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Dose-dependent effects of vitamin C on oxidative stress and kidney function in Albino Rats

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Abstract

Vitamin C is an essential nutrient that plays a crucial role in various body functions. While it is generally considered safe, high-dose administration has raised concerns about potential toxicological effects. This study aimed to investigate the toxicological effects of high-dose vitamin C administration in animal models. 80 male albino rats which were divided into four groups: Control, Low-dose (100 mg/kg), Medium-dose (500 mg/kg), and High-dose (1000 mg/kg) vitamin C administration were used for the study. Vitamin C was administered orally once daily for 28 days. Oxidative stress and kidney damage were assessed using standard biochemical assays. 4-Hydroxynonenal (4-HNE) levels were measured to assess oxidative stress while urea and creatinine levels were measured to assess the possible effects on the kidneys. Our results show that high-dose vitamin C administration caused significant increases in oxidative stress and kidney dysfunction in rats. These findings suggest that high-dose vitamin C administration may have adverse effects on health, and its use should be approached with caution.

Keywords: Vitamin C; High dose; Oxidative stress; Kidney dysfunction; 4-Hydroxynonenal; Toxicity

1. Introduction

Vitamin C is an essential nutrient that plays a vital role in maintaining various bodily functions, including immune response, collagen synthesis, and iron absorption [1]. The recommended dietary allowance (RDA) for vitamin C is 60-90 mg/day for adult men and 75 mg/day for adult women. [2]. Recent studies have investigated the effects of vitamin C on oxidative stress and kidney function, particularly in animal models. For instance, research has demonstrated that vitamin C treatment can significantly ameliorate the progression of proteinuria and albuminuria in diabetic rats by reducing renal malondialdehyde (MDA) accumulation, a marker of oxidative stress [3-5]. Additionally, vitamin C has also been shown to decrease apoptosis of proximal tubular epithelial cells and mitigate glomerular injury in such models [3]. Furthermore, studies have indicated that vitamin C, in combination with other antioxidants like curcumin, can improve renal function and oxidative stress parameters in animal models with induced kidney damage. This combination therapy has been associated with decreased levels of urea, uric acid, and creatinine, alongside improved antioxidant enzyme activities, suggesting a protective effect against renal oxidative damage [6]. Due to its antioxidant properties and other perceived health benefits, many individuals consume vitamin C supplements in excess of the RDA.

However, high-dose vitamin C administration has raised concerns about potential toxicological effects. Previous studies have reported that high doses of vitamin C can cause gastrointestinal disturbances, kidney stones, and oxidative stress [7-9]. The mechanisms underlying these effects are not fully understood, and there is a need for further research to elucidate the potential risks associated with high-dose vitamin C administration.

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The correlation between vitamin C dose and its impact on oxidative stress and renal function seems to be intricate. Although moderate doses may provide protective advantages, high-dose vitamin C therapy has elicited concerns over potential pro-oxidant effects, resulting in heightened oxidative stress and eventual renal dysfunction. This paradoxical outcome highlights the significance of dosage consideration in the therapeutic use of vitamin C.

Considering the prevalent utilization of vitamin C supplements and the continuous investigation of their therapeutic potential, it is essential to comprehend the dose-dependent effects of vitamin C on oxidative stress and renal function. This research seeks to examine these effects in albino rats, offering information that may guide the safe and effective application of vitamin C in clinical contexts.

2. Materials and methods

2.1. Study Design

This study employed a randomized controlled design using 80 male albino rats that weighed between 150-200g. They were housed in metal cages at the animal house of Bayelsa Medical University, Yenagoa, Bayelsa State. All the animals underwent a period of 7 days of observation and acclimatization between the date of arrival and the start of treatment. They were divided into four groups with 20 rats in each group: Control, Low-dose (100 mg/kg), Medium-dose (500 mg/kg), and High-dose (1000 mg/kg) vitamin C administration.

Vitamin C was administered orally to the animals in the different experimental groups once daily for 28 days. The administration volume was 10 ml/kg and the quantity of vitamin C administered to each animal was calculated from its body weight on the day of the treatment. The rats in the control group were treated with distilled water at the same administration volume as the rest of the treatment groups.

2.2. Sample Collection

At the end of the 28th day treatment, samples of blood were withdrawn from the orbital sinus of the rats from each group, under light ether anesthesia. The collected blood samples were centrifuged at 10,000 rpm for 10 minutes to separate the serum.

2.3. Laboratory Procedures

The separated sera were used to evaluate the levels of 4-Hydroxynonenal (4-HNE), urea and creatinine. Fine Test rat 4-Hydroxynonenal ELISA kit was used to determine the levels of 4-HNE. [10] RANDOX Urease-Berthelot Colorimetric method kit was used to measure urea levels [11] and also RANDOX Creatinine kit was used to determine creatinine levels. [12]

2.4. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and Tukey HSD Post-Hoc test using Graph Pad Prism (version 9.5.1) software. The results were expressed as means and standard deviations. Statistical significance was considered at a 95% confidence interval ($P<0.05$).

3. Results

Table 1 represents the means ± standard deviations of 4-HNE (4-Hydroxynonenal), urea, and creatinine measured in animals in the four study groups. The table shows that the Medium-Dose group had the lowest level of 4-HNE (15.22 ± 1.87pg/ml) while the highest level was observed in the High-Dose group (21 ± 1.72pg/ml). The observed differences among the groups (ANOVA) were significant (p-value < 0.001). Urea levels were significantly different between groups, with the High-Dose group having the highest levels (6.12 ± 1.56mmol/l) and the Control group with the lowest levels (4.56± 1.32mmol/l). Creatinine levels were equally significantly different between groups, with the High-Dose group having the highest levels (37.5 ± 2.87 µmol/l) and the Low-Dose group with the lowest levels (26.7 ± 2.11 µmol/l).

Table 1 Mean values of 4-HNE, Urea and Creatinine Levels in Rats Administered with Different Doses of Vitamin C.

N	Group	4-HNE (pg/ml)	Urea (mmol/l)	Creatinine (µmol/l)
20	Control	16.87 ± 1.65	4.56 ± 1.32	27.2 ± 2.53
20	Low-Dose(100mg/kg)	16.40 ± 1.78	4.60 ± 1.44	26.7 ± 2.11
20	Medium-Dose(500mg/kg)	15.22 ± 1.87	5.21 ± 1.41	31.4 ± 3.01
20	High-Dose(1000mg/kg)	21 ± 1.72	6.12 ± 1.56	37.5 ± 2.87
	P Value	< 0.001	0.003	< 0.001

The Tukey Post Hoc analysis (Table 2) shows pair-wise comparisons between Control, Low, Medium, and High dose groups for the three measured parameters. There were no significant differences in the measured levels of 4-HNE between the Control and Low Dose groups ($p = 0.832$) as well as between the Low and Medium Dose groups ($p = 0.155$). Comparisons between Control and Medium Dose ($p = 0.020$), Control and High Dose ($p < 0.001$), Low Dose and High Dose ($p < 0.001$), and Medium Dose against High Dose ($p < 0.001$) all showed significant differences.

Comparison of Urea levels among the four study groups showed significant differences between Control and High Dose ($p = 0.005$) as well as between Low Dose and High Dose ($p = 0.007$) only, with higher urea levels in the High Dose group. Other comparisons were not significant.

The differences in the levels of creatinine were significant among the four groups except for Control versus Low Dose which showed no significant difference ($p = 0.933$).

Table 2 Comparison of the Levels of 4-HNE, Urea and Creatinine among the Four Study Groups.

Study Group	4-Hydroxynonenal (P Value)	Urea (P Value)	Creatinine (P Value)
Control vs Low Dose	0.832	0.100	0.933
Control vs Medium Dose	0.020	0.483	< 0.001
Control vs High Dose	< 0.001	0.005	< 0.001
Low Dose vs Medium Dose	0.155	0.538	< 0.001
Low Dose vs High Dose	< 0.001	0.007	< 0.001
Medium Dose vs High Dose	< 0.001	0.195	< 0.001

4. Discussion

The results show that the High-dose group (1000mg/kg) had significantly higher 4-HNE levels compared to all other groups ($p < 0.001$). This suggests that high doses of vitamin C may induce oxidative stress. 4-HNE is a highly reactive aldehyde that is formed as a result of lipid peroxidation. During lipid peroxidation, the lipid hydroperoxides formed can undergo homolytic cleavage, resulting in the formation of 4-HNE. 4-HNE being highly reactive, can react with proteins, DNA, and other bio-molecules, leading to their modification and potential dysfunction [13].This can activate inflammatory signaling pathways, leading to the production of pro-inflammatory cytokines and the recruitment of immune cells. [14]. Furthermore, 4-HNE has been linked to the development of various diseases, including atherosclerosis, cancer, and neurodegenerative diseases [15-16]. In Contrast with expected antioxidant effects, a high dose of vitamin C (1000mg/kg) exhibited a pro-oxidant effect contradicting the expected antioxidant effects. This highlights the complexity of its mechanisms and potential dose-dependent effects. The observed pro-oxidant effect of high dose vitamin C can lead to the above-mentioned pathological conditions.

The mean value of urea increased with increasing doses of vitamin C, with the highest value observed in the High-Dose group (6.12 ± 1.56). The ANOVA result for urea indicated a P value of 0.003, indicating a significant urea level difference between the three groups. This suggests that high-dose vitamin C supplementation may lead to increased urea

production, potentially indicating kidney dysfunction. The Tukey post hoc analysis showed that high-dose vitamin C administration leads to a significant increase in urea levels compared to the Control and Low-Dose groups. Medium-dose vitamin C administration does not have a significant impact on Urea levels when compared to the Control group. Low-dose vitamin C administration also does not have a significant impact on urea levels. There is no significant difference in urea levels between the Medium-Dose and High-Dose groups. These findings suggest that high-dose vitamin C administration may have adverse effects on kidney function, as indicated by increased urea levels. The results also show that both the Medium Dose and High Dose groups had significantly higher creatinine levels compared to the Control and Low Dose groups ($p < 0.01$). This suggests that medium to high doses of vitamin C (500 and 1000mg/kg) may be associated with kidney damage or impaired kidney function.

Limitations and Future Directions

Despite these findings, this study is not without limitations. Firstly, the use of a single animal model may limit the generalizability of the results to humans. Secondly, the study primarily focused on oxidative stress and renal function markers, without exploring other potential systemic effects of high-dose vitamin C. Future research should investigate the long-term impacts of high-dose vitamin C on other organ systems and its potential synergistic effects with other antioxidants.

5. Conclusion

In summary, this study underscores the importance of understanding the dose-dependent effects of vitamin C on oxidative stress and renal function. While low to moderate doses exhibit protective effects, high doses may lead to adverse outcomes, highlighting the need for caution in clinical applications and supplementation practices.

Compliance with ethical standards

Disclosure of conflict of interest

We declare that there is no conflict of interest.

Statement of ethical approval

Ethical considerations were received from the Faculty of Medical Laboratory Science Research and Ethical Committee, Federal University Otuoke, Nigeria, and all procedures in accordance with the guidelines and regulations approved for the use and care of animals were strictly followed.

References

[1] Carr AC, McCall C. The role of vitamin C in the treatment of the common cold. Am J Lifestyle Med. 2017;11(3):172-176.

[2] Institute of Medicine. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academies Press; 2000.

[3] Lee EY, Lee MY, Hong SW, Chung CH, Hong SY. Blockade of oxidative stress by vitamin C ameliorates albuminuria and renal sclerosis in experimental diabetic rats. Yonsei Med J. 2007;48(5):847-855.

[4] Rehman K, Akash MS, Azhar S, Khan SA, Abid R, Waseem A, et al. A biochemical and histopathologic study showing protection and treatment of gentamicin-induced nephrotoxicity in rabbits using vitamin C. Afr J Tradit Complement Altern Med. 2012;9(3):360-365.

[5] Lee M, Ahn C, Kim K, Jeung EB. Mitochondrial Toxic Effects of Antiepileptic Drug Valproic Acid on Mouse Kidney Stem Cells. Toxics. 2023;11(5):471.

[6] Mehany ABM, Farrag IM, Diab M, Ghoneim MM, El-Sherbiny M, Al-Serwi RH, et al. Curcumin and vitamin C improve immunity of kidney via gene expression against diethyl nitrosamine induced nephrotoxicity in rats: In vivo and molecular docking studies. Heliyon. 2023;9(3):e14126.

[7] Levine M, Conry-Cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A. 1996;93(8):3704-3709.

[8] Auer BL, Auer D, Rodgers AL. The effect of ascorbic acid ingestion on the biochemical and physicochemical risk factors for calcium oxalate kidney stone formation. Clin Chem Lab Med. 1998;36(3):143-147.

[9] Padayatty SJ, Sun H, Wang Y, et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 2004;140(7):533-537.

[10] Li, W., Chen, L., Zhou, Y., Li, M., Yu, X., & Li, P. (2016). Quantification of 4-hydroxynonenal-protein adducts by ELISA and Western blot. Journal of Visualized Experiments, 118, e54755. doi: 10.3791/54755

[11] Fawcett, J. K. and Scott, J.E. 1960; Clin. Path.; 13:156.

[12] Slot, C. Scandinevia Journal of Clinical Laboratory Investigations, 1965; 17: 381–387

[13] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11(1):81-128. doi: 10.1016/0891-5849(91)90192-6

[14] Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress. Free Radic Biol Med. 2002;32(7):1050-1060. doi: 10.1016/S0891-5849(02)00777-1

[15] Lee SH, Blair IA. Oxidative stress and axonal degeneration in neurodegenerative diseases. Curr Med Chem. 2004;11(24):2985-2995. doi: 10.2174/0929867043363929

[16] Negre-Salvayre A, Auge N, Ayala V, et al. Pathological aspects of lipid peroxidation. Free Radic Res. 2008;42(1):28-37. doi: 10.1080/10715760701767191