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Article

Chronic exposure to amoxicillin and its effects on growth, immunity, organ function and residue accumulation in mice

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Abstract: Antibiotic therapy is essential in human and veterinary medicine, but resistance and prolonged exposure to antibiotic residues pose significant risks to health. This study investigated the long-term effects of amoxicillin residue on Swiss Albino male mice. Mice (n=20) were divided into control (no antibiotics) and treated (amoxicillin at the rate of 10 ppm in drinking water for one year; MRL 0.01 ppm) groups. Body weight, physical condition, immune status, blood parameters, ALT and AST enzyme levels, histopathology and residue accumulation in organs was examined. Antibiotics-treated mice exhibited significant weight gain (P<0.05~0.01) from the third month, peaking in the 10th month before declining. Treated mice appeared robust yet lethargic. Blood analysis showed decreased lymphocyte and neutrophil count and while no significant changes were observed in monocyte, eosinophil and basophils counts. ALT and AST levels were elevated but not statistically significant. Histopathology revealed liver steatosis, glomerular atrophy, and inflammatory cell infiltration in the liver and kidneys. TLC analysis confirmed Amoxicillin residue accumulation in the liver, kidneys, spleen, intestine, and muscles. The findings suggest that long-term exposure to antibiotic residues may adversely affect health and highlight the need for controlled antibiotic use to prevent potential hazards.

Keywords: antibiotic; residual effect; mice; health hazards; ALT; AST

1. Introduction

Good health is intrinsically linked to the consumption of safe food, free from harmful substances such as drug residues, toxins, and poisons. As food forms a fundamental basis for human health, its safety has emerged as a critical concern globally. Ensuring safe food is paramount not only for maintaining individual well-being but also for supporting the overall development of societies and nations (Choudhury *et al*., 2022). Among various sources of food, livestock plays a pivotal role in providing animal-derived products such as meat, milk, and eggs, which constitute essential components of the human diet. However, the widespread use of veterinary drugs in food-producing animals has led to the unintended presence of drug residues in these products, posing significant risks to public health (Ame *et al*., 2022).

Antibiotic residues, in particular, are among the most pressing concerns due to their wide-ranging implications for human health. These residues have been associated with hypersensitivity reactions, mutagenicity, teratogenicity, and carcinogenicity. Furthermore, they contribute to the growing problem of antimicrobial

resistance (AMR), which threatens the effectiveness of antibiotics in treating bacterial infections. AMR exacerbates public health challenges by enabling the transfer of resistant pathogens from animal-derived foods to humans, compounding the already critical global health crisis (Bacanlı, 2024). The emergence of antibiotic residues as a food safety issue is especially relevant in developing countries like Bangladesh, where public awareness and institutional mechanisms for monitoring and regulating drug residues remain inadequate. Despite the critical importance of addressing antibiotic residues, there has been limited research in Bangladesh focused on their detection and analysis, leaving a significant gap in understanding their prevalence and associated health risks. The absence of a comprehensive residue monitoring program further compounds the problem, allowing the indiscriminate use of antimicrobials in food-producing animals to continue unchecked. This lack of oversight has far-reaching implications, threatening not only individual health but also the long-term development of the nation. The indiscriminate use of antibiotics in livestock and poultry industries has led to the presence of residues in animal-derived food products, with detrimental effects on human health. These effects include alterations to gut microbiota, hypersensitivity reactions, residual toxicity, and the acceleration of AMR. Prolonged exposure to even low levels of antibiotic residues in food can lead to chronic health conditions and disrupt normal physiological processes, underscoring the need for stricter controls on antimicrobial usage.

Antibiotics, widely used to treat bacterial infections, are derived from microorganisms or synthesized chemically to inhibit or destroy microbial growth. They are essential tools in both human and veterinary medicine, playing a key role in managing infections. However, their overuse and misuse have led to unintended side effects, including cognitive impairments, as reported in multiple studies (Bennett *et al*., 2022). Although the precise mechanisms behind antibiotic-induced cognitive dysfunction remain poorly understood, transient changes in cognitive abilities during antibiotic use have been documented (Wildermuth and Holmes, 2022). These findings highlight the broader consequences of antibiotic exposure beyond their intended therapeutic effects. In recent years, the presence of antibiotic residues in food has garnered significant attention due to mounting concerns over food safety and public health. These residues, when present in animal and poultry products, pose serious health risks, including hypersensitivity reactions, disruption of normal intestinal flora, bone marrow suppression, and potential carcinogenic and teratogenic effects (Agbabiaka *et al*., 2025). Moreover, the contribution of antibiotic residues to the emergence of drug-resistant bacterial strains further underscores the urgency of addressing this issue.

In the context of Bangladesh, the lack of awareness about antibiotic residues among the general population, coupled with insufficient regulatory frameworks, exacerbates the problem. Without effective monitoring and enforcement of residue limits, the indiscriminate use of antibiotics in food-producing animals continues to threaten consumer safety. Addressing this issue requires a multifaceted approach that includes public education, stricter regulations, and robust research to understand the extent of the problem and its implications. The primary danger associated with antibiotic residues lies not only in their direct health impacts but also in their potential to amplify existing challenges in human and veterinary medicine. Residues in food products derived from animals treated with antibiotics can act as reservoirs for resistant pathogens, facilitating their transfer to humans and further complicating the management of bacterial infections.

Given the critical need for evidence-based approaches to address antibiotic residues, the present study was undertaken to investigate the long-term effects of amoxicillin residues in a controlled experimental setup. Amoxicillin, a widely used antibiotic, was chosen for this study due to its extensive application in both human and veterinary medicine. Using a mouse model, this research aimed to assess the potential health hazards of prolonged exposure to low levels of Amoxicillin residue, focusing on physiological, immunological, and histopathological changes. This study seeks to fill a critical knowledge gap by providing insights into the implications of antibiotic residues on health. By exploring the hazards associated with long-term exposure to Amoxicillin, this research aims to contribute to the development of effective strategies for monitoring and controlling antibiotic residues in food, ultimately promoting food safety and public health.

2. Materials and Methods

2.1. Ethical approval

The experiment was done following the ethical and welfare guidelines set by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University [approval number: AWEEC/BAU/2022(15)].

2.2. Selection and preparation of the experimental shed

The experiment was conducted in the Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh (Figure 1). Swiss-albino mice were housed in a controlled

environment at 25 ± 1ºC with a 12:12 h light/dark cycle in polypropylene cages (30×20×13 cm) with soft wood shavings as bedding. They were provided standard feed from International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B) and water ad libitum. The mice were acclimatized to laboratory conditions for at least one week before the experiment.

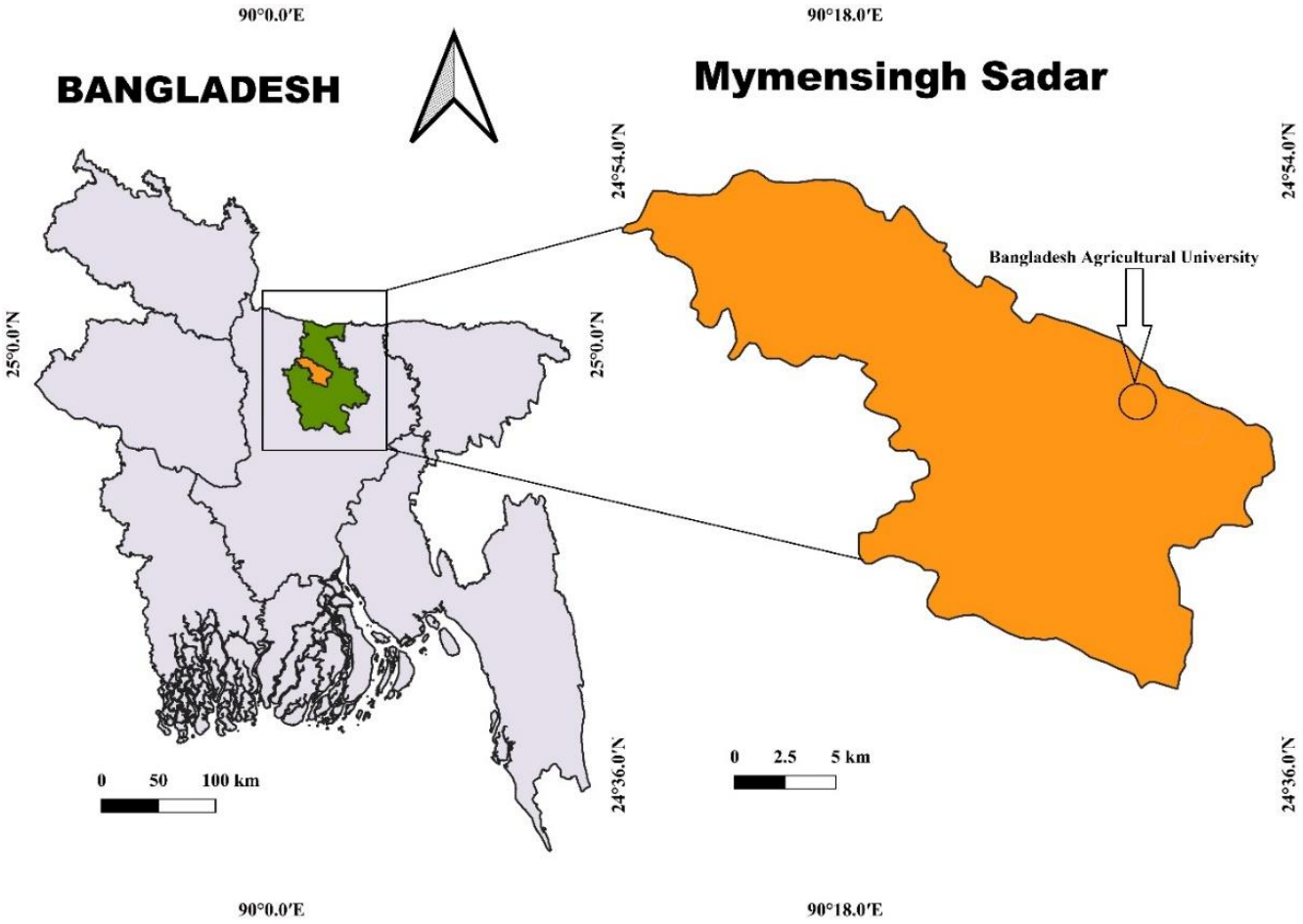

Figure 1. Location of the study area.

2.3. Collection and acclimatization of mice

Twenty-five apparently healthy 7-week-old mice were obtained from ICDDR,B (International Centre for Diarrheal Disease Research, Bangladesh) in Mohakhali, Dhaka. The mice had already been vaccinated by the institute. Upon arrival, the mice were placed in cages, and their initial body weights were recorded. To alleviate transportation stress, they were provided with vitamin C (Rena C) and a 5% glucose solution in drinking water. The mice were acclimatized to laboratory conditions for one week before the experiment.

2.4. Collection of industrial mice feed, feeding and watering

Mice feed was obtained from ICDDR,B and prepared to meet the specific dietary requirements of the mice. The feed was provided twice daily. During the first week, the feed was given in bowls. Each section of the cage was equipped with one feeder and one drinker. Clean, cold, and pure drinking water was supplied twice daily, and feed was also provided twice a day. Both feed and water were available to the mice ad libitum. Feeders and drinkers were washed daily to maintain hygiene.

2.5. Light and biosecurity management

Mice are nocturnal animals, exhibiting peak activity between dusk and dawn. Throughout the experimental period, they were maintained under a 12-hour light and 12-hour dark cycle. A strict biosecurity protocol was implemented both inside and outside the research lab as a crucial measure for disease prevention. Access to the experimental lab was highly restricted.

2.6. Grouping and marking of the mice

Mice were randomly assigned into groups. Each cage housed ten mice, designated as Control (Group A) and Amoxicillin (Group B). To ensure proper identification, each mouse within a group was marked sequentially on the tail with numbers 1 to 10 using a black permanent marker.

2.7. Collection and medication of antibiotics

Amoxicillin (commercial name: Hicomox, generic name: Amoxicillin) was obtained from Opsonin Pharma Limited, Bangladesh. It was supplied in powder form with a strength of Amoxicillin BP 30% and an available pack size of 100 gm. The trade dose was prepared by dissolving 1 gm of powder in 333 mL of water, achieving a concentration of 10 ppm (10 mg Amoxicillin/L water). Group A served as the untreated control and received no medicated water, while Group B was administered amoxicillin at a dose of 10 mg/L drinking water (10 ppm) ad libitum for 365 consecutive days.

2.8. Weight measurement and clinical observation

The mice were weighed weekly, and the data were recorded in a logbook, with the average body weights taken into consideration. At the end of the experiment, the monthly average body weight of each group was presented as mean ± SE. Throughout the study, the mice were closely monitored for clinical signs, including general alertness, feed intake, water intake, locomotion, and mortality, with all observations systematically recorded.

2.9. Sacrificing, sampling, blood and tissue sample collection

At the end of the experiment, the mice were ethically sacrificed for the collection of blood, serum, and tissue samples for various analyses. Blood samples from both the control and antibiotic-treated groups were collected in sterile heparinized and non-heparinized vials and immediately stored in a refrigerator for hematological and enzymatic analyses. Tissue samples, including the liver, kidney, spleen, intestine, breast muscle, and thigh muscle, were carefully collected, washed multiple times with physiological saline to remove clotted blood and debris, and properly labeled. The samples were then stored in zipper bags and preserved at -20 °C for further extraction and analysis.

2.10. Hematological analysis and blood smear examination

White blood cells (WBCs) were analyzed for hematological investigation using manual microscopic classification. Blood samples were collected on the 365th day and transferred to sterile tubes with anticoagulant (1:10 ratio). A thin blood smear was prepared by spreading a drop of blood on a slide at a 30–45° angle, air-dried, and stained with Wright's stain. After rinsing and drying, the smear was examined under 10X for staining quality and 100X with immersion oil for differential leukocyte counting. Data were recorded as mean ± SEM.

2.11. Enzymatic analysis

Mice blood serum was analyzed using the Mispa CXL (Agappe Diagnostics Switzerland GmbH). AST activity was measured by its catalytic reaction with L-Aspartate and α-ketoglutarate, with NADH oxidation monitored at 340 nm. The working reagent was prepared by mixing buffer (R1) and substrate (R2) in a 4:1 ratio. After calibration, 1 mL of reagent and 100 µL of serum were incubated, and absorbance was recorded at 0 and 1-minute intervals for three readings. AST activity was calculated as $\Delta A/min \times 1750 = U/L$. ALT analysis followed a similar procedure, measuring NADH oxidation during the transfer of an amino group from alanine to α-ketoglutarate, catalyzed by ALT.

2.12. Thin Layer Chromatography (TLC) analysis

TLC was performed as described by Islam et al. (2024). Tissue samples (heart, liver, kidney, spleen, thigh muscle, and breast muscle) were stored at -20°C, blended, and 0.5 g was homogenized in 1 mL phosphate buffer (pH 7.2). Proteins were precipitated using 0.25 mL of 30% trichloroacetic acid, followed by centrifugation at 7000 rpm for 20 minutes. The supernatant was extracted with diethyl ether for de-fatation, and after discarding the oily layer, the bottom layer was collected. The extraction was repeated twice, and the extracts were evaporated to dryness before analysis, with each sample tested three times.

2.13. Statistical analysis

Statistical analysis was performed by one-way ANOVA using Graph Pad Prism, version 6. The results were expressed as mean ± standard error mean (SEM).

3. Results

3.1. Long-term exposure of amoxicillin antibiotic residue above the MRL and its effect on body weight and immunity in mice

Control mice displayed a consistent growth pattern, with body weight increasing steadily until the 9th to 10th month, followed by a gradual decline toward the end of the experimental period (Figure 2). In contrast, mice exposed to amoxicillin residues exhibited abnormal growth patterns in body weight. A significant difference ($P < 0.05$) in body weight was observed starting from the 3rd month, which persisted until the end of the experiment. Notably, the highest body weight gain was recorded during the 10th month ($P < 0.01$). These findings suggest that long-term exposure to amoxicillin residues disrupts normal growth patterns and induces body weight abnormalities. The immune system's functionality was assessed by counting various WBC types, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, using microscopy (100X magnification). A slight decrease in neutrophil count was observed in antibiotic residue-exposed mice compared to control mice, though the difference was statistically insignificant. Similar reductions in lymphocyte counts were noted in antibiotic-exposed mice; however, these differences were also statistically insignificant. Counts of monocyte, eosinophil and basophils were marginally higher in antibiotic residue-exposed mice compared to controls, but these differences were not statistically significant.

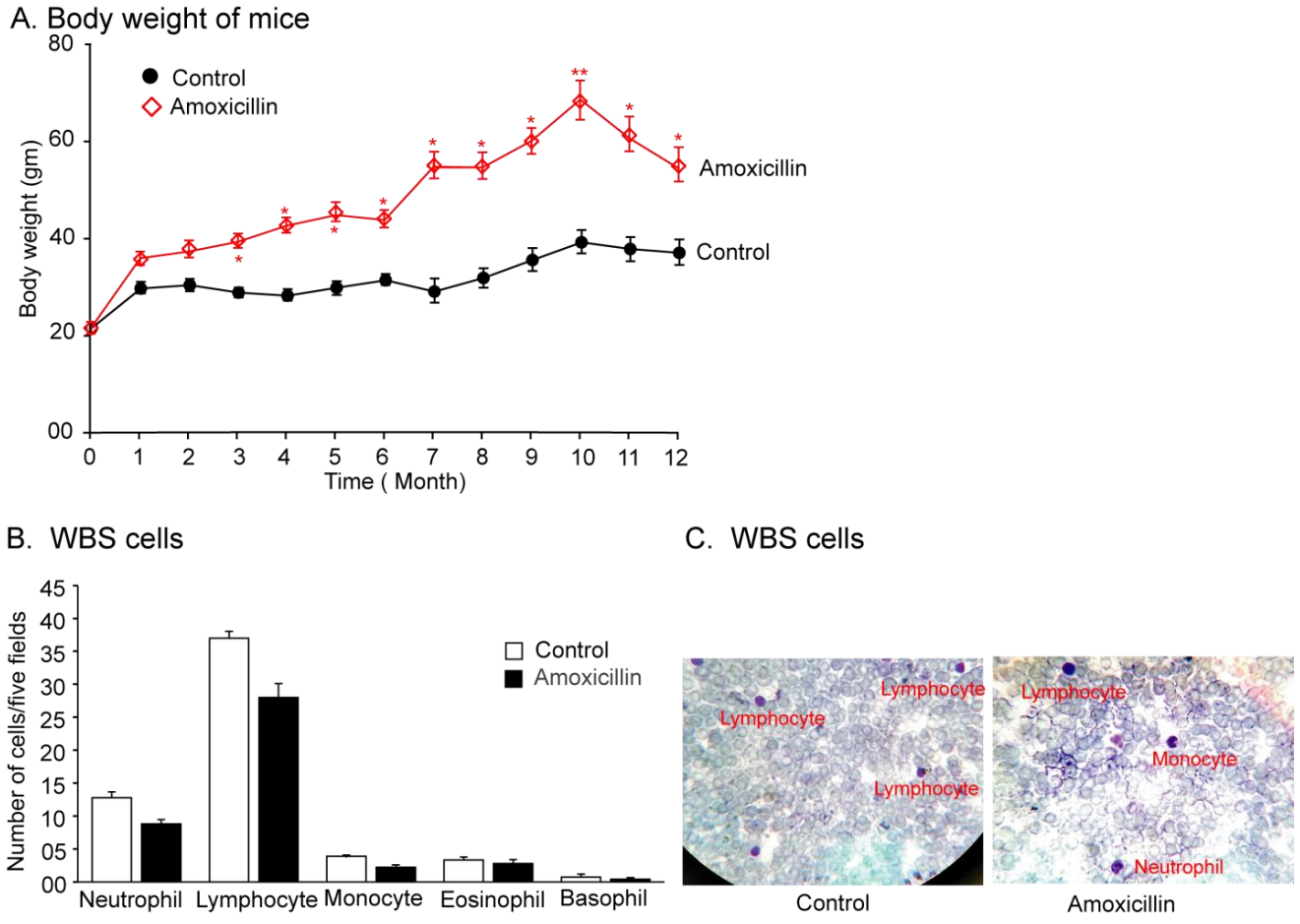

Figure 2. A) The body weight of mice. It represents the body weight of control amoxicillin residue exposure mice. Control mice were supplied drinking water and mice pellet *ab libitum;* amoxicillin (10 ppm) was supplied in drinking water *ab libitum* to treatment groups of mice. B) Blood immune cells count. B demonstrates neutrophil, lymphocyte, monocyte, eosinophil and basophil of control, and amoxicillin residue-induced mice. C) Representative photographs of WBCs of control and amoxicillin residue-induced mice (100X) (n=10) [*, *P*<0.05 and **, *P*<0.01; comparisons between control & amoxicillin. n=10].

3.2. Long term exposure of amoxicillin antibiotics residues above the MRL and its effect on enzymatic analysis SGOT (AST) and SGPT (ALT)

Control mice exhibited AST levels of 65 U/L**,** indicative of normal liver function. In contrast, mice exposed to amoxicillin residues showed elevated AST levels of 88 U/L suggesting potential liver damage or stress induced by prolonged exposure to these antibiotic residues. Similarly, ALT levels in control mice were recorded at 43 U/L**,** while amoxicillin residue-exposed mice demonstrated increased levels of 59 U/L **(**Figure 3). While the elevations in both AST and ALT levels in residue-induced mice did not reach statistical significance, the results strongly suggest that long-term exposure to amoxicillin residues may contribute to liver stress or subclinical hepatic damage.

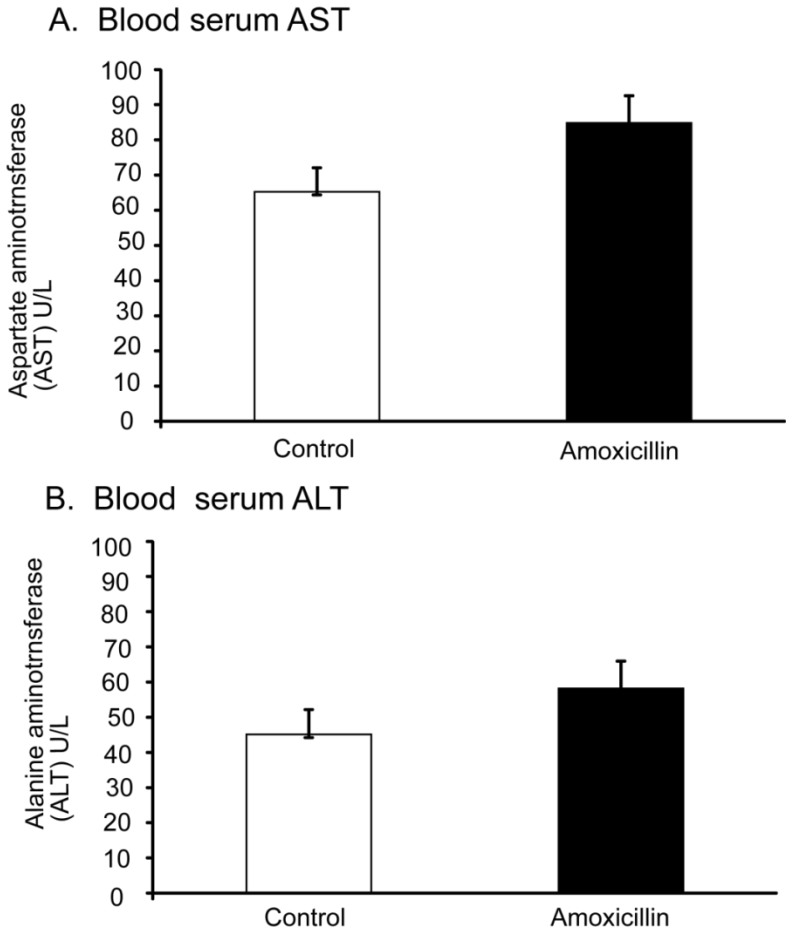

Figure 3. AST and ALT enzyme level in blood serum. A demonstrates blood serum level of AST in control and amoxicillin exposure mice. B demonstrates blood serum ALT of control and Amoxicillin exposure mice respectively. n=10 [*, *P*<0.05 and **, *P*<0.01; comparisons between control & amoxicillin (n=10)].

3.3. Long term exposure of amoxicillin antibiotics residues above the MRL and its effect on histopathology of liver and kidney

Histological examination of liver tissues in control mice showed a normal architecture comprising healthy hepatic cells, intact sinusoids, a well-structured central vein, and functional Kupffer's cells (Figure 4). Conversely, mice exposed to amoxicillin residues demonstrated distinct histopathological changes. Key observations included marked steatosis, an enlarged central vein, and inflammatory cell infiltration into the liver. These findings suggest that prolonged exposure to antibiotic residues can lead to significant structural alterations, potentially impairing liver functionality. The kidneys are essential for maintaining the body's homeostasis by regulating acid-base balance, water and electrolyte levels, blood pressure, erythropoietin production, and waste elimination. Histology of kidney tissues in control mice revealed a normal architecture, including intact glomeruli, Bowman's capsules, and renal tubules. In contrast, kidneys from mice exposed to amoxicillin residues displayed severe abnormalities. This included glomerular atrophy, fragmentation of glomeruli, and inflammatory cells infiltration into kidney.

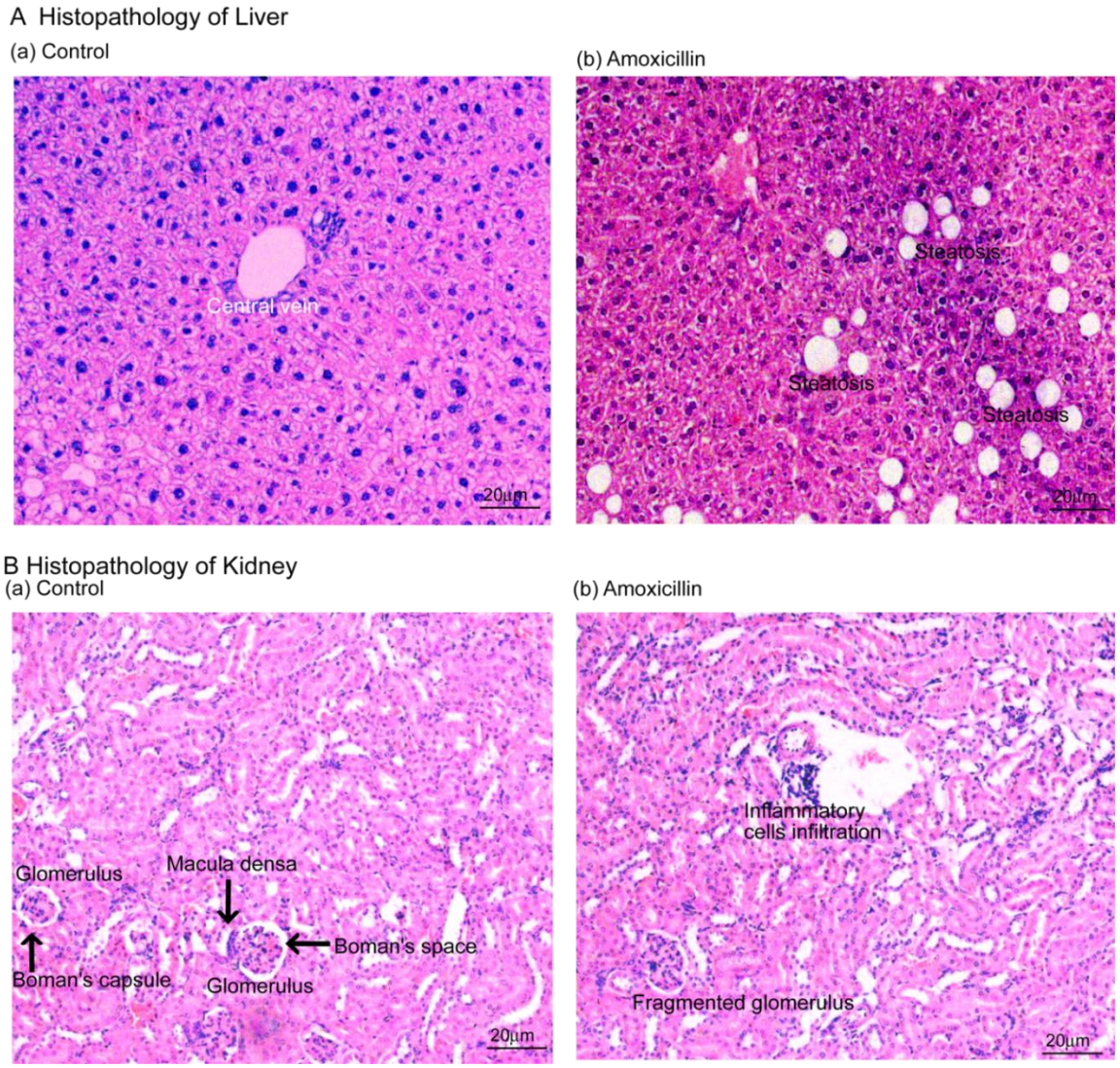

Figure 4. A) Histology of liver. The photomicrographs of liver sections stained with hematoxylin and eosin (scale bar = 20µm), showing (a) control and (c) amoxicillin residue-induced mice; B) Histology of kidney. The photomicrographs of kidney sections stained with hematoxylin and eosin (scale bar = 20µm), (a) control showing normal structure of renal corpuscles with their glomeruli and renal tubules, (b) amoxicillin showing glomerular atrophy, fragmentation of glomeruli inflammatory cell infiltration (n=10).

3.4. Long term exposure of amoxicillin antibiotic residues above the MRL and its residual analysis by TLC

The results revealed the presence of antibiotic residues in liver, kidney, spleen, intestine, breast muscle, and thigh muscle (Figure 5). Residual levels were notably higher in the liver, kidney, and spleen, which are primary organs involved in metabolism and detoxification. The intestine demonstrated moderate residue levels, while the thigh muscle and breast muscle showed relatively lower residue concentrations. These findings suggest that liver and kidney tissues are particularly vulnerable to the accumulation of antibiotic residues, likely due to their roles in filtration and metabolic processing. The presence of residues in edible tissues like thigh and breast muscle, albeit at lower levels, underscores the importance of adhering to withdrawal periods for antibiotics to ensure food safety. This investigation highlights the persistence of amoxicillin residues across multiple tissues in animals subjected to long-term antibiotic exposure. The higher concentrations observed in liver and kidney tissues further affirm their role as primary sites of residue accumulation. To safeguard public health, strict adherence to antibiotic withdrawal periods is essential.

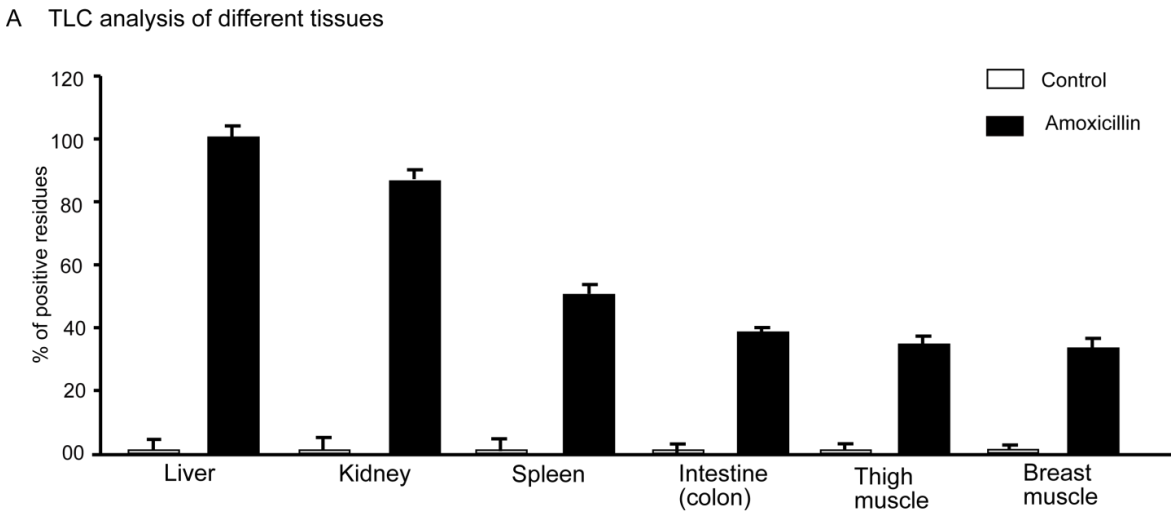

Figure 5. Residual analyses of ciprofloxacin and amoxicillin antibiotic residues in soft tissues in mice. A demonstrates graphical representation of TLC. B demonstrates TLC plate. n=10.

4. Discussion

Antibiotic residues in food chains and animal products have emerged as a critical issue in global public health, with developing countries bearing the brunt of this crisis. The absence of stringent legislation on Maximum Residue Limits (MRLs) for antibiotics, especially in low- and middle-income nations, exacerbates the risks associated with their presence. Despite guidelines provided by the World Health Organization (WHO), Food and Agriculture Organization (FAO), and other relevant authorities, the lack of localized enforcement mechanisms and robust scientific data on antibiotic residues underscores the gravity of the issue. Antibiotics residues in food products infiltrate the human body, interacting with the gut microbiome, which consists of approximately 800-1,000 bacterial species, where 95% are beneficial, and the rest include harmful or opportunistic pathogens (Bacanlı, 2024). This delicate balance is disrupted when antibiotic residues enter the system, potentially reducing beneficial bacteria and enabling harmful pathogens to dominate. This microbiome modulation can have far-reaching effects, including immune dysregulation and the emergence of AMR. Antibiotics have historically been used as growth promoters in livestock, with similar effects observed in humans, particularly pre-pubertal children in middle- and low-income countries (Gough *et al.*, 2014; Ma *et al.*, 2024). Scientific evidence suggests that macrolides, amoxicillin, and fluoroquinolones enhance weight gain in treated individuals, potentially by improving nutrient absorption, modulating immune responses, or increasing appetite (Furlong *et al.*, 2019; Lathakumari *et al.*, 2024). This effect was corroborated by our findings, where long-term exposure to ciprofloxacin and amoxicillin residues above MRL levels significantly increased body weight in mice, with the most pronounced effects observed after three months of continuous exposure.

Fluoroquinolones and amoxicillin are widely prescribed for their broad-spectrum efficacy against infections (Maris *et al.*, 2021). They exert immunomodulatory effects, such as elevated production of cytokines like IL-2 and GM-CSF, and increased white blood cell counts (Lee *et al.*, 2020; Sharma *et al.*, 2024). However, chronic exposure might negatively impact the immune system. Our study found statistically insignificant reductions in neutrophils, lymphocytes, and monocytes, while eosinophils and basophils showed marginally increased levels. These findings hint at subtle immune dysregulation, warranting further investigation into prolonged antibiotic exposure's systemic impacts. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are critical enzymatic markers for evaluating liver function or detecting liver injury. Under normal physiological conditions, these enzymes are confined within liver cells, where they play essential roles in cellular metabolic processes. Liver damage or dysfunction results in the release of AST and ALT into the bloodstream, with elevated levels serving as indicators of hepatic pathology. The liver, a primary organ for metabolism and detoxification, is particularly vulnerable to antibiotic-induced toxicity. Elevated levels of AST and ALT in ciprofloxacin- and amoxicillin-exposed mice indicated hepatocellular damage. Histopathological analysis revealed structural abnormalities such as steatosis, central vein enlargement, and inflammatory cell infiltration (Ajdacic-Gross *et al.*, 2021; Middleton *et al.*, 2024). Similarly, the kidney, another crucial organ for excretion and homeostasis, exhibited marked architectural deviations under prolonged antibiotic exposure. Histological changes included glomerular atrophy and inflammatory cell infiltration. These pathological findings underscore the need for careful antibiotic usage and highlight the potential for long-term exposure to compromise renal

function (Bülow and Boor, 2019). While the spleen plays a pivotal role in immune regulation, chronic antibiotic exposure demonstrated no significant pathological alterations in splenic architecture in our study. However, the slight reduction in lymphocyte counts could imply immunosuppression, a subtle yet concerning outcome of prolonged antibiotic residue exposure. Antibiotics residues are more likely to contribute to AMR development rather than direct structural damage to the body, as indicated by our findings.

Our investigation revealed that antibiotic residues predominantly accumulate in the liver and kidneys, with lower levels detected in thigh and breast muscles. These findings align with previous reports (Sattar *et al*., 2014; Metli *et al*., 2015; Sarker *et al*., 2018), emphasizing the role of these organs in metabolizing and excreting antibiotics. Such residue accumulation underscores the importance of adhering to withdrawal periods for antibiotics in livestock to minimize human exposure through food products. Antibiotic residues present a multi-dimensional threat, encompassing public health risks, environmental contamination, and the propagation of AMR. Non-judicious antibiotic use, whether for growth promotion or therapeutic purposes, has created a feedback loop of resistance and toxicity. The potential for residues to induce subtle yet significant changes in organ function, immune response, and microbial balance calls for immediate regulatory and scientific attention.

5. Conclusions

The findings from our study underscore the pressing need for judicious antibiotic use and stricter residue monitoring protocols. While antibiotics are indispensable in modern medicine, their misuse in agriculture and healthcare poses a significant threat to human health and the environment. By implementing a multi-faceted approach encompassing regulation, education, and research, we can mitigate the hazards of antibiotic residues and safeguard public health. The path forward demands coordinated action, informed decision-making, and a commitment to sustainable practices in antibiotic usage.

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Data availability

The data presented in this study are contained in this manuscript.

Conflict of interest

None to declare.

Authors' contribution

Md. Shafiqul Islam: conceptualization, supervision, reviewing and editing, funding; Md. Shakil Islam: methodology, data analysis, draft writing; Saiful Islam: methodology and data analysis; Md. Ashraful Alam: laboratory animal management, sample collection. All authors have read and approved the final manuscript.

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