Protective effect of Vitamin D against type II diabetic nephropathy in rats: a possible role of adropin

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Abstract

Background: Diabetic nephropathy (DN) is a common complication among type II diabetic patients which may lead to end stage renal disease. Oxidative stress has been found to play a part in the pathophysiology of DN. Several studies have demonstrated the effective nephroprotective role of vitamin D to counteract the progression of DN, although the exact mechanisms are not yet fully understood. Adropin release has been recently linked to be one of the vitamin D effects and was reported to exert its antioxidant effects via nuclear factor erythroid 2-related factor 2 (Nrf2). **Aim:** To examine the nephroprotective effects of Vitamin D focusing on adropin-Nrf2 axis as one of the possible underlying mechanisms of vitamin D in rats. **Materials and Methods:** Thirty two albino male adult rats were used in this experiment. Rats were randomly and equally divided into four groups:(GI) was the control, (GII) received vitamin D, (GIII) was the diabetic model, and (GIV) was the diabetes+ vitamin D group. **Results:** Rats that received vitamin D (0.03 µg/kg/day) for 8 weeks revealed significant lower insulin resistance and oxidative stress state, a significant improvement of kidney dysfunction that was confirmed with histopathological examinations for kidney, and significantly higher levels of serum adropin in association with a significant higher renal mRNA expression of Nrf2. **Conclusion:** Vitamin D administration has a renoprotective effect in DN in type II diabetic rats. The antioxidant effects of vitamin D may be in part related to the adropin- Nrf2 axis.

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1. Introduction

Diabetes Mellitus (DM) is amajor global metabolic disorder with ever-increasing incidence and prevalence. [1]. One of the commonest longstanding microvascular complications of DM is diabetic nephropathy (DN), which affects up to 30-40% of type II diabetic patients [2] [3][4].Studies demonstrated that diabetic patients with early onset kidney involvement can suffer a shortenedlife span by up to sixteen years [5].

The pathophysiology of DN is multifactorial. However, a hyperglycemic state is suggested to be the initiating event. This event causes colossal quantities of reactive oxygen species (ROS) to be generated, which subsequently resultsin reduction in the body's ROS scavenging ability. Ultimately, thisoxidative stress state contributes to the pathogenesis of DN [6].

Vitamin D isa fat-soluble vitamin and a prohormone involved in various bodily functions.Its deficiency plays a part in the pathophysiology of type II DM and its complications including DN [7][8][9]. Some studies noted that the use of vitamin D supplementations reduced the chronic inflammation and albuminuria associated with diabetic kidney disease [10]. On that note, many studies have suggested the role of maintaining vitamin D supplementation in patients with DN, although its mechanisms on how it improves overall kidney function have not yet been fully understood.

The energy homeostasis-associated gene (ENHO) codes adropin, a peptide hormone produced by various bodily tissues including kidney, heart, brain, and the liver [11]. A lot of attention has been centered around adropin due to its effects on glucose homeostasis, lipid homeostasis, and insulin resistance [12]. A link between adropin deficiency, elevated adiposity, as well as insulin resistance had been studied. Adropin has demonstrated protective properties against hepatosteatosis and hyperinsulinemia associated with obesity [13]. Interestingly, recent studies have shown reduced adropin serum levels in vitamin D deficient patients, and that vitamin D supplementations stimulates the expression of ENHO gene [14][15].

A transcription factor that exhibits antioxidant properties is nuclear factor erythroid 2-related factor 2 (Nrf2). In the presence of oxidative stress, Nrf2 will translocate to the cell's nucleus to begin transcription of antioxidant genes and initiate expression of the corresponding proteins, thereby suggesting that Nrf2 is required in the defense against oxidative stress [16]. Various studies have found that Nrf2 can overcome DN induced glucolipotoxicity, and failure to maintain it, suggests that the kidneys have progressed to end stage renal disease (ESRD) [17].Moreover, previous studies show that Nrf2 is one of the antioxidative components that are incorporated within the antioxidant function of adropin [18] [19].

Based on these reports, we hypothesized that vitamin D could induce adropin secretion and enhance Nrf2 expression within the kidney, ameliorating DN in a type II diabetic rat model.

2. Materials and Methods

2.1. Diet and Chemicals

Two types of diets were made, the standard and high-fat diet (HFD). Both diets were prepared in the form of pellets, which were provided by the faculty of Agriculture, Moshtohor,

Egypt. Ingredients % of standard diet were (Soy beans 18.6%, Yellow corn 71.6% and Fat 9.8 %), while ingredients % of high fat diet were (Soy beans 16.5%, Yellow corn 34% and Fat 49.5 %) [20]. Streptozotocin (STZ) powder (Sigma Aldrich, St. Louis, MO, USA). One Alpha, the active ingredient is alfacalcidol was provided as oral drops (2 mcg/ml) (LEO Pharmaceutical Products, Ballerup, Denmark).

2.2. Animals

This study included 32 Wistar Albino adult male rats that were between the ages of 8 - 10 weeks old and weighed between 130-170g. Animals had been bought from the faculty of medicine animal house located in Zagazig University, Egypt. Animals were maintained under normal laboratory and experimental conditions. They were placed in cages and had easy accessibility to water and their assigned diet based on the study. Cages had a stable temperature of 25˚ C with a 12- hour dark and light cycles. Experiments were approved by the Institutional Ethical Committee for Animal Care and Use of the Faculty of Medicine, Benha University(Approval No. MS 27-4-2021). After study completion, animals were disposed of via an incinerator.

All animals were acclimatized to the laboratory setting one week prior to conducting the experiment. Categorization of rats was done randomly into four equal groups of eight.

Group (I):(Control group):rats received normal diet for 12 weeks + olive oil was given orally daily via gavage during last 8 weeks.

Group (II):(Vitamin D group): rats received normal diet for 12 weeks +(0.03 µg/kg/day) vitamin D dissolved in olive oil was given orally via gavage during the last 8 weeks [21].

Group (III):(DM group): A high fat diet (HFD) was administered for 12 weeks to induce DM. After four weeks, rats in this group received a single injection of low dose streptozotocin (STZ, 35 mg/kg) intraperitoneally (i.p)[20].

Group (IV): (DM +vitamin D group): In this group diabetes was induced by the same protocol used in group III + (0.03 µg/kg/day) vitamin D dissolved in olive oil was given orally in the last 8 weeks [21].

2.3. Induction of type II diabetes mellitus

To create a type II diabetic model, rats were given HFD for 12 weeks. After four weeks of diet manipulation, these animals were administered one low dose of STZ (35 mg/kg) dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) via intraperitoneal (i.p) injection [20]. Following 48 hours, blood glucose levels from a tail vein were measured using the Glucotrend 2 glucometer. Moderate hyperglycemic (non-fasting blood glucose $\geq$ 250 mg/dl) animals were labeled as the diabetic model and wereincorporated in the study which lasted for eight weeks after induction of diabetes [21].

2.4. Collection of 24-hour urine

Prior to samples collections and scarification (end of the 12th week), rats were individually placed in a special metallic cage with a tight wire grid floor for 24 hours to calculate urine output (starting from 10:00 am to 10 a.m. next day). A plastic dragger placed underneath the cage was used to obtain urine samples via a collecting bottle. The urine volume was measured and centrifuged for five minutes at 1500 rpm. At a temperature of -20°C, the clear supernatant was stored for further biochemical analysis.

2.5. Blood and tissue sampling

All animals in all experimental groups fasted overnight and weighed prior to undergoing anesthesia. Anesthesia was performed using urethane (1.5 g/kg; i.p.). The abdominal aorta was used to obtain blood samples which were collected in test tubes and left to clot at room temperature. Using the centrifuge (3000 rpm for 15 minutes) the sera were separated. Sera was stored at – 20˚C for further analysis.

Both kidneys were excised rapidly and removed immediately after euthanization via decapitation. Using ice-cold 0.9% saline, the right kidney was washed and subsequently cut into two halves which were stored at -80°C. Half of the kidney was used for measuringthe total antioxidant (TAO) capacity and malondialdehyde (MDA), while the second half was used for quantitative real time PCR analysis of Nrf2 gene expression. Left kidney was preserved in 10% buffered formalin solution (pH 7.8) for histopathological examination.

2.6. Biochemical analysis

Based on manufacturing guidelines, the collected sera and urine were analyzed for the following parameters:

(1)Colorimetric Assay kits (Spinreact, Spain) for serum glucose

(2)INS-ELIZA kits, (Biosource, Belgium) for serum insulin values

(3) Colorimetric commercial kits (Centronic GmbH, Germany) for serum urea and creatinine

(4) Adropin ELISA Kit, (MyBioSource, USA) for serum Adropin

(5)Quantitative diagnostic commercial kits (Sigma Aldrich, St. Louis, MO, USA) for urinary albumin

(6)Urinary creatinine was measured using the colorimetric commercial kits (Centronic GmbH, Germany).

Part of the kidney tissue was stored at -80°C and used to detect oxidative stress markers based on the guidelines of the manufacturer. Kidney total anti-oxidant capacity (TAO) was measured using colorimetric kits (BioDiagnostic, Egypt).Kidney malondialdehyde (MDA) was measured using colorimetric kits (BioDiagnostic, Egypt).

Insulin resistance index was calculated using the homeostasis model assessment index for insulin resistance ($HOMA_{IR}$) using this formula: $HOMA_{IR}$ = Fasting glucose (mg/dL) × Fasting insulin (μU/mL)/ 405 **[22]**.

Creatinine clearance (Ccr) was determined using the following formula: Concentration of creatinine in urine (mg/dl) ×Volume of urine (ml/min) /Concentration of creatinine in serum (mg/dl) as described by **[23]**.

2.7. Quantitative real time PCR analysis of Nrf2Gene expression

Gene expression levels of Nrf2 in kidney samples were estimated using Real-time quantitative reverse transcription–polymerase chain reaction (RT–qPCR). Using the Total RNA Purification Kit Cat No. GZR100, the total RNA extraction from the kidney sample was determined. Reverse transcription (RT) of RNA into complementary DNA (cDNA) was done in a Veriti™ Thermal Cycler (Applied Biosystems), using TOPscript™ RT DryMIX(dN18-dN6 plus) Kit (enzynomics, Korea). To each RT tube supplied; 5 μL RNA template and 20 μL nuclease-free water were added. qRT-PCR for detection of Nrf2 gene expression Using Hera SYBR Green

qPCR kit. The sequences of the primer pairs were as indicated in **Table (1).**Following the correction of GADPH expression, he mRNA expression of each sample was determined. The relative expression was calculated using the $2^{-\Delta\Delta CT}$ method **[24]**, and results were expressed as the n-fold difference relative to the control.

Table (1): RT-PCR primer sequences

Primer	Sequence
Nrf2	Forward 5'-GCTATTTTCCATTCCCGAGTTAC-3' Reverse 5'-ATTGCTGTCCATCTCTGTCAG-3'
GADPH	Forward 5'-AGC TGA ACG GGA AGC TCA CT-3' Reverse 5'-CAT TGA GAG CAA TGC CAG CC-3'

2.8. Histopathological examination

Left kidney samples were initially fixed in a 10 % formalin solution then, dehydrated and embedded in paraffin. 5-µm-thick sections were cut using a microtome. These sections were mounted onto glass slides and stained using two staining techniques: periodic acid-Schiff (PAS) and hematoxylin and eosin (H&E). Samples were examined using light microscopy. PAS stain was used to visualize any glomerular damage, and the glomerulosclerotic injury was graded. Grading system was as follows: **0**: normal glomeruli, **1**: lesions affecting 25% of glomerular area (mild sclerosis), **2**: 25–50% affection (moderate sclerosis), and **3**: 75% affection (severe sclerosis) **[25]**.

2.9. Statistical analysis.

Statistical Package for Social Sciences version 19 (SPSS Inc, Chicago, IL, USA) was used to analyze all data, which were demonstrated as the mean ± standard deviation (SD). Comparisons among the groups were assessed using Bonferroni's Multiple Comparison Test and one-way Analysis of Variance (ANOVA). P value < 0.05 were considered significant.

3. RESULTS:

3.1. Vitamin D improved insulin resistance without affecting body weight in type II diabetic rats:

Regarding body weight and insulin resistance, type II diabetic rats had significantly greater body weight, fasting blood glucose, serum insulin levels, and $HOMA_{IR}$. Administration of 0.03 µg/kg vitamin D daily for 8 weeks in DM+ Vitamin D group caused a significant decline (P < 0.05) in fasting blood glucose, serum insulin level, and $HOMA_{IR}$ without reduction of body weight compared to the DM group **(Table 2)**.

3.2. Vitamin D ameliorated kidney function parameters in type II diabetic rats

Induction of type II diabetes produced a significant increase (P < 0.05) in serum urea and creatinine and urinary albumin excretion associated with a significant decline (P < 0.05) in urinary creatinine and creatinine clearance as compared with control group. Diabetic rats received 0.03 µg/kg vitamin D daily for eight weeks in DM+ Vitamin D group showed a significant reduction (P < 0.05) in serum creatinine, serum urea, and urinary albumin excretion and a significant increase (P < 0.05) in urinary creatinine and creatinine clearance when compared to diabetic group **(Table 3).**

3.3 Vitamin D increased serum adropin and improved renal oxidative stress state:

We found a significant elevation (P < 0.05) in serum adropin in both non-diabetic rats which recieved vitamin D 0.03 µg/kg, and diabetic rats which received 0.03 µg/kg vitamin D daily for 8 weeks in DM+ Vitamin D group. A lower (P <

0.05) level of renal TAO as well as a significant higher (P < 0.05) level of renal MDA were detected in diabetic rats, however these results were ameliorated significantly (P < 0.05) in

diabetic rats received 0.03 µg/kg vitamin D daily for 8 weeks in DM+ Vitamin D group when compared to diabetic group **(Table 4)**.

Table (2): Body weight, fasting glucose, insulin and HOMA$_{IR}$ amongst the study groups:

	Control	Vitamin D	DM	DM+ Vitamin D
Initial weight (g)	153.13±10.67	150±17.53	148.75±13.3	150±16.26
Final weight (g)	213.13±12.23	216.88±9.23	413.75±18.66*	419.88±23.97*
Glucose (mg/dl)	84.04±9.13	79.81 ±6.75	275.20±30.86*	214.36 ±5.59*†
Insulin (µU/ml)	2.08±0.69	2.26±0.97	31.50± 3.12*	6.16±0.86*†
HOMA $_{IR}$	0.43±0.15	0.45± 0.22	21.41 ±3.63*	3.21 ±0.49*†

HOMA $_{IR}$, homeostasis model assessment of insulin resistance. Data is expressed as mean ± standard deviation, (n=8), *P< 0.05* is significant tested by using One-way analysis of variance (ANOVA) test and and post hoc multiple comparisons (LSD). * Significant difference vs the control group † Significant difference vs the diabetic group.

Table (3): Serum creatinine/ urea, urinary creatinine/ albumin excretion, and CCr amongst the study groups:

	Control	Vitamin D	DM	DM+ Vitamin D
Serum creatinine (mg/dl)	0.69±0.25	0.65 ± 0.21	1.95±0.43*	0.93 ±0.13*†
Serum urea (mg/dl)	30.39±8.52	32.09 ± 7.99	52.81±5.49*	40.5 ±4.75*†
Urinary Creatinine (mg/dl)	41.63 ±2.56	40.25±2.05	22.75±2.55 *	33.13±2.64*†
Urinary albumin excretion (mg/24h)	0.4 ± 0.06	0.43 ± 0.12	4 ± 0.31 *	1.1 ± 0.15*†
CCr(ml/min)	0.94±0.17	0.85±0.19	0.15±0.08*	0.66±0.17*†

CCr, Creatinine clearance. Data is expressed as mean ± standard deviation, (n=8), *P< 0.05* is significant tested by using One-way analysis of variance (ANOVA) test and and post hoc multiple comparisons (LSD). * Significant difference vs the control group † Significant difference vs the diabetic group.

Table (4): Serum adropin and renal oxidative stress markers amongst the study groups:

	Control	Vitamin D	DM	DM+ Vitamin D
Serum Adropin (pg/ml)	43.24±2.10	48.12 ±3.04*	32.20±1.54*	49.51 ±2.23*†
TAO (nmol/g tissue)	7.47±0.44	8.64±0.57	1.62±0.28*	5.24±0.66*†
MDA(nmol/g tissue)	16.18±0.87	13.99±0.59	42.7±3.09*	26.01±0.89*†

TAO; total antioxidant, MDA; malondialdehyde. Data is expressed as mean ± standard deviation, (n=8), *P< 0.05* is significant tested by using One-way analysis of variance (ANOVA) test and post hoc multiple comparisons (LSD). * Significant difference vs the control group † Significant difference vs the diabetic group

3.4. Vitamin D increased renal Nrf2 relative mRNA expression in renal tissue in rats

Vitamin D administration 0.03 µg/kg daily for 8 weeks significantly upregulated (P < 0.05) renal Nrf2 mRNA expression in both non-diabetic

rats got vitamin D in vitamin D group and diabetic rats received vitamin D in DM+ Vitamin D group compared with the control group and diabetic group respectively (**Figure 3-a**).

3.4. Effects of vitamin D on histopathological changes of renal tissues in rats

Histopathological examination of kidney specimens using H&E and PAS stains revealed normal architecture, glomerular size and basement membrane thickness in control and non-diabetic rats received Vitamin D (**Figures 1a-b, 2a-b**).According to H&E-stained kidney specimens,the diabetic rats demonstrated marked shrinkage of the glomerular tufts, marked thickening of basement membrane, increased Bowman's space and dilation of the tubular system (**Figure 1-c**). However, diabetic rats received vitamin D (0.03 µg/kg) daily for 8 weeks their kidney specimens' examination revealed mild thickening of basement membrane and glomerular shrinkage (**Figure 1-d**). Kidney specimens stained with PAS exhibited severe glomerulosclerosis (grade 3) in diabetic rats (**Figures 2-c, 3b**).However, in the diabetic rats that received vitamin D, kidney specimens' examination revealed that kidney morphology had been improved; the glomeruli showed minimal to mild sclerosis (grade 1) (**Figures 2-d, 3b**).
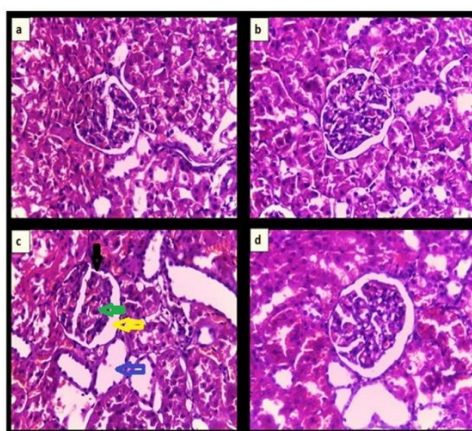

Figure (1) Light microscopy of H & E-stained renal specimens. (Magnification X400). **(a)** Control group, **(b)** Vitamin D group, both showing normal glomerular and tubular structures, **(c)** DM group, rats depict shrinking of the glomerular tufts (green arrow), thickening of basement membrane (black arrow), increase of Bowman's space (yellow arrow) and dilations of kidney tubules (blue arrow), **(d)** DM+ Vitamin D group depict improvement of glomeruli and tubules.
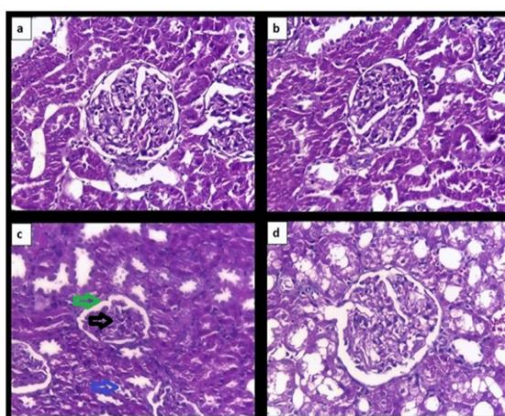

Figure (2) Light microscopy of PAS-stained renal sections. (Magnification X400). (a) Control group, (b) Vitamin D group, both depict normal glomerular and tubular structures, (c) DM group, the rats showed Synechia of the glomerular tufts (severe sclerosis more than 70 %) (black arrow), thickening of basement membrane (green arrow), shedding of tubular epithelium (blue arrow), (d) DM+ Vitamin D group depict improvement of glomeruli and tubules.
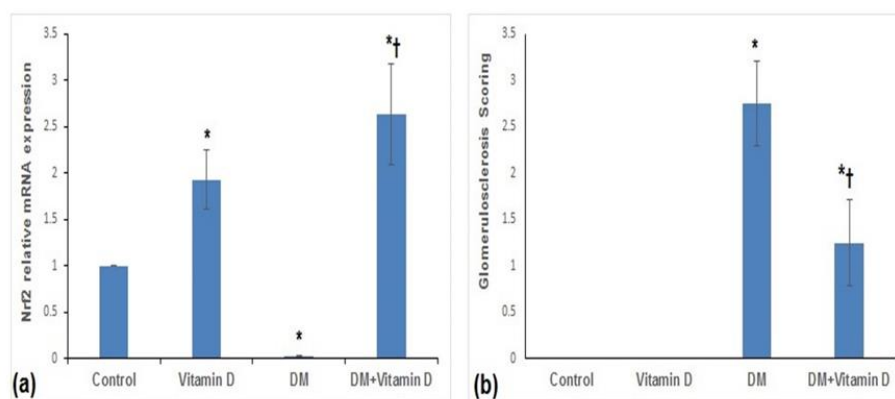
Figure (3): (a) Fold changes in Nrf2 relative mRNA expression, (b) Glomerulosclerosis scoring

Discussion

Complications of diabetes is a rising global health concern socially, economically, and physically on the patient and health care systems. DN has become the second leading cause of ESRD worldwide next to glomerulonephritis **[4],** therefore, prevention or slowing the progression of DN is mandatory for patients with DM.

This study analyzed the renoprotective effects of vitamin D against DN in a HFD/ low-dose STZ-induced type II DM rat model. Our results showed that diabetic rats received vitamin D had improved insulin resistance, reduced renal oxidative stress, enhanced kidney function as well as the pathological renal lesions, increased serum adropin, and increased renal mRNA expression levels of Nrf2 in rats.

Type II DM was confirmed by a significant elevation in serum glucose and insulin levels as well as $HOMA_{IR}$ index, after being fed with the HFD for 12 weeks/ a single low dose of STZ (35 mg/kg) compared to the control rats. According to some studies, the HFD as well as the administration of low dose of STZ to the animals in the study group were capable of inducing insulin resistance and significantly elevating blood glucose values respectively, very similar to the values in human patients with type II diabetes **[26][27].**

Kidney damage was assessed based on the values of renal laboratory parameters including serum creatinine, serum urea, urinary albumin excretion, and creatinine clearance. The animals in the study group demonstrated renal damage which was initially determined by the elevated serum creatinine, serum urea, urinary albumin excretion, and declining values of creatinine clearance when compared to the control rats. A primary indicator of early onset DN induced kidney damage is the presence of albumin in the urine **[28],** also Creatinine clearance is another key indicator of renal function as it correlates with glomerular filtration rate (GFR) **[29]**. Moreover, in the diabetic group, interestingly, histopathological analysis confirmed that damage of the kidney tissue, as it showed marked shrinkage of glomerular tufts, marked thickening of basement membrane, increase of Bowman's space, dilations of kidney tubules and severe glomerular sclerosis, these results are in accordance with previous works **[30] [31]** who reveled deterioration of kidney function tests associated with alterations of the histological architecture in DN rat models.

Oxidative stress is a common product of many pathways that are involved in the pathogenesis of DN[32]. Elevated ROS as a result of hyperglycemia stimulates the production of growth factors, cytokines, and transcription factors implicated in the development of DN [33]. Such factors cause the structural and functional changes of the kidney, which ultimately result in glomerulosclerosis and ESRD [34]. Our study revealed that the diabetic rats had increased oxidative stress, which was determined by the increased levels of lipid peroxidation indicated by higher MDA levels and reduced TAO capacity in the kidney. Our findings coincide with **Ni et al. [35] & Tabassum and Mahboob [36]**, who reported higher renal levels of MDA associated with lower levels of different antioxidant enzymes in type II diabetic rats.

Moreover, results of the current work revealed lower levels of serum adropin and renal Nrf2 mRNA expression levels in diabetic rat model. In support of these results **Wu et al. [37] & Hu and Chen [38]** have demonstrated reduced concentrations of serum adropin in patients with type II DM, which showed a negative correlation with blood creatinine and a positive association with GFR.Interestingly,**Es-Haghi et al. [39]** have demonstrated that reduced adropin levels is associated with renal dysfunction in type II patients and suggested that serum adropin concentrations may be used as a biomarker for early DN detection. **Shelest et al. [40]** also stated similar results and mentioned that the serum adropin values may aid in determining the progression of DN.

In contrast **Ugur et al. [41] &Hosseini et al. [42]** revealed greater adropin levels in type II

diabetic patients and mentioned a positive correlation between adropin and serum creatinine and microalbuminuria levels. They argued that to increased activity of this peptide may be associated with renal homeostasis at the initial phase of DN and also might be due to a feedback mechanism or in response to their medications. Regarding renal Nrf2 expression levels our results coincide with **Kim et al. [37] &Wu et al. [43]** who showed reduced expression levels of Nrf2 and increased oxidative stress in DN of type II diabetic rats, and **Yoh et al. [44]** who reported that Nrf2$^{-/-}$ diabetic mice had elevated ROS production and suffered far greater renal damage in comparison to the Nrf2$^{+/+}$ diabetic mice.

Diabetic rats that received vitamin D (0.03 µg/kg/day) for 8 weeks showed a reduction of the hyperglycemia, hyperinsulinemia and HOMA$_{IR}$ when compared to the untreated diabetic one without affecting body weight. This was similarly observed by**Mohair et al 2020 [45] & Zoair, 2021 [46].** Such effects of vitamin D were explained by **Johansen et al. [47]** who mentioned that Vitamin D has favorable effects on insulin activity directly via insulin receptors or indirectly via extracellular calcium regulation. Calcium is a key component during insulin-mediated intracellular processes in insulin-responsive tissues. Alterations in Ca^{2+} levels, mainly in insulin targeted tissues may contribute to peripheral insulin resistance via impaired insulin signaling, causing reduced glucose transporter-4 (GLUT-4) activity.

Consistent with our results several experimental studies have shown that vitamin D administration protects against progression of DN, **[48] [49] [50],** however, these reports mainly targeted varying mechanisms such as modulation

of autophagy, anti-inflammatory mediators, and antioxidant enzymes.Our results showed amelioration of the kidney dysfunctions, specifically there was a reduction in serum creatinine, serum urea, and urinary albumin excretion, and an elevation in creatinine clearance levels in the diabetic group received vitamin D as compared with diabetic group. Interestingly, all these changes were confirmed by the improvement in the histopathological characteristics of DN, examination of kidney specimens revealed mild thickening of basement membrane and glomerular shrinkage and the glomeruli showed minimal to mild sclerosis, moreover, there was an increase in the TAO capacity and suppression of lipid peroxidation in diabetic rats received vitamin D. Amelioration of the oxidative stress could be related to the improvement of the glycemic state and the well-known antioxidant effects of vitamin D, subsequently the enhancement of the kidney dysfunction may be even in part related to the reversed oxidative stress state observed in the kidney.

We focused on serum adropin and mRNA expression level of renal Nrf2 and found that rats that received vitamin D had higher serum levels of adropin and higher expression levels of renal Nrf2 mRNA when compared to control rats and diabetic rats. The higher serum adropin levels could be explained by the assumption that adropin secretion has been recently linked to be vitamin D, as recognized by **Zorlu et al. [51]**. They have reported a positive connection between vitamin D values and serum adropin, and that vitamin D could show its effects through affecting ENHO gene expression in adropin secreting tissues directly or indirectly. Studies have demonstrated that the effects of vitamin D supplementations on gene expression profiles in a transgenic breast cancer mouse model revealed upregulation of ENHO gene expression **[15].** Moreover, **Onalan et al. [52]** have demonstrated that adropin immunoreactivity was elevated in the kidney glomeruli of type I diabetic rats treated with vitamin D following diabetes development.

The anti-diabetic and antioxidant effects of adropin have gained much attention. Adropin was shown to exert antidiabetic effects via insulin signaling pathway sensitization in diabetic rats as it increases insulin-mediated Akt phosphorylation and augments GLUT4 cell-surface expression, also treatment with adropin stimulates pyruvate dehydrogenase (PDH, a rate-limiting enzyme in glucose oxidation) and down-regulates PDH kinase-4 which is a PDH inhibitor. **Songet al. [53], Gao et al. [54]& Mohamed and Özer [55],** have also revealed that systemic adropin administration reduced the oxidative damage and increased antioxidant levels in diabetes-induced oxidant damage on heart tissue. Furthermore, **Guoet al. [56]** have showed that adropin administration demonstrated nephroprotective effects against the STZ-induced DN via anti-inflammatory and antioxidant pathways.

Nrf2 activation was recently reported as one of the underlying mechanisms of adropin antioxidant actions, **Chen et al. [18]** have revealed that adropin activates Nrf2 signaling, which is also involved in protecting NASH associated liver injury. Concerning higher Nrf2 levels, our results are in agreement with an animal study, demonstrated that Nrf2 aid in ameliorating diabetic renal damage induced by oxidative stress and ROS production **[57].** Moreover, a recent report showed

that Nrf2 contributes to the protection of the kidneys against DN by suppressing oxidative stress [58]. Results of the present work support our hypothesis that the nephroprotective effects of vitamin D may be related in part to enhancement of adropin secretion with subsequent increases in the expression of Nrf2 in diabetic rats.

Conclusion

This study demonstrates the vitamin D associated nephroprotective effects against DN in a type II DM rat model. Alterations in serum adropin levels may play a role in the development and severity of diabetic kidney disease, especially lower levels of adropin. The mechanisms of such protective effect of vitamin D may be associated with glycemic control and anti-oxidative effects that may be related even in part to the increases of serum adropin and the mRNA expression of Nrf2 in kidney tissue.

Conflict of interest

The authors declare that there is no conflict of interest.

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