**Effects of C-Peptide With and Without Antioxidant Supplementation on Diabetic Male Rats**

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**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 axis to water and rat chow bellet, 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 monhydrate (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 Blood samples were withdrawn for determination of blood glucose, insulin, glucagon, total cholesterol (Chol), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein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.

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**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 thatC-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 evaluates 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 axis 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 pyraminndin, 5,6 dioxyuracil) 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 be 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 (μIU/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 determinedaccording 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 ± 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 μIU/ml to 7.28 ± 2.37 μIU/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 ± 10.41mg/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 thatC-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 μIU/ml to14.94 ± 0.78 μIU/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 ± 10.41mg/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 thatadministration 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 μIU/ml to 11.23 ± 0.72 μIU/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 ± 10.41mg/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.**

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| --- | --- | --- | --- |
| **Group**  Parameter | **Group I** | **Group II** | ***P* value** |
| **Bl. glucose (mg/dl)** | **76.4 ± 6.7** | **384.4 ± 31.32** | ***P* < 0.05** |
| **Insulin (μIU / ml )** | **30.18 ± 4.77** | **7.28 ± 2.37** | ***P* < 0.05** |
| **Glucagon (pg/ ml)** | **76.92 ± 4.16** | **70.65 ± 6.18** | ***P* < 0.05** |
| **Total Cho (mg/dl)** | **96.5 ± 7.01** | **131.5 ± 5.54** | *P* **< 0.05** |
| **TG (mg/dl)** | **98.9 ± 9.53** | **119.3 ± 10.41** | *P* **< 0.05** |
| **LDL (mg/dl)** | **37.95 ± 3.8** | **74.36 ± 3.52** | *P* **< 0.05** |
| **HDL (mg/dl)** | **39.9 ± 2.38** | **33.6 ± 3.06** | *P* **< 0.05** |
| **MDA (nmol/ml)** | **6.5 ± 0.85** | **19.4 ± 1.51** | *P* **< 0.05** |

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

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| --- | --- | --- | --- | --- |
| 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** |
| ***P* < 0.05** | *P* **> 0.05** | ***P* < 0.05** |
| **Insulin (μIU / ml )** | **7.28 ± 2.37** | **14.94 ± 0.78** | **11.23 ± 0.72** | **15.42 ± 0.99** |
| ***P* < 0.05** | ***P* < 0.05** | ***P* < 0.05** |
| **Glucagon (pg/ ml)** | **70.65 ± 6.18** | **66.77 ± 2.37** | **71.94 ± 3.32** | **75.42 ± 6.56** |
| ***P* < 0.05** | ***P* > 0.05** | ***P* < 0.05** |
| **Total Cho (mg/dl)** | **131.5 ± 5.54** | **115.7 ± 9.83** | **122.1 ± 8.49** | **115.3 ± 5.12** |
| ***P* < 0.05** | ***P* < 0.05** | ***P* < 0.05** |
| **TG (mg/dl)** | **119.3 ± 10.41** | **106.7 ± 7.48** | **100 ± 7.16** | **97.9 ± 7.02** |
| **P < 0.05** | **P < 0.05** | ***P* < 0.05** |
| **LDL (mg/dl)** | **74.36 ± 3.52** | **60.61± 5.78** | **72.05± 7.25** | **66.12 ± 4.77** |
| **P < 0.05** | **P > 0.05** | ***P* < 0.05** |
| **HDL (mg/dl)** | **33.6 ± 3.06** | **33.5 ± 3.14** | **36.9 ± 2.38** | **37.2 ± 1.55** |
| **P > 0.05** | **P < 0.05** | ***P* < 0.05** |
| **MDA (nmol/ml)** | **19.4 ± 1.51** | **12.7 ± 1.83** | **12.9 ± 1.45** | **12.9 ± 1.52** |
| **P < 0.05** | **P < 0.05** | ***P* < 0.05** |

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| **Figure (1): Glucose Levels in Different Groups.**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I**  **◙**  **◙**  **\***  **mg/dl** |

\* As compared with group I.

◙ As compared with group II.

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| **Figure (2): Insulin Levels in Different Groups.**  **◙**  **◙**  **◙**  **\***  **μIU/ml**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I** |

\* As compared with group I.

◙ As compared with group II.

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| **◙**  **◙**  **\***  **pg/ ml**  **Figure (3): Glucagon Levels in Different Groups.**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I** |

\* As compared with group I.

◙ As compared with group II.

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| **Figure (4): Cholesterol Levels in Different Groups.**  **\***  **◙**  **◙**  **◙**  **mg/ dl**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I** |

\* As compared with group I.

◙ As compared with group II.

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| **Figure (5): TG Levels in Different Groups.**  **\***  **◙**  **◙**  **◙**  **mg/ dl**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I** |

\* As compared with group I.

◙ As compared with group II.

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| **Figure (6): LDL Levels in Different Groups.**  **\***  **◙**  **◙**  **mg/ dl**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I** |

\* As compared with group I.

◙ As compared with group II.

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| **Figure (7): HDL Levels in Different Groups.**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I**  **\***  **◙**  **◙**  **mg/ dl** |

\* As compared with group I.

◙ As compared with group II.

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| **Figure (8): MDA Levels in Different Groups.**  **\***  **◙**  **◙**  **◙**  **nmol/ml**  **Group V**  **Group IV**  **Group III**  **Group II**  **Group I** |

\* As compared with group I.

◙ As compared with group II.

Results of the present study showed thatcombinedadministration 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 ± 15.63mg/dl,significantly increased (P < 0.05) insulin levels from 7.28 ± 2.37 μIU/ml to 15.42 ± 0.99 μIU/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 ± 10.41mg/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 evaluates 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 H2O2 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 glycgenolysis, 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 O2, H2O2 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, glucgon, 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 alsoreported 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 ha 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 depress 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)** whoreported 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 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)** whoreported 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 explained by the rule 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 peroxyl 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.

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