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ORIGINAL ARTICLE

Role of Vitamin D in Hypertension Associated with Experimentally-Induced Diabetic Nephropathy in Rats

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ABSTRACT

Background: Diabetic nephropathy is one of the most common microvascular complications of type II diabetes mellitus that may lead to renal failure. Vitamin D is a fat-soluble vitamin and its deficiency is associated with an increased risk for cardiovascular and diabetic nephropathy. There were controversial studies about the role of vitamin D in diabetic nephropathy. This work aimed to study the protective and therapeutic roles of vitamin D against hypertension accompanied by diabetic nephropathy in type II diabetes mellitus and to find out a possible mechanism of its effect.

Methods: A total of 36 male local strain albino rats. They were randomly divided into Group I: the control group and Group II: The experimentally induced type II diabetic group which was subdivided equally into Group 1(diabetic nephropathy group), Group 2(vitamin D prophylactic group), Group 3(metformin prophylactic group), Group 4(metformin and vitamin D prophylactic group) and Group 5(treated diabetic nephropathy). Fasting serum insulin, glucose, lipid profile, angiotensin II and kidney function tests were estimated. BMI, GFR, and HOMA-IR were calculated in all groups at the end of the experiment. Also, arterial blood pressure was measured.

Results: Vitamin D and metformin alone and in combination or treated diabetic nephropathy group induced a significant increase in SOD, urinary creatinine, and HDL with a significant decrease in serum insulin, glucose, triglycerides, cholesterol, LDL, creatinine, urea, proteinuria, angiotensin II, MDA and ABP. This effect was significant in the vitamin D/metformin combination group compared to the use of either vitamin D or metformin alone.

Conclusions: Vitamin D may have a protective and therapeutic role in hypertension and nephropathy in type II diabetes mellitus.

Keywords: Vitamin D; diabetic nephropathy; hypertension.

INTRODUCTION

Vitamin D insufficiency is a general health problem, that is linked with rickets, dental caries, osteomalacia, osteopenia, osteoporosis, muscle weakness, falls, and increased danger of fracture in adults [1]. Some studies proposed that vitamin D may have extraskeletal effects like lowering oxidative stress levels, neuroprotective action, antimicrobial consequence, immunoregulation, anti-inflammatory, anticancer behavior, and cardiovascular advantage [2].

T2DM may give rise to several microvascular problems like neuropathy, retinopathy, and nephropathy, leading to elevated morbidity and mortality. Diabetic nephropathy is one of the most common microvascular complications and the chief reason for chronic kidney disease and death [3].

The important factors in the pathogenesis of DN are renal inflammation, fibrosis, hyperglycemia, oxidative stress, and renin-angiotensin-aldosterone system activation (RAAS) [4].

Diabetic nephropathy is represented by hypertension, advanced albuminuria, glomerulosclerosis, and a decline in glomerular filtration rate (GFR) leading to ESRD. Hypertension is common among cases with chronic kidney disease (CKD) and diabetes mellitus [5] Hypertension is nearly double as prevalent in patients with diabetes in comparison with healthy people [6].

This study aims to evaluate the protective and therapeutic roles of vitamin D against hypertension accompanied by diabetic nephropathy in type II diabetes mellitus and to find out a possible mechanism of its effect.

METHODS

The study design was approved by Institutional Review Board (IRB) NO.4293-4-2-2018, faculty of medicine, Zagazig University. All animal experiments were with the ARRIVE guidelines and carried out following the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. 36 adult male albino rats 12weeks old with bodyweight 150-200 gm, were received from the animal house - faculty of veterinary Medicine - Zagazig University. The animals were kept in steel wire cages (6 / cage) in the physiology animal house - faculty of Medicine Zagazig University under hygienic conditions. The animals were homed in a well-aerated room and were maintained on a 12 h light/dark cycle and temperature (21-24°C), they had free access to food and water. The rats were accommodated in animal house conditions for 2 weeks before the experiments went on.

Animals were divided into 2 main groups; Group1: Control group (n = 6rats): All animals were fed a diet consisting of mixed commercial rat laboratory chow which was consisted of 25.8 % protein, 62.8 % carbohydrate, and 11.4 % fat and supplied in separate clean containers[7]. Group II: Experimentally induced type II diabetic group (n = 30 rats): In which experimental diabetes was induced as follows: rats were fed a high-fat diet (HFD) (60.3% fat, 18.4% protein, and 21.3% carbohydrate, as a percentage of total kcal) for an initial period of 5 weeks. After 5 weeks of dietary manipulation, the HFD was replaced with a standard rodent diet and animals received a single intraperitoneal injection of a low dose of STZ (35 mg/kg BW; dissolved in normal saline). Plasma glucose level was checked after 7 days of STZ injection. Rats with plasma glucose levels of ≥ 250 mg /dl were included in the study [8].

All diets are obtained from the faculty of agriculture - at Zagazig University. Then, diabetic rats were subdivided equally (n=6) Group 1 (diabetic nephropathy group): This group was maintained on usual care and was left untreated, but received a castor oil injection in equal volume to that of treated rats until the end of the study. Group 2 (vitamin D prophylactic group): This group was given a vitamin D injection intra-peritoneal at a dose of 5 µg/kg body weight intraperitoneal injection twice per week for 8 weeks[9].Group 3 (metformin prophylactic group): This group received treatment with metformin 300 mg/kg / day [10].Group 4 (metformin and vitamin D prophylactic group): Diabetic rats were treated with combined metformin and vitamin D.Group 5 (diabetic nephropathy): this group received treatment with vitamin D 0.03 µg/kg in 0.05 mL castor oil was administrated once daily via gavage for 4 weeks[11].

Anthropometric measures

Measurement of body weight and length: Each rat was put in a closed plastic container and was weighed on the first and the last day of the experiment. The results were written in a record for each rat. Body length was taken as the distance from the nose tip to the anus at the start and the end of the experiment [12].

Calculation of Body Mass Index [BMI]: BMI = body weight (gm) / length2 (cm2). The cutoff value of obesity is BMI more than 0.68 gm/cm2 [12].

Measurement of MABP pressure

MABP is measured in millimeters of mercury (mm Hg) by Non-Invasive Blood Pressure (NIBP) monitor [13].

Urine collection

Urine samples were collected for 24 hours by metabolic cages, measured for volume, and centrifuged for 10 minutes at approximately 3000 rpm to remove insoluble materials. The supernatant was kept at –20°C for further analysis [14].

Measurement of urine total proteins

Estimation of urine total proteins was carried out as described by Nishi and Elin[15] using Urinary Protein Assay Kit [Chondrex, Inc. 2607-151 place NE Redmond, WA 98052, USA].

Measurement of urine Creatinine

Estimation of urine creatinine was carried out as described by Jaffé[16] using Creatinine (Colorimetric) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt].

Blood sampling

Retro-orbital venous plexus blood samples were obtained then serum was separated by allowing the blood samples to clot then centrifuged at 3000 rpm for 20 minutes, kept at (-20o c), and used to measure the serum levels of glucose, insulin, lipids profile, serum urea, creatinine and angiotensin II.

Measurement of serum glucose and insulin

Serum glucose was estimated as described by Tietz[17]using a specific glucose kit (Bioscience, Egypt) and analyzed by spectrophotometers device (URIT-810, China). Insulin was measured by enzyme amplified sensitivity immunoassay (EASIA) as described by Temple et al.[18] using a specific insulin kit (BioSource Belgium) and analyzed by spectrophotometers device.

Calculation of insulin resistance (HOMA-IR)

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula [HOMA-IR = insulin (µU/mL) x glucose (mg/dl) /405[19].

Measurement of serum lipids profile

Total cholesterol (TC) and triglycerides (TG) were

measured by the enzymatic colorimetric method described by Tietz[17]using specific cholesterol and triglycerides kits (Spinreact Spain) and analyzed by spectrophotometers device. High-density lipoproteins (HDLc) were measured by precipitating reagent method described by Tietz[17]using HDLc precipitating reagent kit (Spinreact, Spain) and analyzed by spectrophotometers device.

Measurement of serum creatinine

Estimation of serum creatinine was carried out as described by Jaffé [16]usingCreatinine (Colorimetric) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt].

Measurement of serum urea

Estimation of serum urea was carried out as described by Tietz[20] using a urea/bun (urease) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt].

Measurement of Glomerular filtration rate (GFR)

By using the creatinine clearance formula [21].

Measurement of serum angiotensin II

Estimation of serum angiotensin II was carried out as described by Kumar et al. [22] using rat Angiotensin II Enzyme Immunoassay (EIA) Kit (Catalog Number RAB0010, Sigma-Aldrich Co., Egypt).

Tissue sampling and histopathological examination

Immediately after collecting blood samples, rats were killed by decapitation after light ether anesthesia. Kidneys were immediately excised, the left one was processed for histopathological studies and the right one was homogenated for biochemical estimations of SOD activity and MDA

Renal antioxidant system evaluation

One small section (200 mg) of the right kidney was harvested from the rats and precisely weighed. Subsequently, saline was added according to the tissue weight: Saline (4°C) to exclude the blood cells, blotted and dried with filter paper, then tissue portions from kidneys were kept in 10% buffered formalin - saline at 4°C for at least one week (1ry fixation), then the specimens were dehydrated with a series of ascending grade of ethanol from 75 to 100%. Tissues were placed thereafter in xylol and embedded in paraffin wax. Cross-sections of about 1-2 µm thickness of the kidney were processed on slides and stained with hematoxylin and eosin (H & E) stain to study general microscopic characters of the kidney for routine light microscope assessment at magnification power [400] [23].

Statistical analysis

The data obtained in the present study were expressed as mean ±SD for quantitative variables and statistically analyzed. The statistical analysis is done by using the SPSS program (19) (SPSSInc.Chicago, IL, USA). ANOVA (Post hoc)test was used to compare means among more than two groups. P-value < 0.05 was considered statistically significant.

RESULTS

The present study showed that HFD/STZ significantly increased serum glucose, HOMA-IR, serum TC, TG, LDL-c, serum creatinine, urea, angiotensin II, total urine protein, MABP, and renal MDA levels. There were also significantly decreased serum insulin, HDL-c levels, urine creatinine level, GFR, and renal SOD activities in the diabetic nephropathy group when compared to control (P-value: <0.001 respectively).

However, in diabetic prophylactic treated groups(vitamin D, metformin, vitamin D+metformin) and in diabetic nephropathy vitamin D treated group, there was a significant decrease in serum glucose, HOMA-IR, serum TC, TG, LDL-c, serum creatinine, urea, angiotensin II, total urine protein, MABP, and renal MDA levels as compared to diabetic nephropathy group (P-value: <0.05respectively)

While, there was a significant increase in serum insulin, HDL-c level, urine creatinine, GFR, and renal SOD activities in the same groups (P-value: <0.05respectively).

Moreover, combined administration of vitamin D and metformin produced a significantly higher protective effect than each one alone (P-values were<0.001 and <0.05 respectively).

According to the ANOVA, there was a nonsignificant difference between the prophylactic group treated with vitamin D (Table 2) and the group treated with metformin (Table 3).

In addition, histopathological examination of STZ-induced severe glomerulosclerosis and inflammatory cellular infiltration, tubular dilatation, casts, interstitial fibrosis, and atrophy together with vascular arteriosclerosis and a significant increase in Treatment with either metformin or vitamin D resulted in a significant decrease in glomerulosclerosis, cellular infiltration, and interstitial fibrosis Metformin/Vitamin D combination resulted in significant improvement in the histopathological picture compared to the use of each of these drugs alone (Figure 1-5).

Table 1: Some measured parameters in all studied groups.

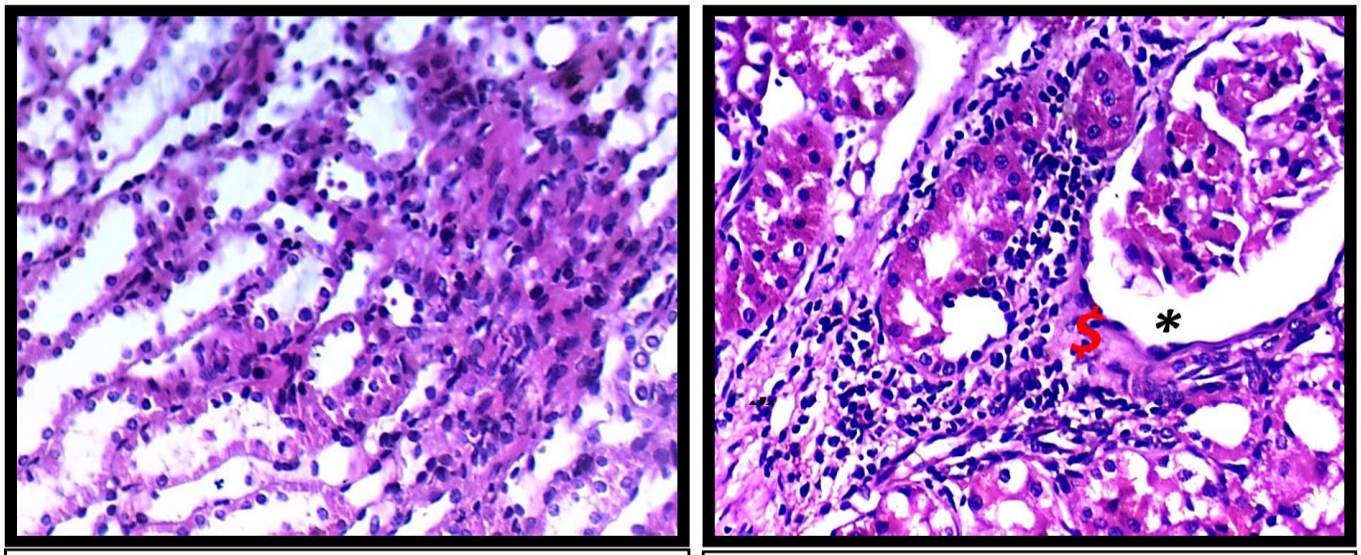
	Control Group (C)	*Diabetic Nephropathy Group (DN)*	*Vitamin D Prophylaxis Group (D)*	*Metformin Prophylaxis Group (M)*	*Combined Vitamin D and Metformin Group (D+M)*	*Treated Group with Vitamin D (TD)*
Glucose (mg/dl)	83.16±7.31	291.66±18.11*	164±8.17**@	151.33±11.18**@	84.83±4.16**	201.33±14.78**
Insulin (µIu/dl)	20.50±2.28	10.40±1.34*	12.54±1.03**@	13.00±0.72**@	19.86±1.52**	13.56±1.09**
HOMA-IR	3.98±0.66	7.47± 1.02*	5.04± 0.35**@	4.81±0.30**@	4.12± 0.17**	6.69± 0.67**
Total cholesterol (mg/dl)	81.00± 10.88	206.55±11.43*	121.16±16.14**@	114.16± 13.55**@	88.00± 6.09**	111.91± 8.63**
HDL (mg/dl)	40.08 ± 2.08	20.94± 3.52*	29.95±2.67**@	31.93±2.84**@	38.33± 3.64**	31.56± 2.12**
MABP(mmHg)	81.68± 3.57	131.43± 4.84*	106.46±8.377**@	104.48± 6.87**@	87.93±5.25**	109.23± 6.89**
LDL (mg/dl)	26.55±2.9	119.76±11.59*	85.58±11.59**@	62.5±12.37**@	28.8±3.75**	102.06±5.51**
Triglyceride (mg/dl)	55.20± 9.33	150.50± 22.39*	90.71± 2.83**@	73.88± 9.64**@	54.66± 2.98**	105.20± 9.17**
S. Creatinine (mg/dl)	0.56± 0.04	2.53± 0.21*	1.13± 0.13	1.02± 0.05	0.61± 0.05**	1.88± 0.21**

*=Significant vs C, **= Significant vs DN, @=Significant vs D+M

Table 2: Some measured parameters in all studied groups.

	Control Group (C)	*Diabetic Nephropathy Group (DN)*	*Vitamin D Prophylaxis Group (D)*	*Metformin Prophylaxis Group (M)*	*Combined Vitamin D and Metformin Group (D+M)*	*Treated Group with Vitamin D (TD)*
Urea (mg/dl)	22.4±1.43	39.52±2.72*	30.3±1.27**@	29.25±1.77**@	24.06±1.42**	34.73±1.69**
U. creatinine (mg/dl)	49.66±4.5	17±2.28*	30.33±3.82**@	28.16±4.07**@	48.5±1.87**	22.16±1.47**
Proteinuria (mg/dl)	4.00±1.41	39.00±6.35*	16.00±1.41**@	13.00±1.78**@	7.00±0.89**	10.33±1.03**
GFR	0.58± 0.057	0.06±0.008*	0.23± 0.048**@	0.22± 0.036**@	0.54± 0.028**	0.11± 0.016**
SOD (u/mg tissue)	10.78±0.74	6.46±0.6*	8.43±0.81**@	8.23±0.71**@	10.18±0.64**	9±0.83**
MDA (nmol/gm tissue)	36.34±3.95	65.45±5.04*	51.07±3.07**@	48.28± 5.41**@	41.19±4.83**	51.03± 2.58**
Angiotensin II (ng/µl)	0.1323±0.009	0.4500±0.0391*	0.2992±0.01179**@	0.293±0.016**@	0.1448±0.006**	0.2428±.0.017**
BMI (mg /cm2)	0.47±0.05	0.39±0.03	0.41±0.03	0.40± 0.03	0.42±0.03**	0.43±0.05**

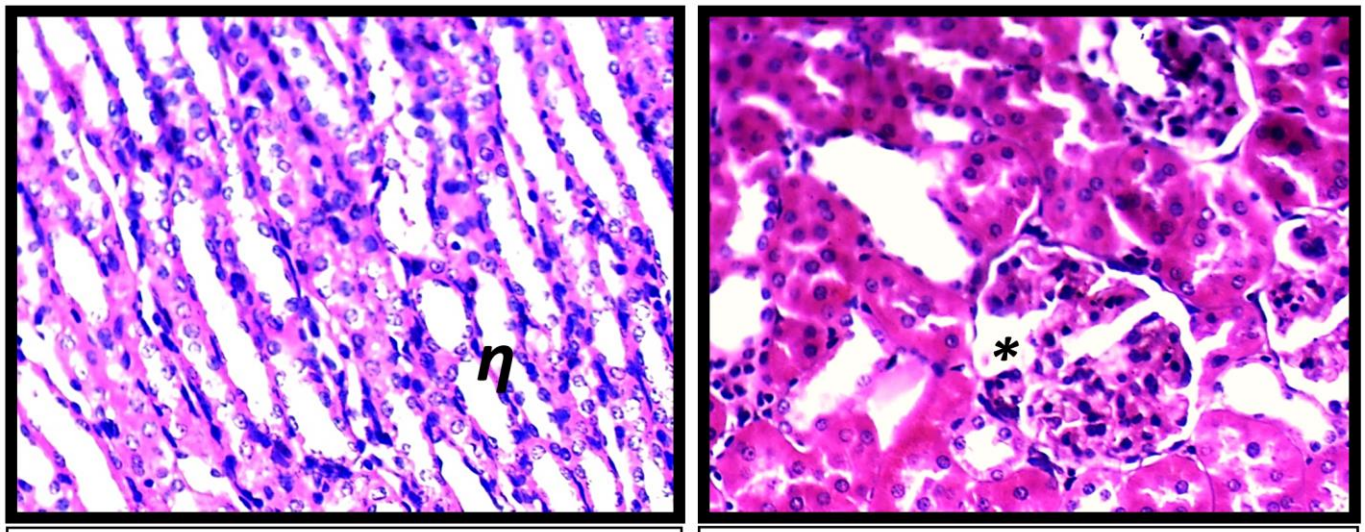
*=Significant vs C, **= Significant vs DN, @=Significant vs D+M



(A) H&E stained sections from the cortical area of the kidney

(B) H&E stained sections from glomeruli of the kidney

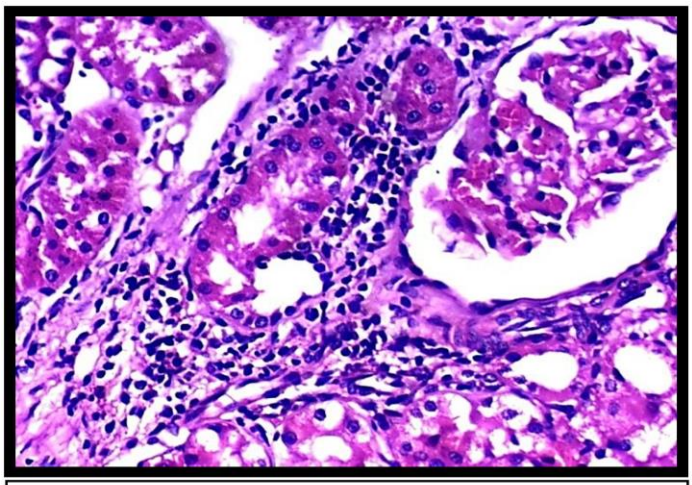
Figure 1: H&E stained section of the kidney in diabetic nephropathy (DN) group; (A, B) showing interstitial inflammatory infiltrate ($)& fibrosis and glomerulosclerosis (*)



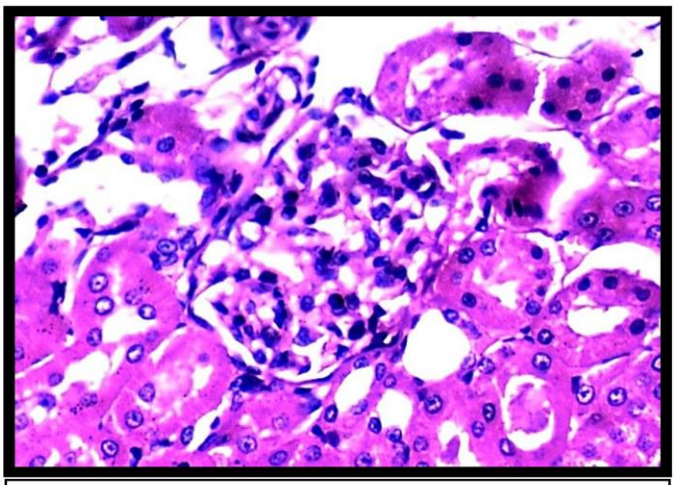
(A) H&E stained sections from the cortical area of the kidney

(B) H&E stained sections from glomeruli of the kidney

Figure 2: H&E stained section of the kidney in metformin (M) group; (A, B) showing apparently normal glomeruli (*), mild interstitial fibrosis, and minimal tubular necrosis (η)

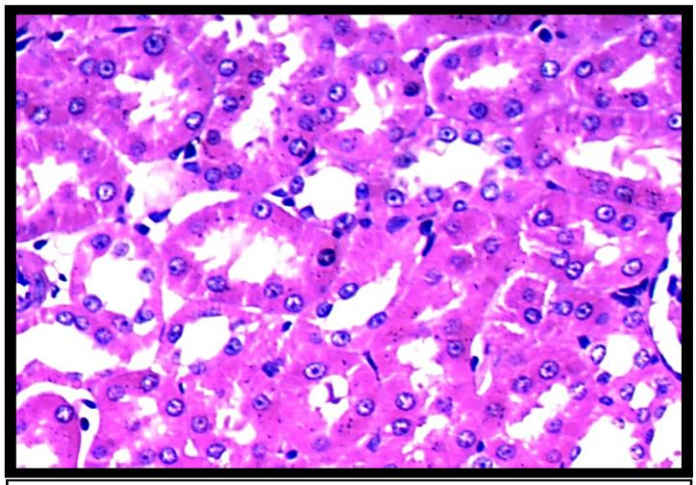


(A) H&E stained sections from the cortical area of the kidney

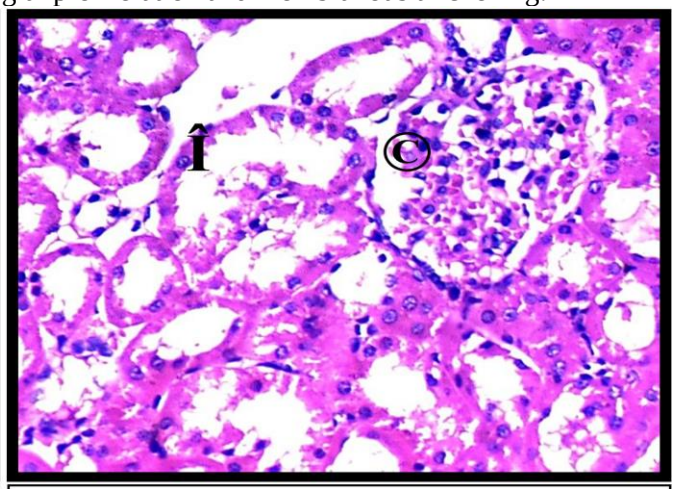


(B) H&E stained sections from glomeruli of the kidney

Figure 3: H&E stained section of the kidney in vitamin D prophylactic (D) group; (A, B) showing marked decrease in cellular infiltration and interstitium with mesangial proliferation and membranous thickening.

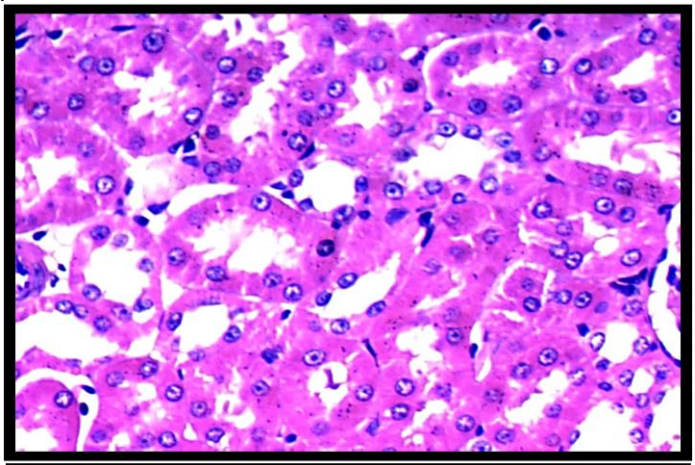


(A) H&E stained sections from the cortical area of the kidney

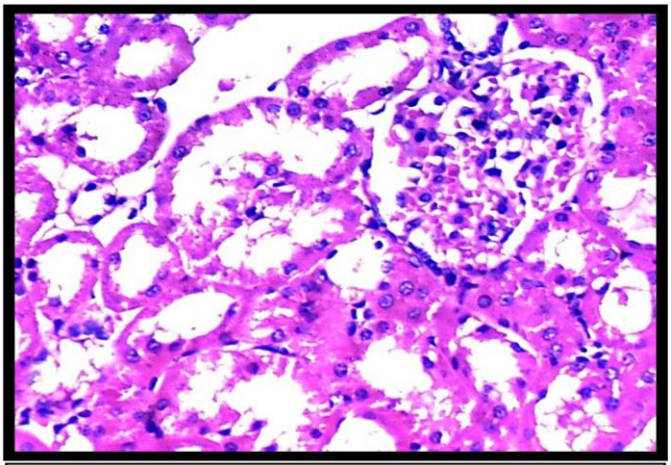


(B) H&E stained sections from glomeruli of the kidney

Figure 4: H&E stained section of the kidney in vitamin D & metformin prophylactic (M+D) group; (A, B) showing minimal cellular infiltration (Î), mild dilatation with few casts (©), and minimal mesangial proliferation.



(A) H&E stained sections from the cortical area of the kidney



(B) H&E stained sections from glomeruli of the kidney

Figure 5: H&E stained section of the kidney in treated diabetic nephropathy (TDN) group; (A, B) showing minimal cellular infiltration, mild dilatation with few casts in the tubules, and minimal mesangial proliferation

DISCUSSION

In the current study, the diabetic nephropathy group showed significantly higher fasting levels of serum glucose in addition to increased HOMA-IR index, and significantly lower fasting insulin levels when compared to control rats. Moreover, the fasting levels of serum TC, TG, and LDL in the diabetic nephropathy group were significantly increased compared to the control group. In addition, HDL level was significantly decreased in the same group.

As regards the role of vitamin D, in the vitamin D prophylactic group, metformin prophylactic group, and combined vitamin D & metformin group fasting serum levels of glucose, TC, TG, LDL, and HOMA –IR significantly decreased while HDL and serum fasting insulin significantly increased when compared with diabetic nephropathy group. The vitamin D and metformin group showed more significant improvement when compared with vitamin D or metformin alone. Also, the treated group with vitamin D showed a significant decrease in fasting serum levels of glucose, TC, TG, LDL, and HOMA –IR and a significant increase in HDL and serum fasting insulin when compared with the diabetic nephropathy group.

The results are in agreement with Gerco et al [24]who reported that vitamin D performs a critical role in keeping glucose levels in T2DM. The active form of vitamin D (1,25(OH)2D) works directly through the activation of transcription of the human insulin receptor gene, enhancing glucose transport. In addition to the decline in triglycerides levels, vitamin D could prohibit free fatty acid-induced insulin resistance, via diminution of JNK;c-jun-N-terminal kinase which act a vital role in metabolism [25]. These detected data were also in agreement with Longenecker et al [26]who found that vitamin D supplementation causes a significant reduction in the serum total cholesterol level and enhanced the lipid profiles.

These results elucidated that insulin has a leading role in controlling fat metabolism as it raises β-hydroxy-β-methylglutaryl coenzyme A reductase activity decreasing cholesterol synthesis [27].

The effect of vitamin D on the lipid profile can be explained by its capability to augment insulin sensitivity, secretion, and action [28]. In addition, vitamin D can increase the transformation of cholesterol into bile acids [29]. Also, Elattar et al. [30] found that 1,25 (OH)2D3 directly increases adipocyte fatty acid synthase expression and activity, enhancing glycerol-3-phosphate dehydrogenase activity and inhibits of lipolysis. These direct effects of 1, 25(OH)2 D3 may be through its modification of calcium ion concentration in the adipocyte, a major factor in the regulation of lipid metabolism.

The current findings are in contrast with those of Deng et al. [31] who found that the blood glucose and lipids of the rats in the vitamin D group did not differ from those of the group DN rats. This suggests that the renal protective effects of vitamin D are attributable to enhancement in oxidative stress, and not to reductions in blood glucose or blood lipids.

Regarding the kidney function parameters, the current study showed that there was a progressive significant increase in both serum urea, creatinine, proteinuria, and tissue MDA while a significant decrease in urinary creatinine, creatinine clearance, and tissue SOD in the diabetic nephropathy group compared to control group which indicates renal impairment.

As regards the role of vitamin D, in the vitamin D prophylactic group, metformin prophylactic group and combined vitamin D and metformin serum urea, creatinine, proteinuria, and renal tissue MDA significantly decreased while urinary creatinine, creatinine clearance, and SOD significantly increased when compared to diabetic nephropathy group. Vitamin D and metformin groups showed more significant improvement when compared with vitamin D or metformin alone. Also, these parameters improved significantly in the treated-diabetic-nephropathy with vitamin D when compared to the diabetic nephropathy group.

The results are in agreement with Kabel et al. [32] who noticed that vitamin D resulted in significant improvement in renal functions. Also, these results are in agreement with Deng et al. [31]who found that vitamin D plays an important role in reducing oxidative stress in DN.

Oxidative stress may be elucidated that hyperglycemia leads to the formation of oxygen free radicals with restriction of the activity of the antioxidant enzymes leading to oxidative stress which is linked with many health problems [33].

On the contrary, Barzegari et al. [34] found no significant variation in vitamin D supplementation on serum total antioxidants capacity (TAC), levels, and antioxidant enzymes activity (SOD, GPX, and CAT), and MDA levels and this may be due to the rat strain difference.

Also, improvement of kidney function parameters may be explained by that1,25(OH)2D3 inhibits podocyte apoptosis and hypertrophy, maintains podocyte structural integrity, and prohibits proteinuria and glomerulosclerosis[35]. These results are supported by the results of the study done by Momeni et al [36] who noticed significantly diminish proteinuria in type II DN patients treated with vitamin D.

Results of this study also revealed a significant

increase in plasma level in angiotensin II and MAP in diabetic nephropathy when compared to the control group. However, vitamin D, metformin, and combined vitamin D & metformin produced a significant decrease in the above-mentioned parameters when compared to the diabetic nephropathy group.

As regards the role of vitamin D in the vitamin D prophylactic group, metformin prophylactic group and combined vitamin D and metformin group serum angiotensin II and mean arterial blood pressure significantly decreased when compared with the diabetic nephropathy group. The combined vitamin D and metformin group showed more significant improvement when compared with vitamin D or metformin alone. Also, the treated group with vitamin D showed a significant decrease in angiotensin II and MAP when compared to the diabetic nephropathy group.

These results are in agreement with Eltablawy et al. [37] who found that vitamin D protects against diabetic nephropathy through inhibition of the renin-angiotensin system. Also, Baradaran et al. [38]demonstrated that oxidative stress mechanisms are involved in the pathogenesis of hypertension and cardiovascular diseases.

On the other hand, the current findings were in contrast with those of Forman et al.[39] who found no relationship between intake of vitamin D from diet and supplements and the risk of hypertension.

In the current study, BMI in the control group steadily increased. In comparison, those of the diabetic nephropathy group showed a significant decrease. The decrease in BMI may be due to increased muscle wasting and loss of tissue proteins, and this is in agreement with Bhutada et al. [40].

However, BMI showed no significant change in the vitamin D prophylactic group, metformin prophylactic group, combined vitamin D & metformin group and treated group when compared with the diabetic nephropathy group.

CONCLUSION

Vitamin D has a protective and therapeutic role in hypertension and nephropathy in type II diabetes mellitus. This occurs through the effect of vitamin D in decreasing insulin resistance and increasing antioxidants in diabetic rats.

Conflict of Interest: None.

Financial Disclosures: None.

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