

## ORIGINAL ARTICLE

**A Laboratory-Based Experimental Study on the Protective Effects Against Isoniazid-Induced Hepatotoxicity in Mice**Ikram Ullah Khan<sup>1\*</sup>, Akbar Waheed<sup>1</sup>, Wasi Ullah Khan<sup>1</sup>, Junaid Aslam<sup>1</sup>, Malik Sikandar Mehmood<sup>1</sup>, Barkat Ullah Khan<sup>2</sup>**ABSTRACT****Objective:** To evaluate the role of Alpha Lipoic Acid (ALA) as a potential antidote to isoniazid-induced hepatitis.**Study Design:** Laboratory-based experimental animal study.**Place and Duration of Study:** The study was conducted at the Department of Pharmacology, Army Medical College, Rawalpindi and The National Institute of Health, Islamabad, Pakistan from January 2020 to June 2021. The study was designed and reported in compliance with the ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines.**Methods:** A total of 90 adult male healthy mice were obtained from NIH and were kept at the animal house of NIH, Islamabad under standard conditions with a daily photoperiod of 12 hours light and 12 hours dark at temp 22–30°C. The mice were acclimatized to the laboratory conditions for 1 week, prior to experimentation, and were provided with a standard diet and water *ad libitum*. Male mice having a weight of 30–50 grams were included in the study. Mice having deranged liver function tests at the start of the experimentation were excluded. Mice were equally divided into three groups. In group A, hepatotoxicity was induced by administration of 100 mg/kg of oral isoniazid, which was indicated by elevated liver enzymes and changes in histo-pathological parameters. Alpha-lipoic acid (ALA) was administered orally at 50mg/kg 1 hour prior to isoniazid for 28 days. Animals were sacrificed on the 29<sup>th</sup> day, and samples of blood and liver were taken for biochemical and histopathological analysis.**Results:** Results of this study indicated that ALA offered protection against isoniazid-induced hepatotoxicity by reducing levels of bilirubin and liver enzymes in the serum. Hepato-protection was further evident from the preservation of liver architecture, which was indicated by reduced necrosis, steatosis, and portal inflammation.**Conclusion:** Alpha-lipoic acid (ALA) proved to be effective in protecting against isoniazid-induced hepatotoxicity.**Keywords:** Alpha Lipoic Acid, Hepatitis, Isoniazid.**How to cite this:** Khan IU, Waheed A, Khan WU, Aslam J, Mehmood MS, Khan BU. A Laboratory-Based Experimental Study on the Protective Effects Against Isoniazid-Induced Hepatotoxicity in Mice. *Life and Science*. 2025; 6(4): 471-476. doi: <http://doi.org/10.37185/LnS.1.1.784>This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license. (<https://creativecommons.org/licenses/by-nc/4.0/>). Non-commercial uses of the work are permitted, provided the original work is properly cited.**Introduction**

Tuberculosis (TB) is one of the significant causes of morbidity and mortality, caused by an acid-fast

bacillus called *Mycobacterium tuberculosis*. Effective diagnosis and treatment of TB has a pivotal role in preventing the mortality and morbidity associated with TB.<sup>1,2</sup>

According to the WHO treatment guidelines 2022, the standard regimen for new cases of TB consists of two phases: the intensive phase and continuation phase. The intensive phase includes administration of four drugs consisting of isoniazid (INH), Rifampicin (RMP), pyrazinamide (PZA), and ethambutol for two months, while the continuation phase consists of administration of INH and RMP for 4 months. The overall success of this regimen is more than 80%

<sup>1</sup>Department of Pharmacology

Army Medical College, Rawalpindi, Pakistan

<sup>2</sup>Department of Physiology

Mohi-ud-Din Islamic Medical College, Mirpur AJK, Pakistan

Correspondence:

Dr. Ikram Ullah Khan

Assistant Professor, Pharmacology

Army Medical College, Rawalpindi, Pakistan

E-mail: [ikramullah2481@yahoo.com](mailto:ikramullah2481@yahoo.com)Received: Aug 02, 2024; 1<sup>st</sup> Revision Received: Mar 25, 20252<sup>nd</sup> Revision Received: Sep 20, 2025; Accepted: Sep 23, 2025

globally.<sup>3</sup> More than 50 million lives have been saved in the last 15 years due to effective antituberculosis treatment (ATT).<sup>4</sup>

Compliance, an essential factor in determining the overall success of ATT, is limited by the variety of adverse drug reactions (ADRs) associated with ATT. One of the major ADRs of ATT is drug-induced hepatotoxicity, having an incidence rate of 2-28% with a mortality of 0.2%.<sup>5</sup> Incidence of hepatotoxicity among ATT drugs is highest with INH (56%), followed by RMP (34%) and PZA (10%).<sup>6,7</sup>

INH-induced hepatotoxicity is caused by the conversion of INH to toxic metabolites by hepatic enzymes N-acetyl transferase 2 (NAT2) and cytochrome P450 2E1 isoform (CYP2E1).<sup>8</sup> These toxic metabolites cause hepatic damage via covalent bonding, oxidative stress and metabolic dysfunction.<sup>8</sup> Multiple risk factors responsible for increased incidence of hepatotoxicity have been identified, such as elderly patients, females, malnutrition, and a history of liver disease.<sup>9</sup> Since metabolism of the drug to toxic metabolites is crucial in the development of hepatotoxicity, the risk of hepatitis is higher in people with slow acetylation isoforms of the enzymes for metabolism of INH.<sup>10</sup>

INH-induced hepatitis has many implications, which may sometimes lead to contraindication to the use of this drug. Overt hepatitis is an indication for switching to alternate treatment regimens which are having potential toxic effects, as well as putting a huge burden on the health care expenditures. The average cost of first line and second line ATT in developing countries is 257 and 3000 USD, respectively.<sup>11-13</sup>

There is a need to investigate a potential remedy to the common and life-threatening adverse effects of these cost-effective and relatively safer first-line agents like isoniazid. Multiple studies have been conducted in recent past in this specific area.<sup>14-16</sup> This study may prove to be promising in devising a cost-effective and safe remedy in the form of Alpha Lipoic Acid (ALA), which is found in natural dietary products. ALA has a strong antioxidant action and has proven to be beneficial in ameliorating different diseases associated with oxidative stress.<sup>17-19</sup> It has shown a protective role in reversing the hepatic damage caused by several drugs and toxins, as well

as isoniazid-induced hepatotoxicity model in rats.<sup>20,21</sup>

However, studies have shown that isoniazid-induced hepatotoxicity in mice closely resembles that of human hepatotoxicity model as compared to that in rats. The contribution of INH and Acetyl Hydrazine (AcHz) covalent binding is determined by the rate of acetylation, affinities of cytochromal enzymes for both agents and their clearance. These steps are species-dependent, and it has been found that INH-induced hepatitis in rats and rabbits does not resemble the human model, as INH-induced hepatitis in humans is delayed onset and is characterized by centrilobular necrosis.<sup>22</sup> This study was carried out in mice to assess the protective role of ALA, which could serve as the basis of its use in humans for the prevention of INH-induced hepatitis.

## Methods

It was an experimental study which was carried out at the Department of Pharmacology, Army Medical College (AMC), Rawalpindi, Pakistan in collaboration with National Institute of Health (NIH), Islamabad from January 2020 to June 2021, after getting approval from the Ethics Review Committee (ERC) of the college vide letter no: ERC/5/19, dated: 10<sup>th</sup> March 2019.

Mice were procured from the animal house of the National Institute of Health (NIH), Islamabad. Isoniazid (100mg) tablets, manufactured by Lisko Pakistan (private) limited, were obtained from a local pharmacy. A stock solution was prepared by dissolving the tablets in water, and the total daily dose was calculated to be 1.07 mL. Commercially available ALA (600mg) manufactured by Natrol USA was obtained from a local pharmacy (Chughtai Health Care Private Limited).

A total of 90 healthy male mice were selected using a convenience sampling technique and equally distributed into three groups, each containing 30 mice, with a mean age of 12 weeks and a mean weight of 30 grams. Group A was labelled as the control group in which mice were fed on a normal diet *ad libitum*. Group B served as the disease control group, in which INH was administered orally at 100mg/kg for 28 days to induce hepatotoxicity.<sup>20</sup> In Group C, ALA was administered orally via gavage tube, half an hour before the administration of INH in a dose of 50mg/kg for 28 days.<sup>21</sup>

On day zero and day 28, blood samples were taken from the tail vein of mice in clot activator tubes for biochemical analysis. Serum levels of bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transferase (AST) were taken as biochemical parameters.

Animals were sacrificed on the 29<sup>th</sup> day, and liver samples were dissected out and preserved for histopathological analysis. Sections were examined under high-power field for the presence or absence of necrosis, steatosis, triaditis, degeneration, fibrosis, and regeneration.

Analysis of data was done using SPSS version 21. Statistical difference between serum levels of liver enzymes on day 0 and day 28 was analyzed using a paired t-test. Histopathological parameters were analyzed using percentages.

## Results

Results of liver enzymes and serum bilirubin are

shown in Table 1 and Figure. 1. Serum levels of enzymes and bilirubin were not raised significantly on day 28 in group A compared to day zero. A significant increase in serum levels of liver enzymes and bilirubin was observed in group B on day 28, indicating the model's accuracy. Serum levels of liver enzymes on day 28 were not significantly different from day zero in group C (intervention group).

Histological findings, shown in Table 2 Figure. 2, were also consistent with the biochemical results. It was observed that administration of INH in group B resulted in ballooning degenerative changes (80% of specimens), necrosis (50% of specimens), fatty degeneration (80% of specimens), and triaditis (50% of specimens). Concurrent administration of ALA in group C resulted in preservation of liver histology characterized by decreased frequency of ballooning degeneration (53%), necrosis (10%), steatosis (60%) and triaditis (10%). (Figure. 2).

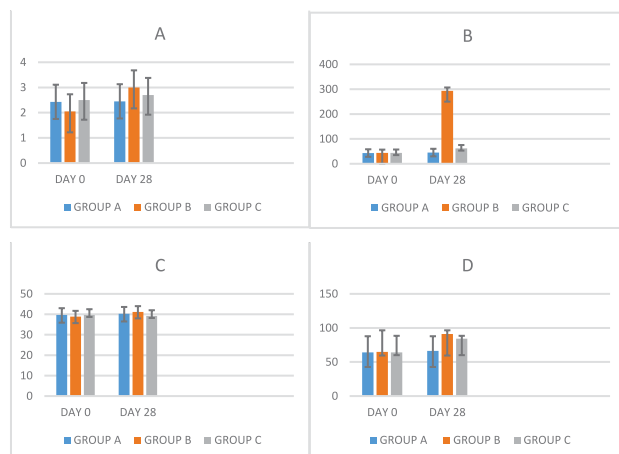
**Table 1: Showing the Comparison of Liver Enzymes in Different Groups using Paired t-tests.**

Parameters	Group A			Group B			Group C		
	Day 0	Day 28	P-Value	Day 0	Day 28	P-Value	Day 0	Day 28	P-Value
Serum bilirubin (umol/L)	2.43 ± 0.68	2.45 ± 0.67	0.371 <sup>#</sup>	2.05 ±0.68	3.0± 0.83	0.026 <sup>*</sup>	2.5 ±0.68	2.7 ±0.79	0.161 <sup>#</sup>
Serum ALT (U/L)	43.3 ±15.1	45.2 ±5.4	0.2 <sup>#</sup>	43.7 ±13.5	293.5± 43.5	<0.001 <sup>*</sup>	43.7 ±13.7	61.8 ±9.9	0.18 <sup>#</sup>
Serum AST (U/L)	39.7 ±3.3	40.3 ±3.8	0.132 <sup>#</sup>	38.9 ±2.9	41.2± 3.2	0.003 <sup>*</sup>	39.7 ±2.9	39.2 ±2.6	0.102 <sup>#</sup>
Serum ALP (U/L)	64.2 ±15.0	66.3 ±14.5	0.5 <sup>#</sup>	64.9 ±13.4	91.1± 10.5	<0.001 <sup>*</sup>	64.2 ±14.1	68.3 ±6.5	0.94 <sup>#</sup>

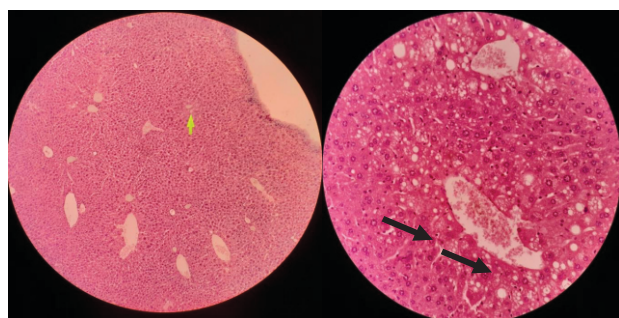
\*Statistically Significant (P value<0.05), # Statistically non-significant (P value>0.05)

**Table 2: Distribution of Histological Parameters among Groups**

Histological Parameters	Group A	Group B	Group C
Ballooning degeneration	0	24 (80%)	16 (53%)
Necrosis	0	15 (50%)	3 (10%)
Steatosis	0	24 (80%)	18 (60%)
Triaditis	0	15 (50%)	3 (10%)
Fibrosis	0	0	0
Regeneration	0	0	0



**Fig.1: Effects of ALA on serum bilirubin(A), ALT (B), AST (C) and ALP (D)**



**Fig.2: Photo-micrograph of slide showing a. Normal architecture (left) b. Ballooning degeneration (right)**

## Discussion

Drug-induced hepatitis is a common and significant adverse effect caused by medications used for the treatment of tuberculosis. Isoniazid (INH) is responsible for most of the cases (56% of all ATT-induced hepatitis cases).<sup>4,7,16</sup> Search for an effective remedy for INH-induced hepatitis has been an active area of research for the last two decades. Studies have been conducted in various animal models using different agents to discover a possible solution to this problem. However, most of these studies were conducted on rat models and a few on rabbit models.<sup>15-18</sup> The discrepancy with these models is that INH-induced hepatitis of human beings resembles that of mice more closely than that of rats or rabbits.<sup>22</sup> The contribution of INH and Acetyl Hydrazine (Ac Hz) covalent binding is determined by the rate of acetylation, affinities of cytochromal enzymes for both the agents, and their clearance. These steps are species-dependent, and it has been found that INH-induced hepatitis in rats and rabbits

does not resemble the human model, as INH-induced hepatitis in humans is delayed-onset and is characterized by centrilobular necrosis.

The purpose of our study was to explore the protective effect of ALA in INH-induced hepatitis in mice, which closely resembles the human model. The contribution of INH and AcHz covalent binding is determined by the rate of acetylation, affinities of cytochromal enzymes for both the agents, and their clearance. These steps are species-dependent, and it has been found that INH-induced hepatitis in rats and rabbits does not resemble the human model, as INH-induced hepatitis in humans is delayed-onset and is characterized by centrilobular necrosis. The current study was conducted in mice model and consequently, the results could justify its use in humans for possible hepato-protection against INH-induced hepatitis.<sup>22</sup>

The dose of INH for induction of hepatitis was 100mg/kg, which is similar to the dose used in a study conducted by Wessam HE et al. INH was administered orally for induction of hepatitis.<sup>23</sup> Serum levels of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and bilirubin were used to assess hepatitis. ALT levels three times the upper limit of normal (ULN) were considered as drug-induced liver injury.<sup>23,24</sup> The degree of hepatic damage was also assessed by microscopic examination of liver samples.

Serum bilirubin levels were raised significantly in the INH-treated group compared to group A on day 28. Bilirubin levels in the ALA-treated group on day 28 were statistically not significant compared to day 0, indicating the ameliorating action of ALA on INH-induced hepatitis. Similar findings were reported in a previous study in which ALA reduced serum bilirubin levels in INH-induced hepatitis in rats.<sup>23</sup> In a study conducted by Saad El et al. a significant reduction in serum levels of bilirubin was observed by the administration of ALA in an INH-induced hepatitis model of rats.<sup>20</sup>

Serum ALT and ALP levels were also raised significantly in the INH-treated group compared to the normal control group on day 28. Levels of ALT and ALP in the ALA-treated group were less than those of the INH-treated group on day 28, indicating the



ameliorating action of ALA on INH-induced hepatitis. The probable reason of these effects is attenuation of free radical generation and scavenging of reactive oxygen species inside the hepatocytes, ultimately preventing the liver injury associated with INH. These findings are in accordance with previous studies in which administration of ALA in rats reduced serum levels of ALT and ALP in INH-induced hepatitis.<sup>19,22</sup>

Serum levels of AST, however, did not rise in our model to clinically significant levels after administration of INH to group B. This is in contrast to previous studies, which showed a clinically significant increase in AST levels in the drug-administered group in the INH-induced hepatitis model in rats and rabbits.<sup>19,20</sup> The probable reason for this might be that INH was administered for 28 days in our model, causing acute liver injury, which is often associated with raised ALT. AST has two predominant isoforms: cytoplasmic and mitochondrial, with the mitochondrial isoform being the dominant one. Raised serum AST is rather more associated with chronic liver injury compared to acute liver injury.<sup>25</sup>

Hepatoprotection and preservation of histopathological changes by INH were obvious from the results. Similar findings were also reported in a study carried out in a rat model of INH-induced hepatitis, in which administration of ALA resulted in marked preservation of liver histology, indicated by reduced inflammatory infiltrates in the portal triad area and necrosis.<sup>23</sup> However, fibrosis was not seen in any of the liver specimens in the current study. The probable explanation may be that fibrosis is more marked in chronic inflammation, while INH-induced liver injury is predominantly of an acute nature. This finding is in accordance with that of previous studies, in which fibrosis was not seen in any of the liver specimens.<sup>21,23</sup> Similarly, the slides were not showing any signs of regeneration. This may be due to the shorter duration of antioxidant treatment.

## Conclusion

Inference can be drawn from the results of this study that ALA has protective effects in INH-induced hepatitis, likely by ameliorating oxidative stress. However, large-scale animal and human studies are needed to strengthen this conclusion.

**Acknowledgement:** None

**Conflict of Interest:** The authors declare no conflict of interest

**Grant Support and Financial Disclosure:** None

## REFERENCES

1. Lee H, Kim J, Choi H. Review on global burden of tuberculosis in 2022. *Public Health Weekly Report*. 2024; 17: 438-51. doi: 10.56786/PHWR.2024.17.11.2
2. Migliori GB, Tiberi SJ. WHO drug-resistant TB guidelines 2022: what is new. *The International Journal of Tuberculosis and Lung Disease*. 2022; 26: 590-1. doi: 10.5588/ijtld.22.0263.
3. Lopes SR, Marçal M, Fernandes N, Silva F, Barbosa P, Vieira M, et al. Update in tuberculosis treatment: a scoping review of current practices. *Breathe*. 2025; 21: 240232. doi: 10.1183/20734735.0232-2024
4. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 4: treatment-drug-resistant tuberculosis treatment, 2022 update. World Health Organization. 2022. Available at: <https://www.who.int/publications/i/item/9789240063129>
5. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, et al. Global Tuberculosis Report 2020—Reflections on the Global TB burden, treatment and prevention efforts. *International journal of infectious diseases*. 2021; 113: S7-12. doi: 10.1016/j.ijid.2021.02.107
6. Allison R, Guraka A, Shawa IT, Tripathi G, Moritz W, Kermanizadeh A. Drug induced liver injury—a 2023 update. *Journal of Toxicology and Environmental Health, Part B, Critical Review*. 2023; 26: 442-67. doi: 10.1080/10937404.2023.2261848
7. Lewis JH, Korkmaz SY, Rizk CA, Copeland MJ. Diagnosis, prevention and risk-management of drug-induced liver injury due to medications used to treat mycobacterium tuberculosis. *Expert Opinion on Drug Safety*. 2024; 23: 1093-1107. doi: 10.1080/14740338.2024.2399074
8. Cavaco MJ, Alcobia C, Oliveiros B, Mesquita LA, Carvalho A, Matos F, et al. Clinical and genetic risk factors for drug-induced liver injury associated with anti-tuberculosis treatment—a study from patients of Portuguese health centers. *Journal of Personalized Medicine*. 2022; 12: 790. doi: 10.3390/jpm12050790
9. Zhuang X, Li L, Liu T, Zhang R, Yang P, Wang X, et al. Mechanisms of isoniazid and rifampicin-induced liver injury and the effects of natural medicinal ingredients: A review. *Frontiers in Pharmacology*. 2022; 13: 1037814. doi: 10.3389/fphar.2022.1037814
10. Zhong T, Fan Y, Dong XL, Guo X, Wong KH, Wong WT, et al. An investigation of the risk factors associated with anti-tuberculosis drug-induced liver injury or abnormal liver functioning in 757 patients with pulmonary tuberculosis. *Frontiers in Pharmacology*. 2021; 12: 708522. doi: 10.3389/fphar.2021.708522
11. Schiuma M, Dinegro S, Battini V, Torre A, Covizzi A, Civati A, et al. NAT2 Acetylation Status Predicts Hepatotoxicity During Antituberculosis Therapy: Cumulative Risk Analysis

- of a Multiethnic Cohort. *International Journal of Molecular Sciences*. 2025; 26: 3881. doi: 10.3390/ijms26083881
12. Mullerpattan JB, Udwadia ZZ, Banka RA, Ganatra SR, Udwadia ZF. Catastrophic costs of treating drug resistant TB patients in a tertiary care hospital in India. *Indian Journal of Tuberculosis*. 2019; 66: 87-91. doi: 10.1016/j.ijtb.2018.04.011
  13. Pooran A, Pieterse E, Davids M, Theron G, Dheda K. What is the cost of diagnosis and management of drug resistant tuberculosis in South Africa? *PloS one*. 2013; 8: e54587. doi: 10.1371/journal.pone.0054587
  14. Jang JG, Chung JH. Diagnosis and treatment of multidrug-resistant tuberculosis. *Yeungnam University journal of medicine*. 2020; 37: 277-85. doi: 10.12701/yujm.2020.00626
  15. Naji KM, Al-Khatib BY, Al-Haj NS, D'souza MR. Hepatoprotective activity of melittin on isoniazid and rifampicin-induced liver injuries in male albino rats. *BMC Pharmacology and Toxicology*. 2021; 22: 39. doi: 10.1186/s40360-021-00507-9
  16. He X, Song Y, Wang L, Xu J. Protective effect of pyrrolidine dithiocarbamate on isoniazid/rifampicin-induced liver injury in rats. *Molecular Medicine Reports*. 2020; 21: 463-9. doi: 10.3892/mmr.2019.10817
  17. Dubiwak AD, Damtew TW, Senbetu MW, Yewhalaw D, Asere TG, Nemo G, et al. Hepatoprotective effect of corm of *Ensete ventricosum* (welw.) cheesman extract against isoniazid and rifampicin induced hepatotoxicity in Swiss albino mice. *Journal of Toxicology*. 2021; 2021: 4760455. doi: 10.1155/2021/4760455
  18. Rincón-Cervera MÁ, Valenzuela R, Hernandez-Rodas MC, Barrera C, Espinosa A, Marambio M, et al. Vegetable oils rich in alpha linolenic acid increment hepatic n-3 LCPUFA, modulating the fatty acid metabolism and antioxidant response in rats. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2016; 111: 25-35. doi: 10.1016/j.plefa.2016.02.002
  19. Maciejczyk M, Żebrowska E, Nesterowicz M, Żendzian-Piotrowska M, Zalewska A.  $\alpha$ -Lipoic Acid Strengthens the Antioxidant Barrier and Reduces Oxidative, Nitrosative, and Glycative Damage, as well as Inhibits Inflammation and Apoptosis in the Hypothalamus but Not in the Cerebral Cortex of Insulin-Resistant Rats. *Oxidative Medicine and Cellular Longevity*. 2022; 2022: 7450514. doi: 10.1155/2022/7450514
  20. Saad El, El-Gowilly SM, Sherhaa MO, Bistawroos AE. Role of oxidative stress and nitric oxide in the protective effects of  $\alpha$ -lipoic acid and aminoguanidine against isoniazid-rifampicin-induced hepatotoxicity in rats. *Food and chemical toxicology*. 2010; 48: 1869-75. doi: 10.1016/j.fct.2010.04.026
  21. Yue J, Peng RX, Yang J, Kong R, Liu J. CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. *Acta Pharmacologica Sinica*. 2004; 25: 699-704.
  22. Metushi IG, Nakagawa T, Uetrecht J. Direct oxidation and covalent binding of isoniazid to rodent liver and human hepatic microsomes: humans are more like mice than rats. *Chemical research in toxicology*. 2012; 25: 2567-76. doi: 10.1021/tx300341r
  23. Wessam HE, Ezzeddin SD, Wessam HE, Somaia AN, Adel B, Naglaa A. Possible protection against isoniazid induced hepatotoxicity by certain natural agents. *Indian Journal of Research in Pharmacy and Biotechnology*. 2013; 2: 345-61.
  24. Biswas A, Santra S, Bishnu D, Dhali GK, Chowdhury A, Santra A. Isoniazid and rifampicin produce hepatic fibrosis through an oxidative stress-dependent mechanism. *International Journal of Hepatology*. 2020; 2020: 6987295. doi: 10.1155/2020/6987295
  25. Iluz-Freundlich D, Zhang M, Uhanova J, Minuk GY. The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. *Annals of hepatology*. 2020; 19: 204-8. doi: 10.1016/j.aohep.2019.08.004

#### Author Contributions

**IUK:** Conception of idea, manuscript writing, validation of data, interpretation, and write-up of results

**AW:** Revising, editing, and supervising for intellectual content

**WUK:** Data acquisition, curation, and statistical analysis

**JA:** Conception and design of the work

**MSM:** Manuscript writing for methodology design and investigation

**BUK:** Writing the original draft, proofreading, and approval for final submission