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Ameliorative Role of Virgin Coconut Oil on Tramadol-Induced Nephrotoxicity in Sprague-Dawley Rats

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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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Original Research Article

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ABSTRACT

This research aims to investigate the effect of Tramadol induced nephrotoxicity and the ameliorative potency of Virgin Coconut Oil (VCO) on the kidneys using Sprague-Dawley rats as experimental models. A total of 40 rats were used for the study and were grouped 1-4 (130±30g; N=10). Group 1 served as the Control group and was given 1ml of Distilled Water (DW). Groups 2-4 received 25, 50 and 100 mg/kg of Tramadol respectively for 4 weeks. Five animals were randomly selected and euthanized, and the kidneys were harvested for histology and to determine oxidative stress levels, and blood sera were used for kidney function test. The remaining rats in each group received 10 mg/kg of VCO for duration of 2 weeks and were euthanized and above parameters were determined. Results showed an increase in the MDA and decrease in SOD and CAT values in the kidney tissue for the groups administered Tramadol. There was an increase in creatinine values in the groups administered Tramadol and an overall decrease in the values of Urea and Albumin. Sections of the tramadol-treated group showed an area of inflammatory infiltration in the interstitial space while groups administered with VCO showed normal histology. There was an increase in the CAT values for the group treated with VCO. There was an increase in creatinine values in the groups administered VCO and a decrease in the values of urea. In conclusion, Tramadol had adverse effects on the kidney however upon administration of virgin coconut oil (VCO), improvement in the cytoarchitecture of the kidney and levels of the oxidant markers was recorded.

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1. INTRODUCTION

There has been misuse and abuse of certain medications other than the therapeutic purpose and this is becoming a global problem [1]. “Commonly abused drugs include cannabis, cocaine, amphetamine, heroin, diazepam, cough medications (codeine, dextromethorphan) and tramadol. Sources where abusers obtain these drugs are pharmacies/patent medicine shops, open drug markets, drug hawkers, fellow drug abusers, friends, and drug pushers” [2].

“Tramadol is a centrally acting weak μ-opioid receptor analgesic and is a racemic mixture of (+)-tramadol and (−)-tramadol enantiomers. It is prescribed to relieve moderate to severe pain in patients” [3]. “It is available in a wide range of pharmaceutical formulations, and it can be administered via the following routes: subcutaneous, intravenous, intramuscular, rectal, sublingual, and oral delivery. The excretion of tramadol occurs almost exclusively via the kidney, as initial studies of tramadol using radioactive isotopes showed that at least 90% of the radiolabel is excreted via the urine, with the residual activity recovered only through the feces” [4]. “Moreover, around 10-30% of this tramadol is excreted as the unmetabolized drug, while 60% is excreted as a metabolite” [5].

“Renal toxicity has been described with tramadol overdoses; however, it is typically associated with rhabdomyolysis, multiorgan failure and/or mortality” [6]. “Nephrotoxicity is defined as a structural and/or functional kidney damage resulting from exposure to any noxious factor of toxic or ischemic character” [7].

The abuse of Tramadol has become a common practice globally due to its availability which has even led to its ban in Nigeria, and it has been proven to have adverse effects on various systems in the body. There are studies that have indicated that the sporadic use of Tramadol cuts across all part of Nigeria [8,9]. “In Kano, Northern Nigeria, a cross sectional study amongst commercial bus drivers reported that 85.2% of respondents misuse Tramadol” [10]. “Another cross-sectional study among ‘Almajiris’ (street children), in Borno Northern Nigeria, reported a 7% prevalence of Tramadol misuse” [11].

“Virgin coconut oil (VCO) is edible oil obtained from the milk of fresh and matured kernel of the coconut (*Cocos nucifera*). VCO contains high saturated fatty acids, which are mostly lauric acid that has a high resistance against oxidation and inhibits rancidity due to its stability and functionality” [11]. “Phenolics compounds of VCO are group of bioactive compounds present in edible oils capable of exerting the antioxidant activities through several mechanisms, mainly hydrogen transfer and reducing power. Several epidemiological studies showed that there is relationship between antioxidant activity and diet containing phenolics compounds. The clinical studies also revealed that VCO rich in polyphenol exhibit beneficial effects due to its high radical scavenging and inhibition of lipid peroxidation properties against cardiovascular disease in recent randomized control trials” [12].

2. MATERIALS AND METHODS

2.1 Source of Drug

Tramadol (50mg) tablets were purchased from Careyard Pharmaceuticals, Iwo, Osun State, Nigeria. The drug was produced by Hovid Berhad. 121, Jalan Tunku Abdulrahman, 30010 ipoh, Malaysia, distributed by Pharmatec Nigeria Limited, Lagos, Nigeria, with NAFDAC No: 04-4036. It was manufactured on 01/09/2020 with an expiry date of 31/08/2023).

2.2 Preparation of Virgin Coconut Oil

The Virgin coconut oil was extracted according to the method of [13].

Fresh coconut fruits were purchased from a commercial market in Iwo, Osun State and used for the extraction of VCO.

2.3 Experimental Animals and Design

A total of forty Sprague-Dawley rats weighing between 130±30g were used for this study. They were housed in standard, well ventilated, wire mesh plastic cages in the animal room of the Department of Anatomy, College of Health Sciences, Bowen University under standard room temperature. The animals were left to acclimatize before the commencement of the experiment. All experimental procedures and techniques were approved by the departmental committee on the use and care of animals and tissue collection. The rats were allowed unrestricted access to water and commercial rat

chow *ad libitum.* Administration was through the oral route with the use of an oral cannula. The body weight of the animals was taken and recorded weekly.

The animals were grouped 1-4 with each group having 10 animals each. Group 1 served as the Control group and was given 1ml of DW. Groups 2-4 received 25, 50 and 100 mg/kg of Tramadol respectively for 4 weeks. Five animals were randomly selected and euthanized, and the kidneys were harvested for histology and to determine oxidative stress levels, and blood sera were used for kidney function test. The remaining rats in each group received 10 mg/kg of VCO for duration of 2 weeks and were euthanized and above parameters were determined.

2.4 Kidney Homogenate Processes for Antioxidant Parameters

"The kidneys were washed in ice cold 1.15% KCl solution, blotted and weighed. They were then homogenized with 0.1 M phosphate buffer (pH 7.2). The tissues were introduced into mortar and laboratory sand was then added. This was crushed using a pestle. The resulting homogenate was centrifuged at 2500 rpm speed for 15 mins. Thereafter, the homogenate was removed from the centrifuge and the supernatant was decanted and stored at -20°C for the analysis" [14].

2.5 Antioxidant Parameters

2.5.1 Superoxide Dismutase (SOD)

Superoxide Dismutase was assayed by its ability to inhibit the autooxidation of epinephrine, determined by the increase in absorbance at 480 nm. The enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min [14].

2.5.2 Catalase (CAT)

Catalase was assayed colorimetrically at 620 nm and expressed as μmoles of H2O2 consumed/min/mg/protein [14].

2.5.3 "Malondialdehyde (MDA)

Malondialdehyde an index of lipid peroxidation was determined using the method of Buege and Aust12. The supernatant was removed, and the absorbance was read at 532 nm. MDA was calculated using the molar extinction coefficient for MDA thiobarbituric acid (TBA) - complex of 1.56 × 105 M-1cm-1" [13].

2.6 Statistics

The data obtained from all the groups were compiled and statistically analyzed using ONE WAY-ANOVA with the Graph pad software version 8. The results of the data were expressed as mean ± SEM (standard error of mean) and $p<0.05$ was taken as significant.

2.7 Histological Procedures

The kidneys were stored in a formalin solution until preparation of microscopic slides. The sections were cut at preselected thickness of 4 microns using LEICA RM2135 microtome. In order to assess the severity of histological damage, the preparations were stained with hematoxylin–eosin (HE).

3. RESULTS

3.1 Effect of Tramadol and Virgin Coconut Oil on Body Weight of Sprague-Dawley Rats

Significant increase in body weight was recorded when post administration was compared to pre-administration in the tramadol group. The same pattern of significant increase was noticed in the VCO group when post administration was compared to pre-administration (Table 1).

3.2 Effect of Tramadol and Virgin Coconut Oil on Kidney Weight of Sprague-Dawley Rats

In the tramadol group, there was a dose dependent increase when treatment groups were compared to control. Significant increase was also noticed when medium dose and high dose were compared to low dose, with an increase recorded when high dose was compared to medium dose. A significant increase was recorded when VCO group was compared to tramadol group (Table 2).

3.3 Effect of Tramadol and Virgin Coconut Oil on Oxidative Stress Markers Kidney of Sprague-Dawley Rats

In the tramadol group, a dose-dependent significant increase was recorded in MDA level

when treatment groups were compared to control. Reverse was recorded in levels of SOD and CAT as significant decrease was seen when treatment groups were compared to control. When the treatment groups of VCO were compared to Tramadol group in MDA levels, significant decrease was recorded, and significant increase was recorded in SOD and CAT values (Table 3).

Table 1. Effect of tramadol and virgin coconut oil on body weight of sprague-dawley rats

Tramadol			
Group	**Pre-Administration (g)**	**Post-Administration (g)**	**% Weight Difference**
Control	141.34 ± 0.26	152.00 ± 1.00*	7.54
Low dose	102.66 ± 1.03	136.00 ± 0.16*	32.48
Medium dose	143.20 ± 0.07	180.00 ± 0.12*	25.69
High dose	150.70 ± 1.13	195.00 ± 0.11*	27.70
Virgin coconut oil			
Group	Pre-Administration (g)	Post-Administration (g)	% Weight Difference
Control	100.02 ± 1.00	187.06 ±1.06*	87.02
Low dose	105.1 ± 1.11	150.04 ± 0.51*	42.65
Medium dose	139.06 ± 0.21	173.63 ± 0.53*	24.86
High dose	101.82 ± 0.71	170.52 ± 1.78*	67.47

Values are expressed as Mean ± Standard Error of Mean (SEM). [a]p<0.05 significant compared to pre-administration

Table 2. Effect on tramadol and virgin coconut oil on kidney weight of Sprague-Dawley rats

Group	**Tramadol**	**VCO**
Control	0.23 ± 0.66	0.60 ± 1.24
Low dose	0.29 ±1.03[a]	0.45 ± 0.04[a*]
Medium dose	0.41 ± 0.25[ab]	0.48 ± 0.21[a]
High dose	0.45 ± 0.23[ab]	0.49 ± 0.19[a]

Values are expressed as Mean ± Standard Error of Mean (SEM). [a]p<0.05 significant compared to control; [b]p<0.05 significant compared with low dose; [c]p<0.05 significant compared with medium dose; [*]p<0.05 significant compared with Tramadol group

Table 3. Effect on tramadol and virgin coconut oil on oxidative stress markers kidney of Sprague-Dawley rats

Oxidative Stress Tramadol			
Groups	**MDA**	**SOD**	**CAT**
Control	131.51 ± 0.33	576.32 ± 1.00	262.42 ± 0.33
Low dose	154.85 ± 1.03[a]	442.25 ± 0.67[a]	244.57 ± 0.67[a]
Medium dose	168.63 ± 0.33[ab]	425.05 ± 1.67[ab]	220.78 ± 0.11[ab]
High dose	174.09 ± 0.33[abc]	411.67 ± 0.03[abc]	159.74 ± 1.02[abc]
Oxidative Stress VCO			
Groups	**MDA**	**SOD**	**CAT**
Control	135.30 ± 0.67	599.78 ± 0.67	256.86 ± 1.03
Low dose	141.06 ± 0.01[a*]	603.11 ± 0.83[a*]	261.97 ± 0.01[a*]
Medium dose	114.12 ± 1.02[ab*]	464.45 ± 0.67[ab*]	263.16 ± 0.67[a*]
High dose	109.35 ± 0.33[abc*]	426.96 ± 0.01[abc*]	272.87 ± 0.33[abc*]

Values are expressed as Mean ± Standard Error of Mean (SEM). [a] p<0.05 significant compared to control; [b] p<0.05 significant compared with low dose; [c]p<0.05 significant compared with medium dose; [*]p<0.05 significant compared with Tramadol group

Table 4. Effect of tramadol and virgin coconut oil on kidney function tests of sprague-dawley rats

Kidney function test for tramadol			
Groups	Creatinine	Urea	Albumin
Control	13.02 ± 0.01	48.99 ± 1.02	26.02 ± 1.05
Low dose	15.96 ± 0.12[a]	40.70 ± 0.15[a]	23.93 ± 0.09[a]
Medium dose	18.12 ± 1.04[a]	36.41 ± 0.12[a]	20.55 ± 0.33[ab]
High dose	21.93 ± 0.03[abc]	30.14 ± 1.16[abc]	18.07 ± 1.02[abc]
Kidney function test for VCO			
Groups	Creatinine	Urea	Albumin
Control	13.80 ± 0.05	49.01 ± 1.01	25.37 ± 1.33
Low dose	14.00 ± 0.11	42.16 ± 1.05[a]	27.74 ± 1.01[a*]
Medium dose	14.50 ± 0.30[*]	40.30 ± 0.01[ab*]	22.87 ± 1.05[ab]
High dose	18.15 ± 1.44[abc*]	37.64 ± 0.32[abc*]	19.40 ± 0.33[abc]

Values are expressed as Mean ± Standard Error of Mean (SEM). [a]p<0.05 significant compared to control; [b]p<0.05 significant compared with low dose; [c]p<0.05 significant compared with medium dose; [*]p<0.05 significant compared with Tramadol group

3.4 Effect of Tramadol and Virgin Coconut Oil on Kidney function tests of Sprague-Dawley Rats

In the tramadol group, when treatment groups were compared to control an increase in the values of Creatinine and decrease in values of Urea and Albumin were recorded. When the treatment groups of VCO were compared to tramadol group, decrease in Creatinine and increase in Urea and Albumin levels were recorded (Table 4).

4. DISCUSSION

Oxidative stress is the state of imbalance between the reacting oxygen species and the ability of a biological system to detoxify readily the reactive intermediates [15]. **"**Malondialdehyde (MDA) is a product of lipid peroxidation and has been used as a biomarker of oxidative stress" [16]. "The main endogenous production of MDA arises from the oxidation of polyunsaturated fatty acids with more than two methylene-interrupted double bonds" [17]. "SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state of O_2-. Decreased activity of SOD leads to increased production of free radicals" [18]. "CAT is a hemoprotein, localized in the peroxisomes and catalyzes the decomposition of H_2O_2 to water and oxygen" [19].

"Lipid peroxidation is the oxidative degradation of lipids, which generates free radicals that cause cell damage. The end product of lipid peroxidation is malondialdehyde, which is known as second messenger of free radicals" [20]. "High concentration of MDA in kidney tissue indicates renal toxicity" [21]. In this study, an increase in the oxidative stress marker MDA in the kidney tissue for the groups administered Tramadol while a decrease in the SOD and CAT values was observed. Tramadol induced oxidative stress results in reduction in antioxidant enzymes including SOD, Catalase [22]. "The inhibition of these antioxidant enzymes observed in this study could be linked to exhaustion of these enzymes as a result of oxidative stress caused by tramadol administration" [23].

"Creatinine is a by-product of creatine phosphate in muscle, and it is produced at a constant rate by the body. For the most part, creatinine is cleared from the blood entirely by the kidney. Decreased clearance by the kidney results in increased blood creatinine" [24]. An increase in serum creatinine is a biomarker for renal damage [25].

The obtained results showed that there was an increase in creatinine values in the groups administered Tramadol when compared with the normal control group. There was also an overall decrease in the values of Urea and Albumin in the groups administered Tramadol compared with the normal group. There was an increase in organ weight of the VCO groups when compared to Tramadol group. The results showed that there was an increase in the CAT values for the group treated with VCO. When MDA values for VCO group was compared to that of the tramadol group, a significant decrease can be observed in the VCO group. When SOD values for VCO group was compared to the tramadol group, a

significant increase in the values for the VCO group was noticed. It can be deduced that VCO reduced the oxidative stress in the kidney.

The results showed that there was an increase in creatinine values in the groups administered virgin coconut oil when compared with the normal control group while there was a decrease in the values of urea in the VCO group when compared with the normal control group. When Albumin for the VCO group was compared with that for Tramadol group, an increase in the values of albumin for VCO group was observed.

5. CONCLUSION

From the results and observations, Tramadol had adverse effects on the kidney however upon administration of Virgin Coconut oil, improvement was seen. Based on the above result, people are advised to abstain from the use of Tramadol except in severe medical cases whereby it has to be prescribed by the medical practitioner. There should be stricter enforcement of policies banning the trade and availability of Tramadol as an over- the- counter medication.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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