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Diallyl trisulfide protects liver against the hepatotoxicity induced by isoniazid and rifampin in mice by reducing oxidative stress and activating Kupffer cells

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Number of figures and tables: six figures and two tables.
ABSTRACT

Background&Aim Diallyl trisulfide (DATS) has been verified to ameliorate hepatotoxicity induced by many drugs, but the protective actions in isoniazid (INH) and rifampicin (RFP) have not been reported. We attempted to elucidate the potential effects and mechanisms of DATS against INH&RFP-caused hepatotoxicity.

Methods Male Kunming mice weighing 18-22g were divided into 6 groups. For the hepatic-protective study, co-administrations of DATS (10mg/kg, 20mg/kg, and 40mg/kg bw, respectively) were orally administered two hours before the INH&RFP (100mg/kg, 100mg/kg bw, respectively) treatments. After 11 days treatments, 10 mice in each group were performed for the carbon clearance test, while the other 10 mice were sacrificed for the collection of serum and livers for further measures, including the levels of serum alanine aminotransferase (ALT), aspartate transaminase (AST) and total bilirubin (T.Bili), the liver index, and liver histopathological examination. Malondialdehyde (MDA), glutathione (GSH), the carbon clearance test, the level of interleukin 1-β (IL-1-β) and the immunohistochemistry of F4/80 marked for activated kupffer cell (KC) were measured to investigate potential mechanisms.

Results DATS co-administration significantly inhibited the increase of liver index and elevation of serum ALT, AST and T.Bili levels induced by INH&RFP, as well as improved hepatocellular structure. The further mechanistic studies demonstrated that DATS co-administration counteracted INH&RFP-induced oxidative stress in mice, which was illustrated by the restoration of GSH levels, and the reduction of MDA levels in liver. Furthermore, DATS co-administration reactivated the KCs inhibited by
INH&RFP, which was illustrated by the increase of carbon phagocytosis, the restoration of the number of activated KCs and IL-1-β levels in liver.

Conclusion DATS effectively protected liver against INH&RFP-induced hepatotoxicity, which might be due to its antioxidant effect and enhancement of KCs’ activities.

KEY WORDS Diallyl trisulfide; Kupffer cell; Isoniazid; Rifampicin; hepatic protection; Immune mechanism
INTRODUCTION

Tuberculosis (TB), an infectious disease induced by infection of *mycobacteria*, remains a major public health problem and leading cause of morbidity and mortality in the world. It is estimated that about 1/3 of the world’s population have been infected with *mycobacterium* TB, and the new infections occur in about 1% of the population every year\(^1\).\(^2\).

Isoniazid (INH) and rifampicin (RFP) are the first-line drugs for the treatment of TB. Unfortunately, these drugs could cause serious adverse effects including drug induced liver injury (DILI)\(^3\). Importantly, the DILI induced by INH&RFP may lead to the termination of the TB treatment, which contributes to the emergence of drug-resistant TB strains\(^4\). Therefore, it’s an urgent task to find protective drugs against the injury and/or find alternative drugs against TB.

Though the damage mechanism remains unclear, classical studies focusing on the INH metabolism assumed that: After entering body, INH is mainly metabolized into acetyl-INH by N-acetyltransferase (NAT2) in the liver and then acetyl-INH is hydrolyzed into acetyl-hydrazine and isonicotinic acid. This pathway of metabolism is mainly oxidized by CYP2E1 accompanied by the production of many reactive hepatotoxins, such as acetyldiazene, ketienoe, ion and radical\(^5\).\(^7\). Additionally, a number of INH undergoes a secondary metabolic pathway hydrolyzed into hydrazine by amidase\(^8\). Both acetylhydrazine and hydrazine will generate the oxidative stress and induce the hepatotoxicity. And a few studies have shown that co-administration of RFP to INH could increase the productions of hepatotoxins such as hydrazine due to its positive effect of CYP2E1 activation\(^9\).\(^11\). Moreover, Metushi et al. have demonstrated that INH also could be oxidized into diazohydroxide. INH itself and the
reactive metabolite form diazohydroxide could covalent bind to the hepatic protein in humans and mice. Both INH-protein and diazohydroxide-protein could induce the increase of INH and an immune hepatotoxicity\textsuperscript{12, 13}.

Kupffer cells (KCs), resident in the liver, are the largest macrophage population in the liver. The major function of KCs is to phagocytize foreign material, including both opsonized and non-opsonized particles as well as to synthesize and release proinflammatory cytokines, such as TNF-\textgreek{a}. Both the macrophage inhibition and increased inflammatory reaction of KCs will induce a liver injury\textsuperscript{14}. Though more and more hepatotoxins have been verified to cause damage via KCs\textsuperscript{15-17}, it’s unclear whether INH&RFP could also induce hepatotoxicity by targeting on KCs.

Diallyl trisulfide (DATS) is an organic sulfide that riches in \textit{S-allyl} cysteine extracted from garlic. It has been well demonstrated that the organosulfur compounds are the major component for the beneficial effects of garlic and its related products such as garlic oil\textsuperscript{5, 11}, which has been reported to have series biological effects, including anti-oxidant, anti-tuberculosis, and anti-inflammation\textsuperscript{12-14}. Moreover, recent studies showed that DATS could alleviate various hepatotoxic effect by ethanol, naphthalene, carbon tetrachloride (Ccl\textsubscript{4}), arsenic et al. through attenuating oxidative stress\textsuperscript{18-21}. More interestingly, DATS was also reported to regulate the immune responses and enhance the function of macrophage\textsuperscript{20, 22, 23}. These all imply that DATS could be a potential drug to prevent the damage caused by INH&RFP.

To address the above question, our study was aimed to investigate the effects of DATS on INH&RFP-induced liver injury. As known, the co-treatment of INH&RFP is a standard regimen for anti-TB therapy clinically. We use a nonlethal, short-term
mouse model with co-administration of INH&RFP to certify it. The dose and time
frame of INH&RFP was defined according to the human-equivalent doses of mouse
as well as reference from previous studies. In those reports, the dose scopes of INH
were given from 50 to 200mg/kg bw, and the dose scopes of RFP were given from
100 to 200mg/kg bw24,25,26,27. To ensure the hepatic injury and no lethal dose of the
cotreatment regimen in mice, a pilot study was performed prior to our formal
experiment and we found the dose of 100mg/kg bw+100mg/kg bw was the best
regimen for the short term model. And based on this model, we found that DATS
effectively protected liver against INH&RFP-induced hepatotoxicity, which might be
due to its antioxidant effect and enhancement of KCs’ activities.
MATERIALS and METHODS

Materials

DATS (purity > 97%) was purchased from Chia Tai of Jiangsu CN (Chia Tai, China); Isoniazid Tablets (C_6H_7N_3O 0.1g INH/tablet) and Rifampicin capsules (C_43H_58N_4O_12 0.15g/capsule), which were produced by Xinyi of Shanghai CN (Xinyi, China), were obtained from Qilu Hospital, and then INH and RFP were 1:1 dissolve into physiological saline; Corn oil was purchased from local market of Jinan CN (GB19111, Gold Embryo CORVOIL Co., Shandong, China); Carbon ink was purchased from Chemical Reagent of Beijing CN (Che Rea, China); ALT kit, AST kit, and T.Bili kit were purchased from Biosino of Beijing CN (Biosino, China); GSH assay kit and MDA assay kit were purchased from Njjcbio of Nanjing CN (Njjcbio, China); Mouse IL-1-β sunny ELISA was purchased from Multi Sci of Beijing CN (Multi Sci, China); Rat anti-mouse-F4/80 serotec was purchased from AbD of Oxford UK (AbD, England); Rat IgG immunohistochemistry kit was purchased from Boster of Wuhan CN (Boster, China).

Animals’ Treatment

SPF male Kunming mice (18-22g) were provided by the Animal Center of Shandong University (Jinan, China), Certificate of Laboratory Animal: SYXX (Jinan China) 20100011. 120 mice were maintained at approximately 22°C with a 12-h light: 12-h dark cycle, and had free access to standard chow and tap water. After 5 days acclimation to the laboratory conditions, the animals were randomly divided into 6 groups (n=20): mice in the INH&RFP+DATS groups and DATS group were treated
with DATS (10mg/kg, 20mg/kg, 40mg/kg, and 40mg/kg bw, respectively) by gavage
every day, while the mice in the control group and INH&RFP group received equal
volume (0.1ml/10g bw) of corn oil. Two hours after the DATS administration, all the
animals except those in the control group and DATS (40mg/kg bw) group orally
received an INH&RFP (100mg/kg and 100mg/kg bw, respectively), the control group
and DATS (40mg/kg bw) group orally got an equal volume (0.2ml/10g bw) of
physiological saline. During the treatments, the body weight was measured at 1, 4, 8,
11 days. After 11 days of co-administration, 10 mice of each group were anesthetized
at 24 hours after the last treatment. Blood was collected by eyeball extract method and
centrifuged at 1500×g for 20 minutes at 4 °C to obtain serum. Liver was stripped and
weighed. A portion of the liver was fixed in paraformaldehyde (4%) for
histopathology and immunohistochemistry, while the other portion of liver tissue was
quickly frozen in liquid nitrogen before storing at -80 °C. The other 10 mice in each
group were injected in India ink through tail vein (i.v.) to measure the phagocytic
capacities by the method of Hudson’s et al10.

All animals procedures were performed according to the National Institutes of
Health Guidelines for the Care and Use of Laboratory Animals which were approved
by the Animal Experimentation Committee of Shandong Uniervstity. All efforts were
made to minimize animal suffering during experiment.

**Measures of Serum Biochemical Index**

The levels of serum alanine aminotransferase (ALT) (rate method, YZB0694, Beijing,
China), aspartate aminotransferase (AST) ( rate method, YZB0693, Beijing, China),
total bilirubin (T.Bili) (diazonium salt method, YZB0121, Beijing, China) were measured by GLAMOUR 1600 random access clinical analyzer (Buenos Aires, Argentina) according to the protocols from manufacturers.

Measures of Mice Phagocytic Capacities----Carbon clearance test

The phagocytosis was measured in vivo using carbon clearance method as previously reported\textsuperscript{16}. In brief, carbon ink diluted with saline injections (dilution 1:3) was injected to mice (i.v., 0.1ml/10g bw) at the 2 minute, 10 minute interval after ink injection, and then 20\(\mu\)l of blood, taken from the inner canthus venous plexus, were added to 2ml 0.1\%\(\text{Na}_2\text{CO}_3\). After that, the mice were sacrificed, and the liver and spleen were stripped, weighted. The absorbance (OD) of the solutions was measured at 600 nm by Infinite M200 PROV of TECAN (Mannedorf, Switzerland) and phagocytic index (a) was calculated as following formula.

\[ K = \frac{\log_{10} \text{OD}_1 - \log_{10} \text{OD}_2}{t_2 - t_1} \]

\[ a = \frac{\text{body weight}}{\text{liver weight} + \text{spleen weight}} \times \frac{\text{body weight}}{K} \]

H&E Staining

Liver histopathological examination was performed using hematoxylin and eosin (H&E) staining. Slices of 5\(\mu\)m were prepared using paraffin slicer (Thermo), and then
deparaffinized, rehydrated, stained with H&E and then viewed by Olympus AX70 microscope of Tokyo JPN (Olympus, Japan).

**Immunohistochemistry of F4/80 marker for KCs**

The activation of KCs was measured using immunohistochemistry detection of F4/80 which is the well characterized and extensively referenced mouse macrophage marker\(^\text{17}\). Briefly, Liver paraffin sections (5\(\mu\)m) were deparaffinized, blocked using 3% H\(_2\)O\(_2\), and 5% normal goat serum, and then were incubated with rat monoclonal antibody against mouse F4/80 (1:200) (serotec, MCA497G, Oxford, UK) at 4\(^\circ\)C overnight. The following steps were performed strictly according to the procedure of a rat IgG immunohistochemistry kit (SABC method, BA1005, Wuhan, China), and then viewed by Olympus AX70 microscope of Tokyo JPN (Olympus, Japan).

**Antioxidant Status Assay in the Liver**

Liver tissues were homogenized in ice-cold 0.8% saline (w/v= 1:9), and then centrifuged at 1000\(\times\)g for 20min. at 4\(^\circ\)C. The supernatant was collected and stored at -80\(^\circ\)C for antioxidant assay.

The levels of T-GSH, GSH and GSSH in the liver were determined by the 5, 5-dithio-bis-2-nitrobenzoic acid assay using the assay kit (Micro ELISA method, A061-1, Nanjing, CN) according to the method described by the manufactures. The reaction products, which were depended on the amounts of T-GSH and GSSH, had an OD at 405nm by Infinite M200 PROV of TECAN (Mannedorf, Switzerland). And the amount of GSH was obtained by subtracting the two T-GSH, GSSH.
Malondialdehyde (MDA) content was measured by the accumulation of thiobarbituric acid-reactive substance and expressed for the Lipid peroxidation (LPO). Briefly, MDA reacted with 2-thiobarbituric acid (TBA) and a pink-colored product, which was developed depended by the concentration of MDA, had an OD at 532 nm by Infinite M200 PROV of TECAN (Mannedorf, Switzerland). The levels of MDA (nmol/ (mg pro)) were analyzed using commercial assay kits (TAB method, A003-1, Nanjing, CN) according to the manufacturer's instructions.

Quantification of IL-1β levels in liver

The levels of IL-1β in liver were determined using an ELISA kit (EK201B2, Multi SCI, CN) employed the quantitative sandwich enzyme immunoassay technique. Briefly, the sample and standards were added to ELISA plate which had been pre-coated with a monoclonal antibody specific for IL-1β, and IL-1β present was bound by the immobilized antibody. The unbound substances were washed away, and a biotin-linked monoclonal antibody specific for IL-1β was added to the wells. After a wash to remove any unbound substances, streptavidin-HRP was added. After washing, substrate solution was added and the color, which has an OD at 450nm measured by Infinite M200 PROV of TECAN (Mannedorf, Switzerland), developed in proportion to the amount of IL-1β.

Statistical analysis

All data were expressed as mean and standard deviation (SD). SPSS18.0 statistical software was used for statistical analysis. Data was analyzed using one-way ANOVA to compare the means among different groups and Tukey. For the comparisons
between two experimental groups (i.e. INH&RFP group versus control group or INH&RFP+DATS groups versus INH&RFP group) was used LSD Test by SPSS 18.0 to be analyzed. A $p$ value <0.05 was considered significant.

RESULTS

Effects of DATS alone on the liver

The DATS only administered group was added, the results there showed that only with DATS no statistically significant differences in body weight and serum ALT, AST, T.Bili were observed compared with control group. Normal lobular architecture with normal cell morphology and no obvious pathological state were founded in both DATS group and control group. These all intimated that DATS is a safe drug in the study dose to the liver. (Figure 1 & Figure 2 & Figure 3 & Table 1 & Table 2)

Effects of DATS on INH&RFP-Induced Hepatotoxicity

Effects of DATS and INF&RFP on the body weight, liver weight and liver index

The body weights of mice were consecutively monitored in 11 days. After INH&RFP treatment, the body weight of mice showed a negative growth in INH&RFP group. At the end of 11 days, the final body weight of INH&RFP group was reduced by 4% of the initial value, while that of control group was increased by 19% ($P$<0.01). By the DATS co-administration, INH&RFP+DATS groups significantly reversed the body weight depression induced by INH&RFP (5%, 10%, 5% in 10, 20, 40 mg/kg bw, respectively) ($P$<0.05, $P$<0.01, $P$<0.01) at the end of 11 days. (Figure 1 & Table 1)
Table 1 showed the final body weight and liver weight of mice in six groups. The relative liver weight in INH&RFP group was increased by 84.76% compared to control group ($P<0.01$). Co-administration of 10, 20 and 40 mg/kg DATS significantly reduced the relative liver weight as compared with INH&RFP group ($P<0.01$) by 12.5%, 17.40%, 24.28%, respectively. (Table 2)

**DATS co-treatment attenuated INF&RFP-induced increase of serum ALT, AST and T.Bili levels**

The levels of serum ALT, AST and T.Bili were elevated in INH&RFP group compared to control group ($P<0.01$) (Figure 2): ALT activity increased from 45.98 ± 6.79 to 123.5 ± 15.89U/L, $p<0.01$; AST activity increased from 100.44 ± 14.89 to 191.85 ± 40.89U/L, $p<0.05$; T.Bili activity increased from 0.38 ± 0.71 to 11.36 ± 2.78, which indicated the hepatic injury. DATS (10mg/kg, 20mg/kg, 40mg/kg, respectively) co-administration groups were ameliorated in the levels of ALT and T.Bili ($P<0.05$ or $P<0.01$) (Figure 2A) (Figure 2C), the levels of AST was also significantly refined in INH&RFP+DATS (20 and 40mg/kg, respectively) compared with INH&RFP group ($P<0.01$ or $P<0.05$) (Figure 2B)

**DATS co-treatment improved the liver histology in INH&RFP-intoxicated mice**

The control and DATS group (40mg/kg bw) had normal lobular architecture with normal cell morphology and no obvious pathological state: cells closely packed in a funicular, the cytoplasm with red dye, the nucleus homogeneous with blue dye. The INH&RFP group emerged typical and obvious pathological characteristics in the portal triad region and other liver regions including loose irregular arrangement of
liver cell, a large number of large, round cavity cells appeared and cell necrosis; nuclei were large and deep dye, central venous blood stasis. Compared with INH&RFP group, INH&RFP+DATS (10mg/kg bw) had a small amount of small vacuoles cells. But in the INH&RFP+DATS (20 and 40mg/kg bw) group, the hepatic injury were obviously recovered. (Figure 3)

**DATS co-treatment effectively blocked INF&RFP-induced decrease of GSH and increase of MDA**

The levels of T-GSH, GSH in INH&RFP had a significant reduction in 31% ($P<0.05$), 51% ($P<0.01$) compared with control group. The contents of GSSH were higher than control group up to 103% ($P<0.01$), but this damage were well reversed by co-administration of INH&RFP and DATS (10, 20 and 40mg/kg bw; respectively). The levels of T-GSH were increased by 17%, 20%, 45% ($P<0.05$), respectively. The levels of GSH were increased by 48% ($P<0.01$), 51% ($P<0.01$), 95% ($P<0.01$), respectively. And the levels of GSSH were declined by 26% ($P<0.05$), 38% ($P<0.05$), 40% ($P<0.01$), respectively. (Table 2)

The levels of MDA are an index of the intensity of lipid peroxidation damage in the liver. The results showed that INH&RFP group had a higher increase of 164% ($P<0.01$) than control group, while with the DATS co-administration, the INH&RFP+DATS (10, 20 and 40 mg/kg bw, respectively) groups were significantly decreased the content of MDA by 47% ($P<0.05$), 56% ($P<0.01$), 59% ($P<0.01$) in comparison with INH&RFP group, respectively, (Table 2)

**DATS co-treatment improved the capacities of KCs induced by INH&RFP**
DATS co-treatment improved the phagocytic capacities by the assay of carbon clearance.

The assay of the carbon clearance test in INH&RFP group was decreased by 39% contrast to the control group, and the results showed that DATS (10, 20, 40 mg/kg bw, respectively) co-administration improved the carbon phagocytic capacities by 62%, 67%, 79% (P<0.01) contrast with the INH&RFP group. (Figure 4)

DATS co-treatment led to the activation percent of the kupffer cells

The immune staining for F4/80 of KCs in the liver showed that a reduction population of activated KCs even despaired in INH&RFP group (Fig 5.). With co-administration of DATS, the KCs in INH&RFP+DATS (10mg/kg bw), INH&RFP+DATS (20 mg/kg bw) and INH&RFP+DATS (40 mg/kg bw) group were in varying degrees of activations than INH&RFP group, and the activations were increased with the increasing dose of DATS. (Figure 5)

DATS co-treatment contributed to the normal secretion of IL-1-β by Kupffer cells

The secretions of IL-1β which was mainly secreted by KCs in the liver were decreased in INH&RFP group contrast with control group (P<0.01). By the DATS (10, 20, 40 mg/kg bw, respectively) treatments, the secretions of IL-1-β were similar with the activation of KCs and was increased by 52% (P<0.05), 82% (P<0.01), 113% (P<0.01), respectively. (Figure 6)

DISCUSSION
DATS has been shown to be an effective drug against many drugs and hepatotoxins induced liver injuries, such as ethanol, naphthalene, carbon tetrachloride (CCL₄), especially due to its powerful antioxidant functions. As a compound purified from garlic, DATS had less toxicity and stronger antioxidant capability than other garlic products. The hepatoprotective ability of DATS has received much studies, and it has been verified to provide multiple benefits, e.g. anti-tuberculosis, immunoregulation. But the effects of DATS on the INH&RFP-induced liver damage have not been reported. Thus, the effect of DATS against the hepatotoxicity induced by INH&RFP is worth to be studied.

ALT and AST are the most commonly indexes to assess the liver injury in vitro/vivo: Serum ALT level is a marker for the hepatotoxic effects while AST level is used to measure the liver function. The co-treatment of RFP also can result in an increase of serum T.Bili level. In this study, the results showed that DATS co-treatment significantly attenuated INF&RFP-induced increase of the serum ALT, AST, and T.Bili levels. Besides, the histological examination showed that INF&RFP treatment led to obvious liver injury shown as irregular arrangement of liver cells, vacuolar degeneration, and necrosis, which were also significantly suppressed by DATS co-treatment. These results strongly suggested that DATS effectively abrogated INF/RFP-induced liver injury.

The mechanism of INH&RFP-induced liver injury has been wildly investigated in the past decades, and the roles of GSH has been highlighted. GSH is the largest percent of non-enzymatic antioxidant in the liver, playing an important role in the antioxidant events. As known, oxidative stress results from an imbalance between

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1. DATS has been shown to be an effective drug against many drugs and hepatotoxins induced liver injuries, such as ethanol, naphthalene, carbon tetrachloride (CCL₄), especially due to its powerful antioxidant functions. As a compound purified from garlic, DATS had less toxicity and stronger antioxidant capability than other garlic products. The hepatoprotective ability of DATS has received much studies, and it has been verified to provide multiple benefits, e.g. anti-tuberculosis, immunoregulation. But the effects of DATS on the INH&RFP-induced liver damage have not been reported. Thus, the effect of DATS against the hepatotoxicity induced by INH&RFP is worth to be studied.

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3. The mechanism of INH&RFP-induced liver injury has been wildly investigated in the past decades, and the roles of GSH has been highlighted. GSH is the largest percent of non-enzymatic antioxidant in the liver, playing an important role in the antioxidant events. As known, oxidative stress results from an imbalance between
oxidants and antioxidants in favor of the oxidants. For the hepatotoxicity of INH&RFP, except for the over-production of oxidative stress, reduced GSH level after INH or hydrazine administration to rats indicates that the decrease of GSH might be also involved in their hepatotoxicity. In the antioxidant activities, the GSH is depleted by free radicals and other oxygen species produced by the hepatotoxins, such as acetyldiazene, ketienoe, ion and radical metabolized by INH&RFP, and oxidized into GSSH$^{10,39}$. In this study, we found a 31% decrease of GSH and 103% increase of GSSH after the INH&RFP administration for 11 days. Parallelly, in consistent with previous studies, it was noticed that INH&RFP exposure also led to significant increase of hepatic MDA levels, which is a biomarker of oxidative stress and lipid peroxidation. The results showed that MDA was increased 164%. However, DATS co-administration significantly elevated the GSH levels by 17%, 20%, 45%, and suppressed the increase of MDA level by 47%, 56%, 59%, which suggested that the restoration of GSH might be a mechanism for the protective effects and the antioxidant ability might be at least partially account for the protection against INH&RFP-induced liver injury.

In addition to the oxidative stress, a number of studies have suggested that immune system was involved in INH&RFP-induced hepatotoxicity$^{40,41}$. Metushi et al. demonstrated that Cb-b$^{-/-}$, PD1$^{-/-}$ mice (which have impaired immune tolerance) and the Rag$^{-/-}$ mice (which lack of T- and B- cells) were more vulnerable to INH-induced hepatotoxicity compared with the wild type C57BL/6 mice, which suggested that INH treatment led to immunosuppression$^{42}$. Several other studies also suggested that TB treatment could result in immune impairment$^{33,44}$. In view of the KCs are the largest
macrophages, and is the first step in the immune response of the liver, we detected the number of activated KCs by using immunochemistry assay of the KCs markers, F4/80, the results showed that INH&RFP exposure led to the significant decrease of the KCs number; And then the clearance of the carbon which was often been represented for the phagocytic activity of macrophages (KCs) and the secretion of IL-1-β were also decreased by 39% and 39.8% after INH&RFP treatment. The results that confirmed the inhibited activity of Kupffer cells reduced by INH&RFP were parallel to the reported impairment of the immune system by INH&RFP as we mentioned. Interestingly, DATS co-treatment led to significant increase of the number hepatic Kupffer cells as well as the increase of the phagocytic capacity. These results suggested that Kupffer cells depletion might be involved in INH&RFP-induced hepatotoxicity, and DATS could protect against INH&RFP-induced hepatotoxicity by activation of Kupffer cells.

In summary, our study demonstrated that DATS could effectively suppress INH&RFP-induced increase of serum ALT, AST, and T.Bili levels and improve the liver morphological changes, which might be associated with the antioxidant capacity and the immunoregulatory capacity. The results of the current study suggested that DATS might be candidate hepatoprotective drug for TB patients receiving INH&RFP.

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DECLARATION OF INTEREST
The authors declare no conflict of interest.
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Figure legends

Figure 1.
Figure 2.

A) 

B)
Figure 3.

Seen in the image 1.

A was for control group, B was for DATS (40mg/kg bw) group, C was for INH/RFP group, D was for INH/RFP+DATS (10mg/kg bw), E was for INH/RFP+DATS (20mg/kg bw), F was for INH/RFP+DATS (40mg/kg bw).

Figure 4.

Figure 5.

Seen in the image 2.
A was for control group, B was for DATS (40mg/kg bw) group, C was for INH/RFP group, D was for INH/RFP+DATS (10mg/kg bw), E was for INH/RFP+DATS (20mg/kg bw), F was for INH/RFP+DATS (40mg/kg bw).

**Figure 6.**

**Figure 1.** The changes of body weight in six groups during the study shown as Mean ± S.D. Compared with control group, †P<0.05, ‡P<0.01; Compared with INH&RFP group, ††P<0.05, ‡‡P<0.01.
Figure 2. (A) The levels of serum ALT (U/L) in the six group shown as Mean ± S.D. Compared with control group, *$P<0.05$, **$P<0.01$; Compared with INH&RFP group, *$P<0.05$. **$P<0.01$.

(B) The levels of serum AST (U/L) in the six group shown as Mean ± S.D. Compared with control group, *$P<0.05$, **$P<0.01$; Compared with INH&RFP group, *$P<0.05$, **$P<0.01$.

(C) The levels of serum T.Bili (μ mol/L) in the six group shown as Mean ± S.D. Compared with control group, *$P<0.05$, **$P<0.01$; Compared with INH&RFP group, *$P<0.05$, **$P<0.01$.

Figure 3. The H&E staining of the livers of different groups. Pictures were original captured at 100× magnification. The bar represents 100μm. A was represented for control group. B was for DATS (40mg/kg bw). C was for INH&RFP group. D was for INH&RFP+DATS (10mg/kg bw). E was for INH&RFP+DATS (20mg/kg bw). F was for INH&RFP+DATS (40mg/kg bw)

Figure 4. The assay of carbon clearance test (a) shown as Mean ± S.D. Compared with control group, *$P<0.05$, **$P<0.01$; Compared with INH&RFP group, *$P<0.05$, **$P<0.01$.

Figure 5. The immune stain for F4/80 of KC in six groups. Pictures were original captured at 200× magnification. The bar represents 100μm.

A was represented for control group. B was for DATS (40mg/kg bw). C was for INH&RFP group. D was for INH&RFP+DATS (10mg/kg bw). E was for INH&RFP+DATS (20mg/kg bw). F was for INH&RFP+DATS (40mg/kg bw)

Figure 6. The levels of IL-1-β (pg/(mg pro)) in the six group shown as Mean ± S.D. Compared with control group, *$P<0.05$, **$P<0.01$; Compared with INH&RFP group, *$P<0.05$, **$P<0.01$. 

25 Tables Legends
Table 1. The comparison of body and liver weight in six groups (mean±S.D.)

<table>
<thead>
<tr>
<th>groups</th>
<th>Final body weight (g)</th>
<th>liver weight (g)</th>
<th>liver index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>29.14±2.03**</td>
<td>1.23±0.13**</td>
<td>4.20±0.35**</td>
</tr>
<tr>
<td>DATS (40mg/kg)</td>
<td>27.66±2.27**</td>
<td>1.24±0.20**</td>
<td>4.45±0.36**</td>
</tr>
<tr>
<td>INH&amp;RFP group</td>
<td>22.75±3.07##</td>
<td>1.82±0.44##</td>
<td>7.76±0.7##</td>
</tr>
<tr>
<td>INH&amp;RFP+D ATS (10mg/kg bw)</td>
<td>27.13±2.44**</td>
<td>1.84±0.29**</td>
<td>#</td>
</tr>
<tr>
<td>INH&amp;RFP+D ATS (20mg/kg bw)</td>
<td>27.83±2.12**</td>
<td>1.80±0.24**</td>
<td>#</td>
</tr>
<tr>
<td>INH&amp;RFP+D ATS (40mg/kg bw)</td>
<td>27.24±2.04**</td>
<td>1.61±0.22**</td>
<td>#</td>
</tr>
</tbody>
</table>
Compared with control group, *P<0.05, **P<0.01; Compared with INH&RFP group, *P<0.05, **P<0.01.

Table 2. The levels of T-GSH, GSH, GSSH and MDA (mean±S.D.)

<table>
<thead>
<tr>
<th></th>
<th>T-GSH</th>
<th>GSSH</th>
<th>GSH</th>
<th>MDA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>µg/(mg pro)</td>
<td>µg/(mg pro)</td>
<td>µg/(mg pro)</td>
<td>nmol/(mg pro)</td>
</tr>
<tr>
<td>Control group</td>
<td>390.69±30.7</td>
<td>50.70±14.23</td>
<td>340.00±29.5</td>
<td>1.56±0.43</td>
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<tr>
<td>DATS (40mg/kg bw)</td>
<td>405.98±31.8</td>
<td>55.80±13.34</td>
<td>350.18±30.4</td>
<td>1.58±0.47</td>
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<tr>
<td>INH&amp;RFP</td>
<td>267.74±57.4</td>
<td>103.01±33.0</td>
<td>164.73±68.6</td>
<td>4.12±0.83</td>
</tr>
<tr>
<td>INH&amp;RFP+ DATS (10mg/kg bw)</td>
<td>312.95±47.4</td>
<td>76.02±26.62</td>
<td>244.64±50.1</td>
<td>2.18±0.57</td>
</tr>
<tr>
<td>INH&amp;RFP+ DATS (20mg/kg bw)</td>
<td>320.37±37.3</td>
<td>63.47±18.67</td>
<td>249.47±37.1</td>
<td>1.81±0.46</td>
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<tr>
<td>INH&amp;RFP+ DATS (40mg/kg bw)</td>
<td>387.42±40.5</td>
<td>61.80±20.39</td>
<td>320.62±43.1</td>
<td>1.69±0.47</td>
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</tbody>
</table>
Compared with control group, *$P<0.05$, **$P<0.01$; Compared with INH&RFP group,  1

* $P<0.05$, ** $P<0.01$.  

2
<table>
<thead>
<tr>
<th></th>
<th>Abbreviations</th>
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<tbody>
<tr>
<td>1</td>
<td>DATS diallyl trisulfide</td>
</tr>
<tr>
<td>2</td>
<td>INH isoniazid</td>
</tr>
<tr>
<td>3</td>
<td>RFP rifampicin]</td>
</tr>
<tr>
<td>4</td>
<td>ALT aminotransferase</td>
</tr>
<tr>
<td>5</td>
<td>AST aspartate transaminase</td>
</tr>
<tr>
<td>6</td>
<td>T.Bili total bilirubin</td>
</tr>
<tr>
<td>7</td>
<td>MDA Malondialdehyde</td>
</tr>
<tr>
<td>8</td>
<td>GSH glutathione</td>
</tr>
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<td>9</td>
<td>IL-1-β interleukin 1-β</td>
</tr>
<tr>
<td>10</td>
<td>KC kupffer cell</td>
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<tr>
<td>11</td>
<td>T-GSH total-glutathione</td>
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<tr>
<td>12</td>
<td>GSSH oxidized glutathione</td>
</tr>
<tr>
<td>13</td>
<td>TB Tuberculosis</td>
</tr>
<tr>
<td>14</td>
<td>DILI drug induced liver injury</td>
</tr>
<tr>
<td>15</td>
<td>TBA 2-thiobarbituric acid</td>
</tr>
<tr>
<td>16</td>
<td>SD standard deviation</td>
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</table>
1 ROS reactive oxygen species
Image 1
16x13mm (600 x 600 DPI)