ORIGINAL ARTICLE

CONTENTS

Histopathological, immunohistochemical and physiological study for the hepatoprotective effect of melatonin against inhrifampicin-induced hepatotoxicity in mice model

Siham Mahmood Al-Rehemi[1], Rasha Hatem Dosh[1], Maha Jamal Frayyeh[2], Rihab Hameed Al Mudhafar[3], Hussein Abdulkadhim[4]

[1]DEPARTMENT OF HUMAN ANATOMY, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, NAJAF, IRAQ
[2]AL-ABRAAR SECONDARY SCHOOL, MINISTRY OF EDUCATION, BAGHDAD, IRAQ
[3]DEPARTMENT OF PATHOLOGY AND FORENSIC MEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, NAJAF, IRAQ
[4]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, NAJAF, IRAQ

ABSTRACT

Aim: The purpose of this study is to assess the hepatoprotective effect of melatonin against isoniazid (INH) and rifampicin (RMP) induced hepatotoxicity in albino mice.

Materials and Methods: Adult male mice were divided into four groups: saline, INH-RMP, INH-RMP+MT and MT were administered for 21 days. Biochemical analyses were performed for the determination of ALT, AST. Histopathological changes in the liver and Immunohistochemical assessment to determine the expression of Caspace3 were also examined.

Results: Biochemical analysis revealed significant increases in serum ALT and AST in INH-RMP group. Histopathological findings demonstrated severe liver damage in INH-RMP group as compared with control group. In contrast, treatment of mice with melatonin (MT) markedly mitigated the liver injury. Immunohistochemical findings demonstrated apoptotic marker caspace3 significantly higher in INH-RMP group as compared with control group.

Conclusions: Experimental findings highlight the potential benefits of melatonin in this model, prompting speculation on its potential application in human therapy.

KEY WORDS: Hepatotoxicity, Isoniazid, Rifampicin, Melatonin, caspace3, Liver damage

Wiad Lek. 2024;77(9):1745-1752. doi: 10.36740/WLek/192268 DOI

INTRODUCTION

Many medications are quite effective in treating target-directed diseases, but their usage is restricted to a brief course of treatment lasting no longer, than 1-2 weeks with a strict dosing schedule. This duration and dosage restriction is linked to their combined harmful effects, which may be caused by direct reactivity or free radicals and oxidants, which are consequences of their metabolism [1]. Tuberculosis is an extremely contagious illness that affects more than a third of the global population, leading to the deaths of over 2 million individuals annually [2]. A meta-analysis investigating the simultaneous administration of isoniazid (INH) and rifampicin (RMP), which are the primary drugs employed in tuberculosis treatment, revealed an association with hepatotoxicity ranging from 2% to 6%, severe liver damage and an elevated mortality rate [3-5], while the majority of instances involving INH hepatotoxicity are mild and can resolve even with the continued use of INH, a small percentage of patients undergoing INH treatment experience severe hepatitis. This severe form may progress to fulminant liver failure and, if INH is not promptly discontinued, can lead to death [6]. Nevertheless, the idiosyncratic nature of INH hepatotoxicity continues to pose a significant safety concern in clinical settings. Currently, there are no specific therapies available for treating INH-induced liver injury. Although corticosteroids, which possess anti-inflammatory and immunosuppressive properties, are frequently employed, their outcomes are not consistently favourable. Consequently, there is an urgent need for mechanism-based strategies in the management of INH-induced liver injury [6]. Melatonin's hepatoprotective potential has been the subject of much interesting research in recent years, especially when it comes to drug-induced liver injury [7, 8]. A

common problem in the treatment of tuberculosis is the combination hepatotoxicity caused by isoniazid and rifampicin. This dual attack on hepatic integrity emphasizes the necessity for cutting-edge treatment approaches to lessen the resulting liver damage [9]. Although melatonin (MT) is most known for its ability to regulate circadian rhythms, it has also been shown to have a variety of other physiological effects, such as anti-inflammatory and antioxidant capabilities [9]. Research indicates that melatonin may provide defence against a range of hepatotoxic attacks, which has led to investigation into its effectiveness with regard to the hepatotoxicity caused by isoniazid-rifampicin.

AIM

The purpose of this study is to assess the hepatoprotective effect of melatonin against isoniazid and rifampicin induced hepatotoxicity in albino mice.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN AND PROCEDURE

Twenty-four male albino mice weighing 18-22 g and 5 weeks old were obtained from the University of Kufa to conduct this investigation. The present study was approved by the ethics committee of the University of Kufa (Reference number: 20578). The mice were kept in a plastic cage throughout the experimentation period under typical lab conditions, which included 13-hour light cycles and 11-hour dark cycles. The mice were fed on commercial food bits and distilled water. In this study, animals were separated into four groups each of six mice. The control group was given normal saline (0.9%), and three intraperitoneal injected groups: isoniazid-rifampicin (INH-RMP) induced hepatotoxicity group were injected with 0.2 ml of INH-RMP 18 mg/kg daily for 21 days; melatonin group were injected with 0.2 ml of melatonin 0.1 mg/kg daily for 21 days; and hepatoprotective group were injected with 0.2 ml of INH-RMP 18 mg/kg and melatonin 0.1 mg/kg daily for 21 days).

PHYSIOLOGICAL AND HISTOPATHOLOGICAL EXAMINATION

On day 21, all of the mice were directly euthanized using intramuscular anaesthesia (0.25 ml xylazine (Arendonk; Belgium) per 100 g and 5 mg ketamine (Alfasan; Holland) per 100 g of the body weight). Blood was immediately aspirated from the heart and centrifuged at 1,370 × g for 10 min at 4°C, the serum was collected and stored at −80°C until use for measurements of alanine aminotransferase (ALT) (GPT) and aspartate aminotransferase (AST) (GOT) activities (Roche diagnostics kit; Mannheim, Germany). The liver was resected after a longitudinal abdominal incision. A section of the liver from the portal hepatic was fixed in 10% neutral buffered formalin. The specimens were gradually dehydrated over a series of alcohol solutions (60%, 80%, 90%, and 100%) for two hours to prepare them for histological analysis. Then, two changes of xylene, which was easily soluble in alcohol, were added to the specimens to replace the alcohol. The specimens were then put in a dish of fresh paraffin wax, which was then moved to an oven that was continuously set at a temperature of 50 to 53°C. To replace the xylene in the tissue with paraffin, the specimens were then changed into two or three sequential plates of paraffin. Later, the specimens were embedded in the paraffin blocks and set on a microtome (Leitz; Germany), where serial sections of 5 µm thickness were cut. Finally, the specimens were stained with hematoxylin and eosin and mounted on glass slides (Leika/China) for histological analysis under a light microscope. The pathologist examined the slides in a blinded method for hepatic injury under the light microscope X100 or X400 magnification. Histopathological changes of the liver were scored as grade 0 indicates no damage, grade 1 indicates mild injury (characterized by vacuoles and focal pyknosis), grade 2 indicates moderate injury (characterized by vacuoles, ballooning, and apoptosis without necrosis), and grade 3 indicates severe injury (characterized by the presence of necrosis) [10].

IMMUNOHISTOCHEMICAL EXAMINATION OF CASPASE3

Formalin-fixed, paraffin-embedded 4 µm sections were subjected to immunohistochemical labelling using caspase3 antibodies at a 1:50 dilution (DAKO, Carpentaria, CA). In every instance, the slides were steam-heated for 30 minutes in a 1 mmol/L EDTA (pH 8.0) solution to retrieve the antigen. An automated immunostainer (DAKO) was used for staining, and a streptavidin-biotin detection system (DAKO) was used for detection. For every experiment, portions of positive and negative controls were employed. Stain intensity in the examined slide is divided into three categories: Score 0 for no stain, Score 1 for weak stain, Score 2 for moderate stain, and Score 3 for strong stain. While the proportion of stained cells and the expression of proteins in the examined slide is divided into the following categories: (Score 0: <10%, Score 1: 10-25%, Score 2: 25-50%, Score 3: 50-75%, Score 4: >75%). The outcome is expressed as a Quick H score, which is calculated by multiplying the

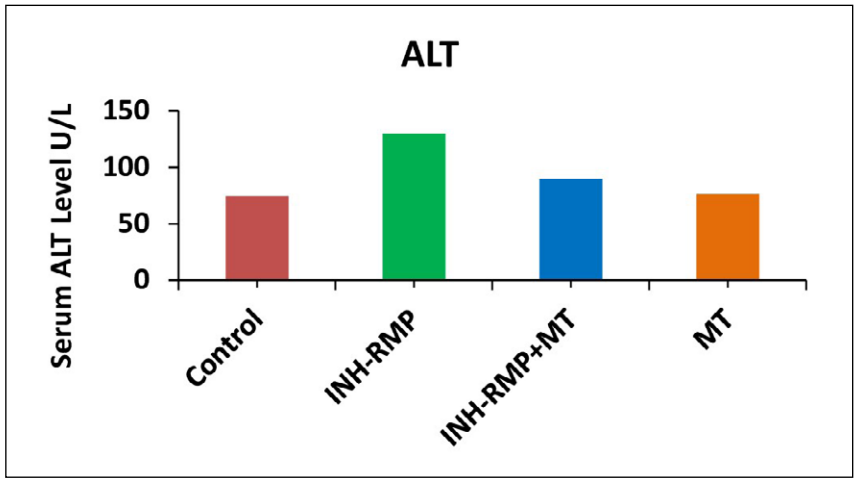

Fig. 1. Serum level of ALT in four experimental groups (six mice in each group). ($P \leq 0.05$).

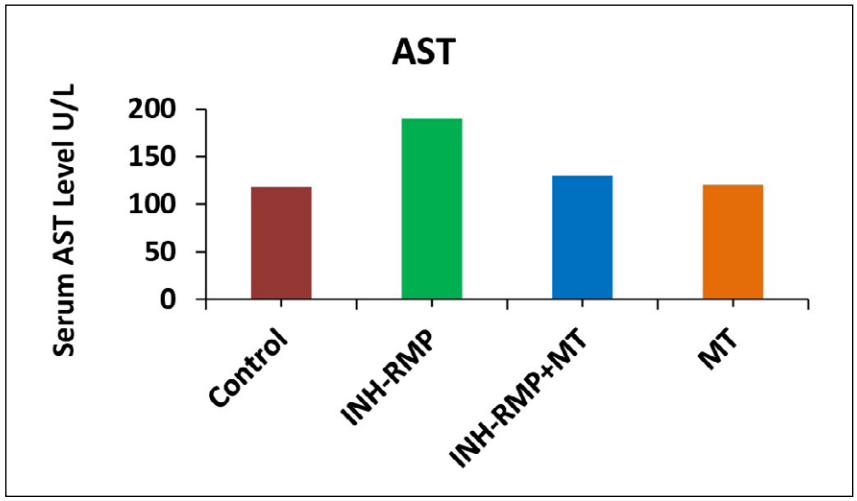

Fig. 2. Serum level of AST in four experimental groups (six mice in each group). ($P \leq 0.05$).

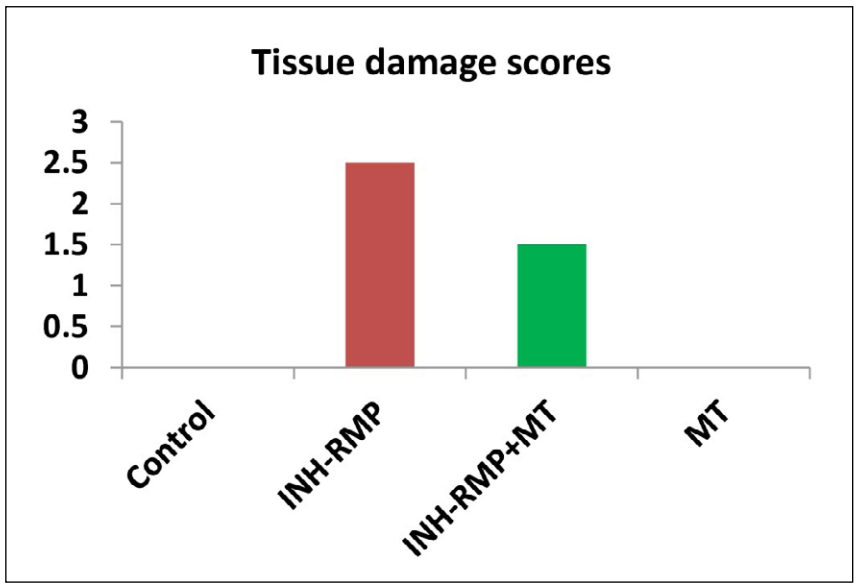

Fig. 3. Tissue damage scores in four experimental groups (six mice in each group). ($P \leq 0.05$).

stain intensity score by the proportion of stained cells on the slide under examination [11].

STATISTICAL ANALYSIS

GraphPad Prism version 8 was used to analyse the data and produce the graphics. To compare the study groups, the Kruskal-Wallis test is a non-parametric method used to assess the levels of ALT, AST, and tissue damage between the study groups as the mean score for histopathological scoring analysis and mean quick H score for immunohistochemical results. Statistical significance was defined as a P-value ≤ 0.05.

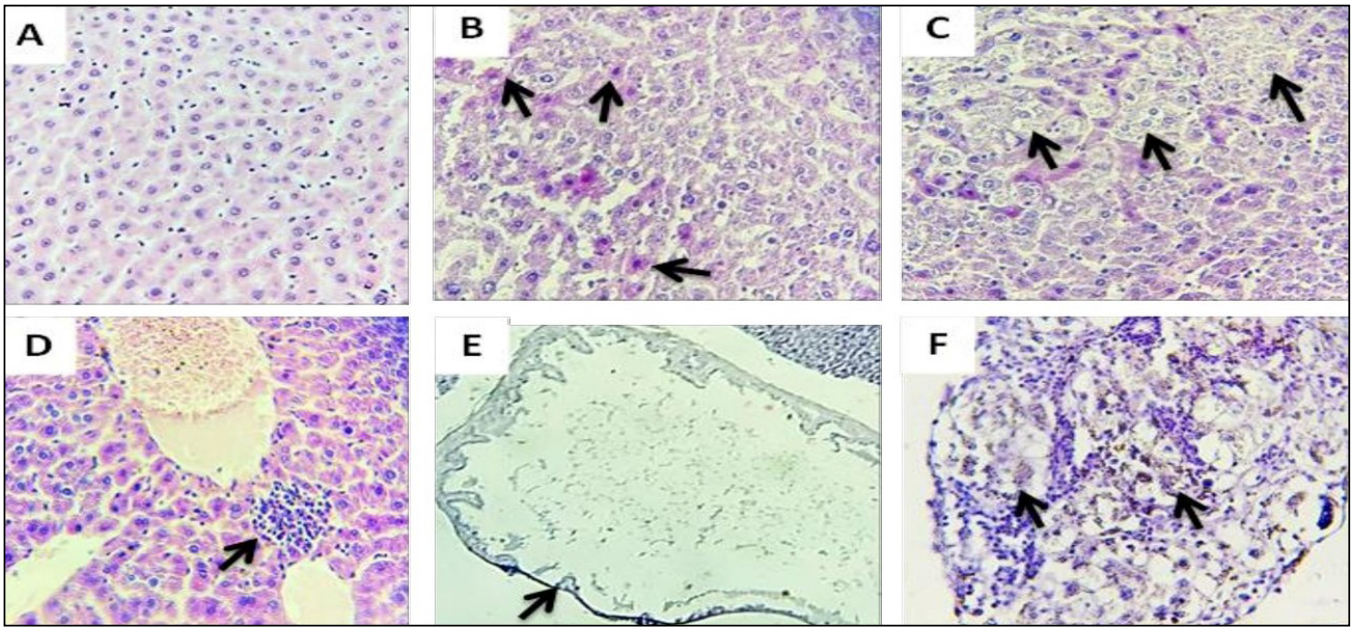

Fig. 4. (A) normal histology of the control group, (B-F) Histological images of liver sections in INH-RMP group showing moderate liver damage (score 2), (B) apoptotic cells with chromatin condensation, pyknotic nuclei, (C) hepatocytes ballooning and intracytoplasmic vacuoles, (D) mild pericentral inflammation, (E) hydatid cyst, (F) multiple granulomas, H&E stain, X400.

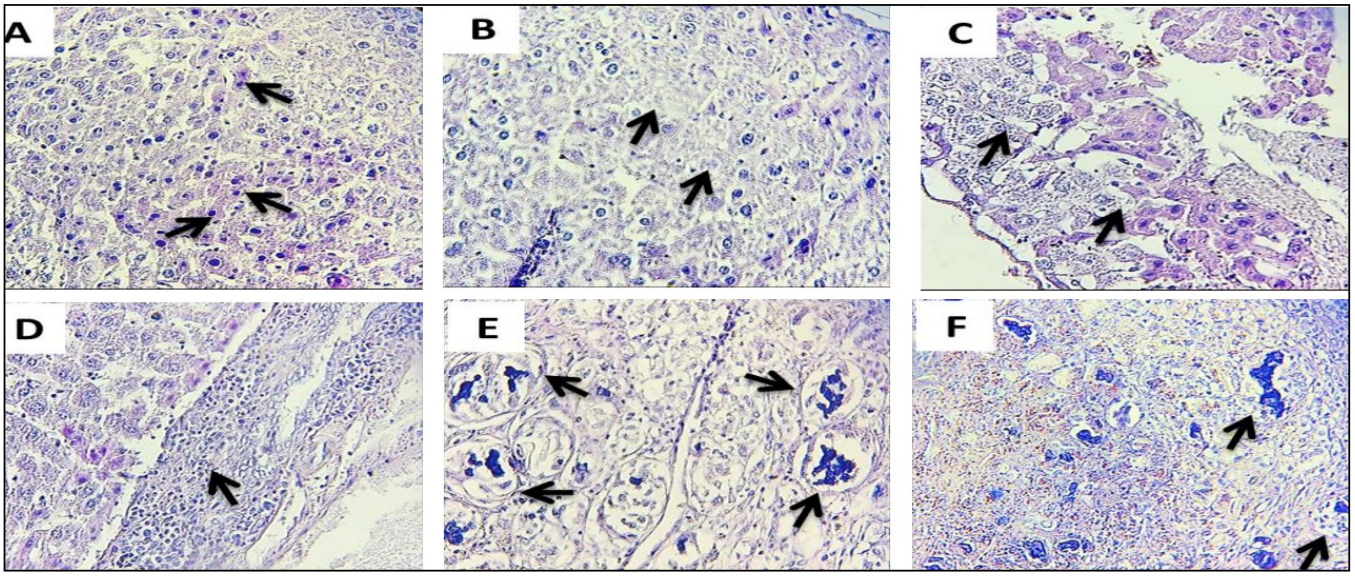

Fig. 5. Histological images of liver sections in INH-RMP group showing severe liver damage (score 3). (A) Apoptotic cells with chromatic condensation, pyknotic nuclei, (B) necrosis, (C) hepatocytes ballooning and intracytoplasmic vacuoles, (D) inflammation, (E &F) multiple granulomas, H&E stain, X400.

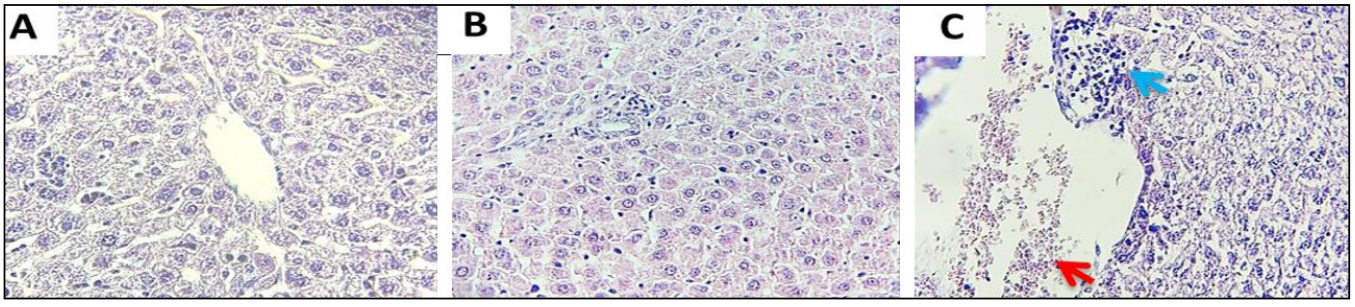

Fig. 6. Histological images of liver sections in (A) MT group showing normal histology. (B&C) INH-RMP+MT group showing: (B) liver with no damage (score 0), (C) Liver sections revealed mild liver damage (score 1) featured with pericentral Inflammation (blue arrow) and vascular congestion (red arrow). H&E stain, X400.

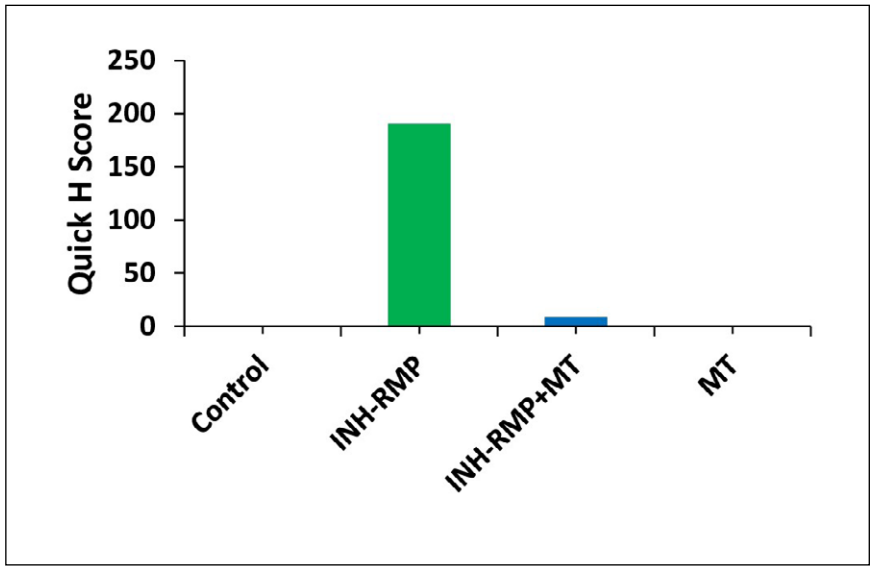

Fig. 7. Mean quick H score in four experimental groups (six mice in each group). ($P \leq 0.05$).

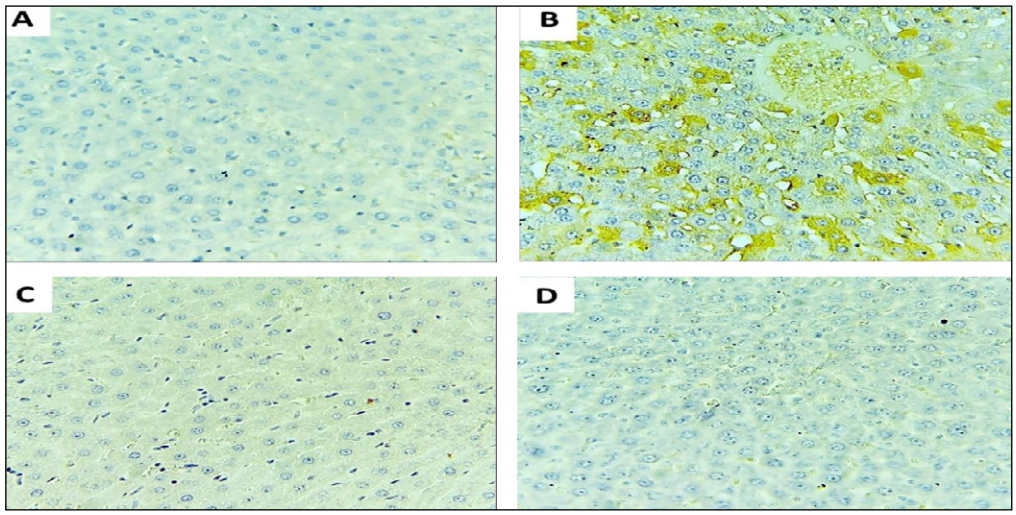

Fig. 8. Immunohistochemistry staining (brown) of Caspase3 for liver tissue: (A) control group showed negative staining, (B) INH-RMP group showed moderate to strong cytoplasmic caspace3 expression in apoptotic hepatocytes condensation in perivascular and around central veins, (C) INH-RMP+MT group showed weak cytoplasmic caspase3 expression, (D) MT group showed negative staining, X400.

RESULTS

PHYSIOLOGICAL ASSESSMENT

Serum levels of ALT and AST were utilized as markers of liver damage to examine liver function. Mice treated with INH-RMP had a significant increase in serum levels of ALT and AST when compared with the control group ($P \leq 0.05$) (Fig. 1, 2). In contrast, treatment with MT significantly reduced the serum levels of ALT and AST in the INH-RMP+MT group when compared with the INH-RMP group (Fig. 1, 2). In addition, there was a significant decrease in the serum levels of ALT and AST in the MT group when compared with the INH-RMP group (Fig. 1, 2). There was a significant decrease in the serum level of ALT in the MT group when compared with the INH-RMP+MT group (Fig. 1), whereas no significant results were found in the serum level of AST between the INH-RMP+MT group and MT group (Fig. 2).

HISTOPATHOLOGICAL SCORES OF LIVER INJURY

Histopathological study was used to investigate the severity of damage in the liver. Mice subjected to the INH-RMP group revealed moderate (score 2) and severe liver damage (score 3) compared to the control and MT groups (Fig. 3-6). By contrast, histological analysis of the liver in the INH-RMP+MT group showed a mild degree of liver damage (score 1), $P \leq 0.05$ (Fig. 3-6).

EFFECT OF INH-RMP ON APOPTOTIC MARKER (CASPACE3)

Quick H score results of cytoplasmic staining of caspace3 demonstrated normal liver tissue and absence of caspace3 (mean H score = 0) in control and MT groups (Fig. 7, 8). In contrast, the INH-RMP group showed moderate to strong positive cytoplasmic caspace3 expression in apoptotic hepatocyte condensation in

perivascular and around central veins. The H score of the INH-RMP group (H score = 190.8) was significantly (p-value less than 0.05) higher than the H score of the INH-RMP+MT group (H score = 8.3) (Fig. 7, 8).

DISCUSSION

The main goal of this current investigation is to provide a detailed understanding of melatonin's hepatoprotective effectiveness against liver injury caused by isoniazid-rifampicin. This research contributes to our understanding of melatonin's function in hepatic resilience and may be useful in the development of focused treatment strategies for hepatotoxicity caused by drugs. In this study, treatment with INH-RMP followed by MT was found to cause a marked reduction in levels of ALT and AST in comparison with the INH-RMP group. These results are in accordance with recent studies indicating that the levels of ALT and AST decreased in patients with liver disease when treated with melatonin [9, 12-13]. AST can be found in the liver, cardiac and skeletal muscles, pancreas, lungs, brain, kidneys, leucocytes, and red blood cells as both cytosolic and mitochondrial isoenzymes. The increase in AST may also be considered secondary to nonhepatic causes because it is not as sensitive or specific for the liver as ALT. The histological changes in hepatic tissues are shown by histopathological investigations, which also clarify the possible moderating effects of melatonin and illuminate the morphological changes caused by INH-rifampicin. Melatonin-induced immune responses can be better understood from a molecular perspective offered by immunohistochemical examination that focuses on certain protein markers. Determining the hepatoprotective mechanisms of melatonin in response to INH-rifampicin exposure requires an understanding of the cellular signaling pathways that it modulates [13]. Isoniazid is an anti-mycobacterial substance employed in the management of active or latent tuberculosis (TB). With nearly seven decades of clinical usage, INH continues to be widely employed as a primary component in anti-TB therapy. Nevertheless, the risk of liver damage and, in severe cases, fulminant liver failure associated with INH-based TB treatment poses a significant hurdle for TB control initiatives globally [14]. The primary manifestation of INH-induced hepatotoxicity is hepatocellular necrosis. Pharmaceuticals can induce liver injury in a foreseeable manner based on both the duration and dosage of their administration. Liver injury caused by INH typically manifests within weeks to months, rather than days to weeks after the onset of treatment [15]. Approximately 60% of INH hepatotoxicity cases in the United States Public Health Service (USPHS) study occurred within the initial 3 months of treatment, with 80% of the incidents taking place in the first 6 months [16], while certain individuals may show no symptoms, others may encounter symptomatic hepatotoxicity. Asymptomatic patients may display up to a three-fold increase above the upper limit of the normal range for serum ALT and AST. The majority of INH hepatotoxicity cases are mild and generally resolve even with the continuation of INH therapy. INH, when administered orally, undergoes rapid absorption through the gastrointestinal tract and is distributed to multiple organs, including the liver, brain, and kidneys [14]. INH is primarily metabolized in the liver. The isonicotinic acyl radical has the potential to form covalent adducts with liver macromolecules, possibly prompting immune responses [17, 18]. Mass spectrometric analysis has identified INH adducts on several proteins within murine livers [19]. Nevertheless, the mechanisms underlying the formation of the INH radical and its interaction with liver proteins remain unclear. These medications facilitate the production of extremely reactive oxygen species (ROS), serving as initiators of lipid peroxidation and a mechanism for damaging the plasma membrane [20-22]. Rifampicin effectively triggers CYP2E1, a cytochrome P450 family member accountable for breaking down environmental chemicals and carcinogens. Additionally, it heightens INH-induced toxicity by promoting the generation of the toxic metabolite hydrazine through the amidase pathway [23]. Hydrazine subsequently interacts with the sulfhydryl group of glutathione (GSH), leading to a depletion of GSH levels within hepatocytes and resulting in cell death [3, 5, 24]. The combined use of INH-RIH in chemotherapy was observed to enhance the transformation of INH into isonicotinic acid, an additional hepatotoxic product. Rifampicin further accelerates the plasma half-life of acetyl hydrazine by rapidly converting it into active metabolites, thereby elevating the likelihood of liver necrosis [17, 25]. Initial research, primarily focused on its positive impact on sleep disorders, stemmed from observations highlighting melatonin's favourable outcomes in various pathological conditions affecting different organs. In the context of the liver, melatonin has been shown to bring about improvements in several experimental models of damage [26-28]. The liver plays a primary role in melatonin catabolism, accounting for 90% of the process through conventional glucuronidation-sulfation pathways. The elimination of melatonin takes place via urine, with excretion occurring in the form of either sulfated metabolites or unchanged melatonin in small quantities [29-30]. Numerous studies have investigated the impact of melatonin on liver injuries and diseases. Melatonin has demonstrated the ability to modulate various molecular pathways, including

those involved in inflammation, metastasis, apoptosis, and autophagy, across different pathophysiological conditions. These findings suggested that melatonin holds the potential for both preventing and treating liver injuries and diseases [11, 31-33].

CONCLUSIONS

In recent decades, numerous significant effects of melatonin have been uncovered. Experimental findings highlight the potential benefits of melatonin in this model, prompting speculation on its potential application in human therapy. Physiological evaluations including liver function tests provided a thorough understanding of the systemic effects of melatonin treatment. The microscopic changes in the hepatic tissues clarified the hypothesized moderating effects of melatonin and illuminated the morphological changes caused by the drug combination. Additionally, immunohistochemistry investigations that concentrated on the Caspace3 marker revealed the molecular landscape, offering insights into the cellular signaling pathways that are modified in response to the INH-rifampicin challenge and the immune responses triggered by melatonin.

REFERENCES

1. Nieuwlaat R, Wilczynski N, Navarro T et al. Interventions for enhancing medication adherence. Cochrane Database Syst Rev. 2014;2014(11):CD000011. doi:10.1002/14651858.CD000011.pub4. DOI
2. Shishoo CJ, Shah SA, Rathod IS et al. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int J Pharm. 2001;228(1-2):53-67. doi:10.1016/s0378-5173(01)00831-6. DOI
3. Tasduq SA, Peerzada K, Koul S et al. Biochemical manifestations of anti-tuberculosis drugs induced hepatotoxicity and the effect of silymarin. Hepatol Res. 2005;31(3):132-135. doi:10.1016/j.hepres.2005.01.005. DOI
4. Wang P, Pradhan K, Zhong XB et al. Isoniazid metabolism and hepatotoxicity. Acta Pharm Sin B. 2016;6(5):384-392. doi:10.1016/j.apsb.2016.07.014. DOI
5. Kapgate SM, Patil AB. Standardization of model of induction of hepatotoxicity with anti-tuberculosis drugs in wistar albino rats. Asian J Pharm Clin Res. 2017;10(6):150-153. doi:10.22159/ajpcr.2017.v10i6.11971. DOI
6. National Institute of Diabetes and Digestive and Kidney Diseases. Liver tox: Clinical and Research Information on Drug-Induced Liver Injury. 2012. https://www.ncbi.nlm.nih.gov/books/NBK547852/. [Accessed 15 October 2023]
7. Zhang JJ, Meng X, Li Y et al. Effects of melatonin on liver injuries and diseases. Int J Mol Sci. 2017;18(4):673. doi:10.3390/ijms18040673.
8. Othman MS, Fareid MA, Abdel Hameed RS et al. The protective effects of melatonin on aluminum-induced hepatotoxicity and nephrotoxicity in rats. Oxid Med Cell Longev. 2020;2020:7375136. doi:10.1155/2020/7375136. DOI
9. Esmaeili A, Nassiri Toosi M, Taher M et al. Melatonin effect on platelet count in patients with liver disease. Gastroenterol Hepatol Bed Bench. 2021;14(4):356-361.
10. Li Z, Liu T, Feng Y et al. PPARγ Alleviates Sepsis-Induced Liver Injury by Inhibiting Hepatocyte Pyroptosis via Inhibition of the ROS/TXNIP/NLRP3 Signaling Pathway. Oxid Med Cell Longev. 2022;2022:1269747. doi:10.1155/2022/1269747. DOI
11. Jin Z, El-Deiry WS. Overview of cell death signaling pathways. Cancer Biol Ther. 2005;4(2):139-163. doi:10.4161/cbt.4.2.1508. DOI
12. Mansoori A, Salimi Z, Hosseini SA et al. The effect of melatonin supplementation on liver indices in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis of randomized clinical trials. Complement Ther Med. 2020;52:102398. doi:10.1016/j.ctim.2020.102398. DOI
13. Terziev D, Terzieva D. Experimental Data on the Role of Melatonin in the Pathogenesis of Nonalcoholic Fatty Liver Disease. Biomedicines. 2023;11(6):1722. doi:10.3390/biomedicines11061722. DOI
14. Lei S, Gu R, Ma X. Clinical perspectives of isoniazid-induced liver injury. Liver Res. 2021;5(2):45–52. doi:10.1016/j.livres.2021.02.001. DOI
15. Mitchell JR, Zimmerman HJ, Ishak KG et al. Isoniazid liver injury: clinical spectrum, pathology, and probable pathogenesis. Ann Intern Med. 1976;84(2):181-192. doi:10.7326/0003-4819-84-2-181. DOI
16. Fountain FF, Tolley E, Chrisman CR et al. Isoniazid hepatotoxicity associated with treatment of latent tuberculosis infection: a 7-year evaluation from a public health tuberculosis clinic. Chest. 2005;128(1):116-123. doi:10.1378/chest.128.1.116. DOI
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. Chem Res Toxicol. 2012;25(11):2567-2576. doi:10.1021/tx300341r. DOI
18. Metushi IG, Sanders C, Lee WM et al. Detection of anti-isoniazid and anti-cytochrome P450 antibodies in patients with isoniazid-induced liver failure. Hepatology. 2014;59(3):1084-1093. doi:10.1002/hep.26564. DOI
19. Koen YM, Galeva NA, Metushi IG et al. Protein targets of isoniazid-reactive metabolites in mouse liver in vivo. Chem Res Toxicol. 2016;29(6):1064-1072. doi:10.1021/acs.chemrestox.6b00098. DOI
20. He X, Song Y, Wang L et al. Protective effect of pyrrolidine dithiocarbamate on isoniazid/rifampicin-induced liver injury in rats. Mol Med Rep. 2020;21(1):463-469. doi:10.3892/mmr.2019.10817. DOI

21. Georgieva N V, Gadjeva V, Tolekova A. New isonicotinoylhydrazones with ssa protect against oxidative-hepatic injury of isoniazid. Trakia J Sci. 2004;2(2):37–43.
22. Lian Y, Zhao J, Xu P et al. Protective effects of metallothionein on isoniazid and rifampicin-induced hepatotoxicity in mice. PLoS One. 2013;8(8):e72058. doi:10.1371/journal.pone.0072058. DOI
23. Ullah U, Badshah H, Malik Z et al. Hepatoprotective effects of melatonin and celecoxib against ethanol-induced hepatotoxicity in rats. Immunopharmacol Immunotoxicol. 2020;42(3):255-263. doi:10.1080/08923973.2020.1746802. DOI
24. Naji KM, Al-Khatib BY, Al-Haj NS et al. Hepatoprotective activity of melittin on isoniazid- and rifampicin-induced liver injuries in male albino rats. BMC Pharmacol Toxicol. 2021;22(1):39. doi:10.1186/s40360-021-00507-9. DOI
25. Tostmann A, Boeree MJ, Aarnoutse RE et al. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. J Gastroenterol Hepatol. 2008;23(2):192-202. doi:10.1111/j.1440-1746.2007.05207.x. DOI
26. Bona S, Fernandes SA, Moreira ACJ et al. Melatonin restores zinc levels, activates the Keap1/Nrf2 pathway, and modulates endoplasmic reticular stress and HSP in rats with chronic hepatotoxicity. World J Gastrointest Pharmacol Ther. 2022;13(2):11-22. doi:10.4292/wjgpt.v13.i2.11. DOI
27. Deng WS, Xu Q, Liu YE et al. Effects of melatonin on liver function and lipid peroxidation in a rat model of hepatic ischemia/reperfusion injury. Exp Ther Med. 2016;11(5):1955-1960. doi:10.3892/etm.2016.3160. DOI
28. Baiocchi L, Zhou T, Liangpunsakul S et al. Possible application of melatonin treatment in human diseases of the biliary tract. Am J Physiol Gastrointest Liver Physiol. 2019;317(5):G651-G660. doi:10.1152/ajpgi.00110.2019. DOI
29. Sankar M, Rajkumar J, Sridhar D. Hepatoprotective activity of heptoplus on isoniazid and rifampicin induced liver damage in rats. Indian J Pharm Sci. 2015;77(5):556-562. doi:10.4103/0250-474x.169028. DOI
30. Oztopuz O, Turkon H, Buyuk B et al. Melatonin ameliorates sodium valproate-induced hepatotoxicity in rats. Mol Biol Rep. 2020;47(1):317-325. doi:10.1007/s11033-019-05134-6. DOI
31. Sadoughi F, Maleki Dana P, Homayoonfal M et al. Molecular basis of melatonin protective effects in metastasis: A novel target of melatonin. Biochimie. 2022;202:15-25. doi:10.1016/j.biochi.2022.05.012. DOI
32. Cho JH, Bhutani S, Kim CH et al. Anti-inflammatory effects of melatonin: A systematic review and meta-analysis of clinical trials. Brain Behav Immun. 2021;93:245-253. doi:10.1016/j.bbi.2021.01.034. DOI
33. Sagrillo-Fagundes L, Bienvenue-Pariseault J, Vaillancourt C. Melatonin: The smart molecule that differentially modulates autophagy in tumor and normal placental cells. PLoS One. 2019;14(1):e0202458. doi:10.1371/journal.pone.0202458. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Rasha Hatem Dosh
University of Kufa
299G+HPX, Kufa Street, Kufa, Najaf Governorate, Najaf, Iraq
e-mail: sgahmed1331962@outlook.com

ORCID AND CONTRIBUTIONSHIP

Rasha Hatem Dosh: 0000-0002-2318-6608 B C
Siham Mahmood Al-Rehemi: 0000-0002-0832-7935 A D
Maha Jamal Frayyeh: 0000-0001-6202-8023 B C D
Rihab Hameed Al Mudhafar: 0000-0003-4997-5549 E
Hussein Abdulkadhim: 0009-0008-2034-2506 A F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 07.06.2024
ACCEPTED: 12.08.2024