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Original article

Therapeutic effect of antihypertensive drug on diabetic nephropathy: Functional and structural kidney investigation

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ABSTRACT

Due to the growth of diabetic mellitus (DM) and diabetic nephropathy as a significant complication for diabetic patients, study on effective treatment with fewer side effects has been fascinated. In this study for the first time carvedilol effects on both function and structure of kidney in diabetic nephropathy treatment were evaluated. Diabetes was induced by injection of streptozotocin (STZ) intravenously in rats and three groups including control, diabetic, and treatment with carvedilol were considered. Biochemical parameters such as, blood glucose level, BUN, creatinine, uric acid, Na^+, K^+ was determined. Results showed that glucose (516 to 291 mg/dl), BUN (42 to 21.67 mg/dl), creatinine (0.75 to 0.6 mg/dl), uric acid (4.45 to 1.36 mg/dl), and K^+ (7.433 to 5.433 mEq/l) level reduced. Decrease in glucose, BUN, creatinine, uric acid, and K^+ and increase in Na^+ level (138 to 146.33 mEq/l) confirmed therapeutic effect of carvedilol. Furthermore, the histopathological study was done for each group. Histopathological results confirmed the data obtained by biochemical parameters. For further investigation, SPECT imaging with ^{99m}Tc-DMSA, which is a gold standard in diabetic nephropathy detection, was done. SPECT imaging showed that accumulation of ^{99m}Tc-DMSA was increased in treated group (5 to 25 kcpm) which means the improvement in renal structure in the treated group compare to the diabetic group (5 kcpm). Finally, obtained results confirmed our hypothesis that carvedilol had a therapeutic effect on diabetic nephropathy.

1. Introduction

Diabetic mellitus (DM (is an endocrine disease that is characterized by a high level of blood glucose (hyperglycemia) and is classified into two types 1 and 2 DM, resulting from defects in insulin action, insulin secretion or both (Kasiewicz and Whitehead, 2017). The prevalence of DM increases in developing countries and is anticipated that the economic burden of DM is rising to USD 845 billion in 2045. Thus, DM is one of the serious disorders that threaten human health and cause a vast socioeconomic burden (Ingelfinger et al., 2017). Hyperglycemia of diabetes is associated with several complications such as tissue-damaging and failure of various organs. Long-term DM will cause cardiovascular diseases, renal failure, and nerve damage. DFUs are a common complication that may occur following the uncontrolled DM and chronic wound and mortality increases as consequences (American Diabetes, 2009). Although sciences have been developed in many aspects (Orooji et al., 2021; Karimi-Maleh et al., 2021; Karimi-Maleh, 2022; Karimi-Maleh, 2021; Ahmadi, 2020; Shirzadi-Ahodashti et al., 2020; Shirzadi-Ahodashti et al., 2020), investigating the drugs for diabetic disorder with few side effects is still required (Souto et al., 2019; Veiseh et al., 2015; Simos et al., 2021). Around 40% of patients with diabetes, faced with diabetic nephropathy when high glucose levels are preserved for long periods. Unique changes happen in kidney structure in diabetic patients, which are the leading cause of kidney disease in patients starting renal replacement (Gross et al., 2005).

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Carvedilol is a nonselective β-blocker improving myocardial function and reversing adverse myocardial remodeling in heart failure. Moreover, carvedilol blocks α_1-adrenergic receptors and declines peripheral vascular resistance (Sica, 2005). Although carvedilol is usually used for hypertension, studies showed that it has efficacy in other diseases. For example, carvedilol affects the sympathetic nervous system, which is overstimulated in diabetes mellitus (Sica and Carvedilol, , 2005). Magadmi & co-workers evaluated the effect of carvedilol on diabetic neuropathy. In this study, an in vitro model of diabetic neuropathy was investigated. Neural viability, morphology, and ATF3 were measured. Results showed that carvedilol protects the neural cells, and reduces the ATF3 expression. They claim that carvedilol demonstrates the neuroprotective effect against high glucose-induced diabetic neuropathy (Magadmi, 2021). Dargie published a clinical trial on the effect of carvedilol on outcome after myocardial infarction. Results showed that cardiovascular mortality and non-fatal myocardial infarctions were also lower on carvedilol than placebo group (Dargie, 2001). Huang *et al.*, reported that carvedilol improved cardiac function in diabetic rats. They claim that carvedilol improved cardiac function by upregulating the expression of Bcl-2 mRNA, recovering activities of antioxidant enzymes (Huang et al., 2007).

With the help of radiology and nuclear medicine, various screening and diagnostic techniques have been developed to improve survival rates. One of the most widely accepted non-invasive sensitive approaches that help physicians to investigate inside the body without surgery is nuclear medicine. It is a potential approach based on physiology and gives us information about the changes in the function of organs (Ilem-Ozdemir et al., 2019; Mirzaei, 2004). Thus, the early diagnosis can occur at an early stage before morphological changes. In most cases, nuclear medicine approaches can identify the abnormalities earlier than other imaging methods like CT, MRI, and Ultrasonic. One of the main advantages of nuclear medicine imaging is the whole-body images, especially when lesions are likely to appear in various places such as cancer (Kramer-Marek and Capala, 2012). Also, nuclear medicine plays an essential role in diagnosing various kidney disorders (Khor et al., 2015; Boubaker, 2006). Because of the ability of the nuclear medicine images to provide information which present the quantification of the function that are not available by anatomical approaches (Mirzaei, 2004). Renal cortical imaging with ^{99m}Tc-DMSA is the gold standard for renal cortical scarring imaging. Gamma radiation, appropriate half-life, availability of ^{99m}Tc made, ^{99m}Tc-DMSA is a radiopharmaceutical commonly used to detect renal scarring or acute pyelonephritis and provide accurate differential renal function (Saleh Farghaly and Mohamed Sayed, 2015).

Since the prevalence of diabetes increases in a developing country, study on a new drug which has lower side effects has fascinated researchers. Although the effect of carvedilol on nephropathy diabetic studied previously (Morsy, 2014), more examination needs to be done to evaluate the effect of carvedilol. For this reason, for the first time we investigated the effect of carvedilol treatment on both structural and functional of diabetic nephropathy by investigating the parameters which are important. Parameters were examined by biochemical factors, histopathology, and SPECT imaging in three groups (control, diabetic, treated with carvedilol). Results showed that carvedilol has a beneficial impact on nephropathy treatment. Also, nuclear imaging confirmed the data obtained by laboratory tests.

2. Material and method

The study was directed in line with the principles of declaration with the number: IR.IAU.AMOL.REC.1400.020. Three normal, diabetic, and diabetic treated by carvedilol groups, in which each group was containing female Wistar rats 180–200 g (animal laboratory-Babol university of medical sciences), were investigated. All experiments were done in triple set.

2.1. STZ-induced diabetic nephropathy

Rats were diabetic after injection of STZ 50 mg/kg, (purchases from Abidi Company) for one week. Then, the blood glucose level of each rat was examined, and the glucose level over 250 mg/dl was considered as a diabetic rat.

2.2. Biochemical, pathological, and SPECT imaging assessment

The study was done in three groups (control, diabetic, and diabetic treated by carvedilol) by following steps: 1- Control group received the normal saline for 30 days (group 1). 2-Diabetic, received the normal saline for 30 days (group 2). 3- Diabetic treated by carvedilol group, received the carvedilol (20 mg/kg) orally for 30 days (group 3). Blood glucose level for each rat was measured by glucometer strip (On Call Plus, China) at the beginning and end of the study. Then each rat was anesthetized by ketamine (80 mg/kg) and xylazine (5 mg/kg) intraperitoneally. Anesthetized rats lying down under SPECT camera (Siemens e.cam gamma camera, equipped with high-resolution collimators, using 128*128 matrix size with a 20% energy window set at 140 keV). SPECT images were acquired 30 min after injection. The rats were scarified 3 h after imaging and different organs were removed and washed with normal saline to clean their surface from blood and residues. Each organ is fixed in formalin buffer 10% (Merck) for 72 h and then dehydrated through a graded-alcohol series. After sectioning, the slides were stained with hematoxylin and eosin (H&E). slides were examined under light microscope and the photomicrographs of them were obtained for assessment of tissue degeneration. For biochemical parameters determination, blood was collected by cardiac puncture and serum was separated and kidney functional factors such as BUN, creatinine, Na^+, K^+, and uric acid were measured.

2.3. Statistical analysis

Statistical analysis was done by SPSS, Microsoft office (2013). For quantitative data analysis one-way analysis of variance followed by Tukey's test was applied. $P < 0.05$ was considered statistically significant.

3. Result

For investigation of carvedilol treatment effect on both structural and functional of diabetic nephropathy many tests which indicate the structure and function performance such as biochemical factors, pathological study, and nuclear medicine imaging were performed.

3.1. Glucose level

The glucose level in each three groups was investigated. Group 1 (control), group 2 (diabetic), and group 3 (diabetic treated by carvedilol) showed the (109 and 84.33 mg/dl), (399.33 and 516 mg/dl), and (551.75 and 291.25 mg/dl) glucose blood concentration at the first day and after one month respectively (Fig. 1). All groups showed a statistically significant difference between others and in each group. ($P < 0.05$).

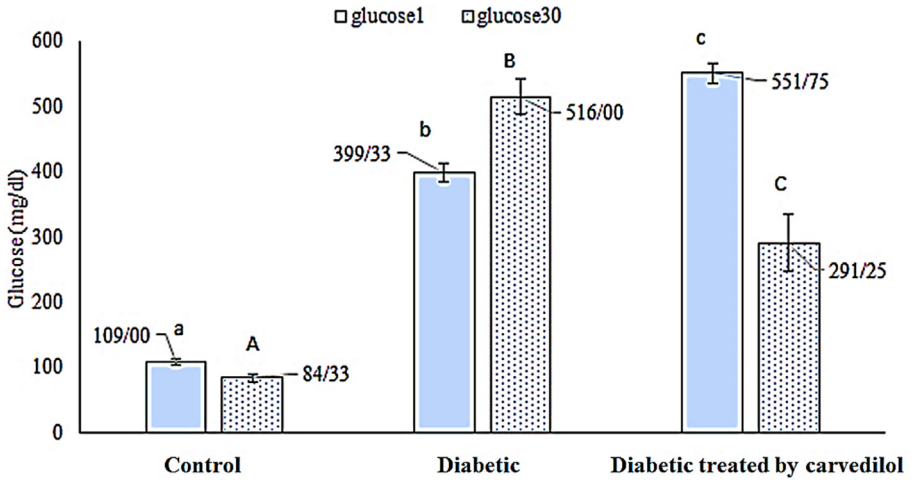

Fig. 1. Blood glucose level in control (109 to 84.33 mg/dl), diabetic (399.33 and 516 mg/dl), and treated by carvedilol (551.75 and 291.25 mg/dl) at first and end of the study (n = 3).

3.2. BUN level

BUN in each control, diabetic, and diabetic treated by carvedilol was determined 19.67, 42, and 21.67 mg/dl respectively. All groups showed a significant difference statistically (P < 0.0001) (Fig. 2).

3.3. Creatinine level

In each three groups, creatinine parameter was estimated 0.5, 0.75, and 0.6 mg/dl in control, diabetic, and treated groups respectively (Fig. 3). P-value < 0.0001 was determined between control and diabetic and P-value < 0.003 was estimated between diabetic and treated groups. The significant difference was observed between groups.

3.4. Uric acid level

Uric acid level in the diabetic group (4.45 mg/dl) was higher than control (1.5 mg/dl) and treated (1.36 mg/dl) groups. There was a significantly difference between control/diabetic and diabetic/treated groups (p < 0.0001), but there was no difference between control and treated groups (Fig. 4).

3.5. Serum sodium level

Fig. 5, shows the serum sodium level estimated in control (144.67 mEq/l), diabetic (138 mEq/l), and treated (146.33 mEq/l) groups. Significant difference was observed between control/diabetic (P-value = 0.006) and diabetic/treated (P-value = 0.002) groups. However, no difference was observed between the control and treated groups.

3.6. Potassium serum level

Fig. 6 shows the potassium serum level including 4.967, 7.433, and 5.433 mEq/l for control, diabetic, and treated groups respectively. Significant difference was observed between control/diabetic (P-value = 0.005) and diabetic/treated (P-value = 0.013) groups. No significant difference was observed between control and treated groups.

3.7. Histopathology study

A kidney histopathology study was done and presented in Fig. 7. Histopathological study with an optical microscope showed that diabetes leads to necrosis in kidney tissue. Also, tubular cells dam-

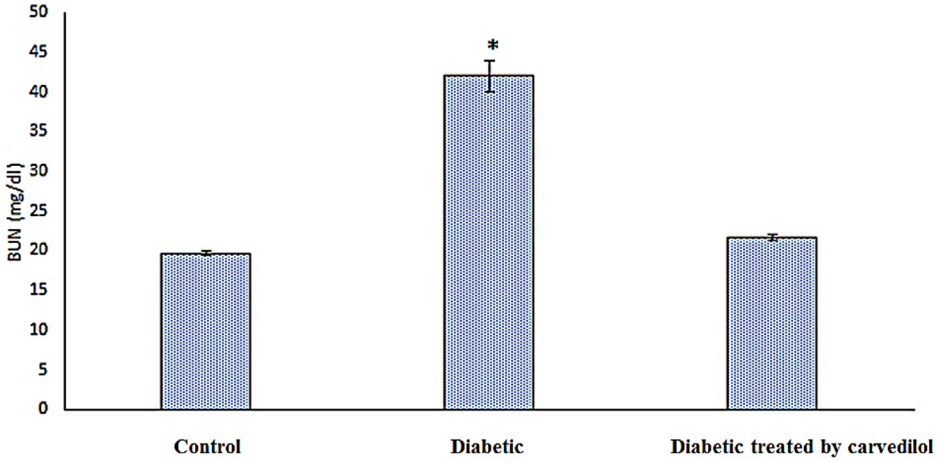

Fig. 2. Blood urea nitrogen (BUN) in control (19.67 mg/dl), diabetic (42 mg/dl), and treated by carvedilol (21.67 mg/dl) groups (n = 3).

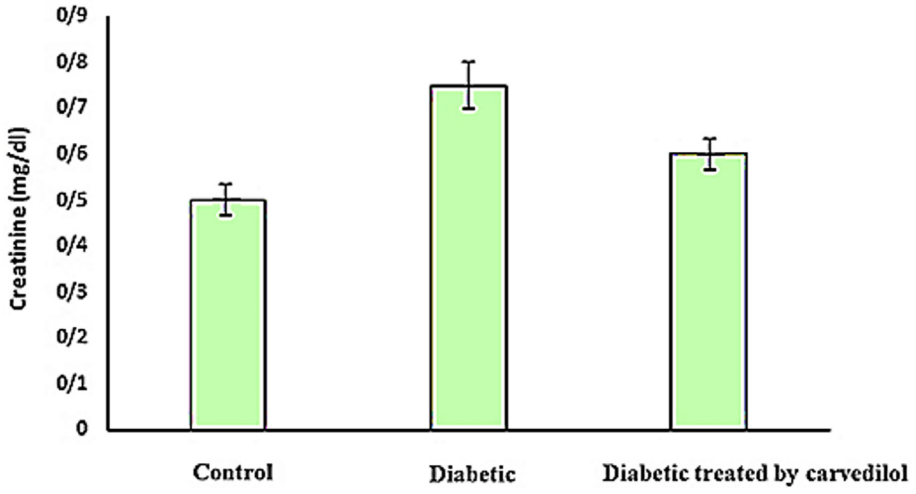

Fig. 3. Creatinine in control (0.5 mg/dl), diabetic (0.75 mg/dl), and treated by carvedilol (0.6 mg/dl) groups (n = 3).

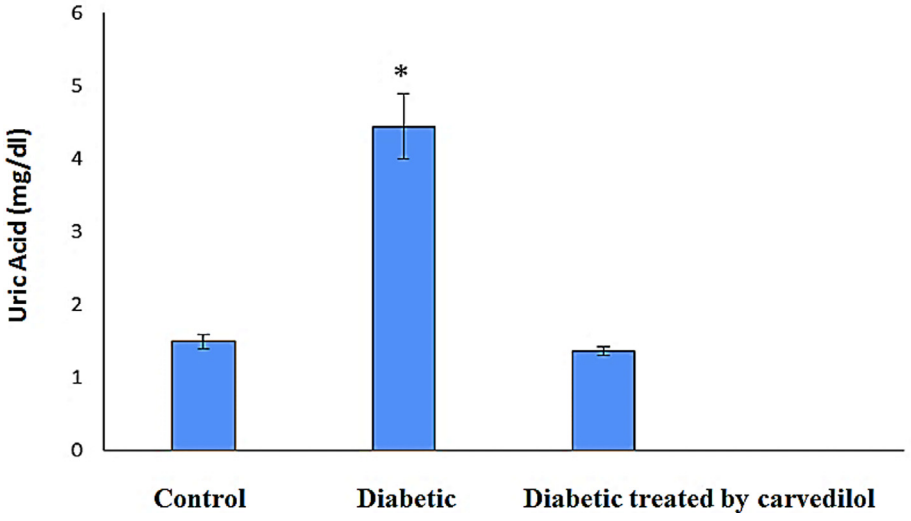

Fig. 4. Uric acid in control (1.5 mg/dl), diabetic (4.45 mg/dl), and treated by carvedilol (1.36 mg/dl) groups (n = 3).

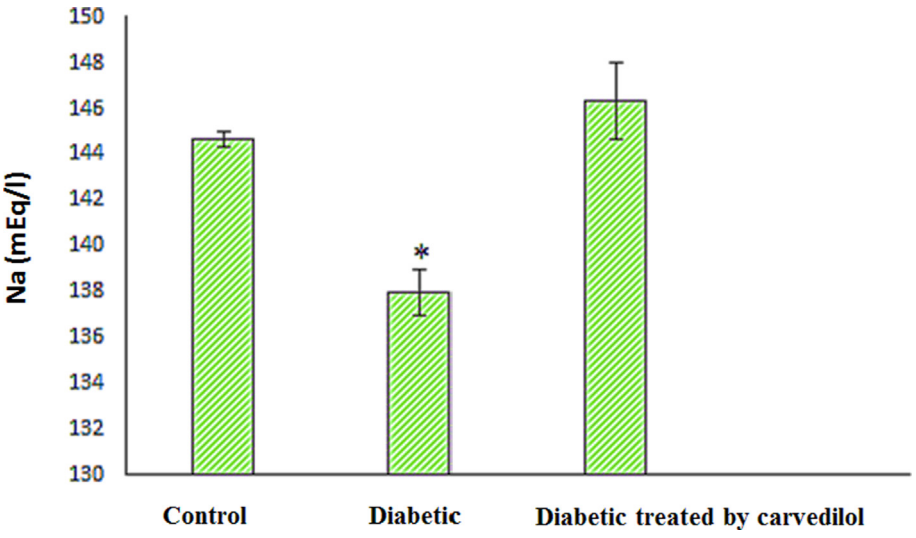

Fig. 5. Serum sodium level in control (144.67 mEq/l), diabetic (138 mEq/l), and treated by carvedilol (146.33 mEq/l) groups (n = 3).

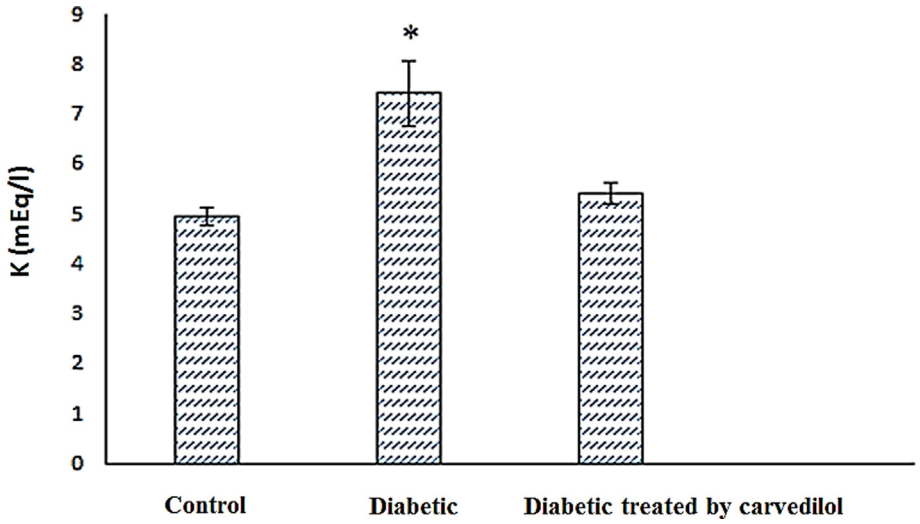

Fig. 6. Serum potassium level in control (4.967 mEq/l), diabetic (7.433 mEq/l), and treated by carvedilol (5.433 mEq/l) groups (n = 3).

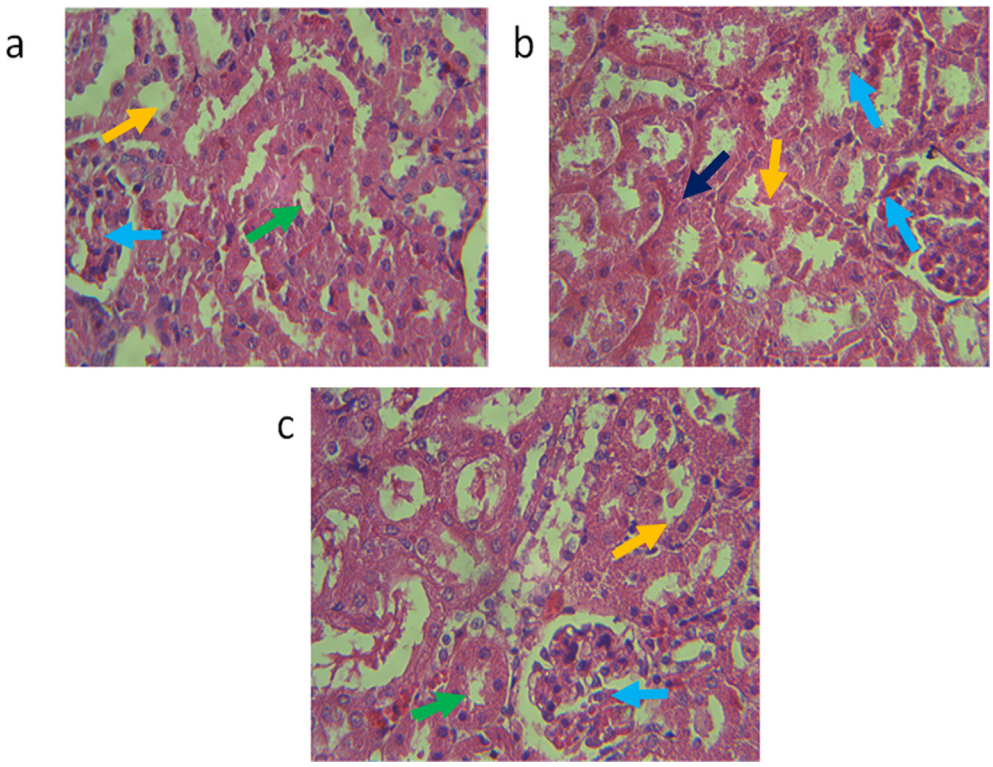

Fig. 7. Kidney histopathology study control (a), diabetic (b), treated by carvedilol (c). Blue, green, yellow arrows indicate the glomerulus, proximal tubule, and distal tubule cells respectively (×40) (n = 3).

aging in proximal and distal tubule and present inflammatory cells are other side effects of diabetes. Treatment with carvedilol decreases the tissue damage and inflammation in tubular cells.

3.8. SPECT imaging

For further investigation, ^{99m}Tc-DMSA renal cortical imaging, which is the gold standard for renal structural imaging was done and presented in Fig. 8. Fig. 8a shows the SPECT imaging in each three groups. As it is apparent, the control group showed acceptable accumulation in both kidneys. In the diabetic group, uptake in both kidneys was reduced due to the nephropathy and accumulation in liver and intestine increased. SPECT imaging by the diabetic treated by carvedilol group, showed that accumulation in both kidneys increased and back to normal range. Also, uptake in the liver and intestine decreased. Fig. 8b, demonstrated the amount of radioactivity accumulated in both (left and right) kidneys. The amount of radioactivity of each organ obtained by selecting a region of interest (ROI) in SPECT images. The significant difference was observed among control/diabetic and diabetic/treated groups. No difference was observed among the treated and control groups.

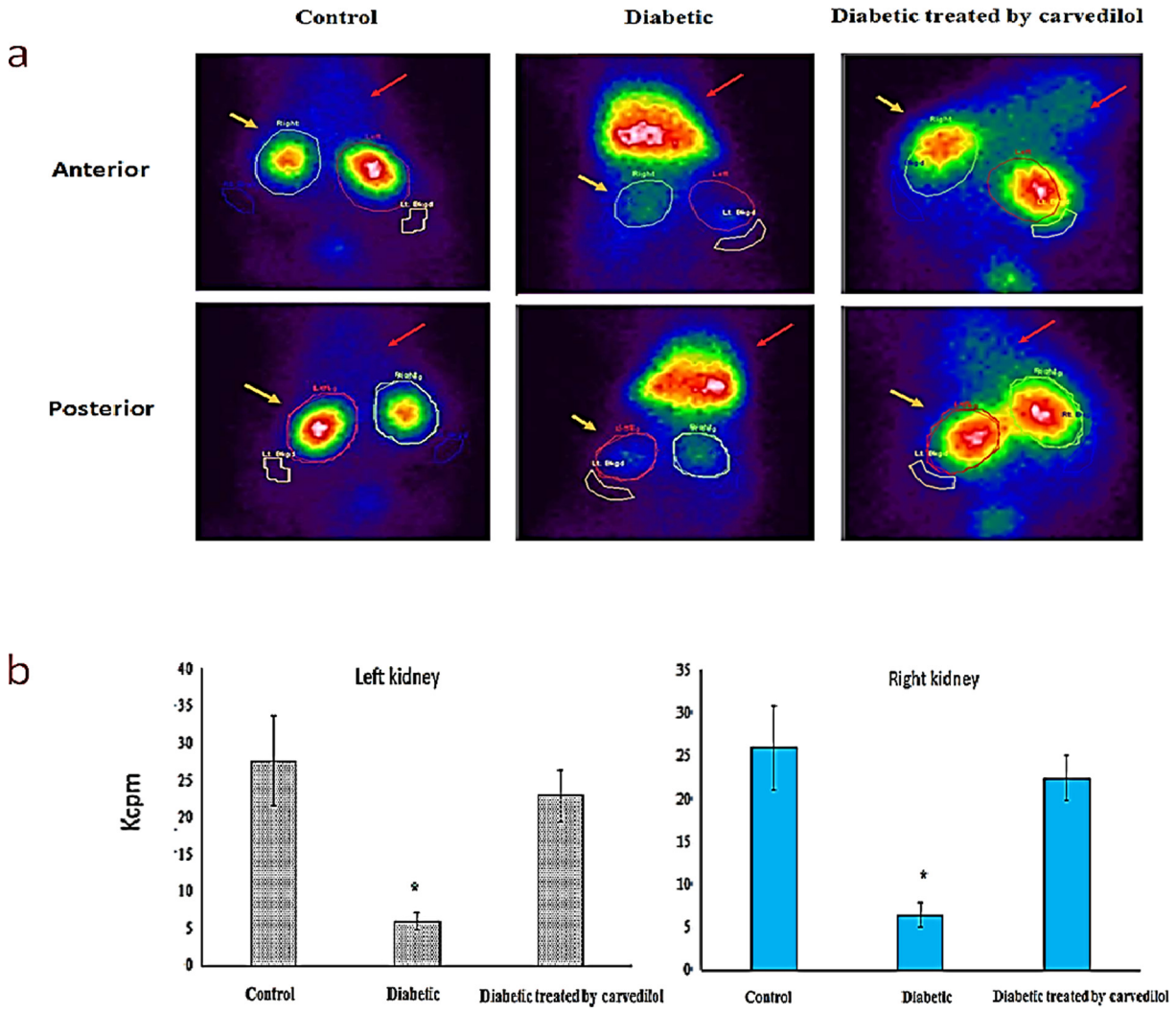

Fig. 8. (a) SPECT imaging in control, diabetic and treated groups at 30 min after injection. Yellow arrow indicate the kidney position and red arrow indicate the heart, liver, intestine and lung. (b) Radioactivity accumulation which is present the uptake of ^{99m}Tc-DMSA in left and right kidney (n = 3).

4. Discussion

Since, DM is one of the serious disorders that threaten human health and cause a vast socioeconomic burden, investigating the cost effective drugs for diabetic disorder with a few side effects is still needed. Great deal of research showed that carvedilol has antioxidant properties (Peeraer, 2011; Arab and El-Sawalhi, 2013). Moreover, incidence of hypertension increases among diabetic patient (Sowers et al., 2001). For those interesting features, carvedilol as an antihypertensive drug was chosen for diabetic nephropathy treatment. In this research, the effect of carvedilol on diabetic nephropathy was investigated by evaluating biochemical parameters, pathological study, and nuclear medicine approach. Statistically significant difference of glucose level was observed between groups and in each group. Differences in glucose level among the control groups maybe because of the changes in the daily diet, while the glucose level were in the normal range. Glucose levels in the diabetic group changed from 399.33 to 516 mg/dl. Increasing in the glucose level confirmed the diabetic process. In contrast to the diabetic group, reduction in glucose level in the treated groups presented the therapeutic effect of carvedilol on diabetic nephropathy. Determination of blood urea nitrogen (BUN) is a simple way to observe kidney function. Excretion of urea nitrogen by the kidney, which is a normal metabolic waste product, disturb in kidney disease and BUN level increases as a consequence (Dabla, 2010). As it is evident in Fig. 2, the BUN level increased in the diabetic group. The treated group showed a reduction in BUN level, and this indicated that carvedilol had a therapeutic effect on diabetic nephropathy. Furthermore, serum creatinine indicates skeletal muscle mass. Although lower serum creatinine is related to improving in the diabetes process (Harita et al., 2009); in diabetic nephropathy, serum creatinine level growth according to the previous study (Roett et al., 2012). The rise in serum creatinine level in diabetic and reduction in the treated group showed that carvedilol had a therapeutic effect on a diabetic nephropathy. Moreover, uric acid is another biochemical parameter that is investigated in diabetic patients. Uric acid is synthesized in the liver by various pathways. Uric acid that is produced by liver, releases to blood circulation and then filtered by glomerulus. Undersecretion of uric acid in diabetic nephropathy leads to an elevation in uric acid serum level (Jalal et al., 2011). Notable decrease in uric acid level in the treated group compared to the diabetic group indicated that carvedilol had a therapeutic effect on diabetic nephropathy. According to the previous study (Liamis, 2014); hyperglycemic leads to an increase in osmolality, which results in water movement out of the cell, and then dilution causes a reduction in serum sodium levels. As it is clear in Fig. 5, carvedilol caused elevation in serum sodium level in the treated groups and

turn the level back to normal. Additionally, serum potassium level was estimated in each three group. As it is apparent in Fig. 6, the serum potassium level increased in the diabetic group. Hypertonicity and insulin deficiency cause by hyperglycemia contributes to the reducing of potassium transport to the intracellular space (Bianchi et al., 2019). Reduction in serum potassium level in the treated group and return the potassium level to the normal, illustrated that carvedilol had a therapeutic effect on diabetic nephropathy. Histopathological investigation with an optical microscope showed that diabetes leads to necrosis in kidney tissue. Moreover, cells damaging in proximal and distal tubules and the presence of inflammatory cells are other side effects of diabetes. It is evident from the data obtained by pathological study that treating with carvedilol reduces the diabetic side effect. The nuclear medicine approach, which is a gold standard test in diabetic nephropathy structural detection, was used to investigate the renal cortical structure in each three groups. All data obtained by nuclear imaging confirmed our hypothesis that carvedilol had a therapeutic effect and improve structural of diabetes nephropathy and lead to reducing glucose level. All in all, our results showed that, treatment with carvedilol had a promising effect on both functional and structural diabetic nephropathies. Antioxidant, vasodilatory, and anti-inflammatory properties of carvedilol which are published previously are likely to be the mechanism of nephropathy prevention (Hayashi et al., 2010).

5. Conclusion

In conclusion, our results showed that carvedilol had a renal therapeutic effect on STZ-induced diabetic nephropathy in rats. Histopathological study and biochemical factors were in agreement. SPECT imaging with ^{99m}Tc-DMSA, which is the gold standard in the detection of nephropathy, confirmed that carvedilol had a therapeutic effect on diabetic nephropathy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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