EFFECT OF VITAMIN C AND ZINC ON SOME BIOCHEMICAL PARAMETER IN ALLOXAN INDUCED DIABETIC RABBITS

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ABSTRACT

Diabetes mellitus (DM) a disease which is characterized by hyperglycemia, lipoprotein abnormalities and oxidative stress. This study was undertaken to investigate the beneficial effect of vitamin C and Zinc on some biochemical parameter when induced diabetic in rabbits by alloxan. Male rabbits groups divided into four groups: Group I (Negative control) received normal saline orally, alloxan diabetic rabbits : Group II (Positive control) received normal saline orally , while Group III were orally administered 50 mg/kg body weight of vitamin C and group IV were orally administered 100 mg/kg body weight Zinc for 15 days. The results showed that vitamin C administration and zinc significantly ($P< 0.05$) reduced the serum glucose, total cholesterol, triglyceride, ALT and AST concentration, while there were significant changes ($p< 0.05$) was observed on the serum levels of total protein, albumin, globulin and albumin/ globulin ratio in diabetic group treated with vitamin C, Zinc respectively.

INTRODUCTION

Diabetes mellitus is heterogeneous metabolic disorder by hyperglycemia resulting from defective insulin secretion, resulting to insulin action or both (1). Type I diabetes is the consequence of autoimmune mediated destruction of pancreatic β cell leading to insulin deficiency .Type II diabetes is characterized by insulin resistance and relative rather than absolute insulin deficiency(2).

Alloxan is used to induced experimental diabetes by selectively destroying pancreatic β_cell (3). In diabetes mellitus oxidative stress seems mainly due to an increased production of free radicals and or a sharp reduction of antioxidant defenses (4).Antioxidant supplement or food rich in antioxidant may used in reducing oxidative damage by free radicals and oxygen ,and can protect the body cells against lipid

peroxdation (5). Vitamin C (ascorbic acid) is an antioxidant substance soluble in water which keeps most of metal cofactors in reduction status. In physiological conditions it appears in the form of dehydroascorbic acid and can react with amino groups and form schiff bases. Its further oxidation product, called diketogulonic acid shows the same reaction (6) Zinc (Zn) is an essential micronutrient which has an important role in the functioning of hundreds of enzymes (6), in insulin metabolism and acts as an efficient antioxidant (7,8). Zn is considered important mainly because it plays a major role in the stabilization of insulin hexamers and the pancreatic storage of the hormone(9) and it is inefficient antioxidant(10),while oxidative stress is considered to be a main component in initiation and progression of insulin resistance and diabetes (11) .The aim of this study was to evaluating the effect of vitamin C and zinc on blood glucose level, serum cholesterol, serum protein and some liver enzymes in animals induced diabetes.

MATERIAL AND METHODS

Twenty-four healthy male domestic rabbits brought from local market /Basra, weighting (1200-1750) grams. The rabbits kept under observation for week. They were provided with feed and tap water *adlibitum.*

Induction of diabetes mellitus Diabetes mellitus induced according to the method of Al-aumar (12), in eighteen rabbits. The animals were overnight fasting. And injected single dose of alloxan (Fluka Biochemika,UK) at dose 100mg/kg body weight in to marginal ear vein. Each 100mg of alloxan was diluted in 1ml of normal saline .Immediately after alloxan injection 10 ml of 20%glucose was injected to the rabbits in order to overcome sudden decrease blood glucose level.

Experimental design: After ten days of alloxan treatment fasting blood glucose was measured the level greater than 200mg/dl were consider as diabetic (13). Then rabbits were divided in to 4 groups (6 rabbits in each group):

Group I (negative control): normal rabbits were served as control which was treated with 2ml of normal saline orally daily.

Group II (positive control): diabetic rabbits were treated with 2ml of normal saline orally daily.

Group III: diabetic rabbits were treated with100mg/kg vitamin C (Aleppo-Syria) orally daily for 15 days.

Group IV: diabetic rabbits were treated with 50mg/kg zinc (Alhavi-Iran) orally daily for 15 days.

Blood collection: At end of experiment the blood samples were collected from heart by using syringe (3ml) and then blood sample was centrifuged to isolate blood serum to estimate the biochemical measurement.

Biochemical assay: The serum glucose was determined by using commercial kits (GOD-POD Trinder reaction -Plasmatec,UK).The total cholesterol(TC) was determined by using commercial kits(CHOD-PAP/Biolabo reagents ,France). The triglyceride (TG) was determined by using commercial kits (Biolabo reagents, France).While the Alanine aminotransferase (ALT) and Aspartate aminotransferase(AST) were obtained by using the colorimetric method (Biolabo reagents ,France)test kits.

The serum total proteins were determined by the Biuret method using commercial kits (Biolabo reagents, France). While albumin value was obtained by Bromocresol green method (Biolabo shows reagents, France) test kits. The globulin and albumin/globulin ratio were determined to the method of close (14).

Statistical analysis The results were analyzed by using two-way covariance (ANOVA) test. The data were expressed as mean ± stander error. Lest significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) SPSS 2012.

RESULTS AND DISCUSSION

The results of daily oral administration (100mg/kg body weight) of vitamin C dailly on the blood glucose concentration in male rabbits shows significant decreased ($p \leq 0.05$)in diabetic treated group as compared with diabetic group and negative control after 15days of the administration (table 1.).

Table (1): Effect of vitamin C and Zinc on serum glucose, total cholesterol, triglyceride, AST and ALT concentration.

Group / Parameter	Negative Control	Positive Diabetic	Treated with vitamin C	Treated with Zinc
Glucose concentration mg/dl	104.583 ± 3.34469 b	265 ± 17.1602 a	134.783 ± 10.714 b	124.8 ± 7.2087 b
Total cholesterol mg/dl	108.966 ± 1.2854 b	185.956 ± 13.532 a	126.466 ± 4.828 b	121.343 ± 7.518 b
Triglyceride concentration mg/dl	108.233 ±0.9405 c	218.716 ±7.5811 a	129.666 ±8.5466 b	136.33 ±7.6753 b
AST concentration µl/l	33.5 ± 0.90034 c	58.3 ± 1.6 a	39.183 ± 1.7758 b	38.084 ± 1.6951 b
ALT concentration µl/l	50.8433 ± 0.2749 b	77.54 ± 1.1531 a	56.7833 ± 1.99 b	47.866 ± 8.69221 b

*****Values are expressed as mean ± stander error. Small letters denote differences between groups, ($P \leq 0.05$) vs. control.

The results are in agreement with (15, 16) who suggested that vitamin C may be prevents the accumulation of sorbitol intracellularly by inhibiting the aldose reductase enzyme. (17) And (18) noted that Vitamin C an aqueous phase antioxidant has been reported to improve whole body glucose disposal in healthy subjects and in diabetic patients and animals .

Vitamin C play important role in reducing the blood glucose through decreasing the oxidative stress have different ways including their damaging effect on the blood vessels and preventing the lipid peroxidation, their ability to regulate nitric oxide synthase that generates the nitric oxide a potent vasodilator that play a key role in controlling the cardiovascular system. There is an ability to decrease the activity of (NADPH) oxidase and (ROS) production, their ability to regulate the antioxidant enzyme including superoxide dismutase and glutathione and the increased tetrehydrobiopterin an important cofactor of nitric oxide synthase enzyme by preventing its oxidation (19).On the other hand (20) suggest that the vitamin C supplementation in humans and animal regulates

blood glucose due to the decreases of the resistance to insulin and the reduced oxidative damage in the tissue by reducing free radicals and the decreasing of glycosylation to protein.

The results revealed significant decreased ($p \leq 0.05$) in blood glucose concentration in diabetic group treated with zinc 50mg/kg body weight (table1) this result agreement with (21)who showed that the zinc is capable of modulating insulin action and improving hepatic binding of insulin.

The results of diabetic treated group with vitamin C caused a significant decreased ($p \leq 0.05$) in lipid profile (total cholesterol, triglyceride, ALT. and AST were compared with diabetic group (table1.) .These finding are similar to that of (15, 22). The hypercholesterolemia effect of vitamin c that prevents LDL-cholesterol from oxidative damage and aids in degradation of cholesterol (18). Oxidative damage caused by toxic free radicals at early stage. The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may partially prevent certain types of hepatic cellular damage (23, 24). Moreover vitamin C is required for regeneration of α-tocopherol, may do preventing LDL-C oxidation (25).

The statistic analysis demonstrates a significant decreased ($p \leq 0.05$) in lipid profile concentration in the diabetic treated group with Zinc (50mg/kg) for15days (table1). This results consist with(26)who found that in diabetic patients from type II demonstrated when treatment with Zinc resulted to reduction of TC and TG and increase of HDL-C concentrations but did not decrease LDL-C concentration significantly which indicate that its treatment improved the individuals metabolic condition. Zinc plays an important role in the structure and function of biological membranes (27). Zinc has also been shown to have an antioxidant potential through the non-enzymatic stabilization of biomembrane and biostructures. The protective effects of zinc could be attributed to its ability to reduce collagen accumulation in liver and also it exert critical physiological role in regulating the structure and function of cells (28) While (29) concluded that Zinc is required for the adequate Formation and function of the antioxidant enzyme copper zinc superoxide dismutase and various metallothioneins .Moreover metallothionein plays a role in the detoxification of heavy metals and stabilize membrane (30).

Table (2): Effect of vitamin C and Zinc on serum total protein, albumin, globulin, ratio (albumin/ globulin)

Group / Parameter	Negative Control	Positive Diabetic	Treated with vitamin C	Treated with zinc
Total protein g/dl	6.8733 ± 0.6396 a	5.4333 ± 0.1165 c	5.985 ± 0.8992 b	5.82 ± 0.0698 B
Albumin g/dl	3.6983 ± 0.05974 b	3.96 ± 0.8485 a	3.671 ± 0.09821 b	3.6283 ± 0.13313 B
Globulin g/dl	3.1783 ± 0.814 a	1.4733 ± 0.1075 c	2.803 ± 0.19205 b	2.205 ± 0.17316 b
Ratio (Albumin/ Globulin)	1.2202 ± 0.11534 b	2.74 ± 0.2647 a	1.4125 ± 0.2084 b	1.5033 ± 0.15947 B

Values are expressed as mean ± stander error. Small letters denote differences between groups, ($P \leq 0.05$) vs. control.

Table (2) shows the effect of vitamin C and Zinc on total protein, albumin, globulin and albumin /globulin ratio. There were significant increased ($p \leq 0.05$) in the diabetic treated group as compared with the diabetic group. These results may reflect the effect of vitamin C and Zinc by stimulating glucose dependent insulin secretion from the pancreatic beta cells (31).This increase in insulin concentration decrease protein catabolism, amino acid degradation and increase protein synthesis (32). Some of the previous observation has shown that vitamin C can compete with glucose for binding to protein and thereby inhibit glycation of them (33). Also, (34) and (35) reported that oral vitamin C inhibited the glycosylation of proteins. It seems that due to structural similarity to glucose, ascorbic acid can be replaced with glucose and affects the glycation of proteins. Moreover, (36) proposed that the Zinc is an essential trace element is closely involved in general metabolism of lipids, carbohydrates, and proteins.

Also the obtained result revealed increase in serum of total protein, albumin and globulin after Zinc treatment, which may be attributed to importance trace mineral zinc in metabolism of protein and agreed with (37)who is investigated that the total protein levels were decreased in Zinc-supplemented animals compared with control.

تأثير فيتامين سي والزنك على بعض المعايير الكيموحيوية في الأرانب المصابة بداء السكري المستحدث بالالوكسان

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الخلاصة

داء السكري هو مرض يتميز بأرتفاع مستوى سكر الدم و اضطرابات البروتينات الدهنية بالاضافة الاجهاد التأكسدي. اجريت الدراسة الحالية لمعرفة تأثير فيتامين سي والزنك على بعض الاختبارات البيوكميائية في الارانب المستحدثة بداء السكري بواسطة الالوكسان.وقد قسمت ذكور الارانب الى اربعة مجاميع :المجموعة الاولى (سيطرة سالبة) ثم قسمت الارانب المصابة بالسكري الى ثلاث مجاميع ،المجموعة الثانية (سيطرة موجبة)) اعطيت كل منهما المحلول فسلجي عن طريق الفم، المجموعة الثالثة اعطيت فيتامين سي 100ملغم/كم عن طريق الفم، والمجموعة الرابعة واعطيت زنك 50 ملغم/كم من وزن الجسم عن طريق الفم ولمدة 15 يوماً. واظهرت النتائج انخفاض معنوي في كل من مستوى سكر الدم والكوسترول الكلي والدهون الثلاثية وانزيمي الكبد AST و ALT بينما اشارت الدراسة الى زيادة معنوية في بروتينات الدم (بروتين الدم كلي ا و والالبومين والكلوبيولين ونسبة الالبومين /الكلوبيولين) عند مقارنة مجموعة السكري مع مجموعة المعالجة بفيتامين سي والمجموعة المعالجة بزنك.

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