Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 8 Issue 7 July 2024

Research Article

Antioxidant Effects of Supplementation with Choice Vitamins C (Ascorbic Acid) And E (Tocopherol) on Rohypnol-Induced Kidney Damage of Male Wistar Rats

Hyacinth Tochukwu Eze[1], Ezejindu Damian Nnabuihe[2], Joshua Izuchukwu Abugu[2]* and Ifeanacho Ezeteonu Abireh[3]

[1]Department of Internal Medicine, Nnamdi Azikiwe University, College of Medicine Nnewi Campus, Anambra State, Nigeria

[2]Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

[3]Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria

***Corresponding Author:** Joshua Izuchukwu Abugu, Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

Received: May 27, 2024

Published: June 11, 2024

Abstract

This study was principally carried out to investigate the effect of Vitamins C and E on the rohypnol-induced kidney damage in male wistar rats. Twenty five adult male wistar rats were randomly divided into four groups with five rats in each group (A, B, C, D and E). Group A, the control group received distilled water. Groups B, C and D received 1 mg/kg of Rohypnol, 1 mg/kg of Rohypnol+100 mg/kg of vitamin C, 1 mg/kg of Rohypnol+100 mg/kg of vitamin E and 1 mg/kg of Rohypnol+100 mg/kg of vitamin C and E of Rohypnol respectively. The animals were anaesthesized under ketamine followed by cardiac puncture after three weeks of administration and the kidney were harvested and fixed in 10% formal saline for histological processing, blood were collected via cardiac puncture for biochemical studies. The results obtained in this study following the administration of Vitamin C&E and increasing dosage of rohypnol shows a progressive improvement in the test group when comparing to the other group. The crucial role of Vitamin C and E in renal functions, as well as responses to the histoarchitecture is well supported by the evidences presented in this study.

Keywords: Flunitrazepam Vitamin C and E (Rohypnol), Kidney; Histoarchitecture; Benzodiazepine

Introduction

Rohypnol, also called flunitrazepam, is a rapid-acting sedative–hypnotic benzodiazepine that is about ten times more potent than diazepam [1,2]. Its medical uses include premedication prior to surgery, treatment of insomnia, sedative, anxiolytic, muscle-relaxing, and anti-convulsant [1] Its ability to produce a relaxed feeling explains its high rate of abuse. It is used to enhance the effects of marijuana, heroin, or alcohol and to minimize the stimulant effects of cocaine [3]. Due to its hypnotic and amnesic properties including its ability to cause dis-inhibition, it is often used as a drug to incapacitate [4] usually with the intent to commit rape or other violent criminal acts (Drug-Induced Rape Prevention and Punishment Act of 1996). It is popularly called date rape, Rope, Roches, Roofies, wolfies, Mexican valium, and Pingus [5]. The illicit use of drugs among adolescents and adults for recreational reasons is an increasing public health issue globally [6-10]. Global prevalence of illicit drug use-related mortality increased by about 60% between year 2000 and 2018 [11]. The negative impacts of illicit drug use include addiction [12] accidents [13], raised criminal tendencies and activities [14], mental health disorders [15,16], risky sexual behavior [17,18], and non-communicable diseases [19], such as cardiorenal collapse.

The kidneys are two bean-shaped organs found on the left and right sides of the body in vertebrate, They are the waste filtering and disposal system of the body. As much as 1/3 of all blood leaving the heart passes into the kidneys to be filtered before flowing to the rest of the body tissues. The ovoid kidneys remove excess water, salts, and wastes of protein metabolism from the blood [20].

Vitamins are organic compounds that people need in small quantities. Each has a different role in maintaining health and bodily function. Some people need supplements to boost their supply, but this depends on their lifestyle and overall health [21]. It has been nearly 100 years since the essential micronutrient, vitamin A, was first described. In 1913 McCollum and Davis reported that the addition of an ether extract from egg yolk or butter, but not lard or olive oil, could reinstate growth in rats maintained for several months on a purified ration of casein, carbohydrates and salt mixtures.

Vitamin C is an important dietary antioxidant which significantly decreases the adverse effects of ROS formed in the cell. Many biochemicals, clinical and epidemiologic studies have indicated that vitamin C may be of benefit in chronic diseases such as cardiovascular disease, cancer, and cataract, probably through antioxidant mechanisms [22]. The chemical name for vitamin C is called ascorbic acid [21]. It is water-soluble. It contributes to collagen production, wound healing, and bone formation. It also strengthens blood vessels, supports the immune system, helps the body absorb iron, and acts as an antioxidant [21], Deficiency of vitamin C may result in scurvy, which causes bleeding gums, a loss of teeth, and poor tissue growth and wound healing. Good sources include fruit and vegetables, but cooking destroys vitamin C [21].

Vitamin E is an antioxidant that can help the body destroy free radicals. Free radicals are unstable atoms that can cause oxidative stress. Oxidative stress can lead to cell damage, and this can result in cancer and other diseases. Vitamin E may help protect the body from a range of health issues. Deficiency of vitamin E is rare, but it may cause hemolytic anemia in newborns. This condition destroys blood cells [21]. Good sources of vitamin E include wheat germ, kiwis, almonds, eggs, nuts, leafy greens, and vegetable oils [21]. There are eight forms of vitamin E, but only alpha-tocopherol meets humans' needs, according to the ODS. Some reasons why the body needs vitamin E are: as an antioxidant, to boost the immune system, to dilate blood vessels and help prevent clotting.

Materials and Methods

Location and duration of the study

This study was carried out in Anatomy Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The rats were made to acclimatize for a period of two weeks after which the test substance was administered for 14 days; the entire experiment lasted for four (4) weeks.

Procurement of drugs

The vitamins C and E and Rohypnol were purchased from a pharmacy, Index Pharmacy, opposite the Nnamdi Azikiwe University Teaching Hospital, Nnewi Anambra state, Nigeria. The feed used was normal Grower mesh, a product of Premier Feed Mills Co. Limited (a subsidiary of Flour Mills Nigeria Plc) in Sapele Delta state, Nigeria.

Experimental animals and design

The research was done with Twenty-five (25) adult Wistar rats weighing between 100-200g. The rats were bought from Onos farm, Nnewi-Anambra state, and moved to the site of the experiment. They were divided into 5 groups (A to E) and were housed in five standard cages. The weight of each rat was measured using an analytical weighing balance. They were fed with feed and water for a period of two weeks to enable them acclimatize with their new environment before the experiment began. During the acclimatization period, the rats were fed with normal grower's mesh of known weight of about 100g daily and given water. The group A served as the Control group while group B, C, D and E served as the Test groups.

Exposure of the animals to test substances

The rats were weighed before the administration of the test substances commenced and thereafter weighed once weekly on Fridays of the week before feeding. The administration of the seed extract was done as follows:

- Group A was given feed and water only
- Group B was given 1 mg/kg of rohypnol only
- Group C was given 1 mg/kg of rohypnol and 100 mg/kg of vitamin C
- Group D was given 1 mg/kg of rohypnol and 100 mg/kg of vitamin E
- Group E was given 1 mg/kg of rohypnol and 100 mg/kg of both vitamin C and E.

Harvesting of the organs and collection of samples

The rats were anesthetized under chloroform vapor by inhalation after 14 days of administration. Blood sample was collected using Randolph kit method and was done by cardiac puncture. The kidneys were then harvested, weighed and fixed in 10% formal saline to maintain normal physiological conditions for its histological studies.

Tissue processing

After weighing the organs, a small part of the Amygdala tissues were cut out and immediately fixed in 10% formal saline in order to preserve the various constituents of the cells in their normal micro anatomical position and to prevent autolysis and putrefaction. After fixation the tissues were dehydrated to remove water and other substances. This was carried out in different percentages of alcohol 50%, 70% and 95% absolute. In each grade of alcohol, tissues were changed twice for two (2) hours, one (1) hour for each change. After dehydration, tissues were cleared in xylene for two (2) hours after which infiltration was done in molten paraffin wax at a temperature of 60°c for two (2) hours, each in two changes. When the paraffin wax cools, it sets as a hard block which allows for easy sectioning of the tissues. The tissue sections were produced by normal histochemical methods of dehydration, clearing, impregnation, embedding, sectioning and staining (with H&E). The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope.

Statistical analysis

The data were analyzed using SPSS version 23. Values were represented as MEAN and SEM, Relative Organ weight (Brain) were analyzed using One way ANOVA, followed by Post Hoc LSD multiple comparison. Body weight was analyzed using Student dependent T-test. Values were considered significant at $P < 0.05$.

Results

Physical and behavioral observation

The Wister rats of Group B were seen to exhibit reduced anxiety, loss of appetite and staggering movement. This could be as a result of the hypnotic and relaxant effect of the rohypnol which was administered to them.

Analysis of the effect on body weight

The weight of all the animals were taken before and after the period of the experiment and are recorded below in Table 1.

Groups	Initial body weight	Final body weight	P < 0.05	Weight change
A	180.051.05	215.302.53	0.0001*	35.251.48
B	218.2813.07	174.289.07	0.0245	-44.004.00
C	198.206.24	223.209.24	0.0552	25.003.00
D	185.706.86	218.086.98	0.0107	32.380.12
E	210.107.48	231.1812.10	0.1767	21.084.62
F-RATIO	89.230	48.510		18.211
PROB.OF SIG.	<0.05	<0.05		<0.05

Table 1: Comparison of mean initial and final body weight and weight change in all the groups (A, B, C, D and E).

Mean ± SEM given for each measurement. Data analyzed using the student dependent T-test followed by Post HOC Turkey's multiple comparison, and data was considered significant at $P < 0.05$. * means that data is significant.

Analysis of the effect on the kidney weight

Groups	Relative kidney weights (%)
A	1.310.03
B	1.330.04
C	1.350.18
D	2.200.44
E	1.350.06
F-RATIO	27.351
PROB. OF SIG.	<0.005

Table 2: Comparison of mean relative kidney weight for group A (control) and experimental groups (B, C, D and E).

Mean ± SEM given for each measurement. Data analyzed using the one-way ANOVA followed by Post HOC Turkey's multiple comparison, and data was considered significant at $P < 0.05$.

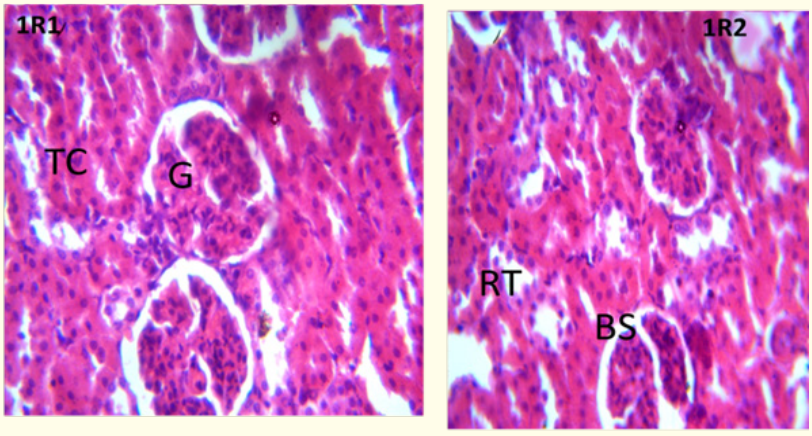
Analysis of the serum levels of urea and creatinine

Groups	Urea	Creatinine
A	24.862.28	1.500.00
B	25.401.99	1.540.14
C	25.520.98	1.561.47
D	38.741.59	2.650.14
E	27.790.95	1.650.09
F-RATIO	7.448	1.421
PROB. OF SIG.	<0.005	<0.005

Table 3: Activities of Serum Levels of Creatinine and Urea.

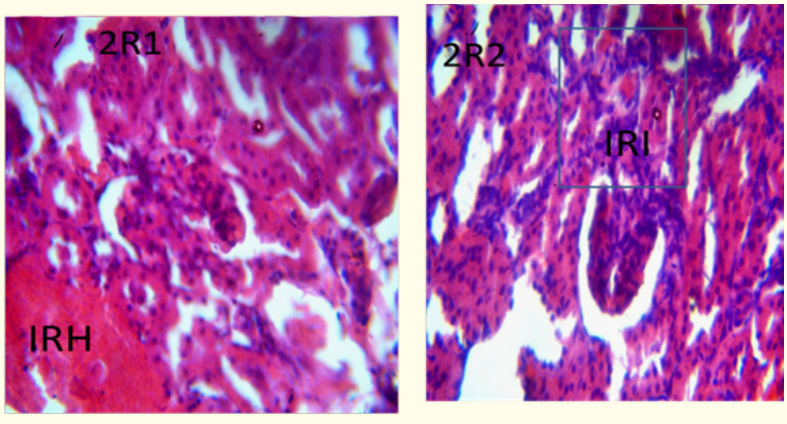
Mean ± SEM given for each measurement. Data analyzed using the one-way ANOVA followed by Post HOC Turkey's multiple comparison, and data was considered significant at $P < 0.05$.

Histopathological findings



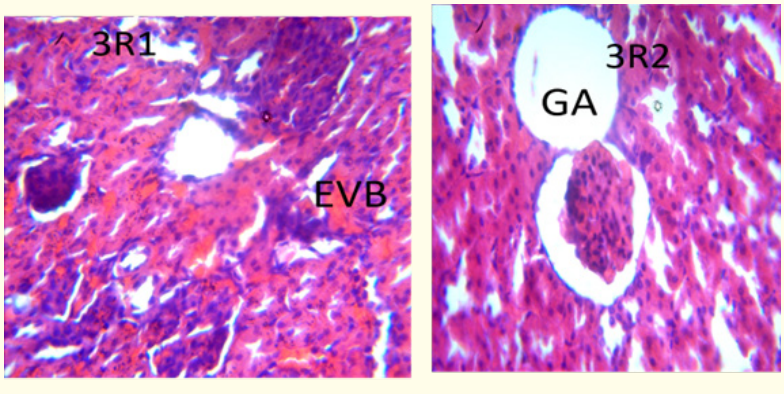
Photomicrograph of 1R1 and 1R2 control section of kidney (X400)(H/E) shows normal renal architecture with glomeruli (G), bowman space (BS), renal tubules (RT) and tubular cell (TC).

Plate 1



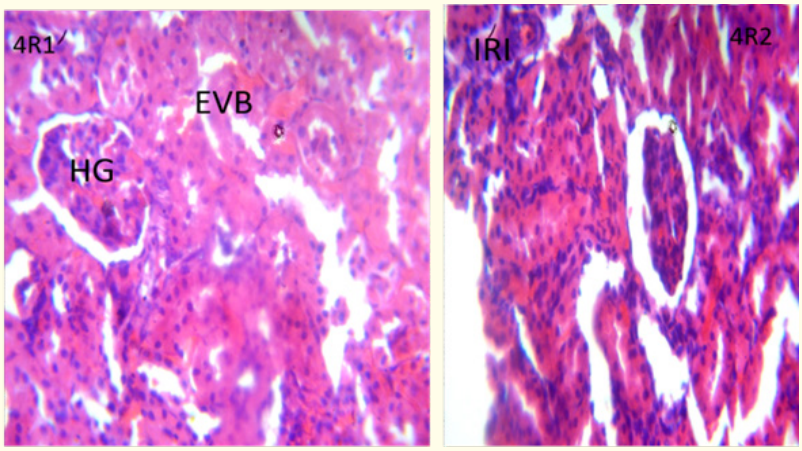
Photomicrograph of 2R1 and 2R2 section of kidney administered with Rohypnol shows moderate effect on the renal tissue [moderate intra renal hemorrhage (IRH), moderate intrarenal inflammation (IRI)]. H&E stain. Magnification: ×400

Plate 2



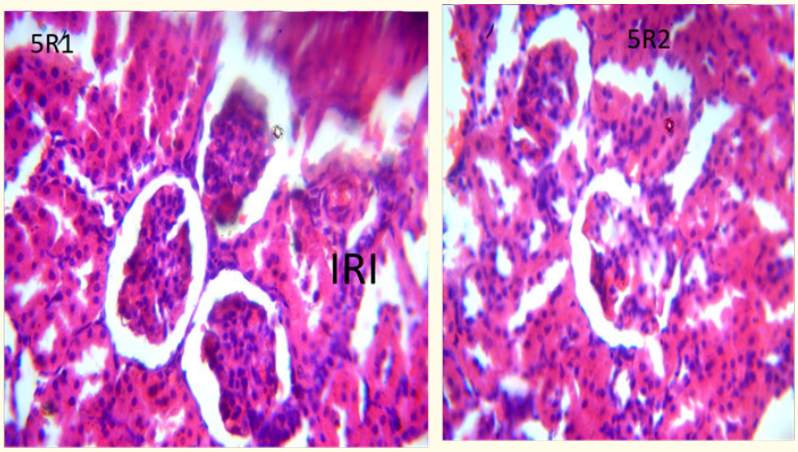
Photomicrograph of 3R1 and 3R2 section of kidney administered with Rohypnol and Vitamin C shows mild healing with moderate extravasated blood (EVB) and glomerular atrophy (GA). H&E stain. Magnification: ×400

Plate 3



Photomicrograph of 4R2 and 4R2 section of kidney administered with Rohypnol and Vitamin E shows moderate healing with mild extravasated blood (EVB) and mild intra-renal inflammation (IRI) with hemorrhagic glomeruli (HG). H&E stain. Magnification: ×400

Plate 4



Photomicrograph of 5R1 and 5R2 section of kidney administered with Rohypnol and Vitamin C and E shows moderate healing with mild intra-inflammation (IRI) otherwise normal. H&E stain. Magnification: ×400

Plate 5

Discussion

This study was principally carried out to investigate the effect of Vitamins C and E on the rohypnol-induced kidney damage in male wistar rats. To my knowledge, this is the first study investigating the effects of Vitamins C and E against kidney damage caused by Rohypnol.

During the course of this study, the Wister rats of Group B were seen to exhibit reduced anxiety, loss of appetite and staggering movement. This could be as a result of the hypnotic and relaxant effect of the rohypnol administered to them. This is synonymous to a study done by Udodi and Ezejindu [23] where the animals were observed to have slow and staggered movement when administered rohypnol at graded dosage.

Observation showed that from the period of acclimatization to the commencement of the administration, there was insignificant increase in body weight of the animals in all groups when the initial weight was compared to the final weight in group A (control group). During the experiment, the control group experienced no reduction in weight. The body weight of rats in Group B decreased, differing from the body weight change of other experimental groups which increased.

Also, the final body weight of group B decreased significantly when compared to its initial body weight. This could be as a result of the impact of Rohypnol that is known from previous study to cause a decrease in appetite of its subject on continuous intake, which could result in significant weight loss. It is, however, in contrast to a recent study done by Akhigbe [24] who stated that there was no significant change in body weight of animals administered graded dosage of Rohypnol for the same duration as this study. The increase in body weight of experimental group E probably indicates the improving or healing effect of the vitamins C and E being administered.

Group C shows slightly insignificant increase when compared to group D which shows a significant increase in weight. This suggests that Vitamin E may have a better ameliorative impact than Vitamin C or its combination with vitamin E.

The kidney weight of group B showed decrease when compared with those of groups C, D and E, which increased when compared with the control group (A). This is in line with the findings of the current study, which revealed that rohypnol exposure led to significant reductions in cardiac and renal weights as well as relative cardiac and renal weights despite without affecting body weight. This might infer that rohypnol induces cardiorenal toxicity possibly by depressing cardiorenal metabolism and growth [24].

The relative kidney weight is given as 100 (kidney weight/final body weight). In the table, that of group D increase significantly when compared the other experimental groups, suggesting an improvement as a result of the antioxidant effect of vitamin E.

Urea and creatinine levels are used to monitor renal function and their levels will not rise until at least half of the kidney nephrons are destroyed [25]. Creatinine is the major catabolic product of the muscles and it is excreted in the kidneys. Also, Creatinine levels are used as indicator in renal failure [26]. Increased level of urea is an indicator of azotemia. High blood urea is associated with increased tissue protein catabolism, excess break down of blood protein and excretion of urea.

Considering this in the biochemical assay of the kidney parameters, there was significant increase in serum levels of Urea and creatinine across the experimental groups when compared with the control group. This suggests that the vitamins had little to no positive effect on the urea and creatinine levels.

The histopathological findings show that Group B had intrarenal haemorrhage with extravasated blood and intrarenal inflammation, due to the rohypnol.

Groups C, D and E show mild to optimum healing, having less intra renal hemorrhage and mild inflammation. This could be attributed to the antioxidant and healing effect of the vitamins C and E administered after damage to the kidney by rohypnol. Group D showed a reduction in intrarenal hemorrhage on administration with vitamin E only, showing it blood clotting ability. Group C showed a reduction in intrarenal inflammation, which could be attributed to its antioxidant effect, eliminating the toxic effect of rohypnol's major metabolite, 7-Aminoflunitrazepam. The glomerular atrophy, however, suggests that there was a stop of blood flow to the kidney during the administration which hypothetically, could be a feedback mechanism in healing the injured kidney. Coadministration of both vitamins in Group E shows that cytoarchitecture has improved to a degree.

Conclusion

From this study, it can be concluded that:

- Rohypnol is a drug that could potentially compromise kidney functions as well as have effects on body and organ weight.
- Vitamins C and E could have antioxidant and possible ameliorative effect on the kidney when damaged by continued use of rohypnol, eliminating the oxidative stress on the kidney caused by 7-Aminoflunitrazepam which is the toxic metabolite of Flunitrazepam (Rohypnol).
- Both vitamins may not have positive effects on the serum urea and creatinine levels.

Recommendations

Following the results of this study, it is recommended that:

- Further studies should be carried out over a longer period of time with higher doses to ascertain the potency of the vitamins C and E on kidney damage caused by rohypnol.
- Studies on effects of rohypnol could be done on other related organs like the spleen and liver to evaluate the possible spread of damage.

Bibliography

1. Mattila M A K and Larni H M. "Flunitrazepam: A review of its pharmacological properties and therapeutic use". *Drugs* 20 (1980): 353-374.
2. George KA and Dundee J W. "Relative amnesic actions of diazepam, funitrazepam and lorazepam in man". *British Journal of Clinical Pharmacology* 4 (1977): 45-50.
3. Calhoun S R., *et al*. "Abuse of funitrazepam (Rohypnol) and other benzodiazepines in Austin and South Texas". *Journal of Psychoactive Drugs* 28 (1996): 1-7.
4. Maxwell J and Hall J. "Memorandum to members, NIDA Community Epidemiology Work Group". May 2 (1995).
5. Badiye A and Gupta M. "Rohypnol: A review on abuse as date rape drug". *Helix* 3 (2012): 161-164.
6. Akhigbe RE., *et al*. "Codeine exerts cardiorenal injury via upregulation of adenine deaminase/xanthine oxidase and caspase 3 signaling". *Life Sciences* 273 (2021): 118717.
7. Hall W D., *et al*. "Why young people's substance use matters for global health". *The Lancet Psychiatry* 3.3 (2016): 265-279.
8. Buck J M and Siegel JA. "The effects of adolescent methamphetamine exposure". *Frontiers in Neuroscience* 9 (2015): 151.
9. Abio A., *et al*. "Substance use and sociodemographic correlates among adolescents in a low-income sub-Saharan setting". *Journal of Injury and Violence Research* 12.1 (2020): 21.
10. Mehanović E., *et al*. "Correlates of cannabis and other illicit drugs use among secondary school adolescents in Nigeria". *Drug and Alcohol Dependence* 206 (2020): 107457.
11. Grant CN and Belanger RE. "Cannabis and Canada's children and youth". *Paediatrics and Child Health* 22.2 (2017): 98-102.
12. Blanco C., *et al*. "Cannabis use and risk of psychiatric disorders: Prospective evidence from a us national longitudinal study". *JAMA Psychiatry* 73.4 (2016): 388-395.
13. Degenhardt L and Hall W. "Extent of illicit drug use and dependence, and their contribution to the global burden of disease". *The Lancet* 379.9810 (2012): 55-70.
14. Pierce M., *et al*. "Insights into the link between drug use and criminality: Lifetime offending of criminally-active opiate users". *Drug and Alcohol Dependence* 179 (2017): 309-316.
15. Volkow ND., *et al*. "Adverse health effects of marijuana use". *New England Journal of Medicine* 370.23 (2014): 2219-2227.
16. Di Forti M., *et al*. "Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users". *Schizophrenia Bulletin* 40.6 (2013): 1509-1517.
17. Ritchwood TD., *et al*. "Risky sexual behavior and substance use among adolescents: A meta-analysis". *Children and Youth Services Review* 52 (2015): 74-88.
18. Doku D. "Substance use and risky sexual behaviours among sexually experienced Ghanaian youth". *BMC Public Health* 12.1 (2012): 571.
19. Qureshi AI., *et al*. "Cocaine use and the likelihood of cardiovascular and all-cause mortality: Data from the Third National Health and Nutrition Examination Survey Mortality Follow-up Study". *Journal of Vascular and Interventional Neurology* 7.1 (2014): 76-82.
20. Chaurasia BD and Garg K. "Human anatomy: regional and applied dissection and clinical". New Delhi, India: Cbs Publishers & Distributors (2010): 1471- 2458- 12- 571.
21. Alexandra P., *et al*. "Gendered Dietary Supplements: Does the Marketing Reflect Different Formulations?" *International Journal of Exercise Science: Conference Proceedings* 2.15 (2023): 19.
22. Carr AC and Frei B. "Toward a New Recommended Dietary Allowance for Vitamin C Based on Antioxidant and Health Effects in Humans". *American Journal of Clinical Nutrition* 69 (1999): 1086-1107.

23. Udodi Princewill Sopuluchukwu., *et al*. "A Study on the Neurotoxicity of Flunitrazepam (Rohypnol) Administration on the Cerebral Cortex of Adult Wistar Rats". *Advances in Pharmacology and Pharmacy* 9.2 (2021): 26-32.

24. Akhigbe RE., *et al*. "Upregulation of Uric Acid Production and Caspase 3 Signaling Mediates Rohypnol-Induced Cardiorenal Damage'. *Cardiovascular Page toxicology* (2022): 13-19.

25. Ajilore Bamidele., *et al*. "Protective effects of Moringa oleifera Lam. on cadmium-induced liver and kidney damage in male Wistar rats". *International Journal of Phytotherapy Research* 2 (2012): 1-50.

26. Aliyu R., *et al*. "The Effects of Ethanolic Leaf Extract of Commiphora africana (Burseraceae) on Rat Liver and Kidney Functions". *Journal of Pharmacology and Toxicology* 2 (2007): 373-379.