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Effects of Tramadol on Antioxidant Enzymes, Total Antioxidant Capacity, and Some Vitamins in Tramadol Abusers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Tramadol is a fully synthetic opioid pain reliever or analgesic that works by changing the way the body senses pain. There is, however, insufficient report on the role exerted by tramadol towards causing a depletion in the serum antioxidant enzymes— superoxide dismutase (SOD), catalase

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(CAT), glutathione (GPX) and total antioxidant vitamins— vitamin A, C, D and E in tramadol abusers especially in Nigeria. This study was therefore carried out to evaluate the blood levels of the antioxidant enzymes— superoxide dismutase, catalase, and glutathione, the total antioxidant capacity and the blood levels of the antioxidant vitamins— vitamins A, C, D and E in tramadol abusers in a Nigerian population. At first, the study subjects' ethical approval and informed consent were obtained. A total of 90 healthy male adult samples who are within the ages of 20 to 40 years were selected. This consists of 45 tramadol abusers and 45 non-abusers who served as control. Venous blood samples were collected, and the serum levels of the antioxidant enzymes and vitamins were estimated. Significant decreases in glutathione peroxidase and SOD but no significant difference in catalase and TAC activity were observed in tramadol abusers when compared with the control group. There was also a significant increase in serum vitamin A in all tramadol users compared to control. There was a significant decrease in serum vitamin C levels in all tramadol users compared to the control group. Furthermore, there was no significant difference in serum vitamin D and E in Tramadol Users compared to controls. In this study, serum vitamin C was significantly negatively correlated with serum E in Tramadol Users. At the same time, there was no significant correlation between vitamin C and vitamins A and D in Tramadol users. There was no significant correlation between vitamin A and vitamins C, D, and E in Tramadol users. This means that the blood level of vitamin A may not affect the blood level of vitamins C, D and E in tramadol users. The findings in this study are essential to the Nigerian population on the use of tramadol as this may predispose them to hypervitaminosis A and vitamin C deficiency. However, more studies are required to elucidate the present report.

Keywords: Tramadol; synthetic opioid pain reliever; antioxidant enzymes.

1. INTRODUCTION

"Tramadol is a centrally-acting opioid analgesic which is mainly used for the treatment of moderate to severe pain" [1]. "Tramadol has high oral bioavailability in the range of 70- 80%. Peak blood levels are reached in about 2 hours after an oral dose. The drug is converted in the liver to at least one active metabolite (O- desmethyl-tramadol), which is 2 to 4 times more potent than tramadol" [2,3]. "Tramadol metabolism occurs in the liver by the cytochrome P450 enzyme system, and its by-products are excreted unchanged through the kidneys. Tramadol exerts its effect of pain relief by binding to the μ- opioid receptor that is important in transmitting pain throughout the body", thereby reducing the pain sensation. [4]

"Antioxidants are substances that significantly inhibit or delay the oxidative process at low concentrations while often being oxidised themselves" [5]. "Enzymatic antioxidants mainly include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, and glutathione reductase. SOD enzymes are present in almost all aerobic cells in the extracellular fluids. There are three major families of SOD depending on their metal cofactor: Cu/Zn (binds both copper and zinc), Fe and Mn (binds either iron or manganese) and Ni type (which binds nickel). Superoxide dismutases (SODs) are referred to as a class of closely related enzymes that catalyses the breakdown of the superoxide anion into oxygen and hydrogen peroxide" [6]. "Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyses the decomposition of hydrogen peroxide i n to water and oxygen" [7]. "Glutathione peroxidase is a tetrameric enzyme with isoforms found in the cytosol, mitochondria, nucleus, and extracellular space. These enzymes can break down both free and organic forms" of H_2O_2 [8].

"Total antioxidant status, designated as total antioxidant capacity (TAC), is a test done to measure the antioxidant capacity of all antioxidants in a biological sample, not just the antioxidant capacity of a single compound. Different antioxidants in the plasma can be measured separately, but this needs to provide an overall measure of cumulative antioxidant status. Several methods have been developed to measure TAC" [9]. "Several studies have underlined the clinical importance of GPx. Chabory et al. [10] postulated that individuals with lower GPx activity are predisposed to impaired antioxidant protection, leading to oxidative damage to membrane fatty acids and functional proteins and neurotoxic damage by inference" [10]. "Oxidative stress is an imbalance between the Reactive Oxygen Species (ROS) formation and the biological system's ability to neutralise or

repair the resulting damage. Oxidative stress from oxidative metabolism causes base damage and strand breaks in DNA. The base damage is primarily indirect and caused by reactive oxygen species", e.g. O^{-} (superoxide radical), OH (hydroxyl radical) and HO (hydrogen peroxide) [11].

"Vitamins are essential nutrients required for various biochemical and physiological processes in the body. It is well known that most vitamins cannot be synthesised in the body, and hence, supplementation in the diet is essential" [12]. "Vitamins are classified based on their solubility as water-soluble (C and B complexes) and fat-soluble vitamins (A, D, E, K) [12]. Water soluble vitamins can dissolve in water, and excess are removed from the body via urine. In contrast, fat-soluble vitamins can dissolve in fats and oils and excess are stored in fatty tissues. Vitamins have various biochemical functions. Some, such as vitamin D, have hormone-like functions as regulators of mineral metabolism or cell and tissue growth and differentiation (e.g., some forms of vitamin A). Others function as antioxidants (for example, vitamins E and C) [13]. Some, such as vitamin D, have hormone-like functions as regulators of mineral metabolism or cell and tissue growth and differentiation (e.g., some forms of vitamin A). "Vitamins are required in small amounts for the body's normal functioning and maintenance of metabolic integrity" [14].

"Vitamin A is an essential micronutrient comprising a group of unsaturated nutritional organic compounds that include retinol, retinal, retinoic acid, and several pro-vitamin A carotenoids, of which beta-carotene is the most notable" [15]. "This means that our bodies cannot manufacture it, so it must be included in our diet. Vitamin A from food is stored in the liver until required by the body and is bound to protein before being transported to where it is needed. Vitamin A is essential for many physiological processes, including maintaining the integrity and function of all surface tissues (epithelia), such as the skin, the lining of the respiratory tract, the gut, the bladder, the inner ear, and the eye. Vitamin A is essential for growth, development, immune system maintenance and good vision" [16]. "The retina requires vitamin A of the eye in the form of retinal, which combines with protein to form rhodopsin, the light-absorbing molecule" [17]. "It is essential for both low-light (scoptic vision) and colour vision. Vitamin A also functions in a very different role as retinoic acid (an irreversibly oxidised form of retinol), an important hormone-like growth factor for epithelial cells and other cells" [16]. "Dietary sources include dark green leafy vegetables, for example, amaranth (red or green), spinach, carrots, squashes/pumpkins, yellow maize, mangoes, papaya, liver, eggs, milk (including breast milk), oils, red palm oil or biruti palm oil" [18].

"Vitamin D, a fat-soluble vitamin, is now recognised as a prohormone because the body can synthesise vitamin D from its precursor (7-dehydrocholesterol) when exposed to ultraviolet light at a wavelength between 290-315 nm. Adequate circulating 25-hydroxy vitamin D concentrations need to be maintained for the functioning of the metabolic, immune, reproductive, muscular, skeletal, respiratory and cutaneous systems of men and women of all ages" [19]. "It is present naturally in a few foods: fish, fish liver oils, and egg yolks and in fortified dairy and grain products. Cholecalciferol and ergocalciferol can be ingested from the diet and supplements" [20].

"Vitamin C (ascorbic acid) is a water-soluble vitamin which cannot be synthesised by humans owing to the loss of an enzyme in the biosynthetic pathway" [21]. "Vitamin E is the major lipid-soluble component in the cell antioxidant defence system and is exclusively obtained from the diet. γ-tocopherol is the most common form found in diet. It can be found in corn, soybean, margarine, and dressings" [22]. "Vitamin E refers to a group of compounds, including tocopherols and tocotrienols. Vitamin E is the major lipid-soluble component in the cell antioxidant defence system and is exclusively obtained from the diet. γ- tocopherol is the most common form found in diet. It can be found in corn, soybean, margarine, and dressings" [23]. It has numerous essential roles within the body because of its antioxidant activity.

Tramadol has extensive tissue distribution and different modes of administration, including oral administration, rectal administration, intramuscular administration, and intravenous administration. When absorbed, it has a harmful effect on both serum antioxidants and antioxidant vitamins. Previous report have shown that vitamin C (ascorbic acid) is synergistic with tramadol, and administration of the two has a greater potency and as a result, serum vitamin C is used up more quickly [24]. Vitamin C enhances the activity of tocopherol (vitamin E), the principal lipid-soluble antioxidant. It reacts

with the tocopheroxyl radicals that arise in cell membranes as a result of vitamin E antioxidant activity and regenerates tocopherols [25,26].

It is likely that the decrease in the antioxidant enzymes, therefore, leads to an imbalance between the ratios of the oxidant/antioxidant, which makes the system susceptible to oxidative stress in tramadol users. The significant components of tramadol reported to induce oxidative stress [27,28] in tramadol users include iron oxide [IONPs (iron oxide nanoparticles)] and titanium dioxide [TiO2 NP (titanium dioxide nanoparticles)], which are nanoparticles. “Due to their small size and surface properties, nanoparticles may cross biological barriers to reach different tissues; hence, the accumulation of metal nanoparticles was previously observed in many other organs” [29]. Although there are literature reports that support the fact that tramadol induces oxidative stress by causing or affecting the levels of the serum antioxidant enzymes, there are, however, scanty available literature reports suggesting if there is any correlation between the serum antioxidant enzymes and the antioxidant vitamins in tramadol users within the Nigerian population.

2. MATERIALS AND METHODS

2.1 Study Design

The time frame for subject enrolment, classification, and sample collection, as well as the evaluation of serum antioxidants, TAC, and vitamins A, C, D, and E, and the generation of data for this basic study, lasted from May 2018 to September 2018. The study was conducted in Owerri Municipal Local Government Area, Owerri, Imo State, Southeastern part of Nigeria, which consists of different ethnic groups: Igbos and Hausas.

2.2 Study Population

A total of 90 healthy male adult samples who are within the ages of 20 to 40 years were selected. This consists of 45 tramadol abusers and 45 non-abuserswho served as control.

2.3 The Selection Criteria for Patients

2.3.1 Inclusion criteria for patients

These include patients who have been identified as tramadol users through the aid of a questionnaire that was given to all the subjects selected for the exercise.

Subjects between the ages of 20 and 40 who had no history of chronic disease and were willing to participate in the exercise were also selected.

2.3.2 Exclusion criteria for patients

The exclusion criteria for this exercise were based on the following: male subjects who are not tramadol users, subjects who are not willing to partake in the exercise, and subjects below the age of 20 or above the age of 40 were not also selected. Lastly, subjects who were identified with any history of chronic diseases were also excluded from the study.

2.3.3 Inclusion criteria for control

This basically comprises adult males between the ages of 20 and 40 who have not used tramadol for the past 10 years, those who willingly agreed to partake in the exercise, and healthy adult males without any history of chronic diseases.

2.3.4 Exclusion criteria for control

This includes those who were unwilling to partake in the study and also those who were reported to have any history of chronic diseases.

2.4 Sample Collection

Ten (10mls) of fresh venous blood was collected from all the participants by venepuncture using a sterile needle and syringe. It was dispensed into a clean, plain sample container and allowed to cloth and retract. Care was taken to prevent the blood sample from hemolysis. The blood samples in the plain container tubes were centrifuged for 10 minutes at 3000rev/min. The serum was separated into plain containers and stored at -20°c prior to use. All samples were analysed within four days of sample collection.

2.5 Methods

An assay method, according to Kakkar et al. [30], was used for the assay of SOD, assay method according to Luck [31], was used for the assay of CAT, assay method according to Habig et al. [32], was used for assay of GPX and lastly method assay according to Jayaprakasha et al. [33], was used for the assay of TAC. The enzymes were first extracted from the sample. Then,

procedures stated by the above methods were followed to analyse the samples, and the absorbance readings were taken at 560nm, 240nm, 340nm and 695nm wavelengths, respectively, with an APEL PD-3000UV Spectrophotometer.

Also, an assay method, according to Bayfield and Cole [34], was used for the assay of Vit A, method according to Roe and Keuther [35], was used for the estimation of Vit C, method according to Brockmann [36], was used for the analysis of vit D and also method according to Rosenberg [37], was used for the estimation of vit E. the samples were analysed following the methods stated by the above methods and absorbance readings were taken at 620nm, 540nm, 464nm and 520nm wavelengths respectively with APEL PD-3000UV Spectrophotometer.

2.6 Statistical Analysis

All data generated from this study were subjected to statistical analysis; means, standard deviations, Student t-tests, and Correlation studies were analysed using IBM SPSS Statistical Package software for Windows Version 21. Results were expressed as Mean ± SD. The 5% ($P<0.05$) significance level was adopted for significance.

3. RESULTS

According to Table 1, serum Glutathione Peroxidase and SOD levels were significantly lower (p= 0.015 and p=0.011, respectively) in tramadol users compared to Controls. However, there were no significant differences in serum Catalase and TAC levels (p= 0.555 and p= 0.973, respectively) in tramadol users compared to Controls.

Also, as shown in Table 2, there was no significant correlation of serum TAC with Glutathione Peroxidase, SOD and Catalase (r= -0.300 p= -0.107, r= 0.061 p= 0.749 and r=-0.200 p=0.289 respectively) in Tramadol Users.

As shown in Table 3, there was no significant correlation of serum SOD with Glutathione Peroxidase, Catalase and TAC (r= -0.246 p= 0.189, r= -0.091 p= 0.632 and r= 0.061p= 0.749 respectively) in Tramadol Users.

Table 1. Serum antioxidant enzymes and TAC in tramadol users versuscontrols

Variables Mean ±SD	Tramadol Users (n=30)	Controls (n=30)	t-value	p-value
Glutathioneperoxidase (µMol/min)	6.474 ±8.534	13.402 ±11.622	2.587	0.015
Lower 95% C.I	3.287	9.062		
Upper 95% C.I	9.661	17.742		
SOD (µMol/min**)**	1.590±0.406	1.806±0.150	2.722	0.011
Lower 95% C.I	1.438	1.750		
Upper 95% C.I	1.741	1.862		
Catalase (µMol/min**)**	0.830±0.306	0.765±0.407	-0.596	0.555
Lower 95% C.I	0.715	0.612		
Upper 95% C.I	0.944	0.917		
TAC (%)	9.443±4.500	9.412±1.952	-0.034	0.973
Lower 95% C.I	7.762	8.683		
Upper 95% C.I	11/123	10.141		

Table 2. Correlation of serum TAC with antioxidant enzymes in tramadol users

Dependent Variables	N	r-value	p-value
Glutathione Peroxidase	30	-0.300	-0.107
SOD	30	0.061	0.749
Catalase	30	-0.200	0.289

Table 3. Correlation of serum SOD with glutathione peroxidase, catalase andTAC in tramadol users

Dependent Variables	N	r- value	p-value
Glutathione Peroxidase	30	-0.246	0.189
Catalase	30	-0.091	0.632
TAC	30	0.061	0.749

Table 4. Serum antioxidant vitamins A, C, D and E in tramadol usersversus controls

VARIABLES Mean ± SD	Tramadol Users (n=30)	Controls (n=30)	t-value	p-value
Vitamin A(mg/dl)	215.85±39.87	177.97±69.04	2.868	0.008
Lower 95% C.I	200.96	152.19		
Upper 95% C.I	230.74	203.76		
Vitamin C(mg/dl)	140.21±27.04	171.01±68.55	-2.238	0.033
Lower 95% C.I	130.11	145.41		
Upper 95% C.I	150.31	196.61		
Vitamin D(mg/dl)	3.72±2.85	5.31±3.74	-1.958	0.068
Lower 95% C.I	2.65	3.92		
Upper 95% C.I	4.78	6.71		
Vitamin E(mg/dl)	2.75±1.62	2.53±1.70	0.716	0.480
Lower 95% C.I	2.14	1.89		
Upper 95% C.I	3.35	3.17		

Table 5. Correlation of serum vitamin C with vitamin A, D and E in tramadol users

Dependent Variables	N	r-value	t-value
Vitamin A	30	-0.265	0.157
Vitamin D	30	-0.194	0.304
Vitamin E	30	-0.782	0.000

Table 6. Correlation of serum vitamin A with vitamin C, D and E in tramadol users

Dependent Variables	N	r-value	t-value
Vitamin C	30	-0.265	0.157
Vitamin D	30	0.011	0.953
Vitamin E	30	-0.111	0.559

As shown in Table 4, Serum vitamin A was significantly higher (p=0.008), while serum vitamin C was significantly lower (p=0.033) in Tramadol users compared to controls. There were no significant differences in serum vitamin D and E (p=0.068 and p=0.480, respectively) in Tramadol Users compared to Controls.

As shown in Table 5, Serum vitamin C was negatively correlated with serum E (r= -0.782, p=0.000) in Tramadol Users. There was no significant correlation between vitamin C and vitamins A and D (r=-0.265, p=0.157 and r=-0.194, p=0.304, respectively) in Tramadol Users.

As shown in Table 6, There was no significant correlation of vitamin A with vitamins C, D, and E (r=-0.265 p=0.157, r=0.011 p=0.953, and r=-0.111 p=0.559, respectively) in Tramadol users.

4. DISCUSSION

Tramadol is a potent analgesic drug which provides pain relief by interacting with the mu-opioid receptors while producing euphoric effects

just like other opioids. Although tramadol is a prescription medication, there is a risk of dependence as with other opioids due to the euphoric effects it provides along with pain relief [29]. Irrational use of some drugs may lead to transient or chronic dependency [38]. Drug abuse has become a major social problem in the modern world as it is widespread and involves the lifetime exposure of about 46% of the general population [39]**.** Addiction is a chronic disease characterised by compulsive drug-seeking and use [40]. Recently, Tramadol Abuse is increasingly becoming a concern in Nigeria.

The major components of tramadol reported to induce oxidative stress [26,27] in tramadol users include iron oxide [IONPs (iron oxide nanoparticles)] and titanium dioxide [TiO2 NP (titanium dioxide nanoparticles)], which are nanoparticles. The unique physicochemical and structural properties of engineered nanoparticles (ENPs) make them an attractive ingredient for drug delivery systems and therapeutics. It has provided resources for various applications in the medical field, leading to significant advances in diagnosis, biological detection, and therapy [41,42]. Due to their small size and surface properties, nanoparticles may cross biological barriers to reach different tissues; hence, the accumulation of metal nanoparticles was previously observed in many other organs [28]. When taken up by cells via endocytosis, IONPs accumulate in the lysosomes and are degraded in iron ions. Theoretically, the ions could cross the membranes and reach regions such as the cell nucleus and mitochondria, reacting with hydrogen peroxide and oxygen, thus generating ROS [43,44]. TiO2 NP-induced oxidative DNA damage has been attributed to the formation of OH [45].

Oxidative stress following TiO2 exposure can lead to reductions in hepatic malondialdehyde levels and reduced glutathione [46]. At the same time, exposure to TiO2 NPs could deplete hepatic glutathione [47]. It may also lead to imbalances of intracellular Na+ and extracellular K+ in hippocampal neurons [48]. TiO2 NPs can promote the generation and accumulation of ROS, inducing indirect oxidative DNA damage in cells. TiO2 NPs have been shown to cause apoptosis in mammalian cells [49].

In this present study, there were significant decreases in glutathione peroxidase and SOD but no significant difference in catalase and TAC activity when compared with the control group. This is in accordance with a previous report by Alaifi et al. [26], who reported a significant decrease in antioxidant enzymes and a significant increase in the production of ROS and MDA. This present finding may be due to the production of ROS by some of the nanoparticles used in the drug's manufacturing, which, in turn, leads to the depletion of the named enzymes [26]. It is likely that the decrease in the antioxidant enzymes leads to an imbalance between the ratios of the oxidant/antioxidant, making the system susceptible to oxidative stress in tramadol users.

Also, this study showed a significant increase in serum vitamin A in all tramadol users compared to the control group. There was a significant decrease in serum vitamin C levels in all tramadol users compared to the control group. There was no significant difference in serum vitamin D and E in Tramadol Users compared to controls. Previous reports have shown that vitamin C (ascorbic acid) is synergistic with tramadol. Administration of the two has a greater potency, so serum vitamin C is used up more quickly [23].

In this present study, serum vitamin C was significantly negatively correlated with serum E in Tramadol Users. While there was no significant correlation of Vitamin C with vitamins A and D in Tramadol Users. Previous studies have shown that vitamin C enhances the activity of tocopherol (vitamin E), the principal lipid-soluble antioxidant. It reacts with the tocopheroxyl radicals that arise in cell membranes due to vitamin E antioxidant activity and regenerates tocopherols [24,25].

In this study, there was no significant correlation of vitamin A with vitamins C, D, and E in Tramadol users. This means that the blood level of vitamin A may not affect the blood level of vitamins C, D, and E in tramadol users. Studies have shown that higher doses of tramadol may affect bone metabolism and consequently lead to higher serum vitamin A levels [50].

The findings in this study are important to the Nigerian population regarding the use of tramadol, as this may predispose them to hypervitaminosis A and vitamin C deficiency. However, more studies are required to elucidate the present report.

5. CONCLUSION

This study emphasises the effects of tramadol use on vitamin levels and oxidative stress in the body. Despite its effectiveness in relieving pain, some data suggest tramadol users have elevated oxidative stress due to a significant drop in vital antioxidant enzymes, including glutathione peroxidase and superoxide dismutase (SOD). Furthermore, tramadol usage causes changes in the levels of some vitamins. In Nigeria, tramadol is widely used and abused, therefore these findings are especially important for public health. They demand further education and investigation to properly grasp the long-term effects of tramadol use. In order to reduce the hazards connected with tramadol and enhance patient outcomes, it is imperative to address these difficulties.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as large language models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during manuscript writing or editing.

CONSENT AND ETHICAL APPROVAL

The Institutional Research Ethics Committee of the Faculty of Health Science, Imo State University, Owerri, approved the research protocol. All subjects' informed consent and approval were obtained before the experiment. Each participant was also required to sign a consent form after the procedure, and a special instructor explained the implications to the subjects in English or Igbo dialect and the Hausa dialect. Participation in the exercise was voluntary, and each participant was free to withdraw from it if they so wished.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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