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Evaluation of anti-diabetic and antioxidant properties of vitamin E and vitamin C in alloxan induced diabetic rats

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Abstract

Diabetes is a burning health issue worldwide. Hyperglycemia is the characteristic feature of diabetes and this elevated blood glucose caused oxidative stress. Vitamin E and C are well known because of their anti-oxidant properties and only few studies have been done on the antidiabetic properties of Vitamin E and C, so we selected these two vitamins to evaluate the anti-diabetic and anti-oxidative properties. A single dose of alloxan (120mg/kg-BW) in over fasting rats was used as a diabetogenic agent and rats showed blood glucose level higher than 250mg/dl were considered as diabetic. Rats were divided into three groups (08 rats/group). Group A contain diabetic rats, Group B contain diabetic rats treated with vitamin E (600mg/kg BW) and group C contain diabetic rats (1000 mg/kg BW). The duration of trial was 21 days. We found a significant increase in the body weight at day 21 in both treated groups as compared to day 1. A significant reduction in fasting serum glucose was found for vitamin E (210.6±8.48) and C (187±4.18). A significant reduction, in total cholesterol (248.64±14.14 for vitamin E and 210.6±21.80 for vitamin C) and LDL (44.63±8.87 vitamin E; 73.34±6.99 vitamin C) was found. However, significant increased for HDL was found in vitamin E (40.4±5.58) group. We also found great anti-oxidant properties of both these vitamins by studying catalase (CAT) enzyme and found a significant (18.18±0.17) decrease for CAT in group B and (18.92±0.23) in group C. In conclusion, both vitamin E and vitamin C showed good anti-hyperglycemic, anti-hypercholesterolemic and anti-oxidative properties.

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Introduction

Diabetes mellitus is metabolic disorder, causes cardiovascular disorders, heart stroke, myocardial infarction, oxidative stress and hyperglycemia etc and is the leading cause of death worldwide (Jonathan *et al.,* 2017;Takeuchi *et al.*, 2018). Hyperglycemia in diabetes is associated with increased production of reactive oxygen species (ROS): a major cause of imbalance oxidants and antioxidants entities inside the body (Lin *et al.*, 2008; Baynes *et al.*, 1991), thus body leads into oxidative stress. Basically, oxidants are made inside the body, in reaction of aerobic metabolic process but their amount can be exceeded in many pathological and physiological conditions (Sies, 1993; Fang *et al.*, 2002). Fatal and non-fatal cardiovascular risk increased when the blood postprandial hyperglycemia is associated with postprandial hyperlipemia. Thus higher the blood glucose concentration would cause the reduction of anti-oxidant status of the body (Erlinger and Brancati, 2001; Gagliardino, 2005). Chronic-hyperglycemia is the leading reason of β cell dysfunctioning /β-cell apoptosis thence insulin level in blood begins to decrease in type II diabetic state (Ibrahim *et al.*, 2008; Chen *et al.*, 2012).

Body has manly two types of anti-oxidant systems i.e. endogenous defenses includes catalase (CAT), Melanoaldehyde (MDA), glutathione peroxidase (GPX) and superoxide dismutase (SOD) and exogenous originating from diets includes Vitamins B, C, E etc. to scavenge the ROS (Andersen and Markham, 2006). Whereas, this anti-oxidant defense system of the body fails to meet the protection demand if the level of ROS increases as in many disorders, such as diabetes mellitus. Many clinical studies were conducted previously on the anti-oxidative properties of vitamin C and E (Jain and Levine, 1995; Davies., 1995; Lonn *et al.*, 2001). Purpose of this study was to observe whether vitamins C and E are good source to decrease the high levels of glucose in diabetes mellitus and where they have any role to decreasing the high lipid levels by its antioxidant action. The data on these aspects of vitamin C and E is not very rich.

Materials and methods

Experimental design

30 adult male-wister rats (150-180g) were taken from UHS (university of health sciences) and were kept in the environmental control temperature (Amin *et al.*, 2011) in the shed of UVAS (university of veterinary and animal sciences Lahore). Free access for standard chow feed and water was given to all rats. Alloxan monohydrate dissolved in 0.5ml saline solution was used as a diabetogenic agent (120-130 mg/kg body weight) in over-night fasting rats (Ebuehi *et al.*, 2010). The animals showing blood glucose levels greater than 250mg/dl were included in the trial (Shankar *et al.*, 2005) and the supplementation of vitamin C and E (Merck) was started.

Animal grouping

Rats were divided into three groups (08 rats/group)

Group A: This group includes rats surfing with diabetic rats.

Group B: This group includes diabetic rats and giving supplementation of vitamin E at the dose of 600 mg/kg BW.

Group C: The rats included in this group were also diabetic but treated with vitamin C at the dose of 1000 mg/kg BW.

The duration of experiment was 21 days.

Blood collection and biochemical parameters

At the end of experimental trial the blood was collected from the heart of each rat, centrifuged at 3000 rpm for 10 minutes and the clear serum was collected for further biochemical parameters. Fasting serum glucose (FSG), total cholesterol (TC), lipid profile (VLDL, LDL, HDL), triglyceride (TG) and Catalase (CAT) were determined using commercially available kits.

Statistical analysis

Data was expressed as Mean ± SD. ANOVA was applied followed by Duncan Multiple Range Testing

by using SPSS (statistical package for social sciences, SPSS Inc., Chicago, IL, USA).

Results and discussion

Diabetes is a leading metabolic disorder of fat, carbohydrate and protein (Amin *et al.*, 2011), affecting approximately 3% of the total population globally. Type-II diabetes is characterized by insulin resistance, pancreatic β-cell dysfunction, hyperinsulinemia, hyperglycemia, dyslipidemia and inflammation (Wild *et al.*, 2004). Scientific literature provides very rich experimental data for patho-physiological relationship between diabetes and its associated disorders. Because the pharmacological drugs have certain limitation and it also have adverse effects on health so, the interest in natural remedies and food that contain vitamins/or anti-oxidant properties has been increased in the last few decades.

Table 1. Effect of Vitamin E (600 mg/KG) and Vitamin C (1000 mg/kg) on FSG, serum lipid concentration (TC, VLDL-C, LDL-C, HDL-C, TG and VLDL-TG) and oxidative Stress (CAT) in alloxan-induced diabetic rats (n=08).

Parameters	Group A	Group B	Group C
FSG (mg/dl)	362.33±29.19	210.6±8.48	187±4.18
TC (mg/dl)	331.66±22.45	248.64±14.14	210.6±21.80
VLDL-C (mg/dl)	20.4±0.61	21.68±2.81	20.00±3.99
LDL-C (mg/dl)	163.26±8.94	44.63±8.87	73.34±6.99
HDL-C (mg/dl)	21.00±1.52	40.4±5.58	37.00±13.52
TG (mg/dl)	198.66±5.81	196.78±2.13	196.4±2.15
CAT (KU/L)	21..43±0.53	18.18±0.17	18.92±0.23

Data is presented as Mean ± S.E.M. Significant difference between the groups in a row at $P < 0.05$. Group A: Diabetic rats, Group B: vitamin E (600 mg/kg/BW) treated rats and Group C Vitamin C (1000 mg/kg/BW) treated rats.

FSG (Fasting Serum Glucose), TC (Total Cholesterol), VLDL-C (Very Low Density Lipoprotein-Cholesterol), LDL-C (Low Density Lipoprotein-Cholesterol), HDL (High Density Lipoprotein-Cholesterol), TG (Total triglyceride), CAT (Catalase).

Effect of vitamin E and C on Growth Performance

Rats were weighed on the day 1 and then at the end of trial. Our data showed a significant increase in the body weight ($p<0.05$) in Vitamin E and Vitamin C treated groups as compared to diabetic group (Fig. 1). The results are not in agreement with other might be due do difference in dose (Vajro *et al.*, 2004).

Effect of vitamin E and C on Serum glucose concentration

We found a significant reduction ($p<0.05$) for serum glucose level in both treated groups however, our data showed more significant reduction in vitamin C treated group as compared to Vitamin E (Table1). Vitamin E found to reduce free oxygen species that commonly found to be increased in diabetes (Weykamp *et al.*, 1995). The results for vitamin C (Ness *et al.*, 1999) and Vitamin E (Jain and Levine, 1995) are agreed with other. It is studies by many researchers that people who take enough Vitamin C in their diets are more resistant to diabetes as compared to the people who take less Vitamin C (Collier *et al.*, 1990; Will and Byers, 1996; Will *et al.*, 1999). Vitamin C has a significant role to enhance insulin secretion from pancreatic β- cells and thus have important role to reduce blood glucose (Wells *et al.*, 1995; Dou *et al.*, 1997). Thus by increasing the use of vitamins in diet showed positive influence on the blood glucose level.

Effect of vitamin E and C on Lipid profile

The total cholesterol found to be significant ($p<0.05$) decrease in both treated groups which previously found to be increased in diabetic condition (Table 1). Furthermore, no significant reduction was found for VLDL, but we found a significant ($p<0.05$) reduction

for LDL in both treated groups. For HDL, a non-significant increase was reported in vitamin E treated group. No significant change was found for triglyceride in any of the treated group.

Since, vitamin E is fat-soluble vitamin and have key role in preventing damage to the lipids by the oxygen free radicals. When this highly-reactive species attack the lipids, these oxygen free radicals; set off the chain reaction of lipid per oxidation.

Vitamin E breaks this chain reaction, e.g. it acts as a chain breaking inhibitor of lipid per oxidation (Jain *et al.*, 1995).

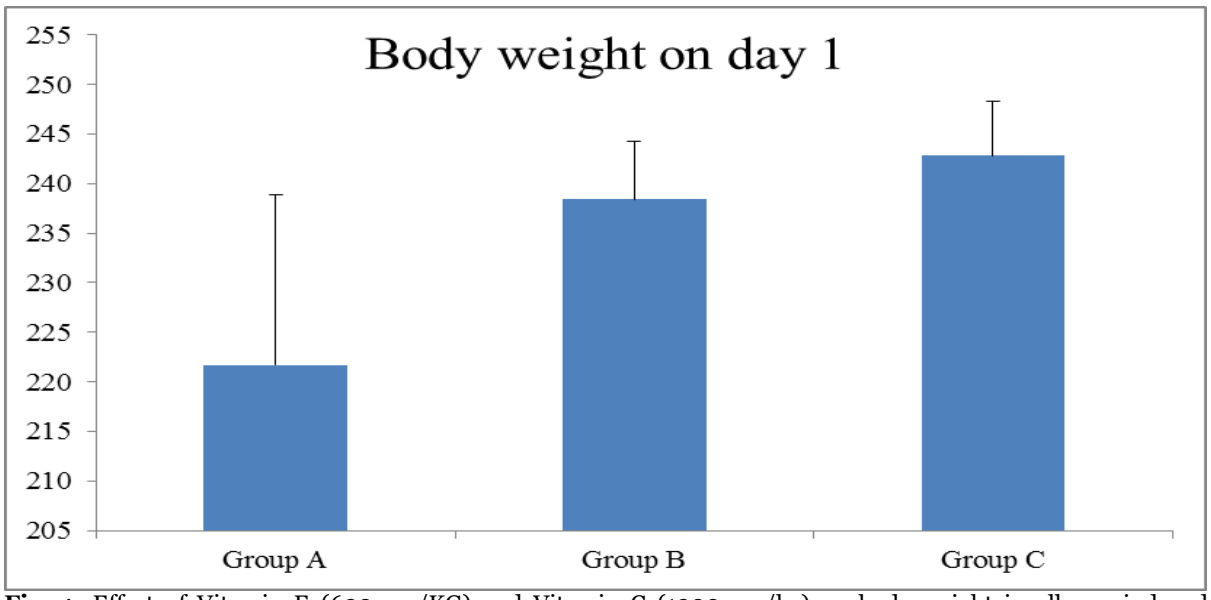

Fig. 1. Effect of Vitamin E (600 mg/KG) and Vitamin C (1000 mg/kg) on body weight in alloxan-induced diabetic rats (n=08) on day 1. Group A: Diabetic rats, Group B: vitamin E (600 mg/kg/BW) treated rats and Group C Vitamin C (1000 mg/kg/BW) treated rats.

As lipids are hydrophobic, so a complex mechanism of lipoproteins is involved to transport it into the cells. These lipoproteins are amphiphilic in nature, the hydrophobic core of lipoprotein contains cholesterol esters and triglycerides and peripheral envelope is made up of protein (apolipoproteins), cholesterol and phospholipids. There are three important lipoproteins found in the body i.e. very low density lipoproteins (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). The increase in the density of the lipoproteins is an effect of depletion of triglycerides alone with enrichment of cholesterol. Dysregulation of cholesterol packaging and elevated blood TG level commonly occurs in diabetic patients (Frunhbeck *et al.*, 2001; Gillian *et al.*, 2002). It is well documented fact that dyslipidemia (high TG and LDL and Low HDL) is the leading factor of cardiovascular disorder and arterial blockage (Witzum, 1994; Benzi and Morretti, 1995). Insulin resistance enhances lipolysis and free fatty acid concentration in the blood; a leading causes of hypercholesterolemic and hypertriglyceridemia in diabetes (Naseem *et al.*, 2016).

Effect of vitamin E and C on antioxidant status

Hyperglycemia causes inefficiency of glycosylation for anti-oxidant enzymes (Lalla *et al.*, 2000). Our data represented a significant decreased for catalase in vitamin E and vitamin C treated groups (Table 1), this showed antioxidant properties of vitamin E and C.

Vitamin E and C is known to be an antioxidant in its action so it ameliorate the insulin sensitivity as the action of insulin is impaired in oxidative stress. Might be due to the measurement error in dietary intake assessment, no such association was found between vitamin E and C in diet during population based study (Vajro *et al.*, 2004; Akindele *et al.*, 2014).

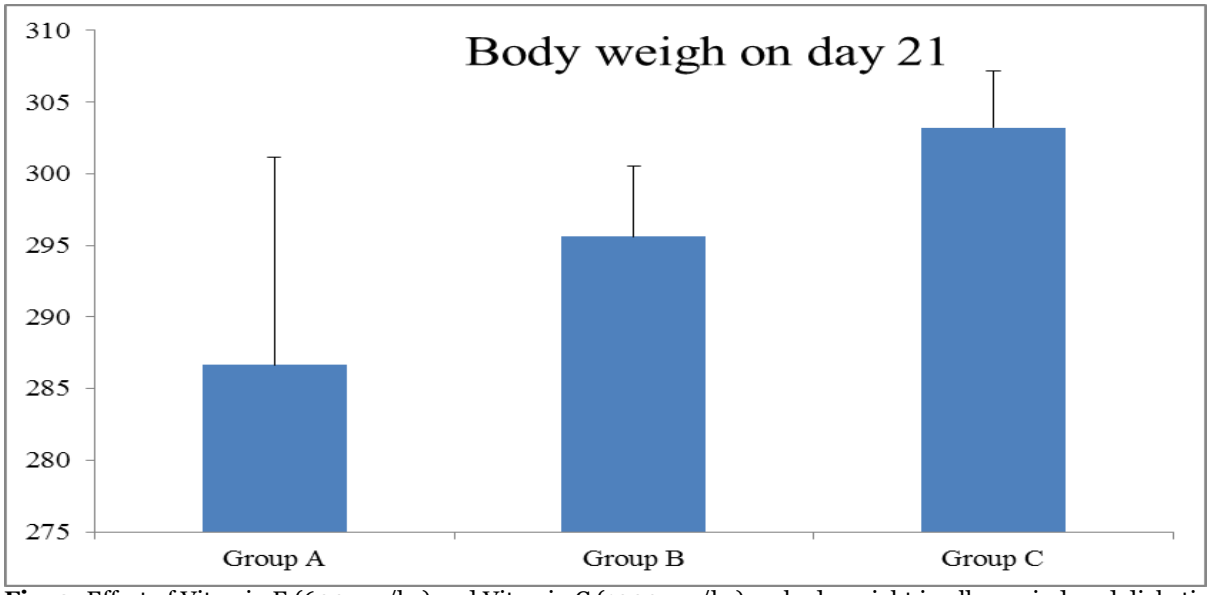

Fig. 2. Effect of Vitamin E (600 mg/kg) and Vitamin C (1000 mg/kg) on body weight in alloxan-induced diabetic rats (n=08) on day 21. Group A: Diabetic rats, Group B: vitamin E (600 mg/kg/BW) treated rats and Group C Vitamin C (1000 mg/kg/BW) treated rats.

The cellular damage due to increased ROS is regulated by complex cascade reaction of antioxidant defense system of the body (Rahimi *et al.,* 2005). In addition, certain antioxidants are obtained from the diet. An imbalance which results from an increased production and/or the reduced scavenging of these free radicals leads to a metabolic state of oxidative stress, which consequently leads to tissue damage. Auto glycosylation reactions, alterations in the sorbitol pathway and hyperglycemia have been proposed as some of the mechanisms which are responsible for this increased oxidative stress (Bambolkar and Sainani, 1995).

Conclusion

In conclusion we found that both vitamin E and C showed good hypoglycemic, anti-hypercholesterolemic and anti-oxidant properties, however it also showed some anti-hyperlipidemic activities as well. So, by increasing the intake of these vitamins in the diet would be helpful to overcome diabetic issues specially the oxidative stress.

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