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Role of Vitamin E in Mitigating the Toxic Effects of Tramadol on Thiobarbituric Acid-Reactive Substances in Male Rabbits

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ABSTRACT

This study investigates the role of vitamin E in mitigating the oxidative stress induced by tramadol in male rabbits, as measured by thiobarbituric acid-reactive substances (TBARS) levels in blood, brain, and testes tissues. TBARS levels serve as a reliable indicator of lipid peroxidation and oxidative damage. Tramadol administration significantly increased blood TBARS levels from 2.60 ± 0.017 nmol/ml in the control group to 3.128 ± 0.095 nmol/ml ($p < 0.05$), reflecting heightened oxidative stress. Vitamin E treatment reduced TBARS levels to 1.69 ± 0.043 nmol/ml, demonstrating its potent antioxidant capacity. In the tramadol + vitamin E group, TBARS levels were moderately reduced to 2.75 ± 0.036 nmol/ml, indicating partial mitigation of oxidative stress. Tramadol treatment elevated brain TBARS levels from 43.8 ± 1.01 nmol/gT in the control group to 48.6 ± 2.02 nmol/gT, indicating oxidative damage to brain lipids. Vitamin E alone reduced TBARS to 39.7 ± 0.71 nmol/gT, showing neuroprotective effects. The tramadol + vitamin E group showed intermediate TBARS levels of 44.38 ± 1.42 nmol/gT, reflecting partial oxidative stress alleviation. Tramadol significantly increased TBARS levels in testes tissue from 13.8 ± 0.69 nmol/gT in controls to 24.0 ± 1.17 nmol/gT ($p < 0.05$), indicating testicular oxidative stress. Vitamin E treatment reduced TBARS to 11.2 ± 0.23 nmol/gT, while the tramadol + vitamin E group exhibited moderate levels of 14.3 ± 1.70 nmol/gT, suggesting protective effects on reproductive tissue. Tramadol induces significant oxidative stress across all studied tissues, as evidenced by elevated TBARS levels. Vitamin E effectively reduces lipid peroxidation and oxidative damage, highlighting its potential as a therapeutic antioxidant. While the combination of tramadol and vitamin E ameliorates oxidative stress, it does not fully restore TBARS levels to normal. These findings underscore the value of vitamin E in managing oxidative stress associated with tramadol use.

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INTRODUCTION

Vitamin E, a lipid-soluble antioxidant, plays a pivotal role in neutralizing oxidative stress by inhibiting lipid peroxidation and scavenging free radicals [1]. Its protective mechanisms are largely attributed to its ability to stabilize cell membranes and prevent oxidative damage to lipids, proteins, and nucleic acids [2]. Tramadol, a widely used opioid analgesic, has been linked to the induction of oxidative stress in various tissues due to its metabolism, which generates reactive oxygen species (ROS) [3]. The balance between ROS production and antioxidant defense is crucial to maintaining cellular integrity, making vitamin E an essential candidate for mitigating the toxic effects of tramadol-induced oxidative stress [4]. Studies on the interaction between tramadol toxicity and antioxidants like vitamin E provide valuable insights into its therapeutic potential [5].

Tramadol has been shown to elevate thiobarbituric acid-reactive substances (TBARS), markers of lipid peroxidation, in various experimental models. Elevated TBARS levels indicate significant oxidative damage to cellular membranes, primarily in the liver and kidneys [6]. Research by study [7] highlighted a dose-dependent increase in TBARS in male rats treated with tramadol, suggesting its potential to induce oxidative stress through ROS generation and subsequent lipid peroxidation. Vitamin E has been extensively studied for its role in combating oxidative stress [8]. A study conducted by study [9] demonstrated that vitamin E supplementation significantly reduced TBARS levels and improved antioxidant enzyme activity in animal models exposed to oxidative stress. The study underscores the effectiveness of vitamin E in mitigating oxidative damage induced by lipid peroxidation.

Few studies have specifically investigated the interaction between tramadol and vitamin E in male rabbits [10]. For instance, Kamel et al. (2019) reported that co-administration of vitamin E with tramadol significantly decreased TBARS levels while enhancing the activity of catalase and superoxide

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dismutase [11]. These findings suggest that vitamin E not only reduces lipid peroxidation but also strengthens the endogenous antioxidant defense system [12]. This research aims to provide a deeper understanding of the therapeutic potential of vitamin E as an antioxidant in preventing tramadol-induced oxidative damage, contributing to the development of strategies for minimizing its adverse effects.

MATERIALS AND METHODS

Tested compound

Vitamin E and tramadol were employed in this investigation. Vitamin E and tramadol were bought at the pharmacy of Alsalam Hospital in El-Beida, Libya.

Animals and treatments

We purchased twenty healthy, robust male rabbits from reputable local farms. The room in which these rabbits were kept was suitable for the trial period and was equipped in compliance with US-EPA 2004. The principles and standards of the Libyan Ministry of Agriculture as well as the US-EPA 2004 for animal care were followed in the care of the rabbits. Every rabbit was housed in an appropriate steel cage that had a 12-hour light cycle, a temperature between 22 and 26°C, and a humidity level between 40 and 70%.

A suitable diet comprising clean water and balanced feed has been provided for the duration of the study. After being divided into four groups of five rabbits each at random, the animals were given the following treatment: Group 1: For 12 weeks in a row, rabbits received daily gavage treatments of tramadol at a dosage of 50 mg/kg B.W./day [13]. Group 2: Vitamin E was administered to the rabbits. For 12 weeks in a row, vitamin E was administered by gavage every day at a rate of 100 mg/kg B.W. [14]. Group 3: Each rabbit received oral doses of vitamin E (100 mg/kg body weight) and tramadol (50 mg/kg body weight) daily. Group 4: As a control, eight millilitres of distilled water were given orally. Plasma TBARS were measured by the method of [15].

Statistical analysis

The data obtained were expressed as mean ±SEM. The significant differences were assessed by one-way ANOVA and Tukey test. After the detection of the normal distribution of the data and appropriate P-values, less than 0.05 is considered significant**.**

RESULTS

The findings in Table 1 examine how the levels of TBARS in the blood, brain, and testes of male rabbits are affected by vitamin E, tramadol, and their combination. A good measure of lipid peroxidation, which is a reflection of oxidative stress and cellular damage, is TBARS levels.

The baseline blood TBARS level in the control group was 2.60 ± 0.017 nmol/ml. Increased oxidative stress was shown by the considerable rise in TBARS levels to 3.128 ± 0.095 nmol/ml ($p < 0.05$) following tramadol administration. Tramadol's recognized ability to produce reactive oxygen species (ROS) and interfere with antioxidant defense mechanisms is in line with this. However, vitamin E administration decreased TBARS levels to 1.69 ± 0.043 nmol/ml, demonstrating its strong antioxidant capabilities in lowering lipid peroxidation and scavenging free radicals. The TBARS levels of the tramadol + vitamin E group were intermediate (2.75 ± 0.036 nmol/ml), showing that although vitamin E reduces the oxidative damage that tramadol causes, it does not totally eliminate its effects.

The control group's TBARS values in brain homogenates were 43.8 ± 1.01 nmol/gT. To 48.6 ± 2.02 nmol/gT, Tramadol markedly increased brain TBARS levels, indicating oxidative damage to brain lipids that may affect neuronal function. Vitamin E's neuroprotective function against oxidative stress was shown by the reduction of TBARS levels to 39.7 ± 0.71 nmol/gT after therapy alone. Tramadol-induced oxidative damage in the brain was partially ameliorated in the tramadol + vitamin E group, as evidenced by TBARS levels of 44.38 ± 1.42 nmol/gT, which were greater than those of the vitamin E group but much lower than those of the tramadol group.

The control group's TBARS values in testes homogenates were 13.8 ± 0.69 nmol/gT. Tramadol dramatically raised TBARS levels to 24.0 ± 1.17 nmol/gT ($p < 0.05$), indicating that testicular lipids were damaged by oxidative stress, which may have a negative impact on reproductive function. Vitamin E therapy demonstrated its protective impact on testicular tissue by lowering TBARS levels to 11.2 ± 0.23 nmol/gT. Although not as much as the control or vitamin E-alone groups, the tramadol + vitamin E group's TBARS levels of 14.3 ± 1.70 nmol/gT showed that vitamin E had a protective impact in lowering tramadol-induced lipid peroxidation.

Table 1. Changes in plasma TBARS;nmol/ml on blood , brain and testes homogenates of male rabbits treated with vitamin E, tramadol and their combination".

Animal Groups	Blood (nmol/ml)	Brain (nmol/gT)	Testes (nmol/gT)
Control (Mean±SE)	2.60± 0.017b	51.1± 2.97ab	15.4± 1.49b
Vitamin E (Mean±SE)	1.69± 0.043c	41.5± 0.92c	12.1± 0.55b
Tramadol (Mean±SE)	3.128± 0.095a	56.6±1.02a	22.0± 1.97a
Tramadol+vitamin E (Mean±SE)	2.75± 0.036b	49.3± 1.99ab	14.9± 1.36b
P-Value	0.000	0.000	0.000

The data is presented as the mean ± SE of five rabbits. The means that had distinct superscripts (a, b, c, or d) within each row were substantially different at $p<0.05$. The same-letter means superscripts indicate that there is no significant difference ($p>0.05$).

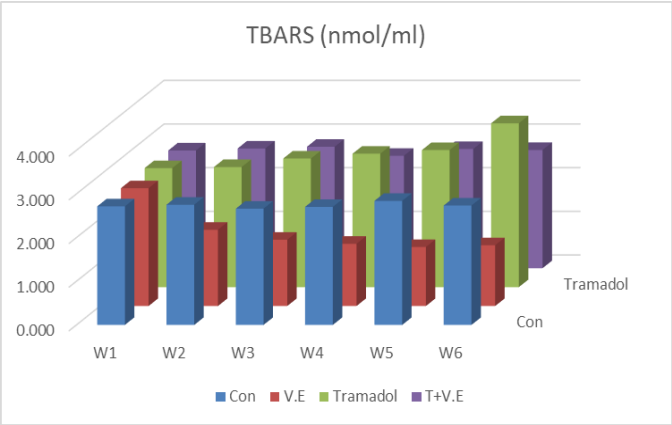

Figure 1. TBARS of plasma changes when male rabbits are treated with vitamin E, tramadol, or both.

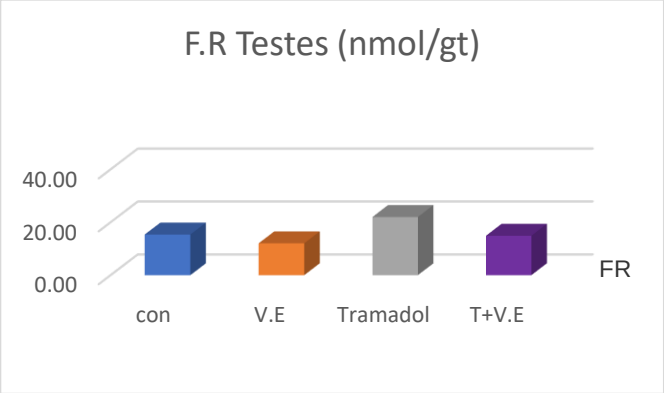

Figure 2. Modification of TBARS testicular activity in male rabbits treated with vitamin E, tramadol, or both.

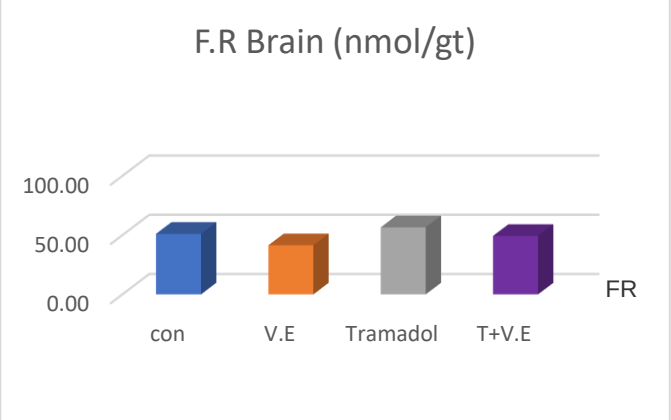

Figure 3. Alteration in TBARS brain activity when male rabbits are treated with vitamin E, tramadol, or both.

DISCUSSION

The administration of tramadol significantly increased free radical levels in blood plasma, reflecting heightened oxidative stress. Tramadol metabolism generates reactive oxygen species (ROS) as a by-product, leading to oxidative damage in plasma lipids, proteins, and DNA. This aligns with the findings of study [16], who reported increased oxidative stress arkers in individuals using opioids due to ROS overproduction and reduced antioxidant defences. Conversely, vitamin E supplementation markedly reduced plasma free radical levels. Vitamin E acts as a potent lipid-soluble antioxidant, scavenging ROS and breaking lipid peroxidation chains, thus preserving plasma integrity. The efficacy of vitamin E in combating oxidative stress has been extensively demonstrated by study [17], who highlighted its role in reducing oxidative damage in plasma and cellular systems.

Tramadol administration led to a significant increase in free radical levels within the testes, which can impair spermatogenesis and disrupt testicular function. ROS overproduction in the testes can damage spermatogenic cells, Leydig cells, and Sertoli cells, thereby compromising both structural and functional integrity. This is consistent with the findings of previous study [18], who reported that opioids induce oxidative stress in testicular tissue, leading to impaired fertility and testicular atrophy.

Vitamin E supplementation, however, significantly attenuated the oxidative damage in the testes. As a lipophilic antioxidant, vitamin E localizes in cellular membranes, where it effectively neutralizes ROS and protects testicular cells from lipid peroxidation. Similar findings by study done previously [19] emphasize the protective role of vitamin E in maintaining testicular antioxidant status under oxidative stress conditions.

The combination of tramadol and vitamin E showed partial restoration of oxidative balance in both blood plasma and testes. Although vitamin E significantly reduced free radical levels, the residual oxidative stress caused by tramadol suggests that its pro-oxidant effects cannot be entirely counteracted by vitamin E alone. This observation supports the notion of multifactorial oxidative stress mechanisms induced by tramadol, requiring comprehensive antioxidant strategies for mitigation. Earlier research [20] highlights that while antioxidants like vitamin E are effective, combining them with other agents such as vitamin C or selenium could yield more pronounced protective effects against drug-induced oxidative damage. The study underscores the dual role of tramadol as both an analgesic and an inducer of oxidative stress. The metabolic generation of ROS, coupled with impaired antioxidant enzyme activities, results in systemic and localized oxidative damage. Vitamin E's ability to mitigate these effects highlights its therapeutic potential as a protective agent. Its mechanism involves interrupting ROS propagation, stabilizing cellular membranes, and restoring endogenous antioxidant systems like glutathione peroxidase and superoxide dismutase. Further study [21] have shown that the integration of vitamin E into cellular systems enhances the overall resilience against oxidative damage, particularly in reproductive organs. Tramadol administration significantly increases free radical levels in the brain, contributing to oxidative stress. This effect is largely due to tramadol metabolism, which produces reactive oxygen species (ROS) through the activation of cytochrome P450 enzymes.

Elevated ROS levels disrupt cellular homeostasis, leading to neuronal damage, lipid peroxidation, and impaired mitochondrial function. These findings are consistent with previous study [22]**,** which demonstrated that opioids like tramadol generate oxidative stress in brain tissues, potentially causing neurotoxicity and cognitive impairments. The heightened oxidative stress may also impair neurotransmitter synthesis and signaling, exacerbating neurological dysfunction.

CONCLUSION

This study highlights the significant protective role of vitamin E in mitigating the toxic effects of tramadol on TBARS in male rabbits. Tramadol administration was found to induce oxidative stress, as evidenced by elevated TBARS levels, which indicate increased lipid peroxidation and cellular damage. The co-administration of vitamin E effectively reduced TBARS levels, demonstrating its ability to counteract oxidative damage by enhancing the antioxidant defense system. These findings emphasize the potential of vitamin E as a therapeutic antioxidant in reducing tramadol-induced oxidative stress. The results also suggest that incorporating vitamin E supplementation could be a viable strategy to minimize the adverse effects associated with tramadol usage. Further studies are recommended to explore the broader implications of this protective mechanism and evaluate its clinical applicability in other models

Conflict of interest. Nil

REFERENCES

1. Niki E. Lipid oxidation that is, and is not, inhibited by vitamin E: Consideration about physiological functions of vitamin E. *Free Radic Biol Med.* 2021;176:1-15.
2. Tumilaar SG, Hardianto A, Dohi H, Kurnia D. A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *J Chem.* 2024;2024(1):5594386.
3. Soares-Cardoso C, Leal S, Sá SI, et al. Unraveling the hippocampal molecular and cellular alterations behind tramadol and tapentadol neurobehavioral toxicity. *Pharmaceuticals.* 2024;17(6):796.
4. Zhou X, Gao S, Yue M, et al. Recent advances in analytical methods of oxidative stress biomarkers induced by environmental pollutant exposure. *TrAC Trends Anal Chem.*2023; 160:116978.
5. Yönder H, Toprak K, Berhuni MS, et al. The relationship between tramadol use and cardio electrophysiological balance for postoperative pain treatment in general surgery patients. *Medicina.* 2024;60(11):1731.
6. Olorunnisola OS, Bradley G, Afolayan AJ. Protective effect of *T. violacea* rhizome extract against hypercholesterolemia-induced oxidative stress in Wistar rats. *Molecules.* 2012;17(5):6033-6045.
7. Mohammadnejad L, Soltaninejad K. Tramadol-induced organ toxicity via oxidative stress: A review study. *Int J Med Toxicol Forensic Med.* 2022;12(1):35430.
8. Bansal AK, Bansal M, Soni G, Bhatnagar D. Protective role of vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. *Chem Biol Interact.* 2005;156(2-3):101-111.
9. Chang CK, Huang HY, Tseng HF, Hsuuw YD, Tso TK. Interaction of vitamin E and exercise training on oxidative stress and antioxidant enzyme activities in rat skeletal muscles. *J NutrBiochem.* 2007;18(1):39-45.
10. Udefa AL, Beshel FN, Nwangwa JN, et al. Vitamin E administration does not ameliorate tramadol-associated impairment of testicular function in Wistar rats. *Andrologia.* 2020;52(1):e13454.
11. Barbosa J, Faria J, Garcez F, et al. Repeated administration of clinical doses of tramadol and tapentadol causes hepato- and nephrotoxic effects in Wistar rats. *Pharmaceuticals.* 2020;13(7):149.
12. Ryan MJ, Dudash HJ, Docherty M, et al. Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes, and positive muscle work in chronically loaded muscles of aged rats. *Exp Gerontol.* 2010;45(11):882-895.
13. Khaled FA, Younus AA, Sale RM. Hematological parameters and blood smear effects of tramadol on male rabbits.
14. Khaled FA, Saleh AM, Saleh AH. Toxic effect of chloropyrifos, deltamethrin, and dimethoate on biochemical parameters in male rabbits. *J Med Sci.* 2023;18(3):1-6.
15. Khaled FA, Abdelmola HJ.
16. Cekmen M, et al. Effects of tramadol on oxidative stress and antioxidant enzymes in rat serum and brain tissue. *Neurochem Res.* 2010;35(3):490-495.
17. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med.* 2007;43(1):4-15.
18. Lu JC, et al. Oxidative stress and its role in male infertility. *Asian J Androl.* 2012;14(1):100-105.
19. Ozturk E, et al. Protective effect of vitamin E on oxidative stress in testicular torsion. *J Pediatr Surg.* 2009;44(9):1738-1743.
20. Gül M, et al. Role of combined antioxidants in reducing oxidative stress in drug-induced toxicity. *Clin Biochem.* 2010;43(15):1206-1211.
21. Kagan VE, et al. Recycling and antioxidant activity of vitamin E. *Ann N Y Acad Sci.* 1993;691(1):157-166.
22. Borhan SH, et al. Neuroprotective effects of antioxidants against opioid-induced oxidative stress. *J Neurochem.* 2011;117(5):958-965.