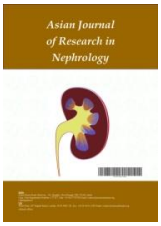

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Hepatotoxicity in the Liver of Wistar Rats Administered with a Combination of Tramadol and Rophynol

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Authors' contributions

This work was carried out in collaboration among all authors. Author EUE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VEO and SBI managed the analyses of the study. Author EFE managed the literature searches. All authors read and approve the final manuscript.

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ABSTRACT

Objectives: The objective of this research was to investigate the histopathological effects of combined oral administration of Tramadol and Rohypnol on the liver and kidney of adult Wistar rats.

Materials: Thirty two (32) adult Wister rats weighing 120 ± 20 g - 180 ±30 g comprising sixteen (16) males and sixteen (16) females were procured from the animal house of the Department of Pharmacology, College of Health Sciences Niger Delta University, Bayelsa state. Nigeria, assigned into four (4) major groups with four male, 4 females animals in each group after the period of acclimatization: a control group "A" and three test groups (B, C and D), Group A (control), test Groups B:Tramadol only. Group C: Rohypnol only. Group D received a combined oral dose of Tramadol and Rohypnol. At the end of the treatment, the liver and the kidney of each sacrificed rat were processed for paraffin sectioning and stained with Harris hematoxylin and eosin.

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Results: Photomicrograph of Groups B, C and D show moderate inflammatory cells and obliteration of the central vein which are features of hepatic injury.
Conclusion: The present study concluded that oral combination of Tramadol and Rohypnol induces hepatoxicity and inflammation of the liver but produces no effect on the kidney at the dose and time.

Keywords: Liver; kidney; tramadol; rophynol; oral; hepatotoxicity.

1. INTRODUCTION

Substance abuse, also known as drug abuse, is a patterned use of a drug in which the user consumes the substance in amounts or with methods which are harmful to themselves or others, and is a form of substance-related disorder. Widely differing definitions of drug abuse are used in public health, medical and criminal justice contexts. In some cases, criminal or anti-social behaviour occurs when the person is under the influence of a drug, and long term personality changes in individuals may occur as well [1]. Tramadol is a widely used therapeutic alternative to other opioid analgesics since it has a low potential for abuse, dependence and tolerance, and low probability to cause adverse effects, including substance abuse, is not clear, with the two predominant theories being respiratory depression [2]. However, the number of cases reporting dependence, abuse, intentional overdose or intoxication by tramadol is increasing. Fatal intoxications due to tramadol alone also exist but are not common [3,2]. The administration of toxic doses of tramadol concomitantly with other central nervous system depressants is one of the most common causes of severe or lethal acute intoxication [4-6]. Tramadol is a centrally acting opioid analgesic, which has a dual mechanism of action: it is a partial agonist of μ-opioid receptors and inhibits serotonin and noradrenaline reuptake at the synapses of the spinal cord, acting on the pain transmission mechanism [3]. After oral administration, tramadol is rapidly and almost completely absorbed [7]. The increase in serum creatinine and BUN levels with acute and chronic tramadol dosing is also in accordance with results of Atici et al. [8]. In rats receiving morphine for a month. Similarly, El-Gaafarawi [9] stated that morphine and codeine administration for a long period resulted in an increased creatinine and BUN levels. The above observations may be confirmed by the suggestions of Wu [10] who stated that liver and kidney are responsible for tramadol metabolism and excretion, so it may cause hepatotoxicity and nephrotoxicity. The main pharmacological effects of flunitrazepam are the enhancement of GABA at various GABA receptors. Rohypnol (Flunitrazepam) is a central nervous system depressant in a class of drugs called benzodiazepines. Benzodiazepines are sedative-hypnotics used to treat anxiety, insomnia and sleep disorders, and seizure disorders; they are also used as skeletal-muscle relaxants [11]. Flunitrazepam has a long half-life of 18–26 hours, which means that flunitrazepam's effects after night time administration persist throughout the next day [12]. Flunitrazepam is lipophilic and is metabolized hepatically via oxidative pathways. The enzyme CYP3A4 is the main enzyme in its phase 1 metabolism in human liver microsomes [13]. The present histopathological results could be attributed to the toxic effect of the drug and its metabolites on the liver. Oxidative stress disrupts lipids, proteins and DNA, induces necrosis and apoptosis of hepatocytes and amplifies the inflammatory response and it stimulates the production of pro-fibrogenic mediators from Kupffer cells and circulating inflammatory cells resulting in the initiation of fibrosis [14]. Also Seqin, et al. [15] stated that chronic administration of diazepam caused an increase in malondialdehyde levels and a decrease in glutathione content and that diazepam markedly lowered Ca2+ATPase activity. Thus, increased lipid peroxidation together with alteration in Ca2+ -ATPase activity may play a role in diazepam induced hepatic injury [15]. The objective of this research was to investigate the histopathological effects of combined oral administration of Tramadol and Rohypnol on the liver and kidney of adult Wistar rats.

2. MATERIALS AND METHODS

2.1 Location of Study

This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State of Nigeria.

Table 1. Experimental layout

Groups	A (Control)	B (Tramadol)	C (Rohypnol)	D (Tram & Rohypnol)
Number of Animals	8	8	8	8
Male	4	4	4	4
Female	4	4	4	4

2.2 Substance of Study

The Tramadol, produced by Exus Pharmaceutical (Nig.) LTD with NAFDAC registration number: A4-9419 and Rohypnol tablets produced by CHEPLAPHARM Meaekenhagen, Germany with NAFDAC registration number: 04-0150 were used and administered per oral route twice daily for 3 weeks. These details were checked to ensure the authenticity of the products purchased.

2.3 Experimental Animals

Thirty two (32) adult Wister rats weighing 120 ± 20 g - 180±30 g comprising sixteen (16) males and sixteen (16) females were procured from the animal house of the Department of Pharmacology, College of Health Sciences Niger Delta University, Amassoma Bayelsa state and moved to the animal house of the department of Medical Laboratory Science Niger Delta University Amassoma, Bayelsa State, Nigeria, where they were housed under standard condition of temperature (27 ± 2ºc) with twelve hours light/dark periodicity in plastic cages. The rats were allowed to acclimatize for two weeks and were fed ad libitum during this period, with water and grower mesh feed. Animals where handled throughout study according to institutions guidelines for an experiment involving the use of laboratory animals.

2.4 Experimental Design

The animals were weighed and assigned into four (4) major groups with four male, 4 females animals in each group after the period of acclimatization: a control group "A" and three test groups (B, C and D).

2.5 Substance Administration

All the animal groups were fed with feed (growers mash) plus water given ad libitum. However, Group A (control) received distilled water and feed (grower mash) only, test Groups B: Received 7.2 mg dose of Tramadol. Group C: 0.72 mg dose of Rohypnol. Group D received a combined oral dose of 7.2 mg Tramadol and 0.72 mg Rohypnol. The route of administration was oral with the aid of orogastric tube. The substance administered/time duration and the groups and doses are below tabulated.

2.6 Sample Collection

At the end of three weeks of administration, the rats were sacrificed by administering chloroform as anesthesia. The rats were then dissected to harvest the liver and the testes which were then fixed immediately in 10% formalin.

2.7 Tissue Processing

The tissues were processed using automatic tissue processor according to standard histological processing schedule using rotary microtome (Heitz 150, Cambridge model) and stained with haematoxylin and eosin (H&E) staining technique at the histopathology laboratory department of the Niger Delta University Teaching Hospital (NDUTH), Okolobri.

2.8 Statistical Analysis

Analysis of variance (ANOVA) was used for all statistical calculations. Differences were considered probably significant at a P value of <0.05 and significant at a value of <0.01.

3. RESULTS

3.1 Microscopy and Photomicrography

Microscopy was done using an Olympus binocular light microscope at magnification x400 and the sections where then photomicrograph using a digital Samsung camera (Samsung 55850 model) attached to the microscope.

3.2 Histology Photomicrograph Plates

Plate 1 shows the liver sections stained with heamatoxylin and eosin x 400 magnification. Sections show normal slides with central vein (CV), portal vein (PV), blood vessels (PA), hepatocytes (HC), kuffer cell (KC), and sinusoids consistent with normal histology of the liver.

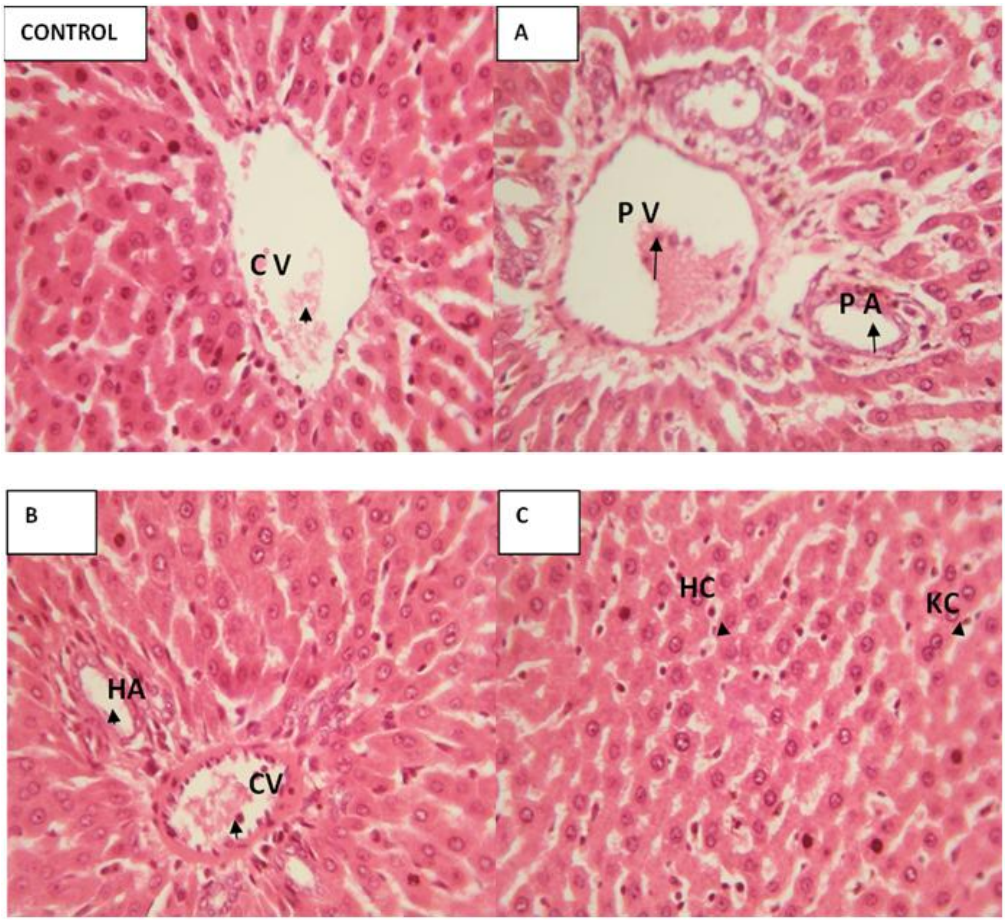

Plate 1. Female liver

Photomicrographs of the liver sections stained with haematoxylin and eosin x 400 magnification. Sections show normal slides with central vein (CV), portal vein (PV), blood vessels (PA), hepatocytes (HC) , kuffer cell (KC), and sinusoids consistent with normal histology of the liver

Remarks: Substance administered is hepatotoxic at the concentration administered

The substance administered is hepatotoxic at the concentration administered.

Plate 2 shows kidney sections stained with heamatoxylin and eosin. Sections show normal glomerulus (GN), with Bowman's capsule (BC). The tubules (RT) are all normal consistent with normal histology. The substance has no effect on the kidney at the concentration administered.

Plate 3 shows kidney sections stained with heamatoxylin and eosin. Sections show normal glomerulus (GN), with Bowman's capsule (BC). The tubules (RT) are all normal consistent with normal histology. The substance has no effect on the kidney at the concentration administered.

Plate 4 shows liver sections stained with haematoxylin and eosin. Control shows a liver with the normal central vein (CV) with radiating sinusoids (S). Group B and C show normal liver section displaying a portal vein (PV), artery (PA) and a central vein (CV) all consistent with normal histology. Group D shows moderate inflammatory cells and obliteration of the central vein.

4. DISCUSSION

The Plates labeled 1-4 shows the photomicrograph of the Liver and Kidney of the animals used in this study. The slide labeled Control represents animals in the control group (Group A) that were given normal feed. The slide labelled B represents animals in Group B that were administered with Tramadol. The slide labeled C represents animals in Group C that were given Rohypnol. The slide labeled D

represents animals in Group D that were administered with oral combination of Tramadol and Rohypnol.

Plate 1 shows the morphology of the Liver after administration of Tramadol and Rohypnol. The slide labelled 'Control' shows normal slides with central vein (CV), portal vein (PV), blood vessels (PA), hepatocytes (HC), kuffer cell (KC), and sinusoids consistent with normal histology of the liver. The slide labeled B and D shows certain changes in liver tissue. A study carried out by Heba, et al. [16] on the effects of acute and chronic tramadol drug toxicity revealed that histopathological examination of liver tissues displayed necrosis and cytolysis, and showed complete cell membrane degeneration in the adult male albino rats. These results could be explained by the fact that the liver is responsible for the metabolism and excretion of tramadol [17]. In the present study, the Tramadol administered was found to be hepatotoxic at the concentration administered. The slide labelled C also shows changes in the histology of the liver. This supports the study carried out by Chatterajee, et al. [18] who noticed pre-necrotic and necrotic changes in the liver. The study showed an increase in the infiltration of inflammatory cells inside the sinusoids. There were degeneration and necrosis of hepatocytic cells and congestion of the central vein.

Plates 2 and 3 show the morphology of the kidney after administration of Tramadol and Rohypnol. The slides show normal glomerulus (GN), with Bowman's capsule (BC). The tubules (RT) are all normal consistent with normal histology. The Slides labeled B, C and D showed normal kidney histology when compared with the

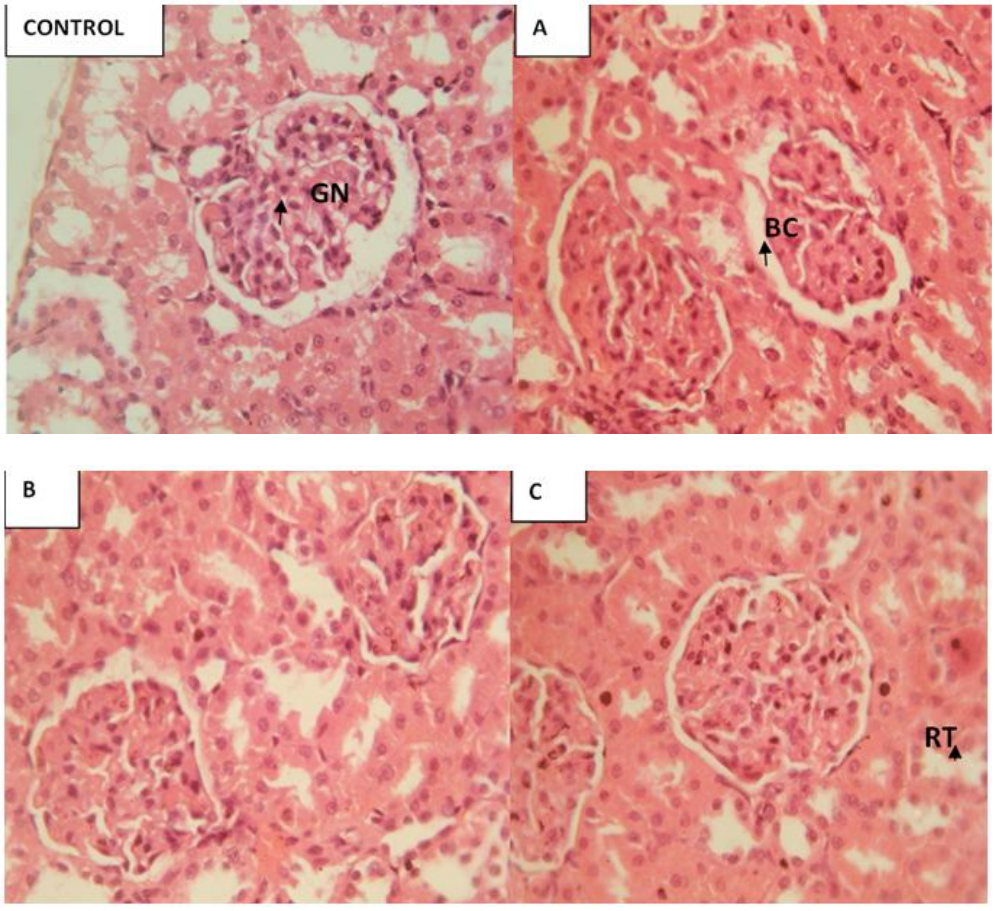

Plate 2. Female kidney

Photomicrograph of kidney sections stained with heamatoxylin and eosin. Sections show normal glomerulus (GN), with Bowman's capsule (BC). The tubules (RT) are all normal consistent with normal histology
Remarks: Substance has no effect on the kidney at the concentration administered

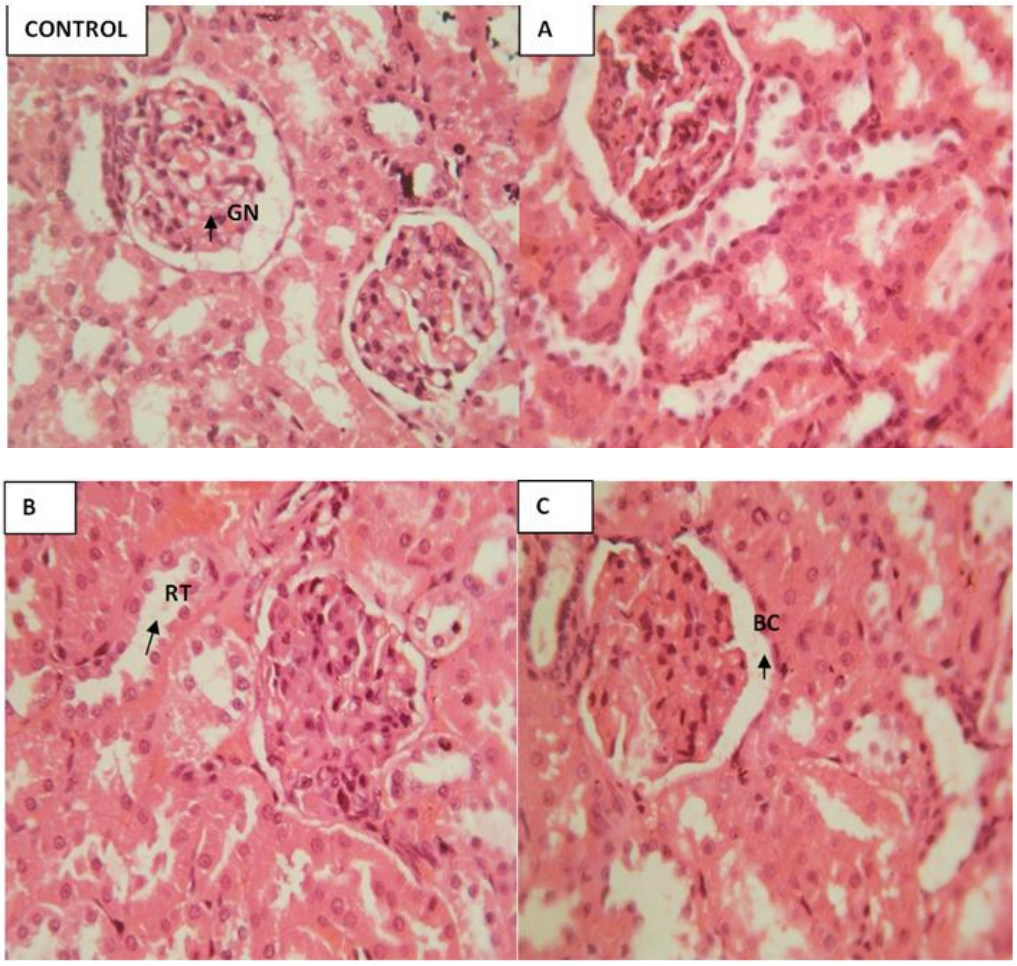

Plate 3. Male kidney

Photomicrograph of kidney sections stained with heamatoxylin and eosin. Sections show normal glomerulus (GN), with Bowman's capsule (BC). The tubules (RT) are all normal consistent with normal histology
Remarks: Substance has no effect on the kidney at the concentration administered

slide labeled Control. Tramadol and Rohypnol were shown to have no effect on the kidney at the concentration administ.

Plate 4 shows the morphology of the liver after administration of Tramadol and Rohypnol. The slide labelled Control shows a liver with the normal central vein (CV) with radiating sinusoids (S). Slides B and C show normal liver section displaying a portal vein (PV), artery (PA) and a central vein (CV) all consistent with normal histology. Slide D shows moderate inflammatory cells and obliteration of the central vein. This confirms that an oral combination of Tramadol and Rohypnol can lead to hepatotoxicity as both substances have been confirmed to cause hepatic injury. In a study carried out by Zuhtu Utku, et al. [19], the study demonstrated that the postoperative effects of morphine and tramadol on the histopathology of liver in rabbits which had hepatocyte degeneration include central vein dilatation and mononuclear cellular infiltration. Smith, et al. [14] carried out a similar study and the histopathological results obtained could be attributed to the toxic effect of Rohypnol and its metabolites on the liver. Oxidative stress disrupts lipids, proteins and DNA, induces necrosis and apoptosis of hepatocytes and amplifies the inflammatory response and it stimulates the production of pro-fibrogenic mediators from Kupffer cells and circulating inflammatory cells resulting in the initiation of fibrosis.

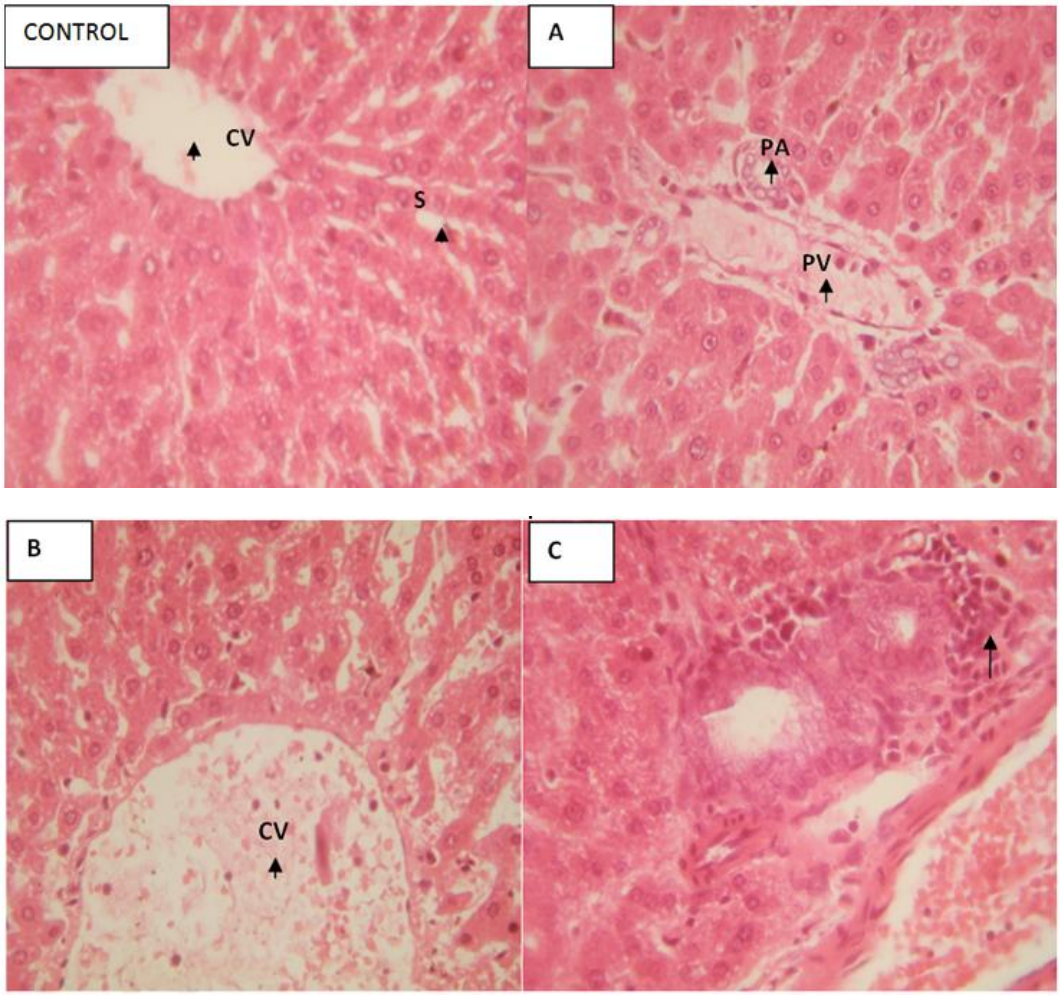

Plate 4. Male liver

Photomicrographs of liver sections stained with heamatoxylin and eosin. Control shows a liver with the normal central vein (CV) with radiating sinusoids (S). Group B and C shows normal liver section displaying a portal vein (PV), artery (PA) and a central vein (CV) all consistent with normal histology Group D shows moderate inflammatory cells and obliteration of the central vein

5. CONCLUSION

The present study conclude that oral combination of Tramadol and Rohypnol induces hepatoxicity and inflammation of the liver but produces no effect on the kidney at the dose and duration of administration.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethics committee approval has been taken and preserved by the author for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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