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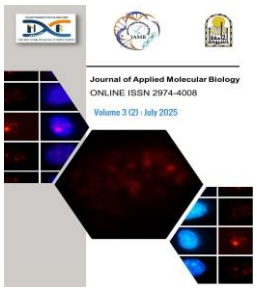

The Impact of Deoxycholate Amphotericin B and Vitamin E on Kidney Structure in Mice

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ABSTRACT

The present study aimed to investigate the potential protective effects of vitamin E on kidney damage induced by deoxycholate deoxycholate amphotericin B (D-Amb) in adult male mice. Twenty-eight adult male mice (Mus musculus) were allotted into four groups: control, D-Amb, vitamin E, and D-Amb + vitamin E groups. Each individual in the D-Amb group received a dose of 3 mg/kg of body weight per day via intravenous injection, while the vitamin E group received 200 IU orally per day for a month. D-Amb administration significantly increased serum creatinine (0.98 ± 0.13 mg/dL), urea (48.6 ± 5.2 mg/dL), and malondialdehyde (MDA; 5.1 ± 0.7 nmol/mg protein), but decreased superoxide dismutase (SOD) activity (6.5 ± 1.0 U/mg protein) compared to the control group ($p < 0.05$). Co-administration of vitamin E with D-Amb substantially improved these measures (creatinine: 0.56 ± 0.09 mg/dL; urea: 29.6 ± 3.7 mg/dL; MDA: 3.0 ± 0.5 nmol/mg protein; SOD: 9.2 ± 1.1 U/mg protein), but values remained statistically different from the control group. Vitamin E alone had no significant effect on these indicators compared to controls ($p < 0.05$). The histological examination showed that D-Amb causes kidney damage: glomeruli atrophy, kidney necrosis, inflammatory cell infiltration, and internal bleeding. On the other hand, vitamin E does not hurt the kidneys on its own, and when combined with D-Amb, it mitigates some of its negative effects. These results indicated that vitamin E partially reverses some harmful effects of D-Amb on the kidney. More research is needed to validate the conditions required to maximize the renal-protecting activity against D-Amb toxicity.

1. INTRODUCTION

Invasive fungal infections remain a global health concern, especially for immunocompromised patients [1]. Deoxycholate amphotericin B (D-Amb) has been a

basic treatment in antifungal therapy for many years, especially in preserving the deoxycholate formulation (amphotericin B), even with its well-known nephrotoxic effects. Its clinical practice is often restricted by overwhelming adverse reactions, with nephrotoxicity being the most concerning [2]. D-Amb-induced nephrotoxicity is characterized by a multitude of renal tissue damage responses, through oxidative stress, inflammation, and membrane injury [3,4]. The cell membrane cholesterol interacts with the amphotericin B and ends up damaging the proximal tubular cells, which guarantee the kidney's filtration system [5]. Studies have shown that up to 80% of patients receiving D-Amb treatment experience some degree of renal dysfunction, ranging from mild electrolyte imbalances to acute kidney injury [6]. Other studies have sought out protective agents for D-Amb nephrotoxic effects, while still sustaining its antifungal effects [7,8]. As a powerful antioxidant, vitamin E has shown potential as a protective factor due to its effects on free radical scavenging and preservation of cellular membranes [9]. The antioxidant effects of vitamin E have been studied more deeply on drug toxicity at the level of kidney lesions [10,11]. The connection between oxidative stress and nephrotoxicity caused by D-Amb has already been demonstrated [8]. The generation of free radicals and lipid peroxidation are primary pathways of D-Amb's renal injurious effects [12]. Vitamin E functions as a preventive antioxidant and is relevant to the protection of cellular functions and restraining the damage to membranes [9]. The present work was performed to study the mitigating effects of vitamin E on D-Amb-induced toxicity in adult Mice. In particular, this study aims to explore the D-Amb impact on kidney morphology and assess the protective role of Vitamin E.

2. MATERIAL and METHODS

2.1 Experimental animals

The present work was carried out on 28 Adult (8–12 weeks old mice, weight: 30±10 g) that were purchased from Animal House at Veterinary Medicine of Mosul University, Iraq. All mice were male and allowed for one week of adaptation (taking food & water without any medications) in their new environment.

2.2 Chemicals

D-Amb drug (fungizone powder for intravenous injection, each vial contains 25 mg of amphotericin B diluted with dextrose 5% to a volume of 0.25 mL) was manufactured and obtained from Sun Pharmaceutical Industries Ltd (Goregaon, Mumbai, India). Vitamin E was obtained from Chemical Co (Jamestown, RI, USA).

2.3 Study design and Animal groups

The research was planned as an open-ended, randomized, interventional study. The animals were randomly assigned to four groups (seven per group) at the start of the trial.

-Group I: the control group monitored basic parameters, and they had unlimited access to food and distilled water throughout the trial.

-Group II: mice received D-Amb (3 mg/kg of body weight/day, given slowly intravenously) for one month [13].

-Group III: mice received orally vitamin E, 200 IU/day for one month [14].

-Group-IV: mice received a daily intravenous dose of D-Amb (3 mg/kg of body weight/day) and a daily dose of vitamin E (200 IU) for one month.

2.4 Blood Sampling

Blood samples were collected from the vena cava under anesthesia using heparinized syringes, and the animals were then slain with ether. Plasma samples were centrifuged at 1500 × g for 15 minutes and cooled− 20 °C.

2.5 Serum Creatinine and Urea

Serum creatinine and urea concentrations were evaluated using commercially available Creatinine (plasma) colorimetric test kits (e.g., BioSystems S.A., Spain) and Urea test Kit (Urease-Berthelot) (e.g., Randox Laboratories, Crumlin, UK) according to the manufacturer's instructions. Creatinine (510 nm) and urea (578 nm) absorbances were measured using a spectrophotometer (BioSpectrometer® Kinetic by Eppendorf, Germany).

2.6 Malondialdehyde (MDA)

MDA levels, an indicator of lipid peroxidation, were evaluated in kidney tissue homogenates using the thiobarbituric acid reactive substances (TBARS) method (e.g., Cayman Chemical TBARS Assay Kit), as reported by Mariutti[15]. The resulting red chromogen's absorbance was measured at 532 nm.

2.7 Superoxide Dismutase (SOD) Activity

SOD activity in kidney tissue was measured using a commercial SOD assay kit (e.g., Cayman Chemical). The inhibition of the reduction of nitroblue tetrazolium (NBT) by Durak et al. [16].

2.8 Histological Examination:

At the end of the experiment, each group of animals was killed by cervical dislocation while anesthetized with light diethyl ether. The abdomen was next opened, and both kidneys were retrieved and cleaned in phosphate-buffered saline to remove any excess blood and debris. Pieces of renal tissues were removed and stored in labeled sample vials in 10% formalin. The renal tissues were treated with graded alcohol and then embedded in paraffin wax. The renal tissue slices were cut to 5 μm thickness and stained with hematoxylin and eosin [17]. An experienced pathologist examined 28 processed pieces via a light microscope.

2.9 Kidney Tissue Homogenization

Kidney samples were rinsed with cold PBS and chopped into small pieces (10-20 mg). For frozen tissues: Use a pre-chilled mortar and pestle to grind under liquid nitrogen. Added 5-10 liters of cold homogenization buffer (PBS or 50 mM potassium phosphate buffer, pH 7.8). Homogenization was performed using a rotor-stator homogenizer (10,000-15,000 rpm, 3×10-sec bursts) with ice freezing in between. OR bead beater (2-5 minutes at 4°C). The centrifuge ran at 12,000×g for 15 minutes at 4°C. Collect the supernatant and store it at -80°C for later MDA measurement and SOD activity assays.

2.10 Statistical analysis

The data was statistically analyzed with GraphPad Prism version 8 (GraphPad Software Inc., USA). Before starting parametric analysis, the Shapiro-Wilk test was used to check that the data had a normal distribution. Data are reported as mean ± SD. A one-way ANOVA was used to compare serum creatinine, urea, malondialdehyde (MDA), and superoxide dismutase (SOD) activity in the four experimental groups (Control, D-Amb, Vitamin E, and D-Amb + Vitamin E). Following a significant F-statistic from the ANOVA, Dunnett's post hoc test was used to perform specific pairwise comparisons, which revealed significant differences between each treatment group and the control group. A p-value of less than 0.05 ($p < 0.05$) indicated statistical significance.

3. RESULTS

D-Amb Group:Mice treated with D-Amb showed significantly higher levels of serum creatinine (0.98 ± 0.13 mg/dL), urea (48.6 ± 5.2 mg/dL), and malondialdehyde (5.1 ± 0.7 nmol/mg protein) than the control group ($p < 0.05$). However, this group had considerably lower superoxide dismutase (SOD) activity (6.5 ± 1.0 U/mg protein) than the control group ($p < 0.05$).

D-Amb + Vitamin E Group: The combination of D-Amb and vitamin E resulted in higher values for these parameters than the D-Amb alone group. Creatinine: 0.56 ± 0.09 mg/dL; urea: 29.6 ± 3.7 mg/dL; MDA: 3.0 ± 0.5 nmol/mg protein; and SOD: 9.2 ± 1.1 U/mg protein). However, these values remained statistically different from those of the control group ($p < 0.05$), showing that vitamin E only provided partial protection.

Vitamin E Alone Group: In comparison to the control group, vitamin E showed no statistically significant effect on blood creatinine, urea, malondialdehyde (MDA), or superoxide dismutase (SOD) activity ($p > 0.05$).Table (1).These findings demonstrate that D-Amb causes considerable renal impairment and oxidative stress, while vitamin E supplementation provides partial but significant protection.

Table 1. Shows the effects of deoxycholate, amphotericin B, and vitamin E on renal physiology parameters in adult male mice.

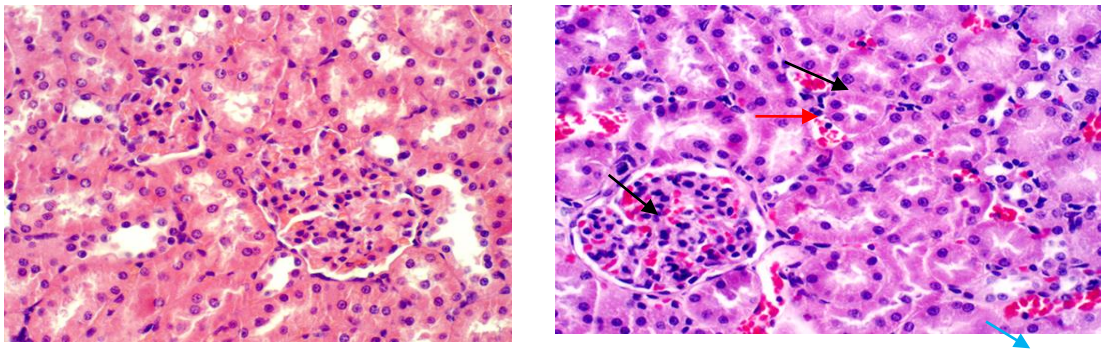
Parameter	Serum Creatinine(mg/dL)	Serum Urea(mg/dL)	MDA (nmol/mg protein)	SOD Activity (U/mg protein)
Control group	0.35 ± 0.05	21.8±2.7	1.9 ± 0.3	13.1± 1.3
D-Amb group	0.98 ± 0.13*	48.6 ± 5.2*	5.1 ± 0.7*	6.5 ± 1.0*
Vitamin E group	0.34 ± 0.06	22.5 ± 2.4	2.0 ± 0.4	12.7 ± 1.2
D-Amb+Vitamin E group	0.56 ± 0.09*	29.6 ±3.7*	3.0 ± 0.5*	9.2 ± 1.1*

*Values are expressed as mean ± SD (n = 7 per group). * Indicates $p < 0.05$ versus control group.

3.1 The histological observations in control group and group-III

The histological evaluation of kidney slices from the control group (Group I) demonstrated that the renal architecture was fully normal. The glomeruli, blood arteries, and renal tubules seemed healthy and well-organized, with no visible pathological changes. Similarly, kidney sections from mice treated only with vitamin E (Group III) exhibited general histological features similar to the control group. Vitamin E delivery

had no adverse effects on glomerular tufts, blood arteries, or kidney-specific structures. (Figure 1 A and B) .



(A) (B)

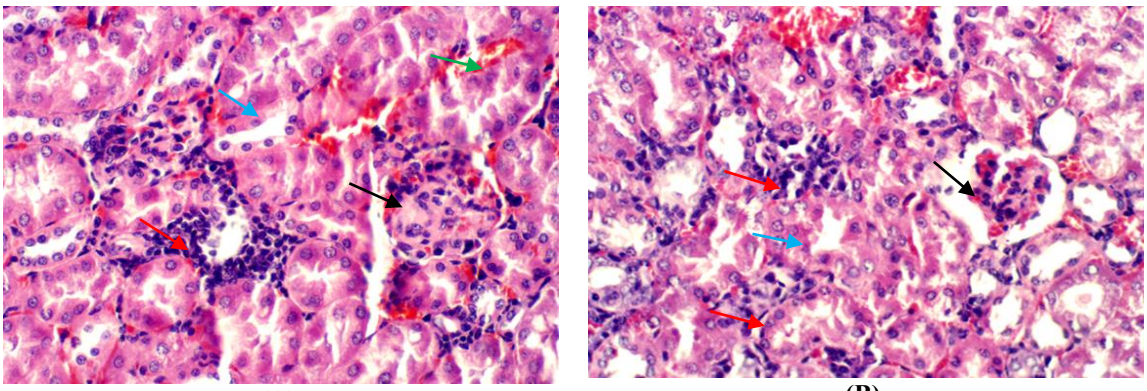
Figure 1. Photomicrographs of renal tissues in (A) the control group and (B) the vitamin E-treated group showing normal glomerular tuft (black arrow), blood vessels (blue arrow), and renal tubules (red arrow). Magnification: 400×, staining: hematoxylin and eosin.

3.2 The histological observations in group-II

In contrast, D-Amb-treated animals (Group II) showed significant histological alterations. These abnormalities included glomerular tuft shrinkage and total destruction, coagulative necrosis in neo-tubuli, inflammatory cell infiltration surrounding the afflicted ducts, and interstitial hemorrhage, all of which indicated significant glomerular and tubular injury. The renal tissues also showed vacuolar degeneration of the collection ducts and coagulation necrosis in other channels. Inflammatory cell infiltration and internal hemorrhage were also seen (Figure 2 A and B). The presence of hyaline casts in several collection ducts showed tubular obstructions and protein material accumulation, which are signs of acute tubular injury. Widespread coagulative necrosis in the renal ducts, the deposition of dead cells as cellular debris, interstitial hemorrhage, and hypercellularity of glomerular tufts highlighted the D-Amb-induced kidney injury's versatility and severity, impacting both glomerular and tubular components (Figure 3 A, B and C).

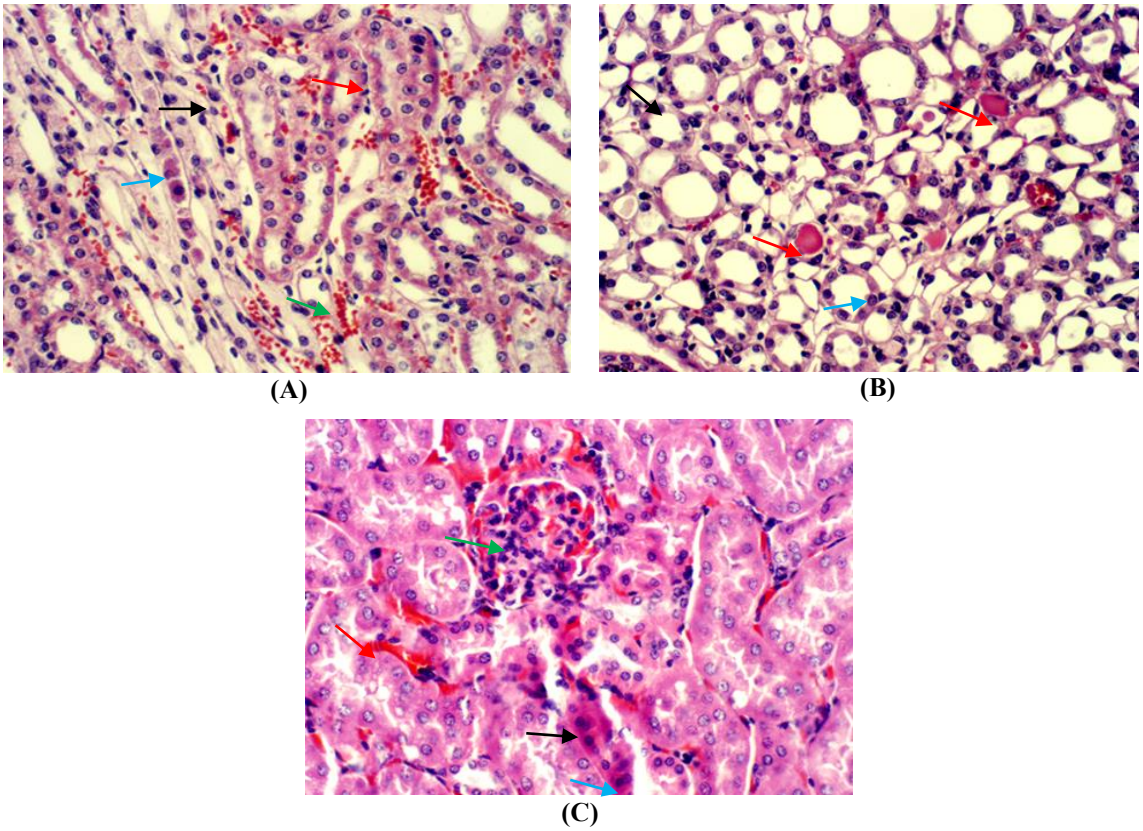
3.3 The histological observations in group-IV

According to histological investigation, the D-Amb group suffered the most severe kidney impairment, as demonstrated by glomerular shrinkage, substantial tubular necrosis, and inflammatory infiltration. In contrast, the D-Amb + vitamin E group (Group IV) had less severe necrosis and inflammation, indicating that vitamin E provided some histologic protection. Cloudy cell enlargement in epithelial cells, interstitial hemorrhages, and vacuolar degeneration were all observed in collecting tubule epithelial cells. Despite vitamin E's protective benefits, some kidney changes persisted in the D-Amb plus vitamin E group, indicating that vitamin E cannot completely protect against D-Amb-induced nephrotoxicity (Figure 4 A and B).



(A) (B)

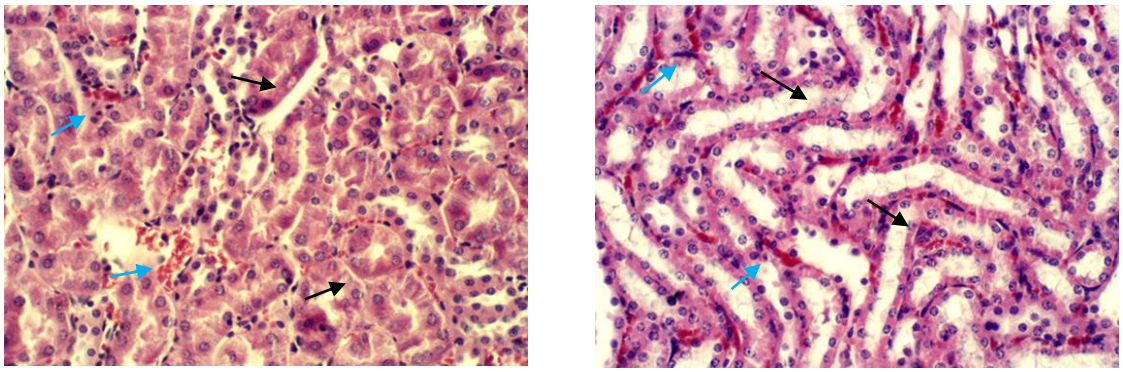
Figure 2. Photomicrographs of renal tissues in deoxycolate amphotericin B-treated group showing: (A) atrophy of glomerular tuft (black arrow), cogaulative necrosis in renal tubules (blue arrow), and infiltration of inflammatory cells around affected tubules (red arrow) with interstitial hemorrhages (green arrow); (B) the glomerular tuft was completely destroyed (black arrow), the renal tubules had cogaulative necrosis (blue arrow), and inflammatory cells had infiltrated the afflicted tubules (red arrow). Magnification: 400×, staining: hematoxylin and eosin.



(A) (B)

(C)

Figure 3. Photomicrographs of renal tissues in deoxycolate amphotericin B-treated group showing: (A) interstitial hemorrhages (green arrow), cogaulative necrosis in other tubules (blue arrow), and vacuolar degeneration in collecting tubules (black arrow); (B) vacuolar degeneration in collecting tubules (black arrow), cogaulative necrosis in other tubules (blue arrow), presence of hyaline casts in other collecting tubules (red arrow); (C) cogaulative necrosis affected tubules (black arrow), deposition of dead cells as cellular debris (blue arrow), interstitial hemorrhages (red arrow), and hypercellularity of glomerular tuft (green arrow). Magnification: 400×, staining: hematoxylin and eosin.



(A) (B)

Figure 4. Photomicrographs of renal tissues in deoxycolate amphotericin B + vitamin E treated group showing: (A) cloudy cell swelling in epithelial cells (black arrow), as well as interstitial hemorrhages (blue arrow); (B) vacuolar degeneration in the epithelial cells of collecting tubules (black arrow), interstitial hemorrhages (blue arrow). Magnification: 400×, staining: hematoxylin and eosin.

4. DISCUSSION

The current study found that administering deoxycholate amphotericin B (D-Amb) to mice resulted in large increases in serum creatinine, urea, and malondialdehyde (MDA) levels, as well as a significant reduction in superoxide dismutase activity. These findings support previous reports that D-Amb causes acute renal injury by interrupting glomerular filtration and inducing tubular damage, as evidenced by higher creatinine and urea levels. The rise in MDA and decrease in SOD activity further corroborate the major role of oxidative stress in D-Amb-induced nephrotoxicity, which is consistent with studies indicating that amphotericin B enhances reactive oxygen species (ROS) formation and lipid peroxidation in renal tissues [18,7]. The partial improvement in these indicators following vitamin E co-administration reflects the supplement's well-known antioxidant capabilities. Vitamin E supplementation significantly lowered serum creatinine, urea, and MDA levels while increasing SOD activity relative to the D-Amb group, albeit values did not fully return to normal. This finding is consistent with prior research showing that vitamin E reduces drug-induced oxidative kidney damage by scavenging free radicals and stabilizing cellular membranes. For example, Abdelhalim et al. (2020) discovered that vitamin E and other antioxidants protect against nephrotoxic damage and lipid peroxidation in experimental animals [11]. Similarly, Nasiri et al. (2020) found that vitamin E administration decreased cisplatin-induced nephrotoxicity in juvenile patients [19].

The current results evaluated the renal destructive effects of D-Amb and the protective impact of vitamin E on renal histology in mice. Based on our findings, it was observed that D-Amb administration resulted in considerable histopathological changes such as glomerular atrophy, necrosis of tubules, inflammatory cell accumulation, and hemorrhage in the interstitial region. These findings corroborate the previous concerns showing that the inflected nephrotoxicity of D-Amb is too great a hurdle for its appropriate clinical application [20,21]. The reasons for the nephron damage caused by D-Amb are many and varied. D-Amb is well documented to act with cholesterol present in the membranes of renal cells, forming pores, and making membranes more permeable,

which causes damage to the cells, especially to the proximal tubule cells [22]. D-Amb also causes an increase in oxidative stress through the elevation of reactive oxygen species production, creating further damage through lipid peroxides, and destructive cellular components [18,8]. The inflammatory cell infiltration and interstitial hemorrhage shown in the current study in the D-Amb-treated group inflicted renal injury as previously reported [4] reduces drug-induced oxidative kidney damage by scavenging free radicals and stabilizing cellular membranes. For example, Abdelhalim et al. discovered that vitamin E and other antioxidants protect against nephrotoxic damage and lipid peroxidation in experimental animals [11]. Similarly, Nasiri et al. found that vitamin E administration decreased cisplatin-induced nephrotoxicity in juvenile patients [19].

Suppression of renal injury induced by D-Amb was shown in the current study with vitamin E supplementation. From a histological perspective, mice that received both D-Amb and vitamin E demonstrated less glomerular and tubular damage, inflammatory infiltration, and hemorrhage relative to the D-Amb group, indicating improved outcome. Vitamin E maybe exerted this protective effect because of its strong antioxidant capacity, which allows it to remove free radicals and inhibit lipid peroxidation, therefore stabilizing membranes and reducing oxidative stress [23,24]. Galli et al. (2022) also documented its nephroprotective effects and showed that vitamin E modifies inflammatory responses in chronic kidney disease [25]. Our results corroborate with prior literature that demonstrated the effects of vitamin E and other antioxidants in reversing drug-induced renal damage. For example, in a clinical trial, Abo-Elmaaty et al. demonstrated that vitamin E lessened renal injury caused by cisplatin [26]. Abdelhalim et al. [11] showed that in other ex-vivo experiments, the combination of vitamin E and other antioxidants protected against nephrotoxic inflammatory damage.

As shown in the present study, despite the protective effects of vitamin E, some kidney alterations persisted in the D-Amb plus vitamin E group, demonstrating that vitamin E cannot fully protect against D-Amb-induced nephrotoxicity. This suggests that other techniques, such as using lipid-based totals of amphotericin B or combination treatments with multiple antioxidants, may be required to further minimize renal toxicity [27,8]. Recent findings from this study show that vitamin E only provided modest protection against D-Amb-induced nephrotoxicity, as indicated by biochemical and histological markers. Although co-administration of vitamin E and D-Amb resulted in significant improvements in these markers, they did not fully recover to normal levels. These findings are consistent with recent research, such as Zhang et al. which found that antioxidants like vitamin E can reduce oxidative stress and kidney damage caused by nephrotoxic drugs, but they do not always completely prevent injury, especially in cases of severe damage or high drug doses .Similarly, Galli et al. found that the renoprotective efficacy of vitamin E is dependent on a number of factors, including treatment time, dosage, and the animal model employed, and that combining vitamin E with other antioxidants may provide higher protection against nephrotoxicity.

5. CONCLUSION

In conclusion, vitamin E supplementation partially reduces the nephrotoxic effects of deoxycholate amphotericin B in mice. Further research is needed to establish the best circumstances for maximum kidney protection.This study had limitations, including

Focus on vitamin E only, without testing other antioxidants, Short experimental duration and Absence of quantitative histopathological measurements.

Ethics statement

The local Ethical Committee of Alnoor University College approved this study with the following number: UNM-REC/AAH-11-2024-33.

Authors' contributions

M.H.A. and N.T.A. contributed to the conceptualization, methodology, practical work, and writing of the original draft. All authors have read and approved the final manuscript and agree to its submission.

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Data Availability Statement

The datasets used in this investigation are accessible from the corresponding author on reasonable request.

Conflicts of Interest

There are no conflicting interests.

Consent to Participate

All of the study's subjects provided informed consent.

Consent to Publish

Because this study did not include human participants or personal data, no agreement for publishing was required.

6. REFERENCES

[1] Firacative, C. , 2020 . Invasive fungal disease in humans: are we aware of the real impact? Memórias Do Instituto Oswaldo Cruz, 115.

[2] Fanos, V., Cataldi, L., 2000 . Amphotericin B-Induced Nephrotoxicity: A Review. Journal of Chemotherapy, 12(6), 463–470.

[3] Mesa-Arango, A. C., Scorzoni, L., Zaragoza, O., 2012. It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. Frontiers in Microbiology, 3.

[4] Abdel-Rahman, D. M., Messiha, B. A. S., Ali, F. E. M., Azouz, A. A., 2023. Regulation of renal nitric oxide and eNOS/iNOS expression by tadalafil participates in the mitigation of amphotericin B–induced renal injury: Down-regulation of NF-κB/iNOS/caspase-3 signaling. Naunyn-Schmiedeberg's Archives of Pharmacology, 397(5), 3141–3153.

[5] Cohen, B. E., 2016. The Role of Signaling via Aqueous Pore Formation in Resistance Responses to Amphotericin B. Antimicrobial Agents and Chemotherapy, 60(9), 5122–5129.

[6] Tuon, F. F., Koenig, F., Jacometto, D., Rocha, J. L., 2013. Are there risk factors for acute renal failure in adult patients using deoxycholate amphotericin B? Revista Iberoamericana de Micología, 30(1), 21–24.

[7] Ferreira, D., de Bragança, A. C., Volpini, R. A., Shimizu, M. H. M., Gois, P. H. F., Girardi, A. C. C., Seguro, A. C., Canale, D., 2019. Vitamin D deficiency is a potential risk factor for lipid Amphotericin B nephrotoxicity. PLOS Neglected Tropical Diseases, 13(7), e0007567.

[8] Magalhães, E. P., Silva, B. P., Aires, N. L., Ribeiro, L. R., Ali, A., Cavalcanti, M. M., Nunes, J. V. S., Sampaio, T. L., de Menezes, R. R. P. P. B., Martins, A. M. C. , 2022. (−)-α-Bisabolol as a protective agent against epithelial renal cytotoxicity induced by amphotericin B. Life Sciences, 291, 120271.

[9] Niki, E., 2014 . Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radical Biology and Medicine, 66, 3–12.

[10] Azarfar, A., Ravanshad, Y., Golsorkhi, M., Azarfar, A., Ravanshad, S., seyedin, Z., Bagheri, S., Seyedkaboli, S., 2020 . Ten Year Retrospective Review of Bartter Syndrome at Sheikh Hospital 2008-2018. Clinical and Experimental Investigations, 1–5.

[11] Abdelhalim, M. A. K., Qaid, H. A. Y., Al-Mohy, Y. H., Ghannam, M. M., 2020 . <p>The Protective Roles of Vitamin E and α-Lipoic Acid Against Nephrotoxicity, Lipid Peroxidation, and Inflammatory Damage Induced by Gold Nanoparticles</p> International Journal of Nanomedicine, Volume 15, 729–734.

[12] Schlottfeldt, F. D. S., Fernandes, S. M., Martins, D. M., Cordeiro, P., Fonseca, C. D. D., Watanabe, M., Vattimo, M. D. F. F., 2015 . Prevenção da nefrotoxicidade da anfotericina B por meio do uso de fitomedicamentos. Revista da Escola de Enfermagem da USP, 49(spe), 74-79.

[13] Gradoni, L., Davidson, R., Orsini, S., Betto, P., Giambenedetti, M. , 1993. Activity of liposomal amphotericin B (AmBisome) against Leishmania infantum and tissue distribution in mice. Journal of Drug Targeting, 1(4), 311-316.

[14] Conei, D., Rojas, M., Santamaría, L., Risopatrón, J., 2021 . Protective role of vitamin E in testicular development of mice exposed to valproic acid. Andrologia, 53(8), e14140.

[15] Mariutti, L. R. B., 2022 . Lipid Peroxidation (TBARS) in Biological Samples. In Basic Protocols in Foods and Nutrition (pp. 107-113). New York, NY: Springer US.

[16] Durak, I., Yurtarslanl, Z., Canbolat, O., Akyol, Ö., 1993 . A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. Clinica Chimica Acta, 214(1), 103-104.

[17] Kiernan, J. ,2015. Histological and histochemical methods. Scion publishing ltd.

[18] Mesa-Arango, A. C., Trevijano-Contador, N., Román, E., Sánchez-Fresneda, R., Casas, C., Herrero, E., ... Zaragoza, O., 2014 . The production of reactive oxygen species is a universal action mechanism of amphotericin B against pathogenic yeasts and contributes to the fungicidal effect of this drug. Antimicrobial agents and chemotherapy, 58(11), 6627-6638.

[19] Nasiri, M., Farhangi, H., Badiee, Z., Ghasemi, A., Golsorkhi, M., Ravanshad, Y., Azarfar, A., 2020 . The effect of vitamin E on cisplatin induced nephrotoxicity: a clinical trial study. Int J Pediatr, 8(1), 10767-773.

[20] Tonomura, Y., Yamamoto, E., Kondo, C., Itoh, A., Tsuchiya, N., Uehara, T., Baba, T., 2009 . Amphotericin B-induced nephrotoxicity: characterization of blood and urinary biochemistry and renal morphology in mice. Human & experimental toxicology, 28(5), 293-300.

[21] Gursoy, V., Ozkalemkas, F., Ozkocaman, V., Yegen, Z. S., Pinar, I. E., Ener, B., ... Pinar, I. E., 2021 . Conventional amphotericin B associated nephrotoxicity in patients with hematologic malignancies. Cureus, 13(7).

[22] Delhom, R.; Nelson, A.; Laux, V. et al., 2020 . The antifungal mechanism of amphotericin B elucidated in ergosterol and cholesterol-containing membranes using neutron reflectometry. Nanomaterials (Basel), 10(12): 2439

[23] Tappel, A. L., 1962 . Vitamin E as the biological lipid antioxidant. In Vitamins & Hormones (Vol. 20, pp. 493-510). Academic Press.

[24] Kilicarslan You, D., Fuwad, A., Lee, K. H., Kim, H. K., Kang, L., Kim, S. M., Jeon, T. J., 2024 . Evaluation of the protective role of vitamin E against ROS-driven lipid oxidation in model cell membranes. Antioxidants, 13(9), 1135.

[25] Galli, F., Bonomini, M., Bartolini, D., Zatini, L., Reboldi, G., Marcantonini, G., ... Di Pietro, N., 2022 . Vitamin E (alpha-tocopherol) metabolism and nutrition in chronic kidney disease. Antioxidants, 11(5), 989.

[26] Abo-Elmaaty, A. M., Behairy, A., El-Naseery, N. I., Abdel-Daim, M. M., 2020 . The protective efficacy of vitamin E and cod liver oil against cisplatin-induced acute kidney injury in rats. Environmental Science and Pollution Research, 27(35), 44412-44426.

[27] Steimbach, L. M., Tonin, F. S., Virtuoso, S., Borba, H. H., Sanches, A. C., Wiens, A., ... Pontarolo, R., 2017 . Efficacy and safety of amphotericin B lipid-based formulations—A systematic review and meta-analysis. Mycoses, 60(3), 146-154.