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Original Article

# Possible Inhibition of Renal Protein Glycation by Carnosine and Tocopherol in Diabetic Rat

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## ABSTRACT

**Background:** Carnosine is a biological active dipeptide in different tissue exert physiological role as buffer in muscle and cell cycle division. Tocopherol (vitamin E) is the potent antioxidant natural product. This study investigated the role of carnosine supplementation with vitamin E in protection against nephropathy in diabetic rats. **Materials and Methods:** This study was carried out on 75 rats grouped into five groups; control and the other rats groups (II-V) were received a single dose of streptozocine *i.p*, at dose of (65 mg/kg/b.w) for diabetogenesis. GPI were considered as diabetic untreated. Rats in groups 3-6 were treated *i.p* daily with carnosine (1mg/kg b.w), vitamin E (50,00 IU/kg b.w) or combined (10 mg/kg b.w) respectively for 12 weeks. Fasting serum was subjected for assay of glucose, $HbA^{1c}$, AGEs, MDA, SOD, total antioxidant activity and inflammatory markers (TNF-α and IL-6). **Results:** Data obtained showed that, diabetic rats treated with carnosine and Vitamin E showed improvement in glucose, glycated hemoglobin, antioxidant enzymes, decreased inflammatory mediators and AGEs ($p$ <0.001) versus untreated diabetic. In addition, combined treatment is better than individual treatment ($p$ <0.05). **Conclusion:** Promising positive results were spot a new strategy of carnosine and Vitamin E rich food supplements for protection against diabetic complications. It can be used as complementary and alternative therapy for nephropathy, neuropathy and cardiomyopathy.

**Keywords:** Diabetic nephropathy, AGEs, Inflammation, Carnosine, Vitamin E, Rat.

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## INTRODUCTION

Diabetes Mellitus (DM) is a serious chronic disease characterized by sustained elevated plasma glucose level. It affects the body organs and functions.[1] The rapid and wide spread of the disease creates an enormous burden on the society, with regard to mortality and morbidity, as well as the coast of the health care. The most complications of DM include macro and microvascular disorders as nephropathy, retinopathy and neuropathy. The prevention/treatment or attenuation of Diabetic Nephropathy (DN) in early stage are of greatest importance and are scope of numerous current research studies.[2-5] Improving the lifestyle, including medical nutritional therapy and a suitable physical activity is the base of DM mangment and attenuating the late diabetic complications. In addition, type II diabetic patients require pharmacologic therapy to reach a controlled level. Most of these patients should be able to achieve desired glucose control by using the available pharmacologic agents together with improving their lifestyle[6-9] Although all the known hypoglycemic agents as well as insulin are often efficient and successful in controlling the blood glucose level, they have many well-known side effects and fail to markedly prevent and/or alter the late diabetic complications.[10] Thus, the rational of this study was to find a natural product which can prevent or at least attenuate the diabetic complications is an interesting field of DM treatment. Signal transduction is involved in living cell function,[11] development,[12] differentiation[13] and cell death. Signaling molecules including hormones, neurotransmitters and growth factors.[14] Carnosine is a biological active dipeptide in different tissue exert physiological role as buffer in muscle and cell cycle division. Carnosine is synthesized from histidine amino acid. Carnosine plays an important role as biological buffer of vital organs as kidney, eye, heart and lung. The DN is a result of non-enzymatic glycation of nephron protein that lead to formation of advanced glycated end products (AGEs) with nonfunctional of nephron, several factors are reported to contribute this complication is release of inflammatory mediators.[15-17] The AGEs and inflammatory mediators increased production of free radicals and decreased antioxidant efficacy of

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**Table 2: Serum and renal malondialdehyde, reduced glutathione level and antioxidant enzymes activities (CAT and SOD) (Mean +SD).**

| | | Renal | | | |
|---|---|---|---|---|---|
| Groups | Serum MDA µ mol /L | MDA µ mol/mg protein | GSH µg /mg protein | CAT U/mg protein | SOD U/mg protein |
| Group I Mean+S.D. | 25+2.4 | 43.7+ .8 | 212+16 | 980+87 | 1135+154 |
| Group II Mean+S.D. | 95+2.6 [a] | 162+8 [a] | 113.6+11 [a] | 312+35 [a] | 732+68 [a] |
| Group III Mean+S.D. | 73+ .2 [a, b] | 112+9 [a,b] | 159+9 [a, b] | 631+74 [a,b] | 832+86 [a, b] |
| Group IV Mean+S.D. | 53+4.3 [a, b] | 83+8 [a,b] | 180+ 1.1 | 549+ 6 [a,b] | 943+90 [a, b] |
| Group V Mean+S.D. | 58+3.4 | 81+9 | 1 78+20.2 | 801+ 6 | 989+93 |

[a] *p* vs control; [b] *p* vs untreated.

the cell, consequence of these events are cell aging and damage. This study targeting to investigate the inhibition of kidney AGEs production and evaluate the role of carnosine synergize with Vitamin E in diabetic rats of diabetic complications is the main target of therapeutic regime, For that, we Evaluated the role of carnosine and Vitamin E on the formation of AGEs and diabetic nephropathy in rat model.

## MATERIALS AND METHODS

### Animals

Carnosine and Vitamin E, streptozocine were obtained from Sigma, Aldrich. This study was carried out on 75 male Albino rats, (80±10g) obtained from animal house at King Abdulaziz University. The handling of animals was according to ethical committee of KAU. The Rats were sorted into 5 groups (each 15); Control (GPI). Rats in groups (2-6), were received a single dose of STZ *i.p*, at dose of 65 mg/kg for induction of diabetes. If blood glucose (≥250 mg/dl) were considered as diabetic. Rats in group II were considered diabetic untreated. Rats in groups III-V were treated *i.p* daily with carnosine (1mg/ kg b.w) or Vitamin E (50,00 IU/kg b.w) or combined for 12 weeks.

### Methods

At the end of experiment, animals were fasted for 14 hr, water only was available, Blood was collected from, serum were used for the measurement of glucose, $HbA^{1c}$, AGES, MDA, inflammatory markers as tumor necrosis factor (TNF-α) and interleukine (IL-6) levels were determined by using ELISA kit, BIORAD, Jeddah.

One Kidneys from were removed washed with ice-cold phosphate-buffered saline, and frozen in liquid nitrogen. Malondialdehyde, reduced glutathione, superoxide dismutase and catalase were measured by using kits from BIORAD.

### Statistical analysis

Results were statistically analyzed using SPSS program version 15.0. If $p<0.05$, it was considered as significance. Pearson correlation was applied between different parameters among different groups.

## RESULTS

Data presented in Table 1 showed that, diabetic rats revealed a significant reduction in body weight ($p<0.001$) compared with control group. Diabetic rats treated with carnosine /or with Vitamin E increased in body weight compared with untreated, the effect was dose dependent. Diabetic rats showed a significant increase in blood, $HbA^{1c}$, MDA, levels compared with the control group ($p<0.001$). Treatment of diabetic with carnosine and or with Vitamin E resulted in a significant reduction in blood glucose, $HbA^{1c}$ and MDA compared with the untreated diabetic animals ($p<0.001$) (Table 1). The higher dose effect is more potent than lower dose (Table 2). The hypoglycemic effect exerted by carnosine and Vitamin E was better than individual treatment ($p<0.01$).

Table 2 revealed that, in kidney tissue homogenate, the levels of GSH, catalase, and SOD were significantly decresed while MDA level was increased significantly in diabetic animals versus control ($p<0.001$). Diabetic rats treated with carnosine and or with Vitamin E showed a significant reduction in MDA and a significant increase in GSH ($p<0.001$) level, catalase and SOD ($p$ <0.01) respectively. Figure 1 showed that, the levels of inflammatory mediators TNFα and IL-1 and AGEs were significantly elevated in diabetic rats' comparison with control group ($p<0.001$) respectively. Treatment with carnosine and or with Vitamin E resulted in a significant reduction of their levels ($p<0.001$) versus untreated. Correlation study showed a positive correlation between IL-6, TNF and AGEs (r=0.5 and 0.54)

**Table 1: Changes in body weight, glucose and HbA$^{1c}$ levels in all groups are represented as mean + SD.**

| Parameters | Gp I | GP II | GP III | GP IV | GP V |
|---|---|---|---|---|---|
| Body weight (start) | 120.2+8.5 | 125.6+15.5 | 129.12+7.5 | 132+8.2 | 121.3+ .5 |
| Body weight (Final) | 199.5+13.2 | 130.8+16.2[a] | 167.5+ .5[a, b] | 175.41+10.9[a, b] | 196+11.3[a, b] |
| Glucose (mg/dl) | 89.18+6.3 | 335.95+36.33[a] | 275.92+ 4.4[a, b] | 199.81+ 1.3[b] | 198.81+9.9[a, b] |
| HbA$^{1c}$ (%) | 5.44+0.41 | 10.12+0.64[a] | 8.8+0.54 [b] | 7.4+0.48 [b] | 7.1+0.32 [b] |

[a] *p vs* control; [b] *p* vs untreated.

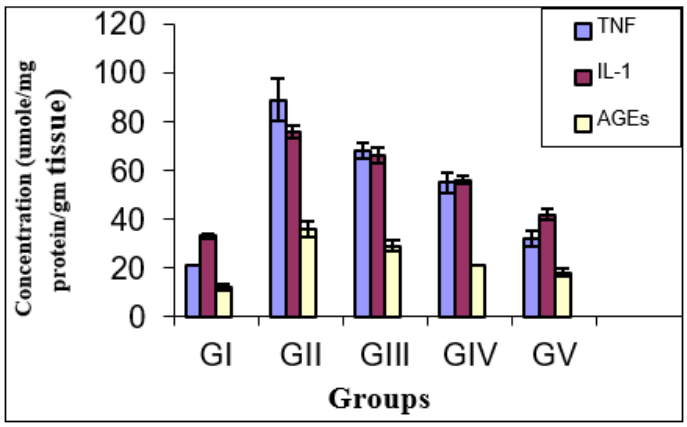


**Figure 1:** Serum TNF- α, IL-1 and AGEs levels in all studied groups (Mean +SD).

respectively. and a negative correlation between HbA$^{1c}$ and SOD, GSH (r=-0.56 and 0.61) respectively.

## DISCUSSION

Foods rich in active ingredients are the sole line of defense against chronic diseases. Carnosine and Vitamin E are considered as functional foods due to their impact on health status.[18] Carnosine plays an important role in prevention of damage of vital organs as kidney, eye, heart and lung.[19] The action of Vitamin E on protein glycation (AGEs) is related to their antioxidant potential, they prevent the oxidation and the subsequent formation of AGEs, sometimes referred to as autoxidation. Inhibition the formation of AGEs in diabetic will delay or prevent the late irreversible complications of diabetes. It was found that, carnosine and Vitamin E exert a potent hypoglycemic effect by lowering blood glucose level and glycated hemoglobin in diabetic rats compared with untreated group. The combined treatment is better than individual treatment. It was found that, these compounds reduced release of inflammation mediators in renal cells, thereby delaying the progression to diabetic nephropathy.[20-22] The action of these compounds on protein glycation (AGEs) is related to their suppression of free radicals' release, they inhibit the oxidation and the subsequent formation of AGEs. However, it enhances antioxidant capacity. Due to presence of these compound carnosine and Vitamin E naturally, it is being to be no toxic for human use.[23,24]

The action of carnosine may be due to high affinity to glucose and inhibit glycation with protein tissue as kidney. The Vitamin E was known as potent antioxidant to inhibit peroxidation and AGEs formation.

The present study also demonstrated that inhibation the overproduction of free radicals by carnosine and Vitamin E in the diabetic rats was indicated by the reduction of malondialdhyde level as compared to the untreated group. Moreover, carnosine and Vitamin E neutralize the parameters of oxidant/antioxidant in nephron of diabetic rats and suppress the activation of pathways involved in hyperglycemia-induced nephrone vascular damage.[25] The prevention of free radical overproduction may be an indirect AGE-inhibiting effect. The results obtained are simialar to those exerted by.[26] Data obtained showed that, renal IL-6 and TNF-α were found to be significantly elevated in diabetic rats in comparison with control rats. However, carnosine and Vitamin E treatment modulated the expression of IL-6 as compared to the untreated group.[11]

The TNF-α implicated in the development of diabetic nephropathy and significantly elevated in diabetes subjects relative to control subjects. It was found that, TNF-α contribute in the apoptotic pathway of renal endothelial cells during different stages of diabetic nephropathy. In the currrent study, TNF-α was significantly elevated in the diabetic rats in comparison to treated with either carnosine and Vitamin E. However, it showed a significant reduction in TNF-α levels in relation to the untreated diabetic group. Carnosine and Vitamin E was found to reduce f aldose reductase in diabetic rats that convert fructose to sorbitol. It was demonstrated that carnosine and Vitamin E possess a broad beneficial cell biological effects due to its ability to lower the hyperglycemia-induced free radical production.[12,13]

To protect renal basement protein from glycation, carnosine and Vitamin E trap the glucose and prevent glycation. It is promising carnosine and Vitamin E give positive results that spot a new strategy for protection against diabetic complications. It can be used as complementary and alternative therapy for nephropathy, neuropathy and cardiomyopathy.

## CONCLUSION

It was concluded that, supplementation of natural products as Vitamin E and carnosine in diabetic subjects ameliorate protein glycation and consequently delay or prevent diabetic complications.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**AGEs:** Advanved glycated end products; **HbA$^{1c}$:** Glycated hemoglobin; **MDA:** Malondialdhyde; **SOD:** Superoxide dismutase; **TNF-α:** Tumor necrosis factor; **IL-6:** interlukine-6.

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