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Histological and some biochemical markers of the liver and kidney of female albino wistar rats exposed to rohypnol (Flunitrazepam)

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Abstract
Rohypnol (flunitrazepam) is a type of benzodiazepine medication with sedating, anti-anxiety and hypnotic properties that act as a central nervous system depressant, which may potentiate neurotoxicity hence prescribed only for short term in some countries to treat insomnia. The tolerance, dependency from the illicit use of this drug can develop very quickly, creating a euphoric sensation, including serious and deadly side effects arising from abuse and addiction especially amongst adolescents and adults for recreational purposes. This study was designed with the general aim of investigating the effect of varied dosages of rohypnol on the functionality of the liver and kidney by assessing relevant biochemical parameters alongside basic histological investigations. A total number of 20 adult female albino rats of the Wistar strain that weighed between 180g - 200g were used for this study. The experimental animals were randomly selected into four groups of five rats each, namely- Groups A-D. Group A was used as the control group and was treated with distilled water while groups B- D were treated with 0.5mg, 1.0mg, 1.5mg and 2.0mg per kilogram body weight respectively. The administration was done per os, two times daily and for 14days. Results obtained from this study showed statistically significant (p<0.05) increases in the serum levels of total and conjugated bilirubin, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) relative to the control. The serum levels of urea and creatinine decreased significantly (p<0.05), and in a dose dependent manner. The histological assessments carried out on the renal (kidney) tissues of rohypnol treated female albino rats revealed mild to severe vacuolations, disrupted renal tubules, vascular congestion and reductions of the glomeruli in the kidneys of treated rats relative to the control. In the liver several spots of vascular congestion, mild to severe vacuolations were also recorded. The above results (biochemical and histological) are indicative of injurious effects on the kidney and liver tissues, and can be predicative to significant liver and kidney damage.

Keywords: Rohypnol, urea and creatinine, liver enzymes, histology, wistar rat

1. Introduction
Rohypnol is the brand name by which flunitrazepam a benzodiazepine is marketed in countries where it is legalized. It is a nitro-benzodiazepine and the fluorinated *N*-methyl derivative of nitrazepam. Benzodiazepines generally function as skeletal-muscle relaxants that act as sedative-hypnotics and can serve as pre-anesthetic agents also used for the treatment of insomnia or severe sleep disorders, anxiety, and seizure disorders (Carson-DeWitt, 2001; Kusuri-no-Shiori Drug Information Sheet, 2015) [11, 27]. It is advisable to prescribe flunitrazepam only for short-term use since it is a central nervous system depressant which may potentiate neurotoxicity in situations of overdose. Some of the after effects of overdosing include respiratory depression, impairment of balance, slurry speech, excessive sedation, loss of composure, coma, depression and eventual death. Flunitrazepam is frequently involved in drug intoxication and known to induce anterograde amnesia in sufficient doses where individuals are unable to remember certain events they experienced while under its influence, hence complicates investigations (Liljesson, 1999) [28]. This effect could be particularly dangerous if it is used to aid in the commission of sexual assault; victims may be unable to clearly recall the assault, the assailant, or the events surrounding the assault (Kiss *et al*., 2016) [25]. It is widely known for its use as a date rape drug with the following street names: “floonies”, "roofie", "ruffie" Circles, R2, Forget Me Pill, lunch money drug, La Rocha, Pingus, Mexican valium, roll and fall, roofenol, Roach 2 etc (Schwartz *et al*., 2000; Center for Substance Abuse Research, 2013; Gautam *et al*., 2014; United States Drug Enforcement Administration, 2020) [33, 12, 14, 34].

Rohypnol was formulated by Leo Sternbach in 1960 under Roche as a benzodiazepine and it was first sold in 1972 (Bryan, 2009) [9]. In 1998, the formulation was modified to lower doses due to incessant abuse of the drug and by early 2016 the drug had to be completely taken off the markets in countries where the abuse was more endemic including Germany, Norway, Spain, France and United Kingdom. Worthy of note is the fact that Rohypnol has never been market in the United States of America (Kiss *et al*., 2016) [25]. Drug tests to detect rohypnol utilize blood samples, urine, hair and saliva. However urine is usually the preferred specimen for routine substance monitoring purposes (Kiss *et al*., 2016) [25].

The liver is a vital and highly specialized organ in humans as well as other higher animals consisting mostly of hepatocytes (Abdel-Misih and Bloomston, 2010) [1]. It is uniquely placed for handling dietary compounds since it receives all the blood from the synthesis of many metabolically important compounds from diet derived precursors and for their interconversions. It plays a vital role in several metabolic processes, which includes regulating glycogen metabolism, alcohol breakdown, hormone production, detoxification, synthesis of plasma proteins and red blood cells decomposition (Abdel-Misih and Bloomston, 2010) [1]. The liver is also responsible for converting some toxic compounds both from the systemic circulation and the portal blood and channeling it for excretion (Baron *et al*., 1989) [40]. Liver function tests (LFTs) involves the use of certain biomarkers that are relevant in monitoring the functional integrity of the liver to perform routine tests. Some of the biomarkers include alkaline phospahatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), albumin, bilirubin and gammaglutamyl tranferase (GGT) (McClatchy, 2002). Liver diseases usually present with minimal symptoms at its onset, therefore regular routine liver functions tests are advised (Johnston, 1999) [23].

The kidneys are described as bean shaped organs located retroperitoneal aspect of the abdomen usually protected by a tough capsular irregularly dense connective tissue whose septa ramifies into the substance of the kidneys thus lobulating them. Each kidney houses over a million functional units called nephrons whose major functions include but not limited to the filtration of plasma, reabsorption and excretion of liquid waste products like urea and creatinine. The kidney is also concerned with osmoregulation which is the maintenance of ionic balance between plasma and other fluids from the various compartments of the body.

Rohypnol has been reported as having the propensity to depress the functionality of the nervous system. Reports have shown that it can also cause organ damage, blackouts, respiratory disorders, hyperthermia and in some cases outright death when abused (Daderman, 1999; Druid *et al*., 2000; Bramness, 2005) [13, 15, 8]. Based on the known wide range uses of rohypnol in most countries of the world, it is necessary to study the effect of this substance at various graded dosages (low, moderate to high) on the liver and the kidney. Although extensive studies have been done on the effect rohypnol, very little literature is available on the combined effects of rohypnol on the liver and kidney. This study was designed to evaluate and elucidate the possible changes that rohypnol could cause on the liver and kidney tissues of female albino rats as well as possible alterations in the levels of the liver and kidney marker enzymes

2. Materials and methods

2.1 Drugs

Rohypnol (Flunitrazepam 90 mg) was purchased from Don Joe Pharmacy, Okada, Edo State, Nigeria. To homogenize the pills, a laboratory grade mortar and pestle were used until a smooth powdery consistency was obtained. The rohypnol powder was dissolved in distilled water obtained from the Igbinedion University Teaching Hospital (IUTH), Okada, Edo State to form a homogenous mixture for administration to the rats.

2.2 Experimental design

Twenty adult female albino rats of the Wistar strain with weights between 180-200 g were used for this investigation. These experimental animals were supplied by the animal holding unit, Igbinedion University, Okada. The experimental animals were housed in plastic cages under temperatures between 25 °C to 27 °C, with 12hours light and dark cycles. Food and water were also given freely to the animals. The rat pellets used for feeding the animals was procured from Bendel feed and flour mills, Ewu, Edo state. Acclimatization of the experimental animals lasted for one week and the animals were weighed before they were randomly placed in groups of four rats each such that we had groups A, B, C, D and E. The animals in group A were used as control and received distilled water as a form of placebo, while groups B to E were treated with rohypnol dissolved in distilled water at graded dosages as follows: Group B received 0.5mg/kg body weight, Group C was given 1mg/kg body weight, Group D was given 1.5mg/kg body weight, and Group E received 2mg/kg body weight. The drug was administered twice daily, first at 8am and later at 5pm for two weeks. The permission to use animal model for this research was obtained from the Director, Animal House and Ethics, Igbinedion University, Okada; who ensured through supervision that animal handling was done in line with acceptable global best practices. The animals were cared for by ensuring they had free and liberal access to 12 hours light, 12 hours darkness, food and water, the cages were regularly cleaned, and the laboratory was properly ventilated.

2.3 Experimental Animal Sacrifice and Tissue collection

On completion of treatment, the animals were weighed and sacrificed by cervical dislocation 24hours after the last administration. The whole blood from all the animals were carefully collected by cardiac puncture and placed in heparinized sample bottles. The blood samples were centrifuged at 5000 revolutions per minute and the serum aspirated for the liver and kidney function tests respectively. A laparotomy procedure was carried out to excise the liver and the kidneys from the experimental animals. The organs were blotted dry with cotton wool, weighed and processed using the standard tissue processing procedure (Avwioro, 2010) [6]. Sections were mounted on slides with DPX and stained with Hematoxylin and Eosin.

2.4 Statistical Analysis

Statistical analysis was carried out by collating all the respective data and inputting it into the IBM statistical analysis package for social science version 20. The results obtained were expressed as Mean ± standard error of mean (SEM). The relevant statistical significance was obtained through the analysis of variance (ANOVA). Finally the Duncan Post-hoc test was carried out to determine the significant differences between the groups at $p<0.05$.

3. Results

Twenty adult female albino rats of the Wistar strain were used for this study. The animals were weighed at the beginning of the experiment and only rats that weighed between 180 g-200 g were used for the study. Body and relative organ weights, some liver function tests (Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), albumin, total and conjugated bilirubin), kidney function tests (urea and creatinine) and histological evaluations of both the control group given distilled water and groups treated with varied dosages of rohypnol (0.5, 1.0, 1.5 and 2.0 mg/kg body weight) through a 14 day period was analyzed. These results are shown below.

3.1 Effect of rohypnol on the body weight (g) of normal female Wistar albino rats

The mean changes and percentage change in the body weights of the normal female Wistar albino rats after treatment with different dosages of rohypnol are represented in Figures 1a and 1b. According to the results obtained from the weight analysis, there was a significant (p<0.05) decrease in the final weight of all the treatment groups relative to the initial weight. However for the control group there was rather a significant (p<0.05) increase in the final weight relative to the initial weight.

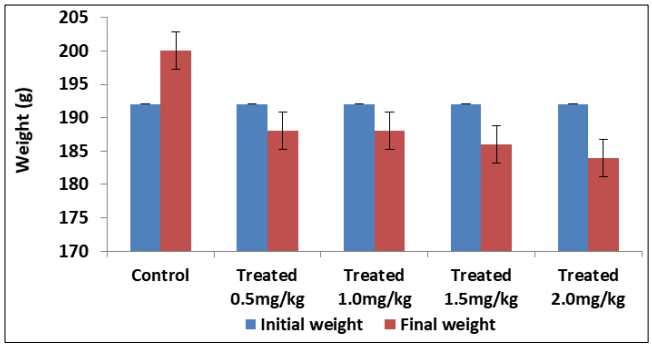

Fig 1a: Mean changes in initial and final body weight (g) of normal female Wistar albino rats treated with rohypnol over a 14 day period

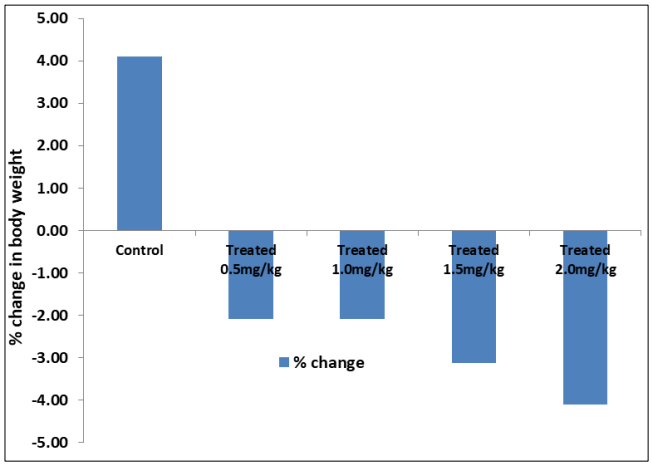

Fig 1b: Percentage change in the body weight of normal female Wistar albino rats treated with rohypnol over a 14 day period

3.2 Effect of rohypnol on the mean organ weight (g)

Figures 2a and 2b represents the mean organ weight changes and relative organ/body weight ratios respectively. It was generally observed that there was a significant (p<0.05) increase in the mean absolute liver and kidney weights as well as their relative organ to body weight ratios in the rohypnol treated groups relative to the control. However there was no significant (p>0.05) change in the brain weight for all the rohypnol treated groups compared to the control.

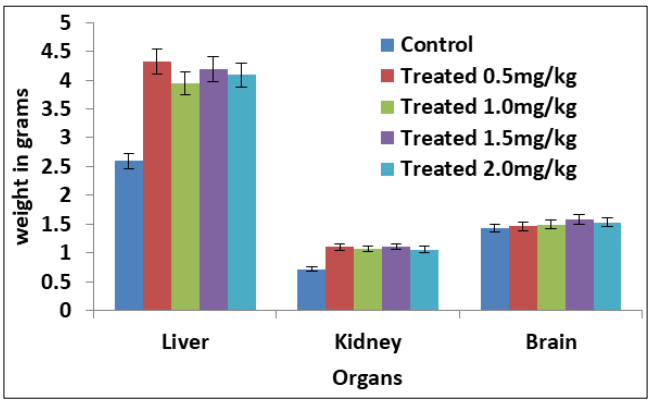

Fig 2a: Mean absolute organ weights in normal female Wistar albino rats treated with rohypnol

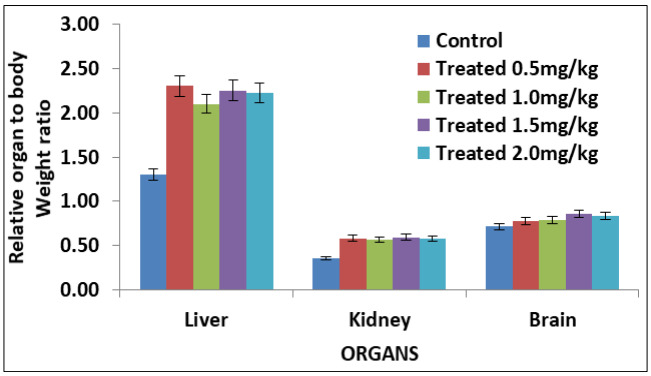

Fig 2b: The effect of rohypnol on the mean changes of the relative organ to body weight ratio of normal female Wistar albino rats

3.3 The effect of rohypnol on some liver function indices in normal female Wistar albino rats

Figures 3a, 3b and 3c represents a summary of the mean values of some serum liver function indices obtained in the experimental animals treated with varied dosages of rohypnol. Statistical evaluation of the serum levels of alkaline phosphatase (ALP) aspartate transaminase (AST) and alanine transaminase (ALT) activities showed statistically significant increases (p<0.05) in all the rohypnol experimental groups. These increases occurred in a dose based manner when compared with the control group. The serum level of albumin, total bilirubin and conjugated bilirubin showed statistically significant increases (p<0.05) in all the rohypnol treated groups, also in a dose dependent manner relative to the control.

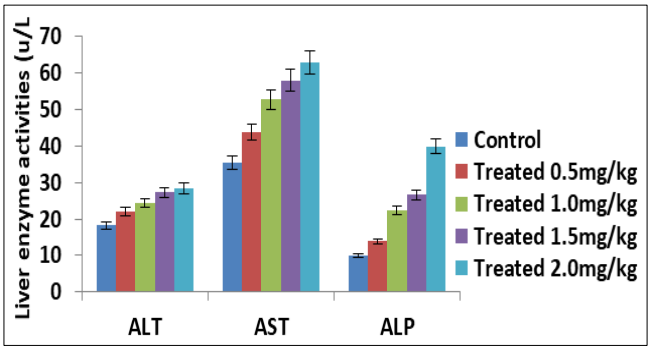

Fig 3a: Mean activities of some serum liver marker enzymes (ALT, AST and ALP) in normal female Wistar albino rats treated with rohypnol

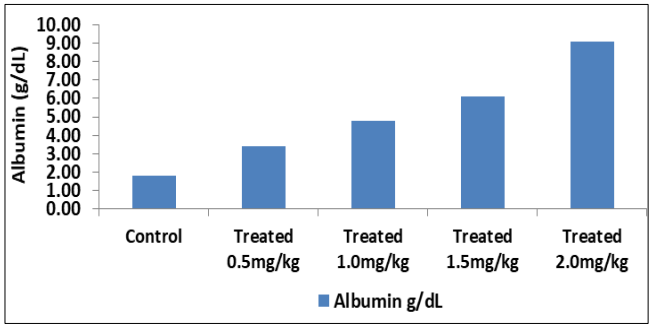

Fig 3b: Mean albumin level in normal female Wistar albino rats treated with rohypnol

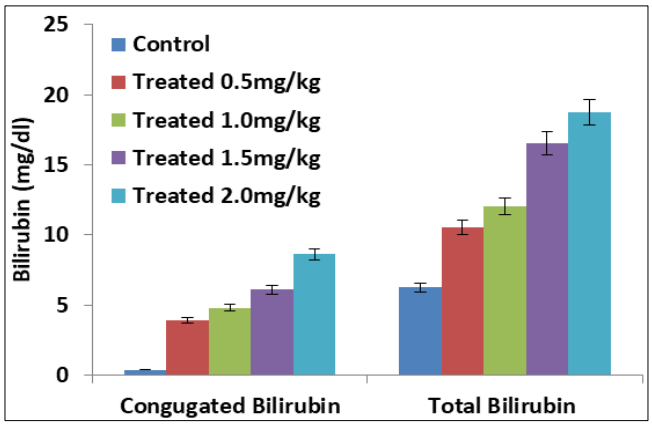

Fig 3c: Mean level of serum conjugated and total bilirubin (mg/dl) in normal female Wistar albino rats treated with rohypnol

3.4 The effect of rohypnol on some kidney function indices in normal female Wistar albino rats

Figures 4a and 4b represents a summary of the mean values of some serum kidney function indices (urea and creatinine respectively) obtained in the experimental animals treated with varied dosages (0.5, 1.0, 1.5 and 2.0 mg/kg body weight) of rohypnol. Evaluation of the serum levels of urea and creatinine showed statistically significant decreases (p<0.05) in all the rohypnol treated groups in a dose based manner when compared with the control

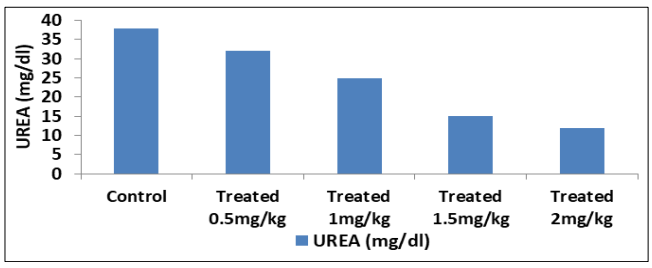

Fig 4a: Mean level of serum urea (mg/dl) in normal female Wistar albino rats treated with rohypnol

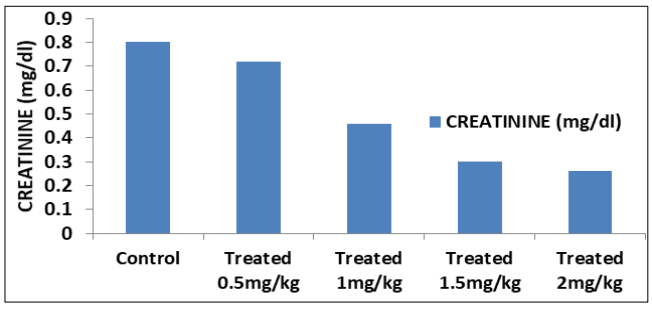

Fig 4b: Mean level of serum creatinine (mg/dl) in normal female Wistar albino rats treated with rohypnol

3.5 Histological evaluations

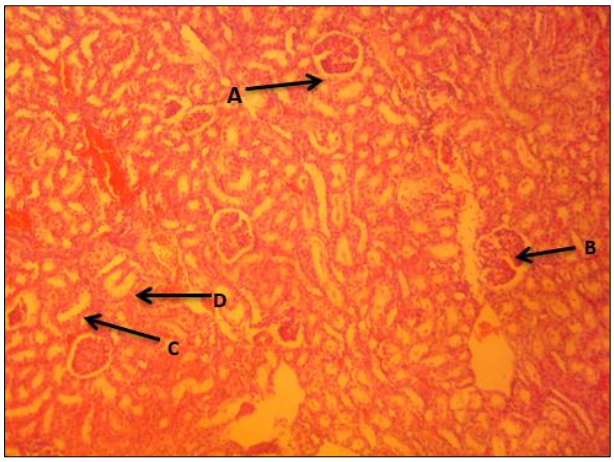

Plate 1: Photomicrograph of kidney sections in female albino rats administered distilled water that served as control showing normal histoarchitecture with several renal corpuscles with Bowman's capsule = A, glomeruli = B, proximal convoluted tubules = C and distal convoluted tubules = D (H&E X100)

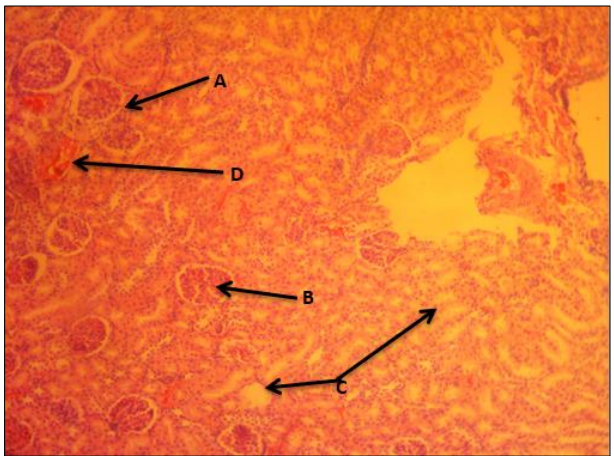

Plate 2: Photomicrograph of kidney in female *Wistar* albino rats treated with 0.5mg/kg body weight of rohypnol showing fairly normal histoarchitecture with Bowman's capsule = A; glomeruli =B; fairly normal tubules = C; and mild vascular congestion D (H&E X100)

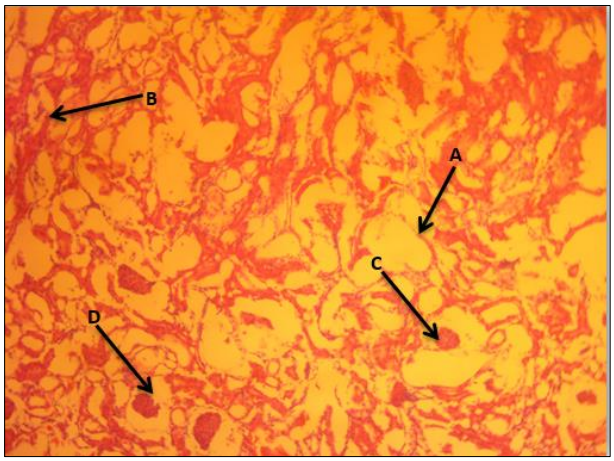

Plate 3: Photomicrograph of kidney in female *Wistar* albino rats treated with 1.0mg/kg body weight of rohypnol showing severely distorted renal parenchyma with enlarged tubules = A, vascular congestion = B, enlarged bowman's capsule = C and shrunken glomeruli = D. (H&E X100)

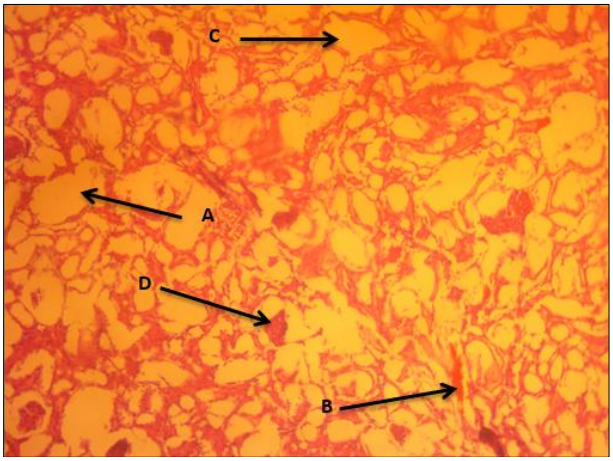

Plate 4: Photomicrograph of kidney in female *Wistar* albino rats treated with 1.5mg/kg body weight of rohypnol showing severely distorted renal parenchyma with vacuolations =A, vascular congestion = B, enlarged bowman's capsule = C and shrunken glomeruli = D. (H&E X100)

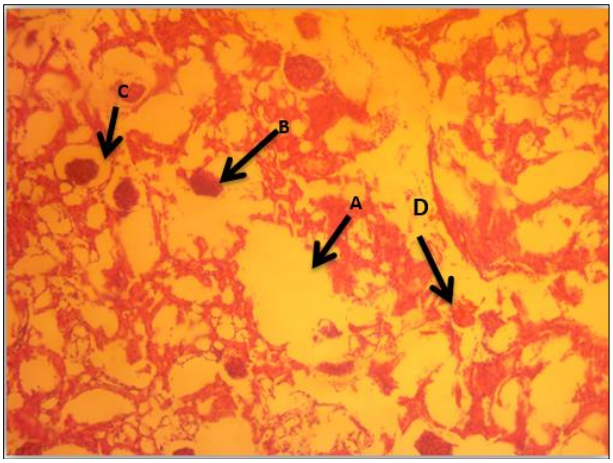

Plate 5: Photomicrograph of kidney in female *Wistar* albino rats treated with 2.0mg/kg body weight of rohypnol showing severely distorted renal parenchyma with enlarged tubules = A, vascular congestion = B, enlarged bowman's capsule = C and shrunken glomeruli = D. (H&E X100)

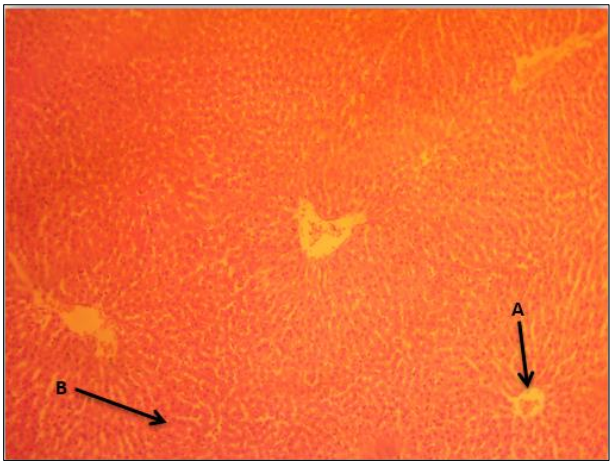

Plate 6: Photomicrograph of liver tissue in female *Wistar* albino rats administered distilled water that served as control showing normal histoarchitecture with central vein = A, sinusoids and hepatocytes = B (H&E x100)

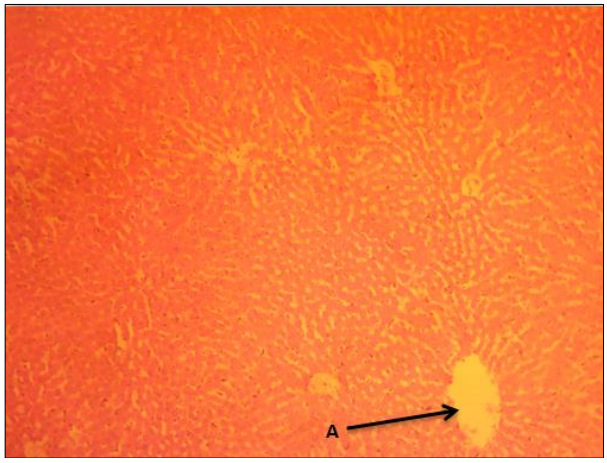

Plate 7: Photomicrograph of liver tissue in female *Wistar* albino rats treated treated with 0.5mg/kg body weight of rohypnol showing focal area of central vein enlargement = A, and fair distortion of liver tissue with (H&E x100)

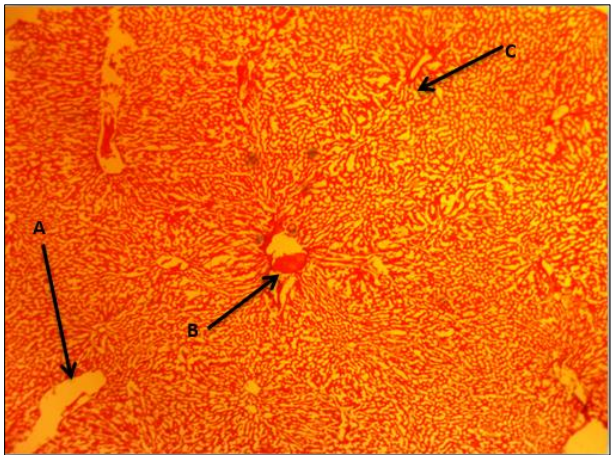

Plate 8: Photomicrograph of liver tissue in female *Wistar* albino rats treated with 1.0mg/kg body weight of rohypnol showing fairly distorted liver tissue with enlarged central vein = A, mild vascular congestion= B and disrupted hepatocytes =C (H&E x100)

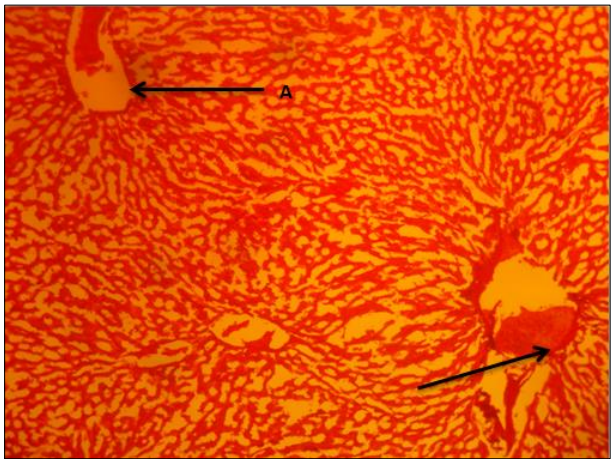

Plate 9: Photomicrograph of liver in female *Wistar* albino rats treated with 1.5mg/kg body weight of rohypnol showing severe distortion of the liver parenchyma, enlarged central vein = A, severe vascular congestion = B (H & E X100)

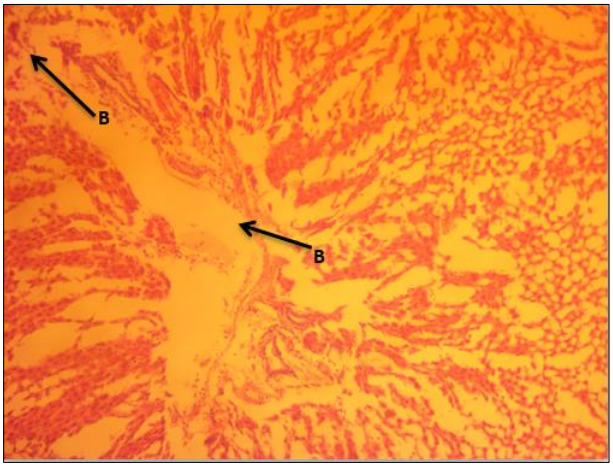

Plate 10: Photomicrograph of female *Wistar* albino rats treated with 2.0mg/kg body weight of rohypnol showing severe degeneration of the liver parenchyma, vascular congestions and focal area of mild edema = A, and severe enlargement of liver sinusoids = B (H & E X100)

4. Discussion

Rohypnol with generic name flunitrazepam is commonly tagged a date-rape-drug and reportedly implicated in drug overdose, intoxication as well as abuse. It is mostly bubble packaged by the manufacturer and sold in pill form (Druid *et al*., 2000; Zevzikovas, *et al*., 2002; Jonasson and Saldeen, 2002) [15, 39, 24]. Rohypnol when taken orally is absorbed almost completely (80%) and in suppository form its bioavailability is up to 50% (Cano *et al*., 1977) [10]. According to a report by Vermeeren in 2004, the effect of rohypnol taken at night time can persist through the next day owing to its long half-life of about 18-26 hours. The reason behind this is the production of active metabolites which enhances the duration of its activity compared to other benzodiazepines that rather produces non-active metabolites (Griffin *et al*., 2014) [19]. Rohypnol is lipophilic in nature and often metabolized by the liver through oxidative pathways with CYP3A4 being the principal enzyme that catabolizes its phase 1 metabolism in human liver microsomes (Hesse *et al*., 2001) [20]. Its main pharmacological effect is the enhancement of GABA, which is an inhibitory neurotransmitter that exerts its effects on GABA receptors (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2015) [16].

The results obtained from the present study as represented in figures 1a and 1b respectively, showed a significant (p<0.05) decrease (dose dependently) of the mean absolute body weights and percentage change in the body weight of all the rats that received varied dosages (0.5, 1.0, 1.5 and 2.0 mg/kg body weight) of rohypnol twice daily through a 14 day period relative to the control group. The weight loss by the treated groups may have been as a result of loss of appetite caused by the persistent use of rohypnol that could be likened to its abuse hence leading to malnutrition. Body weight changes (gain or loss) may result from the limited intake of balanced diets at the acceptable standard proportion or deficiency. During such instances most essential nutrients such as vitamins and minerals are not retained for bodily functions. Consequently, the body may become vulnerable leading to other physical conditions, psychological effects, emotional setbacks and several medical disorders. Flunitrazepam as well as other benzodiazepines is among the numerous substances lacking in nutrients which may eventually cause changes in body weights. Some of these substances can cause vomiting, nausea and diarrhea in most persons under the influence of the drugs resulting in loss of appetite as well as poor absorption of essential vitamins and minerals, and potentially leading to malnutrition as well as extreme weight loss over a long time **(**US Drug Enforcement Administration, 2018) [34].

The mean changes in the organ (liver, kidney, brain) weights as well as the relative organ to body weight ratio of the normal female Wistar albino rats treated with varied dosages of rohypnol are represented in Figures 2a and 2b respectively. It was generally observed that there was a significant (p<0.05) increase in the mean absolute liver and kidney weights as well as their relative organ to body weight ratios in the rohypnol treated groups when compared to the control. However there was no significant (p>0.05) change in the brain weight for all the rohypnol treated groups compared to the control. In 2014, Nirogi and colleagues reported that the change in body weight of animal models may arise as a result of disruptions in hormonal status, growth related change, and alterations in neurotransmitters that control consumption of food. The results of the weight comparison between substance treated animals and the untreated ones is often a useful diagnostic tool to determine the extent of tissue damage in histopathological studies. Histopathological assessments no doubt is standard for identifying treatment related changes in most organs, however organ weights are also vital indicators of target organs (Nirogi *et al*., 2014) [31]. It is therefore worthy of note that organ weight changes are accepted as a sensitive indicator of chemically induced organ damage (Nirogi *et al*., 2014) [31]. In this study, there were significant increases in absolute mean organ weights of both liver and kidney relative to the control. This may be as a result of residual blood that may have been retained in the organs at the point of sacrifice (Wilson *et al*., 2000) [38].

The liver is relevantly involved in the metabolism and elimination of xenobiotics and that often makes it prone to toxicity. Several biochemical parameters are readily measurable in blood, which helps to detect, quantify and clarify the significance of exposure to hepato-toxicants and their attendant structural and functional effects (Ramaiah, 2011) [32]. Commonly performed liver function tests to help determine the health status of the liver are alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), albumin and bilirubin tests. In this study, Figures 3a, 3b and 3c respectively showed that, the levels of ALT, AST, ALP, albumin, conjugated and total bilirubin increased significantly (p<0.05) amongst the rohypnol treated groups when compared to the control group. The dose dependent serum increase in the levels of the liver marker enzymes, protein and bilirubin could be attributed to the outright destruction of the liver hepatocytes by the toxic effect of rohypnol (Adachi *et al*., 1995; Krans and Cafasso, 2018) [2, 16]. The manifestation of oxidative stress may result to hepatocellular damage, necrosis, vascular dysfunction, cirrhosis etc. (Arika *et al*., 2016) [4], hence this could be the reason behind the changes in the levels of liver marker enzyme, bilirubin and protein as recorded in this study. Hepatotoxicity can arise from direct toxicity of primary compounds as well as from an immunologically-mediated activity against hepatocytes or from reactive metabolites (Jaeschke *et al*., 2002) [22].

The kidney function indices evaluated in this study are serum urea and serum creatinine, which are the major catabolic byproducts of protein and carbohydrate metabolism in the liver. These markers are required for the purpose of assessing the normal excretory capacity and/or potentials of different segments of the functional unit (nephron) of the kidney

(Musabayane, 2012) [30]. Figures 4a and 4b summarizes the results obtained in the current investigation, which showed statistically significant decreases (p<0.05) in levels of serum urea and serum creatinine in all the rohypnol treated groups. The decreases were seen to be dose dependent when compared with the control group. The present study has found a significant tie between the severity of liver dysfunction and some parameters of renal dysfunction. Most diseases that affect the kidneys or liver can also affect the amount of urea present in the blood. This is because it is a known fact that urea is produced in the liver and gets to the kidney through blood circulation where it is excreted as a component of urine. Therefore it is expected that any liver pathology that causes reduction in urea synthesis will cause an attendant decrease in blood/serum urea concentration. Results obtained from the histopathological investigation of this study show a breakdown of the renal parenchyma which may be due to the toxicity. The renal tissue breakdown may have snowballed into the reduction in urea excretion, therefore leaving decreased levels of urea in blood serum.

The histological evaluation of this study showed that the renal cortex and medulla of most of the rohypnol treated rats had severe vacuolations, vascular congestions, and disrupted renal architecture when compared to the control groups where tissue architecture was maintained. The results of this current study corroborates those of Atacci *et al.* (2005) [5] who reported that tramadol elicited significant histopathological alterations in kidney tissues including tissue degeneration, glomerular chamber enlargement and swelling of the lining epithelium, mononuclear cell infiltration, damaged proximal convoluted tubule brush borders, necrotic lesions, and pyknotic nuclei of the urinary tubules. The disruptions in the serum levels of the kidney markers are further confirmed by the histological lesions recorded in this study.

The liver also showed spots of vascular congestions, severe and mild vacuolations. Thus, changing the hepatic tissue architecture of the treated animals compared to the normal group. According to Albarakai, 2017 [3], triple analgesic dose of Nalbuphine-HCl caused dilated congested blood and marked stagnant blood in the portal vein, inflammatory infiltration around the bile duct, and vacuolated ballooning cytoplasm with pyknotic nuclei. The vacuolations recorded in this study may be an indication of early cellular degeneration due to injury to the hepatocytes (hydropic degeneration) due to exposure to rohypnol. Vascular congestion is accumulation of blood due to circulatory disturbances which may be in the form of decreased velocity of blood flow within the liver parenchyma (Daiane Cristina Marques dos Santos., *et al* 2015) [14]. In severe cases of congestion, adjacent hepatocytes can suffer atrophy which may in turn lead to decreases in the rate of metabolic exchange between blood plasma and tissue with the resultant cellular damage (Verlog, 1982) [36]. The histological changes recorded clearly indicates that rohypnol at the doses used in this study has injurious and deleterious effects on the histoarchitecture of kidney and liver tissues. It was also found that there were marked alterations in the levels of liver and kidney marker enzymes across the treated groups. According to Arika., *et al* 2016 [4], hepatocellular damage arises with manifestation of oxidative stress and/or reactive metabolites (Jaeschke *et al*., 2002) [55]. This may explain the mechanism of action by which the various dosages of rohypnol acted on the organs of study.

5. Conclusion

The present study clearly displays an obvious relationship exhibited by the dose dependent rohypnol administration regarding the body weights, organ to body weight ratio, severity of liver and renal dysfunction. This study further emphasizes the fact that excessive consumption or abuse of rohypnol may impair liver and renal function. There is therefore a need to always carry out liver and renal function tests for proper screening, prevention measures as well as treatment of liver and renal dysfunction can decrease morbidity and mortality amongst consumers of this drug. There is need for more awareness campaigns amongst the youth in our societies to highlight the underlying dangers of rohypnol abuse to avoid kidney and/or liver damage as well as death.

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