



The effect of C-peptide with and without antioxidant complementary in diabetic

Sufi Rikhoa¹

¹Medical Physiology Departments, Faculty of Medicine, Cairo, England

Abstract: Diabetes mellitus is one of the most common endocrine disorders in all populations causing major health problem with long-term complications responsible for its mortality and morbidity. Oxidative stress has been suggested to be one of the factors in the development of both types of diabetes and its disabling chronic complications. So, in addition to insulin and oral hypoglycemic, it is necessary to deal with diabetes mellitus by a poly-therapy regimen including drugs, diet, exercise and other new lines of treatment required to improve symptoms and preventing future complications. The present work was designed to evaluate the possible effects of c-peptide administration with and without antioxidant supplementation as a new line of treatment of diabetes mellitus in male rats. Fifty adult local strain male albino rats were chosen to be the model of the present study. They were left for two weeks in the laboratory room before any experimental interference for acclimatization with free access to water and rat chow pellet, then they were divided into five equal groups as follow; (Group I) received intraperitoneal saline injection and served as control group, (Group II) were subjected to induction of diabetes by subcutaneous injection of alloxan monohydrate (120mg/kg body weight), (Group III) were subjected to induction of diabetes as group II followed by intraperitoneal injection of C-peptide (50 nmol/kg/day) for four weeks. (Group IV) were subjected to induction of diabetes as group II followed by oral administration of vitamin C (200mg/kg/day) and vitamin E (14.4 IU/kg/day) for four weeks. (Group V) were subjected to induction of diabetes as group II then received C-peptide plus vitamin C and vitamin E for four weeks. Blood samples were withdrawn for determination of blood glucose, insulin, glucagon, total cholesterol (Chol), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and malondialdehyde (MDA) levels. It was noted that treatment of diabetic rats by C-peptide showed a lower levels of glucagon, LDL, glucose, cholesterol, triglycerides and MDA in addition to higher level of insulin level versus diabetic group.

Keywords: Diabetes mellitus. Insulin. Alloxan. C-Peptide. Antioxidants,

1. Introduction

Diabetes mellitus is one of the most common endocrine diseases in all populations. It is a syndrome of disturbed metabolism caused by inadequate insulin secretion, impaired insulin action or both resulting in abnormalities of glucose, protein and lipid metabolism with acute or chronic complications (Franconi *et al.*, 2008 and Alejandro *et al.*, 2011).

Hyperglycemia accelerates lipid oxidation and formation of advanced glycation end products (Sampson *et al.*, 2004). Hyperglycemia also induces a decrease in the antioxidant enzymes levels in the human endothelial cells (Ascan and Thomas, 2005). So, oxidative damage is an integral part of diabetes mellitus and its complication (Ortega, 2012).

Increased lipid peroxidation and reduced antioxidant status may contribute to the development of complications in diabetes (Armstrong *et al.*, 2006). Reduced lipid peroxidation and improved antioxidant status may be one mechanism by which dietary antioxidant supplementation contributes to the avoidance of diabetic complications (Armstrong *et al.*, 2006 and Giacco, 2010).

Proinsulin C-peptide was considered to be without biological activity of its own (Al-Rasheed, 2006). The knowledge that insulin biosynthesis provides the release of equimolar amounts of both insulin and C-peptide into the blood stream has stimulates looking for any significant biological activity related to C-peptide (Marques *et al.*,

2004). C-peptide is considered a reliable marker of residual β -cell function in patients with type I diabetes during the long-lasting process of immune destruction of β -cells which may assist in differentiating type I from type II diabetes (Chailurkit *et al.*, 2007).

Walenciak *et al.* (2007) has reported that C-peptide, although not influencing blood sugar control, might play role in preventing or potentially reversing some of the chronic complications of type I diabetes.

C-peptide might play a role in stabilizing secretory granules (Steiner *et al.*, 2000). Furthermore, C-peptide molecules facilitates its own excision from proinsulin during the maturation into insulin that exposes the COOH terminal part of the insulin's β -chain, allowing the appropriate conformational change for effective interaction with the insulin receptors (Liu *et al.*, 2003).

In type I diabetes, a significant portion of β -cell mass is still preserved and remains functional during the first year of diagnosis. The initial C-peptide level is moderately low in these patients, but it decreases significantly during the second and third year after diagnosis (Picardi *et al.*, 2006).

Antioxidants may improve β -cell function, increase plasma insulin and C-peptide levels, possibly by influencing the antioxidant capacity of the organism and blocking the ability of the immune system to recognize β -cells (Song *et al.*, 2005).

The present work was designed to evaluate the effects of C-peptide with and without antioxidant supplementation in diabetic male rats.

2. Material and Methods

* Animals:

Fifty adult local strain male albino rats weighted 200-250 gm were chosen to be the model of the present study. They were left for two weeks in the laboratory room before any experimental interference for acclimatization with free access to water and rat chow pellets, then they were divided into five equal groups as follow;

1- Control group (group I): rats were received saline by intraperitoneal injection / day for 4 weeks.

2- Diabetes group (group II): the overnight fasted rats were received a single subcutaneous injection of alloxan monohydrate 120 mg/kg of the rat body weight with glucose infusion to avoid fatal hypoglycemia (Maduka *et al.*, 2003).

3- Diabetes with C-peptide (group III): rats were given alloxan as above and C-peptide 50 nmol/kg/day by intraperitoneal injection for 4 weeks (Rebsomen *et al.*, 2006).

4- Diabetes with antioxidants (vitamins C & E) (group IV): rats were received alloxan as above, vitamin C 200 mg/kg/day and 14.4 IU/kg/day of vitamin E in drinking water for 4 weeks (Gokkusu *et al.*, 2001).

5- Diabetes with C-peptide and antioxidants (vitamins C & E) (group V): rats were received combined C-peptide and vitamins C and E in drinking water for 4 weeks with alloxan as above.

* Drugs:

1- Alloxan monohydrate: (2, 4, 5, 6- tetra-oxy pyrimidin, 5,6 dioxuracil) was obtained by Nile pharmaceutical company in a powder form which was dissolved in cold saline and given immediately after preparation to the overnight fasted animals (Kumawat *et al.*, 2010).

2- C-peptide: was obtained from Sigma (Antwerpen, Belgium) in a powder form which was dissolved and diluted in physiological saline before use. It was used in a dose of 50 nmol/kg/day by intraperitoneal injection for 4 weeks (Rebsomen *et al.*, 2006).

3- Vitamin C: was purchased in the form of tablets provided by Memphis Co. for Pharm & Chemical Ind. Cairo. Each tablet contains 500 mg ascorbic acid. The dose was calculated according to the rat's body weight 200 mg/kg/day. The daily dose was dissolved in 0.5ml distilled water and immediately administered orally via intra-esophageal tube (Paget and Barnes, 1964).

4- Vitamin E was purchased in the form of capsules derived from Pharco Pharmaceuticals, Alexandria, Egypt. The dose was calculated according to the rat's body weight as 14.4 IU/kg/day i.e. (14.4 IU/day = 13mg as 1mg= 1.1 IU) dissolved in a 0.5 ml vegetable oil. Every capsule of 100 mg is enough for 7 rats. The solution immediately administered orally via intra-esophageal tube after preparation and put in dark bottle away from air and light (Paget and Barnes, 1964).

* Induction of Diabetes Mellitus:

A single subcutaneous injection (120 mg/ kg body weight) of alloxan with glucose infusion 3 g/kg body weight by gastric intubation to all diabetic rats to overcome fatal hypoglycemia caused by transient hyperinsulinemia after alloxan injection due to destruction of β -cells. The injection was repeated in the 2nd day to obtain response according to Maduka *et al.* (2003).

At the end of the experimental period (4 weeks), food was withheld for 12-13 hours and a large drop of blood from the rats tails were taken to determine the blood glucose level. Rats with blood glucose level equal or higher than 200 mg/dl were considered diabetic.

* Blood Sampling:

Rats were lightly anesthetized by ether and venous blood samples were withdrawn from the retro-orbital plexus by heparinized capillary tubes. Plasma was separated for determination of blood glucose, insulin, glucagon, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and malondialdehyde (MDA) levels.

* Biochemical assay:

1. Blood glucose levels (mg/dl) were determined by enzymatic calorimetric method according to Tietz (1986).
2. Plasma insulin levels ($\mu\text{IU/ml}$) were determined by radioimmunoassay according to Burrin (1994).
3. Plasma glucagon levels (pg/ml) were determined by radioimmunoassay (RIA) according to Saito *et al.* (1979).
4. Plasma levels of total cholesterol were determined according to Allain *et al.* (1974).
5. Plasma levels of high density lipoproteins (HDL) were determined according to Groove (1979).
6. Plasma levels of low density lipoproteins (LDL) were determined according to Friedewald *et al.* (1972).
7. Plasma levels of triglycerides (TG) were determined according to Fossati and Prencipe (1982).
8. Plasma levels of malondialdehyde (MDA) were determined according to Erdelmeier (1997).

* Statistical analysis:

Data input and analysis were done using SPSS computer program. All results were expressed as the mean \pm standard error. Mean values of the different groups were compared using a one way analysis of variance (ANOVA). Least significant difference (LSD) post hoc analysis was used to identify significantly different mean values. *P* value < 0.05 was accepted to denote a significant difference (Bortz *et al.*, 2000).

3. Results

* Effects of induction of DM on the measured parameters (Table: 1 Figures 1-8):

Results of the present study showed that induction of DM led to significant increase ($P < 0.05$) of blood glucose levels from 76.4 ± 6.7 mg/dl to 384.4 ± 31.32 mg/dl, significantly decreased ($P < 0.05$) insulin levels from 30.18 ± 4.77 $\mu\text{IU/ml}$ to 7.28 ± 2.37 $\mu\text{IU/ml}$, significantly decreased ($P < 0.05$) glucagon levels from 76.92 ± 4.16 pg/dl to 70.65 ± 6.18 pg/ml, significantly increased ($P < 0.05$) total cholesterol levels from 96.5 ± 7.01 mg/dl to 131.5 ± 5.54 mg/dl, significantly increased ($P < 0.05$) triglyceride levels from 98.9 ± 9.53 mg/dl to 119.3 ± 10.41 mg/dl, significantly increased ($P < 0.05$) LDL levels from 37.95 ± 3.8 mg/dl to 74.36 ± 3.52 mg/dl, significantly decreased ($P < 0.05$) HDL levels from 39.9 ± 2.38 mg/dl to 33.6 ± 3.06 mg/dl and significantly increased ($P < 0.05$) MDA levels from 6.5 ± 0.85 nmol/ml to 19.4 ± 1.51 nmol/ml.

* Effects of C-peptide and antioxidant supplementation on the measured parameters (table 1, figures 1-8):

Results of the present study showed that C-peptide administration led to significant decrease ($P < 0.05$) of blood glucose levels from 384.4 ± 31.32 mg/dl to 272.2 ± 26.49 mg/dl, significantly increased ($P < 0.05$) insulin levels from 7.28 ± 2.37 $\mu\text{IU/ml}$ to 14.94 ± 0.78 $\mu\text{IU/ml}$, significantly decreased ($P < 0.05$) glucagon levels from 70.65 ± 6.18 pg/ml to 66.77 ± 2.37 pg/dl, significantly decreased ($P < 0.05$) total cholesterol levels from 131.5 ± 5.54 mg/dl to 115.7 ± 9.83 mg/dl, significantly decreased ($P < 0.05$) triglyceride levels from 119.3 ± 10.41 mg/dl to 106.7 ± 7.48 mg/dl, significantly decreased ($P < 0.05$) LDL levels from 74.36 ± 3.52 mg/dl to 60.61 ± 5.78 mg/dl, insignificant change ($P > 0.05$) of HDL levels from 33.6 ± 3.06 mg/dl to 33.5 ± 3.14 and significantly decreased ($P < 0.05$) MDA levels from 19.4 ± 1.51 nmol/ml to 12.7 ± 1.83 nmol/ml.

Results of the present study showed that administration of both vitamin C and E led to insignificant decrease ($P > 0.05$) of blood glucose levels from 384.4 ± 31.32 mg/dl to 356.5 ± 25.2 mg/dl, significantly increased ($P < 0.05$) insulin levels from 7.28 ± 2.37 $\mu\text{IU/ml}$ to 11.23 ± 0.72 $\mu\text{IU/ml}$, insignificant increase ($P < 0.05$) of glucagon levels from 70.65 ± 6.18 pg/ml to 71.94 ± 3.32 pg/dl, significantly decreased ($P < 0.05$) total cholesterol levels from 131.5 ± 5.54 mg/dl to 122.1 ± 8.49 mg/dl, significantly decreased ($P < 0.05$) triglyceride levels from 119.3 ± 10.41 mg/dl to 100 ± 7.16 mg/dl, insignificant decrease ($P > 0.05$) of LDL levels from 74.36 ± 3.52 mg/dl to 72.05 ± 7.25 mg/dl, significantly increased ($P > 0.05$) HDL levels from 33.6 ± 3.06 mg/dl to 36.9 ± 2.38 and significantly decreased ($P < 0.05$) MDA levels from 19.4 ± 1.51 nmol/ml to 12.9 ± 1.45 nmol/ml.

* Table (1): Effects of induction of DM on the measured parameters.

Parameter	Group	Group I	Group II	<i>P</i> value
Bl. glucose (mg/dl)		76.4 ± 6.7	384.4 ± 31.32	$P < 0.05$
Insulin ($\mu\text{IU} / \text{ml}$)		30.18 ± 4.77	7.28 ± 2.37	$P < 0.05$

Glucagon (pg/ ml)	76.92 ± 4.16	70.65 ± 6.18	<i>P</i> < 0.05
Total Cho (mg/dl)	96.5 ± 7.01	131.5 ± 5.54	<i>P</i> < 0.05
TG (mg/dl)	98.9 ± 9.53	119.3 ± 10.41	<i>P</i> < 0.05
LDL (mg/dl)	37.95 ± 3.8	74.36 ± 3.52	<i>P</i> < 0.05
HDL (mg/dl)	39.9 ± 2.38	33.6 ± 3.06	<i>P</i> < 0.05
MDA (nmol/ml)	6.5 ± 0.85	19.4 ± 1.51	<i>P</i> < 0.05

* Table (2): Effects of C-peptide and antioxidant supplementation on the measured parameters.

Groups Parameter	Group II	Group III	Group IV	Group V
Bl. glucose (mg/dl)	384.4 ± 31.32	272.2 ± 26.49	356.5 ± 25.2	264.2 ± 15.63
		<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> < 0.05
Insulin (μIU / ml)	7.28 ± 2.37	14.94 ± 0.78	11.23 ± 0.72	15.42 ± 0.99
		<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05
Glucagon (pg/ ml)	70.65 ± 6.18	66.77 ± 2.37	71.94 ± 3.32	75.42 ± 6.56
		<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> < 0.05
Total Cho (mg/dl)	131.5 ± 5.54	115.7 ± 9.83	122.1 ± 8.49	115.3 ± 5.12
		<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05
TG (mg/dl)	119.3 ± 10.41	106.7 ± 7.48	100 ± 7.16	97.9 ± 7.02
		<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05
LDL (mg/dl)	74.36 ± 3.52	60.61 ± 5.78	72.05 ± 7.25	66.12 ± 4.77
		<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> < 0.05
HDL (mg/dl)	33.6 ± 3.06	33.5 ± 3.14	36.9 ± 2.38	37.2 ± 1.55
		<i>P</i> > 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05
MDA (nmol/ml)	19.4 ± 1.51	12.7 ± 1.83	12.9 ± 1.45	12.9 ± 1.52
		<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05

- * As compared with group I.
- ☐ As compared with group II.

- * As compared with group I.
- ☐ As compared with group II.

- * As compared with group I.
- ▣ As compared with group II.

- * As compared with group I.
- ▣ As compared with group II.

* As compared with group I.
■ As compared with group II.

* As compared with group I.
■ As compared with group II.

* As compared with group I.
■ As compared with group II.

* As compared with group I.
■ As compared with group II.

Results of the present study showed that combined administration of C-peptide and antioxidants led to insignificant decrease ($P > 0.05$) of blood glucose levels from 384.4 ± 31.32 mg/dl to 264.2 ± 15.63 mg/dl, significantly increased ($P < 0.05$) insulin levels from 7.28 ± 2.37 μ IU/ml to 15.42 ± 0.99 μ IU/ml, significant increase ($P < 0.05$) of glucagon levels from 70.65 ± 6.18 pg/ml to 75.42 ± 6.56 pg/dl, significantly decreased ($P < 0.05$) total cholesterol levels from 131.5 ± 5.54 mg/dl to 115.3 ± 5.12 mg/dl, significantly decreased ($P < 0.05$) triglyceride levels from 119.3 ± 10.41 mg/dl to 97.9 ± 7.02 mg/dl, significantly decreased ($P > 0.05$) LDL levels from 74.36 ± 3.52 mg/dl to 66.12 ± 4.77 mg/dl, significantly increased ($P > 0.05$) HDL levels from 33.6 ± 3.06 mg/dl to 37.2 ± 1.55 and significantly decreased ($P < 0.05$) MDA levels from 19.4 ± 1.51 nmol/ml to 12.9 ± 1.52 nmol/ml.

4. Discussion

Diabetes mellitus is a disease of epidemic proportion affecting millions of population. Diabetes is recognized as a major problem worldwide with substantial impact on morbidity and mortality (Khoo *et al.*, 2009).

The present work was designed to evaluate the effects of C-peptide with and without antioxidant supplementation as a new line of treatment of diabetes mellitus in male rats.

In the present work, alloxan was used for induction of diabetes due to its effectiveness and production of irreversible β -cell damage within hours. The mortality rate with alloxan-induced diabetes is high due to severe hypoglycemia occurs as a result of release of insulin from injured β -cells so, glucose infusion was given to avoid this fatal effect (Zobali *et al.*, 2002).

Alloxan induced diabetes led to significant higher level of blood glucose and reduced insulin level. These results are in agreement with Green *et al.* (2004) who mentioned that reactive oxygen species produced by alloxan administration causes breakdown of DNA strands. Such damaged DNA activates nuclear poly-synthetase which depletes the cellular pool of NAD^+ resulting in β -cell damage. Also Bromme *et al.* (2001) stated that β -cell damage induced by alloxan is produced through the effect of noxious oxygen free radicals such as H_2O_2 and MDA. In addition, Eleazer, (2003) reported that giving a single dose of alloxan (120 mg/kg) subcutaneously elevates blood glucose level through selective pancreatic β -cell damage and Eleazer, (2003) elucidated three phases response to alloxan which are immediate hyperglycemia lasting for 2 hours probably due to hepatic glycogenolysis, transient hypoglycemia for about 6 hours which is due to the release of insulin from the damaged β cells and permanent hyperglycemia which is due to insulin deficiency after about 12 hours.

Results of the present work showed that induction of diabetes led also to disturbed lipid profile in the form of higher levels of cholesterol, triglycerides, LDL and MDA associated with lower levels of HDL, insulin and glucagon.

These results are compatible with that of Irshaid, (2012) who revealed that diabetes mellitus lead to elevated plasma levels of cholesterol, triglycerides and LDL while depressing HDL. These effects could be due to initiation of reverse cholesterol transport from cells to the liver for excretion (Parthasarathy *et al.*, 2000). In addition, the plasma LDL-cholesterol levels increase in DM possibly because insulin stimulates LDL receptors (Sampson *et al.*, 2004).

Bromme *et al.* (2001) reported that β -cell damage induced by alloxan occurs through the noxious oxygen free radicals such as O_2 , H_2O_2 and MDA. Sethi *et al.* (2012) also reported that alloxan causes liberation of oxygen radicals associated with reduced antioxidant status.

In the present work, administration of C-peptide led to showed a lower level of blood glucose, glucagon, cholesterol, triglycerides, LDL and MDA levels with higher insulin and HDL levels.

These results are in agreement with Nordquist *et al.* (2007) who reported that C-peptide given to diabetic rats resulted in reduced blood glucose levels. This could be referred to improved glucose utilization, renal function and capillary diffusion capacity in type I diabetic patients Sato *et al.*(2004). Also, Wallerath *et al.*(2003) demonstrated that in vitro studies confirming that C-peptide stimulates glucose transport in skeletal muscle.

It has been reported that C-peptide, derived from proinsulin, secreted in equimolar concentrations to insulin and therefore depleted in type I diabetes mellitus (Zhong *et al.*, 2005). Moreover, Meyer *et al.* (2008) reported that C-peptide facilitates glucose clearance and the release of a nitric oxide stimulus via the GLUT1 transporter. In addition, Chailurkit *et al.* (2007) has stated that C-peptide could enhance the function of β -cell to secrete insulin. Kärvestedt *et al.* (2002) has also reported that C-peptide elevates insulin and depresses glucose by the same mechanism.

C-peptide might play a role in insulin secretion through auto-feedback mechanism activating the insulin-signaling pathway as stated by Shafqat *et al.* (2006). And might play a role in preventing and potentially reversing some of the chronic complications of diabetes mellitus as mentioned by Walenciak *et al.* (2007).

It has been reported that insulin and/or C-peptide and glucagon dominance over each other might be due to feedback mechanism i.e. when insulin and/or C-peptide increase glucagon decreases and vice versa. So that insulin and hence C-peptide suppresses glucagon release (XU *et al.*, 2006). In addition, Sima *et al.* (2004) reported that C-peptide circulates at plasma concentrations five times higher than that of insulin. So, by feedback inhibition, C-peptide depresses the plasma glucagon hormone level and improvement of glucose utilization, renal function and capillary diffusion capacity (Sato *et al.*, 2004).

Hills and Brunskill, (2008) demonstrated that C-peptide appears to bind in nanomolar concentrations to a cell surface receptor which is most likely G-protein coupled. Binding of C-peptide initiates multiple cellular effects, evoking a rise in intracellular calcium and stimulation of the Na⁺/K⁺-ATPase and increased endothelial nitric oxide synthase (eNOS) transcription. These raise the possibility that C-peptide may serve as a potential therapeutic agent for the treatment or prevention of long-term complications associated with diabetes mellitus including dyslipidemia.

Results of this work are incompatible with Bo *et al.* (2012) who reported that patients with higher C-peptide levels had higher baseline triglyceride and lower HDL-cholesterol levels. These results were obtained after a 14-years follow-up study.

This discrepancy could be due to inefficient control of hyperglycemia. Wu *et al.* (2012) reported that C-peptide stimulates insulin secretion and significantly reduces the blood glucose level which leads to improved lipid profile and explained by elevated plasma cholesterol levels in both type I and type II diabetes mellitus and tend to fall toward the normal level with the control of hyperglycemia.

In accordance with results of the present work, Scalia *et al.* (2000) reported a significant elevation in endothelial nitric oxide synthase mRNA level in rats injected with C-peptide and so, elevation of NO and subsequent depression of MDA level.

Results of the present work are incompatible with Martin *et al.* (2004) who revealed that the diabetes-induced elevation in lipid peroxidation (MDA level) was unaffected by C-peptide treatment and both superoxide dismutase (SOD) and catalase activity were decreased in diabetic rats were unaffected by C-peptide treatment.

Results of this work showed that administration of antioxidants (vitamin C and E) associated with slight decrease in blood glucose level, decreased cholesterol, triglyceride, LDL and MDA levels while increasing insulin, glucagon and HDL levels.

These results are compatible with Farvid *et al.* (2011) who reported that over 4 months treatment, vitamins C showed no significant changes in glycemic control. But, with other minerals they might ameliorate diabetic neuropathy symptoms. This could be explained by improvement in insulin-stimulated glucose metabolism and increase insulin-mediated glucose utilization by vitamins C and E administration and therefore, in order to produce glucose lowering effect by vitamins C and E a proper amount of insulin should be present (Ceriello and Motz, 2004).

The elevation of insulin hormone level with vitamins C and E explained by effects of vitamins C and E in maintaining residual β -cell function through they act as a free oxygen radical scavenger hence, prevent β -cell cytotoxicity. So, they elevate insulin and depress glucagon below control group level. Also vitamins C and E may improve the functions of β -cells, elevate plasma insulin and C-peptide levels possibly by increasing the antioxidant capacity. In addition, antioxidants may also block the ability of the immune system to recognize β -cells (Gokkusu *et al.*, 2001).

The results of this work are also compatible with Ceriello and Motz, (2004). They reported that vitamins C and E produce improvement in insulin-stimulated glucose metabolism and increase insulin-mediated glucose utilization. These results are also in agreement with Abdel-sattar, (2004) who stated that diabetic rats receiving antioxidants (vitamins C, E and zinc) from 15 up to 60 days encountered a significant reduction of blood glucose, cholesterol, TG and with significant elevation of HDL-C.

On the other hand, results of the present work are incompatible with Rizzo *et al.* (2008) who reported that vitamins C and E depress blood glucose serum level by increasing glucose clearance. The results of this work are also incompatible with those of Ardekani, (2007). Who reported that vitamin C might lead to better glucose clearance and so reduce serum insulin hormone level and increase plasma glucagon hormone level significantly in comparison to diabetic rats. Moreover, Saudek *et al.* (2006) reported that vitamins C and E administration reduce blood glucose serum level by lowering glycosylated hemoglobin (HbA1c) level, glycation and shortening erythrocyte life span and so reduce insulin hormone level.

Results of this work are incompatible with the above study in the plasma LDL level. According to Miccoli *et al.* (2008), the decline in the plasma LDL is mainly due to the change in plasma TG and HDL which are essentially associated with the improvement in insulin action. This could be time dependant as it has been reported that the antioxidants vitamins C and E effect on lipid profile of rats increase as long as the study period (Parildar *et al.*, 2008).

Data in the present study are in strong accordance with Armstrong *et al.* (2006) who stated that reduced lipid peroxidation revealed by reduced MDA and improved antioxidant status may be one mechanism by which dietary treatment in the form of vitamins C and E, contributes to the reduction of MDA level and prevention of diabetic complications. EL-Seady and EL-Deeb, (2012) concluded that vitamins C and E treatment may potentiate insulin action on lipid peroxidation in diabetic dogs and so lower serum MDA. Also Naziroglu and Butterworth (2005) reported that vitamins C and E can help lower the markers indicative of oxidant stress and lipid peroxidation in diabetic subjects and animals. In addition, Farvid *et al.* (2004) have found that supplementation with vitamins C and E depresses MDA level in type II diabetic patients.

These effects could be explained by the role of vitamin C as it reacts directly with super-oxide, (OH) radicals and singlet oxygen in addition it reduces the tocopheroxyl radical back to α -tocopherol. It is likely that vitamins C and E act by synergistic manner where vitamin E primarily oxidized to the tocopheroxyl radical and then reduced back to tocopherol by vitamin C. Tocopherol inhibits lipid peroxidation because they scavenge lipid peroxy radicals much faster than these radicals can react with fatty acid side chain or with membrane proteins (Azzi *et al.*, 2000).

In the present work, despite the significant reduction of MDA levels by different treatment when compared to the diabetic group, it was noted that there was insignificant changes between different lines of treatment. Hence, there is no apparent interaction between C-peptide with and without vitamins C and E supplementation.

Finally, it could be concluded that the remarkable finding of therapy in this study are significantly better than diabetic group levels but did not reach the levels of the control group i.e. no complete cure. C-peptide administration showed one of the best results regarding significant reduction in glucagon and LDL, blood glucose, cholesterol, triglycerides and MDA in addition to elevated plasma insulin levels. Vitamins C and E improved serum lipid profile and level of MDA as an oxidative stress indicator. So, use of C-peptide might help to avoid or reverse diabetic complications as hyperlipidemia.

A poly therapy regimen may be a recommended guideline for diabetic patients in order to reach euglycemia (control level goal) and to postpone or even avoid the development of the serious complications of diabetes mellitus.

Further researches with more than 4 weeks are required to intensify the actions of vitamins C and E on MDA and lipid profile.

References

1. Abdel-sattar, M. H. (2004): Effect of some antioxidants on diabetes mellitus in albino rats. MD thesis, Physiology Dep., Fac. of Med., AL-Azhar University, Cairo, Pages:57-69.
2. Alejandro, U., Lim, G., Mehran, E., Hu, X., Taghizadeh, F., Pelipecyenko, D., Baccarini, M. and Johnson, D. (2011): Pancreatic beta cell Raf-1 is required for glucose tolerance, insulin secretion and insulin 2 transcription. *FASEB, J.*, 25 (11): 3884-95.
3. Allain, C., Poon, L., Chan, C., Richmond, W. and Fupc, C. (1974): Enzymatic determination of total serum cholesterol. *Clin. Chem.* 20:470-475.
4. Al-Rasheed, N. M., Willars, G. B. and Brunskill, N. J. (2006): C-peptide signals via G α i to protect against TNF- α -mediated apoptosis of opossum kidney proximal tubular cells. *J. Am. Soc. Nephrol.* 17:986-995.
5. Ardekani, A. M. S.A. (2007): Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients. *Indian J. Med. Res.* 126(5):471-4.
6. Armstrong, A. M., Chestnutt, J. E., Gormley, M. J. and Young, I. S. (2006): The effect of dietary treatment on lipid peroxidation and antioxidant status in newly diagnosed noninsulin dependent diabetes. *Free Radical Biology and Medicine (USA)*, 21(5): 719-726.
7. Ascan, W. and Thomas, M. (2005): Why do antioxidants fail to provide clinical benefit? *Curr. Control trials cardiovascular med.*, 1:38.
8. Azzi, A., Breyer, I. and Fener, M. (2000): Specific cellular responses to α -tocopherol. *J. Nutr.* 130:1649.
9. Bo, S., Gentil, L., Castiglione, A., Prandi, V., Canil, S., Ghigo, E. and Ciccone, G. (2012): C-peptide and the risk for incident complications and mortality in type 2 diabetic patients: a retrospective cohort study after a 14-year follow-up European society of endocrinology. 10.1016-10.026.
10. Bortz, J., Lienert, G.A. and Boehnke, K. (2000): Verteilungsfreie Methoden inder Biostatistik, Vol. 2. Korrigierte und Aktualisierte Auflage, Springer-Verlag, Berlin/Heidelberg/New York.
11. Bromme, H. J., Weinandy, R., Peschke, D. and Peschke, E. (2001): Simultaneous quantitative determination of alloxan, GSH and GSSG by HPLC. Estimation of the frequency of redox cycling between alloxan and dialuric acid. *Horm Metab. Res.*, 33:106-109.
12. Burrin, D. (1994): Immunotechnical technique in principles and technique of practical biochemistry. Wilson K. and Walker J. eds, 4th edition; chap.2:65-109.

13. Ceriello, A. and Motz, E. (2004): Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler. Thromb. Vasc. Biol.* May 24 (5): 816-823.
14. Chailurkit, L. O., Jongjaroenprasert, W., Chanprasertyothin, S. and Ongphiphadhanakul, B. (2007): Insulin and C-peptide levels, pancreatic beta cell function and insulin resistance across glucose tolerance status in Thais. *J. Clin. Lab. Anal.* 21(2): 85-90.
15. Eleazer, S. (2003): Diabetes in animal: Contribution to the understanding of diabetes study of its etiopathology in animal models. In: D.M, 6th edition, chapter16, P.231-232.
16. EL-Seady, Y. and EL-Deeb, W. (2012): Effect of vitamin C and vitamin E administration on lipoproteins and lipid peroxidation markers in natural diabetic dogs. *International research journal of biochemistry and bioinformatics.* Vol. 2(3): pp.69-74.
17. Erdelmeier, I. (1997): Reactions of N-Methyl-2-phenylindole with malondialdehyde and 4-Hydroxyalkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation, *Chemical research in Toxicology*, 11:10, 1184-1194.
18. Farvid, M. S. Homayouni, F., Amiri, Z., Adelmanesh, F. (2011): Improving neuropathy scores in type 2 diabetic patients using micronutrients supplementation. *Diabetes research and clinical practice.* Volume 93, Issue 1, Pages:86-94.
19. Farvid, M. S., Jalali, M., Siasi, F., Hosseini, M. (2004): The impact of vitamins and/ or mineral supplementation on blood pressure in type 2 diabetes. *J. Am. Coll. Nutr.* 23(3): 272-79.
20. Fossati, P. and Prencipe, L. (1982): Triglycerides determination after enzymatic hydrolysis. *Clin. Chem.* 28:2077-2080.
21. Franconi, F., Seghieri, G., Canu, S., Straface, E., Campesi, I. and Malorni, W. (2008): Are the available experimental models of type 2 diabetes appropriate for a gender perspective? *Pharmacol. Res.* 57:6-18.
22. Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, June, vol.18 no.6:499-502.
23. Giacco, F., Brownlee, M. and Schmidt, A. M. (2010): Oxidative stress and diabetic complications from the diabetes research center. *American Heart Association, Inc.*10461-1602.
24. Gokkusu, C., Palanduz, S., Ademoglu, E. and Tamer, S. (2001): Oxidant and antioxidant systems in NIDDM patients: influence of vitamin E supplementation. *Endocr. Res.* 27(3):377-386.
25. Green, K., Brand, M. D. and Murphy, M. P. (2004): Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes*, 53:S110-118.
26. Groove, T. (1979): The effect of reagent ph on the determination of high density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clin.Chem*; 25:560-564.
27. Hills, C. E. and Brunskill, N. J. (2008): Intracellular signalling by C-peptide. *Exp diabetes Res.* 6351-58.
28. Irshaid, F. I., Mansia, K., Bani-Khaleda A. and Aburjiab, T. (2012): Hepatoprotective, cardioprotective and nephroprotective actions of essential oil extract of *Artemisia Siberia* in alloxan induced diabetic rats. *Iranian journal of pharmaceutical research.* 11(4):1227-1234.
29. KÄrvestedt, L., Andersson, G., Effndic, S. and Gill, V. (2002): A rapid increase in β -cell function by multiple insulin injections in type 2 diabetic patients is not further enhanced by prolonging treatment. *Diabetes*, 4: 1365-1396.
30. Khoo, J., Rayner, C. H. K., Jones, K. L. and Horowitz M. (2009): Incretin-based therapies: new treatments for type-2 diabetes in the new millennium. *Therapeutic and clinical risk management* 5:683-698.
31. Kumawat, N. S., Chaudhari, S. P., Wani, N. S., Deshmukh, T. A., Patil, V. R. (2010): Antidiabetic activity of ethanol extract of *Colocasia esculenta* leaves in alloxan induced diabetic rats. *International Journal of Pharm.Tech. Research, CODEN (USA):* Vol., 2 (2): pp:1246-1249.
32. Liu, M., Ramos-Castaneda, J. and Arvan, P. (2003): Role of the connecting peptide in insulin biosynthesis. *J. Biol. Chem.*287:14798-14805.
33. Maduka, H., Obi, F. and Mamza, Y. (2003): Effect of chloroquine on blood glucose and cholesterol levels in alloxan- induced diabetic rabbits. *J. of boil. Sciences*, 3 (10): 875-881.
34. Marques, R. G., Fontaine, M. J. and Rogers, J. (2004): Much more than a byproduct of insulin biosynthesis. *Pancreas.* 29:231-238.
35. Martin, J., Weixian, S., Li, Z. F., and Sima, A. F. (2004): C-peptide corrects endoneurial blood flow but not oxidative stress in type 1 BB/Wor rats. *AJP. Endo.* September 1, vol. 287 no. 3

36. Meyer, J. A., Froelich, J. M., Reid, G. E., Karunarathne, W. K. and Spence, D. M. (2008): Metal-activated C-peptide facilitates glucose clearance and the release of a nitric oxide stimulus via the GLUT1 transporter. *Diabetologia*. 51:175-182.
37. Miccoli, R., Bianchi, C., Penno, G., Del Prato, S. (2008): Insulin resistance and lipid disorders. *Future Lipidology*. 3(6):651-664.
38. Naziroglu, M. and Butterworth, P. (2005): Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. *Can. J. Appl. Physiol.* 30(2):172-85.
39. Nordquist, L., Moe, E., and Sjöquist, M. (2007): The C-peptide fragment reduces glomerular hyperfiltration in streptozotocin-induced diabetic rats. *Diabetes/Metabolism Research and Reviews*, 23(5):400-405.
40. Ortega, L. M. and Arora, S. (2012): Metabolic acidosis and progression of chronic kidney disease: incidence, pathogenesis, and therapeutic options. *Nefrología. Madr.* vol.32 no.6.
41. Paget, G. and Barnes, J. (1964): Evaluation of drug activities: Toxicity tests. *Pharmacometrics*, Laurance, D.R. and Bachorach, A.L. Pbl. Academ. Press. London and New York. Ed. 4:135.
42. Parildar, H., Dogru-Abbasoglu, S., Mehmetçik, G., Ozdemirler, G., Koçak-Toker, N. and Uysal, M. (2008): Lipid peroxidation potential and antioxidants in the heart tissue of beta-alanine or taurine-treated old rats. *J. Nutr. Sci. Vitaminol. (Tokyo)*. Feb; 54(1):61-5.
43. Parthasarathy, S., Santanam, N., Ramachandran, S. and Meilhac, O. (2000): Potential role of oxidized lipids and lipoproteins in antioxidant defense *Free Radic. Res.* 33:197-215.
44. Picardi, A., Visalli, N., Lauria, A., Suraci, C., Buzzetti, R., Merola, R., Manfrin, S., Guglielmi, C., Gentilucci, U. V., Pitocco, D., Crinò, A., Bizzarri, C., Cappa, M. and Pozzilli, P. (2006): Metabolic factors affecting residual beta cell function assessed by C-peptide secretion in patients with newly diagnosed type-1 diabetes. *Horm. Metab. Res.* Oct; 38(10):668-872.
45. Rebsomen, L, Pitel, S., Boubred, F., Buffat, C., Feuerstein, J. M., Raccach, D., Vague, P. and Tsimaratos, M. (2006): C-peptide replacement improves weight gain and renal function in diabetic rats. *Diabetes Metab. Jun*;32(3):223-228.
46. Rizzo, M.R. (2008): Evidence for anti-inflammatory effects of combined administration of vitamin C and E in older persons with impaired fasting glucose: Impact on insulin action. *J. Am. Coll. Nutr.* August vol. 27 no. 4:505-511.
47. Saito, K., Yaginuma, N. and Takahashi, T. (1979): Differential volumetry of A, B, and D cells in the pancreatic islets of diabetic and nondiabetic subjects. *Tohoku J. Exp. Med.* 192:273-283.
48. Sampson, M., Davies, I., Gavrilovic, J., Sussams, B., Brown, J., Astley, S. and Hughes, D.A. (2004): Plasma matrix metalloproteinases, low-density lipoprotein oxidisability and soluble adhesion molecules after a glucose load in Type 2 diabetes. *Cardiovasc. Diabetol.*, 3(1): 7.
49. Sato, Y., Oshida, Y., Han, YQ., Morishita, Y., Li, L., Ekberg, K., Jörnvall, H. and Wahren J.(2004): C-peptide fragments stimulate glucose utilization in diabetic rats. *Cell Mol. Life Sci.* 61:727-732.
50. Saudek, C. D., Derr, R.L. and Kalyani, R. R. (2006): Assessing Glycemia in Diabetes Using Self-monitoring Blood Glucose and Hemoglobin A1c. *Clinical Review, JAMA.* 295(14):1688-1697.
51. Scalia, R., Coyle, K. M., Levine, B. J., Booth, G. and Lefer, A. M. (2000): C-peptide inhibits leukocyte-endothelium interaction in the microcirculation during acute endothelial dysfunction. *FASEB. J.* Nov; 14(14):2357-64.
52. Sethi, J., Gupta, A., Sood, S., Dahiya, K., Singh, G. and Gupta, R. (2012): Antioxidant effects of Aloe Vera in experimentally induced diabetes mellitus. *Asian J. Phar. Biol. Res.* 2(2):147-149.
53. Shafqat, J., Juntti-Berggren, L., Zhong, Z., Ekberg, K., Kohler, M., Berggren, P. O., Johansson, J., Wahren, J. and Jörnvall, H. (2006): Proinsulin C-peptide and its analogues induce intracellular Ca²⁺ increases in human renal tubular cells. *Cell. Mol. Life. Sci.* 59:1185-1189.
54. Sima, A. A., Kamiya, H. and Li, Z. G. (2004): Insulin, C-peptide, hyperglycemia and central nervous system complications in diabetes. *European J. Pharm.* 490:187-197.
55. Song, Y., Manson, J. E., Buring-Howard, J. E., Sesso, D. and Liu, S. (2005): Associations of dietary flavonoids with risk of type 2 diabetes and markers of insulin resistance and systemic inflammation in women: A prospective study and cross-sectional analysis. *Am. Coll. Nutr.* October, vol. 24 no. 5:376-384.
56. Steiner, D., Rubenstein, A. and Chan, S. (2000): Biosynthesis of insulin. In: *Handbook of physiology, The Endocrine System*, Edited by Jefferson, L., and Cherrington, A. Vol. II:49-77.
57. Tietz, (1986): *Textbook of clinical chemistry*. W.B. Saunders. Co., London, Philadelphia. p:796.
58. Walenciak, L., Fendler, W. and Mlynarski, W. (2007): Proinsulin C-peptide the bioactive peptide with a huge promise. *Endokrynol. Diabtol.* 13(2):95-98.

59. Wallerath, T., Kunt, T., Forst, T., Closs, E., Lehmann, R., Flohr, T., Gabriel, M., Schäfer, D., Göpfert, A., Pfützner, A., Beyer, J. and Förstermann, U. (2003): Stimulation of endothelial nitric oxide synthase by proinsulin C-peptide. *Nitric Oxide*. 9:95-102.
60. Wu, L., Olverling, A., Huang, Z., Jansson, L., Chao, H., Gao, X. and Sjöholm, Å. (2012): GLP-1, exendin-4 and C-peptide regulate pancreatic islet microcirculation, insulin secretion and glucose tolerance in rats. *Clin. Sci. (Lond)*. Apr; 122(8):375-84.
61. Xu, E., Kumar, M., Zhang, Y., Ju, W., Obata, T., Zhang, N., Liu, S., Wendt, A., Deng, S., Ebina, Y., Wheeler, M.B., Braun, M. and Wang, Q. (2006): Intra-islet insulin suppresses glucagon release via GABA-GABAA receptor system. *Cell Metab*. 3(1):47-58.
62. Zhong, Z., Davidescu, A., Ehren, I., Ekberg, K., Jornvall, H., Wahren, J. and Chibalin, A.V. (2005): C-peptide stimulates ERK1/2 and JNK MAP kinases via activation of protein kinase C in human renal tubular cells. *Diabetologia*, 48:187-197.
63. Zobali, F., Avci, A., Canbolat, O. and Karasu, C. (2002): Effects of vitamin A and insulin on the antioxidative state of dia- betic Rrt Hhart: A comparison study with combination treatment. *Cell Biochem. Funct*. 20:75-80.