ORIGINAL ARTICLE

Laboratory-Based Experimental Study of the Protective Effects of Alpha-Tocopherol On Isoniazid-Induced Hepatitis in Mice

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ABSTRACT

Objective: To investigate the role of alpha tocopherol in preventing isoniazid-induced hepatitis.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: This study was conducted at the Pharmacology Department of the Army Medical College, National University of Medical Sciences, Rawalpindi, in collaboration with the National Institute of Health, Islamabad, Pakistan, from January 2020 to June 2021.

Methods: It was an animal-based experimental study conducted in male mice. A sample size of ninety mice was selected and randomly distributed into three groups. Hepatitis model was induced by oral administration of 100mg/kg of isoniazid for 28 days, which was indicated by elevated levels of serum bilirubin and liver enzymes, and changes in histo-pathological parameters in liver samples. Alpha-tocopherol was administered orally 30 minutes prior to isoniazid at a dose of 100mg/kg for 28 days. Blood samples were collected from the tail vein of mice on day 0 for baseline levels and day 29 via intra-cardiac puncture after euthanasia, for post-intervention comparison. Liver samples were also collected and preserved for histopathological analysis. The biochemical parameters were analyzed within groups using a paired t-test, while histopathological parameters were analyzed for frequency distributions across the three groups.

Results: This study showed that alpha-tocopherol protected against isoniazid-induced hepatotoxicity, which was indicated by decreased levels of liver enzymes and bilirubin 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 tocopherol is effective in protecting against isoniazid-induced hepatotoxicity.

Keywords: *Alpha-Tocopherol, Hepatitis, Isoniazid.*

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Introduction

One of the most lethal diseases mankind has suffered from since ancient times is Tuberculosis (TB).[1] The causative pathogen for the disease is Mycobacterium tuberculosis. Early diagnosis and anti-tuberculous therapy are important for early recovery and prevention of mortality and morbidity accompanying the disease.[2]

The standard treatment regimen for TB consists of two phases, the intensive phase and the continuation phase, with two- and six-month durations, respectively. The drugs administered in the intensive phase are isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA), and ethambutol (E), while INH and RMP are administered during the continuation phase for four months. This regimen is the gold standard for primary TB since long and is associated with excellent results as far as the elimination of pathogen and disease symptoms are

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concerned, with some studies showing more than 80% cure rate.[3] Million lives have been saved in the last decade with effective anti-tuberculosis treatment (ATT).[4]

A variety of adverse drug reactions (ADRs) are associated with ATT, which limits the compliance to these medications and hence the overall success rate of the regimen. One of the major ADRs of ATT is drug-induced hepatotoxicity. The incidence of this side effect ranges from 2 to 40 percent, with a mortality of 2 per thousand patients with hepatitis.[5] INH is the most notorious ATT agent, responsible for more than 50% cases of ATT-induced hepatitis.[6,7]

The probable mechanism of INH-induced hepatitis is conversion of INH to toxic metabolites by hepatic enzymes N-acetyl transferase 2 (NAT2) and cytochrome P450 2E1 isoform (CYP2E1), which leads to hepatocyte damage by covalent bonding, free radical formation, and metabolic dysfunction. Increased age, female gender, poor nutritional status, slow acetylator status, and prior hepatic dysfunction are major risk factors for developing INH-induced hepatitis.[8-10]

Multiple implications are associated with INH-associated hepatitis, culminating in the contraindication of further use of INH and switching to second-line treatment regimens. This leads to ten times increase in the overall treatment cost and exposure to much toxic side effects of the second line drugs.[11-13]

Reversing isoniazid-induced hepatitis is an active area of research for a long time, and different studies have been conducted in this regard.[14-16] Alpha tocopherol, a biologically active form of vitamin E, is a potent antioxidant and reduces oxidative stress by preventing lipid peroxidation and scavenging free radicals. Multiple studies have been conducted to explore the potential role of alpha-tocopherol in reversing ATT-induced hepatitis. However, these studies are conducted on a rabbit model, while studies have shown that the mouse model closely resembles the human model of ATT-induced hepatitis.[17] Our study is designed to explore the effects of alpha tocopherol in a mouse model so that the results can be replicated in humans as well.

Methods

It was an experimental study that was conducted at the Pharmacology Department from January 2020 to June 2021. The procedures were performed after obtaining approval from the College's Ethics Review Committee (ERC) vide letter no: ERC/6/19, dated: 11th March 2019. Mice of C3HeB/FeJ strain, having a weight of 30-50 grams, were procured and kept in the animal house of NIH, Islamabad, at room temperature, maintaining a 12-hour dark and light cycle and diet ad libitum. Isoniazid (100mg) tablets, manufactured by Lisko Pakistan (private) limited, were obtained from a local pharmacy. Tablets of isoniazid were dissolved in water, and a stock solution was prepared with a total daily dose calculated as 1.07 ml. Alpha tocopherol was purchased from the local market, manufactured by Merck Pharmaceuticals, as gel capsules. A total of 90 healthy male mice were selected by a convenience, non-probability, random sampling technique and distributed in three groups with 30 mice in each group. The first group was labelled as group A and taken as the control group. In this group, mice were fed a normal diet ad libitum. Group B was used as the disease control group, in which oral INH was given for 28 days at a 100mg/kg dosage for induction of hepatotoxicity. In Group C, oral alpha-tocopherol was given via gavage tube 30 minutes prior to INH administration at a 100mg/kg dose for 28 days.[18]

Blood samples were collected from the tail vein of the mice on day 0 and via intracardiac injection on day 28 in clot activator tubes for biochemical analysis. Serum 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 triaditis, steatosis, necrosis, degeneration, fibrosis, and regeneration.

Data was analyzed using SPSS version 21. Statistical difference between the serum levels of liver enzymes within the groups on day 0 and day 28 was analyzed using a paired t-test. Histopathological parameters were analyzed for their frequency distributions.

Results

Results of liver enzymes and serum bilirubin are shown in Table 1 and Figure 1. Paired t-test showed a significant rise in the serum levels of liver enzymes and bilirubin (P=0.026) in group B from day zero to day 28. Serum enzyme levels showed no changes in group A (P=0.371) and C (P=0.61) from day zero to day 28.

Table 1: Results of paired t-test showing the differences of biochemical parameters in three groups

Parameters	Group A Day 0	Group A Day 28	*P-value*	Group B Day 0	Group B Day 28	*P- value*	Group C Day 0	Group C Day 28	*P-value*
Serum bilirubin	2.43 ± 0.68	2.45 ± 0.67	0.371#	2.05 ± 0.68	3.0 ± 0.83	0.026*	2.5 ± 0.68	2.1 ± 0.55	0.61**
Serum ALT	43.3 ± 15.1	45.2 ± 15.4	0.21*	43.7 ± 13.5	293.5 ± 43.5	<0.001*	43.9 ± 13.6	51.0 ± 25.4	0.14**
Serum AST	39.7 ± 3.3	40.3 ± 3.8	0.132#	38.9 ± 2.9	41.2 ± 3.2	0.003*	39.5 ± 2.8	41.4 ± 2.7	0.135**
Serum ALP	64.2 ± 15.0	66.3 ± 14.5	0.5#	64.9 ± 13.4	91.1 ± 10.5	<0.001*	65.5 ± 13.5	69.1 ± 14.1	0.52**

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

Table 2: Distribution of histological parameters among groups

Histological Parameter	Group A	Group B	Group C
Ballooning degeneration	0	24 (80%)	3 (10%)
Necrosis	0	15 (50%)	1 (3%)
Steatosis	0	24 (80%)	9 (30%)
Triaditis	0	15 (50%)	0 (0%)
Fibrosis	0	0	0
Regeneration	0	0	0

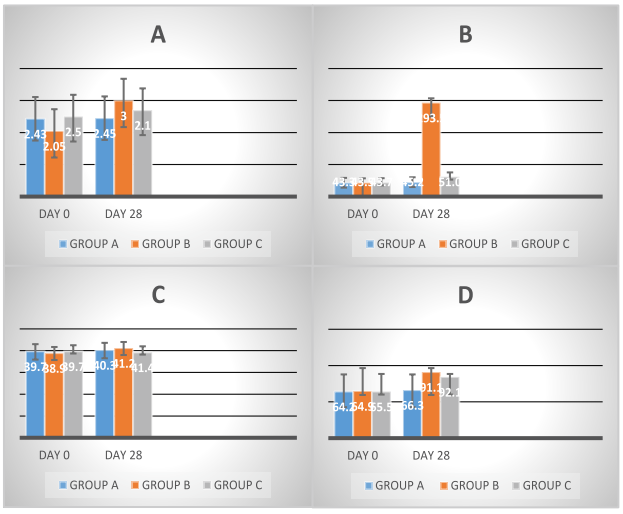

Fig.1: Effects of Alpha Tocopherol on serum bilirubin(A), ALT (B), AST (C), and ALP (D)

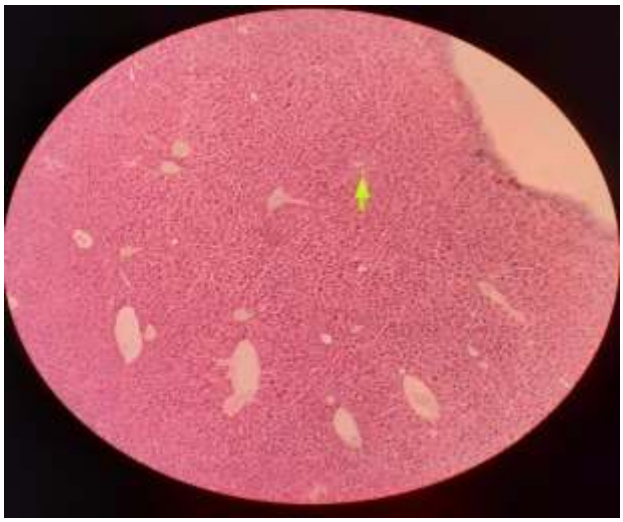

Fig.2: Photomicrograph of slide showing normal architecture in group A

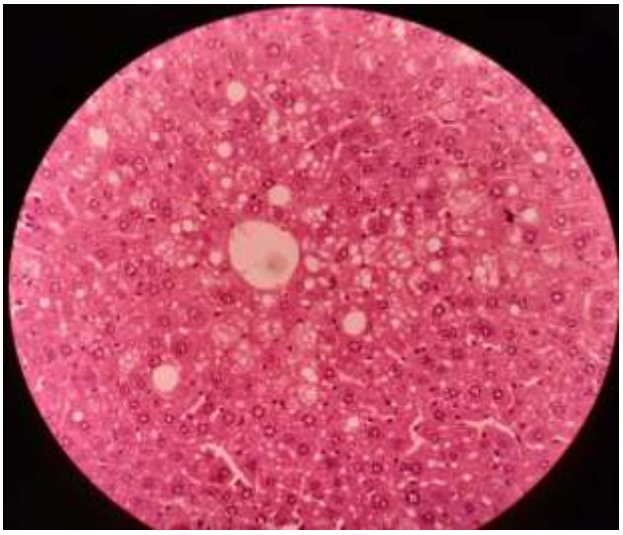

Fig.3: Photomicrograph of slide showing steatosis in group B (INH-treated group)

Results of the histologic findings, shown in Table 2 and Figure 2, were also consistent with the biochemical results. It was observed that administration of INH resulted in ballooning degeneration (80% of specimens), necrosis (50% of specimens), steatosis (80% of specimens, Figure 3), and triaditis (50% of specimens) in group B on day 28. Concurrent administration of Alpha tocopherol in group C resulted in preservation of liver histology characterized by decreased frequency of ballooning degeneration (10%), necrosis (3%), steatosis (30%), and triaditis (0%).

Discussion

Hepatotoxicity is a common and most sinister side effect occurring with ATT, with INH responsible for most of the cases.[1,4,19] Isoniazid is one of the most common causative agents in this condition.[6] Prevention of hepatitis with ATT is an active research area with multiple studies conducted to decrease the occurrence of concurrent hepatitis. 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.[13-15] The discrepancy with these models is that the human hepatotoxicity model is delayed-onset and characterized by centrilobular necrosis, which differs from the rat and rabbit models of hepatitis. The probable reason is the difference in the acetylation affinities of cytochrome enzymes for both INH and acetyl hydrazine. These steps are species-dependent, with research showing that INH-induced hepatitis in rats and rabbits does not resemble the human model.[20] The results of these studies cannot justify the use of alpha tocopherol in humans.

The purpose of our study was to explore the potential protective role of alpha tocopherol in a mouse model that closely resembles the human hepatitis model, as the relative concentrations of INH to AcHz are similar to those in humans.[20] Consequently, the results of the study will justify the use of alpha tocopherol in humans for its possible hepatoprotective effect.

Raised levels of liver enzymes and bilirubin in the serum were the biochemical parameters used for assessing hepatitis. ALT levels three times the upper limit of normal (ULN) were considered as drug-induced liver injury.[21] The degree of hepatic damage was also assessed by microscopic examination of liver samples.

INH was used in a 100 mg/kg dose in the study, which is similar to the dose used by Wessam H et al. and administered orally.[22]

Serum bilirubin levels were raised significantly in the INH-treated group compared to group A on day 28. Bilirubin levels in the Alpha tocopherol treated group on day 28 were statistically not significant compared to day 0, indicating the ameliorating action of alpha tocopherol on INH-induced hepatitis. These findings are similar to the study conducted by Tayal V et al., which showed ameliorating action of tocopherol in albino rabbits.[23]

The INH-treated group also revealed a significant rise in serum levels of ALT and ALP compared to the normal control group on day 28. Serum levels were significantly lower in the alpha tocopherol-treated group, which shows amelioration in the hepatotoxicity by INH. Probable mechanisms include scavenging free radicals and reduced free-radical formation in hepatocytes. These findings are also similar to the previous studies, which showed the protective effects of alpha-tocopherol.[13,14]

One of the unique findings in our study was the lack of a clinically significant rise in serum AST levels following an INH 100mg/kg dosage, unlike previous studies.[14,18] The probable explanation for this is that administration of INH for 28 days leads to acute liver injury, which is characterized by a rise in ALT levels

compared to AST. Raised serum AST is rather more associated with chronic liver injury compared to acute liver injury.[24]

The liver injury in the INH-treated group was evident from the histopathology of liver samples as well, which showed ballooning degeneration, steatosis, triaditis, necrosis, and fibrosis, observed under high and low power microscopy. Alpha tocopherol administration led to preservation of liver architecture, which was shown by reduction in ballooning degeneration, steatosis, triaditis, and necrosis. These results are similar to those of Naji KM et al. and Tayal V et al., which showed reduced inflammatory infiltrates in the portal triad area and reduced necrosis.[15,23] Dubiwak et al. also reported similar findings in which quercetin was administered as a remedy for INH-induced liver injury.[18] However, fibrosis was not seen in any of the liver specimens in our study, as the injury induced was of an acute nature, with fibrosis being more common in models of chronic liver injury. This finding is in accordance with that of previous studies, in which fibrosis was not seen in any of the liver specimens.[19,23] Similarly, regeneration was not observed in our model, probably because of the shorter duration of treatment with the antioxidants.

The study had certain limitations that should be acknowledged. Assessment of oxidative stress biomarkers and inflammatory cytokines was not possible due to resource and time constraints. Due to the short study duration, the chronic effects of the antioxidants were not evaluated. Blinding the assessors was not possible due to technical constraints.

Conclusion

Hence, it can be concluded that alpha tocopherol has a protective effect against isoniazid–induced hepatitis. Further large-scale animal studies and human studies are required to support this finding, which may prove alpha tocopherol to be a potent remedy against INH-induced hepatitis.

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REFERENCES

1. MacNeil A, Glaziou P, Sismanidis C, Maloney S, Floyd K. Global epidemiology of tuberculosis and progress toward achieving global targets—2017. Morbidity and Mortality Weekly Report. 2019; 68: 263-6. doi: 10.15585/mmwr.mm6811a3
2. Van Cutsem G, Isaakidis P, Farley J, Nardell E, Volchenkov G, Cox H. Infection control for drug-resistant tuberculosis: early diagnosis and treatment is the key. Clinical Infectious Diseases. 2016; 62: S238-43. doi: 10.1093/cid/ciw012
3. Obeng G. Tuberculosis: Early Diagnosis and Treatment. 2019. Available at: https://digitalcommons.otterbein.edu/stu_msn/367
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. Yew WW, Chang KC, Chan DP. Oxidative stress and first-line antituberculosis drug-induced hepatotoxicity. Antimicrobial agents and chemotherapy. 2018; 62: e02637-17. doi: 10.1128/AAC.02637-17
7. Mahmood K, Hussain A, Jairamani KL, Talib A, Abbasi BU, Salkeen S. Hepatotoxicity with antituberculosis drugs: the risk factors. Pakistan Journal of Medical Sciences. 2007; 23: 33-8.
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%2Fjpm12050790
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. Suvichapanich S, Fukunaga K, Zahroh H, Mushiroda T, Mahasirimongkol S, Toyo-Oka L, et al. NAT2 ultra-slow acetylator and risk of anti-tuberculosis drug-induced liver injury: a genotype-based meta-analysis. Pharmacogenetics and genomics. 2018; 28: 167-76. doi: 10.1097/fpc.0000000000000339
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, Pieterson 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. 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
18. 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
19. 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
20. Maciejczyk M, Żebrowska E, Nesterowicz M, Żendzian-Piotrowska M, Zalewska A. α-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
21. Saad EI, El-Gowilly SM, Sherhaa MO, Bistawroos AE. Role of oxidative stress and nitric oxide in the protective effects of α-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
22. Wessam H, Ezzeddin S, Wessam H, Somaia A, Adel B, Naglaa A. Possible protection against isoniaizd induced hepatotoxicity by certain natural agents. The International Journal of Pharmaceutical Research and Bio-Science. 2013; 2: 345-61.
23. Tayal V, Kalra BS, Agarwal S, Khurana N, Gupta U. Hepatoprotective effect of tocopherol against isoniazid and rifampicin induced hepatotoxicity in albino rabbits. 2007; 45: 1031-6.
24. Hyder MA, Hasan M, Mohieldein AH. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. European journal of experimental biology. 2013; 3: 280-4.

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MSM: Revising, editing, and supervising for intellectual content

WUK: Data acquisition, curation, and statistical analysis

JA: Conception and design of the work

AS: Manuscript writing for methodology design and investigation

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