Protective Effects of Resveratrol on Hepatotoxicity Induced by Isoniazid and Rifampicin via SIRT1 Modulation

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · OCTOBER 2014

Impact Factor: 3.8 · DOI: 10.1021/np5003143 · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

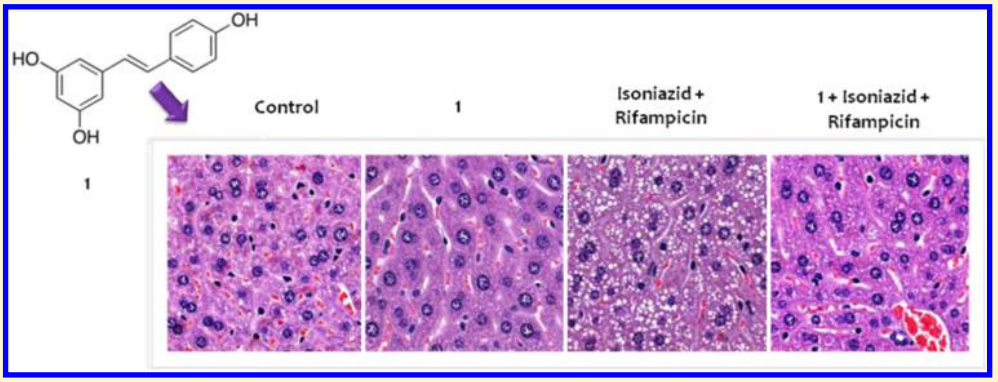
Article

pubs.acs.org/jnp

Protective Effects of Resveratrol on Hepatotoxicity Induced by Isoniazid and Rifampicin via SIRT1 Modulation

Natália F. Nicoletti,[†,‡] Valnês Rodrigues-Junior,[†,§] André A. Santos, Jr.,[†,‡] Carlos E. Leite,[∥] Ana C. O. Dias,[†] Eraldo L. Batista, Jr.,[⊥] Luiz A. Basso,[†,‡,§] Maria M. Campos,[†,§,∥,⊥] Diógenes S. Santos,[†,‡] and André A. Souto*[,‡,#]

[†]Instituto Nacional de Ciência e Tecnologia em Tuberculose, Centro de Pesquisas em Biologia Molecular e Funcional, [‡]Programa de Pós-Graduação em Biologia Celular e Molecular, [§]Programa de Pós-Graduação em Medicina e Ciências da Saúde, [∥]Instituto de Toxicologia e Farmacologia, [⊥]Faculdade de Odontologia, and [#]Faculdade de Química, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, RS Brazil



ABSTRACT: Acute liver injury was induced in male BALB/c mice by coadministering isoniazid and rifampicin. In this work, the effects of resveratrol (**1**) were investigated in the hepatotoxicity caused by isoniazid−rifampicin in mice. Compound **1** was administered 30 min prior to isoniazid−rifampicin. Serum biochemical tests, liver histopathological examination, oxidative stress, myeloperoxidase activity, cytokine production (TNF-α, IL-12p70, and IL-10), and mRNA expression of SIRT1−7 and PPAR-γ/PGC1-α were evaluated. The administration of **1** significantly decreased aspartate transaminase and alanine aminotransferase levels, myeloperoxidase activity, and cytokine levels. Furthermore, **1** reverted the decrease of catalase and glutathione activities and ameliorated the histopathological alterations associated with antituberculosis drugs. Modulation of SIRT1 and PPAR-γ/PGC1-α expression is likely involved in the protective effects of **1**. The results presented herein show that **1** was able to largely prevent the hepatotoxicity induced by isoniazid and rifampicin in mice, mainly by modulating SIRT1 mRNA expression.

Tuberculosis (TB) is a major cause of death from a curable infectious disease. About 8.7 million new TB cases occurred in 2011, and 1.4 million people died from TB during that year.[1] The recommended treatment for TB precompiled by the World Health Organization is a regimen of isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months, followed by 4 months of isoniazid and rifampicin. The bioactive metabolites of isoniazid generated by the drug-metabolizing enzymes have been implicated in isoniazid-induced hepatotoxicity in humans.[2,3] Up to 20% of patients taking anti-TB drugs experience hepatic injury, and mortality in such cases is not rare.[4,5] Importantly, the hepatotoxicity evoked by anti-TB drugs diminishes the treatment effectiveness, contributing to nonadherence and emergence of drug-resistant *Mycobacterium tuberculosis* strains.[6]

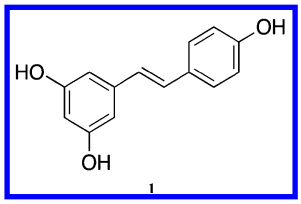
Resveratrol (*trans*-3,5,4′-trihydroxystilbene, **1**) is found in a wide variety of plants including grapes, berries, and peanuts.[7] Compound **1** has been demonstrated to display protective effects in age-related disorders, diabetes, stroke, cancer, and cardiovascular and inflammatory diseases and disturbs lipid metabolism.[8−12] Concerning the possible mechanisms of action, **1** appears to be a complex molecule, as it interacts with various unrelated targets, including sirtuins (SIRTs).[13] SIRTs constitute a highly conserved family of class-III histone deacetylases.[11] Mammals have seven SIRTs (1−7) characterized by differences in subcellular localization, substrate preference, and biological function.[16] SIRT1 plays an essential role in the liver lipid metabolism pathways and is able to either inhibit or stimulate the activities of several molecules, such as peroxisome proliferator-activated receptor-γ (PPARγ) and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1-α). Furthermore, SIRT1 also exerts anti-inflammatory effects by regulation of proinflammatory cytokines.[10−12] Compound **1** produces SIRT1-dependent effects that are

Received: April 23, 2014

dx.doi.org/10.1021/np5003143 | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

consistent with improved cellular function and organism health.[10−12,14−16]

Previous in vivo studies have demonstrated beneficial effects for **1** in animal models of hepatic insult.[9] A protective action for **1** has been documented in acetaminophen-induced liver failure in mice.[17] The treatment with **1** also protected against liver damage induced by ethanol.[18,19] Additionally, **1** reduced mortality and liver damage induced by ischemia-reperfusion injury,[20] transplants, and surgical procedures.[21,22] Accordingly, this study was designed to evaluate, for the first time, the effects of **1** on the hepatotoxicity caused by isoniazid and rifampicin in mice and to investigate the potential molecular mechanisms of action of **1**. It was hypothesized that SIRT1−7 and (PPARγ)/(PGC1-α) expression and inflammatory cytokines might represent essential regulatory mechanisms implicated in the effects of **1**.



1

RESULTS AND DISCUSSION

The coadministration of isoniazid and rifampicin for the chemoprophylaxis and treatment of TB has been largely associated with severe hepatotoxicity.[23] Herein, the protective effects of **1** were demonstrated in the hepatotoxicity caused by isoniazid−rifampicin in mice. Furthermore, the beneficial actions of **1** were assessed in terms of modulation of the sirtuin system and inflammatory markers.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were analyzed as indications of hepatic function. The levels of AST and ALT were significantly higher in the isoniazid−rifampicin groups (109 ± 6 and 51 ± 5 U/mL) when compared to the control groups (62 ± 3 and 26 ± 4 U/mL). The administration of **1** significantly decreased AST and ALT levels (36 ± 8% and 58 ± 4%, respectively), as assessed in the protocol of isoniazid−rifampicin-induced hepatotoxicity (Figure 1).

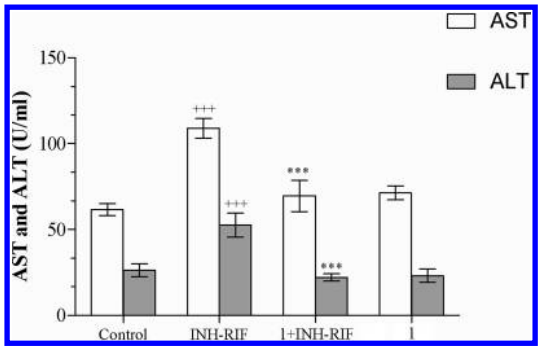


Figure 1. AST and ALT serum levels. Effects of resveratrol (**1**) treatment (100 mg/kg) on serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in isoniazid (INH, 50 mg/kg) plus rifampicin (RIF, 100 mg/kg)-induced liver injury. Each column represents the mean of 6 animals, and the vertical lines show the SEM. ***Significantly different from INH-RIF ($p < 0.0001$); $^{+++}$significantly different from control ($p < 0.0001$).

The impact of antioxidant systems in the hepatoprotective effects of **1** was evaluated. Data presented in Figure 2A and B indicate that both glutathione (GSH) and catalase (CAT) levels in the liver were significantly decreased after isoniazid−rifampicin administration (1.5 ± 0.3 and 8 ± 0.7 mmol/mg protein) when compared to the control groups (2.9 ± 0.2 and 14 ± 0.9 mmol/mg protein). Pretreating mice with **1** significantly reversed isoniazid−rifampicin-induced GSH depletion and reduced CAT activity (89 ± 15% and 34 ± 7%, respectively), as assessed following hepatotoxicity induction by isoniazid−rifampicin (Figure 2A and B).

Neutrophil infiltration is an important hallmark of the inflammatory process. Hepatic myeloperoxidase (MPO) activity was significantly increased in the isoniazid−rifampicin group (0.2 ± 0.008 OD/mg tissue), as compared to the control group (0.1 ± 0.003 OD/mg tissue). Interestingly, treatment with **1** significantly prevented this effect (19 ± 3%) (Figure 2C).

Isoniazid−rifampicin administration caused a significant increase in the serum levels of transaminases AST and ALT and oxidative damage of the liver tissue, as evidenced by a marked decrease in the antioxidant enzymatic systems GSH and CAT. Moreover, liver oxidative injury was accompanied by neutrophil accumulation, as demonstrated by high tissue myeloperoxidase activity. Of note, this series of hepatic alterations were significantly prevented by **1**. Analyzed together, these results suggest that antioxidant and free-radical-scavenging activities of **1** may contribute to the modulation of neutrophil influx to the liver. The results of the present study are in accordance with previous reports[17,18,20,24] showing that **1** is a free radical scavenger and a potent antioxidant, due to its ability to activate a variety of antioxidant enzymes.[25,26]

Liver histological analysis revealed microvesicular steatosis and increased apoptosis in the isoniazid−rifampicin-treated animals (Figure 3C). Conversely, fatty accumulation was rarely observed in livers of control animals (Figure 3A) or in mice treated with **1** alone (Figure 3B). Importantly, the treatment with **1** (Figure 3D) was able to completely prevent hepatic lesions induced by isoniazid−rifampicin administration (Figure 3C). Histological observations support biochemical findings, as they clearly show morphological alterations related to fatty accumulation in isoniazid−rifampicin-treated groups. In addition, the administration of **1** was able to almost completely prevent development of isoniazid−rifampicin liver steatosis, which is consistent with previous studies by others who used different hepatotoxins such as acetaminophen,[17] methotrexate,[27] ethanol,[18,19] cadmium,[24] or carbon tetrachloride.[28]

Liver injury commonly involves the generation of some inflammatory mediators. In the current study, isoniazid−rifampicin evoked a marked increase in the levels of TNFα, interleukin-12p70 (IL-12p70), and interleukin-10 (IL-10) (Figure 4A−C, respectively). Notably, treatment with **1** significantly prevented the increased production of these cytokines when compared to the isoniazid−rifampicin-administered group (Figure 4). Nevertheless, the levels of interleukin-6 and monocyte chemoattractant protein-1 were not significantly different among the groups, whereas IFNγ was undetectable in all the samples (results not shown). Both clinical and experimental studies have shown that noxious events in the liver are preceded by an influx of inflammatory cells, which in turn secrete cytokines.[29] The aforementioned studies on **1**[17,30,31] demonstrate anti-inflammatory effects, as evidenced by decreased production of pro-inflammatory

dx.doi.org/10.1021/np5003143 | J. Nat. Prod. XXXX, XXX, XXX−XXX

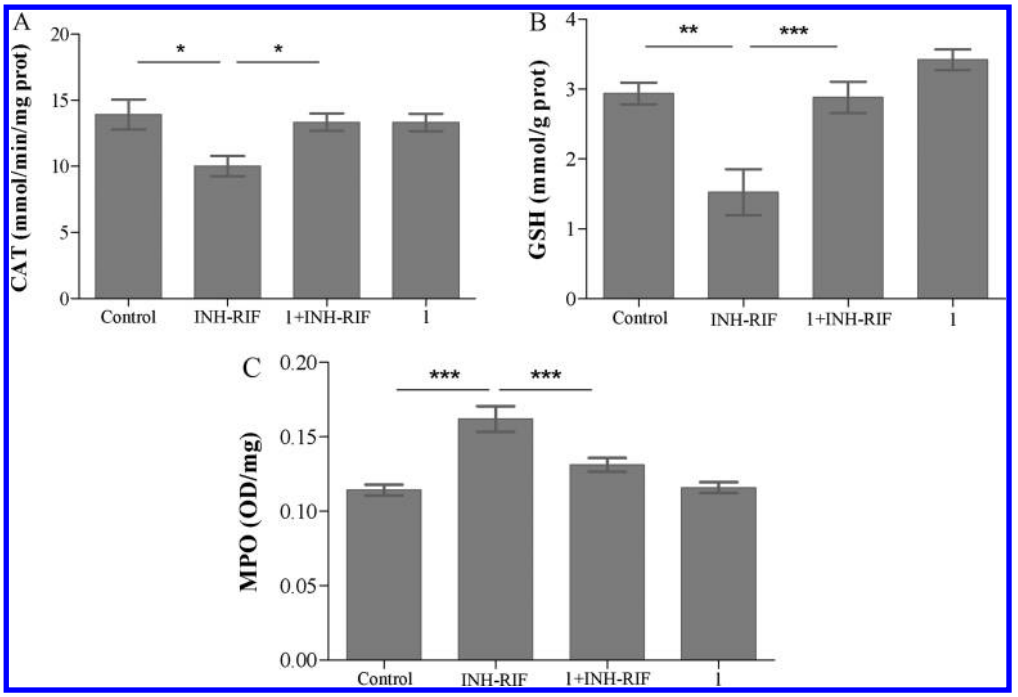


Figure 2. CAT, GSH, and myeloperoxidase levels. Effects of resveratrol (**1**) treatment (100 mg/kg) on (A) catalase (CAT), (B) glutathione (GSH), and (C) myeloperoxidase (MPO) levels in isoniazid (INH, 50 mg/kg) plus rifampicin (RIF, 100 mg/kg)-induced liver injury. Each column represents the mean of 6 animals, and the vertical lines show the SEM. ***$p < 0.0001$, **$p < 0.002$, *$p < 0.07$ significantly different between the indicated groups.

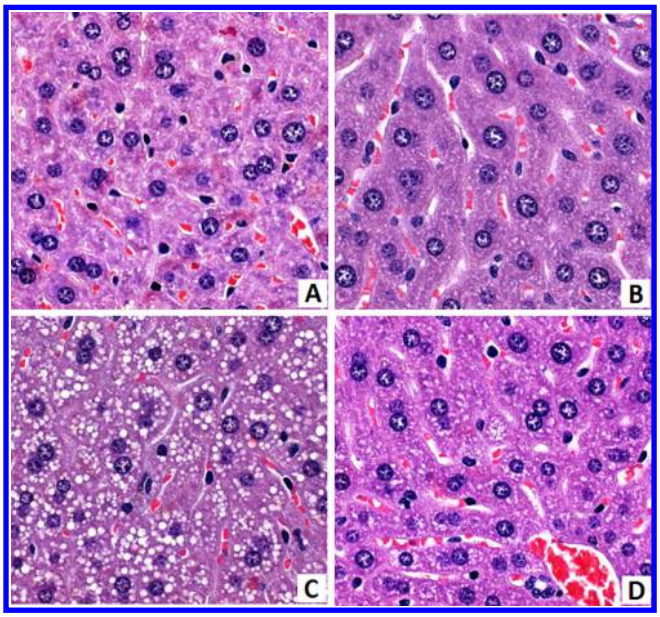


Figure 3. Liver histology. Liver sections from mice in control (A) and **1** (B) groups showed normal liver histology. Liver sections from a mouse in isoniazid–rifampicin (INH-RIF) group (C) revealed fatty accumulation and steatosis, which was prevented with resveratrol (**1**) administration (D).

cytokines, including TNF-α, interleukin-6, and IL-12p70, associated with an increase of anti-inflammatory cytokines, such as IL-10. In the current study, isoniazid–rifampicin administration resulted in augmented levels of TNF-α, IL-12p70, and IL-10, an effect that was abolished by **1**. TNF-α and IL-12 are pro-inflammatory cytokines that have been previously correlated with liver injury elicited by several chemical agents, such as ethanol,[18,19] carbon tetrachloride,[28] and acetaminophen.[17] Otherwise, the development of hepatotoxicity has been commonly correlated to decreased levels of IL-10. The decrease of IL-10 in the livers of **1**-treated animals was unexpected, since most investigations suggest that **1** leads to IL-10 upregulation.[32−34] It is feasible that **1** may change the requirement for an endogenous anti-inflammatory cytokine such as IL-10, obviating its absolute need for the resolution of the process and protection of the organ.

Sirtuins constitute a family of regulatory molecules that have been shown as main targets of **1**. Thus, we investigated how the expression of these proteins might be modified in this experimental protocol. It has been suggested that oxidative stress downregulates SIRT1 expression by triggering SIRT1 mRNA decay and by reducing SIRT1 protein levels.[35,36] Accordingly, this work shows that treatment with isoniazid–rifampicin led to a marked reduction of SIRT1 mRNA expression in the liver, which was almost completely restored by **1** (Figure 5A). Therefore, SIRT1 may contribute to the protective effects of **1** against hepatotoxicity induced by anti-TB drugs. These pieces of evidence confirm previous literature data indicating the ability of **1** in modulating SIRT1 expression in different biological systems.[10−12,15,16,37] On the other hand, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, and SIRT7 mRNA expression remained unchanged in all evaluated groups (data not shown).

Literature evidence shows that SIRT1 knockdown led to an increase of inflammatory gene expression.[38,39] Furthermore, hepatic-specific deletion of SIRT1 alters fatty acid metabolism, resulting in hepatic steatosis and inflammation.[40] Additionally, it was demonstrated that overexpression of SIRT1 resulted in a protective effect against high-fat-induced steatosis and glucose intolerance.[10,12,16,41,42] Therefore, SIRT1 might be crucial for maintaining normal liver functions.

dx.doi.org/10.1021/np5003143 | J. Nat. Prod. XXXX, XXX, XXX−XXX

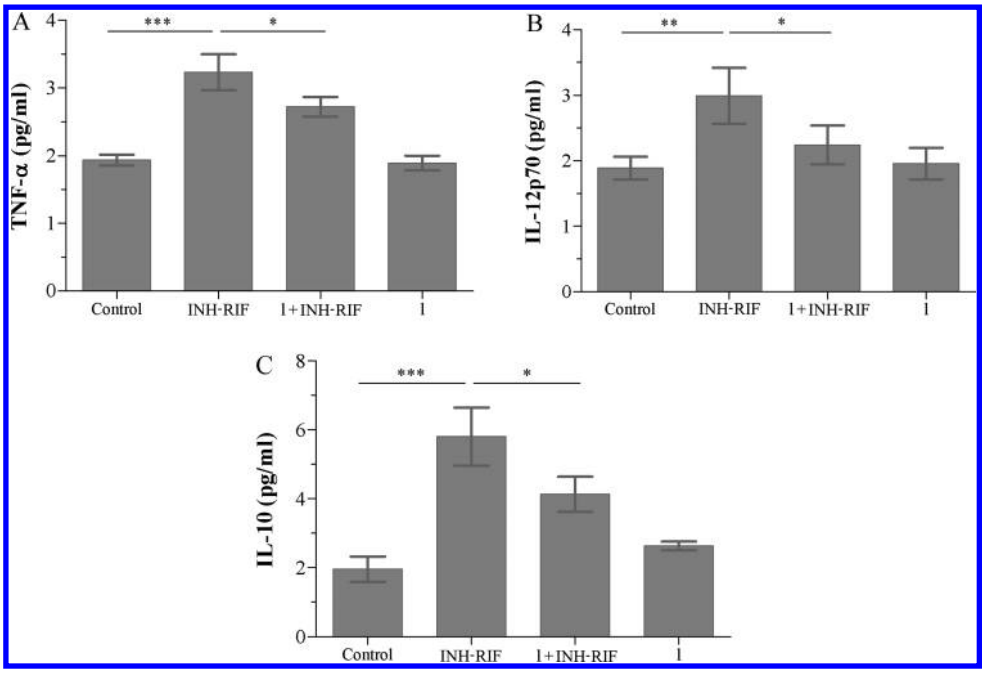


Figure 4. TNF-α, IL-12p70, and IL-10 levels. Effects of resveratrol (**1**) treatment (100 mg/kg) on (A) tumor necrosis factor α (TNF-α), (B) interleukin-12p70 (IL-12p70), and (C) interleukin-10 (IL-10) levels in isoniazid (INH, 50 mg/kg) plus rifampicin (RIF, 100 mg/kg)-induced liver injury. Each column represents the mean of 6 animals, and the vertical lines show the SEM. ***$p < 0.0001$, **$p < 0.005$, *$p < 0.03$ significantly different between the indicated groups.

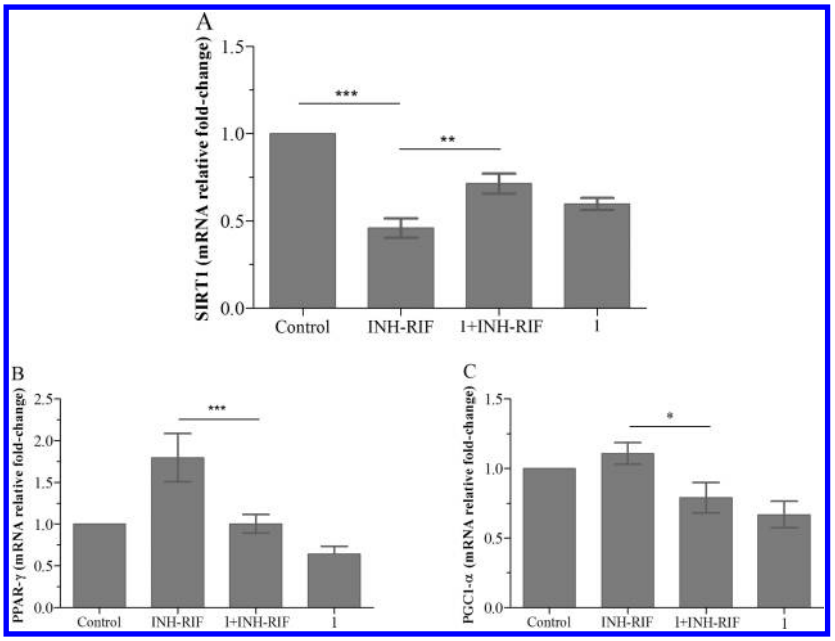


Figure 5. SIRT1, PPAR-γ, and PGC1-α mRNA expression. Effects of resveratrol (**1**) treatment (100 mg/kg) on (A) sirtuin 1 (SIRT1), (B) peroxisome proliferators-activated receptor-γ (PPAR-γ), and (C) peroxisome proliferators-activated receptor-γ coactivator 1α (PGC1-α) mRNA relative levels in isoniazid (INH, 50 mg/kg) plus rifampicin (RIF, 100 mg/kg)-induced liver injury. Each column represents the mean of 6 animals, and the vertical lines show the SEM. ***$p < 0.0001$, **$p < 0.003$, *$p < 0.02$ significantly different between the indicated groups.

It was found that SIRT1 is expressed in both the cytoplasm and nuclei in many metabolically active tissues, where it regulates the activity of histone and non-histone proteins, such as the PPAR-γ receptor and its coactivator PGC1-α.[43,44] In the liver, SIRT1 has been shown to control the glycolytic pathway through the transcriptional coactivator PGC1-α, while PPAR-γ has been proposed as an important target for hepatic steatosis. In this study, the administration of isoniazid−rifampicin caused a significant increase of hepatic PPAR-γ mRNA expression, and this rise was virtually prevented by **1** (Figure 5B). Furthermore, the expression of PGC1-α mRNA was significantly reduced by **1** administration (Figure 5C). The decrease of PPARγ may be dependent on the regulation of SIRT1 by **1**. These data are somewhat different from studies demonstrating that chronic ethanol feeding of mice led to decreased PGC1-α and PPAR-γ expression[45,46] and the treatment with **1** increased PGC1-α, and PPAR-γ mRNA expression.[37,44] However, several earlier studies demonstrated an increase in PPARγ−ubiquitin

dx.doi.org/10.1021/np5003143 | J. Nat. Prod. XXXX, XXX, XXX−XXX

conjugate formation in the presence of **1**[47,48] and that overexpression of PPARγ leads to adipogenic hepatic steatosis.[49]

NAD^+ is a necessary co-substrate for deacetylase activity of sirtuins.[37] Changes in the expression of nicotinamide phosphoribosyltransferase, an enzyme involved in the salvaging of NAD^+ from nicotinamide, lead to significant alterations of SIRT1 activity.[50] However, we found no significant difference of nicotinamide phosphoribosyltransferase mRNA expression or NAD^+ and NADH concentrations (results not shown). Thus, these pathways do not seem to be relevant for **1** actions, at least in our experimental paradigm.

Altogether, the results presented here demonstrate, for the first time, that treatment with **1** can prevent the hepatotoxicity induced by isoniazid−rifampicin. The protective effects of **1** can be mostly attributed to the modulation of SIRT1 mRNA expression. Furthermore, decreased production of cytokine levels of TNF-α, IL-12p70, and IL-10 might also be involved in the effects of **1**. **1** might well represent a useful strategy in the treatment of liver failure due to isoniazid−rifampicin-induced toxicity.

EXPERIMENTAL SECTION

Chemicals. Compound **1** was purchased from Pharma Nostra (Rio de Janeiro, Brazil; >98% purity, determined by HPLC). Isoniazid was obtained from Acros Organics (Morris Plains, NJ, USA), and rifampicin was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals are from standard commercial suppliers, having analytical grade quality.

Experimental Design. The experimental protocol was approved by the local Animal Ethics Committee (09/00107). Male BALB/c mice (25−30 g; 6 animals/group) were used. Animals were fed with standard chow diet (Nuvilab CR1, Sogorbo, São Paulo, BR) and water ad libitum. The animals were distributed into the following groups: (i) control, (ii) **1**-treated control, (iii) isoniazid−rifampicin, (iv) **1**-treated + isoniazid−rifampicin. The liver damage was induced by isoniazid (50 mg/kg) and rifampicin (100 mg/kg), dissolved in a 1% DMSO solution in saline 0.9%, administered orally, for three consecutive days. **1** (100 mg/kg) was administered orally 30 min prior to isoniazid−rifampicin administration, and two times daily, each 6 h, until the third day after hepatotoxicity induction. Mice were sacrificed 24 h after the last dose of isoniazid−rifampicin to collect blood and liver tissue. The samples were stored at −80 °C.

Biochemical Analysis. To assess liver function, serum AST and ALT levels were determined using commercial kits (Labtest, Lagoa Santa, Brazil).

Catalase Activity. Livers were collected and homogenized in saline solution (0.9%) in the proportion of 1:9 w/v liver/saline. The samples were centrifuged at 3500*g* for 10 min, at 4 °C, and the supernatant was used for the estimation of catalase activity. For this purpose, the enzymatic decomposition of hydrogen peroxide was followed continuously at 240 nm. Briefly, 50 μL of samples and 0.65 mL of 50 mM PBS (pH 7.0) were added to a quartz cuvette, and 0.3 mL of 20 mM hydrogen peroxide was used as the substrate. Enzymatic activity was expressed as mmol hydrogen peroxide decomposed/min/mg protein, according to the method described by Aebi.[51]

Determination of Reduced Glutathione. Hepatic glutathione content was determined by using 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) according to the method described by Shim et al.[28] with some modifications. Livers were collected and homogenized in saline solution (0.9%) in the proportion of 1:9 w/v liver/saline. The homogenate was centrifuged at 3500*g* for 20 min, at 4 °C, and the supernatant was used for the determination of GSH. Next, 0.5 mL of 4% sulfosalicylic acid was added to 0.5 mL of the supernatant and centrifuged at 3000 rpm for 10 min. The supernatant (500 μL) was mixed with 2 mL of 0.1 M phosphate buffer and 10 μL of 0.01 M DTNB. Absorbance of the mixture was measured at 412 nm. GSH content was determined using a standard curve and expressed as micromoles per microgram of protein.

Myeloperoxidase Activity. Neutrophil recruitment to the liver was quantified indirectly by determining tissue myeloperoxidase activity, according to the method described by Paszcuk et al.,[52] with minor modifications.

Histological Preparation and Analysis. Samples of liver tissues were fixed in 10% formaldehyde and processed for embedding in paraffin. Paraffin sections (4 μm) were stained with hematoxylin and eosin and examined with a Zeiss Axiolmager M2 light microscope (Carl Zeiss, Göttingen, Germany) by an experienced veterinary pathologist (CRMV-RS-Brazil 5756) blinded to the experimental groups.

NAD^+/NADH Assay. NAD^+ and NADH concentrations were measured using a commercial kit (BioChain, Newark, CA, USA), according to the manufacturer's recommendations. The intensity of the reduced product color was measured at 565 nm, which is proportional to the NAD^+/NADH concentration in the sample.

Detection of Cytokines. The homogenized tissue samples were analyzed using a flow cytometer (FACSCanto II, BD Biosciences, San Jose, CA, USA) with a 488 nm laser and fitted with a high-throughput sampler. The BD CBA mouse inflammation kit (BD Biosciences,) was used to determine the protein levels of interleukin-6, IL-10, monocyte chemoattractant protein-1, interferon-γ (IFN-γ), tumor necrosis factorα (TNFα), and IL-12p70. Sample data were acquired using BD FACSDiva V6.1.3 (BD Biosciences), and experimental data were analyzed using FCAPArray v1.0.1 (BD Biosciences/Soft Flow Hungary Ltd.) analysis software. The results are expressed in pg/mL.

Real-Time PCR. Total RNA was extracted from liver tissues using RNeasy protect mini kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The conversion of total RNA to cDNA was carried out using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) with random primers and 60 ng of total RNA. Real-time PCR was performed using 180 ng of cDNA and amplified using a thermocycler (AB 7500 Applied Biosystems). The TaqMan probes (Assays-on-Demand, Applied Biosystems) were Mm01168521_m1 (mouse SIRT1), Mm01149210_m1 (mouse SIRT2), Mm00452129_m1 (mouse SIRT3), Mm01201917_m1 (mouse SIRT4), Mm0066372_m1 (mouse SIRT5), Mm01149042_m1 (mouse SIRT6), Mm00461895_m1 (mouse SIRT7), Mm01184322_m1 (mouse PPAR-γ), Mm00731216_m1 (mouse PGC1-α), and Mm00451938_m1 (mouse nicotinamide phosphoribosyltransferase). The probe for Mm4352932 (mouse GAPDH 20x) was used as an endogenous control. Reactions were run in duplicate. Gene expression values were calculated based on the $2^{\Delta\Delta C}$ relative expression method.[53]

Statistical Analysis. Data are expressed as mean ± SEM. Statistical analysis was carried out by means of one-way analysis of variance (ANOVA) followed by Newman−Keuls' test (GarphPad Prism 5.0, USA). Values of $p < 0.05$ were regarded as significant.

AUTHOR INFORMATION

Corresponding Author

*Tel: + 55 51 3320 3549. E-mail: arigony@pucrs.br.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Dr. D. Driemeier, from Federal University of Rio Grande do Sul (Brazil), for the technical assistance in histopathological analysis. This work was supported by the National Institute of Science and Technology on Tuberculosis (DECIT/SCTIE/MS-MCT-CNPq-FNDCT-CAPES) and the Millennium Initiative Program (CNPq). A.A.S., D.S.S., L.A.B., and M.M.C. are Research Career Awardees of the National Research Council of Brazil (CNPq). D.S.S. also acknowledges a grant awarded by BNDES. N.F.N. acknowledges an MSc

scholarship awarded by CNPq (Ed152008 INCTs DI FB). V.R.J. acknowledges a scholarship awarded by FAPERGS-CAPES (DOCFIX, 05/2013). This work was also supported by the FINEP research grant "Implantação, Modernização e Qualificação de Estrutura de Pesquisa da PUCRS" (PUCRSINFRA) no. 01.11.0014-00.

REFERENCES

(1) WHO. *Global Tuberculosis Report*. World Health Organization Report, 2012.
(2) Huang, Y. S.; Chern, H. D.; Su, W. J.; Wu, J. C.; Chang, S. C.; Chiang, C. H.; Chang, F. Y.; Lee, S. D. *Hepatology* **2003**, *37*, 924−930.
(3) Yue, J.; Peng, R.; Chen, J.; Liu, Y.; Dong, G. *Pharmacol. Res.* **2009**, *59*, 112−119.
(4) Cho, H. J.; Koh, W. J.; Ryu, Y. J.; Ki, C. S.; Nam, M. H.; Kim, J. W.; Lee, S. Y. *Tuberculosis* **2007**, *87*, 551−556.
(5) Haroon, M.; Martin, U.; Devlin, J. *Rheumatol. Int.* **2012**, *32*, 33−37.
(6) Tostmann, A.; Boeree, M. J.; Aarnoutse, R. E.; Lange, W. C. M.; Van der Ven, A. J. A. M.; Dekhuijzen, R. *Gastroenterol. Hepatol.* **2008**, *23*, 192−202.
(7) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. *Clin. Biochem.* **1997**, *30*, 91−113.
(8) Baur, J. A.; Sinclair, D. A. *Nat. Rev. Drug Discovery* **2006**, *5*, 493−506.
(9) Bishayee, A.; Darvesh, A. S.; Politis, T.; McGory, R. *Liver Int.* **2010**, *30*, 1103−1114.
(10) Kelly, G. S. *Altern. Med. Rev.* **2010**, *14*, 313−328.
(11) Chung, S.; Yao, H.; Caito, S.; Hwang, J.; Arunachalam, G.; Rahman, I. *Arch. Biochem. Biophys.* **2010**, *501*, 79−90.
(12) Hubbard, B. P.; Sinclair, D. A. *Trends Pharmacol. Sci.* **2014**, *35*, 146−154.
(13) Pacholec, M.; Bleasdale, J. E.; Chrunyk, B.; Cunningham, D.; Flynn, D.; Garofalo, R. S.; Griffith, D.; Griffor, M.; Loulakis, P.; Pabst, B.; Qiu, X.; Stockman, B.; Thanabal, V.; Varghese, A.; Ward, J.; Withka, J.; Ahn, K. *J. Biol. Chem.* **2010**, *285*, 8340−8351.
(14) Picard, F.; Kurtev, M.; Chung, N.; Topark-Ngarm, A.; Senawong, T.; Machado De Oliveira, R.; Leid, M.; McBurney, M. W.; Guarente, L. *Nature* **2004**, *429*, 771−776.
(15) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. *Nature* **2006**, *444*, 337−342.
(16) Lomb, D. J.; Laurent, G.; Haigis, M. C. *Biochim. Biophys. Acta* **2010**, *1804*, 1652−1657.
(17) Sener, G.; Toklu, H. Z.; Sehirli, A. O.; Velioğlu-Oğünç, A.; Cetinel, S.; Gedik, N. *Hepatol. Res.* **2006**, *35*, 62−68.
(18) Kasdallah-Grissa, A.; Mornagui, B.; Aouani, E.; Hammami, M.; El May, M.; Gharbi, N.; Kamoun, A.; El-Fazaâ, S. *Life Sci.* **2007**, *80*, 1033−1039.
(19) Ajmo, J. M.; Liang, X.; Rogers, C. Q.; Pennock, B.; You, M. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *295*, G833−842.
(20) Gedik, E.; Girgin, S.; Ozturk, H.; Obay, B. D.; Ozturk, H. *World J. Gastroenterol.* **2008**, *14*, 7101−7106.
(21) Wu, S.; Yu, L.; Meng, K.; Ma, Z.; Pan, C. *World J. Gastroenterol.* **2005**, *11*, 4745−4749.
(22) Kirimlioglu, V.; Karakayali, H.; Turkoglu, S.; Haberal, M. *Transplant. Proc.* **2008**, *40*, 293−296.
(23) Wong, W. M.; Wu, P. C.; Yuen, M. F.; Cheng, C. C.; Yew, W. W.; Wong, P. C. *Hepatology* **2000**, *31*, 201−206.
(24) Eybl, V.; Kotyzova, D.; Koutensky, J. *Toxicology* **2006**, *225*, 150−156.
(25) Ignatowicz, E.; Baer-Dubowska, W. *Polym. J. Pharmacol.* **2001**, *53*, 557−569.
(26) Parvaiz, S. *FASEB J.* **2003**, *17*, 1975−1985.
(27) Tunali-Akbay, T.; Sehirli, O.; Ercan, F.; Sener, G. *J. Pharm. Pharmaceut. Sci.* **2010**, *13*, 303−310.
(28) Shim, J.; Kim, M.; Kim, H.; Ahn, J.; Yun, Y.; Song, J. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 318−325.
(29) Lin, H. I.; Chu, S. J.; Wang, D.; Feng, N. H. *J. Microbiol. Immunol. Infect.* **2004**, *37*, 8−15.
(30) Masubuchi, Y.; Sugiyama, S.; Horie, T. *Chem. Biol. Interact.* **2009**, *179*, 273−279.
(31) Hassan-Khabbar, S.; Vamy, M.; Cottart, C. H.; Wendum, D.; Vibert, F.; Savouret, J. F.; Thérond, P.; Clot, J. P.; Waligora, A. J.; Nivet-Antoine, V. *Biochimie* **2010**, *92*, 405−410.
(32) Imler, T. J. J.; Petro, T. M. *Int. Immunopharmacol.* **2009**, *9*, 134−143.
(33) Sánchez-Fidalgo, S.; Cárdeno, A.; Villegas, I.; Talero, E.; de la Lastra, C. A. *Eur. J. Pharmacol.* **2010**, *633*, 78−84.
(34) Svajger, U.; Obermajer, N.; Jeras, M. *Immunology* **2010**, *129*, 525−535.
(35) Wu, A.; Ying, Z.; Gomez-Pinilla, F. *Eur. J. Neurosci.* **2006**, *23*, 2573−2580.
(36) Furukawa, A.; Tada-Oikawa, S.; Kawanishi, S.; Oikawa, S. *Cell. Physiol. Biochem.* **2007**, *20*, 45−54.
(37) Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; Geny, B.; Laakso, M.; Puigserver, P.; Auwerx, J. *Cell* **2006**, *127*, 1109−1122.
(38) Yang, Z.; Kahn, B. B.; Shi, H.; Xue, B. Z. *J. Biol. Chem.* **2010**, *285*, 19051−19059.
(39) Yoshizaki, T.; Schenk, S.; Imamura, T.; Babendure, J. L.; Sonoda, N.; Bae, E. J.; Oh, D. Y.; Lu, M.; Milne, J. C.; Westphal, C.; Bandyopadhyay, G.; Olefsky, J. M. *Am. J. Physiol. Endocrinol. Metab.* **2010**, *298*, 419−428.
(40) Purushotham, A.; Schug, T. T.; Xu, Q.; Surapureddi, S.; Guo, X.; Li, X. *Cell. Metab.* **2009**, *9*, 327−338.
(41) Banks, A. S.; Kon, N.; Knight, C.; Matsumoto, M.; Gutiérrez-Juárez, R.; Rossetti, L.; Gu, W.; Accili, D. *Cell. Metab.* **2008**, *8*, 333−341.
(42) Pfluge, P. T.; Herranz, D.; Velasco-Miguel, S.; Serrano, M.; Tschöp, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9793−9798.
(43) Lieber, C. S.; Leo, M. A.; Wang, X.; Decarli, L. M. *Biochem. Biophys. Res. Commun.* **2008**, *370*, 44−48.
(44) Chaudhary, N.; Pfluger, P. T. *Curr. Opin. Clin. Nutr. Metab. Care* **2009**, *12*, 431−437.
(45) Fischer, M.; You, M.; Matsumoto, M.; Crabb, D. W. *J. Biol. Chem.* **2003**, *278*, 27997−28004.
(46) Nakajima, T.; Kamijo, Y.; Tanaka, N.; Sugiyama, E.; Tanaka, E.; Kiyosawa, K.; Fukushima, Y.; Peters, J. M.; Gonzalez, F. J.; Aoyama, T. *Hepatology* **2004**, *40*, 972−980.
(47) Liao, W.; Nguyen, M. T.; Yoshizaki, T.; Favelyukis, S.; Patsouri, D.; Imamura, T. *Am. J. Physiol. Endocrinol. Metab.* **2007**, *293*, 219−227.
(48) Floyd, Z. E.; Wang, Z. Q.; Kilroy, G.; Cefalu, W. T. *Metabolism* **2008**, *57*, 32−38.
(49) Yu, S.; Matsusue, K.; Kashireddy, P.; Cao, W. Q.; Yeldandi, V.; Yeldandi, A. V.; Rao, M. S.; Gonzalez, F. J.; Reddy, J. K. *J. Biol. Chem.* **2003**, *278*, 498−505.
(50) Dominy, J. E. J.; Lee, Y.; Gerhart-Hines, Z.; Puigserver, P. *Biochim. Biophys. Acta* **2010**, *1804*, 1676−1683.
(51) Aebi, H. *Methods Enzymol.* **1984**, *105*, 121−126.
(52) Paszcuk, A. F.; Quintão, N. L. M.; Fernandes, E. S.; Juliano, L.; Chapman, K.; Andrade-Gordon, P.; Campos, M. M.; Vergnolle, N.; Calixto, J. B. *Eur. J. Pharmacol.* **2008**, *581*, 204−215.
(53) Livak, K. J.; Schmittgen, T. D. *Methods* **2010**, *25*, 402−408.