ORIGINAL ARTICLE

A Laboratory-Based Experimental Study on the Protective Effects Against Isoniazid-Induced Hepatotoxicity in Mice

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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 3^0C. 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 29th 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*

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.[1,2]

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%

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Received: Aug 02, 2024; 1st Revision Received: Mar 25, 2025
2nd Revision Received: Sep 20, 2025; Accepted: Sep 23, 2025

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

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%.[5] Incidence of hepatotoxicity among ATT drugs is highest with INH (56%), followed by RMP (34%) and PZA (10%).[6,7]

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).[8] These toxic metabolites cause hepatic damage via covalent bonding, oxidative stress and metabolic dysfunction.[8] Multiple risk factors responsible for increased incidence of hepatotoxicity have been identified, such as elderly patients, females, malnutrition, and a history of liver disease.[9] 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.[10]

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.[11-13]

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.[14-16] 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.[17-19] 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.[20,21] 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.[22] 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: 10th 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.[20] 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.[21]

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 29th 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#	2.05 ±0.68	3.0± 0.83	0.026*	2.5 ±0.68	2.7 ±0.79	0.161#
Serum ALT (U/L)	43.3 ±15.1	45.2 ±5.4	0.2#	43.7 ±13.5	293.5± 43.5	<0.001*	43.7 ±13.7	61.8 ±9.9	0.18#
Serum AST (U/L)	39.7 ±3.3	40.3 ±3.8	0.132#	38.9 ±2.9	41.2± 3.2	0.003*	39.7 ±2.9	39.2 ±2.6	0.102#
Serum ALP (U/L)	64.2 ±15.0	66.3 ±14.5	0.5#	64.9 ±13.4	91.1± 10.5	<0.001*	64.2 ±14.1	68.3 ±6.5	0.94#

**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

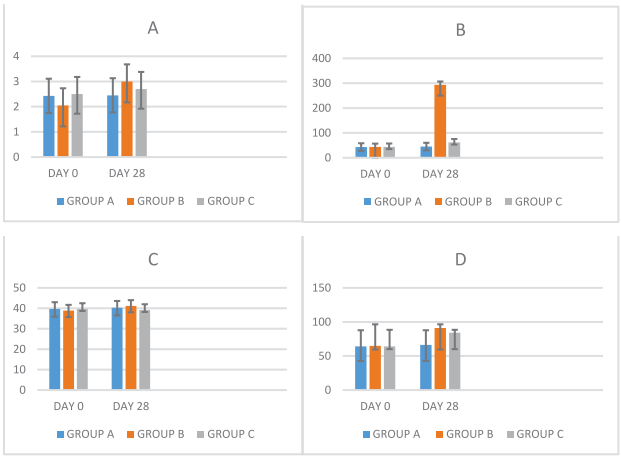

Fig.1: Effects of ALA on serum bilirubin(A), ALT (B), AST (C) and ALP (D)

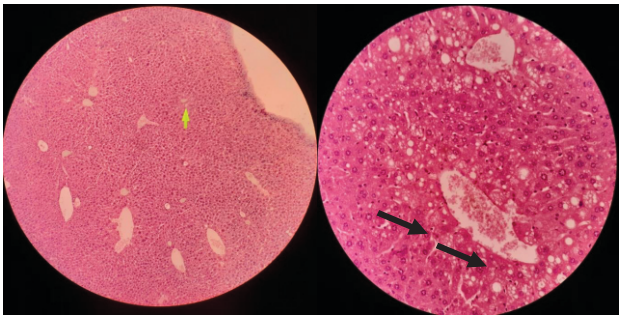

Fig.2: Photo-micrograph of slide showing a. Normal architecture (left) b. Ballooning degeneration (right)

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).[4,7,16] 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.[15-18] 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.[22] 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.[22]

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.[23] 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.[23,24] 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.[23] In a study conducted by Saad EI 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.[20]

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.[19,22]

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.[19,20] 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.[25]

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.[23] 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.[21,23] 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

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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