D_C, D_E and D_{C+E}) compared to D group. Vitamin treated groups (D_C,D_E and D_{E+C}) showed significantly lower plasma MDA levels compared to D_G group and was even significantly lower in D_{C+E} group compared to either D_C and D_E groups. Liver MDA increased significantly (P< 0.001) in D, D_G,D_C and D_E groups compared to C group and became significantly lower in all treated diabetic groups (D_G, D_C, D_E and D_{C+E}) compared to D group. However, it showed significant decrease in all vitamin treated diabetic groups (D_C, D_E and D_{C+E}) compared to D_G and in D_{C+E} group compared to either D_C or D_E groups (Table 1). TAC decreased significantly in diabetic group (P < 0.001). All treated diabetic group showed significant increase (P < 0.01, Table 2)

Changes in lipid profile

Mean total cholesterol, LDL-C, VLDL-C and triacylglycerol were significantly higher in D groups in comparison to the C group, while the mean value of HDL-C and phospholipids were significantly lower in D group. On the other hand,, mean total cholesterol, LDL-C, VLDL-C and triacylglycerol were significantly decreased in treated diabetic groups (D_C, D_E, D_{C+E} ,P< 0.001), while the mean value of HDL-C and phospholipids were significantly higher (increased) in D_C, D_E,

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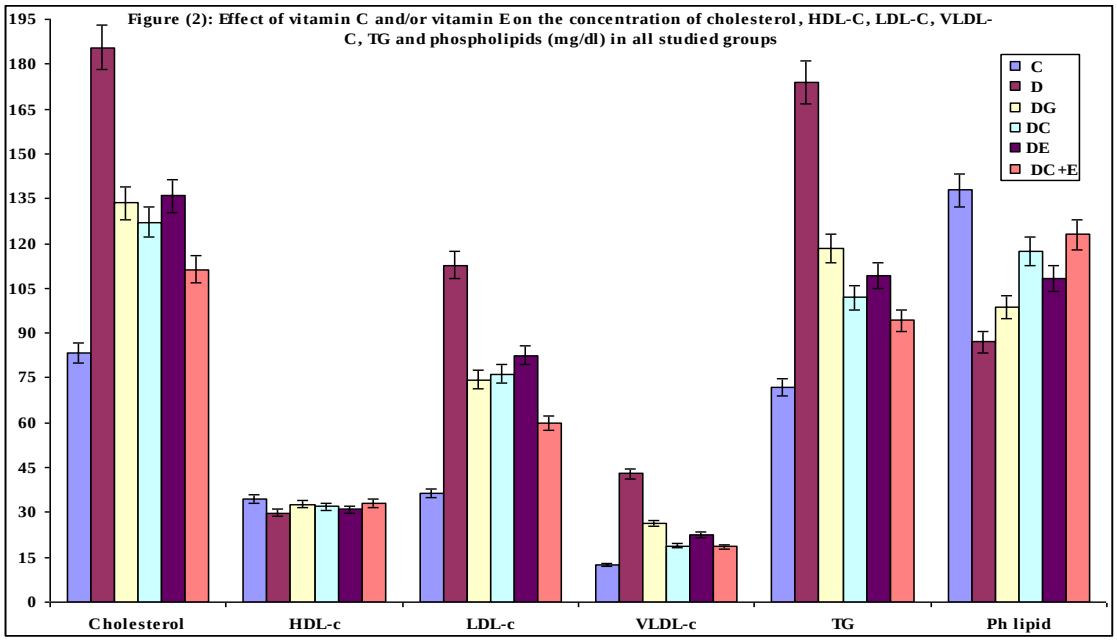
D_{C+E} groups in comparison to D group returning to near normal level of normal control (C) group. Phospholipids levels were significantly decreased in D groups but after treatment with vitamins, it begins to be significantly higher ($P < 0.001$, Table 3, figure 2).

Table (3): Mean values of serum lipid profile and atherogenic index of normal controls (C), untreated diabetic (D), diabetic treated with glibenclamide (D_G), diabetic treated with vitamin C (D_C), diabetic treated with vitamin E (D_E) and diabetic treated with vitamin C and E (D_{C+E})

Rats	Total Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Triacylglycerol (mg/dl)	Phospholipid (mg/dl)	Atherogenic index
C	83.39± 1.19	34.62± 0.75	36.49± 0.70	12.28± 0.57	71.73± 0.83	137.76± 1.67	1.41± 0.03
D	185.53± 4.56 [a]	29.90± 0.55 [a]	112.64± 2.45 [a]	42.99± 3.43 [a]	173.93± 1.76 [a]	87.00± 0.89 [a]	5.22± 0.17 [a]
D_G	133.46± 1.25 [abdf]	32.71± 0.58 [b]	74.42± 1.23 [abef]	26.34± 1.22 [abdef]	118.53± 6.28 [abde]	98.74± 2.39 [abde]	3.09± 0.05 [abef]
% change vs D	-28.06	9.38	-33.93	-38.74	-31.85	13.50	
D_C	127.16± 2.55 [abcf]	31.95± 0.69 [ab]	76.40± 1.57 [abef]	18.82± 0.69 [abcef]	102.01± 3.79 [abcf]	117.38± 2.52 [abcef]	2.99± 0.06 [ac]
% change vs D	-31.46	6.84	-32.18	-56.23	-41.35	34.93	
D_E	135.97± 3.68 [abf]	30.94± 0.69 [af]	82.61± 3.35 [abcdf]	22.42± 0.64 [abcdf]	109.11± 1.67 [abcf]	108.25± 2.23 [abcdf]	3.41± 0.14 [ac]
% change vs D	-26.71	3.47	-26.66	-47.84	-37.27	24.43	
D_{C+E}	111.36± 1.72 [abcde]	32.96± 0.62 [b,c]	59.84± 1.54 [abcde]	18.56± 0.42 [abce]	94.20± 1.63 [abce]	99.20± 2.23 [abde]	2.38± 0.06 [abcde]
% change vs D	-39.98	10.23	-46.88	-56.83	-45.84	14.02	

Results are expressed as Mean±SEM

a: significance with C; b: significance with D; c: significance with D_G; d: significance with D_C, ; e: significance with D_E, by ANOVA and Duncan posttest



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Correlation study

Table (4) showed significant and inverse correlations between serum vitamin C and $HbA1_C$ in all experimental groups (P< 0.01), vitamin C and serum cholesterol in all groups except D_E (P< 0.01), vitamin C and MDA in C. D, D_G,D_C (P< 0.01), vitamin C and HOMA-IR in C and D; glucose and total antioxidant capacity (P < 0.01). HbA1c showed significant direct correlation with plasma MDA in C, D and D_G. Significant and inverse correlations between TAC and glucose in all experimental groups were found.

Table (4): Correlations of serum levels of vitamin C versus HbA1c, total cholesterol, MDA and HOMA-IR; correlations of MDA versus HbA1c and HOMA-IR; total antioxidant capacity versus glucose in normal controls (C), untreated diabetic (D), diabetic treated with glibenclamide (D_G), diabetic treated with vitamin C (D_C), diabetic treated with vitamin E (D_E) and diabetic treated with vitamin C and E (D_{C+E}) .

Correlation		Experimental groups					
		C	D	D_G	D_C	D_E	D_{C+E}
Vit. C vs HbA1c	r	-0.84*	-0.60*	-0.77*	-0.74*	-0.89*	-0.78*
	P	0.01					
Vit. C vs Total cholesterol	r	-0.61*	-0.76*	-0.74*	-0.94*	-0.12	-0.86*
	P	0.01				NS	0.01
Vit. C vs MDA	r	-0.77*	-0.61*	-0.67*	-0.64*	-0.06	-0.14
	P	0.01				NS	
Vit C vs HOMA-IR	r	-0.41*	-0.54*	-0.22	-0.16	-0.24	-0.30
	P	0.05		NS			
MDA vs HOMA-IR	r	0.67*	0.60*	0.61*	0.65*	0.50*	0.55*
	P	0.01				0.05	
MDA vs HbA1c	r	0.56*	0.81*	0.53*	0.17	0.28	0.39
	P	0.05	0.01	0.05	NS		
TAC vs Glucose	r	-0.83*	-0.90*	-0.89*	-0.87*	-0.47*	-0.86*
	P	0.01				0.05	0.01

DISCUSSION

Streptozotocin is a naturally occurring nitrosamide used to develop animal models of diabetes by exerting cytotoxic effect on pancreatic β-cells possibly by generating lipid peroxides and excess reactive oxygen species (ROS), interfering with glucose transporter GLUT-2 and causing DNA damage either by alkylation or peroxynitrite formation [24, 25]. The DNA strand breakage by streptozotocin activates poly ADP-ribose polymerase (PARP) and causes ATP depletion leading to cell death and drop in insulin level [26]. Whereas a single diabetogenic dose of STZ (70-250 mg/kg, b.w.) had been demonstrated to induce complete destruction of β cells in most species within 24-72 hour leading to diabetes [27] , yet lower doses were found to cause partial destruction of β cells, thus inducing type II diabetes [28, 29, 2]. In the present study, we chose STZ dose 50 mg/kg because higher doses were associated with high mortality rate, while lower doses were associated with spontaneous recovery. All diabetic rats developed significant decrease in insulin level rather than complete depletion as well as mild hyperglycemia which indicates incomplete destruction of pancreatic β cells. To assess

therapeutic efficacy of vitamin C and/or E we chose glibenclamide, a member of sulfonylurea drugs used in treatment of type II diabetes. The mechanism of action of glibenclamide was reported to be inhibition of a K_{ATP} channel leading to depolarization of pancreatic β cells and stimulation of insulin release [30].

In the present study, we used intraperitoneal injection of vitamin C and vitamin E at doses of 1000 and 600 mg/kg BW/day respectively. The used dose of vitamin C in this study give best results as agreed with [11] since using lower doses (500 mg, 800 mg) of vitamin C did not show any change in type II diabetic patients [31,32]. Moreover, Errikson and Kahvakka (1995) 33 reported that the use of 2000 mg vitamin C for 90 days give better results. However, It must be recognized that continual high intake in the range of several grams daily can produce adverse effects as uricosura, kidney stones and gout which were reported to occur with vitamin C doses between 3-12 g for some days [34, 35], while doses of 0.5-2 g were reported to have no observable effect.

Although vitamin E in mega doses is promoted as beneficial to the aged, there is no scientific evidence that this is true [36]. While thousands of people take in vitamin E daily in quantities 100 or more times greater than recommended, there is little evidence of undesirable secondary effects [37], which indicates that vitamin E is highly tolerated.

In the present study, diabetes induced significant weight loss (-26.0±0.47 gm) which agrees with [38] where they found 8% loss, or due to excessive breakdown of tissue proteins [39] as well as muscle wasting, dehydration and catabolism of fats [40]. Administration of glibenclamide, vitamin C and /or vitamin E to diabetic rats minimized body weight loss which suggests interruption, at least partially, of the previously mentioned metabolic derangements.

Glycemic control manifested by serum glucose and HbA_{1c} level and liver glycogen content was better in all treated diabetic groups compared to untreated diabetics which might indicate either sparing of more pancreatic islet cells, improved insulin sensitivity or an insulin–like action of these drugs. Glibenclamide exerted the best glycemic control possibly via increasing insulin secretion as mentioned previously [30]. However, combined vitamin C and E treatment were observed to increase liver glycogen to a value comparable to that observed with glibenclamide treatment although serum glucose level remained significantly higher which denotes that vitamin C and E exerted a hypoglycaemic effect maybe through suppressing glycogenolysis rather than by enhancing peripheral tissue uptake of glucose (this require more experiments as liver perfusion test for conformation). Whether this effect of vitamin C and E is direct or indirect (via enhancing insulin release or sensitivity) is difficult to speculate but the possibility that early administration of vitamin C and E might have spared more pancreatic β–cells with more insulin availability in these rats cannot be excluded. The Hypoglycemic effect of vitamin C and E was reported by many authors [41-43]. Vitamin C was reported by [44] to stimulate insulin–like mechanism.

Our results demonstrated that increased insulin resistance in the untreated diabetic group (D) was significantly decreased in all treated diabetic groups and that D_G and D_C groups had the maximal decrease in insulin resistance. Vitamin C intake was found to improve insulin sensitivity in conditions with increased oxidative stress and insulin resistance as in cigarette smokers [45] but in type II diabetic patients , the results were not encouraging [46]. Our data showed that all treated diabetic groups had serum insulin levels significantly higher than the untreated diabetic group although still lower than normal control values which indicates that early therapeutic intervention in type II diabetes might have protected and spared more pancreatic β cells than the untreated diabetic group with consequent improved insulin resistance. It seems that oxidative stress might be involved in induction of insulin resistance as shown by the significant positive correlation between plasma malondialdehyde and HOMA-IR (Homeostasis model assessment of insulin resistance) [47], the significant inverse correlation between total antioxidant capacity and serum glucose as well as the significant inverse correlation between serum Vitamin C and HOMA-IR which would point to the rational of the use of antioxidant therapy to minimize insulin resistance, the main criterion in type II diabetes. It was of interest to observe that the highest value of total antioxidant capacity among all diabetic groups was in D_G group that showed the best glycemic control although the improvement in dyslipidemia and oxidative stress were less evident than vitamin-treated groups.

The significant decrease of HbA_{1C} in all treated diabetic groups can be attributed to amelioration of hyperglycemia as well as the free radical scavenging activity of vitamin C and E as reported by [11,47]. Also The hypoglycaemic action of combined vitamins C and E in diabetic rats may be due to increase of antioxidant enzymes expressions and/or activities [48], or due to inactivation of the circulating free radicals [free radicals quench nitric oxide (NO) before it reaches pancreatic β-cells, causing its damage and/or death] [48].

The highly significant inverse relationship between Vitamin C and HbA1c further highlights the role of vitamin C supplementation in optimizing long term glycemic control which was reported to reduce both the development and progression of microvascular diabetic complications [49]. Our findings agree with the findings of [50] and suggest that vitamin C was strongly implicated in glycemic control and that restoring the normal level of vitamin C in diabetics might be crucial for long term euglycemia.

In contrast to humans, healthy adult rats synthesize their own vitamin C, and standard rat diets do not contain any vitamin C [51]. Nevertheless, diabetic rats exhibit the same abnormalities in vitamin C metabolism that are seen in diabetic patients [52, 53, and 54] where a sharp reduction of the plasma level of vitamin C is observed. In our study, vitamin C level decreased significantly in all diabetic rats which agree with [55] (whose diabetes was induced by alloxan and vitamin C were given orally in drinking water) and [56] and increased significantly in all treated diabetic groups.. Experiments with diabetic rats found that the increased turnover of ascorbic acid was probably due to increased oxidation of ascorbate to dehydroascorbate in tissue mitochondria [57]. The significant inverse relationship between

serum vitamin C and HOMA-IR suggest that diabetes- induced reduction in vitamin C level would team up with insulin deficiency to worsen hyperglycemia, dyslipidemia and oxidative stress in type II diabetes.

It is well established that diabetes induces dyslipidemia with excess LDL-C, VLDL-C and decreased HDL-C [58, 59]. In the present study, it was observed that all treatments brought about almost complete normalization of HDL-C. However, total cholesterol, LDL-C, VLDL-C and triacylglycerol remained significantly higher than normal control values. The maximum lowering of these lipids was observed with combined vitamin C and E treatment which indicates that these vitamins specifically targeted the mechanisms involved in diabetes –induced dyslipidemia as evidenced by the strong inverse (–ve) relationship between vitamin C and total cholesterol observed in Dc group. This favorable effect of combined vitamin C and E cannot be attributed merely to improved glycemic control because glibenclamide induced better glycemic control than combined vitamin C and E therapy. Vitamin C was reported to enhance cholesterol excretion as bile salts [60] and vitamin E was reported to stimulate lipoprotein lipase activity in STZ diabetic rats [61].

Our results demonstrated increased plasma and liver lipid peroxides levels in all diabetic groups, a finding previously reported in several studies [62, 54, and 63]. Elevated levels of lipid peroxides in plasma could be the consequence of increased production and liberation into the circulation of tissue lipid peroxides as indicated by the significantly elevated liver peroxides [64]. The significant inverse (-ve) correlation between vitamin C and MDA explain increased lipid peroxides in diabetic state [65]. Moreover, the significant positive correlation between HbA_{1C} and MDA indicates that long term hyperglycemia was implicated in enhancing lipid peroxidation by generating free radicals, hydrogen peroxide, and reactive ketoaldehydes by the auto-oxidation of glucose or from glycated proteins as reported by [66]. Vitamin therapy was more superior to glibenclamide as regards normalization of serum and liver peroxides and the best result was observed when vitamin C and E were combined. This favourable effect of vitamins can be partially attributed to their hypoglycaemic, hypolipidemic as well as their scavenging potential, thus interrupting the interlacking mechanisms involved in production of lipid peroxides at multiple points. Vitamin E is a lipid soluble chain-breaking antioxidant that protects LDL particles from oxidative attack (LDL particles are small and dense in type II diabetes, which render it susceptible to oxidation) [5]. Vitamin C is required for regeneration of α-tocopherol and may thus prevent LDL oxidation [67]. Enhanced lipid peroxidation is a risk factor for atherosclerosisas as shown by the significant increase in atherogenic index in all diabetic groups compared to normal controls and its significant decrease in all treated diabetics. Combined vitamin C and E therapy supervened glibenclamide as regards antiatherogenic potential despite a significantly elevated blood glucose level.

Our results demonstrated decreased plasma TAC in diabetic groups than controls which agree with [58, 59] who found the same with diabetic NIDDM patients. A significant, inverse correlation between TAC and fasting plasma glucose was found which agree with [68] ($P < 0.01$). Treated diabetic groups showed a significant increase in TAC which agree with [69] who found

that treating Type II diabetic patients with Gliclazide (a sulfonylurea derivative) for 12 weeks increased plasmatic TAC.

CONCLUSION

The present study demonstrated that combined vitamins C and E therapy was the most effective in ameliorating diabetes-induced dyslipidemia and oxidative stress. However, it was not as effective as glibenclamide as regards glycemic control. Combined vitamin C and E could be of value for those patients who suffer from poor glycemic control to confer them protection against atherosclerosis. These findings could be intriguing to investigate the outcome of using higher doses of vitamin C and E for longer durations or combining them with glibenclamide as an optimum regimen for treatment of type II diabetes mellitus.

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