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Antioxidant Role of Carnosine in Type-II Diabetic Wistar Rats

KEYWORDS: Carnosine, diabetes mellitus type-II, pancreatic ß-cells.

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ABSTRACT *Background: Antioxidant effect of carnosine against metabolic disorders and oxidative stress in Streptozotocin diabetic–induced models was studied.*

Methods: Streptozotocin induced diabetes was done in male Wistar rats and low-density lipoprotein cholesterol, high density lipoprotein cholesterol and triacyglycerol, serum glucose and insulin analyzed weekly. At fourth week end, serum catalase, superoxide dismutase and glutathione levels were measured, accompanied by histopathological and immunohistochemical studies.

Results: Diabetic rats showed significant increased levels of serum glucose, insulin, triacylglycerol and low density lipoprotein, but, high density lipoprotein was decreased. Antioxidant enzymes levels, tissue glutathione, superoxide dismutase and Catalase were decreased. Histopathological and immunohistochemical alterations were found. Carnosine induction, was effective in ameliorating serum glucose and insulin levels of diabetic animals and improving studied parameters of lipid profile and antioxidant enzymes activity. Also, recovery of histopathological and immunohistochemical alterations.

Conclusion: These results suggest that carnosine may be used as potential multi-protective agent for diabetic complications .

Abbreviations: DM-II: Diabetes Mellitus type-II, HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, TG: triacylglycerol, SOD: superoxide dismutase , GSH: glutathione , STZ : streptozotocin.

INTRODUCTION

Diabetes is the world's largest endocrine disease with deranged carbohydrate, fat and protein metabolisms [1]. The prevalence of diabetes mellitus type-II (DM-II) continues to increase recently, so the global burden of this disease will progress between 2000 to 2030 from 171 million to 366 million patients [2]. In Kingdom of Saudi Arabia, the rise in the prevalence of (DM-II) has been started to gain attention after rapid industrialization in the country [3].

Oxidative stress is a condition in which cellular production of reactive oxygen species exceeds the physiological capacity of antioxidant defense system [4]. There is considerable evidence suggesting that oxidative stress plays a role in tissue damage associated with diabetes since persistent hyperglycemia causes an increased production of oxygen free radicals through auto oxidation of glucose and non-enzymatic protein glycation [5]. Oxidants are counteracted by antioxidant enzyme systems such as catalase, superoxide dismutase (SOD) and glutathione (GSH) [6]. The efficiency of this defense mechanism is altered in diabetes [7].

Antioxidant has been defined as "any substance exogenous or endogenous in nature that delays or inhibits oxidative damage to a target molecule [8] and protects biologically important molecules as DNA, proteins, and lipids from oxidative damage [9]. Carnosine (beta-alanyl-L-histidine) is a biological dipeptide predominating in long lived tissues as skeletal muscle and brain as it was has a protective effect on diabetes for its antioxidant effects (pre and post consecutive doses of carnosine) [10]. Keeping in view of oxidative stress in diabetes and carnosine medicinal properties, the present investigation was undertaken to assess carnosine influence as potential antioxidant on oxidative stress in diabetes mellitus type-II.

MATERIALS AND METHODS

1. Animals

The experiments were carried out on male (aged 75-90 days old) Wistar rats which weight about 200-250 grams and were obtained from King Saud University, KSA. During the experiment, animals were housed in clean properly ventilated cages under constant controlled climatic conditions: temperature (23°C) and lighting conditions (12L/12D). Rat food and filtered tap water were provided ad libitum. They were acclimatized to their environment at least two weeks before starting the experiment. Practical part of the study were done in Northern Borders University, KSA and King Abdelaziz University, KSA.

2. MODEL DESIGN

Streptozotocin diabetic rat model

Animals were injected intravenously (i.v.) by streptozotocin (STZ) (60 mg/kg) as it was dissolved by 0.01 M citrate buffer immediately before used [11]. Streptozotocin induces diabetes within consecutive 3 days by destroying ß-cell. STZ Injected rats were considered as diabetic when fasting blood glucose levels was > 200 mg dL^{-1} [11]. Blood glucose levels were determined in all collected blood samples from tail tip after animals anaesthetization (0.1 ml i.p. of 1% sodium barbiturate).

Carnosine treatment rat model

Animals were injected intramuscularly with 20mg/100g for 4-weeks [12].

GROUP DESIGN:

GROUP 1 (n=6) : Male Wistar rats not diabetic, nor Carnosine administration.

GROUP 2 (n=6): Male Wistar rats not diabetic, with Carnosine administration only.

GROUP 3 (n=6): Male Wistar rats, with STZ induction for DM-II, without Carnosine administration.

GROUP 4 (n=6): Male Wistar rats with STZ induction for DM-II, with Carnosine administration.

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3. Methods

1.Body mass index determinations

Animals were anaesthetized (0.1 ml i.p. of 1% sodium barbiturate) for measurement of body length (nose-anal length) and body weight [13]. Body weight and body length were used to determine body mass index, (gm./ cm^2) weekly.

2.Biochemical studies:

Animals were anaesthetized (0.1 ml I.P. of 1% sodium barbiturate) and blood was collected intravenously (i.v.) of rats tails using capillary tubes containing lithium heparin at same day for measurements: (LDL) low-density lipoprotein cholesterol, (HDL) high density lipoprotein cholesterol and (TG) triacyglycerol [14], serum glucose [15] and insulin [16] and that was repeated weekly. The food was withdrawn 10 hours before blood collection.

By the end of experiment (week 4), pancreas were excised, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper. Homogenates (1:10 w/v) were prepared by 100 mM KCl buffer (7:00 p H) containing EDTA 0.3 Mm, centrifuged at 600g for 60 minutes/4°C as supernatant was used for biochemical assays. 10µl of BHT (butylated hydroxytoluene) (0.5 M in acetonitrile) was added to prevent homogenate from oxidation and was stored at -70°C for measurements of : catalase [17], SOD [18] and GSH levels [19].

4.Histopathological and immunohistochemical studies

By the end of experiment, rats were anaesthetized by ether and sacrificed by decapitation. Pancreas samples were quickly removed, immerse in neutral buffered 10 % formalin for 24 hours, dehydrated cleared and embedded in paraffin, sectioned at 3µm, for light microscopic examination, then stained with routine hematoxylin and eosin stain (H&E) [21]. Immunohistochemical staining occurred by anti-insulin antibodies stain; which stain ß-cells (endocrine secretary portion in pancreatic islets of langerhans) as: Ab-7842 added at 1/1000 dilution. Sections were fixed with formaldehyde and blocked with 5% serum prior for incubation with primary antibody for 12 hours. A Cy2 conjugated goat polyclonal antibody was used as secondary antibody. ß-cells, unit of insulin secretion, were stained in green or reddish brown [22].

3.Statistical Analysis

Results were presented as means ± standard deviations (SD). Statistical significance and differences from control and test values were evaluated by Student's 't'-test [20].

RESULTS

1.Body mass index determination

Table 1 summarize body mass index (gm/cm2) during study period (4-weeks). All groups started with a similar body weight at week 0. Diabetic rats showed weight increasing, but carnosine treated gained their start weight. Significant separation of body weight between diabetic and carnosine treated groups; but a high significant separation were observed between Non-diabetic and diabetic groups. There was no significant separation between Non-diabetic and carnosine treated groups.

Table 1: showing body mass index (gm/cm).Values were expressed as means ± standard deviation. (*)= Significant; P ≤ 0.05 and (**)= High significant; P < 0.01.

	Group(1)	Group(2)	Group(3)	Group(4)
Week(1)	1.874 ±0.4685	1.873 ±0.4682	1.874 ±0.4685	1.873 ±0.4682
Week(2)	1.876 ±0.469	1.874 ±0.4685	1.885** ±0.4525	1.869* ±0.462
Week(3)	1.877 ±0.4692	1.875 ±0.4687	1.9** ±0.4471	1.868* ±0.461
Week(4)	1.877 ±0.4692	1.875 ±0.4687	1.931** ±0.4312	1.869* ±0.462

2.Biochemical studies

2.1.Determination of Diabetes

Baseline values of glucose and insulin were relatively similar in day 0 in all groups. Fasting serum glucose and insulin levels showed high significant increasing in diabetic group as was compared with non-diabetic group, and a significant increasing from carnosine treated group. There was not a significance variation in serum glucose and insulin levels between carnosine treated and non-diabetic groups. After carnosine treatment, a high significance decreasing in serum glucose and insulin levels was noticed between carnosine treated and diabetic groups to be in demanded balance (relatively similar to control groups) (Tables 2,3).

	Group(1)	Group(2)	Group(3)	Group(4)
Week(1)	56.28± 4.02	56.5 ±7.035	56.37 ±7.026	56.74 ±4.053
Week(2)	56.35± 4.025	56.34 ±7.04	57.48** ±7.18	57.92* ±7.24
Week(3)	56.24± 4.017	56.48 ±7.06	62.36** ±7.79	58.35* ±7.26
Week(4)	56.31± 4.022	56.68 ±7.08	66.18** ±8.27	58.16* ±7.29

Table 2: showing mean of serum glucose levels (mg/dl), Values expressed as means ± standard deviation. (*)= Significant; P ≤ 0.05 and (**)= High significant; P < 0.01.

Table 3: showing the mean of serum insulin levels (mg/dl). Values expressed as means ± standard deviation. (*)= Significant; P ≤ 0.05; (**)= High significant; P < 0.01.

	Group(1)	Group(2)	Group(3)	Group(4)
Week(1)	0.621 ±0.077	0.623 ±0.077	0.622 ±0.077	0.624 ±0.077
Week(2)	0.627 ±0.078	0.631 ±0.077	0.668 ** ±0.083	0.64 * ±0.082
Week(3)	0.632 ±0.79	0.634 ±0.078	0.681 ** ±0.085	0.638 * ±0.08
Week(4)	0.634 ±0.79	0.635 ±0.079	0.694 ** ±0.086	0.635 * ±0.079

2.2.Lipid Profile

Baseline values of serum levels of TG, HDL and LDL levels not varied in day 0 in all groups. In diabetic group, there was a high significances increasing for TG and LDL levels and a high significances decreasing in HDL level when compared with non-diabetic group, but there was a significance increasing for TG and LDL levels and a significances decreasing in

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HDL level when compared with carnosine treated group. After carnosine induction, a high significance decreasing in serum LDL and TG levels and a high significance increasing in serum HDL levels was noticed when compared to diabetic group to be in demanded balance (relatively similar to control groups). There was not a significance variation in TG, HDL and LDL levels between carnosine treated group and non-diabetic group (Tables 4,5,6).

Table 4: showing the mean serum LDL levels (mg/dl). Values expressed as means ± standard deviation. (*)= Significant; P ≤ 0.05; (**)= High significant; P < 0.01.

	Group(1)	Group(2)	Group(3)	Group(4)
Week(1)	32.62 ±4.07	33 ±4.125	33.6 ±4.2	32.8 ±4.1
Week(2)	32.87 ±4.11	33.14 ±4.2	39.67** ±4.95	37.95* ±4.74
Week(3)	33 ±4.12	32.12 ±4.26	40.73** ±5.09	34.92* ±4.36
Week(4)	33.25 ±4.15	32.89 ±4.36	41.19** ±5.14	34.64* ±4.2

Table 5: showing the mean serum HDL levels (mg/dl). Values expressed as means ± standard deviation. (*)= Significant; P ≤ 0.05; (**)= High significant; P < 0.01.

	Group(1)	Group(2)	Group(3)	Group(4)
Week(1)	44.5 ±5.64	44.85 ±5.84	44.56 ±5.8	44.23 ±5.54
Week(2)	43.42 ±5.44	46.47 ±5.81	38.41** ±4.8	42.85* ±5.34
Week(3)	42.23 ±5.21	45.62 ±5.75	37.74** ±4.73	43.5* ±5.44
Week(4)	43.6 ±5.42	44.21 ±5.53	35.53** ±4.44	43.6* ±5.42

Table 6: showing mean serum TG levels (mg/dl). Values expressed as means ± standard deviation. (*)= Significant; P ≤ 0.05 and (**)= High significant; P < 0.01.

	Group(1)	Group(2)	Group(3)	Group(4)
Week(1)	92.15 ±11.51	92.22 ±11.53	92.13 ±11.52	92.23 ±11.53
Week(2)	92.32 ±11.54	92.73 ±11.59	95.41** ±11.93	94.82* ±11.85
Week(3)	92.47 ±11.55	92.15 ±11.64	96.35** ±12.04	93.68* ±11.71
Week(4)	92.56 ±11.57	92.81 ±11.72	98.44** ±12.3	92.92* ±11.61

2.3. Antioxidant enzymes Profile

Table 7 showed a high significant decrease in tissue SOD, GSH and catalase levels in diabetic rats when compared by non-diabetic one. In carnosine treated group SOD, GSH and catalase levels were restored and showed a significant increase when compared with diabetic group. Carnosine alone showed no significant effects when compared with non-diabetic groups.

Table 7: showing mean tissue SOD, GSH and catalase levels (mmol/g of tissue). Values expressed as means ± standard deviation. (*)= Significant; P ≤ 0.05 and (**)= High significant; P < 0.01.

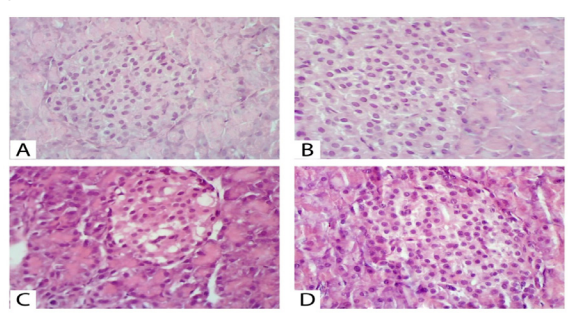
	Group(1)	Group(2)	Group(3)	Group(4)
SOD	28.76 ±3.056	29.36 ±5.76	19.83** ±2.67	24.04* ±4.51
GSH	4.257 ±0.91	2.63 ±0.38	1.508** ±0.66	2.73* ±0.92
Catalase	0.537 ±0.05	0.467 ±0.17	0.258** ±0.02	0.379* ±0.14

3.Histopathological and immunohistopathological studies

3.1.Histopathological studies

For histopathological studies of pancreatic tissue sections by routine hematoxylin and eosin stain (H&E) (Figure 1). Comparison between sections of control, carnosine and diabetic rats showed that pancreatic Langerhans islets of diabetic rats degeneration with necrotic changes. There was a sever vaculation of pancreatic islets with an extensive fibrosis and loss of architecture. Also, there were hyaline and amyloidal droplets which were due to extracellular proteinaceous materials deposition. Control and carnosine groups have normal histological appearance. After carnosine treatment, there were regeneration for pancreatic islet cells and a reduction in vaculation and fibrosis, also hyaline and amyloidal droplets.

Figure 1: The histopathology of pancreatic islet of Langerhans. Normal appearance in control group (a). Normal appearance in carnosine/without diabetes group (b). Diabetic rats/without carnosine treatment (c). Carnosine treated group(d). (H&Ex 400).



3.2. Immunohistochemical studies

For a comparison of insulin content, anti-insulin antibody stain was applied. Strongly positive immune-reaction was observed at control and carnosine groups. After carnosine administration for diabetic group were showed moderate positive immune-reaction of insulin in pancreatic β-cells; as carnosine helped in regeneration of pancreatic β-cells then insulin secretion became in demanded balance (Figure 2). On the other hand, at diabetic group, but without carnosine induction; showed a light to null immune-reaction of insulin in pancreatic β-cells.

Figure 2: Immunohistochemicaal studies of pancreatic islet of Langerhans. Control group (a). Carnosine/without diabetes group (b). Moderate staining β-cells in Langerhans

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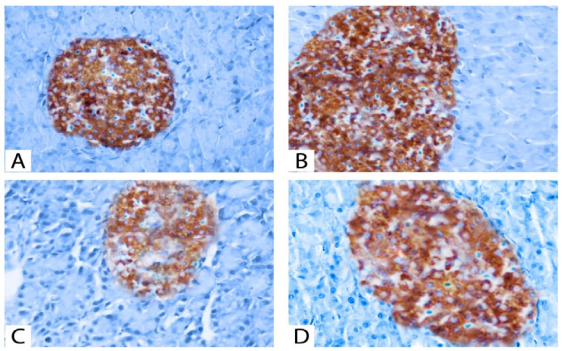
islets in carnosine treatment groups (d). There is light to null staining β-cells in Langerhans islets in diabetic/without carnosine treatment group(c). (Anti-insuline antibody stain X400).



DISCUSSION

Carnosine, an antioxidant, was shown to be an important protective factor in human diabetes [23] which were proved to be a consequence of oxidative stress [24]. It is known that carnosine is an endogenously synthesized dipeptide which is present in brain, cardiac muscle, kidney, stomach, olfactory bulbs and in large amounts in skeletal muscle [25].

In the pathogenesis of DM-II, progressive deterioration of ß-cell function leads to an inability to secrete sufficient insulin to compensate for insulin resistance [26] and other oxidative stress symptoms as it may be source of ROS activation (reactive oxygen species) [27]. DM-II is accompanied by chronic insulin resistance and progressive decline in ß-cell function [28]. Dysfunction of ß-cells or increased rates of ß-cells death (apoptosis) would result in reduced capacity to produce insulin [29]. The purpose of this study is to evaluate effect of association of carnosine administration with DM-II oxidative stress symptoms as: high glucose and insulin levels, lipid profile and antioxidant enzymes (catalase, SOD and GSH levels decrease) impairment. These biochemical parameters were correlated with pancreatic ß-cells histopathological and immunohistochemical results.

The administration of carnosine as DM-II therapy in experimental animals was accompanied by marvelous improvement of serum glucose and insulin levels [30]. Antioxidants administration (pre-or post-) DM-II injury caused significant improvement in function of pancreas compared to rats treated with STZ alone [31]. The most important intracellular antioxidant enzymes systems are GSH, SOD and catalase. They are essential compounds for maintaining cell integrity [32]. The depletion in their levels has been observed in diabetic rats in response to oxidative stress [33]. Pre- or Post-treatment of carnosine significantly restored endogenous antioxidant enzymes [34]. At present study, LDL levels increased, HDL levels decrease and triglyceride levels increased that because saturated fat which were resulted due to blood cholesterol increasing. There was noticed recovery after carnosine administration in lipid profile. Histopathological and immunohistochemical examinations of pancreatic langerhans islets showed neogensis of pancreatic islets ß-cells with noticed insulin immune reaction.

Uniquely, carnosine was proved to posses antioxidant action in scavenging ROS. It was reported to stimulate proteins and enzymes which may produce direct effects on many cellular enzymes [35]. In addition, it preserves protein functional properties via. preventing the combination of deleterious radicals with protein molecules and removing already formed harmful protein products by forming non-functional protein-carbonyl-carnosine complexes which are gotten rid of by lysosomes [36]. Furthermore, carnosine was proved to have an immunostimulatory action with increased natural immune response via stimulating growth factors and cytokines, which promotes tissue recovery [37]. Moreover, carnosine has an anti-inflammatory effect by its ability to suppress histamine release from mast cells, which is a part of the inflammatory reaction. Protecting cells from deleterious transformation of both lipids and protein molecules, preserve molecular cell constituents and organelles thus, decreasing apoptosis [38]. These promising results suggests the possibility of combined type-II diabetes therapy with carnosine as pharmacological approach. From clinical point of view, human studies with recombinant carnosine administration will be necessary.

In Conclusion:

These results suggest that carnosine can be used as potential multi-protective agent for diabetic complications.

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