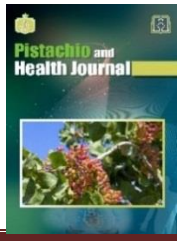

Nephroprotective Activity of Pistachio Kernels in STZ-Induced Diabetic Rats

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Abstract

Introduction: Different parts of pistachio are precious sources for preparing new candidates for bio-molecule drugs to manage diseases such as cancer, obesity and diabetes. According to our previous study, the protein hydrolysates of pistachio kernels (PHPK) are capable of inhibiting the mechanism of the diabetes. The aim of this study was to investigate the effect of PHPK on kidney biochemical and histopathological indices in STZ induced diabetic and high-sugar diet-fed rats.

Materials and Methods: Ninety-six Wistar male rats weighing approximately 250-300g were randomly divided into 12 groups of eight: three control groups and nine target groups (including rats with normal diet, rats with high-sugar diet and streptozotocin-diabetic rats with a normal diet), which were fed with different doses of PHPK for 8 weeks. Then the kidney function was measured using biochemical and histopathological assays.

Results: The results demonstrated a reduction in the level of creatinine due to recovery in the glomerular filtration and repair of the cell damage caused by STZ. The PHPK and the ingredients showed therapeutic potential for kidney injuries preceded by STZ. In all dosages of PHPK, urea and creatinine levels decreased significantly, which is possibly correlated with the better protection of the kidneys ($P \leq 0.05$). After PHPK treatment, the levels of urea and creatinine decreased and the renal function improved.

Conclusions: Treatment with PHPK demonstrated a potential diabetes mellitus therapeutic effect. Specifically, higher treatment with dose corresponds to better renal function and better activity through the mechanistic action to reduce creatinine and urea levels in STZ-injected Wistar rats.

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1. Introduction

Diabetes mellitus is the most common disease of the endocrine glands, characterized by hyperglycemia (increased blood sugar) and impaired metabolism of carbohydrates, lipids, and proteins [1]. More than 3.6% of the world's population has diabetes mellitus [2]. It is predicted that the incidence of diabetes will increase to 592 million people in the world until 2035 [3, 4]. Defects in insulin secretion and insulin resistance lead to increased blood glucose (hyperglycemia), the symptoms of which include polyuria, polydipsia, weight loss, polyphagia, blurred vision, growth retardation. and ketoacidosis occurs with diabetes [3].

Diabetic nephropathy increases protein excretion in the urine in the absence of other causes despite diabetes [5]. It is one of the most critical and severe complications of diabetes, which has several stages, but finally, it can lead to kidney failure [6]. The most critical diabetic nephropathic lesions in the kidney's glomerular part include sclerosis, maxillary matrix diffusion, and thickening of the basement membrane [7]. Based on blood sugar levels and the complications of diabetes, a variety of medications have been developed, including Tolazamide, Tolbutamide, Choloropropamide, Glyburide, Glibenclamide, Metformin and so on [8]. Unfortunately, these drugs have side effects such as nausea, vomiting, weight gain, cardiovascular complications, and so on [9]. Today, due to the side effects and high cost of chemical drugs, on the other hand, the ease of access, effectiveness, cheapness, and fewer side effects of plants, medical scientists have considered using plants as an alternative [6]. Oral delivery and injection of bioactive peptides are two new ways of diabetes management [10]. Bioactive peptides are short-chain acids with biological activity in the human body. Some of the valuable advantages of using these compounds are antimicrobial [10], antihypertensive, immunomodulatory, antithrombotic [11], antioxidant [12] and antidiabetic effects [13, 14].

Pistachio has been used as a traditional remedy for a variety of diseases. Pistachio is one of the flowering plants grown in the desert, and tolerant of the sun and saline soil. Pistachio kernels are one of the richest sources of fat-soluble antioxidants [15]. It has been shown that pistachio consumption can be useful in reducing the harmful acute phase proteins and lowering the level of hemoglobin A1c in diabetic patients [16]. Many studies have reported several therapeutic effects for pistachio, such as reducing LDL and cholesterol and increasing HDL levels [17, 18], protecting against cardiovascular diseases [19], changing the metabolism of lipids and carbohydrates [20, 21] and reducing blood glucose [22].

So far, the extracted protein compounds from specific plants and animal sources have been used in the treatment of diabetes in the form of bioactive peptides or enzymes. Due to the prevalence of diabetes and subsequently nephropathy, and considering lack of research on the impact of pistachio peptides on the renal parameters of male Wistar diabetic rats, and also with regard to the findings on the valuable role of these compounds in controlling blood sugar, this study was conducted to evaluate the effects of protein hydrolysate of pistachio kernels (PHPK) on kidney biochemical and histopathological indices in STZ induced diabetic and high sugar diet rats.

2. Materials and Methods

2.1. Preparation of protein hydrolysate of pistachio kernels (PHPK)

In this research, Ohadi pistachio cultivar was selected and obtained from Rafsanjan, Iran. The PHPK were prepared using the enzymatic hydrolysis protocol described by Mohamadi et al., (2019) [23].

2.2. Animal groups

Ninety-six Wistar male rats weighing approximately 250-300 grams were randomly divided into 12 groups of eight. Before the animals entered the study, care was taken to ensure that the rats were differentiated into different groups in terms of body weight. Then, all animals were kept for eight weeks at 22.2 °C, between 25 - 30 % humidity, and in a 12-hour dark and 12-hour light cycle. The ethical standards of working with animals were observed during the experiments, and the code of ethics was obtained from the ethics committee of Rafsanjan University of Medical Sciences (IR.RUMS.REC.1398.186).

2.3. Experimental procedure

In this experimental research, a total of 96 male Wistar rats weighing 250-300 g were randomly divided into 12 groups (N=8): 2 control groups, one sham group, and 9 target groups. All groups are in accordance with our previous research and the complete characteristics of the groups are explained in detail in our previous study [23].

The study groups are as follows.

C0: Normal control rats receiving physiological saline by intraperitoneal injection

C1: Control rats receiving high-sugar diet

C2: STZ-induced diabetic control rats

T1-T3: Normal rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively.

T4-T6: High-sugar diet-fed rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively.

T7-T9: STZ-induced diabetic rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively.

2.4. Experimental protocol

Standard pellet diet and water were provided for the animals. The rats were injected intraperitoneally using streptozotocin (STZ) at a dose of 60 mg/kg body weight. After three days, they developed classic symptoms of diabetes, such as hypnosis and paradigm, and on the fourth day, their blood flow increased. Fasting was evaluated, and blood sugar above 300 mg/dl was considered as a measure of diabetes [24].

In this work, 30% sucrose in the drinking water of the rats was used as a high-sugar diet [25]. The PHPK were used freshly and was given to treated rats by gavage once a day for 8 weeks in three concentrations 5, 50 and 500 mg/kg.

2.5. The measurement of biochemical parameters

After eight weeks of treatment, the rats were anesthetized with ketamine. Blood samples were taken from the corners of their eyes, and their blood serum was removed. The levels of blood urea, creatinine, uric acid and albumin serum were measured in Pathobiology Laboratory of Rafsanjan University of Medical Sciences.

2.6. Histopathology

Tissue samples were taken from the kidney and fixated in 10% buffered formalin solution for 48 h. After routine tissue follow-up, as dehydration through an ascending alcohol series and clearing with xylene, the samples were embedded into paraffin blocks; 4 µm-sections were taken using microtome (Leica RM 2135); stained with hematoxylin-eosin and examined under light microscope (Olympus CX33, Tokyo, Japan). These tissue sections were assessed by a blinded pathologist.

2.7. Statistical Analysis

SPSS software version 19 was used for the statistical analysis of the data. After describing the various variables, one-way analysis of variance (One-Way ANOVA) was used to perform inferential statistical analysis of the data to see if there are any statistically significant differences among group means. If significant, the Post Hock test was used to determine the differences between the groups. The mean, standard deviation (SD) was expressed, and the significance level in all tests was considered 0.05.

3. Results

3.1. The effects of PHPK on blood urea level in diabetic and high-sugar diet-fed rats

Our results revealed that blood urea levels in C1 group that drank sucrose dissolved in water and C2, the diabetes group, were significantly higher than the control group (C0) ($P<0.05$). Blood urea with PHPK consumption in doses of 5, 50 and 500 mg/kg (T1, T2 and T3 groups, respectively) was not significant compared to control group (C0). The blood urea

levels in high-sugar diet rats that received the doses of 50 and 500 mg/kg (T5 and T6 groups) and also in diabetic rats that received the doses of 5, 50 and 500 mg/kg of PHPK (T7, T8 and T9 groups), were significantly lower than C1 and C2 groups (P<0.05).

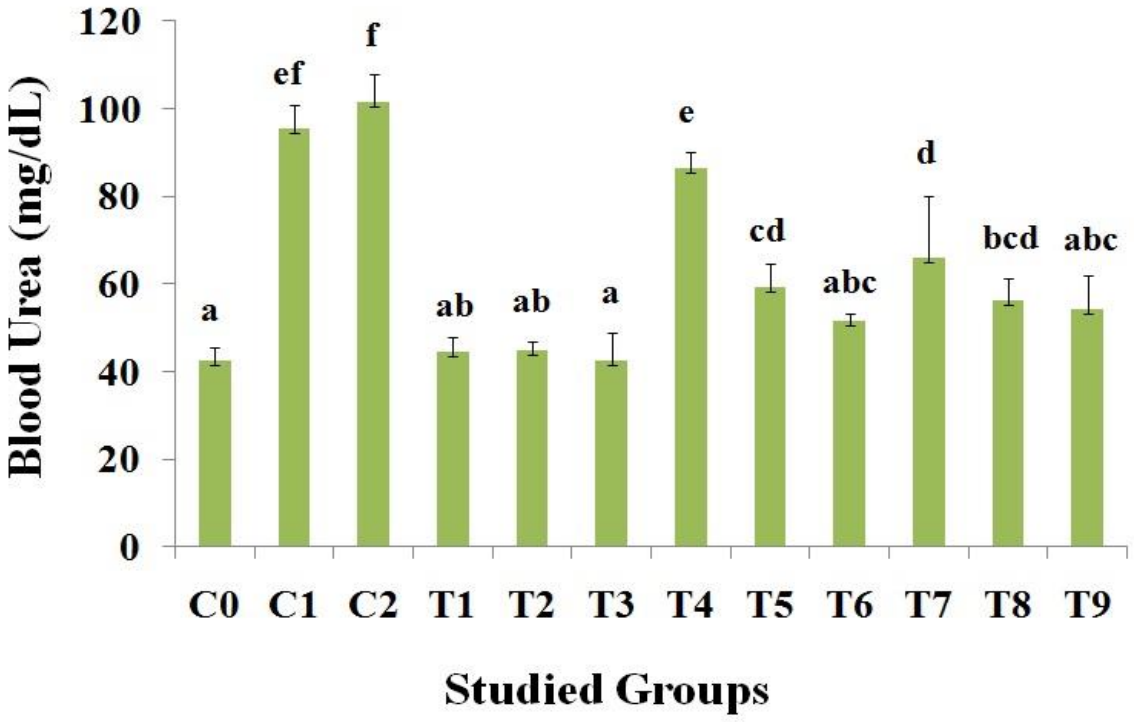

Fig. 1- The effects of PHPK on blood urea level in diabetic and high-sugar diet-fed rats

C0: Normal control rats received physiological saline by intraperitoneal injection. **C1**: Control rats receiving high-sugar diet. **C2**: STZ-induced diabetic control rats. **T1-T3**: normal rats receiving PHPK at concentration of 5 mg/kg at concentration of 5, 50 and 500 mg/kg, respectively. **T4-T6**: High-sugar diet-fed rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. **T7-T9**: STZ-induced diabetic rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. Data are demonstrated as means ± SD (*P<0.05).

3.2. The effects of PHPK on creatinine level in diabetic and high-sugar diet-fed rats

Our results showed that just in diabetes group (C2), creatinine level was significantly higher than the control group (C0) ($P<0.05$). Creatinine level in T1, T2 and T3 groups was not significant compared to control group (C0). There is no significant difference between treated groups (T4 -T9) and C1 and C2 groups.

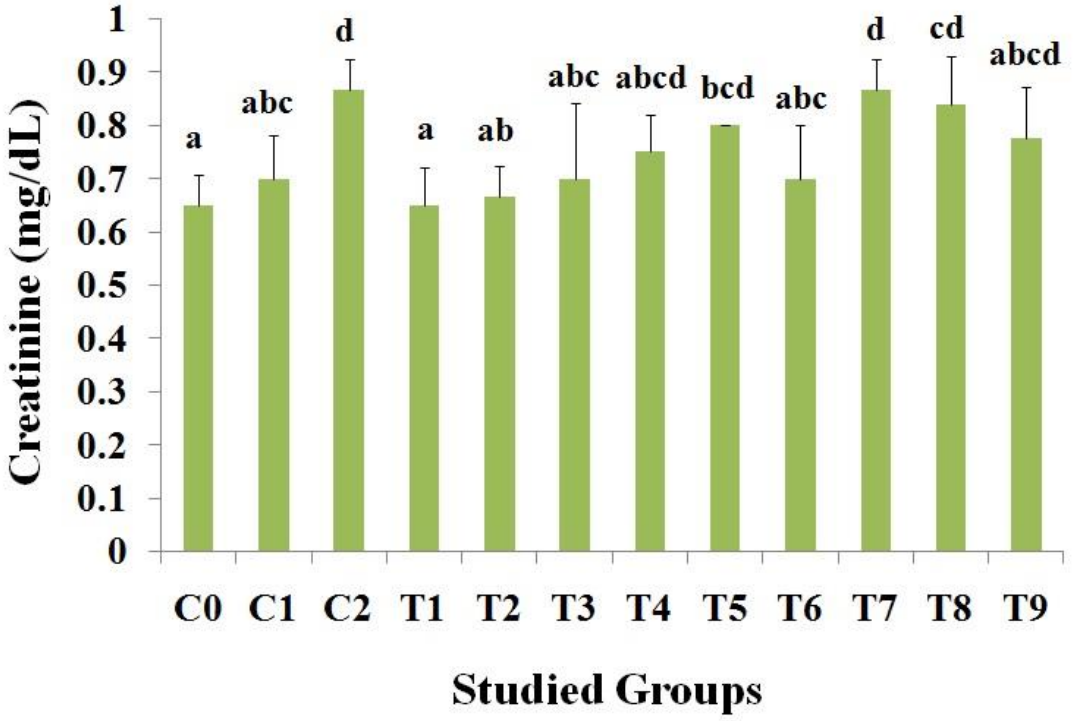

Fig. 2 -The effects of PHPK on creatinine level in diabetic and high-sugar diet-fed rats

C0: normal control rats received physiological saline by intraperitoneal injection. **C1**: control rats receiving high-sugar diet. **C2**: STZ-induced diabetic control rats. **T1-T3**: normal rats receiving PHPK at concentration of 5 mg/kg at concentration of 5, 50 and 500 mg/kg, respectively. **T4-T6**: high-sugar diet-fed rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. **T7-T9**: STZ-induced diabetic rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. Data are demonstrated as means ± SD (*$P<0.05$).

3.3. The effects of PHPK on Uric Acid level in diabetic and high-sugar diet-fed rats

Our results showed that uric acid level in C1 group and C2 group, the diabetic rats, was significantly higher than the control group (C0) ($P<0.05$). The uric acid level in T1, T2 and T3 groups was not significant compared to control group (C0). In high-sugar diet-fed rats (T4, T5 and T6 groups), the uric acid level was significantly lower than the C1 group. Also in diabetic rats (T7, T8 and T9 groups), the uric acid level was significantly lower than the C2 group ($P<0.05$).

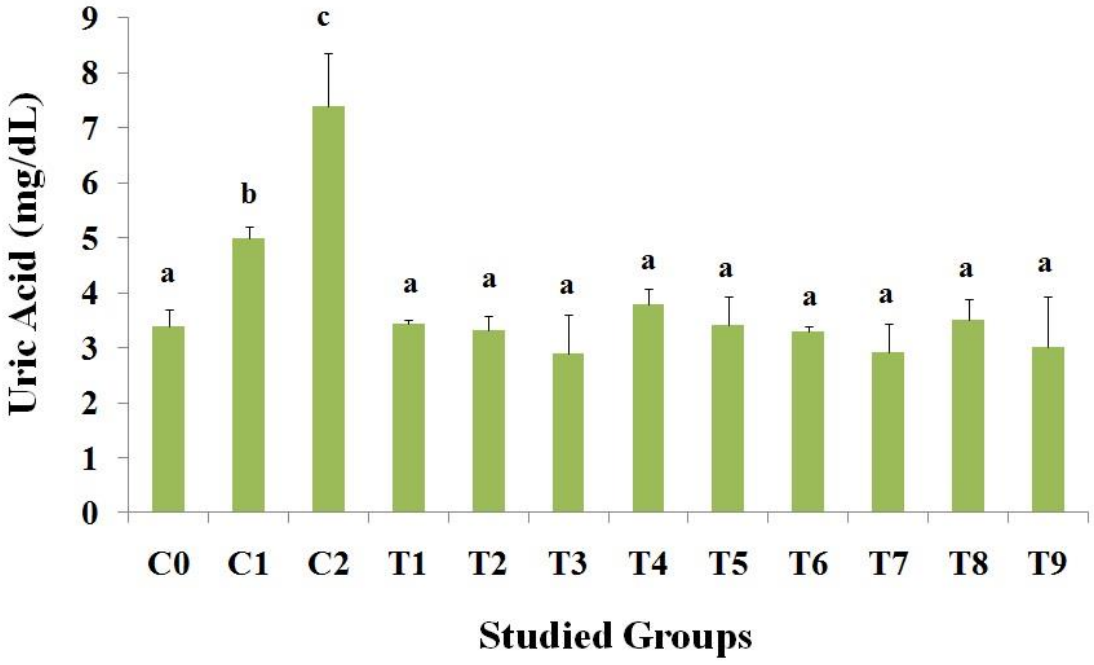

Fig.3- The effects of PHPK on Uric Acid level in diabetic and high-sugar diet-fed rats

C0: Normal control rats received physiological saline by intraperitoneal injection. **C1**: Control rats receiving high-sugar diet. **C2**: STZ-induced diabetic control rats. **T1-T3**: Normal rats receiving PHPK at concentration of 5 mg/kg at concentration of 5, 50 and 500 mg/kg, respectively. **T4-T6**: High-sugar diet-fed rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. **T7-T9**: STZ-induced diabetic rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. Data are demonstrated as means ± SD (*$P<0.05$).

3.4. The effects of PHPK on Albumin serum level in diabetic and high-sugar diet-fed rats

Our results showed that just in C2, diabetes group, albumin serum level was significantly lower than the control group (C0) ($P<0.05$). The albumin serum level in T1, T2 and T3 groups was not significant compared to control group (C0). In T6 group, rats with high-sugar diet and receiving 500 mg/kg PHPK, the serum albumin level was significantly higher than the C1 group. Also in diabetic rats (T7, T8 and T9 groups), the albumin serum level was significantly higher than the C2 group ($P<0.05$).

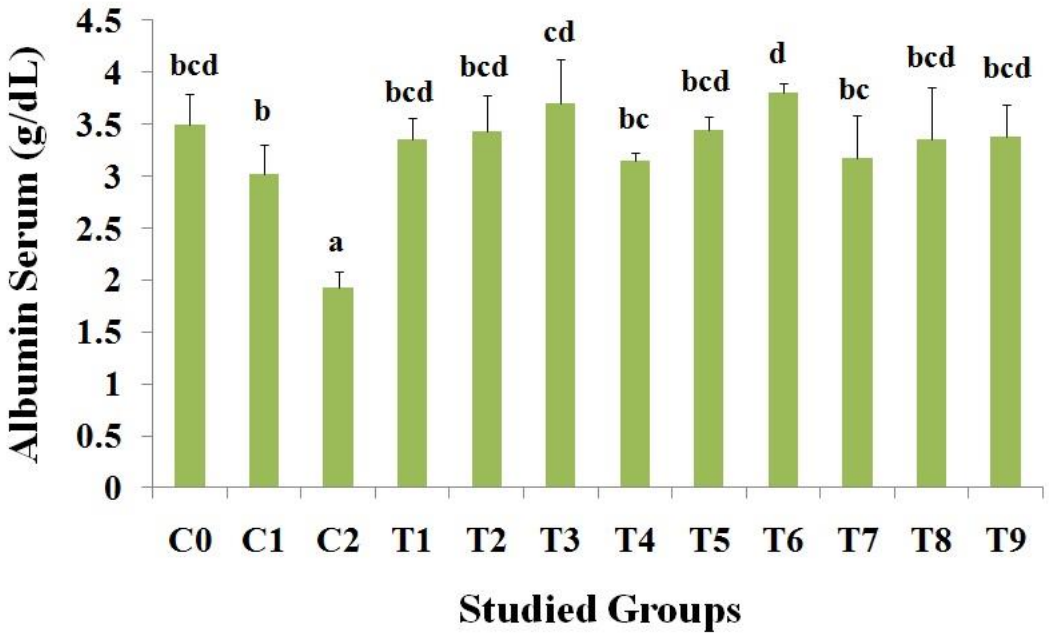

Fig.4- The effects of PHPK on Albumin serum level in diabetic and high-sugar diet-fed rats **C0**: Normal control rats received physiological saline by intraperitoneal injection. **C1**: Control rats receiving high-sugar diet. **C2**: STZ-induced diabetic control rats. **T1-T3**: Normal rats receiving PHPK at concentration of 5 mg/kg at concentration of 5, 50 and 500 mg/kg, respectively. **T4-T6**: High-sugar diet-fed rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. **T7-T9**: STZ-induced diabetic rats receiving PHPK at concentration of 5, 50 and 500 mg/kg, respectively. Data are demonstrated as means± SD (*$P<0.05$).

3.5. Histopathological examinations

Rats in sham group show normal structure in their kidneys. No pathologic changes and normal glumeruli was observed in group C1. Glomeruli from the diabetic group C2 that included STZ-induced diabetic rats fed with normal diet showed expanded Bowman's space and deposition of eosinophilic material plus distorted glomeruli and dilated glomerular capillaries. Moreover, the tubular epithelial lining displayed many cells with pyknotic nuclei and others with vacuolated cytoplasm.

Rats in T1 group show preserved architecture. In T2 group, in some of the tubules, preserved architecture and protein deposition was observed. In T3 group, in some of the tubules, preserved architecture, vascular congestion and protein deposition was observed.

Rats in T4 group showed degeneration of tubular cells and destroyed brush border (less than C2 &T7 groups), along with scattered chronic inflammatory cells in interstitium and congestion of capillaries.

Rats in T5 group showed preserved architecture, vascular congestion, tubular dilatation, minimal degeneration of tubular cells and destroyed brush border (less than T4 Group) and eosinophilic secretory materials deposition.

Rats in T6 group showed preserved architecture, vascular congestion, tubular dilatation and eosinophilic secretory materials deposition.

Rats in T7 group showed congested blood vessels, mild dilatation of glomerular capillaries and capsular space, intratubular protein deposition. Furthermore, tubular epithelial lining cells displayed many degenerative cells with pyknotic nuclei and others with vacuolated cytoplasm (less than C2 group), mild focally mononuclear cells infilteration in interstitium.

Rats in T8 group showed congested blood vessels, only mild dilatation of glomerular capsular spaces, some intratubular protein deposition and also tubular epithelial lining cells displayed some degenerative cells with pyknotic nuclei.

Rats in T9 group showed mild tubular dilatation, intratubular protein deposition. In addition, tubular epithelial lining cells displayed few degenerative cells with pyknotic nuclei (less than T7&T8 groups). Overall, the results of this study showed that PHPK markedly improved the renal lesions in diabetic rats.

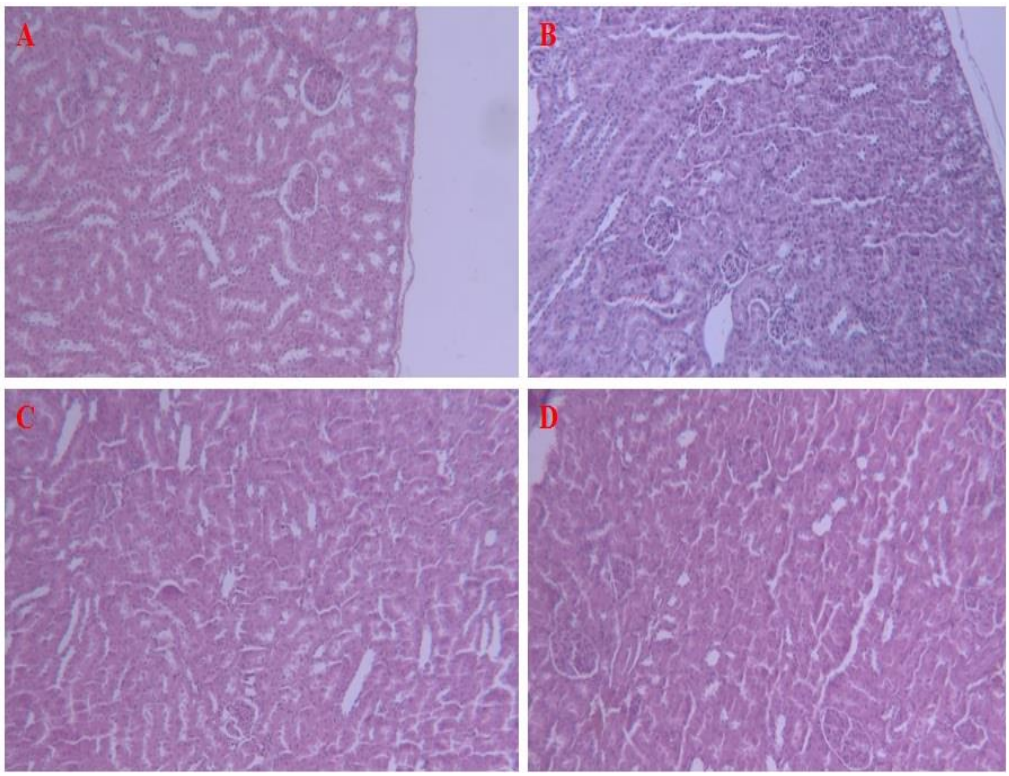

Fig.5- Histopathological findings in the kidney in control and normal treated rats with PHPK. (H&E staining, ×100 magnification) **C0**: Rats received physiological saline by intraperitoneal injection on the first day and fed with normal diet. T1, T2 and T3: Target groups 1, 2 and 3 are normal rats that were fed with normal diet and treated daily with 5 mg/kg, 50 mg/kg and 500 mg/kg of the PHPK, respectively.

C0 Show normal structure (A). **T1-** Show preserved architecture (B). **T2-** Show preserved architecture and protein deposition in some of the tubules (C). **T3** Show preserved architecture, vascular congestion and protein deposition in some of the tubules (D).

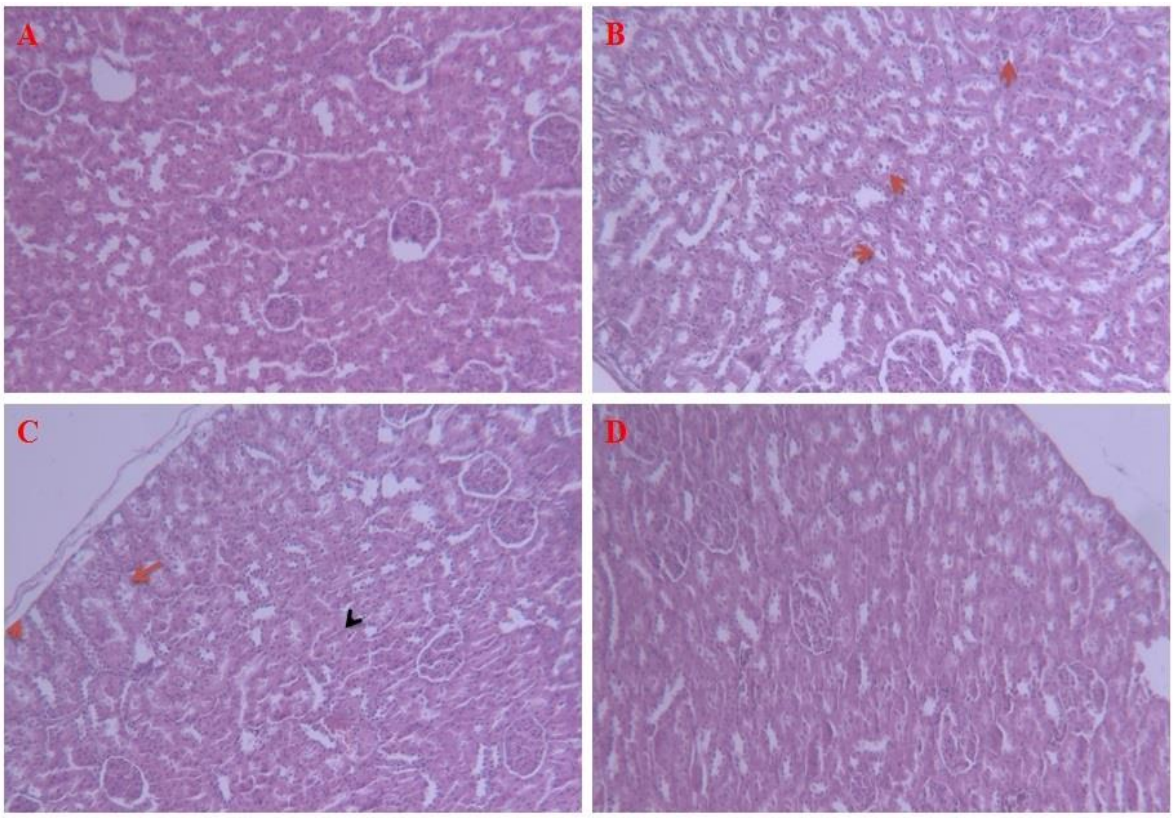

Fig.6- Treatment with PHPK in control and high diet groups (H&E, ×100 magnification_)

C1: Rats were fed with normal diet and received 30% sucrose in the daily drinking water. T4, T5 and T6: Target groups 4, 5 and 6 are normal rats that were received 30% sucrose in their daily drinking water and treated daily with 5 mg/kg, 50 mg/kg and 500 mg/kg of the PHPK, respectively.

C1- Show no pathologic changes, normal glumeruli & tubules (A). **T4**-: Show degeneration of tubular cells and destroyed brush border (less than C2 & T7 groups) (red arrows), along with scattered chronic inflammatory cells in interstitium and congestion of capillaries (B). **T5**- Show preserved architecture, vascular congestion (black arrow), tubular dilatation, minimal degeneration of tubular cells and destroyed brush border (less than T4 Group) (Red arrows) and eosinophilic secretory materials deposition (C). **T6**- Show preserved architecture vascular congestion, tubular dilatation and eosinophilic secretory materials deposition (D).

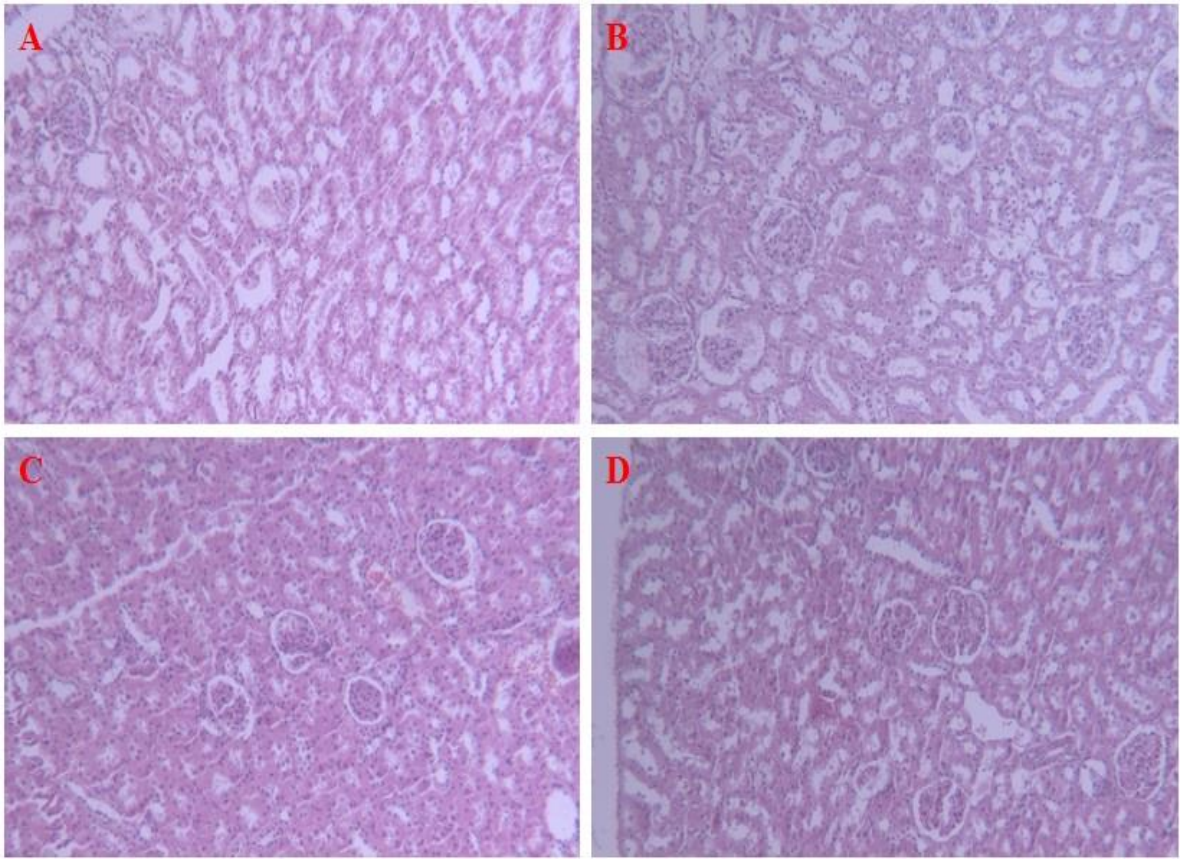

Fig.7- Histopathological findings in the kidney in control and STZ-induced diabetic rats treated with PHPK. (H&E staining, ×100 magnification).

C2: Included STZ-induced diabetic rats fed with normal diet and receiving physiological saline. T7, T8 and T9: Target groups of 7, 8 and 9 that included STZ-induced diabetic rats fed with normal diet and treated daily with 5 mg/kg, 50 mg/kg and 500mg/kg of the PHPK, respectively.

C2: Glomeruli from the diabetic group showed expanded Bowman's space and deposition of eosinophilic material plus distorted glomeruli and dilated glomerular capillaries, and also the tubular epithelial lining displayed many cells with pyknotic nuclei and others with vacuolated cytoplasm (A). **T7-**: Show congested blood vessels mild dilatation of glomerular capillaries and capsular space, intratubular protein deposition and also tubular epithelial lining cells displayed many degenerative cells with pyknotic nuclei and others with vacuolated cytoplasm (less than C2 group), mild focally mononuclear cells infilteration in interstitium (B). **T8-**: Show congested blood vessels only mild dilatation of glomerular capsular space, some intratubular protein deposition and also tubular epithelial lining cells displayed some degenerative cells with pyknotic nuclei (C). **T9-**: Show mild tubular dilatation, intratubular protein deposition and also tubular epithelial lining cells displayed few degenerative cells with pyknotic nuclei (less than T7&T8 groups) (D).

4. Discussion

The PHPK were prepared by using a simple and almost quick enzymatic hydrolysis. Various kinds of plant-derived peptides have been reported in recent years, and even some of them have successful marketing as peptide-based drugs. They are safe compared to other small molecules and drugs for the synthesis of the favorable peptide [26].

Different parts of pistachio are precious sources for preparing new candidates for bio-molecule drugs due to application of bioactive peptides and proteins in the management of diseases such as cancer, obesity and diabetes [27]. Previous studies reported functional effects of plant hormones through different signaling pathways and cellular mechanisms [28].

Proteins were used as the bioactive peptides with more tissues such as kidney affinity to a specific receptor. Besides, most of the bioactive peptides used for the treatment of diabetes have been produced from the recombinant technology, which additionally increases the cost of the treatment [29].

In this study, an economic biomolecule and peptide therapy was conducted using plant-based peptide. We used bioactive protein hydrolysates isolated from Pistachio kernels capable of inhibiting the mechanism of the diabetes. The PHPK are assumed to contribute to insulin secretion. Because in diabetes mellitus, beta cell destruction occurs in the pancreas. Results demonstrated a reduction in the level of creatinine due to recovery in the glomerular filtration and repair of cell damage caused by STZ. The PHPK and the ingredients showed therapeutic potential for kidney injuries preceded by STZ. The hydrolysate protein of green peas protein had therapeutic effects on renal function by antioxidant activity [30].

In all dosages of PHPK, the levels of urea and creatinine were reduced significantly, which is possibly correlated with the better protection of kidneys. The PHPK, like some other protein hydrolysates from natural sources, are widely used in the treatment of many disorders, such as diabetes, because they are safe and efficient and no side effects were observed in the treated animals.

Pistachio has a high level of protein content. In five Iranian pistachio cultivars protein content varies from 16.2% to 20.7 % [31].

In vivo experiments indicated that the protein digestibility of pistachio kernel was high and it acted effectively in renal problems in diabetes.

Increased level of creatinine showed that glomerular filtration rate (GFR) decreased in STZ-treated group. However, the levels of urea and creatinine decreased after PHPK treatment and improvement in the renal function.

Furthermore, on the last day of PHPK treatment in the present experiment, all doses of PHPK treatment groups indicated lower mean creatinine levels in comparison to creatinine level in the STZ-treated group. Our result can be due to the fact that the STZ injection caused severe kidney injuries, and PHPK treatment showed an improvement in renal function.

It has been demonstrated that a meal containing walnut, controls insulin levels in obese people [32].

In another study, Hernández-Alonso et al. (2014) demonstrated that in pre-diabetic patients, fasting glucose, insulin, and HOMA (homeostatic model assessment) of insulin resistance decreased after the consumption of pistachio-supplemented diet compared with the Control Diet [33].

Diabetes mellitus is a global problem that affects a large number of people. Therefore, many herbal medicines have been used to treat this disease in different countries, because herbal remedies can be effective to lower blood sugar alone or in combination with chemicals. Treatment with PHPK improved renal function better than negative and positive control groups.

5. Conclusion

Treatment with PHPK demonstrated a potential diabetes mellitus therapeutic effect. Specifically, higher treatment with dose corresponds to better renal function and better activity through the mechanistic action to reduce creatinine and urea levels in STZ-injected Wistar rats. It is necessary to speed up investigations into the mechanism and molecular targets of PHPK, as plant-based peptides, to use these PHPK as potential drug candidates for further investigations.

Conflict of Interest

All authors declare that they have no conflict of interest.

Acknowledgments

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