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Time Course Effects of Dietary Vitamin D on Diethylnitrosamine-Induced Oxidative Stress in Rat Kidney

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ABSTRACT

The kidney is an essential organ known for its role in the endogenous elimination of the body's waste products. The kidney also functions in metabolic activities, including vitamin D metabolism. Other kidney functions involves transport and reabsorption of solutes exposing the organ to oxidative stress induced by toxicants. The study, therefore, examined the effects of dietary vitamin D (Vit D) on diethylnitrosamine (DEN)-induced oxidative stress in rat kidney. Eighty-four male Wistar rats were divided into four groups of 21 animals each, and 30 mg/kg body weight of DEN was administered twice weekly for 11 weeks. Each group received either DEN + Vit D deficient diet or Vit D diet, and normal saline + Vit D deficient diet, or a Vit D diet. Oxidative stress/antioxidant parameters, including Glutathione S-transferase (GST), Thiobarbituric acid reactive substances (TBARS), Glutathione (GSH), Superoxide dismutase (SOD), and Nitric Oxide (NO), were measured using spectrophotometric methods after weeks 6, 12, and 20. Results showed an early significant ($p < 0.05$) increase in GST activity with DEN exposure, which was significantly ($p < 0.05$) reduced with the Vit D diet. In the DEN + Vit D deficient diet group, a significant ($p < 0.05$) decrease in nephrotic NO concentration and increased SOD activities were observed in the 20th week of evaluation. Nephrotic tubular hyalinization and inflammation were noticeable after 11 weeks of DEN exposure from histopathology results. The findings showed that dietary Vit D could, in part, alleviate kidney oxidative stress effects through oxidative stress modulation.

Keywords: Oxidative stress, Antioxidant, Vitamin D, Diethylnitrosamine, Kidney.

Introduction

The kidney is an essential organ involved in the endogenous elimination of waste products from the body. Other vital functions elucidated by the kidney include the mediation of endocrine functions, acid-base balance, and electrolyte maintenance.[1] Due to its role in metabolism, transport, and reabsorption of solutes, typical exposure to toxic effects of ingested toxicants, drugs, or chemicals are common.[1] In the kidney, proximal tubules contain a high amount of mitochondria used for the energy-demanding processes involved in the reabsorption of filtrates passing through the glomerulus.[2] This abundance of mitochondria in the kidney also exposes the organ to the production of intracellular free radicals,[3] and possibly nephrotoxicity.

Interestingly, nephrotoxicity is a significant cause of acute kidney disease (AKD).[4] Globally, approximately 2 million people die annually from AKD,[5] and many survivors of AKD are even at a higher risk of developing end-stage renal diseases and chronic kidney disease (CKD).[5] Various conditions and nephrotoxic agents related to kidney failure are strongly associated with oxidative stress induction.[6] This association suggests oxidative stress plays a vital role in the pathophysiology of kidney diseases.[3]

Nitrosamines, including diethylnitrosamine (DEN), are potent carcinogens formed through the reaction of nitrite produced-nitrosating agents with secondary or tertiary amines.[7] Nitrosamines are found in food, including meat, milk, seafood, oil, alcoholic beverages, and other products.[8-11] DEN has previously been used to induce oxidative stress either as single administered toxicants[12-14] or alongside other toxicants like carbon tetrachloride (CCL-4).[15] Besides its hepatocarcinogenic role, DEN has also been reported to cause nephrotoxicity.[16] Nephrotoxic effects of DEN occurs by altering kidney functions. [12,17] Inclusively, DEN induces oxidative stress during Cytochrome P450 (CYP450) metabolic activation. This induction can lead to carcinogenesis, mutagenesis, and cytotoxicity.[18] Since oxidative stress is associated with various pathological conditions reports suggest it could be essential to use exogenous antioxidants and vitamins[19-22] to augment the endogenous antioxidant capacity. The augmentation may reduce the risk factors of these conditions.

Vitamin D (Vit D) is a critical hormone principally known for the regulation of mineral homeostasis. However, it also mediates autocrine functions aside from its usually known classical roles.[23] Over the years, scientific evidence has reportedly associated vitamin D deficiency with CKD even though Vit D's roles in the progression of these diseases are largely unknown.[24-27] In recent years, Vit D supplementation in different conditions indicates that Vit D could perform other crucial functions. Therefore, Vit D maintenance is essential to regulate the hormone's classical and non-classical functions, thus improving clinical outcomes in some CKD patients.[23] Suggested non-classical roles of vitamin D include regulating physiological processes involved in inflammation, apoptosis, cell differentiation, and oxidative stress.[28]

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Given the suggested antioxidant roles of vitamin D and its deficiency in kidney diseases, it is essential to understand Vit D's non-classical function in alleviating oxidative stress, a necessary factor in kidney diseases' pathogenesis. Therefore, this study investigated the role of vitamin D in DEN-induced oxidative stress in rats. In contrast to previous reports, this study explored the role of vitamin D in DEN-induced kidney toxicity.[29]

Materials and Methods

Chemicals/reagents
Diethylnitrosamine (DEN) used was a product of TCI America, USA. Vitamin D mix, vitamin D deficient mix, and mineral mix used was from Dyets Inc. Bethlehem, PA, USA.

Experimental design
Eighty-four male Wistar rats were divided into four groups of twenty-one animals each. Each group was further subdivided into three groups of seven animals each. Rats were housed at room temperature and kept away from direct sunlight. Food was supplied with water *ad libitum*. The experiment was carried out according to the Covenant University Ethical Committee animal care guidelines with reference number CHREC/022/2019. The experimental groups with respective feeding routines are described below with the feed composition shown in Table 1:
Group 1: Diet deficient in vitamin D + DEN (D-VDD)
Group 2: Control diet + DEN (D-V)
Group 3: Diet deficient in vitamin D+ Normal saline (C-VDD)
Group 4: Control diet + Normal saline (C-V)
The experiment was carried out in three stages;
Stage 1: 6 weeks DEN administration and sacrificed after week 7
Stage 2: 11 weeks DEN administration and sacrificed at week 12
Stage 3: 11 weeks DEN administration and sacrificed at week 20
Feed composition, including vitamin and mineral mix used in the experiment and endogenous Vit D depletion, was initially described by Adelani et al. (2020).[30] In stage 1, animals in groups 1 and 2 were administered 30 mg/kg DEN twice weekly for six weeks through intraperitoneal injection and sacrificed a week after while groups 3 and 4 received normal saline. In stage 2, animals in groups 1 and 2 were administered 30 mg/kg DEN twice weekly for eleven weeks through intraperitoneal injection, while animals in groups 3 and 4 received normal saline. However, stage 3 rats were left for an additional eight weeks following administration of 30 mg/kg DEN twice weekly for eleven weeks before sacrifice using the method described by Ding et al. (2017).[29] Rats were sacrificed by euthanizing using 5 mg xylazine and 50 mg ketamine.

Blood and tissue collection
Blood samples were collected in heparinized tubes while kidney tissues were collected, washed in cold saline, and stored at -20ºC before analysis. Red blood cells (RBC) were separated from plasma for further research.

Blood and kidney antioxidant/oxidative stress parameters
At different experimental stages, oxidative stress parameters in the rat kidney, including Thiobarbituric acid reactive substances (TBARS), Glutathione (GSH), Superoxide dismutase (SOD), Nitric Oxide (NO), and Glutathione S-transferase (GST), were assayed. NO, TBARS, GSH, SOD, and GST were quantified using methods described by Yucel *et al*.[31] Buege and Aust,[32] Ellman,[33] Marklund and Marklund,[34] and Habig *et al*.[35] respectively.

Kidney histopathology
Hematoxylin and eosin (H&E) staining were used in kidney histopathology. A portion of rat kidney tissues was collected, fixed in adequate buffered formalin, processed and embedded in paraffin wax. A slice of the tissues (5 microns) was cut using a microtome, air-dried and exposed to heat to remove paraffin. Staining was carried out using haematoxylin (pH higher than 5) with the cytoplasmic part stained with eosin Y .[36,37]

Statistical analysis
Statistical analysis was carried out using R software (version 4.0.2).[38-41] Significance was measured with one-way Analysis of Variance (ANOVA), and values with $p < 0.05$ were considered significant. Post hoc analysis was done using Tukey HSD, and results shown as mean ± SD.

Table 1: Feed composition in g/kg

Composition	D-VDD C-VDD	D-V C-V
Maize starch	500	500
Soy Bean	350	350
Oil	50	50
Vitamin D mix	-	10
Vitamin D deficient mix	10	-
Minerals	35	35
Fibre	50	50
Methionine	5	5

*In Vitamin D mix is composed of 1000IU of vitamin D_3
Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V)

Results and Discussion

Toxic implications generated from DEN administration could have influenced the alterations of kidney and RBC oxidative balance. However, dietary vitamin D modulated an early stage GST activity and mediated late-stage NO concentration in the kidney and RBC after prolonged exposure to DEN. In the past, the adaptation of vitamins as antioxidants in kidney-related diseases resulted in little success. Despite the relatively low level of success in using vitamins as antioxidants, their roles in alleviating oxidative stress in kidney injuries cannot be underestimated.[23] Furthermore, vitamins, including vitamins C and E, have been previously reviewed for their antioxidant roles[42] in renal injuries and CKD.[43]
At the first stage of kidney oxidative stress evaluation, GST activity was significantly ($p < 0.0001$) increased in group 1 compared to the control groups (Figure 1d). This increase was significantly ($p < 0.0001$) reduced with dietary Vit D. Meanwhile, there were a slight increase and decrease in SOD activity and NO concentrations with DEN administration (Figure 1). Furthermore, other oxidative stress parameters showed no significant ($p > 0.05$) variations among groups observed. Incidentally, a reversed observation of events occurred after 11 weeks of DEN administration (Figure 2). MDA concentration was significantly ($p < 0.05$) reduced in the D-VDD group when compared to the control group, C-V (Figure 2c). Also, group 1 showed no significant ($p > 0.05$) increase in NO concentration and GSH levels (Figure 2a & 2b). Dietary Vit D reduced the increased NO concentration and GSH levels non-significantly ($p > 0.05$). After 11 weeks, the D-VDD group showed reduced GST and SOD activities (Figure 2d & 2e). At stage 3, oxidative stress evaluation was indicated by a decreased NO concentration ($p = 0.007$), which correlates with an increased TBARS level in group 1 (Figure 3). Besides, increased SOD activity ($p = 0.003$) was observed in group 1 compared to the control (C-V) in group 4. The inclusion of dietary Vit D upregulated the NO concentration, decreased SOD activity, and the TBARS level, although these differences were not significant ($p > 0.05$), as seen in Figure 3. However, in the RBC, increased NO concentration and reduced TBARS levels were observed at an early stage (6 weeks) of DEN administration (Figure 4). GST activity was also significantly ($p = 0.012$) upregulated in the RBC after stage 1 (Figure 4d).
However, at the late stage (20 weeks), increased GST activity and reduced GSH levels were observed in group 1 (Figure 6). Interestingly,

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reduction (p = 0.056) of NO concentration in group 1 was significantly (p = 0.0077) increased with dietary Vit D inclusion (Figure 6). In correlation with the observation at stage 2 in the kidney, RBC TBARS levels were also reduced in the DEN-administered group void of vitamin D diet compared to the control groups. From the 6th week, although decreased NO, alongside increased TBARS and SOD, were not distinctively observed after six weeks in this study (Figure 1), there was a reversed observation of the result after eleven weeks (Figure 2). TBARS, however, was significantly reduced compared to the control groups. This observation corresponded to the increased SOD activity and increased NO concentration. Results at this stage could suggest a possible alleviation of oxidative stress effects through organ-specific endogenous responses. In histopathology results, all groups of kidney tissues showed no major pathological changes except for the vascular congestion reported in the D-VDD group (Figure 7). However, after 11 weeks of DEN administration, tubular hyalinization was observed in both the D-VDD and D-V groups. In addition to the hyalinization, the D-VDD group also showed some inflammation and interstitial haemorrhages (Figure 8). Furthermore, after 20 weeks, the tubular hyalinization in D-VDD was accompanied by dysplastic epithelial cells. However, D-V groups showed tubular hyalinization and fibrosis (Figure 9a-b).

Overproduction of free radicals and subsequent reduced production of antioxidants are important risk factors involved in the progression of many renal ailments. Reduced NO concentration after twenty weeks, as observed in the study, is a common phenomenon in CKD. For example, increased production of free radicals has been reported as a significant risk factor in CKD.[3] This finding could be attributed to DEN administration thus, triggering oxidative stress.[12-14] Increased oxidative stress reduces NO bioavailability by upregulating endothelial Nitric Oxide synthase (eNOS) in a mechanism that simultaneously reduces NOS through feedback inhibition.[44] The results suggest that kidney endothelial dysfunction might have caused the reduced NO concentration observed in the study.[45] Also, since renal NO concentration plays a critical role in sodium balance, the reduced NO concentration observed from the study could have altered the tubular sodium transport[44] and kidney functions. The results also indicate there could be a case of nitrosative stress through the reduced NO production or disruption of the NO signalling pathway. This disruption of the signalling pathway is a usual occurrence in the development of various kidney diseases.[45-47] The administration of nitric oxide decreased the occurrence of acute kidney injuries and CKD.[48] Besides, there was a time-dependent reduction of nitric oxide concentration in the RBC, which was evident after twenty weeks. Furthermore, increased SOD activities observed from the study at the later stage of the experiment corroborate the endogenous kidney response to increased oxidative stress. Since SOD plays a vital role in alleviating oxidative stress by converting oxygen radicals to hydrogen peroxide, the endogenous response to stress could have triggered the increased SOD activity.[49] Also, it is suggested that the high SOD activity, which correlates with reduced NO concentration, could result from increased superoxide anions. The increased anions due to oxidative stress are vital in NO degradation by reacting to produce peroxynitrite (ONOO).[42] Additionally, increased GST activity observed after six weeks of DEN administration in both kidney tissue, and RBC could be attributed to internal response mechanisms in alleviating DEN-induced oxidative stress. Furthermore, GSH synthesis is generally triggered as an adaptive mechanism in eliminating a slight increased oxidative stress. However, a high burden of oxidative stress could eliminate the adaptive mechanism and alter oxidation reactions of GSH to GSSG, thus, reducing the GSH levels.[50] In fact, increased SOD and reduced GSH has previously been reported as an indicator of kidney oxidative stress.[51] Furthermore, the decreased GSH reported in oxidative stress could result from the conjugation of DEN with GSH, a reaction possibly mediated by GST.[50,52] This line of thought correlates with this study outcome, as seen in the early and late stages of DEN exposure.

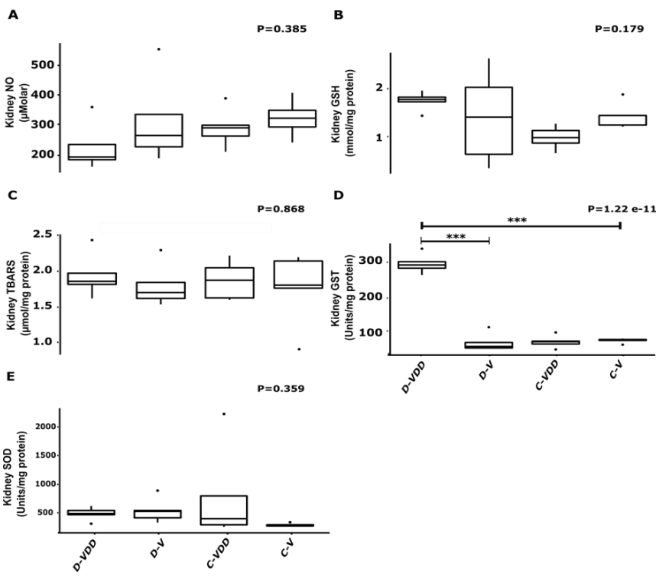

Figure 1: Effects of vitamin D on Kidney DEN-induced oxidative stress after six weeks

(A) NO concentration, **(B)** GSH level, **(C)** TBARS, **(D)** GST activity, (E.) SOD activity. Plots represent mean ± SD (n = 5). Significance is indicated as *** = < 0.001 Thiobarbituric acid reactive substances (TBARS); Glutathione (GSH); Superoxide dismutase (SOD); Nitric Oxide (NO); Glutathione S-transferase (GST); Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

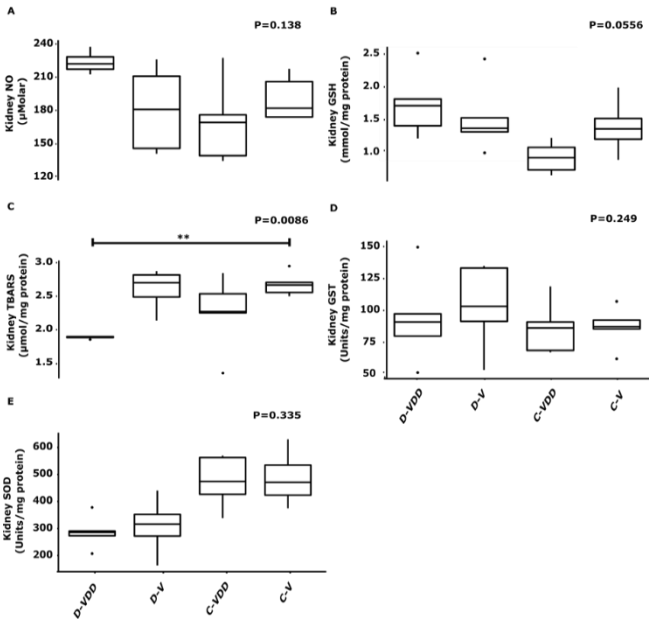

Figure 2: Effects of vitamin D on Kidney DEN-induced oxidative stress after eleven weeks

(A) NO concentration, **(B)** GSH level, **(C)** TBARS, **(D)** GST activity, (E.) SOD activity. Plots represent mean ± SD (n=5). Significance is indicated as ** = < 0.01. Thiobarbituric acid reactive substances (TBARS); Glutathione (GSH); Superoxide dismutase (SOD); Nitric Oxide (NO); Glutathione S-transferase (GST); Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

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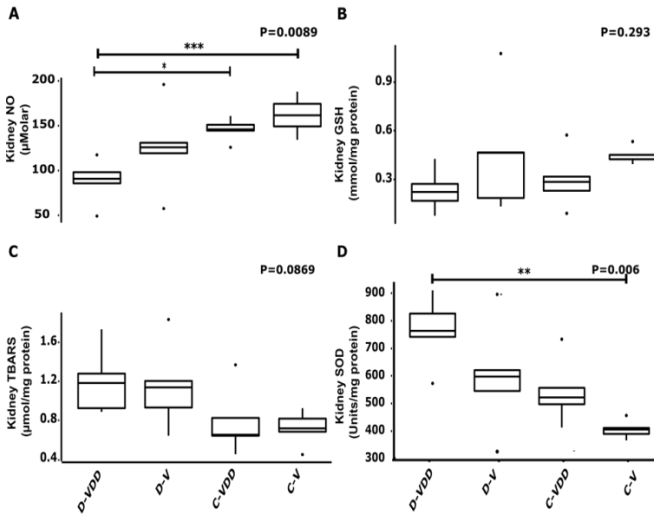

Figure 3: Effects of vitamin D on Kidney DEN-induced oxidative stress after twenty weeks.
(A) NO concentration, **(B)** GSH level, **(C)** TBARS, **(D)** SOD activity. Plots represent mean ± SD (n=5). Significance is indicated as *** = < 0.001; * = <0.05. Thiobarbituric acid reactive substances (TBARS); Glutathione (GSH); Superoxide dismutase (SOD); Nitric Oxide (NO); Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

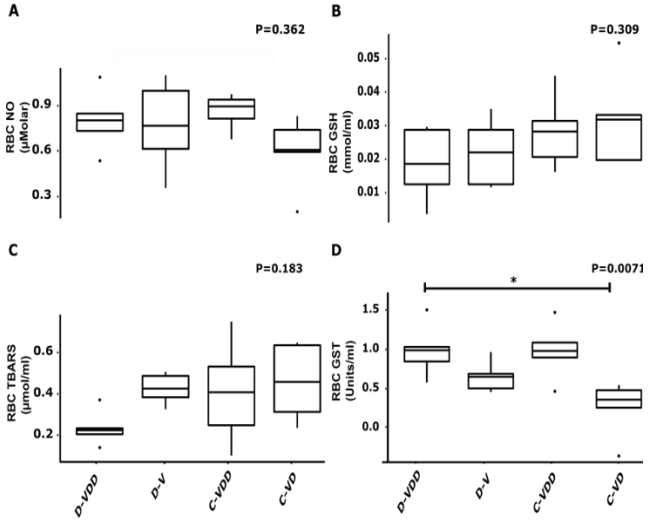

Figure 4: Effects of vitamin D on RBC DEN-induced oxidative stress after six weeks.
(A) NO concentration, **(B)** GSH level, **(C)** TBARS, **(D)** GST activity. Plots represent mean ± SD (n = 5). Significance is indicated as * = < 0.05. Thiobarbituric acid reactive substances (TBARS); Glutathione (GSH); Nitric Oxide (NO); Glutathione S-transferase (GST); Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

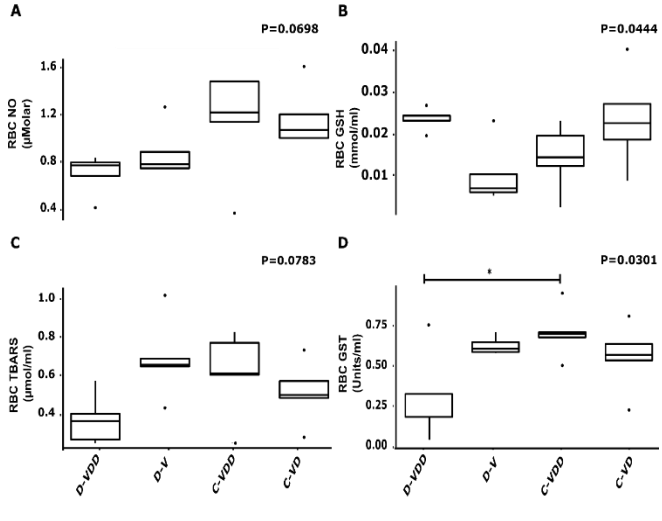

Figure 5: Effects of vitamin D on RBC DEN-induced oxidative stress after eleven weeks.
(A) NO concentration, **(B)** GSH level, **(C)** TBARS, **(D)** GST activity. Plots represent mean ± SD (n=5). Significance is indicated as * = < 0.05. Thiobarbituric acid reactive substances (TBARS); Glutathione (GSH); Nitric Oxide (NO); Glutathione S-transferase (GST); Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

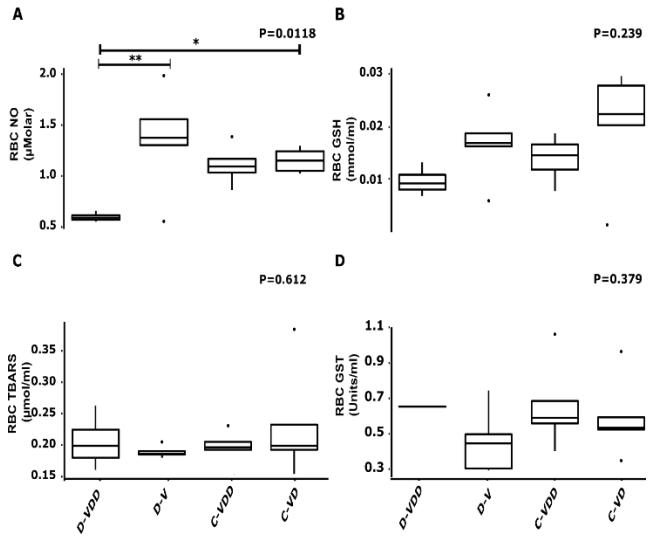

Figure 6: Effects of vitamin D on RBC DEN-induced oxidative stress after twenty weeks.
(A) NO concentration, **(B)** GSH level, **(C)** TBARS, **(D)** GST activity. Plots represent mean ± SD (n = 5). Significance is indicated as * = < 0.05; ** = < 0.01. Thiobarbituric acid reactive substances (TBARS); Glutathione (GSH); Nitric Oxide (NO); Glutathione S-transferase (GST); Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

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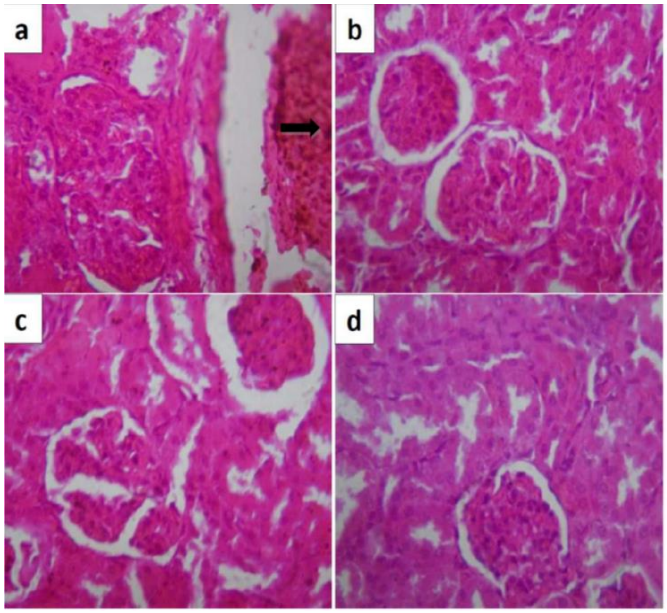

Figure 7: Histopathology of kidney tissues after six weeks of DEN administration.
(a) D-VDD group indicating congested blood vessels; vascular congestion, **(b)** D-V group indicating normal kidney, **(c)** C-VDD group indicating normal kidney, **(d)** C-V group indicating normal kidney. Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

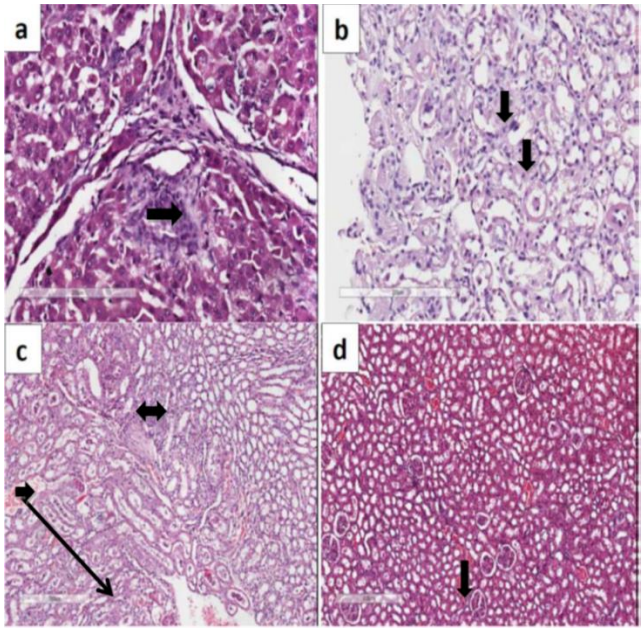

Figure 8: Histopathology of kidney tissues after eleven weeks of DEN administration
(a) D-VDD group indicating inflammation, Tubular hyalinization, and interstitial haemorrhage **(b)** D-V group showing focal tubular hyalinization, **(c)** C-VDD group indicating interstitial haemorrhage and inflammation with a focal area of fibrosis, **(d)** C-V group indicating an essentially normal kidney with focal interstitial haemorrhage Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V)

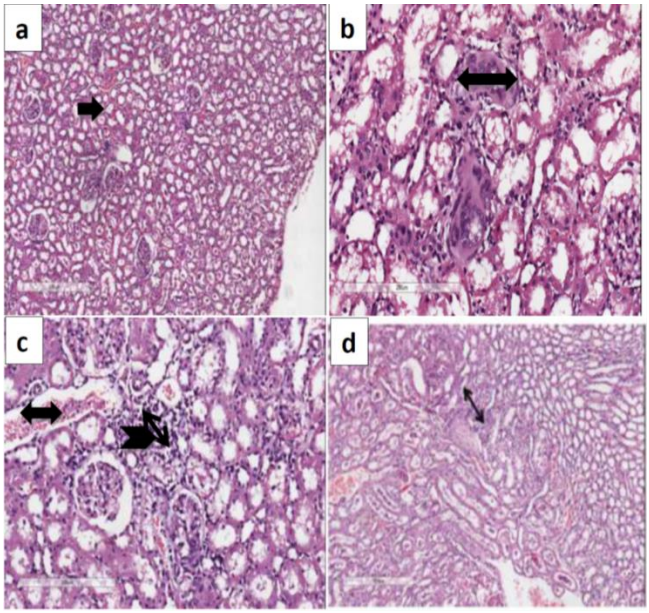

Figure 9: Histopathology of kidney tissues after 20 weeks of the experiment.
(a) D-VDD group indicating tubular hyalinization and presence of atypical dysplastic epithelial cells **(b)** D-V group indicating focal tubular hyalinization and fibrosis; with few atypical cells **(c)** C-VDD group indicating interstitial inflammation and haemorrhage **(d)** C-V group indicating focal tubule-interstitial inflammation with hyalinization. Diet deficient in vitamin D + DEN (D-VDD); Control diet + DEN (D-V); Diet deficient in vitamin D+ Normal saline (C-VDD); Control diet + Normal saline (C-V).

From the histopathology results, dietary Vit D showed possible ameliorative effects with the absence of vascular congestion and a reduced interstitial haemorrhage after six weeks of DEN administration. The observed congested blood vessels in DEN administered group with vitamin D deficiency could result from increased blood flow, which correlates with the observation of Abdel-Moneim et al.[16] in a DEN/2AAF induced nephrotoxicity study. Additionally, thrombosis, a risk factor in hepatocellular carcinoma patients, could also be associated with the findings' vascular congestion.[53] The role of dietary Vit D as an anti-inflammatory agent could also attribute to the observations recorded after eleven weeks of DEN administration.[30] Unlike the group fed dietary Vit D, tubular hyalinization accompanied by intestinal haemorrhage and inflammation were characteristics of the Vit D deficient group. Finally, increased nephrotoxic effects through DEN accumulation during its reabsorption and transportation in the proximal convoluted tubules could have caused the inflammation.[54]

Conclusion

In conclusion, the study shows that dietary Vit D could, in part, alleviate kidney oxidative stress effects through oxidative stress modulation.

Conflict of Interest

No conflict of interest.

Authors' Declaration

The authors with this declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Hosohata K. Role of oxidative stress in drug-induced kidney injury. Int J Mol Sci. 2016; 17:1826.
2. Bhargava P and Schnellmann RG. Mitochondrial energetics in the kidney. Nat Rev Nephrol. 2017; 13(10):629-646.
3. Gyurászová M, Gurecká R, Bábíčková J, Tóthová Ľ. Oxidative stress in the pathophysiology of kidney disease: implications for noninvasive monitoring and identification of biomarkers. Oxid Med Cell Longev. 2020; 2020(5478708):1-11.
4. Sales TGM and Foresto RD. Drug-induced nephrotoxicity. Rev Assoc Med Bras. 2020; 66(1):82-90.
5. Chawla LS, Bellomo R, Bihorac A, Goldstein SL, Siew ED, Bagshaw SM. Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 workgroup. Nat Rev Nephrol. 2017; 13:241-257.
6. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol. 2012; 2012(465897):1-9.
7. Scanlan RA. Formation and occurrence of nitrosamines in food. Cancer Res. 1983; 43(5):2435s-2440s.
8. Scanlan RA and Issenberg P. N - nitrosamines in foods. Crit Rev Food Technol. 1975; 5(4):357-402.
9. Park J, Seo J, Lee J, Kwon H. Distribution of Seven N-nitrosamines in food. Toxicol Research. 2015; 31(3):279-288.
10. Maduagwu EN and Bassir O. Detection of dimethyl- and diethylnitrosamine in a Nigerian fermented milk product (nono). Toxicol Lett. 1979; 4(3):169-173.
11. Maduagwu, Joaquim KA, Bassir O. Contamination of some fermented Nigerian beverages by carcinogenic nitrosamines. Trop Geogr Med. 1979; 31(2):283-290.
12. Hassanen NHM, Fahmi A, Shams-Eldin E, Abdur-Rahman M. Protective effect of rosemary (*Rosmarinus officinalis*) against diethylnitrosamine-induced renal injury in rats. Biomarkers. 2020; 25(3):281-289.
13. Elguindy NM, Yacout GA, El Azab EF. Amelioration of DENA-induced oxidative stress in rat kidney and brain by the essential oil of *Elettaria cardamomum*. Beni-Suef Univ J Basic Appl Sci. 2018; 7(3):299-305.
14. Ahmed RR, Mahmoud AM, Ashour MB, Kamel AM. Hesperidin protects against diethylnitrosamine-induced nephrotoxicity through modulation of oxidative stress and inflammation. Natl J Physiol Pharm Pharmacol. 2015; 5(5):391-397.
15. Aly MS, Galaly SR, Moustafa N, Mohammed HM, Khadrawy SM, Mahmoud AM. Hesperidin protects against diethylnitrosamine/carbon tetrachloride-induced renal repercussions via up-regulation of Nrf2/HO-1 signaling and attenuation of oxidative stress. J Appl Pharm Sci. 2017; 7(11):7-14.
16. Abdel-Moneim A, Ahmed OM, Fahim HI, Zaky MY. Ameliorative Effects of Quercetin and Naringenin on Diethylnitrosamine/2-acetyl aminoflourene-Induced Nephrotoxicity in Male Wistar Rats. Am J Biochem. 2016; 6(5):113-121.
17. Pashmforoosh M, Rezaie A, Haghi-karamallah M, Fazlara A, Shahriari A, Najafzadeh H. Effects of caffeine on renal toxicity induced by diethylnitrosamine. Zahedan J Res Med Sci. 2015; 17(1):7-9.
18. Pradeep K, Mohan CVR, Gobianand K, Karthikeyan S. Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. Eur J Pharmacol. 2007; 560(2007):110-116.
19. Marcadenti A and Coelho RCLA. Dietary antioxidant and oxidative stress: Interaction between Vitamins and Genetics. J Nutr Heal Food Sci. 2015; 3(1):1-7.
20. Pehlivan FE. Vitamin C: An antioxidant agent. In: Intech. IntechOpen; 2017. 23-35p. Available from: http://dx.doi.org/10.1039/C7RA00172J%0Ahttps://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0Ahttp://dx.doi.org/10.1016/j.colsurfa.2011.12.014
21. Paolini M, Sapone A, Canistro D, Chieco P, Valgimigli L. Antioxidant vitamins for prevention of cardiovascular disease. Lancet. 2003; 362:920.
22. Olubukola Sinbad O, Folorunsho AA, Olabisi OL, Abimbola Ayoola O, Johnson Temitope E. Vitamins as antioxidants. J Food Sci Nutr Res. 2019; 2(3):214-235.
23. Williams S, Malatesta K, Norris K. Vitamin D and chronic kidney disease. Ethn Dis. 2009; 19(5):8-11.
24. McCarron DA and Drueke TB. Vitamin D deficiency and chronic kidney disease risk: cause or merely association? Am J Clin Nutr. 2018; 108:1164-1165.
25. Filipov JJ and Dimitrov EP. Vitamin D Deficiency in Renal Disease. In: IntechOpen. 2019. 1–24p. Available from: http://dx.doi.org/10.1039/C7RA00172J%0Ahttps://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0Ahttp://dx.doi.org/10.1016/j.colsurfa.2011.12.014
26. Gois PHF, Wolley M, Ranganathan D, Seguro AC. Vitamin D deficiency in chronic kidney disease: Recent evidence and controversies. Int J Environ Res Public Health. 2018; 15:1773.
27. Al-Badr W and Martin KJ. Vitamin D and kidney disease. Clin J Am Soc Nephrol. 2008; 3:1555-1560.
28. Umar M, Sastry KS, Chouchane AI. Role of vitamin D beyond the skeletal function : a review of the molecular and clinical studies. Int J Mol Sci. 2018; 19(1618):1-28.
29. Ding Y, Zhen-hui W, Wei Y, Shu L, Peng Y. Hepatic inflammation-fibrosis-cancer axis in the rat hepatocellular carcinoma induced by diethylnitrosamine. J Cancer Res Clin Oncol. 2017; 143(5):821-834.
30. Adelani IB, Ogadi EO, Onuzulu C, Rotimi OA, Maduagwu EN, Rotimi SO. Dietary vitamin D ameliorates hepatic oxidative stress and inflammatory effects of diethylnitrosamine in rats. Heliyon. 2020; 6(2020):e04842.
31. Yucel AA, Gulen S, Dincer S, Yucel AE, Yetkin GI. Comparison of two different applications of the Griess method for nitric oxide measurement. J Exp Integr Med. 2012; 2(2):167-171.
32. Buege JA and Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52:302-310.
33. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82(1):70-77.
34. Marklund S and Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47:469-474.
35. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. J Biol Chem. 1974; 249(22):7130-7139.
36. Rolls G. An introduction to specimen preparation. [Online]. [Cited 2021 March 24]. Available from: https://www.leicabiosystems.com/knowledge-pathway/an-introduction-to-specimen-processing/
37. Sikandar A. Histopathology: An old yet important technique in modern science. In S. Srivastava (Ed.). Intech; 2018; 32:1-10 p.
38. Team RC. R: A language and environment for statistical computing. R Found Stat Comput Vienna, Austria. 2020; Available from: https://www.r-project.org/.
39. Wickham H, François R, Henry L, Müller K. dplyr: A grammar of data manipulation. R package version 1.0.0. 2020.
40. Kassambara A. ggpubr: "ggplot2" Based Publication Ready Plots. R Packag version 040 https//CRANR-project.org/package=ggpubr. 2020; Available from: https://cran.r-project.org/package=ggpubr
41. Wickham H. ggplot2: Elegant graphics for data analysis. Springer-Verlag New York. 2016.
42. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative / nitrosative stress: current

state. Nutr J. 2016; 15:71.
43. Ling XC and Kuo K-L. Oxidative stress in chronic kidney disease: diet and exercise. Ren Repacement Ther. 2018; 4:53.
44. Lee JU. Nitric oxide in the kidney: Its physiological role and pathophysiological implications. Electrolyte Blood Press. 2008; 6(1):27-34.
45. Reddy YS, Kiranmayi VS, Bitla AR, Krishna GS, Srinivasa Rao PVLN, Sivakumar V. Nitric oxide status in patients with chronic kidney disease. Indian J Nephrol. 2015; 25(5):287-291.
46. Baylis C. Nitric oxide deficiency in chronic kidney disease. Am J Physiol - Ren Physiol. 2008; 294:F1-F9.
47. Singh RR, Easton LK, Booth LC, Schlaich MP, Head GA, Moritz KM, Denton KM. Renal nitric oxide deficiency and chronic kidney disease in young sheep born with a solitary functioning kidney. Sci Rep. 2016; 6(26777):1-11.
48. Lei C, Berra L, Rezoagli E, Yu B, Dong H, Yu S, Hou L, Chen M, Chen W, Wang H, Zheng Q, Shen J, Jin Z, Chen T, Zhao R, Christie E, Sabbisetti VS, Nordio F, Bonventre JV, Xiong L, Zapol WM. Nitric oxide decreases acute kidney injury and stage 3 chronic kidney disease after cardiac surgery. Am J Respir Crit Care Med. 2018; 198(10):1279-1287.
49. Cabaña-Muñoz ME, Parmigiani-Izquierdo JM, Bravo-González LA, Kyung HM, Merino JJ. Increased Zn/glutathione levels and higher superoxide dismutase-1 activity as biomarkers of oxidative stress in women with long-term dental amalgam fillings: Correlation between mercury/aluminium levels (in hair) and antioxidant systems in plasma. PLoS One. 2015; 10(6):e0126339.
50. Wu HY, Xu HX, Hong YB, Zhang JF, Wu JC. The use of biomarkers in the antioxidant responses of Daphnia magna to the acute and chronic exposure to no. 20 diesel oil and 2,4-dichlorophenol. Chem Speciat Bioavailab. 2011; 23(2):80-87.
51. Rotimi SO, Bankole GE, Adelani IB, Rotimi OA. Hesperidin prevents lipopolysaccharide-induced endotoxicity in rats. Immunopharmacol Immunotoxicol. 2016; 38(5):364-371.
52. Prione LP, Olchanheski LR, Tullio LD, Santo BCE, Reche PM, Martins PF, Carvalho G, Demiate IM, Pileggi SAV, Dourado MN, Prestes RA, Sadowsky MJ, Azevedo RA, Pileggi M. GST activity and membrane lipid saturation prevents mesotrione‐induced cellular damage in Pantoea ananatis. AMB Express. 2016; 6(70):1-12.
53. Zanetto A, Campello E, Spiezia L, Burra P, Simioni P, Russo FP. Cancer-associated thrombosis in cirrhotic patients with hepatocellular carcinoma. Cancers (Basel). 2018; 10(450):1-19.
54. Jarrar BM. Histological and histochemical alterations in the kidney induced by lead. Ann Saudi Med. 2012; 23(1–2):10–15.