# EVALUATING CARDIOTOXICITY UPON CHRONIC ADMINISTRATION OF GRADED DOSES OF PENTAZOCINE IN WISTAR RATS

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## ABSTRACT

**BACKGROUND**: Common analgesics such as pentazocine are frequently used for the management and treatment of pain in patients suffering from sickle-cell disease in Nigeria. **AIM OF THE STUDY**: The current study aimed to investigate the toxicological effects of chronic use of pentazocine on the heart of Wistar rats. **METHODS**: Twenty-eight (28) female albino wistar rats (120 – 160g) were used for the study. They were grouped into four; control group (1ml normal saline) while experimental groups 2, 3 and 4 were given intramuscular administrations of 30 mg/kg, 60 mg/kg, and 90 mg/kg pentazocine (PZ), respectively for a period of 14 days. Heart samples were obtained and analyzed for biochemical parameters such as catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA). Histopathological analyses of cardiac tissues were also carried out and photomicrographs were obtained. A one-way analysis of variance (ANOVA) was used to analyze the differences between the groups using the Statistical Package for Social Sciences (SPSS) version 23.0. **RESULTS**: The activities of CAT, and SOD in the PZ-treated groups were decreased while that of MDA increased significantly in comparison with the control group ($p < 0.05$). Histopathologically, there was evidence of elevated mononucleated inflammatory cardiocytes, with increased depositions of fibrous tissues, and hemorrhages in the PZ-treated rat groups. **CONCLUSION**: Chronic pentazocine use could induce cardiotoxicity in pentazocine-treated rats.

**Keywords**: Pentazocine, cardiotoxicity, chronic administration.

## INTRODUCTION

Generally, analgesics help patients to recover from operations and medical procedures more quickly by relieving pain thereby mitigating its detrimental effects on their physical and mental health **(Garland, 2016; Hylands-White et al., 2017; El-Tallawy et al., 2021).** One opioid analgesic from the synthetic family that is frequently used to treat pain is pentazocine. Pentazocine functions as a mixed agonist-antagonist, showing antagonistic action at mu opioid receptors and agonistic activity at kappa opioid receptors. It binds to opioid receptors in the central nervous system to modify neurotransmission. It produces analgesia by preventing the release of excitatory neurotransmitters **(Van Niel et al., 2016; Gress et al., 2020)**.

Opioids are an essential component of pain treatment for sickle cell disease. They, however, may be linked to physical as well as psychological dependency among young people. Pentazocine is the most widely accessible opioid analgesic for moderate to severe pain of sickle cell disease management in Nigeria **(Adewoyin et al., 2019; Mba et al., 2024).** While this drug has proven efficacy in pain relief, its use is not without potential side effects. In particular, concerns have been raised regarding their toxicological effects on the heart of experimental animals.

After being administered either orally or intramuscularly, pentazocine is later absorbed in the muscle or gastrointestinal system before eventually reaching the circulation **(Devadasu et al., 2018).** Although the heart plays a key role in the circulation of pentazocine within the blood, the impact of its administration on cardiac tissue morphology and cardiac functioning is yet to be fully understood. When examining the toxicological effects of similar analgesics on the heart, animal models are essential. Because of their physiological similarities to humans and the availability of a variety of genetic and pharmacological methods, rodents like rats and mice are frequently employed **(Burma et al., 2017).**

With the use of these models, scientists may evaluate the short and long-term effects on cardiac morphology and physiology of related analgesics **(Lindsey et al., 2018; Regmi and Shah, 2020).** Assessments of electrocardiography, echocardiography, and hemodynamics are major examples of in-vivo procedures that offer a better understanding of the alterations in heart tissue architecture brought on by these drugs **(Santos et al., 2015; Wang et al., 2022)**.

In line with related literature, analgesic-induced cardiotoxicity have been shown to influence oxidative stress and programmed cell death (apoptosis). The chronic administration of these analgesics may either elevate or decrease markers of oxidative stress, such as reactive oxygen species (ROS), as well as apoptotic markers **(Gan and Karmazyn, 2018; Barbosa et al., 2021).** Furthermore, related studies on similar analgesics have performed histopathological examination of cardiac tissue samples to assess morphological changes such as signs of inflammation, fibrosis, necrosis, or other pathological alterations **(Barbosa et al., 2021).** However, this study aims to investigate the toxicological effects of chronic use of graded dosages of pentazocine on the heart tissues of Wistar rats.

## MATERIALS AND METHODS

### Drugs and Chemicals

Injections of pentazocine (PZ) (marketed as Pentabeta-30) were obtained from Dooka Pharmacy, Port-Harcourt. The drug was dissolved in normal saline (0.9% NaCl) and administered to the experimental animals (albino rats) orally. Normal saline served as a placebo drug.

### Animals and Animal Handling

This study involved an experimental design. In line with **Festing et al. (2006)**, the resource equation method based on the degrees of freedom (E) was used to determine the rat sample size for the study. The study made use of four (4) groups (k) with a total number (n) of seven rats per group. Therefore, E was calculated as; thus, $kn - k = (28 - 4) = 24$. Since the value of E is more than 20, it was considered sufficient to use seven (7) rats per group to determine the sample size. Upon approval, twenty-eight (28) female albino Wistar rats with a mass range of 100 – 140g served as experimental animal models. Then, they were nurtured in the Zoo-research Laboratory of Basic Medical Sciences, University of Port-Harcourt to be placed inside four individual wooden confines (seven animals each) at room temperature of about 25°C, with a relative humidity of 40 – 48% daily, and allowed to undergo acclimatization for 2 weeks before the start of the experiment. In addition to their normal diet, unlimited amounts of water to drink had to be provided for subjects. Experimental animal procedures and handling techniques will be in agreement with the directives of the Animal Use and Care Committee of the National Veterinary Research Institute, Vom, Nigeria, and ethical approval was gotten from the Research Ethics Committee of the University of Port Harcourt.

### Experimental Model and Drug Treatment

According to the study earlier reported by **Haot et al. (1964)**, the lethal dose that was capable of killing fifty percent (50%) of the laboratory rats was 175 mg/kg. Therefore, wistar rats were randomly grouped into four groups with one of the groups serving as the control group which were administered 1 ml of normal saline (0.9% NaCl) while groups 2, 3, and 4 were given intramuscular administrations of 30 mg/kg (0.1ml PZ), 60 mg/kg (0.25ml PZ) and 90 mg/kg (0.4ml PZ) pentazocine, respectively for 14 days.

The low dose was measured for the group 2 using the formulae of calculating dose volume from 30 mg/ml;

$$\text{Dosage (mg)} = \frac{\text{Average Body weight of animal (g)}}{1000\text{g}}$$

$$\text{x dose} = \frac{120\text{ g}}{1000\text{g}} \text{x } 30\text{mg} = 3.6\text{ mg}$$

If 30 mg of stock solution = 1 ml of dosage; therefore, 3.6 mg of stock solution will give a calculated volume of 3.6mg divided by 30 mg of stock solution which is approximately 0.1ml.

The medium dose was measured for the group 3 using the same formulae as shown above to calculate dose volume from 60 mg/ml to give 7.2 mg. If 30 mg of stock solution gives 1 ml of dosage, 7.2 mg of stock solution will give a calculated volume of approximately 0.25ml.

Finally, the high dose was measured for the group 4 using the above formulae of calculating dose volume from 90 mg/ml to give 10.8 mg. If 30 mg of stock solution gives 1 ml of dosage, 10.8 mg of stock solution will give an approximate volume of 0.4ml.

Daily, all chemicals were administered once between 11 am to 12 pm, and experimental rats were monitored for 3 hours

after administration. Upon the completion of a daily administration, the animals were given drinking water as usual; however normal dietary routine was lowered while as well supervised. On the final day of experiment after the animals have been administered the various doses, they were provided only drinking water and later carefully examined for the next 24 hours till they were all sacrificed the next day. During the sacrifice, the heart samples were surgically obtained and homogenized using an Ultra-Turrax homogenizer with 1:4 (m/v) of ice-cold 50 mM phosphate buffer for further biochemical analysis.

**Assessment of Biochemical Parameters**

Catalase (CAT) activity was analyzed by the **Sinha (1972)** method, superoxide dismutase (SOD) activity was assayed according to **Misra and Fridovich (1972),** while assessment of lipid peroxidation marker, malondialdehyde (MDA) through the technique of reactive constituents of malonylurea **(Buege and Aust, 1978).**

**Histopathological Analysis of the Heart**

Heart samples were removed from sacrificed rats in order to perform standard histopathological procedures. These samples were later preserved in 10% formaldehyde and hydrated using ethanol grades (75%, 90%, 95%, and 100%). The samples were then cleared in two changes of xylene after dehydration. After that, samples were embedded and blacked out after being impregnated with melted paraffin wax. Using a sled microtome, paraffin slices 5 µm thick were cut, mounted on glass slides, and stained using both hematoxylin and eosin in a stepwise manner; after which were analyzed, and slide images were contrasted. With the aid of an Accu-Scope 3000 digital microscope, photomicrographs were produced.

**Methods of Data Analysis**

This was carried out using the Statistical Package for Social Sciences (SPSS) version 23.0. Both descriptive and inferential statistical methods were employed in analyzing obtained data. To examine the differences between the groups, a one-way analysis of variance (ANOVA) was performed, followed by the utilization of the least significant difference (Post hoc) test. A significant p-value is less than 0.05, and a 95% confidence interval will be used.

## RESULTS

In Table 1, there were significant decreases in the activities of CAT (in low and medium PZ doses) and SOD (in low and high doses) in comparison with the control groups of CAT (1.27 ± 0.04) and (0.36 ± 0.04). However, there were significant increases in the activities of MDA (in low and medium doses) in comparison with the control group (0.45 ± 0.04).

Based on the histopathological examination as shown in Figure 1, the cardiac tissues of the low and medium-dose PZ-rat groups showed milder mononucleated inflammatory cardiocytes and deposition of fibrous tissues. However, the high-dose PZ-rat groups showed more manifestations of mononucleated inflammatory cardiocytes, increased deposition of fibrous tissues, and hemorrhages.

**Table (1):** Effects of pentazocine treatment on antioxidant enzyme activity and lipid peroxidation status in heart of rats.

| Treatment Groups | CAT (nmol/g) | SOD (nmol/g) | MDA (nmol/g) |
|---|---|---|---|
| | **Mean ± S.E.M** | **Mean ± S.E.M** | **Mean ± S.E.M** |
| **1mL saline (control)** | 1.27 ± 0.04 | 0.36 ± 0.04 | 0.45 ± 0.04 |
| **0.1mL PZ (low dosage)** | 0.97 ± 0.10[a] | 0.27 ± 0.03[a] | 0.52 ± 0.03[a] |
| **0.25mL PZ (medium dosage)** | 1.09 ± 0.11[a] | 0.32 ± 0.04 | 0.48 ± 0.04[a] |
| **0.4mL PZ (high dosage)** | 1.02 ± 0.10 | 0.30 ± 0.04[a] | 0.50 ± 0.03 |

**PZ** = Pentazocine, **CAT** = Catalase, **SOD** = Superoxide dismutase, **MDA** = Malondialdehyde.
**a** = denotes least significant difference compared with control group at $p < 0.05$

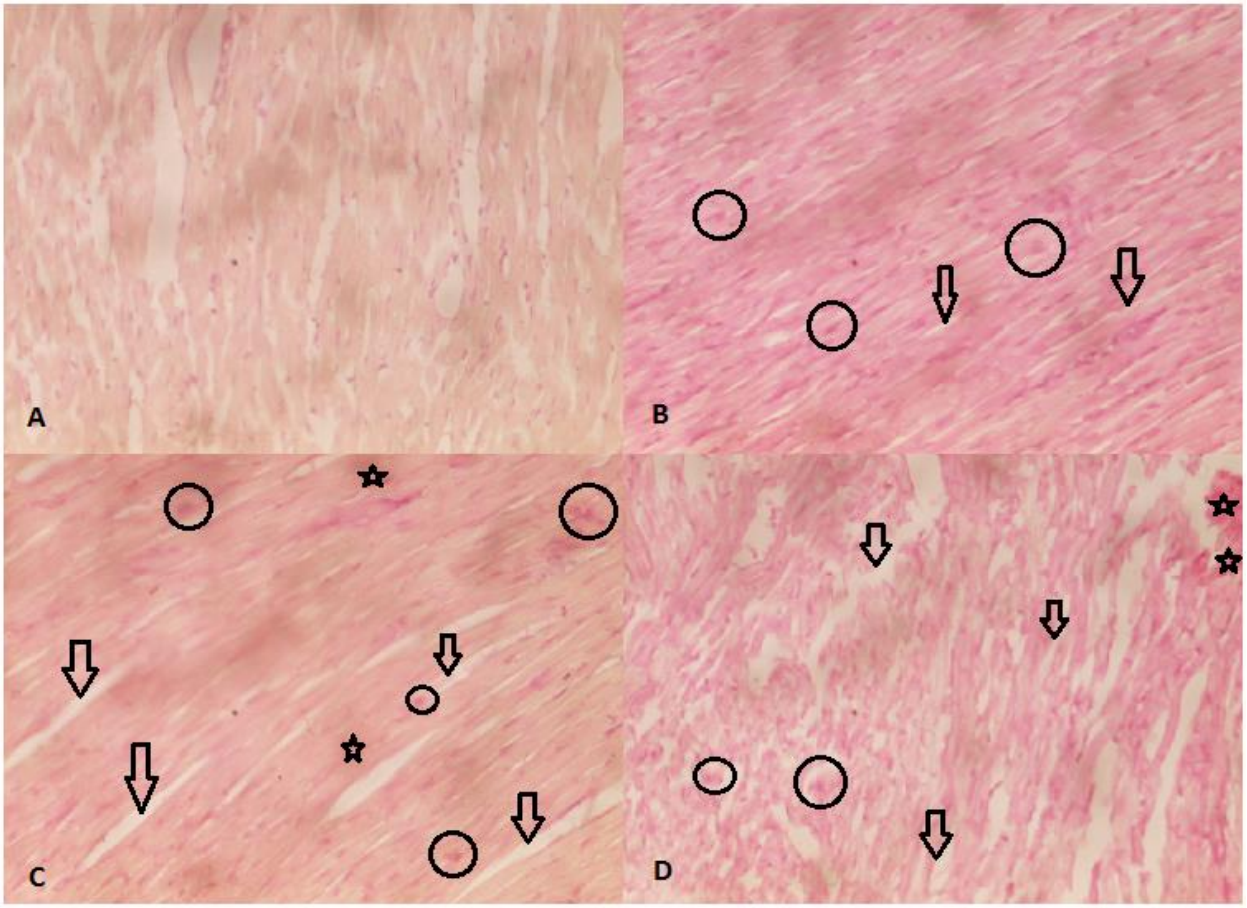


**Figure (1)**: Photomicrograph of heart tissues (using H&E at x100 magnification) showing the comparisons between control group (A) and the pentazocine (PZ) treated groups at doses of 0.1ml PZ (B), 0.25ml PZ (C), and 0.4ml PZ (D). **A** – normal histoarchitecture of cardiac tissue. **B** – mononucleated inflammatory cardiocytes (circled), and deposition of fibrous tissues (arrows). **C** – Presence of mononucleated inflammatory cardiocytes (circled), deposition of fibrous tissues (arrows), and haemorrhages (stars). **D** - Presence of mononucleated inflammatory cardiocytes (circled), increased deposition of fibrous tissues (arrows), and haemorrhages (stars).

## DISCUSSION

The present study was done to investigate the toxicological effects of the administration of graded doses of pentazocine on the heart of female wistar rats. As revealed in prior literature, the chronic administration of related analgesics could influence the concentrations of markers of oxidative stress, such as reactive oxygen species (ROS) **(Gan and Karmazyn, 2018; Barbosa et al., 2021).** This current study showed that there were significant decreases in the activities of CAT and SOD while there were increases in the activities of MDA in comparison with the control groups. According to a related experimental investigation, administering tramadol and tapentadol was found to cause oxidative impairment, specifically protein oxidation, in heart tissues **(Faria et al., 2017).** This was probably because of declined antioxidant activity as well as intensified free radicals generation **(Singh et al., 2010)**. Diclofenac is a widely used analgesic that is used to relieve inflammation and discomfort. Moreover, cardiotoxicity is a result of the generation of free radicals during the biochemical (metabolic) processing of these drugs **(Deavall et al., 2012)**.

In another related study, there were significant increases in malondialdehyde (MDA) as well as significant decreases in catalase (CAT) enzymatic activities in cardiac tissue in experimental rats that were treated with isoproterenol when compared to the control group **(Ojha et al., 2011)**. However, in contrast to this current study results, **Chaudhary et al. (2020)** reported significant increases in superoxide dismutase (SOD) activities. Increased MDA levels are an important indicator of reactive oxygen species (ROS) generation which might be associated

with oxidative tissue damage **(Priscilla and Prince, 2009).** Similarly, reductions in antioxidant enzyme activities such as SOD and CAT, and markers of lipid peroxidation markers are greatly associated with the indicators of heart failure **(Li et al., 2012).** Despite differences in CAT and MDA levels of high-dose PZ-rat groups in this present study, they were not statistically significant in comparison with the control group when compared to other experimental groups. This could be ascribed to differences in the metabolism of the drug doses and the production of reactive oxygen species in the rat groups.

Finally, the present study showed from its histopathological findings that the cardiac tissue architecture of the PZ-treated groups was impacted by the varying drug doses as it revealed the presence of mononucleated inflammatory cardiocytes, as well as increased deposition of fibrous tissues, and hemorrhages. Histopathological changes in cardiac muscle histoarchitecture are evidence of abnormal cardiovascular functions **(Chan et al., 2011).** A similar investigation found that administering naproxen at a modest dosage increases the cardiac damage caused by doxorubicin **(Pathan et al., 2010).** In a related study where the rats were treated with cyclophosphamide showed vacuolization of the cardiomyocytes, infiltration of inflammatory cells, myocardial tissue separation, and myofibril loss **(Bhatt et al., 2017).** Also, **Ahmed et al. (2018)** investigated the effects of the administration of celecoxib, an NSAID, on the cardiac tissues of Wistar rats and it revealed that the tissue architecture was predominantly made up of collagen depositions. **Barbosa et al. (2021)** reported from their study that there were indications of visibly scattered heart muscle cells, the disarray of fiber filament, heterogeneous coloration, striatal disappearance, and deposits of fibrous tissue. A previous study reported by **Faria et al. (2017),** found that the results upon examination of cardiac tissues when subjected to high amounts of tramadol and tapentadol included visibly scattered heart muscle cells, cellular vacuolations, and striatal disappearance in these cells – which is similar to the findings of this present study.

## CONCLUSION

Conclusively, chronic pentazocine (PZ) administration was shown to induce cardiotoxicity in the PZ-treated rat groups. Rat groups administered with low and medium doses of PZ were shown to be associated with significant changes in anti-oxidative activities as well as pathological changes in cardiac tissue histology. Hence, there is a need for caution on the use of pentazocine for the management and treatment of pain-related health issues to prevent possible cardiovascular-related problems.

## RECOMMENDATION

A limitation of the present study was that the rats selected for this study were only females. Therefore, it is recommended that further, comprehensive investigations of the comparisons of the effects of pentazocine administration in the hearts of between male and female rats should be considered due to differences in hormonal metabolic activities.

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Nil

## CONFLICTS OF INTEREST

There exists no form of conflicting interest among authors.

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