**Sub-acute Pentazocine-Induced Toxicity: Biochemical and Histopathological Study of Rat Intestinal Tissues**

**ABSTRACT**

**Background**: Pentazocine addiction in Nigeria has emerged as a health issue among young people dealing with various types of pain, despite the drug's widespread use among sickle-cell sufferers. **Objective**: This study aimed to evaluate the toxicological effects of pentazocine on the intestines. **Methods**: Twenty-eight (28) adult Wistar rats were acclimatized and grouped into four. Group 1 (control) was administered 1ml of normal saline (0.9% NaCl) while groups 2 – 4 served as the experimental groups and administered graded doses of 30 mg/kg, 60 mg/kg, and 90 mg/kg pentazocine (PZ) intramuscularly for 14 days. Both intestines were obtained and analyzed for biochemical parameters such as catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA), as well as histopathological analyses of sample tissues. 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**: In the intestines, there were significant increases in CAT and SOD enzymes, as well as substantial decreases in MDA levels in PZ-treated groups. Intestinal histopathology of PZ-treated groups displayed notable glandular distortions, and infiltration of inflammatory cells within the mucosa epithelia. **Conclusion**: Sub-acute pentazocine administration was shown to cause moderate toxicity and could lead to associated gut-brain inflammatory responses.

**Keywords**: Pentazocine, Toxicity, Intestines, Sub-acute

**INTRODUCTION**

Medical practitioners usually prescribe a class of drugs known as analgesics to treat and reduce different forms of pain (Sarzi-Puttini et al., 2012; Manchikanti et al., 2017). Through the activation of certain receptors in the central nervous system, these chemicals change how pain is perceived and start the healing process (Uniyal et al., 2020). Although, opioid analgesics aid in relieving pain by acting majorly in the nervous system, their peripheral action at the site of inflammation is crucial in some conditions like inflammatory bowel disease (IBD) (Philippe et al., 2006; Niccum et al., 2021; Thomas et al., 2022). They exert these actions by binding to their unique receptors usually located on sensory nerve endings in the intestine that in turn, reduces the perception of pain by inhibiting the release of pro-inflammatory neurotransmitters (Sobczak et al., 2014). Already, certain opioids have been shown to increase the permeability of the intestinal barrier, thereby allowing substances to pass through that would normally be restricted, thereby causing a “leaky gut” (Rueda-Ruzafa et al., 2020; Thomas et al., 2022).

Pentazocine is a mixed agonist-antagonist opioid analgesic primarily used mostly among patients suffering from sickle-cell disease (SCD) for pain relief. It works as an agonist by binding to μ-opioid receptors, while also acting as an antagonist at by binding with κ-opioid receptors to cause an analgesic effect ((Mori et al., 2015; Cruz and Granados-Soto, 2022).). The regular abuse of common opioids is closely associated with various side effects that impact the gastrointestinal systems such as constipation, reduced gut motility, and potential mucosal damage and these can cause gastrointestinal toxicity resulting in inflammation, ulceration, and damage to the mucosal lining of the intestines. (Ghosh et al., 2022; Jalodia et al., 2022). Furthermore, opioid-induced inhibition of gastrointestinal motility caused by the activation of μ-opioid receptors could present certain complications in gut health (Sobczak et al., 2014; Rueda-Ruzafa et al., 2020).

Despite some literature that has been reported on the toxic effects of pentazocine in selected organ systems such as cardiovascular and nervous systems (Hart and Adheke, 2024 & 2025), there remains the knowledge gap regarding the specific impacts of pentazocine on intestinal health. However, previously reported literature has clearly shown that both long- and short-term uses of certain analgesics such as non-steroid anti-inflammatory drugs (NSAIDS) can develop inflammatory processes, stenosis, and ulcers (non- or perforated) in the small intestine or colon (Gonçalves Junior et al., 2012; Boelsterli et al., 2013; Bjarnason et al., 2018). The purpose of this study was to examine both the biochemical and histological effects of pentazocine administration on the intestines of Wistar rats. This study is highly relevant as it broadens the scope of the pathophysiology of gut-brain axis by providing a novel understanding of the mechanism of action of pentazocine on the sensory nerve endings of the intestine.

**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) intramuscularly. Normal saline served as a placebo drug.

**Animals and Animal Handling**

The current study used a randomized controlled trial (RCT) research methodology. The experimental rat sample size was determined by using the resource equation technique, utilizing degrees of freedom (E) as specified in the procedures defined by Festing et al. (22). The study included four groups, each consisting of seven rats, denoted as “k”. Hence, the mathematical representation for E was derived as kn-k = (28-4) = 24. Given that the value of E is more than 20, it was considered sufficient to use seven (7) rats per group to determine the ideal sample size. After receiving authorization, a cohort of twenty-eight (28) albino Wistar rats, with weights ranging from 100 to 140g, were used as experimental animal models. The animals were then accommodated at the Zoo-research Laboratory of Basic Medical Sciences, situated at the University of Port-Harcourt. All the animals were separately housed in four wooden cages, each particularly built to fit seven creatures. In line with the regulations for the protection of animals used in scientific procedures in the Directive 2010/63/EU, proper handling of cages was done and the conditions under which animals are kept were favorable throughout the research process. Cages should be designed to provide adequate space, comfort, and enrichment for the animals. To maintain thermal equilibrium, the temperature of the surroundings was consistently regulated at roughly 25°C, while the daily relative humidity fluctuated between 40% and 48%. Trained personnel were responsible for the handling of animals and the maintenance of their cages. The animals had 2 weeks of physiological adaption before the trial began. Furthermore, apart from their usual meals, they were mandated to be provided with unlimited amounts of water for consumption. It is essential that the management techniques and procedures for experimental animals strictly comply with the criteria established by the Animal Use and Care Committee of the National Veterinary Research Institute in Vom, Nigeria. Authorization has been granted by the University of Port Harcourt's Research Ethics Committee, under reference number UPH/CEREMAD/REC/MM95/023.

**Experimental Model and Drug Treatment**

In a prior study conducted by Hart and Adheke (2024), it was determined that the terminal dose necessary to induce mortality in fifty percent (50%) of the laboratory rats was 175 mg/kg. Therefore, Wistar rats were randomly allocated to four groups. One cohort was assigned as the control group and administered a 1 ml dosage of a normal saline solution containing 0.9% sodium chloride concentrate. Over 14 days, pentazocine was delivered intramuscularly to treatment groups 2, 3, and 4 at dosages of 30 mg/kg (0.1ml PZ), 60 mg/kg (0.25ml PZ), and 90 mg/kg (0.4ml PZ) accordingly.

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

Dosage (mg) = x dose = = 3.6 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 0.1 ml.

The medium dose was measured for group 3 using the same formulae as shown above to calculate the 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 0.25 ml.

Finally, the high dose was measured for 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 0.4 ml.

All treatments were administered once daily between 11 am and 12 pm, and the experimental rats were monitored for 3 hours after the drug delivery. After the regular daily meal, the animals were furnished with their customary access to drinkable water. Nevertheless, their usual dietary routine was decreased and carefully supervised. Upon completion of the experiment, after the animals had been given each dose, they were only provided with water for drinking and thereafter subjected to rigorous monitoring over the next 24 hours until they were all executed the next day. Following the sacrificial process, surgical acquisition of neurological and intestinal tissue samples was performed. These samples were then homogenized using an Ultra-Turrax homogenizer with a cold 50 mM phosphate buffer in a 1:4 (m/v) ratio for further biochemical analysis.

**Assessment of Oxidative Stress Parameters of the Intestines**

The Sinha (23) method was used to quantify the activity of catalase (CAT). Here, the tissues of were obtained and homogenized in phosphate buffer (at pH 7.0) to create a tissue homogenate of about 1.0 mg protein/ml. The homogenate was then centrifuged to remove debris to obtain a clear supernatant. Later, the Bradford assay was used to determine the protein concentration in the supernatant. A dichromate/acetic acid reagent was prepared by mixing a 5% solution of potassium dichromate (K₂Cr₂O₇) with glacial acetic acid in the ratio of 1:3 (by volume). Then, a 0.2 M hydrogen peroxide (H₂O₂) solution was also prepared. Inside small test tubes, we add a specific volume of the tissue supernatant to a series of tubes containing varying amounts of hydrogen peroxide. The reaction was monitored for about 2 minutes at room temperature. Subsequently, the reaction was stopped by adding the dichromate/acetic acid reagent to the mixture and heated for some time. Finally, the absorbance of the resulting chromic acetate was measured at a wavelength of between 570-610 nm using a spectrophotometer. Then, the catalase activity (K) was determined based on the decrease in hydrogen peroxide concentration.

The Misra and Fridovich (24) method was used to compute the activity of superoxide dismutase (SOD). The tissues were homogenized in phosphate buffer to extract the superoxide dismutase. The homogenate was then centrifuged to remove debris, and the supernatant was collected for analysis. The protein concentration of the tissue extract was determined using the Bradford assay method. Later, the SOD activity was assessed by measuring its ability to inhibit the autoxidation of epinephrine. Here, a specific concentration of epinephrine is prepared in a buffered solution (e.g., Tris or sodium carbonate buffer). The tissue extract containing the SOD is added to the epinephrine solution. The reaction was monitored spectrophotometrically by measuring the increase in absorbance at 480 nm. The rate of increase in absorbance was recorded, and the percentage of inhibition of epinephrine oxidation is calculated. The results were plotted to determine the concentration of SOD.

The methodology of reactive components of thiobarbituric acid (TBA) (25) was employed to evaluate the lipid peroxidation marker, malondialdehyde (MDA). Here, the tissues were first homogenized in phosphate buffer to create a tissue extract to help release lipids and proteins from the tissue cells to produce a homogenate. The homogenate was then subjected to a solvent extraction process using a mixture of chloroform and methanol to separate the lipids from proteins and other cellular components. Later, the solution is allowed to settle, and the lower organic phase (containing the lipids) was collected. The protein concentration in the remaining aqueous phase can be quantified using the Bradford assay. The TBA reagent was prepared and combined with the lipid extract. The mixture is then heated using a boiling water bath for about 2 minutes. After heating, the solution was cooled and centrifuged to remove any precipitate. Later, the absorbance of the supernatant was measured at 535 nm using a spectrophotometer. The concentration of MDA was calculated using a standard curve generated from known concentrations of MDA.

**Histopathological Analysis of the Brain and Intestines**

To conduct standard histopathology procedures, intestinal samples were obtained from sacrificed rats. After being preserved in a 10% formaldehyde solution, the samples were hydrated using ethanol at different percentages, namely 75%, 90%, 95%, and 100%. After the dehydration process, the materials underwent further purification by two cycles of xylene extraction processing. The samples were then submerged and made translucent by being covered with molten paraffin wax while being subjected to pressure. Divisible paraffin slices, each measuring 5 µm in thickness, were generated using a sled microtome. Moreover, the slices were positioned on glass slides and subjected to a sequential staining procedure using hematoxylin and eosin-colored dyes. Following an exhaustive examination of the stained slides, their images were then compared. Utilizing an Accu-Scope 3000 digital microscope, photomicrographs were produced.

**Methods of Data Analysis**

This study was conducted using Version 23.0 of the Statistical Package for Social Sciences (SPSS). An exhaustive examination of the obtained data was carried out using both descriptive and inferential statistical methods. Next, the comparisons between the groups were evaluated using the least significant difference (Post hoc) test after doing a one-way analysis of variance (ANOVA). Statistical significance is shown by a p-value below 0.05, and a 95% confidence range will be used.

**RESULTS**

**Changes in oxidative stress parameters of the small intestine**

The findings presented in Table 2 demonstrate significant differences in the antioxidant enzyme activities of CAT and a little increase in SOD levels across all experimental groups in comparison to the control groups. With mean CAT levels of 0.96 nmol/mg in the control group, the experimental groups in this study, which were administered PZ at low, medium, and high dosages, had mean CAT levels of 1.19, 0.91, and 0.81 nmol/mg, respectively. In addition, the control group averaged 0.16 nmol/mg of SOD levels, whereas the experimental groups, administered PZ at low, medium, and high dosages, produced mean SOD levels of 0.16, 0.21, and 0.26 nmol/mg, respectively.

Also, all experimental groups showed significant reductions in the lipid peroxidation activities of MDA as compared to the control group. Compared to the mean MDA levels of 0.60 nmol/mg in the control group, the experimental groups that received low, medium, and high doses of PZ had mean MDA values of 0.59, 0.56, and 0.49 nmol/mg, respectively.

**Changes in oxidative stress parameters of the large intestine**

By comparison to the control groups, all experimental groups demonstrated significant enhancements in the antioxidant enzyme activities of CAT and considerably elevated levels of SOD, as seen in Table 3. The experimental groups in this study, which were administered PZ at low, medium, and high dosages, had average CAT levels of 1.33, 1.56, and 0.93 nmol/mg, respectively, compared to the control group's average CAT level of 0.86 nmol/mg. A mean SOD level of 0.38 nmol/mg was observed in the control group, whereas the experimental groups, which received PZ at low, moderate, and high doses, had mean SOD values of 0.45, 0.43, and 0.49 nmol/mg, respectively.

Importantly, all experimental groups showed substantial reductions in the lipid peroxidation activities of MDA as compared to the control group. The mean MDA level in the control group was 0.41 nmol/mg, while that of the experimental groups that were treated with low, medium, and high doses of PZ in this study had mean MDA levels of 0.37, 0.37, and 0.26 nmol/mg, respectively.

**Table 1. Effects of pentazocine treatment on antioxidant enzyme activity and lipid peroxidation status in small intestine of rats**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment Groups** | **CAT (nmol/mg)** | | **SOD (nmol/mg)** | | **MDA (nmol/mg)** | |
| **Mean ± S.E.M** | **Mean ± S.E.M** | | **Mean ± S.E.M** | |
| 1mL saline (control) | 0.96 ± 0.20 | 0.16 ± 0.04 | | 0.60 ± 0.03 | |
| 0.1mL PZ (low dosage) | 1.19 ± 0.18 | 0.16 ± 0.02 | | 0.59 ± 0.02**a** | |
| 0.25mL PZ (medium dosage) | 0.91 ± 0.19**a** | 0.21 ± 0.05**a,b** | | 0.56 ± 0.04**a** | |
| 0.4mL PZ (high dosage) | 0.81 ± 0.23**a** | 0.26 ± 0.07**a,b,c** | | 0.49 ± 0.06**a,b** | |

**a** = indicates the least significant difference in comparison with the control group at p < 0.05

**b** = indicates the least significant difference in comparison with 0.1mL PZ group at p < 0.05

**c** = indicates the least significant difference in comparison with 0.25mL PZ group at p < 0.05

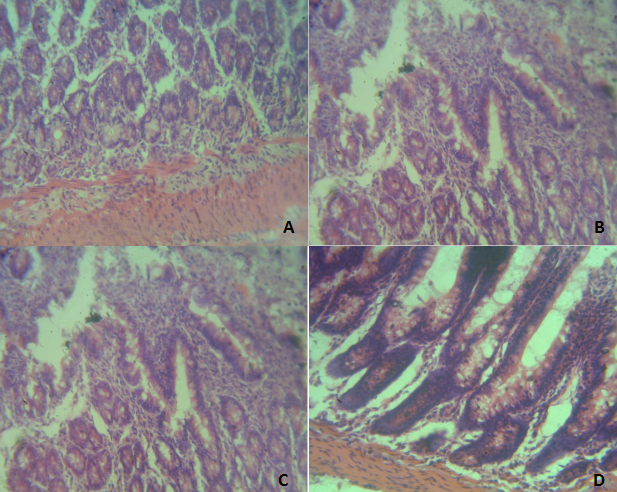
**Table 2. Effects of pentazocine treatment on antioxidant enzyme activity and lipid peroxidation status in large intestine of rats**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment Groups** | **CAT (nmol/mg)** | | **SOD (nmol/mg)** | | **MDA (nmol/mg)** | |
| **Mean ± S.E.M** | **Mean ± S.E.M** | | **Mean ± S.E.M** | |
| 1mL saline (control) | 0.86 ± 0.13 | 0.38 ± 0.06 | | 0.41 ± 0.05 | |
| 0.1mL PZ (low dosage) | 1.33 ± 0.35**a** | 0.45 ± 0.01**a** | | 0.37 ± 0.01**a** | |
| 0.25mL PZ (medium dosage) | 1.56 ± 0.19**a** | 0.43 ± 0.03**a** | | 0.37 ± 0.04**a** | |
| 0.4mL PZ (high dosage) | 0.93 ± 0.21 | 0.49 ± 0.01**a,b** | | 0.26 ± 0.01**a,b,c** | |

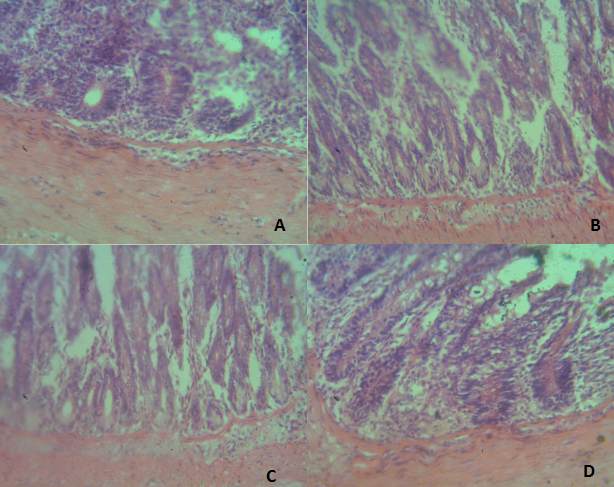
**a** = indicates the least significant difference in comparison with the control group at p < 0.05

**b** = indicates the least significant difference in comparison with 0.1mL PZ group at p < 0.05

**c** = indicates the least significant difference in comparison with 0.25mL PZ group at p < 0.05

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**Figure 1.** Photomicrograph of control (A) and experimental small intestinal groups (B, C, D). Signs shown in B, C, and D include distortions, inflammatory cell infiltrations within the mucosa epithelia, and intervillous spaces of the mucosa wall.

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**Figure 2.** Photomicrograph of control (A) and experimental large intestinal groups (B, C, D). Signs shown in B, C, and D include mild distortions and inflammatory cell infiltrations within the mucosa epithelia.

**DISCUSSION**

Apart from serving as crucial physical and immunological barriers, the gastrointestinal tract is the primary organ accountable for the functions of food digestion, absorption, and metabolism (Kim & Pritts, 2017). The gastrointestinal tract is well acknowledged as a substantial storage site for reactive oxygen species (ROS) because of its inevitable exposure to industrial toxins and microbial diseases (Anik et al., 2022). The results of this study demonstrate significant disparities in the efficacy of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) in the small intestines of the experimental groups in comparison to the control group. Considerable variation in CAT levels was seen across the experimental groups, with the group given a low dose of PZ displaying the highest mean level. Relative to the control group, all experimental groups exhibited slight increases in the levels of superoxide dismutase (SOD). Furthermore, building upon a previous study carried out by Miyazono et al. (2004), the levels of antioxidant enzymes, such as catalase and SOD, in the intestinal mucosa were examined and demonstrated to have significantly increased in the rats administered methotrexate compared to the control rats. In response to heightened levels of reactive oxygen species (ROS), cells typically enhance the synthesis and efficiency of superoxide dismutase (SOD) and catalase as a defense mechanism to mitigate oxidative harm (Birben et al., 2012; Schieber & Chandel, 2014).

In addition, the current study demonstrated significant reductions in lipid peroxidation activity, as measured by malondialdehyde (MDA) levels, in all experimental groups as compared to the control group. The chemical malondialdehyde (MDA) is a byproduct of lipid peroxidation and is often used as a marker to assess the level of oxidative stress. An observed decrease in lipid peroxidation activity suggests a corresponding decrease in the generation of reactive oxygen species and the manifestation of oxidative stress (Jadoon & Malik, 2017). Furthermore, a decrease in MDA levels might disrupt these cycles of redox signaling, leading to alterations in cellular reactivity and redox equilibrium (Sadiq, 2023).

The present study also evaluated oxidative stress markers in the large intestine which showed significant increases in the concentrations of the CAT and SOD in all experimental groups compared to the control group. Also, the study findings revealed substantial decreases in lipid peroxidation activity, measured by malondialdehyde (MDA) levels, in all experimental groups in comparison to the control group. The inhibition of lipid peroxidation may have compromised the ability of the intestinal barrier to counteract the action of PZ by enhancing their permeability and facilitating their penetration into the intestinal mucosa (John et al., 2011; Almousa et al., 2018; Wang et al., 2020). Furthermore, the products of lipid peroxidation can control the immunological activity of the gastrointestinal system. Suppression of lipid peroxidation might disrupt the balance of the intestinal immune system and result in uncontrolled inflammatory responses (Iddir et al., 2020). Alterations in the redox balance and oxidative stress resulting from dysbiosis induced by variations in lipid peroxidation and antioxidative activities may impact the composition and function of the gut microbiota (Kunst et al., 2023).

The present study showed that the groups of rats exposed to different dosages of pentazocine had notable alterations in the structural integrity of their intestinal tissues. The histopathological findings consisted of the distortion of the intestinal glands and goblet cells in the lamina propria, along with the invasion of inflammatory cells into the mucosal epithelium and the existence of puncture holes in the mucosal wall. A concomitant study on the toxicity caused by diclofenac in rats that received treatment showed evidence of tissue necrosis and inflammation of the small intestine lamina propria (Simon et al., 2019). A previous study demonstrated that rats subjected to meloxicam had signs of intestinal histopathologies, such as mucosal and Villar muscle atrophy, necrosis, and desquamation of the epithelial lining, especially at the villar termini. Furthermore, the intestinal glands exhibited localized cellular congestion (Abd El-Mawla & Osman, 2011).

**CONCLUSION**

Rat groups exposed to sub-acute pentazocine (PZ) displayed a moderate level of toxicity. The administration of different dosages of PZ was linked to pathological anomalies in the histology of the intestines as well as noticeable changes in anti-oxidative activity. A study limitation was that systemic inflammatory markers, including cytokines, were not measured in the blood to check for inflammation in organ tissue. Therefore, it is advised that more thorough research be done on the effects of PZ dosing on systemic inflammation. Additionally, pharmaceutical and medical facilities in the area must issue warnings regarding the use of pentazocine for the management and treatment of clinical pain problems.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**ETHICAL APPROVAL**

It is essential that the management techniques and procedures for experimental animals strictly comply with the criteria established by the Animal Use and Care Committee of the National Veterinary Research Institute in Vom, Nigeria. Authorization has been granted by the University of Port Harcourt's Research Ethics Committee, under reference number UPH/CEREMAD/REC/MM95/023.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

Abd El-Mawla, A. M., & Osman, H. E. H. (2011). Effects of Gum acacia aqueous extract on the histology of the intestine and enzymes of both the intestine and the pancreas of albino rats treated with Meloxicam. *Pharmacognosy research*, *3*(2), 114.

Almousa, A. A., Meurens, F., Krol, E. S., & Alcorn, J. (2018). Linoorbitides and enterolactone mitigate inflammation-induced oxidative stress and loss of intestinal epithelial barrier integrity. *International Immunopharmacology*, *64*, 42-51.

Anik, M. I., Mahmud, N., Masud, A. A., Khan, M. I., Islam, M. N., Uddin, S., & Hossain, M. K. (2022). Role of reactive oxygen species in aging and age-related diseases: A review. *ACS applied bio materials*, *5*(9), 4028-4054.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World allergy organization journal*, *5*, 9-19.

Bjarnason, I., Scarpignato, C., Holmgren, E., Olszewski, M., Rainsford, K. D., & Lanas, A. (2018). Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. *Gastroenterology*, *154*(3), 500-514.

Boelsterli, U. A., Redinbo, M. R., & Saitta, K. S. (2013). Multiple NSAID-induced hits injure the small intestine: underlying mechanisms and novel strategies. *Toxicological sciences*, *131*(2), 654-667.

Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In *Methods in enzymology* (Vol. 52, pp. 302-310). Academic press.

Cruz, S. L., & Granados-Soto, V. (2022). Opioids and opiates: pharmacology, abuse, and addiction. In *Neuroscience in the 21st Century: From Basic to Clinical* (pp. 4045-4082). Cham: Springer International Publishing.

Festing, M. F. (2006). Design and statistical methods in studies using animal models of development. *Ilar Journal*, *47*(1), 5-14.

Ghosh, N., Kesh, K., Ramakrishnan, S., & Roy, S. (2022). Opioid use in murine model results in severe gastric pathology that may be attenuated by proton pump inhibition. *The American journal of pathology*, *192*(8), 1136-1150.

Gonçalves Junior, I., Naresse, L. E., Rodrigues, M. A. M., & Kobayasi, S. (2012). Diclofenac sodium and Imipenem action on rat intestinal mucosa: a biomechanical and histological study. *Acta Cirúrgica Brasileira*, *27*, 131-136.

Hart, J. S., & Adheke, O. M. (2024). Evaluating cardiotoxicity upon chronic administration of graded doses of pentazocine in wistar rats. *The Egyptian Journal of Forensic Sciences and Applied Toxicology*, *24*(2), 73-78.

Hart, J. S., & Adheke, O. M. Histopathological and Biochemical Investigations of the Brain upon Sub-Acute Pentazocine Administration. *Asian Journal of Advanced Research and Reports*. 19(2), 150 – 158.

Iddir, M., Brito, A., Dingeo, G., Fernandez Del Campo, S. S., Samouda, H., La Frano, M. R., & Bohn, T. (2020). Strengthening the immune system and reducing inflammation and oxidative stress through diet and nutrition: considerations during the COVID-19 crisis. *Nutrients*, *12*(6), 1562.

Jadoon, S., & Malik, A. (2017). A review article on the formation, mechanism and biochemistry of MDA and MDA as a biomarker of oxidative stress. *Int. J. Adv. Res*, *5*, 811-818.

Jalodia, R., Kolli, U., Braniff, R. G., Tao, J., Abu, Y. F., Chupikova, I., & Roy, S. (2022). Morphine mediated neutrophil infiltration in intestinal tissue play essential role in histological damage and microbial dysbiosis. *Gut Microbes*, *14*(1), 2143225.

John, L. J., Fromm, M., & Schulzke, J. D. (2011). Epithelial barriers in intestinal inflammation. *Antioxidants & redox signaling*, *15*(5), 1255-1270.

Kim, Y., & Pritts, T. A. (2017). The gastrointestinal tract. *Geriatric trauma and critical care*, 35-43.

Kunst, C., Schmid, S., Michalski, M., Tümen, D., Buttenschön, J., Müller, M., & Gülow, K. (2023). The influence of gut microbiota on oxidative stress and the immune system. *Biomedicines*, *11*(5), 1388.

Manchikanti, L., Kaye, A. M., Knezevic, N. N., McAnally, H., Trescot, A. M., Blank, S., & Helm, S. (2017). Responsible, safe, and effective prescription of opioids for chronic non-cancer pain: American Society of Interventional Pain Physicians (ASIPP) guidelines. *Pain physician*, *20*(2), S3.

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*, *247*(10), 3170-3175.

Miyazono, Y., Gao, F., & Horie, T. (2004). Oxidative stress contributes to methotrexate-induced small intestinal toxicity in rats. *Scandinavian journal of gastroenterology*, *39*(11), 1119-1127.

Mori, T., Itoh, T., Yoshizawa, K., Ise, Y., Mizuo, K., Saeki, T., & Suzuki, T. (2015). Involvement of μ‐and δ‐opioid receptor function in the rewarding effect of (±)‐pentazocine. *Addiction Biology*, 20(4), 724-732.

Niccum, B., Moninuola, O., Miller, K., & Khalili, H. (2021). Opioid use among patients with inflammatory bowel disease: a systematic review and meta-analysis. *Clinical Gastroenterology and Hepatology*, *19*(5), 895-907.

Philippe, D., Chakass, D., Thuru, X., Zerbib, P., Tsicopoulos, A., Geboes, K., & Chamaillard, M. (2006). Mu opioid receptor expression is increased in inflammatory bowel diseases: implications for homeostatic intestinal inflammation. *Gut*, *55*(6), 815-823.

Rueda-Ruzafa, L., Cruz, F., Cardona, D., Hone, A. J., Molina-Torres, G., Sánchez-Labraca, N., & Roman, P. (2020). Opioid system influences gut-brain axis: Dysbiosis and related alterations. *Pharmacological Research*, *159*, 104928.

Sadiq, I. Z. (2023). Free radicals and oxidative stress: Signaling mechanisms, redox basis for human diseases, and cell cycle regulation. *Current molecular medicine*, *23*(1), 13-35.

Sarzi-Puttini, P., Vellucci, R., Zuccaro, S. M., Cherubino, P., Labianca, R., & Fornasari, D. (2012). The appropriate treatment of chronic pain. *Clinical drug investigation*, *32*, 21-33.

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current biology*, *24*(10), R453-R462.

Simon, J. P., Parthasarathy, M., Nithyanandham, S., Katturaja, R., Namachivayam, A., & Prince, S. E. (2019). Protective effect of the ethanolic and methanolic leaf extracts of Madhuca longifolia against diclofenac-induced toxicity in female Wistar albino rats. *Pharmacological Reports*, *71*(6), 983-993.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical biochemistry*, *47*(2), 389-394.

Sobczak, M., Sałaga, M., Storr, M. A., & Fichna, J. (2014). Physiology, signaling, and pharmacology of opioid receptors and their ligands in the gastrointestinal tract: current concepts and future perspectives. *Journal of gastroenterology*, *49*, 24-45.

Thomas, K. R., Watt, J., Wu, C. M. J., Akinrinoye, A., Amjad, S., Colvin, L., & Forget, P. (2022). Pain and opioid-induced gut microbial dysbiosis. *Biomedicines*, *10*(8), 1815.

Uniyal, A., Gadepalli, A., Akhilesh, & Tiwari, V. (2020). Underpinning the neurobiological intricacies associated with opioid tolerance. *ACS chemical neuroscience*, *11*(6), 830-839.

Vašková, J., Kočan, L., & Vaško, L. (2016). Oxidative stress and opioids. *Global Journal of Anesthesiology*, *3*(1), 020-029.

Wang, Y., Chen, Y., Zhang, X., Lu, Y., & Chen, H. (2020). New insights in intestinal oxidative stress damage and the health intervention effects of nutrients: A review. *Journal of Functional Foods*, *75*, 104248.