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Effects of vitamin D_3 supplementation on lipid profile and renal indices in rat model of drug induced renal injury

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ABSTRACT

Introduction and aim. Renal injury is associated with decreased renal function, hypovitaminosis D, deranged calcium-phosphate metabolism and dyslipidemia, thus increasing risk for chronic kidney disease and cardiovascular diseases. However, reports on the effects of vitamin D on drug induced renal injury are few. The aim was to investigate the possible role of vitamin D supplementation in reversing deranged lipid profile and renal function post drug induced renal injury.

Material and methods. Wister male rats (36) were randomly divided into group 1, 2 and 3 (n=12). Single dose of Adriamycin was given to all except group 1(control) to induce renal injury. Group 2 left untreated, group 3 given vitamin D_3 for 28 days. Serum urea, creatinine, total protein, total cholesterol (TC), triglycerides (TG), LDL-C, HDL, apolipoprotein (Apo) A and B were measured. C-reactive protein (CRP) and nitric oxide were assessed in kidney homogenate.

Results. Vitamin D_3 significantly brought down levels of serum creatinine, TC, LDL CRP, nitric oxide and increased the levels of Apo A, albumin, HDL. Serum urea, TG and Apo B in group 3 were not significantly different after vitamin D_3 administration. Histological examination revealed improvement in glomerular messangialisation.

Conclusion. Vitamin D_3 may improve renal health, through its positive impact on dyslipidemia, inflammation, and oxidative stress in drug induced renal injury.

Keywords. adriamycin, chronic kidney disease, dyslipidemia, oxidative stress, renal injury, vitamin D

Introduction

Chronic kidney disease (CKD) is a term that includes all degree of decrease in the kidney functions resulting from damage, and can be defined as a persistent abnormality in kidney structure or function (e.g, glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 or albuminuria ≥30 mg per 24 hours) for more than 3 months.[1] Kidney disease is an under-recognized public health crisis.[2] It is a global problem affecting 8–16% of the world population and more common in women (14%) than men (12%).[3] Renal disease affects an estimated 15% of the adult population in the United States of America and its prevalence ranged from 10.7–13.9% in sub-Saharan Africa and approximately 11.4% when adjusted for age and gender in Nigeria.[4]

Renal disease occurs as a result of abnormalities in the structure or function of the kidney and it is linked to several complications including, cardiovascular disease, hypertension, anemia, mineral bone disorders, volume overload, electrolytes and acid-base derangements.[5] Renal injury is most commonly attributed to diabetes and hypertension, other causes include glomerulonephritis, inherited diseases, such as polycystic kidney disease; immune diseases; kidney cancer, kidney stones, frequent untreated and/or long-lasting urinary tract infections, drugs/medications, toxic metal poisoning, renal artery stenosis etc.[6] Regardless of the cause of renal injury, late diagnosis and poor management often results in progression to kidney failure, complications in other organs and death.[7]

Adriamycin (doxorubicin hydrochloride, ADR) an anti-tumor antibiotic important in the treatment of malignancies, such as prostate cancer, breast cancers and leukemia.[8] ADR causes focal segmental glomerulosclerosis in rodents, which is characterized by proteinuria, progressive glomerulosclerosis, and tubulointerstitial injury.[9] As a result, pharmacological research on human chronic renal disorders frequently uses the rodent form of ADR nephropathy as an experimental paradigm as employed in this present study.

Individuals with CKD exhibit an elevated cardiovascular risk manifesting as coronary artery disease, heart failure, arrhythmias, and sudden cardiac death.[5] It is also associated with dyslipidemia and a systemic, chronic proinflammatory state contributing to vascular and myocardial remodeling processes resulting in atherosclerotic lesions, vascular calcification, and vascular senescence as well as myocardial fibrosis and calcification of cardiac valves.[10] The general mechanisms underlying the dyslipidemia of renal disease may be summarized as altered metabolism of postprandial lipoproteins, altered metabolism of other triglyceride-rich lipoproteins, changes in the route of reverse cholesterol transport, structural changes of lipoproteins, postribosomal modifications of lipoproteins, insulin resistance, proteinuria, and increased lipoprotein(a).[11] Regardless of the cause of disease, adverse outcomes include progression to kidney failure, complications in other organs and tissues, and death.

CKD is also commonly associated with disorders of mineral and bone metabolism, manifested by either one or a combination of the following; abnormalities of calcium, phosphorus, parathyroid hormone, fibroblast growth factor 23 (FGF23), and vitamin D metabolism; abnormalities in bone turnover, mineralization, volume linear growth, or strength; extraskeletal calcification.[12] Vitamin D (25(OH)D) deficiency has also been shown by previous studies to be associated with the complications of renal injury.[13] Low serum level of vitamin D is

often associated with hypocalcemia, increased blood pressure, hyperparathyroidism, reduced bone density and cardiovascular disease.[14] Vitamin D might be important in the regulation of lipid metabolism and may have implications for preventing the progression of CKD and aid in the management of CKD and ultimately CVD. Also, lower baseline vitamin D levels have been found to correlate with atherogenic blood lipid profiles, while 25(OH)D supplementation has been found to influence the levels of serum lipids in that it lowers the levels of total cholesterol, triglycerides, and LDL cholesterol and increases the levels of HDL cholesterol in pregnancy complications like gestational diabetes mellitus and preeclampsia.[15,16] Thus, dietary reversal of vitamin D-deficiency and treatment with vitamin D analogs could be therapeutic options for renal and cardiovascular related diseases. Recent studies on the role of Vitamin D in nephropathies reported renoprotective effects of Vitamin D through anti-inflammatory and antifibrotic mechanisms, however little is known on its role on cardiometabolic markers in kidney related pathologies.[17]

Aim

Hence, this study explored the efficacy of vitamin D supplementation in the amelioration of the complications of renal injury via its effects on cardiometabolic and renal markers implicated in the pathology of CKD. Overall, the study's significance lies in the potential of vitamin D supplementation to improve health outcomes in renal injury and prevent development of associated cardiovascular complications.

Materials and Methods

Experimental rats

This is an experimental study carried out at the Lead City Animal House between March, 2023 to June, 2023. Handling of experimental animals was as approved by the institution's ethical committee. Thirty-Six (36) male Wistar albino rats (weight between 190 and 250g) were purchased from Rainbow farms in Ojo, Ibadan Nigeria. Rat pellets (Vital Feed[@], Nigeria) and unlimited water were provided to the animals, which were housed in well-ventilated plastic cages and allowed to get use to a 12-hour light/dark cycle for a week before the treatments started.

Body weight determination

Following their acclimatization, the body weight of rats was measured using weighing balance and initial weight recorded. This was used to calculate the dose of ADR (injection) administered. The body weight was subsequently determined weekly to adjust the doses of the treatment. After treatment in all groups, the final weight was measured.

Animal grouping and administration of ADR, vitamin D_3 (cholecalciferol)

The male Wistar rats were divided into three groups and twelve (12) Wistar rats were randomly selected into each group. Group 1 served as the untreated control and were not given any treatment throughout the duration of the experiment.

After one week of acclimatization, ADR was administered to group 2 and 3 once intravenously at a single dose of 7.5 mg/kg of body weight at the first day to induce nephrotoxicity in the experimental rats. The vehicle for ADR administration was 0.9% NaCl. The group 3 was the ADR treated group and were given oral cholecalciferol (200 IU/day). Vitamin D was administered at the dosage of 200 IU/kg body weight of Cholecalciferol (Vitabiotics$^{@}$) and given orally using a gavage once daily for 28 days between 8:00–10:00 hrs.

Termination of treatment, and collection of organ

On the last day of treatment (28th day), all experimental animals were made to fast overnight with access to water only, and their final body weights taken. All experimental animals were euthanized using an approved method under the institutional guidelines and blood was collected through their orbital plexus into plain bottles and serum separated within 30 minutes of blood collection by centrifuging at 4000rpm for 15 minutes and kept at -20^{0}C prior biochemical analysis. The kidney was surgically harvested for histopathological examination, and then homogenized at a PH of 7.4 with homogenizing buffer (1.15% potassium chloride solution and 50 mM Tris-HCl). For biochemical analysis, the homogenate was centrifuged for 10 minutes at 4^{0}C and 12,500 rpm and kept at -20^{0}C prior analysis.

Biochemical analysis

Serum albumin was determined by dye binding method known as bromocresol green (BCG) method as described by Duomas et al. using Randox Laboratories Limited diagnostic kits, England.[18] Total protein was evaluated by biuret method as described by Tietz using Sigma Diagnostic Kit, USA.[19] Creatinine was determined by alkaline picrate method as described by Murray using Randox Laboratories Limited Diagnostic kits, England.[20] Urea was quantified by enzymatic colorimetric UV method as described by Fawcett et al. using Sigma Diagnostic Kit, USA.[21] C-reactive protein (CRP) was quantified by turbidimetric immunoassay as described by Tietz on an automated chemistry analyzer (LW C 100plus).[22] Nitric oxide was determined by spectrophotometric method as described by Risa A Ridnour.[23] Plasma total cholesterol was analyzed by the cholesterol CHOD-PAP method, an enzymatic end point method as described by Allain et al.[24] HDL-C was quantified by precipitation through the procedure adopted by Lopez-Virella.[25] Serum triglycerides (TG) by GPO-PAP method as described by Trinder.[26] All lipid profile tests were done on an automated chemistry analyzer (LW C 100plus). LDL-C was calculated using Friedwald's formula as shown:

LDL- cholesterol (mg/dl) = Total Cholesterol – HDL-cholesterol-TAG/5.[27]

Apolipoprotein A and B were estimated by immunoturbidimetric method as described by Labeur et al., on an automated chemistry analyzer (LW C 100plus).[28]

Histopathological study

Histology of the kidney was carried out by a method described by Avwioro at the Anatomic Pathology Department, University College Hospital, Ibadan Nigeria.[29]

Statistical analysis

Prism Graphpad, version 6.4, was used for data analysis. The data was subjected to descriptive statistics. One-way analysis of variance (ANOVA) was used to determine the statistical difference in mean between the three groups and level of significance was set at $p<0.05$.

Results

There was a significant decrease in the body weight after treatment in the ADR only and ADR+vitamin D group as shown in Figure 1.

The serum levels of albumin was significantly lower ($p<0.05$) in the ADR group when compared with control, a significant increase occurred after treatment with vitamin D as shown in Figure 2.

The serum levels of creatinine was significantly higher ($p<0.05$) in the ADR group when compared with control while a significant decrease ($p<0.05$) occurred after treatment with vitamin D in the ADR+vitamin D group as shown in Figure 4.

The serum levels of urea was significantly higher ($p<0.05$) in the ADR group when compared with control but there was no significant difference in urea levels after treatment with vitamin D in the ADR+vitamin D group as shown in Figure 5.

Serum TC and TG levels were significantly higher ($p<0.05$) in the Adriamycin group, while a significant decrease in TC occurred after vitamin D treatment in ADR+vitamin D group and there was no difference in TG after vitamin D treatment in ADR+vitamin D group as shown in Figures 6 and 9 respectively.

The serum levels of LDL was significantly higher ($p<0.05$) in the ADR group when compared with control but there was no significant difference in urea levels after treatment with vitamin D in the ADR+vitamin D group as shown in Figure 8.

The serum levels of HDL, Apo A1 were significantly lower ($p<0.05$) in the ADR group, while a significant increase occurred after vitamin D treatment in ADR+vitamin D group when compared with the ADR as shown in Figures 7 and 10 respectively.

There was no significant change in the levels of TP and Apo B in all groups after treatment as shown in Figures 3 and 11 respectively.

The CRP level in kidney homogenates was significantly higher ($p<0.05$) in the ADR group when compared with control while a significant decrease occurred after vitamin D administration in ADR+vitamin D group when compared with the ADR group as shown in Figure 12.

Plate show severe tubular necrosis (black arrow) and glomerular messangialisation (green arrow) in group 2. There was only tubular necrosis (black arrow) seen in Group 3 as shown in Figure 14.

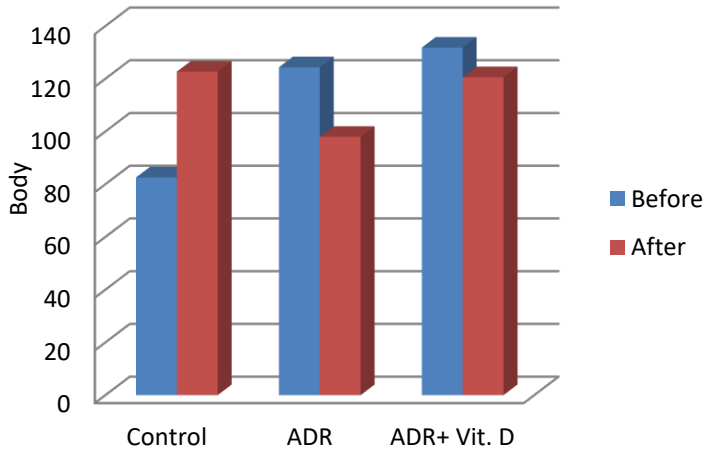

Fig. 1. Effects of vitamin D_3 on bodyweight in ADR-induced nephrotoxic rats

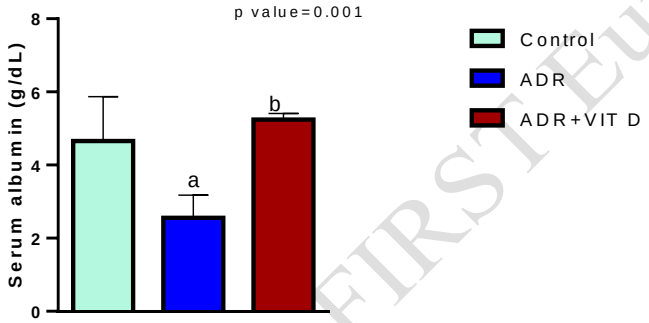

Fig. 2. Effects of vitamin D_3 on serum albumin in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

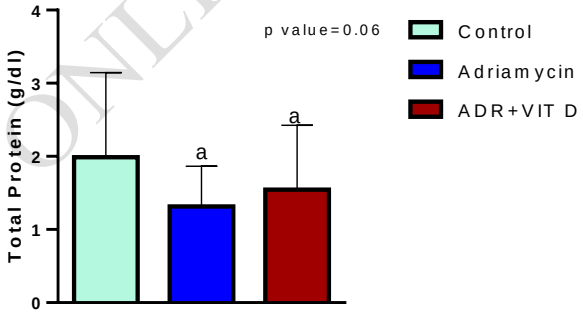

Fig. 3. Effects of vitamin D_3 on serum total protein in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

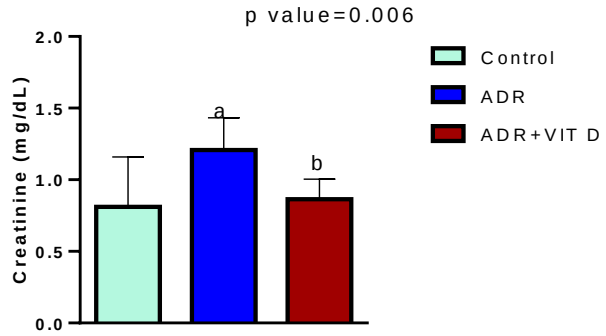

Fig. 4. Effects of vitamin D_3 on serum creatinine in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

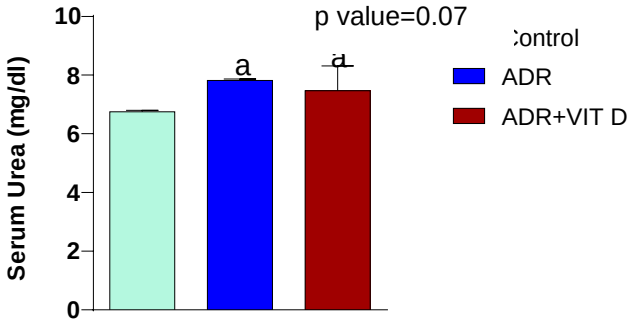

Fig. 5. Effects of vitamin D_3 on serum urea in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

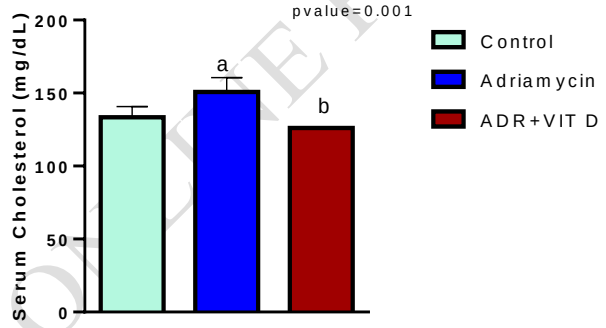

Fig. 6. Effects of vitamin D_3 on serum total cholesterol in in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

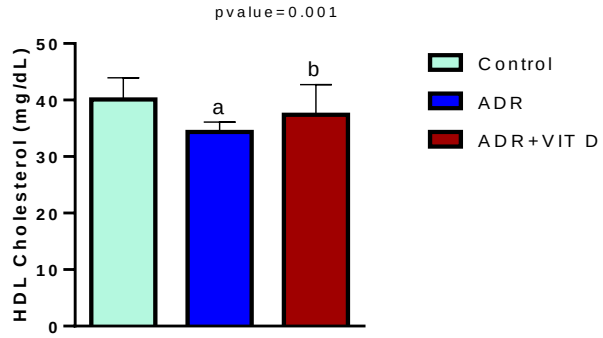

Fig. 7: Effects of vitamin D_3 on serum HDL-C in in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

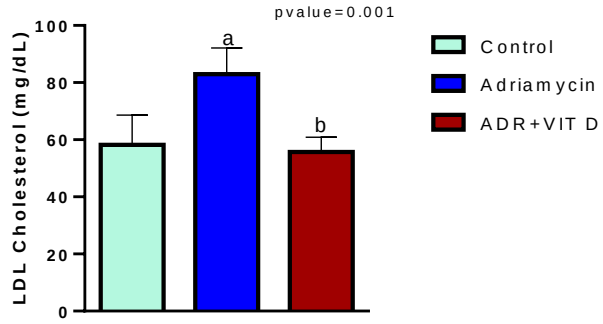

Fig. 8: Effects of vitamin D_3 on serum LDL-C in in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

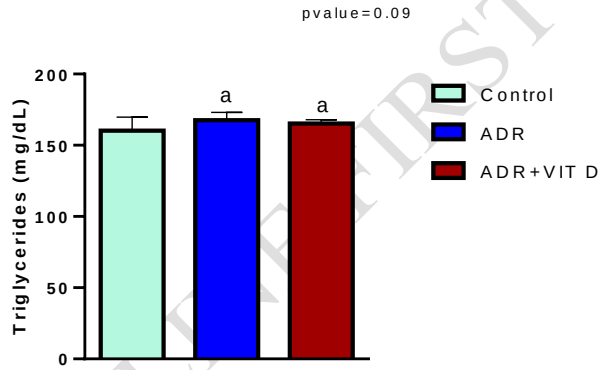

Fig. 9. Effects of vitamin D_3 on serum TG in in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

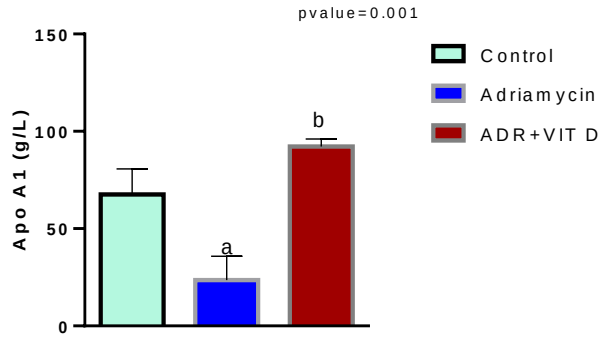

Fig. 10. Effects of vitamin D_3 on serum Apo A in in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

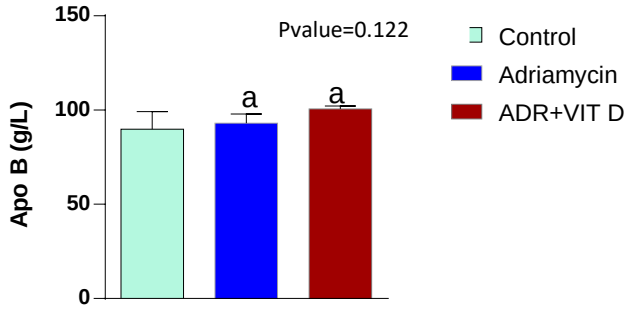

Fig. 11. Effects of vitamin D_3 on serum Apo B in in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

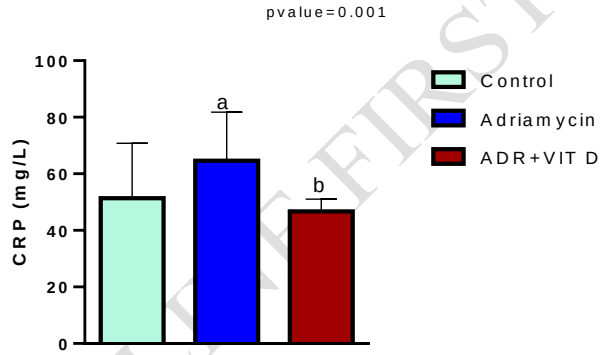

Fig. 12. Effects of vitamin D_3 on CRP levels in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

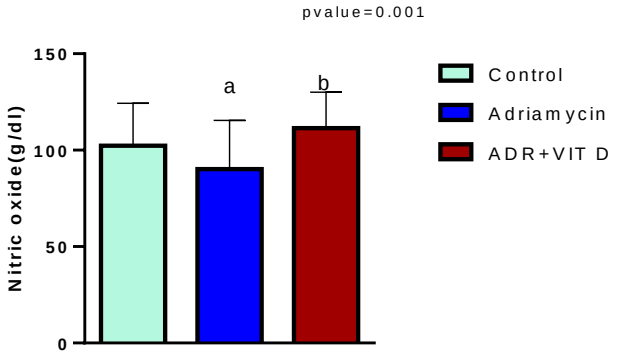

Fig. 13. Effects of vitamin D_3 on nitric oxide levels in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

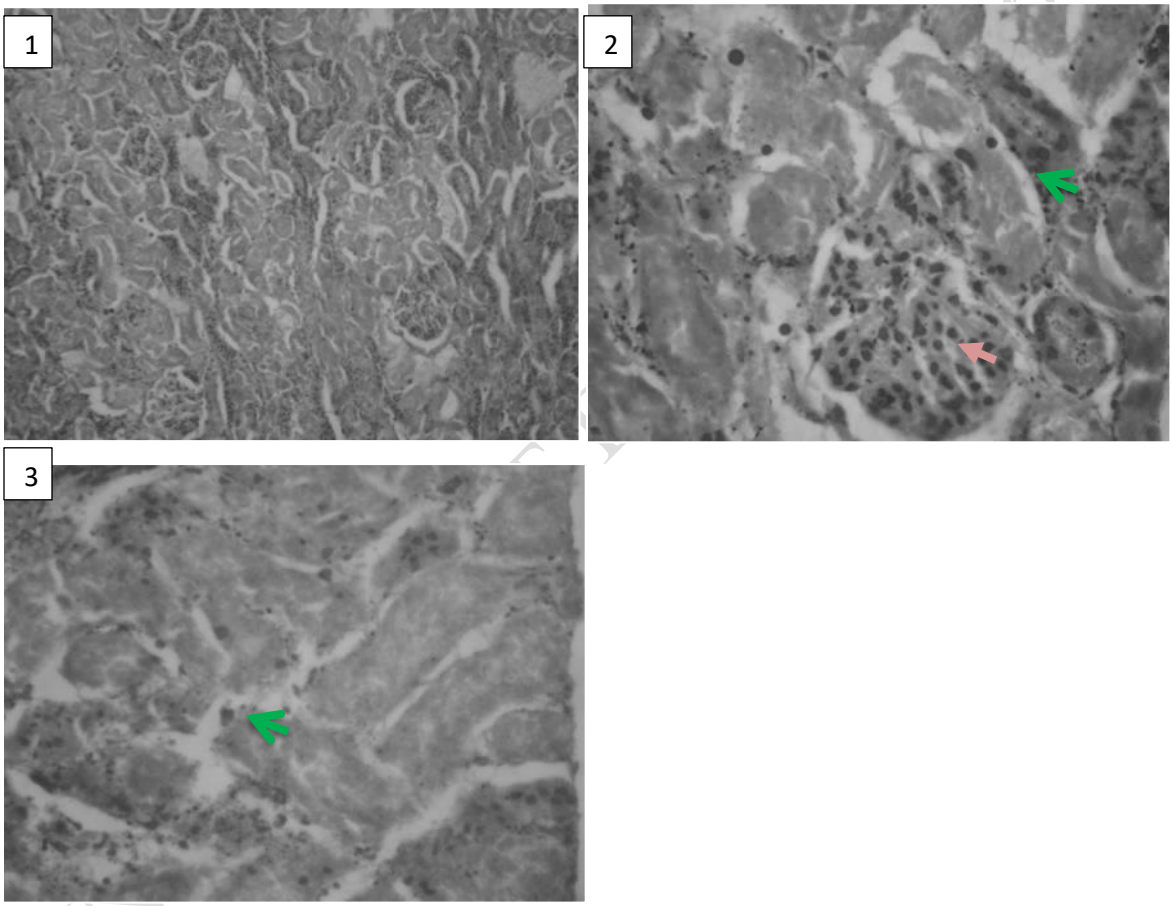

Fig. 14. Photomicrograph of the kidney, plate show severe tubular necrosis (pink arrow) and glomerular messangialisation (green arrow)

Discussion

Our findings shows that vitamin D supplementation has no significant effects on the weight loss associated with nephrotoxicity induced by adriamycin. The weight loss after the ADR treatment might be associated with loss of appetite for food, loss of nutrients, loss of muscle mass, protein energy wasting from the kidney

as a result of the glomerular injury and nephrotoxicity caused by ADR administration in all the test groups.[30,31] Adriamycin can induce weight loss and cause muscle atrophy by influencing lipid and glucose metabolism through induction of peroxisome proliferator-activated receptor gamma (PPARγ) and 5' adenosine monophosphate-AMP-activated protein kinase.[32] The active form of vitamin D can promote and induce apoptosis in adipocytes as reported by previous study.[33] In a double-blind clinical research of overweight and obese women (aged 20–40) taking supplements containing 50,000 IU of vitamin D per week for 6 weeks, it was reported that there was a significant reduction in the BMI, weight, and waist circumference.[34] Studies have shown that there is an inverse relationship between vitamin D and WC and some have reported inability of vitamin D to modify body weight.[35]

High levels of high-molecular-weight proteins are found in the urine of individuals with nephrotoxicity and other cases of renal injury. Ideally, the glomeruli limit the transport of these proteins from the blood to the nephron lumen but this function is affected in the case of renal injury.[36] Such proteins are albumin and urea which are important in the prompt discovery of renal injury and specifically, dysfunctionality in glomerular filtration.[37] In our study, there was a significant reduction in serum albumin as shown in Figure 2 in ADR only group which is expected because high ADR dose induces nephrotoxicity as seen in previous studies.[38] Increased albuminuria, and reduced serum albumin is a marker of kidney injury and damage, albuminuria and the presence of other macromolecules in the tubular fluid may initiate tubular damage and accelerate the progression to CKD. However, we noticed that after vitamin D therapy, serum albumin levels were significantly increased. This is similar to the work of Liyanage et al.[39] Vitamin D may influence albumin levels through a mechanism that involves a decline in renin expression in the kidney and angiotensin II, as well as transforming growth factor-β.[40] In individuals with chronic kidney disease, proteinuria is a surrogate predictive indicator for progression of kidney damage.[41] There was an increase in serum protein level though it was not statistically significant. This might be as a result of the lingering tubular necrosis seen in the photomicrograph (Fig. 14, group 3) which implies that though vitamin D improves renal injury, renal repair is not absolute. Improvements in renal and cardiovascular outcomes have been associated with a decrease in proteinuria.[42] Damage to the glomerular basement membrane and endothelium surface, are glomerular abnormalities that can result in proteinuria. Vitamin D has been reported to preserve the structural integrity of the slit diaphragm which help to protect the podocytes. The glomerulus expresses the VDR, particularly in podocytes and parietal epithelial cells thereby preserving the integrity of the podocyte via the coordination of the activity of heparanase promoter and urokinase receptor during genetic expression of nephrin and podocin.[43] This suggests that vitamin D might be effective in improving the integrity of the glomerulus in renal related diseases.

There have been controversies about the effects of vitamin D on serum creatinine, a clinical trial involving Vitamin D supplementation in 16 patients with renal diseases reported an increase in serum levels of creatinine though GFR remained unchanged, this is as a result of enhanced muscle production of

creatinine.[44] Another study reported increase in creatinine levels with concomitant decrease in GFR.[45] Our study observed a contrary result; a significant decrease in creatinine after vitamin D therapy in ADR induced renal damage though GFR was not assessed. The discrepancies in creatinine levels after vitamin D administration might be as a result of the drug: ADR used in the induction of renal damage in our study. Human studies involving large sample size need to be conducted to resolve these conflicting results.

High creatinine to urea ratio in renal injury has been linked to poor clinical outcomes like chronic heart failure, myocardial infarction and ischemic stroke.[46] Fortunately, our study observed a decrease in creatinine levels after vitamin D treatment though the increased urea levels in the ADR group were not brought down by vitamin D administration as seen in Fig. 5. This is similar to the work of Vanholder et al.[47]

The dyslipideamia observed in the ADR groups was resolved after treatment with vitamin D as seen in Fig. 6-11. This is similar to the findings of Hager et al.[48] There was an increase in HDL, apolipoprotein A, and a decrease in serum levels of TG and LDL after vitamin D treatment in the ADR group. This is an interesting finding, though similar results have been reported in studies involving patients with diabetes mellitus, findings on kidney-related diseases are few.[15] Dyslipideamia in kidney injury is associated with increased risk of cardiovascular complications, development of glomerulosclerosis and progressive kidney disease.[49] Vitamin D has been suggested to increase the amount of calcium absorbed from the intestine, the intracellular calcium in the liver can stimulate microsomal triglycerides transfer protein. This increase in intracellular calcium invariably help in the formation and secretion of VLDL via stimulation of MTP. This action result in the decrease of serum triglycerides levels.[35] Also, the positive effects of Vitamin D on lipid profile might be due to its role in cholesterol synthesis, by increasing the activity of lipoprotein lipase in adipose tissue and through inhibition of SREBP-2 which regulates the level of lipogenic genes and controls lipid synthesis.[50,51] In the skeletal muscle cell, calcitriol alters lipid partitioning and lipid droplet packaging in a way that increases lipid turnover.[45]

CRP is an acute phase reactant and a potent indicator of systemic inflammation produced in response to tissue damage.[52] In our study, the reduced CRP serum levels in nephrotoxic group indicate that there is an increased inflammatory process involved in nephrotoxicity caused by ADR. Previous research has demonstrated the anti-inflammatory properties of vitamin D through its inhibition of the NF-κB pathway, a transcription factor that increases inflammation by modifying the expression of adhesion molecules and cytokines' genes.[53] Vitamin D also affects inflammation mediated by TLRs, (trans-membrane receptors) on mediators of innate immune response like monocytes and macrophages.[53] It has been shown that the infiltration of inflammatory cells in the interstitium correlates directly with proteinuria in renal injury, therefore when there is reduction in inflammation; it impacts positively on proteinuria as seen in our study.[37]

Nitric oxide has a vital role in inhibition of tubular reabsorption of sodium, mediation of natriuresis and glomerular hemodynamics.[54] In cases of renal injury, these functions mediate the beneficial role of NO in

maintaining renal health, however high concentration of serum NO might be detrimental because NO reacts readily with oxygen and nitrogen species thereby contributing to a state of oxidative stress.[54] NO levels were reduced in the ADR group as seen in previous studies involving renal diseases.[55] Interestingly, after treatment with vitamin D, the reduced levels significantly increased to levels similar to the control group, this agrees with the study of Tony wolf who gave college-aged Africa Americans vitamin D supplements for four weeks, and reported improvement in NO mediated microvascular function.[56] Vitamin D has been reported to enhance the production of endothelium enzyme called endothelial NO synthase which helps in the generation of NO essential for prevention of arterial stiffness, regulation of blood pressure and associated cardiovascular issues.[57]

As seen in our study, vitamin D was able to a certain degree resolve the glomerular damage though tubular necrosis was still present (Fig 14, group 3). The renoprotective effects of vitamin D might thus be mediated locally by directly affecting the podocyte, or theoretically other glomerular cells, like the glomerular endothelium. Activated vitamin D has been demonstrated to have a role in the treatment of glomerulonephritis through vitamin D receptor action which regulates the heparanase promoter activity and modulates the urokinase receptor, guaranteeing podocyte preservation.[58] It also controls the podocyte distribution by modulating mRNA synthesis and protein expression of nephrin and podocin.[59,60]

Thus, this present study demonatrated that vitamin D might be beneficial in the reversal of renal injury and amelioration of cardiometabolic complications associated with chronic kidney disease, however there were some limitations associated with our study. The GFR and neutrophil gelatinase associated lipocalin were not determined in the experimental rat because urine samples were not collected, this would have further clarified the role of vitamin D on glomerular function and assessment of renal health. Also, the study did not assess the impact of vitamin D administration on parathyroid hormone, further studies are encouraged in this area.

Conclusion

The results of this study show that vitamin D might have theurapeutic potential in the amelioration of the complications associated with adriamycin induced renal injury or other injuries to the kidney, thereby slowing down the progression into chronic kidney disease and development of cardiovascular diseases. The exact mechanisms involved in the reno-protective and anti-proteinuric effects of vitamin D are not well known. Therefore, human clinical trials involving large sample size is necessary to establish the findings of this study.

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Declarations

Funding

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Author contributions

Conceptualization, A.S.; Methodology, M.A. and O.S.; Software, A.S.; Validation, A.S, O.L and O.S.; Formal Analysis, A.S.; Investigation, M.A.; Resources, O.S; Data Curation, A.S and M.A.; Writing – Original Draft Preparation, A.S. and M.A.; Writing – Review & Editing, A.S, and O.S.; Visualization, M.A, O.S. and O.L.; Supervision, A.S.; Project Administration, A.S. and O.L; Funding Acquisition, A.S. and M.A.

Conflicts of interest

Authors declare no conflicting interest.

Data availability

The datasets generated during and/or analyzed during the current study are available in the repository of the Department of Biochemistry, Lead City University, Ibadan, Nigeria.

Ethics approval

Ethical approval (Reference Number: LCUERB906) was obtained from Lead City University Ethical Review Board (LCU/ERB) to perform this animal research and the animals were treated in accordance with the guidelines of the committee.

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