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Evaluation of the Hepato-Pancreatic Toxicities of Selected Antibiotics in Male Wistar Rats

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ABSTRACT

Background and Purpose: The misuse and toxicity of antibiotics remain significant public health concerns, with implications extending beyond hepatotoxicity to include pancreatic injury. This study investigated the toxic effects of selected antibiotic drugs (amoxicillin, ciprofloxacin, erythromycin and metronidazole) on the liver and pancreas of male Wistar rats.
Methods: Adult male Wistar rats were randomly assigned into five groups (n=5 per group). Group I served as the control and received normal saline. Groups II to V received ciprofloxacin (86 mg/kg), amoxicillin (129 mg/kg), erythromycin (86 mg/kg), and metronidazole (69 mg/kg), respectively. Administration was by the oral route for 21 or 42 days. Plasma insulin, nuclear factor kappa-beta (NFK-β), liver function enzymes (AST and ALT), lipid profile, and caspase-3 activity in liver and pancreas were evaluated. Histology and immunohistochemistry were performed on the liver and pancreas.
Results: Erythromycin significantly elevated serum AST and ALT levels, indicating hepatocellular injury. LDL levels rose following a 21-day metronidazole exposure and a 42-day ciprofloxacin and amoxicillin administration, while HDL levels declined across all antibiotic groups, demonstrating a consistent dyslipidemic effect. Metronidazole altered insulin levels differently in 21 days and 42 days durations, suggesting duration-dependent metabolic modulation. NFK-β levels increased significantly in metronidazole and erythromycin groups, indicating a heightened inflammatory response. In the 21-day treatment, hepatic caspase-3 activity was significantly elevated in the amoxicillin and erythromycin groups, indicating enhanced hepatocyte apoptosis. Conversely, pancreatic caspase-3 levels were significantly reduced in these same groups, suggesting suppression of pancreatic apoptotic activity. In contrast, during the 42-day treatment, pancreatic caspase-3 levels were markedly increased in the ciprofloxacin and metronidazole groups. Immunohistochemistry revealed disrupted hepatic and pancreatic architecture across all treated groups.
Conclusion: Ciprofloxacin, erythromycin, and metronidazole induced significant metabolic, inflammatory, and histopathological alterations in hepatic and pancreatic tissues. These findings underscore the need for cautious use and monitoring of these antibiotics to mitigate organ-specific toxicities.

INTRODUCTION

Antibiotic drugs are essential in modern medicine and are primarily used to treat bacterial infections such as tonsilitis, pneumonia, urinary tract infection, and sexually transmitted infections like gonorrhea and chlamydia, and as prophylactic agents (WHO, 2020; Yonts *et al*., 2018). However, their misuse has led to significant short and long-term consequences. They are ineffective against viral infections such as the common cold or flu and the absence of this knowledge often leads to misuse, which contributes to antimicrobial resistance (AMR).

Alarmingly, global data suggests that up to half of all irrationally prescribed medications are antibiotics (Onah and Idoko, 2022). In Nigeria, the WHO estimates that 60% of antimicrobial prescriptions are irrational (Otuko *et al*., 2023). This misuse significantly drives AMR, a global crisis. Africa, with the world's heaviest infectious disease burden, continues to face high morbidity and mortality from infections (Otuko *et al*., 2023). Antibiotic misuse and toxicity pose a pressing public health challenge, with an estimated 70 billion antibiotic doses consumed globally each year (Mittal *et al*., 2020). There has been a surge in antibiotic consumption in the 21st century with the penicillins and macrolides being the most prescribed (Roberts and Zembower, 2021).

Emerging research has linked metabolic diseases like obesity and type-2 diabetes with changes in microbiota composition and function (Fu *et al*., 2025). For example, erythromycin has been associated with liver toxicity (Jamshed *et al*., 2022), ciprofloxacin with oxidative tissue damage due to free radical production (Ramos *et al*., 2023), and metronidazole with liver toxicity in rat models (Enas, 2017). Additionally, early antibiotic exposure has been linked with altered insulin responses and several chronic diseases (Li *et al.,* 2017).

The liver and pancreas are closely linked anatomically, metabolically, and physiologically (Yang, 2020). The pancreas produces enzymes for digestion and homeostasis and regulates glucose levels through insulin secretion (Röder *et al*., 2016). The liver aids in lipid digestion, glucose release from glycogen, and detoxification by removing harmful substances and cells (Tiniakos *et al*., 2010). This study aimed to evaluate the effects of prolonged treatments with antibiotic drugs (a subclass of antibiotics) on hepato-pancreatic integrity and metabolic functions in male Wistar rats, with a focus on exploring potential links to the increasing prevalence of diabetes mellitus of unknown origin.

MATERIALS AND METHODS

Animals and Treatments

Fifty (50) male Wistar rats weighing 200–220 g were procured from the animal house of Afe-Babalola University Ado-Ekiti (ABUAD). The rats were housed under natural conditions. All rats had unlimited access to standard rat chow and water. The study was conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Ethical Review Board of Afe-Babalola University (Ado-Ekiti, Nigeria). Four (4) different antibiotics (amoxicillin, ciprofloxacin, erythromycin and metronidazole) were used for the study due to their distinct mechanisms of action, broad spectrum, and relevance in treating various bacterial infections. The drugs were procured from the Pharmacy Department of the Afe Babalola University Multisystem Hospital, Ado-Ekiti. The animals were randomly assigned to five groups (n=5 per group) each for the two duration of exposure namely: group 1 (control) given normal saline; group 2 (ciprofloxacin); group 3 (amoxicillin); group 4 (erythromycin), and group 5 (metronidazole). The doses were determined by extrapolating from human dosage guidelines (Reagan-Shaw *et al*., 2008) and were administered *per os* for a 21-day and a 42-day toxicity evaluation. Assays and histology were carried out at the end of each duration of treatment.

Oral Glucose Tolerance Test

The blood glucose was estimated at the end of each duration of treatment (21 or 42 days). Blood was obtained from the tail before glucose load (2 g/kg) and then sequentially at 30, 60, 90 and 120 min after glucose load. Glycemic concentrations were determined with a hand-held glucometer (OneTouch) as previously described (Omoaghe *et al*., 2022).

Sacrifice and Sample Collection

At the end of each duration of treatment, the animals were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg). The rats were cardiac punctured to collect blood samples into heparinized tubes which were centrifuged at 3000 rpm for 15 min at room temperature. Plasma samples were stored frozen until biochemical assays were performed. For the histology, the animals were trans-cardially perfused with phosphate buffered solution (PBS) of pH 7.4 to clear the blood vessels, and with 10% neutral buffer formasaline (NBF) which served as the pre-fixative. For biochemical assays, the animals were perfused with PBS only and thereafter, the liver and pancreas were excised, cleared of adhering connective tissues and homogenized in phosphate buffer.

Biochemical Analysis

High-density lipoproteins (HDL), triglycerides (TG), and total cholesterol (TC) levels in plasma were evaluated by an enzymatic colorimetric method (Dolati *et al.,* 2020). Alanine aminotransferase (ALT) (BXC0212) and aspartate aminotransferase (AST) (BXC0202) levels were evaluated using kits manufactured by Fortress Diagnostics Limited (United Kingdom) (Jain *et al*., 2022). Plasma concentrations of insulin and NF-κB were determined using ELISA kits from Ray Biotech, Inc. (Georgia, USA), according to the manufacturer's protocols. The enzymatic activity of caspase-3 in liver and pancreatic tissues was evaluated using assay kits procured from Fortress Diagnostics Limited (United Kingdom). The methodology was adapted from Thapaliya *et al*. (2014), ensuring consistency with established experimental procedures.

Histological and Immunohistochemical Processing of Tissue Samples

Liver and pancreatic tissue sections were deparaffinized in xylene and rehydrated through descending graded concentrations of ethanol (100%, 95%, and 70%) to distilled water. Hematoxylin staining was performed by dissolving ammonium alum in boiling water, combining it with hematoxylin in absolute ethanol, and enhancing nuclear affinity with mercuric oxide and glacial acetic acid. Sections were immersed in the hematoxylin solution, rinsed, and counterstained with eosin prepared in a distilled water–alcohol–acetic acid mixture. Slides were cleared in xylene, mounted with Permount, and examined microscopically using an OPTO-Edu Industrial Light Microscope (China). Photomicrographs were captured using a high-resolution Moticam X3 digital microscope camera (12.0 MP) mounted on the same microscope. Images were acquired under standardized illumination and magnification using Motic Images Plus software and analyzed with ImageJ. For immunohistochemistry (IHC), tissues were fixed in formalin, dehydrated using ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax prior to sectioning (5 µm). After deparaffinization and rehydration, antigen retrieval was achieved via heat or enzymatic digestion. Primary antibodies specific to target antigens were applied, followed by HRP-conjugated secondary antibodies. Diaminobenzidine (DAB) served as the chromogen, while hematoxylin counterstaining enhanced structural contrast. Slides were examined microscopically to assess protein localization and distribution (Ige *et al*., 2017). For BCL-2 expression analysis, liver tissue sections were subjected to immunohistochemical staining using anti-BCL-2 primary antibodies (1:200 dilution), followed by Horseradish Peroxidase (HRP)-conjugated secondary antibodies. Antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave oven. Detection was carried out with DAB, and hematoxylin was used for counterstaining. Stained sections were examined for cytoplasmic and peri-nuclear localization of BCL-2 (Kelland and Beale, 2001).

Statistical Analysis

All data are expressed as mean ± standard error of mean (SEM). Statistical group analysis was carried out using GraphPad Prism software version 8.5. We compared the mean values of the variables among the groups using one-way analysis of variance. *Post hoc* analysis was performed using Tukey's test. Differences were considered statistically significant at $P<0.05$.

RESULTS

Effect of Antibiotics Administration on Body Weight

No significant weight changes were observed between the antibiotic-administered groups and the control, as illustrated in Figures 1A and 1B.

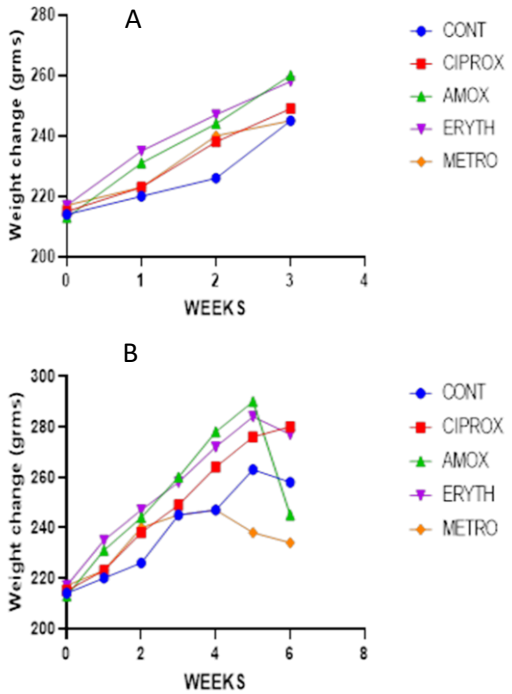

Figure 1: Weight changes in rats after 21-day (A) and 42-day (B) dosing with antibiotics. CONT – Control, CIPROX – Ciprofloxacin, AMOX – Amoxicillin, ERYTH – Erythromycin, METRO – Metronidazole. Weight changes are not statistically significant.

Effect of Antibiotic Drug Administration on Glucose and Insulin Concentrations, Area Under the Curve for Glucose Concentration

In the 21-day duration, there were no significant differences among the groups in the OGTT results as glucose levels were comparable (Figure 2A). Similarly, the area under the curve (AUC) indicated no significant variations (Figure 2B). Plasma insulin concentration was significantly elevated ($P<0.05$) in the metronidazole group, while other groups remained unchanged (Figure 3A). In the 42-day

duration, glucose concentrations remained statistically unchanged, but the AUC for glucose was significantly (P<0.0001) lower in the metronidazole group (Figures 2C, 2D). Insulin concentration significantly decreased in the **0**metronidazole group in the 42-day duration (Figure 3B).

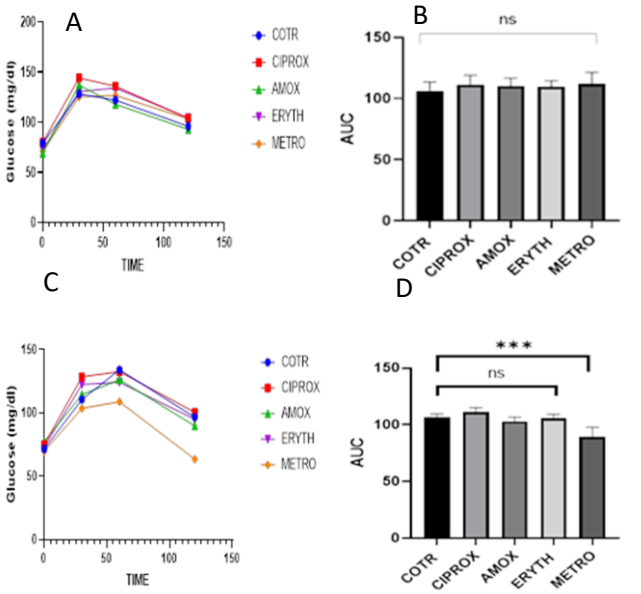

Figure 2: Plasma glucose concentration and area under the curve (AUC) following 21-day (A and B) and 42-day (C and D) treatment of rats with antibiotic drugs. ***P<0.001 versus control, n=5. CONT – Control, CIPROX – Ciprofloxacin, AMOX – Amoxicillin, ERYTH – Erythromycin, METRO- Metronidazole, ns – not significant.

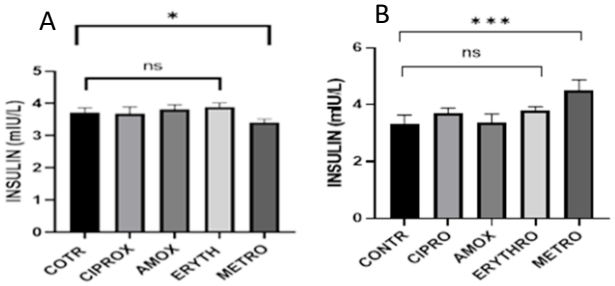

Figure 3: Plasma insulin concentrations following 21 days (A) and 42 days (B) treatment of rats with antibiotic drugs. *P<0.05, ***P<0.001 versus control, n=5. CONT – Control, CIPROX – Ciprofloxacin, AMOX – Amoxicillin, ERYTH – Erythromycin, METRO- Metronidazole, ns – not significant.

Effect of Antibiotic Administration on Liver Enzymes

In the 21-day duration (Figure 4A), ALT levels increased significantly in the erythromycin group but decreased significantly in all other administered groups compared with the control. In the 42-day duration (Figure 4B), ALT levels increased significantly in the erythromycin groups. AST levels in the 21-day duration (Figure 4C) increased significantly in the ciprofloxacin and erythromycin groups but decreased significantly in the metronidazole group, compared with the control. In Figure 4D (42-day duration), AST levels significantly decreased in the ciprofloxacin and amoxicillin groups compared with the control.

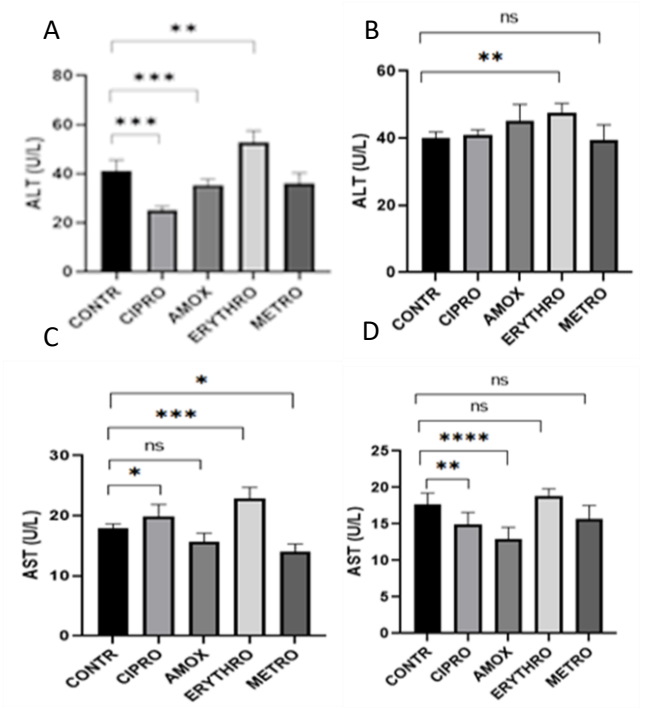

Figure 4: Levels of liver function enzymes (ALT and AST) following 21 days (A, B) and 42 days (C, D) of antibiotic drug administration to rats. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 versus control, n=5. CONT – Control, CIPROX – Ciprofloxacin, AMOX – Amoxicillin, ERYTH – Erythromycin, METRO- Metronidazole, ns – not significant.

Effect of Antibiotic Drug Administration on Plasma Lipids

Total cholesterol levels (Figure 5A) increased significantly in ciprofloxacin and erythromycin groups but were unchanged in amoxicillin and metronidazole groups in the 21 days duration in comparison with the control group. However, in the 42-day evaluation, only the amoxicillin group had significant elevation in total cholesterol levels (Figure 5B). Triglycerides levels (Figures 5C, 5D) did not change significantly in the 21-day and 42-day evaluations in most of the groups except for an increase in the amoxicillin group (Figure 5C), and a decrease in the erythromycin group (Figure 5D). LDL Levels (Figure 5E) significantly increased in the metronidazole group when compared with the control of the 21-day duration, while in the 42-day duration, ciprofloxacin and amoxicillin groups had significant increase when compared with the control (Figure 5F). HDL Levels in the 21-day duration (Figure 5G) significantly decreased in ciprofloxacin, amoxicillin, and metronidazole groups compared with the control group. In the 42-day duration, only the plasma levels of the ciprofloxacin group was significantly reduced (Figure 5H), compared with the control group.

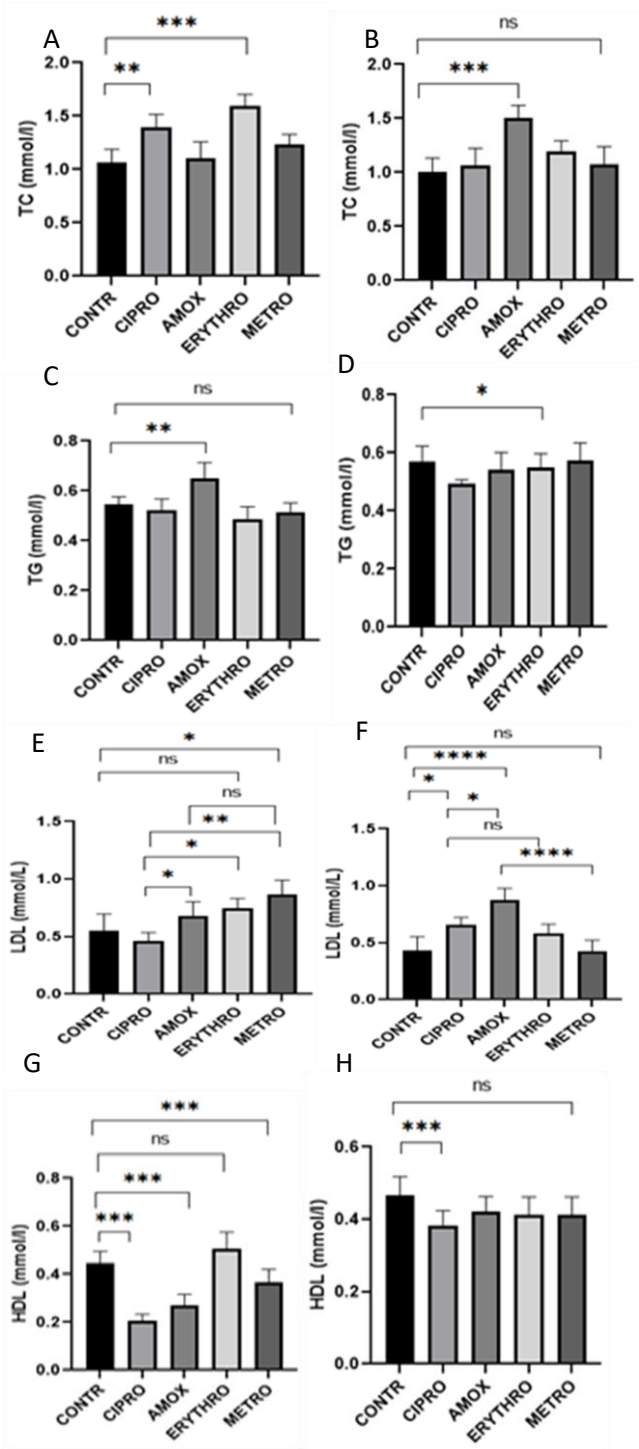

Figure 5: Levels of plasma lipids: TC (A, B), TG (C, D), LDL (E, F), and HDL (G, H) after 21 (first alphabet) and 42 (second alphabet) days of administering antibiotic drugs to rats. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 versus control, n=5. CONT – Control, CIPROX – Ciprofloxacin, AMOX – Amoxicillin, ERYTH – Erythromycin, METRO- Metronidazole, ns – not significant.

Effect of Antibiotic Drug Administration on Plasma NFK-β Levels

Plasma NFK-β levels (Figure 6A) increased significantly in the metronidazole and erythromycin groups, whereas in the ciprofloxacin and amoxicillin groups, the levels were not significantly altered when compared with the control in the 21-day duration of treatment. In the 42-day duration of treatment, plasma NFK-β levels (Figure 6B) were not significantly changed in any antibiotic drug-administered group compared with the control.

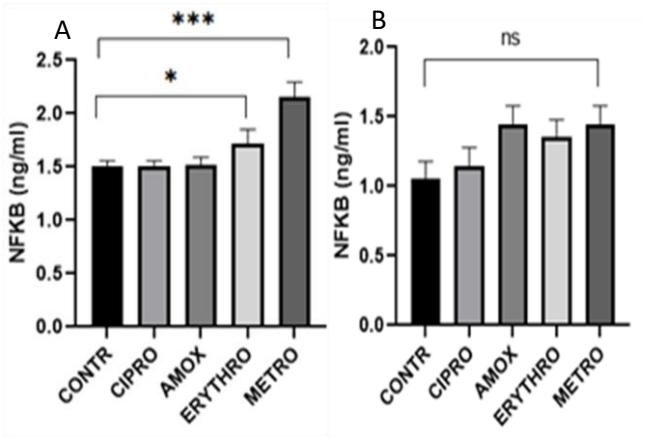

Figure 6: Plasma NFK-β after 21-day (A) and 42-day (B) of antibiotic drug administration to rats. **P*<0.05, ****P*<0.001 versus control, n=5. CONT – Control, CIPROX – Ciprofloxacin, AMOX – Amoxicillin, ERYTH – Erythromycin, METRO- Metronidazole, ns – not significant.

Effect of Antibiotic Drug Administration on Liver and Pancreatic Caspase-3 Levels

Liver caspase-3 levels (Figure 7A) were not significantly changed in any antibiotic drug-administered group compared with the control, in the 21-day duration. However, in the 42-day duration, liver caspase-3 levels (Figure 7B) increased significantly in the amoxicillin and erythromycin groups, while the changes in levels were not significant in ciprofloxacin and metronidazole groups compared with the control.

Pancreatic caspase-3 levels (Figure 7C) significantly increased in the ciprofloxacin and metronidazole groups, while for the other groups, there were no changes in the levels compared with the control group, in the 21-day duration. Pancreatic caspase-3 levels (Figure 7D) significantly increased in the ciprofloxacin and metronidazole groups, whereas for the amoxicillin and erythromycin groups, there were no significant alterations compared with the control in the 42-day evaluation.

Effect of Antibiotic Drug Administration on Liver Histology

Photomicrographs of the liver revealed the presence of perivascular inflammatory cells in the periportal area across all drug-administered groups during both the 21-day (Figure 8) and 42-day (Figure 9) durations. Additionally, there was no significant decrease in hepatocyte count in the erythromycin and amoxicillin groups compared with the control group. However, in the ciprofloxacin and metronidazole groups, there were significant reductions in hepatocyte count relative to the control group (Figure 8).

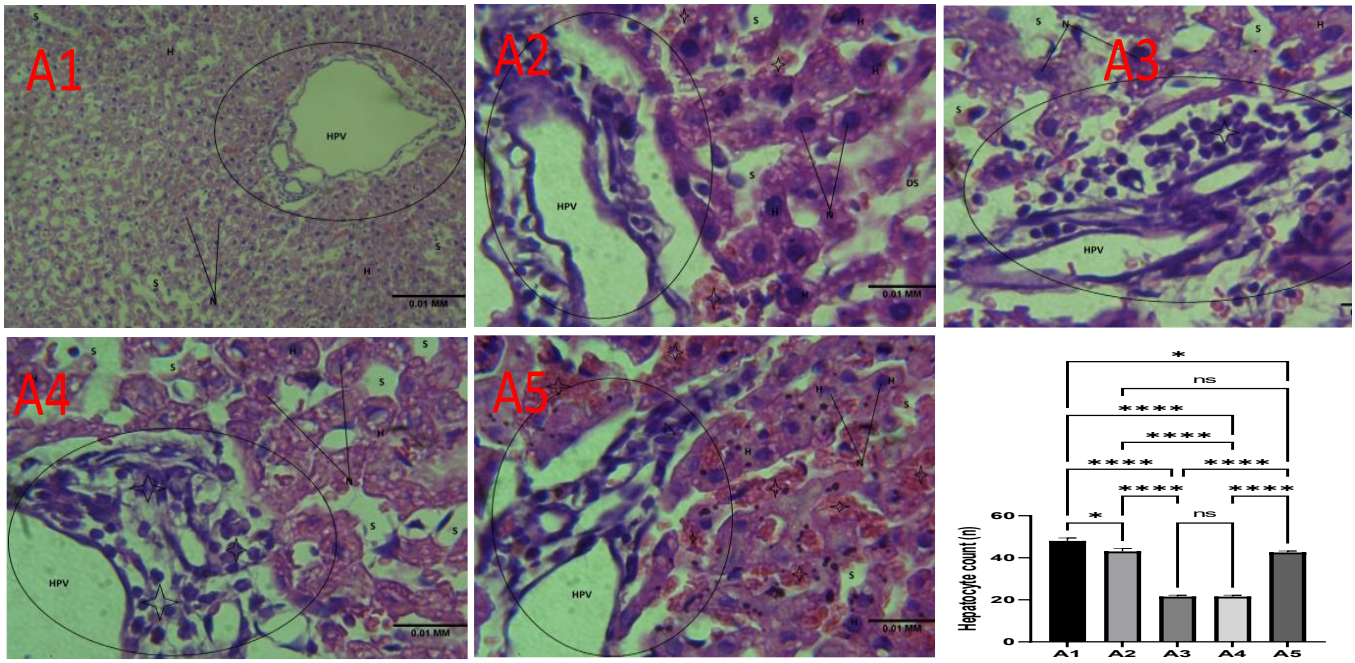

Figure 8: Photomicrographs of liver histology after 21 days of treating rats with antibiotic drugs (H&E stain, ×400 magnification). A1 = control; A2 = ciprofloxacin-treated; A3 = amoxicillin-treated; A4 = erythromycin-treated; and A5 = metronidazole-treated. Statistical significance compared with the control group is as follows: $P<0.05$ (*) and $P<0.0001$(****). ns = not significant.

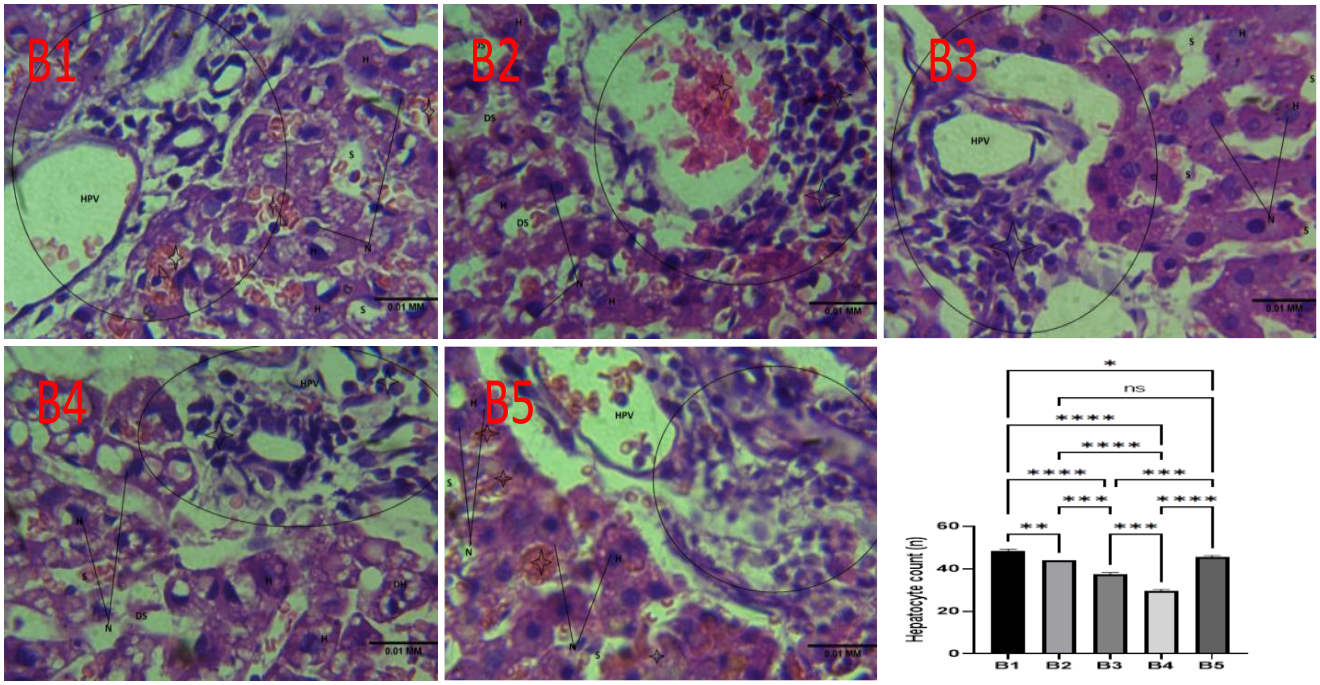

Figure 9: Photomicrograph of liver histology (H&E stain, ×400 magnification) after 42 days of treatment of rats with antibiotic drugs. B1 = control, B2 = ciprofloxacin-treated, B3 = amoxicillin-treated, B4 = erythromycin-treated, and B5 = metronidazole-treated. Statistical significance is denoted as follows: $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and $P<0,0001$ (****). ns = not significant.

Effect of Antibiotic Drug Administration on BCL-2 Expression in the Liver

The control group showed normal BCL-2 expressions. Among the antibiotic drug-administered groups, amoxicillin increased BCL-2 expression in the liver, whereas erythromycin reduced it during the 21-day duration (Figure 10). In the 42-day duration, all drug-administered groups experienced a significant reduction in hepatocyte count compared with the control group (Figure 11).

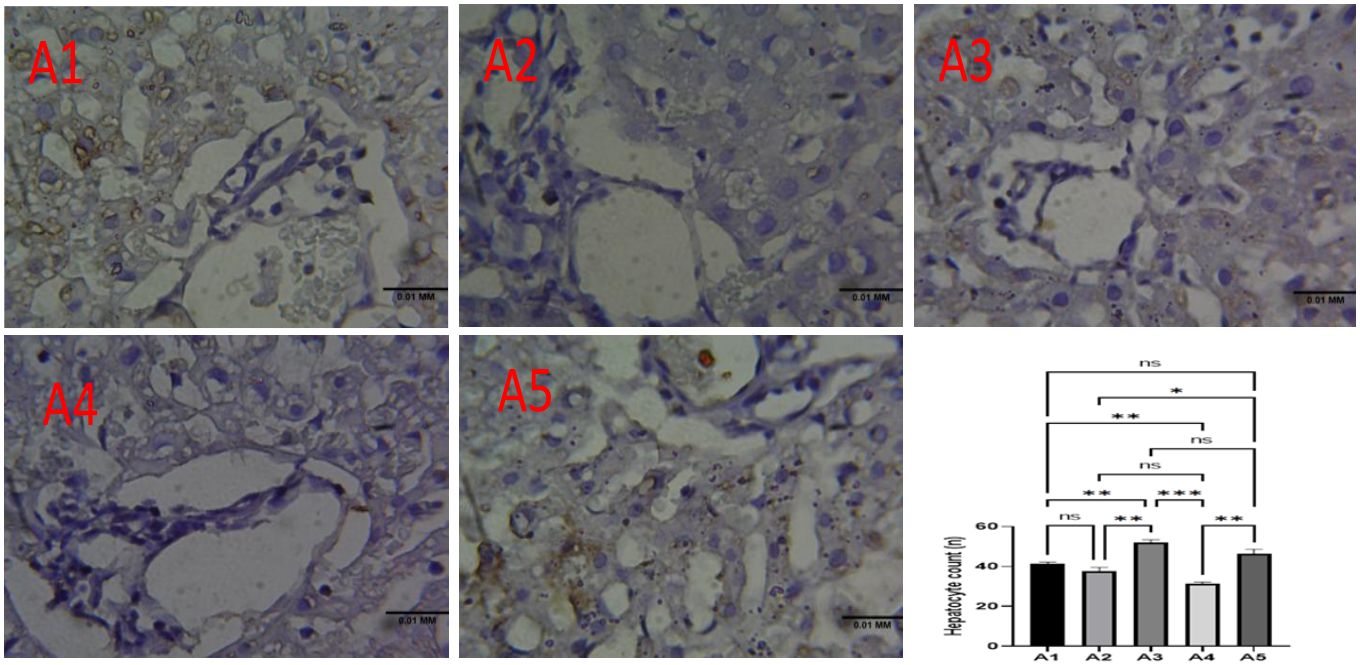

Figure 10: Photomicrograph of BCL-2 expression in liver tissue (H&E stain, ×400 magnification) after 21 days of treatment of rats with antibiotic drugs. A1 = control, A2 = ciprofloxacin-treated, A3 = amoxicillin-treated, A4 = erythromycin-treated, and A5 = metronidazole-treated. Statistical significance is as follows: $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***). ns = not significant.

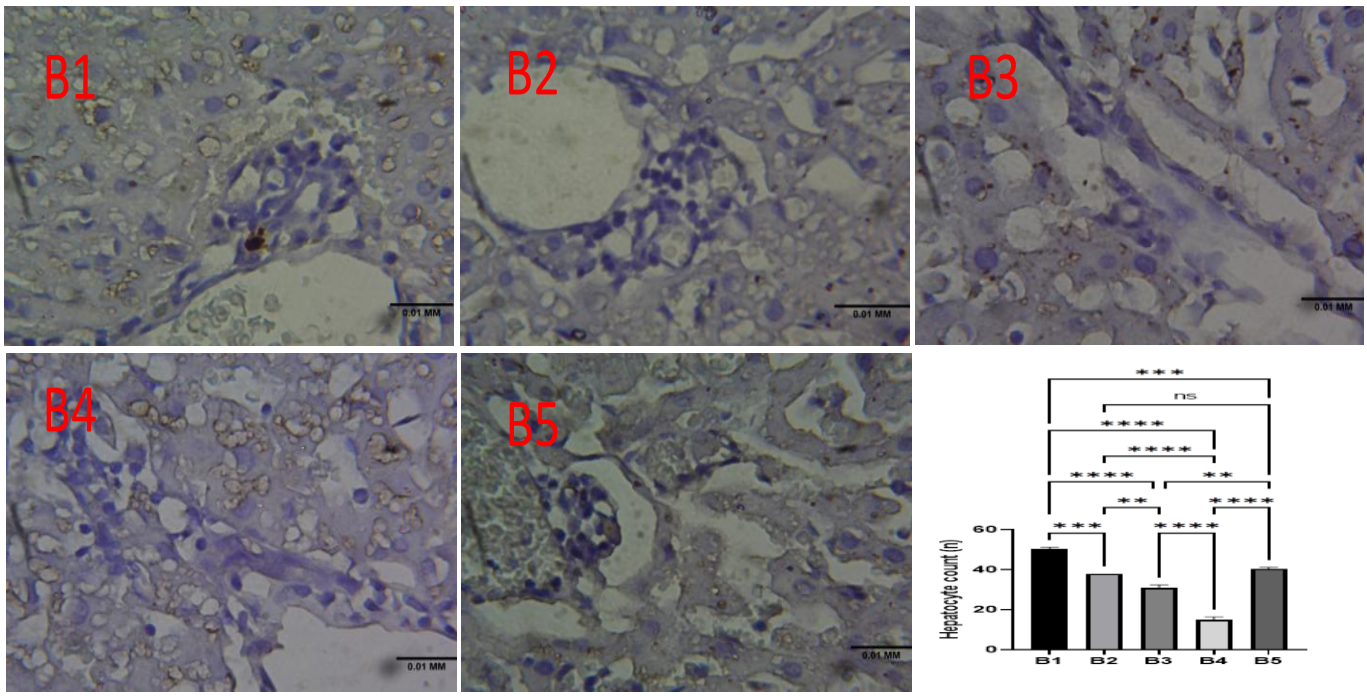

Figure 11: Photomicrograph of BCL-2 expression in liver tissue (H&E stain, ×400 magnification) after 42 days of treatment of rats with antibiotic drugs. B1 = control, B2 = ciprofloxacin-treated, B3 = amoxicillin-treated, B4 = erythromycin-treated, B5 = metronidazole-treated. Statistical significance is indicated as follows: $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and $P<0.0001$ (****). ns = not significant.

Effect of Antibiotic Drug Administration on Pancreas Histology

Histological analysis did not reveal a significant difference in the number of islets of Langerhans in pancreas across all groups (Figure 12) in the 21-day duration. However, in the antibiotic drug-administered groups, decrease in islet count was significant when compared with the control group after 42 days of treatment (Figure 13).

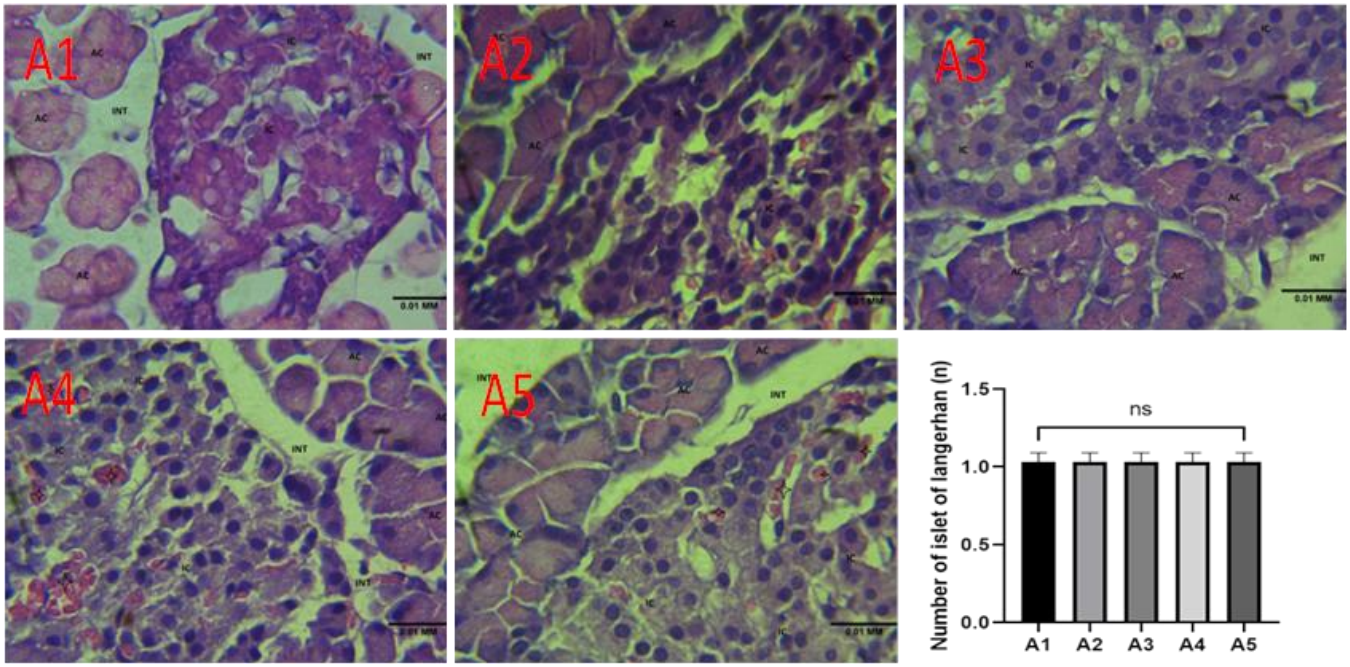

Figure 12: Photomicrograph of pancreatic tissue (H&E stain, ×400 magnification) after 21 days of antibiotic drug treatment to rats: A1= control, A2 = ciprofloxacin-treated, A3 = amoxicillin-treated, A4 = erythromycin-treated, and A5 = metronidazole-treated. ns = not significant.

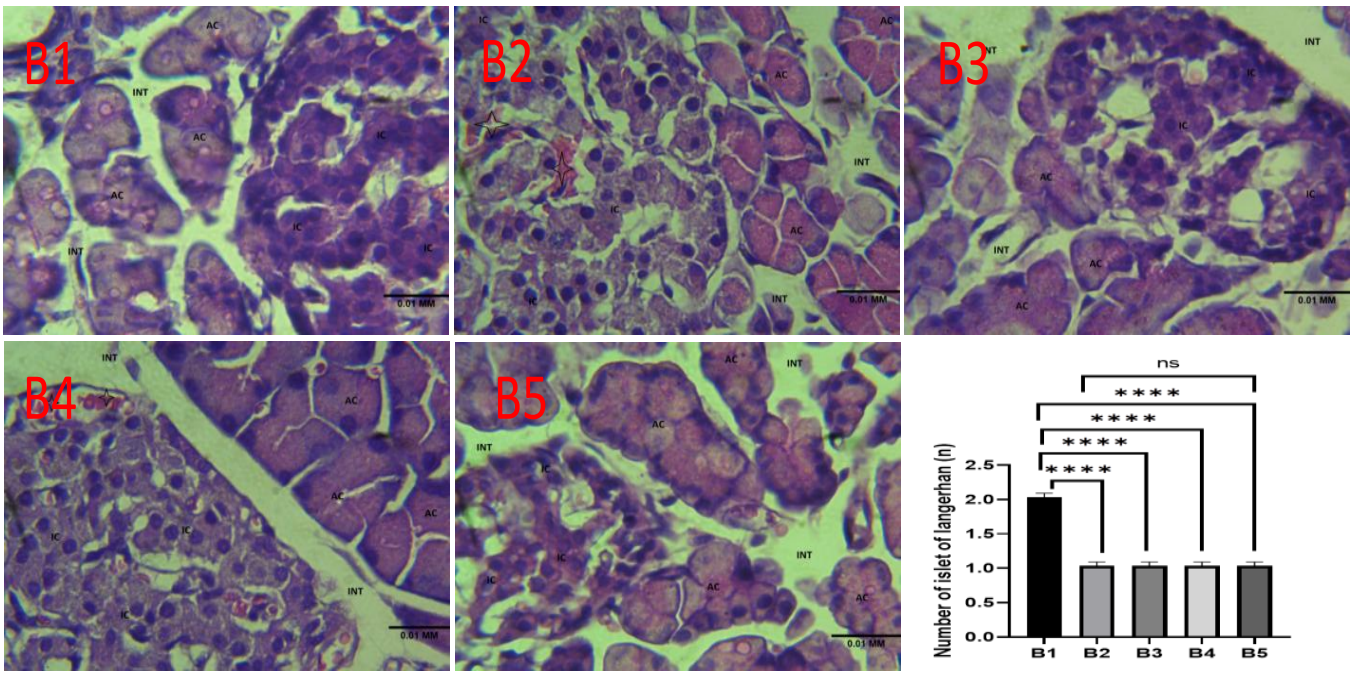

Figure 13: Photomicrograph of pancreatic tissue (H&E stain, ×400 magnification) after 42 days of treating rats with antibiotic drugs. B1 = control, B2 = ciprofloxacin-treatment, B3 = amoxicillin-treatment, B4 = erythromycin-treatment, B5 = metronidazole-treated. $P<0.0001$ (****) compared with control. ns = not significant.

Effect of Antibiotic Drug Administration on Pancreatic BCL-2 Expression

Administration of antibiotic drugs led to mild membranous expression of BCL-2 in the pancreas in both the 21-day (Figure 14) and 42-day (Figure 15) treatment. In contrast, there was normal expression in the control group. There was no significant difference in the islet cell count between the antibiotic drug-treated group and the control group in either duration of treatment.

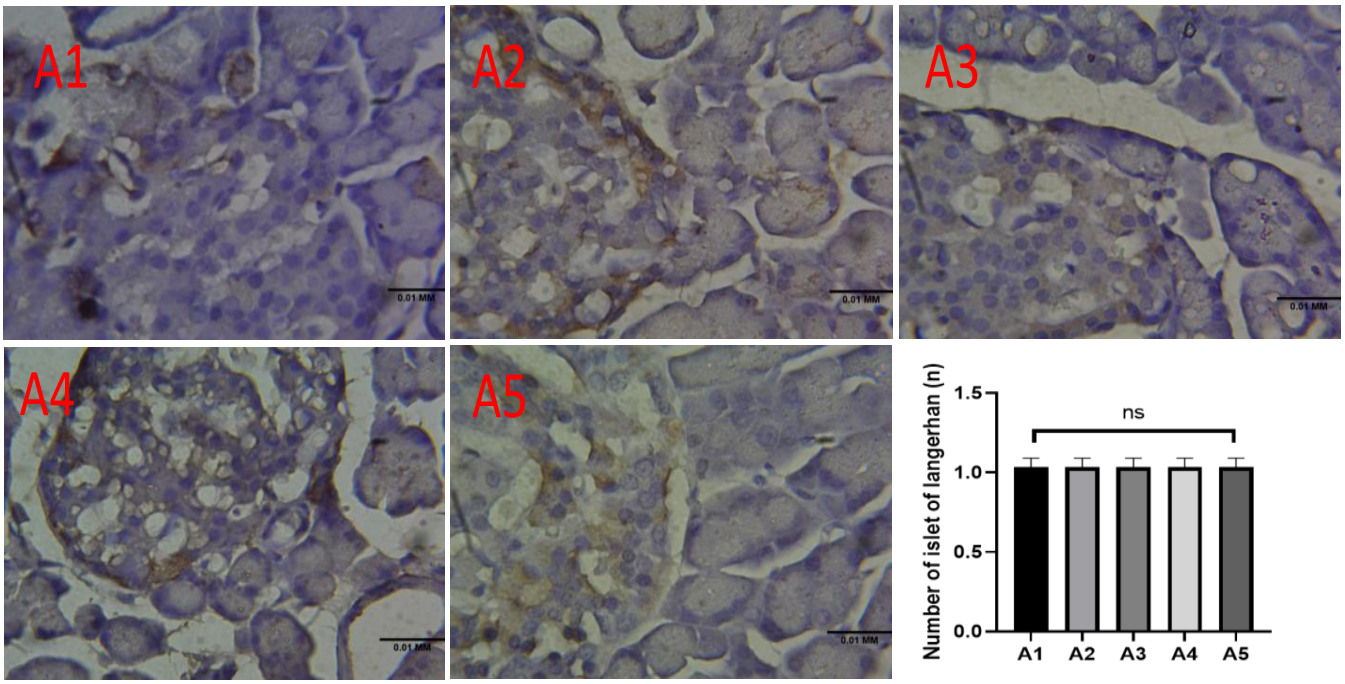

Figure 14: Photomicrograph of BCL-2 expression in pancreatic tissue (H&E stain, ×400 magnification) after 21 days of treating rats with antibiotic drugs. A1 = control, A2 = ciprofloxacin-treated, A3 = amoxicillin-treated, A4 = erythromycin-treated, A5 = metronidazole-treated. ns = not significant.

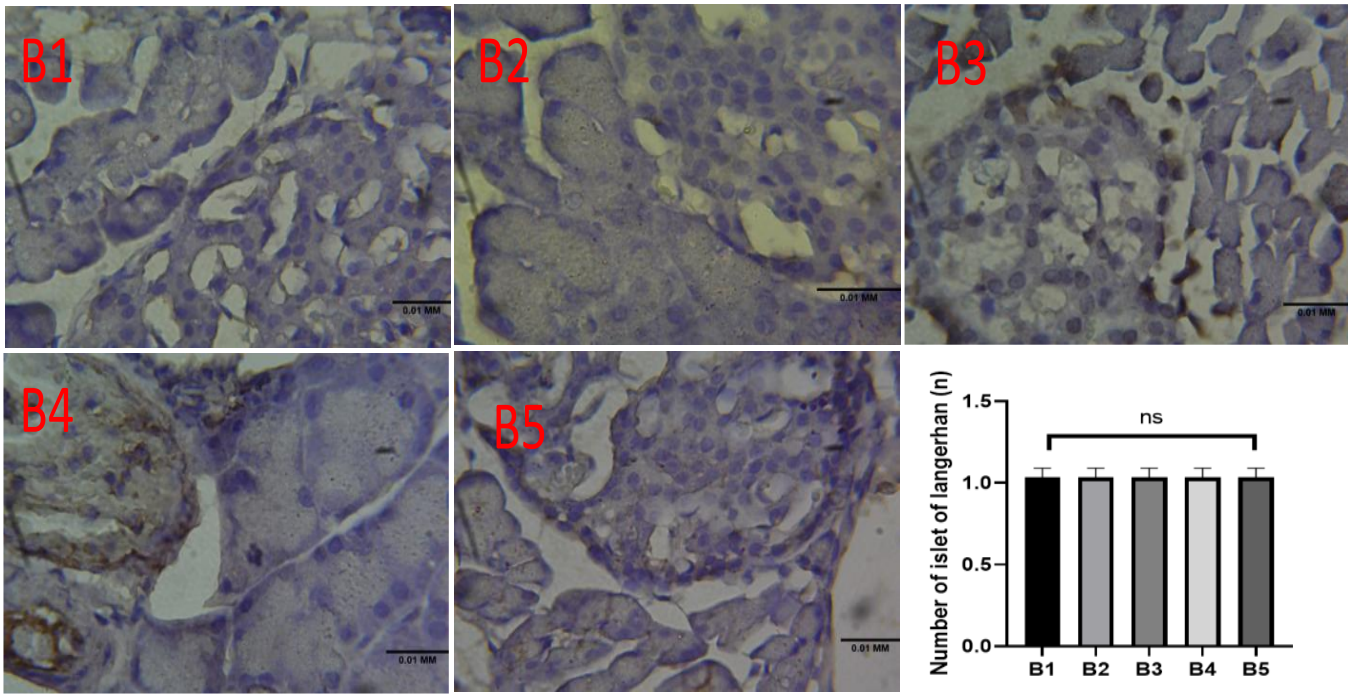

Figure 15: Photomicrograph of BCL-2 expression in pancreatic tissue (H&E stain, ×400 magnification) after 42 days of treating rats with antibiotic drugs. B1 = control, B2 = ciprofloxacin-treated, B3 = amoxicillin-treated, B4 = erythromycin-treated, B5 = metronidazole-treated. ns = not significant.

DISCUSSION

This study provides some insights into the toxic effects of some antibiotic drugs on the liver and pancreas. Weight gain in all antibiotic-treated and control groups during both 21- and 42-day evaluations was not significant, contrasting with Mihalas *et al*. (2018), who reported significant weight gain in rats given amoxicillin. The administration of erythromycin led to elevation in liver enzymes (ALT and AST), indicating potential hepatic dysfunction, and aligning with Adnan (2019). Conversely, ciprofloxacin, amoxicillin, and metronidazole treatments resulted in decreased ALT and AST levels, and consistent with Bilgic *et al*. (2017). However, Priya *et al.* (2024) reported increased liver enzyme levels, highlighting discrepancies

likely due to variations in dosage and duration of drug administration.

Insulin, secreted by beta cells in response to elevated blood glucose, promotes glucose absorption in muscles and adipose tissue, enhancing their glucose storage and leading to glycogen synthesis in the liver (Wollheim and Maechler, 2002). In the present study oral glucose tolerance test (OGTT) and insulin concentration were used to assess proneness to diabetes. In the metronidazole group, the area under the curve significantly decreased compared to the control, but plasma insulin levels markedly increased, indicating hyperinsulinemia. This contradicts the report by Adeoye *et al*. (2022), who found that there were no significant changes in plasma insulin after metronidazole administration.

Inflammation and infections play a crucial role in altering lipid metabolism. While these changes initially support immune responses in combating infections, they may also increase the risk of atherosclerosis and fibrosis, affecting vital organs such as the liver. In this study, total cholesterol (TC) levels were elevated in the ciprofloxacin and erythromycin groups compared to the control. This finding aligns with that of Ogbonna *et al*. (2024), but contrasts with Hamoya *et al*. (2017), likely due to differences in dosage and duration of drug administration.

Our findings also indicate that metronidazole led to a significant increase in low-density lipoprotein (LDL) levels in the 21-day evaluation, while ciprofloxacin and amoxicillin induced similar increases in LDL levels in the 42-day evaluation. This suggests that certain antibiotic drugss influence lipid metabolism over a period of time, highlighting the need for further investigation into their long-term effects. Furthermore, significant reductions in high-density lipoprotein (HDL) levels were observed in the ciprofloxacin, amoxicillin, and metronidazole groups relative to the control. However, Igbayilola *et al*. (2020) reported an increase in HDL levels following ciprofloxacin administration, suggesting that antibiotic effects on lipid metabolism may be influenced by additional factors. Interestingly, the erythromycin group did not exhibit significant HDL changes, differing from the findings by Kong *et al*. (2020), who documented decreased lipoproteins after 28 days of erythromycin treatment.

Previous studies have explored the relationship between antibiotic drugs and lipid profiles, demonstrating that certain antibiotic drugs can alter lipid metabolism and potentially contribute to dyslipidemia. Additionally, interactions between antibiotic drugs and lipid-modifying agents have been reported, reinforcing the importance of monitoring lipid levels during antibiotic drug therapy. Given the association of some antibiotic drugs with increased LDL cholesterol levels, this study provides valuable insights into time-dependent effects of metronidazole, ciprofloxacin, and amoxicillin on lipid metabolism, further enriching the existing body of knowledge in this area.

NFK-β activation, associated with increased pro-inflammatory biomarkers IL-1, TNF-α, and IL-6, correlates with severity of inflammation. In the 21-day evaluation, erythromycin and metronidazole groups showed significant increases in nuclear NFK-β, unlike amoxicillin and ciprofloxacin groups. Chaturvedi *et al*. (2020) suggested metronidazole's role in NFK-β activation, while Silva *et al*. (2022) reported erythromycin down-regulated NFK-β. No significant changes were observed in the 42-day assessment across all groups compared to the control.

Caspase-3, an essential enzyme in apoptosis, was significantly increased in the liver of the erythromycin group, with no significant changes in ciprofloxacin, amoxicillin, and metronidazole groups during regardless of the duration of treatment whether 21 days or 42 days. This contradicts the report by Hemieda *et al*. (2019), who found increased liver caspase-3 levels following ciprofloxacin administration. In addition, the significant increase in pancreatic caspase-3 levels in the ciprofloxacin and metronidazole groups compared to the control during both the 21- and 42-day evaluations. This finding aligns with that of Abdel-Rahman *et al*. (2021), who reported a significant increase in pancreatic caspase-3 following ciprofloxacin administration. However, no significant changes were observed in the amoxicillin and erythromycin groups compared to the control.

Histology results revealed distortions in liver architecture indicated by perivascular inflammatory cell infiltration and congestion, suggesting cellular injury in the drug-administered groups in both the 21-day and 42-day assessments. There was a significant decrease in hepatocyte counts in the amoxicillin and erythromycin groups compared to the control, while there were no significant changes in the ciprofloxacin and metronidazole groups. This aligns with Omodamiro (2018), who observed similar histoarchitecture distortions after 21 days of ciprofloxacin and amoxicillin administration. Immunohistochemistry (IHC) revealed mild membranous BCL-2 expression and decreased hepatocyte counts in the amoxicillin and erythromycin groups, with no changes in BCL-2 expressions in the ciprofloxacin and metronidazole groups (Adeoye *et al*., 2022).

In the pancreas, histological analysis revealed distorted histoarchitecture, characterized by congestion between endocrine and exocrine units, indicating acinar and islet cell injury (acute pancreatitis). This finding supports the involvement of antibiotic drugs in the etiology of acute pancreatitis (Párniczky *et al*., 2019). IHC revealed mild

membranous BCL-2 expression, with no significant differences in islet cell counts between antibiotic drug-administered groups and control groups during both durations of treatment (Adeoye *et al*., 2022).

CONCLUSION

This study sheds light on the adverse hepato-pancreatic effects that may arise following the misuse of antibiotic drugs. Administration of metronidazole can significantly change insulin concentrations, and this has implications for blood glucose concentration. Misuse can also result in altered lipid metabolism such as elevation in the total cholesterol levels (ciprofloxacin and erythromycin) and decrease in HDL levels (ciprofloxacin, amoxicillin, and metronidazole) with possible cardiovascular sequelae. Also, elevation in the levels of some pro-inflammatory enzymes and distortions in the histoarchitecture of the liver and pancreas have been seen with some of the drugs. Therefore, it is important that the dosage and duration of antibiotic therapy be well-considered to mitigate potential adverse effects on liver and pancreatic functions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization, Methodology, Data Collection and Analysis, Writing (Original Draft)**:** IIK, OAO, AOK**.** Data Collection & Analysis, Supervision and Project Administration**:** MYH, ET, EN.

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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