AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Nephrotoxic and Hematotoxin Effects of Sublethal Isoniazid Administration in Albino Rats

Duha K. Qanbar [1], Jaafar B. Algburi [2]

[1,2] *Department of ecology, Faculty of Science, University of Kufa, Kufa, Iraq.*

Article history
Received: 15/7/2025
Revised: 14/8/2025
Accepted: 25/8/2025

*Corresponding Author: *Jaafar Algburi, Department of ecology, Faculty of science,* University of kufa, Kufa, Iraq; Email: jaferb.algabory @uokufa.edu.iq

Abstract: Isoniazid, a synthetic antibiotic developed in 1952, remains a cornerstone of first-line tuberculosis treatment. Its effectiveness and specificity against Mycobacterium tuberculosis have made it a vital medication for decades. Using probit analysis, the LD50 of isoniazid was determined to be 1500 mg/kg. For the chronic exposure study, a sublethal concentration of 150 mg/kg (10% of the LD50) was administered for 30 days. The experimental design involved two groups—a control group and a treatment group—each with three replicates, and five rats per replicate. Hematological and nephrotoxic parameters were evaluated at three time points: after 1, 15, and 30 days. The study's findings revealed a significant decrease ($p<0.05$) in WBC count was observed on first day, but this change was no longer significant on days 15th and 30th. The RBC count showing no significant alterations. A significant decrease in hemoglobin was recorded on 15th day, while no significant changes were noted on days first and last day. Platelet counts showed a non-significant alter. A significant decrease ($p<0.05$) in MCV was observed on the last day of the experiment. while, the kidney functions results show, a significant elevation in urea levels was consistently recorded throughout the experiment, also, creatinine value shows a significant increase in last days and non-significant increase in the 1st and 15th days of experiments. suggesting potential nephrotoxic effects.

Keywords: Isonziad, Hematological markers, kidney functions, Sublethal effects, Albino rats

1. Introduction

The negative effects of human activities with the end of the 20th century on the environment had reached a dangerous point, creating imbalances that threaten both human health and the ability of the planet to support life. In many cases, these effects have gone beyond what natural systems can handle [1]. The environment, acting somewhat like the body's natural ability to stay balanced, works to support life by protecting a variety of resources, both those that can be renewed and those that cannot [2].

Isoniazid (INH), a synthetic antibiotic, has been a key treatment for tuberculosis since 1952, known for its strong and specific action against Mycobacterium tuberculosis [3; 4]. To ensure effective patient care, pharmaceutical analysis and therapeutic drug monitoring of INH in both drug formulations and biological samples are vital for understanding its bioavailability and bioequivalence [5].

However, despite its effectiveness, INH carries considerable risks for non-target species, especially terrestrial mammals. For example, studies in male albino mice have shown that INH treatment can cause hepatotoxicity, damaging liver structures [6], and may

also have immunosuppressive effects [7]. In rats, isoniazid has been linked to various adverse effects, including hematological changes, nephrotoxic dysfunction, and neurotoxicity, due to its bioaccumulation and long-term environmental persistence. Specifically, isoniazid can disrupt hematological parameters such as white blood cell (WBC) count, platelet (PLT) count, and hemoglobin (HGB) levels [8].

According to a review of the literature, Isoniazid-induced kidney damage is a notable cause of acute renal failure, with reported incidence rates ranging from 1.8% to 16% [9]. This condition has a high mortality rate, with some studies indicating an overall mortality of 18%, When renal injury is severe, it is often accompanied by other serious complications, including thrombocytopenia, immune hemolytic anemia, and intravascular hemolysis [10]. This highlights the critical need to evaluate chronic exposure to sub-lethal doses of environmental contaminants, such as pharmaceutical waste, to fully comprehend their cellular damaging mechanisms and associated risks [11].

2. Methodology

Rattus norvegicus var albinus, or albino rats, weighed 150 ± 5g. To become used to the new conditions, rats were brought to the lab in cages and kept in several plastic containers for 14 days. The temperature in the lab stayed at 19 ± 2 °C [12]. The rats were fed commercial rat food during the acclimation and testing period [13]. We conducted a static acute toxicity test after 14 days of acclimatization [14]. The 99% pure isoniazid that was sold by the General Company for the Manufacture of Medicines and Medical Supplies / Samarra was used to create isoniazid test solutions. To determine LD50 values, rats were exposed to six different isoniazid concentrations, including a control group (0, 250, 500, 1000, 2000 and 4000 mg/kg). Mortality rates were recorded at 24, 48, 72, and 96-hours' post-exposure. The LD50 value was subsequently calculated using Probit analysis [15]. The sub-lethal toxicity test was conducted for 30 days. Rats were randomly divided into two groups, with three replicates per group and five individuals per replicate (total of 15 rats per group). The experimental groups were exposed to isoniazid at one sub-lethal concentrations: 150 mg/kg (10% of the determined LD50), These concentrations were selected to evaluate the chronic effects of isoniazid exposure. The blood was collected 3 time through experiment period in (1,15,30) days of experiment for physiological parameters [16].

The auto hematology analyzer was used to obtain all the hematological parameters results [16].

The kidney function marker, urea and creatinine, were determined using commercial diagnostic kits on an automated biochemical analysis (Fujifilm) [17].

3.Results and discussion

The acute toxicity assessment revealed a time-dependent increase in isoniazid lethality in albino rats (Rattus norvegicus var. albinus), The calculated 96-hour LD50 value for albino rats was 1500 mg/kg.

Red blood cells count (RBCs)

The results of RBC count show the highest value was $(7.22 \pm 0.15) \times 10^6$ /µL in the last day of experiment and the lowest value was $(6.64 \pm 0.04) \times 10^6$ /µL in the 15th day of experiment compare with control groups. The results show there are a slight non-significant increase in RBC value through all experiment day, figure (1).

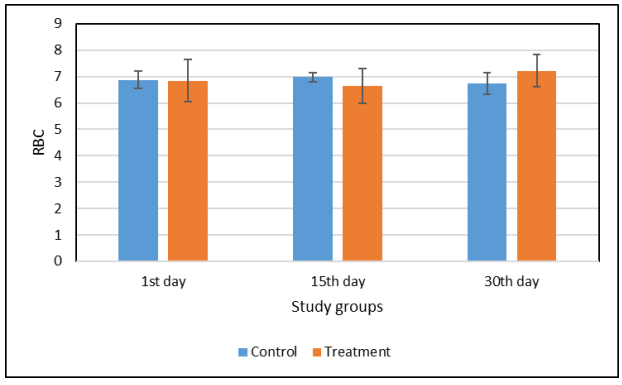

Fig 1: The total count of RBC of *Rattus norvegicus* after exposure to sub lethal concentration of Isoniazid.

There are many studies that indicates the isoniazid treatment groups can negatively impact red blood cell production. Specifically, one study demonstrated that

DOI: https://doi.org/10.36320/ajb/v17.i3.21052

administering isoniazid at a daily dose of 100 mg/kg for 30 days led to a significant reduction in red blood cell counts. This finding suggests that isoniazid may either suppress the formation of new red blood cells (hematopoiesis) or increase the destruction of existing red blood cells (red cell lysis) [18]. Further evidence from another study supports this observation, showing a significant decrease in red blood cell counts in rats given a combination of isoniazid and rifampicin. This consistent finding across studies reinforces the notion that these anti-tuberculosis drugs can have an adverse effect on the body's ability to maintain healthy red blood cell levels [19].

White blood cells (WBC)

The white blood cell (WBC) count result indicates a significant reducing in the first day of experiment, the value was (3.15 ±1.6) * 103 /µL. while there is non-significant decreasing in the 15th and 30th day of experiment when compared with control groups.

This reduction was statistically significant when compared to initial values (0.021), suggesting that the treatment effectively lowered WBC counts. By day thirty, WBC levels showed a partial recovery, reaching (11.58 ± 1.24) * 103 /µL. however, they did not return to the initial day one levels figure (2).

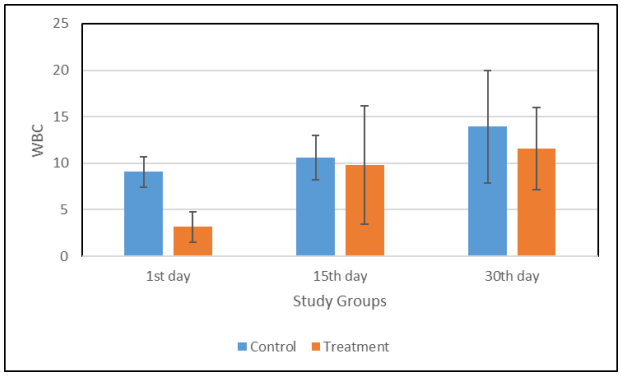

Fig 2: The total count WBC of Rattus norvegicus after exposure to sub lethal concentration of Isonzaide.

The temporary and mild reduction in white blood cell (WBC) counts observed in this study supports the hypothesis of isoniazid-induced leukopenia, a phenomenon well-documented in clinical settings by [20]. This finding aligns with [21] observations regarding the potential ecological impact of anti-tuberculosis drugs on organisms in aquatic ecosystems. Furthermore, the notion that the WBC decrease was linked to treatment exposure is bolstered by broader research, such as that by Daughton and [22], which confirms isoniazid's immunosuppressive properties at certain concentrations in environmental water.

Hemoglobin estimation (Hgb)

The hemoglobin count result was highest value on 1st day (12.70 ±1.4 g/dL). These levels then significantly decreased, reaching their lowest value on 15th day (11.03 ±0.7 g/dL). This notable drop from baseline suggests that the treatment effected hemoglobin concentration during the study, this reduction was statistically significant with a value was (0.007) in 15th day. By last day, hemoglobin count levels showed a partial recovery figure (3).

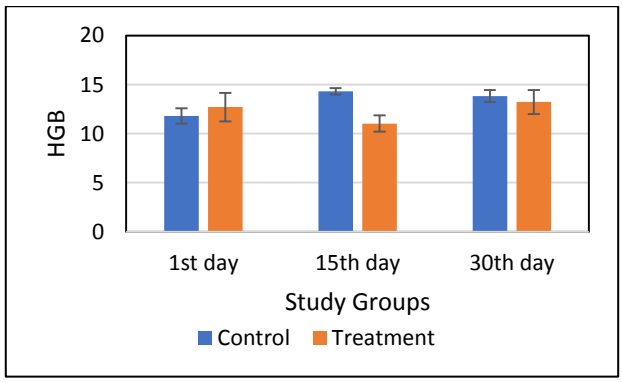

Fig 3: The Hemoglobin (Hb) of Rattus norvegicus after exposure to sub lethal concentration of Isonzaide.

The present study's findings, demonstrating a significant decrease in hemoglobin concentration (from 12.70±1.4 g/dL on day 1 to 11.03±0.7 g/dL on day 15 (P=0.007)), align with existing research on isoniazid's hematological effects. This observed is directly supported by [23], who reported isoniazid-induced anemia in rats. Furthermore, clinical reports by [20], confirm that isoniazid can lead to erythroblastosis or megaloblastic anemia in certain patients. This pattern also resonates with data from [24],

who documented decreased hemoglobin concentrations in tuberculosis patients during intensive treatment. Collectively, these results suggest that isoniazid may cause a temporary yet significant reduction in hemoglobin concentration during the initial phase of treatment.

platelet count (PLT)

The result of platelet counts showed exhibited an increasing pattern. On first day, the lowest value was recorded at (459.67) and platelet counts gradually increases, reaching to the higher of value (675.67) on day 15, and this elevated level was sustained through day 30. These significant differences indicate that the treatment promoted an increase in platelet count throughout the study duration.

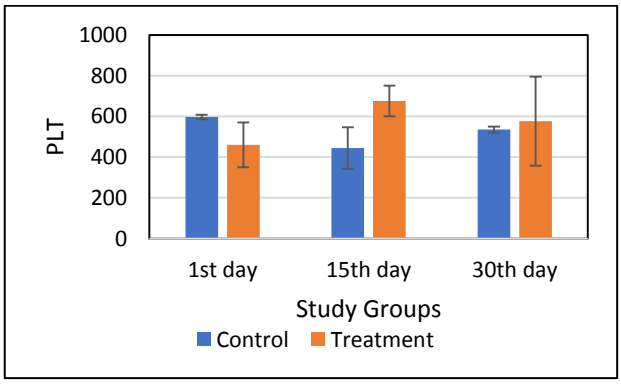

Fig 4: The Platelet (PLT) of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide.

The current study's findings, a notable rise in platelet counts after intensive treatment. This finding is consistent with earlier research, such as that by [26], which observed a similar frequency of elevated platelet counts during comparable exposures. This pattern resembles reactive thrombocytosis, a condition often seen in clinical studies and typically interpreted as a temporary inflammatory response [25]. These observations collectively support the idea that activated platelets circulating in the bloodstream are an important part of the body's immune response to tuberculosis [25]. Therefore, our results suggest that the changes we saw in platelet count are not just a side effect, but rather an indication of shifts in how the body's inflammation and immune system are working.

Mean corpuscular volume (MCV)

The result of highest value (MCV) was observed on first day (54.4 ± 6.0) and MCV gradually decreases reaching its lowest value on last day (53.17 ± 0.7). This sustained decrease indicates that the treatment significantly contributed to a reduction in MCV over time, with statistically significant differences noted particularly on days 15th and 30th compared to baseline values.

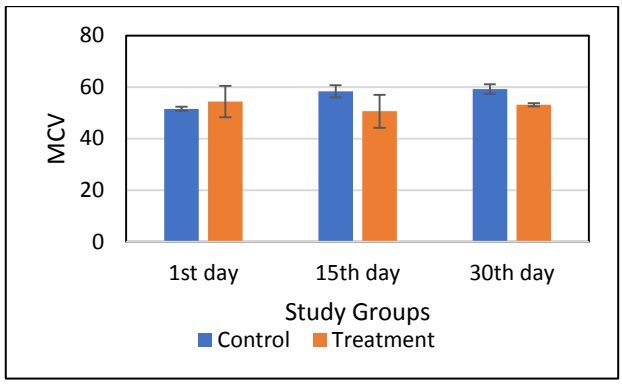

Fig 5: The mean corpuscular volume (MCV) of *Rattus norvegicus* after exposure to sub-lethal concentration of Isonzaide.

The initial slight increase in MCV on the 1st day could be a temporary effect of the compound on bone marrow or red blood cell maturation, possibly affecting the rate of cell division. However, the subsequent decrease in MCV on the 15th and 30th days is a more significant finding. A low MCV, known as microcytosis, indicates that the red blood cells are smaller than normal [27]. This is often associated with conditions such as iron deficiency anemia, thalassemia, or chronic diseases. The sustained drop in MCV in the treatment group suggests that the pharmaceutical compound may be interfering with iron metabolism, hemoglobin synthesis, or the production of red blood cells over a prolonged period. This indicates a potential adverse hematological effect of the compound that requires further investigation [28].

Serum Creatinine

Creatinine results remained largely consistent throughout the study. Values ranged from (0.40 ± 0.08) on 1st day to (0.42 ± 0.05) on the last day, with the lowest

DOI: https://doi.org/10.36320/ajb/v17.i3.21052

measurement occurring on 15th day (0.29 ± 0.1). Statistical analysis revealed no significant differences in the 1st and 15th day, there are a significant alter in the last day of experiment, indicating that the treatment did not significantly impact creatinine levels, just in the last day was positive effects as show in figure (6).

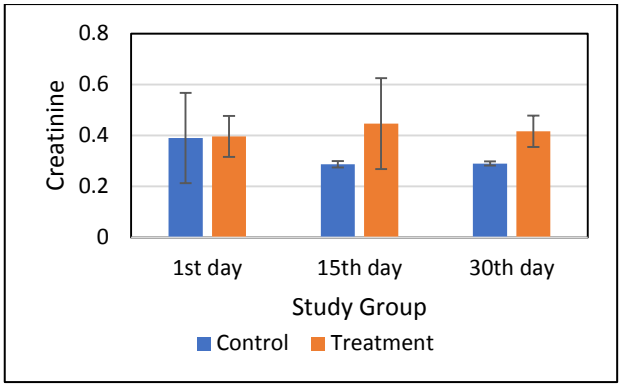

Fig 6: Creatinine of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide.

Creatinine is a waste product of muscle metabolism that, like urea, is filtered from the blood by the kidneys. A rise in blood creatinine levels is a widely accepted and specific marker of impaired kidney function [29]. The initial similarity of the two groups on day 1 confirms that the experiment started with a consistent baseline. The subsequent increase in creatinine levels in the treatment group from 15th day to 30th day is a strong indication of a nephrotoxic effect from the pharmaceutical compound [30]. The compound likely damages the nephrons, reducing the kidneys' ability to filter waste from the blood, leading to the accumulation of creatinine. The fact that the creatinine levels in the control group remained stable and even slightly decreased to a lower, healthy baseline is crucial. This demonstrates that the observed rise in the treatment group is directly attributable to the drug and not due to other external factors. The large error bar for the treatment group on day 15 suggests that the rats may be responding differently to the compound, with some experiencing more severe kidney damage than others. By day 30, while still elevated, the variability has decreased, suggesting a more uniform progression of kidney impairment [31].

Urea

The result of urea showed on the first day the mean urea value was 47.7. This high value to 54.1 by the fifteenth day, and by the thirtieth day, it reached to highest value at 84.9. This observed increase over time strongly suggests a direct effect of the treatment level in elevating urea concentrations. significant increase in urea levels (0.001) throughout the study period as in figure (7).

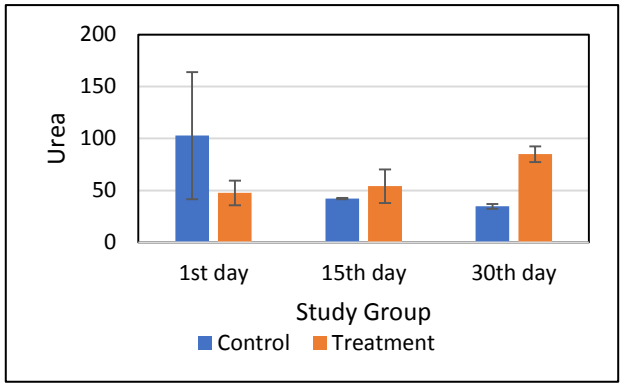

Fig 7: Urea of Rattus norvegicus after exposure to sub-lethal concentration of Isonzaide.

In mammals, including rats, urea is a waste product of protein metabolism that is filtered from the blood by the kidneys and excreted in urine. Therefore, elevated urea levels, a condition known as azotemia, are a common indicator of impaired kidney function or renal toxicity [32]. The initial high variability in the control group on the first day may be due to factors such as diet, hydration status, or stress before the study began. By 15th day, both groups seem to have adapted to the study conditions, as their urea levels become more consistent. The divergence between the two groups on the last day is a critical piece of evidence. The gradual increase in urea in the treatment group, culminating in a high value by the end of the study, strongly suggests that the pharmaceutical compound is toxic to the kidneys [33, 36]. The drug likely interferes with the kidneys' ability to filter urea from the blood, causing it to accumulate. This finding warrants further investigation into the specific mechanism of action and the potential for long-term renal damage [34]. The stable or decreasing urea levels in the control group confirm that the observed increase in the treatment group is a direct result of the compound, not a general change in the rats' health over the study period. This data would be a major concern in the pre-clinical safety assessment of this compound [35].

DOI: https://doi.org/10.36320/ajb/v17.i3.21052

Conclusion

In conclusion Isonzaide has a harmful effect on the blood parameters after exposure to sub-lethal concentration, which was vividly clear on the results and make a renal damage.

Acknowledgement

We would like to thank the chief of Ecology Department in Kufa University for their Advice and support on this research

Funding Information

The department of ecology, faculty of science, university of kufa.

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017).

References

1. Steffen, W., Persson, Å., Deutsch, L., Zalasiewicz, J., Williams, M., Richardson, K., ... & Svedin, U. (2011). The Anthropocene: From global change to planetary stewardship. Ambio, 40(7), 739-761.
2. Schroeder, H. W. (2021). Ecology of the heart: Understanding how people experience natural environments. In Natural Resource Management (pp. 13-27). Routledge.
3. Gegia, M., Winters, N., Benedetti, A., van Soolingen, D., & Menzies, D. (2017). Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. The Lancet Infectious Diseases, 17(2), 223-234.
4. Dartois, V. A., & Rubin, E. J. (2022). Anti-tuberculosis treatment strategies and drug development: challenges and priorities. Nature Reviews Microbiology, 20(11), 685-701.
5. Sen, K. K., Sinha, D., Nayak, A. K., & Sen, S. O. (2024). Contribution of biopharmaceutics and pharmacokinetics to improve drug therapy. In Physico-Chemical Aspects of Dosage Forms and Biopharmaceutics (pp. 231-249). Academic Press.
6. Torfs, E. (2019). In vitro biological investigation of novel anti-tubercular compound classes and the development of improved research tools. University of Antwerp.
7. Brindha, P. (2016). Role of phytochemicals as immunomodulatory agents: A review. International Journal of Green Pharmacy (IJGP), 10(1).
8. Preziosi, P. (2007). Isoniazid: metabolic aspects and toxicological correlates. Current drug metabolism, 8(8), 839-851.
9. Singh NP, Ganguli A, Prakash A. Drug-induced kidney diseases. J Assoc Physicians India 2003; 51:970-9.
10. Covic A, Goldsmith DJ, Segall L, Stoicescu C, Lungu S, Volovat C, et al. Rifampicin-induced acute renal failure: a series of 60 patients. Nephrol Dial Transplant 1998; 13(4):924-9.
11. Muzika, V., Custovic, S., Mornjakovic, Z., Cosovic, E., & Kapic, D. (2016). Histological study of isoniazid-rifampicin related nephrotoxicity in Wistar rats. Folia Medica Fac Med Univ Saraeviensis, 51(1), 4-9.
12. de Boer, S. F., & Koolhaas, J. M. (2024). The laboratory rat. The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, 379-399.

13. Hussein A. Mohammed., & Jaafar B. Algburi (2025). An Investigation into the Hematotoxin Effects of Sublethal Diazinon Doses in Albino Rats (Rattus norvegicus var. albinus). Al-kufa journal for biology, 2025, vol. 17 issue: 2, page 61-67 DOI: 10.36320/ajb/v17.i2.19508.
14. Yuan, G., Dai, S., Yin, Z., Lu, H., Jia, R., Xu, J., ... & Zhao, X. (2014). Toxicological assessment of combined lead and cadmium: Acute and sub-chronic toxicity study in rats.
15. Finney, D. J. (1971). Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of Information in Medicine, 10(04), 237–245.
16. Algburi, J. B., & AL-Amari, M. J. (2023, July). The LC50 of Diazinon and sub-lethal concentration effect of it on hematological properties in Cyprinus Carpio fish. in 4th international scientific conference of engineering sciences and advances technologies (Vol. 2830, No. 1, p. 050009). AIP Publishing LLC.
17. Tanabe, J., Ogura, Y., Nakabayashi, M., Nagai, Y., Watanabe, S., Sugaya, T., ... & Kamijo-Ikemori, A. (2019). The possibility of urinary liver-type fatty acid-binding protein as a biomarker of renal hypoxia in spontaneously diabetic Torii fatty rats. Kidney and Blood Pressure Research, 44(6), 1476-1492.
18. Ben Said, D., Dahmani, I., Ben Ali, R., Bassem, H., El Fekih, M., Chedly, A., ... & El Aidli, S. (2022). Toxicological evaluation of oral exposure to isoniazid: behavioral, biochemical, and histopathological assessments in rats. Drug and Chemical Toxicology, 45(6), 2594-2600.
19. Abdel-Ghaffar, O., Mahmoud, S. T., Said, A. A., & Sanad, F. A. A. Y. (2018). Ameliorative effect of rutin against isoniazid-induced alterations in certain hematological and biochemical parameters of albino rats.
20. Shishido, Y., Nagayama, N., Masuda, K., Baba, M., Tamura, A., Nagai, H., ... & Yotsumoto, H. (2003). AGRANULOCYTOSIS DUE TO ANTI-TUBERCULOSIS DRUGS INCLUDING ISONIAZID (INH) AND RIFAMPICIN (RFP) A Report of Four Cases and Review of the Literature. Kekkaku (Tuberculosis), 78(11), 683-689.
21. Chen, W. Y., Chen, Y. F., Tsai, J. M., Huang, H. M., & Su, Y. C. (2023). Epidemiology-based wastewater monitoring for ecological risks of anti-tuberculosis drugs mixture effects. Science of The Total Environment, 892, 164560.
22. Talhar, S. S., Waghmare, P. J., Ambulkar, P. S., Waghmare, J. E., Pal, A. K., & Narang, P. (2021). Assessment of oxidative stress biomarkers and body mass index in pulmonary tuberculosis patients: A case-control study. Nigerian Medical Journal, 62(3), 122-126.
23. Adebimpe, W. O., Faremi, A. O., & Nassar, S. A. (2015). Effects of treatment on free radicals in patients with pulmonary tuberculosis in South Western Nigeria. African Health Sciences, 15(4), 1256-1261.
24. Ashenafi, G., Tibebu, M., Tilahun, D., & Tsegaye, A. (2023). Immunohematological Outcome Among Adult HIV Patients Taking

Highly Active Antiretroviral Therapy for at Least Six Months in Yabelo Hospital, Borana, Ethiopia. Journal of Blood Medicine, 543-554.

25. Kirwan, D. E., Chong, D. L., & Friedland, J. S. (2021). Platelet activation and the immune response to tuberculosis. Frontiers in Immunology, 12, 631696.
26. Bao, S., Xia, W., Xu, S., Li, Y., Lu, B., Wu, C., ... & Zhou, A. (2020). Multiple metal exposure and platelet counts during pregnancy: A repeated measure study. Environment International, 136, 105491.
27. Sandhya, I., & Muhasin, T. (2014). Study of RBC histogram in various anemias. Journal of Evolution of Medical and Dental sciences, 3(74), 15521-15535.
28. Putra, P. M. (2018). Effect of anti-tuberculosis multi drug resistance regimen on hematological lung tuberculosis patients profile with multi drug resistance. Berkala Kedokteran, 14(1), 59-68.
29. Avila, M., Mora Sánchez, M. G., Bernal Amador, A. S., & Paniagua, R. (2025). The metabolism of creatinine and its usefulness to evaluate kidney function and body composition in clinical practice. Biomolecules, 15(1), 41.
30. Sales, G. T. M., & Foresto, R. D. (2020). Drug-induced nephrotoxicity. Revista da Associação Médica Brasileira, 66, s82-s90.
31. Caravaca-Fontán, F., Azevedo, L., Luna, E., & Caravaca, F. (2018). Patterns of progression of chronic kidney disease at later stages. Clinical kidney journal, 11(2), 246-253.
32. Kumahor, E. K. (2023). The biochemical basis of renal diseases. In Current trends in the diagnosis and management of metabolic disorders (pp. 185-200). CRC Press.
33. Faria, J., Ahmed, S., Gerritsen, K. G., Mihaila, S. M., & Masereeuw, R. (2019). Kidney-based in vitro models for drug-induced toxicity testing. Archives of toxicology, 93(12), 3397-3418.
34. Aprianto, A., Sudarsono, T. A., Wardani, D. P. K., & Rahaju, M. (2022). Comparison of Ureum and Creatinine Levels in Patients with Pulmonary Tuberculosis in the Treatment Phase 0 and 6 Months. Jurnal Ilmu dan Teknologi Kesehatan, 10(1), 14-24.
35. Rathi, M., Bharati, J., Adarsh, M. B., Nada, R., Sharma, K., Sharma, A., & Gupta, K. L. (2020). A Young Female with Fever and Proteinuria. Journal of Postgraduate Medicine, Education and Research, 54(1), 12-14.
36. Wibowo, W., Mulia, Y. S., Sunarno, S., Su'udi, A., Putranto, R. H., Kusumawati, K., & Renitia, G. I. (2023). Analisis Kadar Ureum, Creatinin, Dan Asam Urat Pada Oat Tb-Paru. Jurnal Riset Kesehatan Poltekkes Depkes Bandung, 15(2), 442-449.