The effects of antioxidants supplementation on haemostatic parameters and lipid profiles in diabetic rats

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Abstract: Diabetes mellitus is a complex, progressive disease, which is accompanied by multiple cardiovascular complications. Oxidative stress is significantly increased in diabetic patients and may lead to great haemostatic disturbances existing in these patients. Antioxidants have been reported to reduce oxidative and haemostatic variables by arresting free radical damage .The aim of this study was to assess the role of antioxidants (vitamin E and C) in modulation of the haemostatic parameters and lipid profiles in experimentally-induced diabetic rats. Blood samples are obtained from control rats (no=24) and diabetic rats (no=24) to estimate haemostatic status by platelets aggregation, fibrinogen levels and prothrombin time. Oxidative status was assessed by estimation of the lipid profiles {Triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) cholesterol} and plasma uric acid. Diabetics rats were divided into two sub-groups. The first sub-group (no=12) was orally supplemented with Vitamin E (7mg/rat) daily for 4 weeks and the second sub-group (no=12) was co-administrated Vitamin C (7mg/rat) and Vitamin E daily for 4 weeks. Blood samples are withdrawn from the two sub-groups and the previous parameters were assessed. .Increased levels of TG and LDL cholesterol and reduced levels of HDL cholesterol and plasma uric acid were recorded in the rats after induction of diabetes, compared to prediabetic values. Hypercoagulability state was observed in diabetic rats through percentage increase in platelet aggregation and fibrinogen level. Oral supplementation of Vitamin E to diabetic rats resulted in a significant inhibitory effect on the oxidative stress and partial reduction of the hypercoagulability state, which were more observed by co-administration of vitamin C. It is concluded that hyperglycemia in rats increased oxidative stress which may play a role in induction of hypercoagulable state. Dietary co-administration of vitamin E and C induced protective effects to diabetic rats.

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Key words: Diabetes, oxidative stress ,lipid profile , platelet aggregation, antioxidants.

Introduction:

According to the International diabetes Federation , percent of diabetes in EGYPT was 11.4% in the year 2010 and this likely to increase to 13.7 % by the year 2030 (IDF 2009).Diabetes mellitus is a complex, progressive disease, which is accompanied by multiple complications. It has been recognized as the sole independent risk factor for the development of cardiovascular disease (Jay et al., 2006).

Cardiovascular complications include stroke, myocardial infarction and atherosclerosis which are considered to be important causes of health-related deaths in diabetic patients .Lipid abnormalities in type-2 diabetes are characterized by high triglyceride concentrations, low high density lipoprotein-cholesterol concentrations and normal total and low density lipoprotein-cholesterol concentrations (Valabhji and ElkelesL,2003). Premature atherosclerosis and other vascular disorders are serious complications of diabetes mellitus due to increased peroxidation of LDL leading to foam cell formation, fatty streaks and plaque formation in the arterial wall, and hyper-reactivity of blood platelets leading to increased platelet adhesion and aggregation. Altered platelet morphology and function have been reported in patients with metabolic syndrome, stroke and diabetes mellitus ((Ferreiro et al.,2010).Mean platelet volume is an independent risk factor for atherothrombosis and cardiovascular disease. Many studies have shown that increased mean platelet volume is one of the risk factors for myocardial infarction, cerebral ischemia and transient ischemic attacks (Chu et al., 2010). Oxidative stress is significantly increased in diabetes mellitus, due to prolonged exposure to hyperglycemia, disturbances in capacities of the antioxidant defense system including uric acid, scavenging enzymes such as superoxide dismutase and glutathione reductase, and deficiencies of antioxidants such as vitamin C and E (Abou-Seif and Youssef, 2004). Uric acid is the most important

circulating antioxidant contributing to about 50% of the total radical trapping antioxidant capacity while other antioxidants such as bilirubin, vitamin E , vitamin C and glutathione contribute to the other 50% (Ceriello et al., 1997). Antioxidants have been reported to reduce the complications in DM by arresting free radical damage. This suggests that treatment with vitamins C and E could ameliorate the oxidative stress caused by hyperglycaemia (Kukner et al., 2009).

Thus, this study was aimed to assess the existence of oxidative stress in diabetic rats by estimating the lipid profile (LDL cholesterol, HDL cholesterol and triglycerides) and plasma uric acid, which is one of the major counteracting antioxidant markers in the circulation. And since vitamins E and C were potent antioxidants nutrients, so this work was performed to evaluate their role in inhibiting diabetic LDL oxidation and to verify whether prevention of free radical might have a role in modifying hemostatic variables in diabetes.

Materials and Methods

Experimental Animals:

Sprague Dawley male rats (n=50) were used in this study, weighing 150-200 gm., obtained from the animal house of National Organization of Drug Control and Research (NODCAR). Animals were kept under standard conditions and allowed free access to food and water. The standard guidelines of NODCAR were used in handling the experimental animals.

Methods:

1) Blood samples were obtained retro-orbitally from the control group (24 rats) to assess the following parameters:
a) Lipid and lipoprotein profile: Triglycerides, LDL cholesterol and HDL cholesterol.
b)Markers of hypercoagulability: Platelets aggregation, fibrinogen level and prothrombin time
c) Marker of antioxidant status: Serum uric acid.
2) Diabetes induction :
Diabetes was induced in rats by using Alloxan® (Alloxan tetrahydrate-Sigma) in a dose of 150 mg/kg for each rat (Chetan et al.,2005).The drug was freshly dissolved in distilled water and was given by intraperitoneal route in two divided doses 75mg/kg each, preceded by 18 hours fasting period. Diabetes was confirmed by the development of hyperglycemia within 36 hours of drug administration using commercially available calorimetric kits.
3) Blood samples were collected retro-orbitally from the diabetic rats and assayed for the lipid profile. Platelets aggregation, fibrinogen, prothrombin time and uric acid were also assessed and compared to control values.
4)The diabetic rats were then divided into two groups
Group 1 (no.=12): rats received vitamin E, 7mg /rat daily, orally by gavage, for 3 weeks.
Group 2 (no.=12): rats received vitamin C,7mg /rat daily, orally by gavage , in addition to vitamin E for 3 weeks (Maxwell et al., 1993)
5) At the end of 3 weeks, blood samples were collected retro-orbitally from each group and assayed for the same previous parameters.

Preparation of blood samples:

Citrated plasma was prepared by centrifugation of amixture of nine volumes of freshly drawn blood with one volume of trisodium citrate (0.11mol/L) for 30 min. (1600g) then frozen at -80° until assayed.

Chemical Analysis:

Triglycerides concentration in serum were measured using enzyme colorimetric methods (Fossati and Prencipe 1989) .The high-density lipoprotein cholesterol (HDL-C) concentration was measured using the method described by Warnick et al.(1982). Low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the method of Bergmenyer (1985). Uric acid was measured using an enzymatic method (Trivedi et al., 1978).
Spectrophotometer (UV-120-02) was utilized. Commercially available kits (Boehringer Mannheim, Germany) were used.

-Assessment of Platelet Aggregation :

Blood was prepared and used immediately for the assay of platelet aggregation .Collagen produced by chrono-par was used as an agonist for platelet aggregation in a dose of 4 ul/ml blood sample. Platelet aggregation was assayed at 37° C using a platelet aggregometer coultronics (450 dual channel aggregometer) and 540 dual channel recorded. The whole blood impedance method was the technique used during the study. The change in resistance is recorded on a linear strip chart recorder and calibrated as percentage of extent of Platelet aggregation (Cardinal and Flower, 1980).

Assessment of blood coagulation :

Coagulation assays were carried out in ACL 200, a nephlometric centrifugal analyser which measure the intensity of light, scattered by a plasma sample before, during and after clot formation. The increase in light scatter signal at the beginning of clot formation is related to prothrombin time (PT) while the delta scatter reached at equilibrium is proportional to fibrin and therefore to total clottable

fibrinogen (FIB) (Rossi et al., 1988). PT and FIB were determined by IL test TM PT=FIB (97567) kit.

N.B.: Assessment of Platelet Aggregation and blood coagulation were performed in the Clinical and Chemical Pathology Department of Faculty of Medicine, Cairo University.

Statistical Analysis:

Data were processed and statistics were carried out using the student "t" test for paired and unpaired comparison. Correlations were evaluated with the Spearman rank order correlation. P<0.05 was considered significant.

RESULTS

Effects of diabetes on Oxidative and Antioxidative Markers:

Table1, Fig.1 showed that diabetic rats had significantly (p<0.05) higher levels of TG and LDL cholesterol in comparison to control rats. Meanwhile a significant (p<0.05) lower levels of HDL cholesterol and plasma uric acid (Fig.2) had occurred in diabetic rats.

Effects of diabetes on hemostatic parameters:

Table2, Fig.3 showed that diabetes caused significant (p<0.05) higher percentage of platelets aggregation, higher levels of fibrinogen and shorter prothrombin time compared to control values.

Effects of antioxidant vitamins supplementation on diabetic induced changes:

Table (1) showed that supplementation of vitamin E for 3 weeks improved the picture of oxidative stress in diabetic rats as evidenced by a significant (p<0.05) reduction in triglycerides and in LDL cholesterol, and significant increase in HDL cholesterol and in uric acid levels compared to the control diabetic values. Moreover, vitamin E partially reduced the diabetic-induced hypercoagulability state as evidenced by reduction of platelet aggregation and fibrinogen levels accompanied by prolongation in prothrombin time Table (2). By co-administration of vitamin C and vitamin E significant improvement had occurred as evidenced by significant (p<0.05) decrease in platelet aggregation and fibrinogen levels, and prolongation of prothrombin time compared to diabetic levels.

Table (1): Effect of vitamin supplementation on diabetic induced changes in lipid profile {triglycerides (TG), LDL cholesterol, HDL cholesterol} and plasma uric acid. (Mean± S.D)

Parameters	Control rats (n=24)	Diabetic rats (n=24)	Diabetic rats +Vitamin supplementation	
			Vit E (n=12)	Vit E+C (n=12)
TG(mg/dl)	65.3 ±5.2	124± 6.2 *	85± 4*º	81± 8*º
LDL Cholesterol (mg/dl)	118 ±7	134± 2.2 *	123± 7 º	120± 8.6º
HDL Cholesterol (mg/dl)	35.3 ± 3.5	28.2± 3*	31± 3*	32± 2.5*º
Uric acid(mg/dl)	3.7 ± 0.5	3.1± 0.3*	3.5±0.2º	3.6± 0.5º

*Significant difference between control prediabetic and diabetic values (p<0.05).
º Significant difference between vitamin supplemented and non-supplemented diabetic values (p<0.05).

Table (2): Effect of vitamin supplementation on diabetic induced changes in hemostatic parameters {platelets aggregation, fibrinogen (FIB) and prothrombin time(PT)}.(Mean± S.D.) .

Parameters	Control rats (n=24)	Diabetic rats (n=24)	Diabetic rats + Vitamin supplementation	
			Vit E (n=12)	Vit E+C (n=12)
Platelet aggregation (%)	70.3±8.2	86 ± 7 *	81 ± 6.4*	78 ± 7 º
FIB (mg/dl)	185.2±14.1	222 ± 13*	203 ± 10* º	200 ± 9* º
PT (sec)	13.5±1.2	11.4 ± 1.1*	11.7 ± 1.5*	11.9 ± 1.6*

*Significant difference between control prediabetic and diabetic values (p<0.05).
º Significant difference between vitamin supplemented and non- supplemented diabetic values (p<0.05).

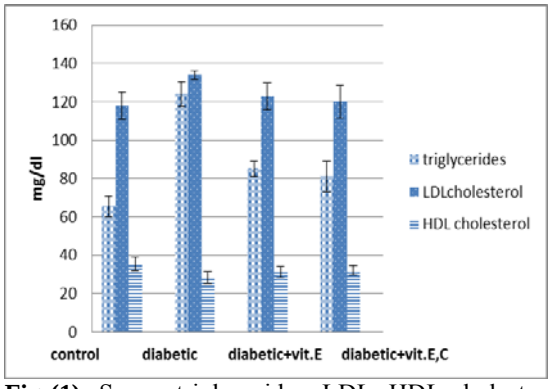

Fig (1): Serum triglycerides, LDL, HDL cholesterol levels in different experimental groups as compared to control.

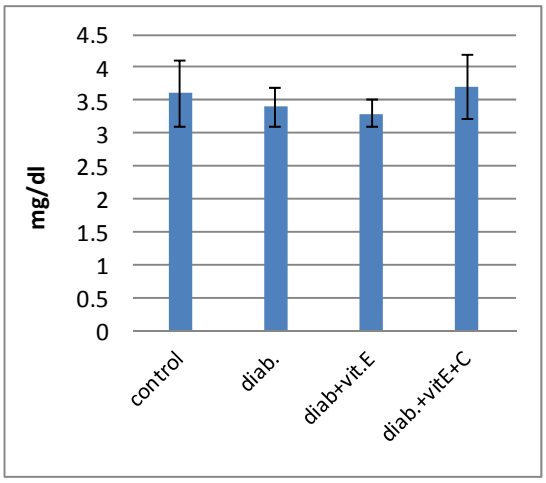

Fig (2): Serum uric acid level in different experimental groups as compared to control.

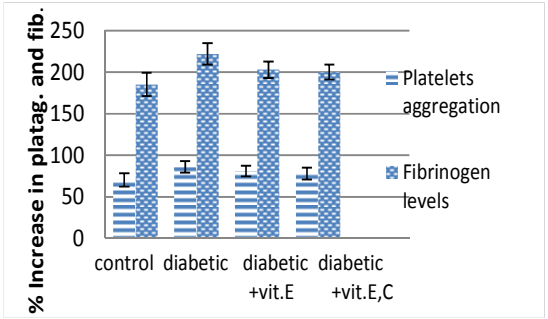

Fig (3): Percentage increase in platelets aggregation and fibrinogen levels in the different experimental groups as compared to control.

Discussion

Hyperglycemia has been accepted as an essential factor in the development of diabetic complications (Rosen et al., 2001). Oxidative stress plays an important role in the development of diabetes complications, both microvascular and cardiovascular (Jay et al., 2006). The results of this study supported the relationship of poor glycemic control and higher risk of cardiovascular complications. Administration of alloxan to rats destroyed pancreatic β-cells, leading to inhibited insulin secretion, thus increasing plasma glucose levels. The present study demonstrated that in diabetes, increased production of triglycerides and LDL cholesterol occurred in association with reduced levels of HDL cholesterol and plasma uric acid. This was in accordance with Budin et al., (2009) who studied that both lipid accumulation particularly triglycerides and reduction in antioxidant activity contributed to the development of oxidative stress in diabetes. Hyperglycemia was found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals (Giacco and Brownlee, 2010) .It appears that hyperglycemia could be a driving force for induction of oxidative stress, enhanced leucocyte endothelial interaction and glycosylation of virtually every protein in the body, including lipoproteins and clotting factors (Al-Rawi 2011). Vitamins E and C play an important role in glucose metabolism (Martini et al., 2010). Reduced levels of antioxidants such as ascorbic acid and vitamin E occurred in diabetes (Ceriello et al.,1998). In this study, oral administration of vitamins E and C for 3 weeks improved the picture of oxidative stress in diabetic rats and reduced the diabetic-induced hypercoagulability. Our results were consistent with Rahimia et al., (2005) who indicated that the use of antioxidants reduces oxidative stress in diabetes.

Both vitamin C and vitamin E decreased lipid peroxidation and augmented the activities of antioxidant enzymes in diabetic rats (Kędziora-Kornatowskaa et al., 2003).Vitamin C was found to significantly decrease the elevated levels of blood hydroperoxide, glucose, cholesterol, triglycerides and low-density lipoprotein (LDL) in diabetic rats (Badr et al., 2011). Several studies in diabetic patients, have shown that supplementation with several hundred IU of vitamin E significantly reduced platelet aggregation and lipid peroxidation, so vitamin E may have a therapeutic role in free radical mediated diseases (Gerster;1993). On the other hand, however, a recent study by de Oliveira et al.,(2011), suggested that vitamin E supplementation alone, did not affect the lipid profile of type 2 diabetic patients. In diabetics, platelet vitamin E levels tend to be reduced with concomitant increase in platelet aggregation, this was reversed by correction of the vitamin E status (Garg et al., 2005). In addition, Vitamin E may play a protective role as membrane

stabilizing agent. In platelets it appears to regulate arachidonic acid metabolism (Pazdro and Burgess , 2010). In this study, Uric acid level was significantly reduced in diabetic rats .Uric acid is the end product of purine metabolism; it can act as a pro-oxidant, particularly at increased concentration and may thus be a marker of oxidative stress.Type-1diabetics showed significantly lower serum uric acid levels in comparison to type-2 diabetics. Elevated serum uric acid occurred in type 2 diabetics may lead to significantly higher incidences of coronary artery disease, carotid atherosclerosis, cerebral infarction, diabetic nephropathy and diabetic retinopathy level (Wu et al., 2011). It was observed that uric acid may act as an antioxidant both by binding iron ions and also by directly scavenging reactive oxygen species. In addition , uric acid adds to the enhanced antioxidant profile by preventing ascorbate oxidation and lipid peroxidation (Waugh; 2008).In this study, vitamin E and C supplementation significantly increased the level of uric acid in diabetic rats. Opara; (2002) recommended, that high doses of micronutrient antioxidant vitamins should be administered in combination rather than as single supplement because micronutrient antioxidants interact with each other in a biochemical chain of defence against free radicals. Zhi et al., (2006) proved that there are synergistic antioxidative effects among the antioxidants. On the other hand, however, vitamin C supplementation in healthy dogs doesn't clearly affect blood level of uric acid (Hesta et al., 2009).

In conclusion, the present study provides evidence that hyperglycemia plays a significant role in induction of a hypercoagulable state. Oxidative stress could be the most important factor in the pathogenesis of diabetic complications. Supplementation of vitamins C and E to diabetic rats might assist endogenous antioxidant capacity and ameliorate the oxidative stress caused by hyperglycemia.

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