Iran Red Crescent Med J. 2018 May; 20(5):e60819. doi: 10.5812/ircmj.60819.

Published online 2018 April 17. **Editorial**

Methyl Palmitate Protection Against Isoniazid and Rifampicin-Induced Oxidative Liver Damage

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Received 2017 August 24; **Revised** 2017 October 18; **Accepted** 2017 December 20.

To the Editor,

Isoniazid (INH) and rifampicin (RFP) are primary antituberculosis drugs. Hepatotoxicity is one of the most critical side effects of these drugs (1). Cessation of INH and RFP, due to hepatotoxicity in patients with tuberculosis, may lead to treatment failure. Therefore, we need novel protective agents against the hepatotoxic side effects of INH and RFP.

Methyl palmitate (MP, methyl ester of palmitic acid) is a fatty acid composed of methyl esters (2). Experimental trials have shown that MP exerts antiphagocytic activity by inhibiting inflammatory cells (3). The protective effects of MP on NF-kappa B decrease the stimulation of the tumor necrosis factor alfa (TNF-alfa), cyclooxygenase-2 (COX-2), nitric oxide (NO), and oxidative stress (3). In this study, we hypothesized that MP could prevent INH/RFP induced oxidative liver damage in mice.

This study was conducted at Adnan Menderes University, Aydin, Turkey, between October 1 to October 21, 2016. A total of 30 pathogen-free female BALB/c mice were obtained from the experimental animal studies center of the Adnan Menderes University. They were kept in hygienic macrolene cages in air-conditioned rooms at 22 ± 3°C on a 12-hour light/dark cycle. Food and tap water were provided ad libitum. All experimental procedures complied with the requirements of the institutional animal care and Ethics committee (64583101/2016/72). INH, RFP, and MP were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). MP was dissolved in corn oil with a vortex.

Animals were randomly allocated into five groups (n = 10) by use of a random number generator (random.org). In Group A, animals were treated with saline only; in Group B, animals were treated with RFP (300 mg/kg) + INH (150 mg/kg) by gastric gavage, and in Group C, animals were treated with RFP (300 mg/kg) + INH (150 mg/kg) by gastric gavage and intraperitoneal MP (300 mg/kg, 3/w). The mice were treated for 21 days and sacrificed 24 hours after the last treatment to collect liver tissue.

The liver tissues were fixed in 10% formaldehyde, embedded in paraffin blocks, and 3.5 micrometer thick liver sections were prepared. These sections were stained with Hematoxylin and Eosin (HE) and enclosed with a coverslip. Single investigator evaluated all histologic sections using a light microscope (BX20, Olympus Corporation, Tokyo, Japan) in a blinded fashion. Hepatic injury was evaluated according to Ashcroft (4).

Malondialdehyde (MDA) level (nmol/mg) was determined by measuring the absorbance of thiobarbituric acid (TBA)-MDA complex with a spectrophotometer at 532 nm according to Draper and Hadley (5). Catalase activity was determined by observing the decrease of H_2O_2 concentration per unit of time spectrophotometrically at 240 nm, according to the Aebi method (6). The glutathione peroxidase (GPx) activity was determined by measuring the rate of NADPH oxidation based on the reduction of peroxide hydroperoxide by GPx in the presence of glutathione (7). Superoxide Dismutase (SOD) activity was measured in liver homogenates using a commercially available kit, according to manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, PR China). All instruments were calibrated before measurements.

The data were analyzed using the SPSS version 20.0 (IBM Corp., Armonk, N.Y., USA). Continuous variables were presented with mean ± standard deviation. One way analysis of variance was used to compare groups. Statistical significance was set at $P < 0.05$.

In this study, we investigated the effects of MP on INH/RFP induced hepatotoxicity. We observed that the INH/RFP administration caused apparent histopathological changes such as hepatocellular swelling, vacuolization, and fatty degeneration (Figure 1A). In the MP group, however, no abnormal histopathological changes were observed (Figure 1B). Control livers showed no signs of apparent abnormality. We concluded that MP treatment prevented histopathological changes caused by INH/RFP in mice livers.

Previous studies have shown that hepatotoxic effects of INH/RFP are based on oxidative stress (8). To evaluate the oxidative injury caused by INH/RFP and protective effects of MP, we measured MDA, the end product of lipid peroxi-

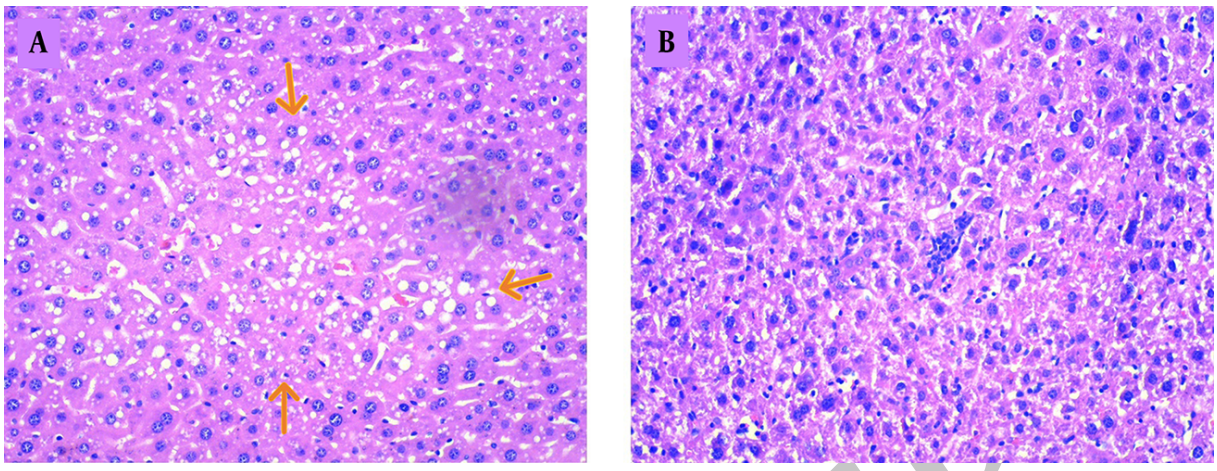

Figure 1. A, Histological analysis of the INH/RFP group hepatic tissue: hepatocellular swelling, vacuolization and fatty degeneration (Arrows) were observed in the hepatic tissue, HE, X200; B, histological analysis of the MPA group hepatic tissue; livers showed no signs of apparent abnormality in mice, HE, X200.

dation, and antioxidant enzyme activities in liver tissues. MDA level was significantly higher in the INH/RFP group (101.53 ± 3.87 nmol/mg) compared to the control (54.66 ± 6.37 nmol/mg) and MP groups (61.68 ± 5.77 nmol/mg) ($P < 0.001$). Compared to the control group (0.62 ± 0.1), INH/RFP treatment significantly reduced catalase activity (0.17 ± 0.05), however, MP treatment increased catalase activity to normal levels (0.89 ± 0.07) ($P < 0.001$). GPx activity was significantly lower in the MP (2.62 ± 0.66 U/mL) and INH/RFP (3.02 ± 0.91 U/mL) groups ($P = 0.018$) compared to the control group (4.93 ± 2.76 U/mL). Compared to the control group (18.32 ± 1.16 U/mL), INH/RFP treatment significantly reduced SOD activity (13.06 ± 0.66 U/mL); however, MP treatment increased SOD activity to normal levels (22.29 ± 4.32 U/ml) ($P < 0.001$).

Our findings showed that increased generation of reactive oxygen species by INH/RFP in livers reduces the antioxidant enzyme availability and henceforth increases lipid peroxidation. On the other hand, MP treatment restores antioxidant enzyme levels that were diminished by INH/RFP and therefore, limits lipid peroxidation. Shoeib et al. investigated the effects of MP in acetaminophen-induced hepatotoxicity (9). Similar to our findings, they reported that the protective effects of MP are based on alleviation of the oxidative stress. Kuppfer cells could be the primary source of reactive oxygen species that cause oxidative liver injury. The inhibitory effect of MP on Kuppfer cells have been demonstrated before (3, 10). We think that hepatoprotective effects of MP against INH/RFP could be mediated via inhibition of Kuppfer cells through NF-cappa B pathway (3).

This study has limitations. First, confirmation of hepatotoxicity with biochemical parameters is lacking. Secondly, due to technical limitations, we were unable to conduct electron microscope analysis. Lastly, NF-cappa B levels were not studied.

In this study, we demonstrated that methyl palmitate prevents INH/RFP-induced oxidative liver injury in mice. Further studies are needed to elucidate the mechanisms of protective effects of MP against INH/RFP induced hepatotoxicity.

Footnote

Conflict of Interests: The authors state that they have no conflict of interest.

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