Original Article

Protective effect against isoniazid induced hepatic-toxicity

Relationship of Oxyresveratrol on Expression Levels of Tumor Necrosis Factor Alpha and Interferon Gamma in Isoniazid Induced Hepatotoxicity in Experimental Model in Mice

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ABSTRACT

Objective: To determine the protective effect against isoniazid induced hepatic-toxicity on expression levels of TNF-α and IFN-γ in immunomodulatory activity of oxyresveratrol.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Pharmacology, University of Health Sciences, Lahore, Pakistan from 1st July 2019 to 31st December 2019.

Materials and Methods: Mice were given a number from 1 to 35 and were assigned to five groups using lottery method. Each mouse was allotted a number that was written on a piece of small paper. A blinded person picked papers and a mouse whose number was written on paper was assigned group.

Results: The mRNA expression levels of TNF-α were significantly high in INH group as compared to control group (795.9 ± 47.45 vs 417.4 ± 6.55). Oxyresveratrol treatment significantly downregulated expression levels of TNF-α as compared to INH group (372.3 ± 10.27 vs 795 ± 47.45). Oxyresveratrol treatment significantly reduced expression levels of IFN-γ as compared to INH group (225.7 ± 15.57 vs 798 ± 36.9). Combination therapy showed a significant decreased in expression levels of IFN-γ as compared to INH group (117.7 ± 33.89 vs 798 ± 36.9). Oxyresveratrol showed a significant reduction in interferon γ expression levels as compared to silymarin (225.5 ± 15.57 vs 368.9 ± 22.80).

Conclusion: Oxyresveratrol possesses a protective effect against the isoniazid induced hepatic-toxicity. The decrease in expression levels of TNF-α and IFN-γ may explain immunomodulatory activity of oxyresveratrol.

Key Words: Tumor Necrosis Factor, Alpha, and Interferon Gamma, Isoniazid Induced Hepatotoxicity

Citation of article: Abid M, Shahani MY, Zaidi SZH, Bangulzai MA, Khan S, Hameed A. Relationship of Oxyresveratrol on Expression Levels of Tumor Necrosis Factor Alpha and Interferon Gamma in Isoniazid Induced Hepatotoxicity in Experimental Model in Mice. Med Forum 2021;32(3):100-104.

INTRODUCTION

The world's most often used for tuberculosis care isoniazid (INH) and rife-ampicin (RFP), but hepatotoxicity is an important issue throughout clinical therapy. Previous studies have shown that these drugs have induced liver oxidative stress and several antioxidants have reduced their effect.[1] Isonazide (INH) and Rifampicin (RFP) are first-line anti-TB therapy drugs, but the hepatotoxicity that comes from using them remains an important clinical problem.[2]

The dichotomy occurring among protective or non-protective immune responses is likely to be linked to cytokine patterns formed during initial surviving stages of the pathogen within macrophagen by various subpopulations of lymphocytes. Interferon acts as a strong macrophage activator, increasing the expression of the key histocompatibility complex of class II and enhancing cell responses, including cytokine production, nitric oxide and increasing cytolytic activity, with a main role in Th1 form[3]

Studies performed with [4] and[5] found that the Mice were unable to battle M-infection without the IFN gene. The illness TB. People with genetic mutations in IFN receptors were found highly susceptible to infections caused by atypical mycobacteria6 in humans, indicating that the IFN plays an important role in protecting TB. [5]

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Received: October, 2020
Accepted: November, 2020
Printed: March, 2021

The purpose of this study is to determine the protective effect against isoniazid induced hepatic-toxicity on expression levels of TNF-α and IFN-γ in immunomodulatory activity of oxyresveratrol**.**

MATERIALS AND METHODS

The Experimental study was carried out at the Department of Pharmacology, Resource lab and Department of Morbid Anatomy and Histopathology, University of Health Sciences, Lahore, Pakistan for six months from 1st July 2019 to 31st December 2019. The present study was approved by Ethical Review Committee and Advanced Studies and Research Board of UHS Lahore. The sample size was calculated to be 35 mice keeping in view the statistical reliability and validity of sample.[7]

It was simple random sampling using lottery method. Mice were given a number from 1 to 35 and were assigned randomly to five groups I, II, III, IV, and V using the lottery method in which each mouse was randomly allotted a number that was written on a piece of small paper. All the papers were folded and mixed. A blinded person randomly picked the papers and the mouse whose number was written on paper was assigned the group. The first seven were assigned control group and so on.

Preparation of Experimental Animals: All mice were housed at controlled room temperature (23±2°C), moisture (50±5 percent) and light and dark cycles of 12 hours each at UHS' Experimental Research Laboratory. The animals had a normal diet and water ad libitum feeding on the rodent. At first, and then regularly in alternative days, the animals' body weight was registered.

Determination of mRNA Expression Levels of TNF-α and IFN- γ

Total RNA was extracted from the liver using a commercially available kit (TRIzol Plus RNA Purification kit). The reverse transcription-polymerase chain reaction was performed to produce cDNA from mRNA.[8] Appropriate primers for TNF-α and IFN-γ were synthesized and used for production of copies by PCR.[8]

RNA isolation from liver tissue by TRIzol method

Homogenization of Liver Tissue[9]

Added 0.75 mL i.e., 750 ul of TRIzol LS Reagent /50–100 mg of the liver tissue sample. Then homogenized the sample using an ultrasonic homogenizer till the color of TRIzol changed, usually, it was 15 to 30 seconds.

Phase Separation[9]

Incubated the sample at room temperature (15-30° C) for 5min, to allow complete dissociation of the nucleoprotein complex. Then added 200ul of chloroform per 0.75 mL of TRIzol LS Reagent used for homogenization. The tube was caped securely. Shook tube by hand for 15 seconds. Then incubated it for 2–15 minutes at room temperature. Centrifuged at 13,500 RPM for 15 minutes at 4°C to obtain the aqueous phase.

RNA Precipitation[9]

Transferred the aqueous phase into new tubes. Then added 0.5 mL of 100% isopropanol to the aqueous phase, of 0.75 mL TRIzol LS Reagent.Incubated the tubes at room temperature for 10 min after vortexing for 15 seconds. Centrifuged at 13,500 RPM for 10 minutes at 4°C to obtain a gel-like RNA pellet on the side and bottom of the tube.

RNA Wash [9]

Removed the supernatant from the tube, leaving behind the RNA pellet.Washed the RNA pellet, with 1 mL of 75% ethanol per 0.75 mL of TRIzol LS reagent. The sample was vortexed to mix. Centrifuged the sample at 10,000 RPM for 5 minutes at 4°C, and discarded the supernatant. Then air dried the RNA pellet for 5–10 minutes.

Polymerase Chain Reaction for mRNA expression Levels of interferon γ and TNFα[9] The cDNA prepared by reverse transcription was further amplified by polymerase chain reaction using gene-specific primers. Added the following to a PCR reaction tube for a final reaction volume of 20 µl and placed in a thermal cycler:

PCR Pre Mix = 10 µl
Template cDNA = 1 µl
Primers (forward)= 1 µl
Primers (reverse) = 1 µl
RNase free water up to= 20 µl[9]

PCR cycle

- Initial denaturation at 95ºC for 10 to 15 min.
- Denaturation at 95º C for 10 sec
- Annealing at 58º C to 62º C for 15 sec
- Elongation at 72º C for 15 to 30 sec
- Number of cycles 35 to 55 times

Specific primers for TNF-α and IFN- γ were synthesized from a commercial manufacturer. GAPDH gene was used as a reference gene. PCR product was visualized by using gel electrophoresis and densitometry was used for semi quantification of PCR product. The relative density of bands represented mRNA expression of inflammatory cytokines .[9]

Primer for TNF-α [10]

Forward 5-CAGGCGGTGCCTATGTCTC-3
Reverse 5-CGATCACCCCGAAGTTCAGTAG-3

Primer for Interferon-γ[11]

Forward 5-CCATCGGCTGACCTAGA-3
Reverse 5-GCCACTTGAGTTAAAATAGTTATTCAGAC-3

Statistical Analysis: All the data was entered and analyzed by using the SPSS version 26.0. Data were

expressed as mean ± SD. One-way ANOVA was applied to observe the difference in groups. Post-Hoc Tukey test was applied to observe which group mean is different from others. A P-value ≤ of 0.05 was considered statistically significant.

RESULTS

Effect of INH, Silymarin, and Oxyresveratrol on mRNA level of TNF-α

The results showed that the mRNA expression levels of TNF-α were significantly high in the INH group as compared to the control group (795.9 ± 47.45 vs 417.4 ± 6.55). Oxyresveratrol treatment significantly down regulated the expression levels of TNF-α as compared to the INH group (372.3 ± 10.27 vs 795 ± 47.45). Similarly, silymarin also significantly reduced the TNF-α expression levels as compared to the INH group (226.4 ± 20.75 vs 795 ± 44.45). Combination therapy resulted in a significantly higher reduction in the expression levels of TNF-α as compared to the INH group (141.9 ± 36.30 vs 795 ± 47.45). Silymarin showed a significant reduction in TNF α expression levels as compared to oxyresveratrol (226.4 ± 20.75 vs 372.3 ± 10.27).

Effect of INH, silymarin, oxyresveratrol on the mRNA expression level of IFN-γ

The graph shows the expression levels of IFN-γ were significantly high in the INH group as compared to the control group (798 ± 36.9 vs 419.9 ± 11.46). Oxyresveratrol treatment significantly reduced the expression levels of IFN-γ as compared to INH group (225.7 ± 15.57 vs 798 ± 36.9). Silymarin also significantly reduced the IFN-γ expression levels as compared to INH group (368.9 ± 22.80 vs 798 ± 36.9). Combination therapy showed a significant decreased in the expression levels of IFN-γ as compared to the INH group (117.7 ± 33.89 vs 798 ± 36.9). Oxyresveratrol showed a significant reduction in interferon γ expression levels as compared to silymarin (225.5 ± 15.57 vs 368.9 ± 22.80).

Table No.1: Result showing PCR values for TNF α and Interferon γ after 30 days of the experimental protocol (n=7).

PCR	Control	INH	INH+OXY	INH+Sily	INH+Oxy+Sily
TNF α	417.4 ± 6.55	795.5 ± 47.45$^{###}$	372.3 ± 10.27***	226.4 ± 20.75***	141.9 ± 36.3***
Interferon γ	412.9 ± 11.46	798.0 ± 36.9$^{###}$	225.7 ± 15.7***	368.9 ± 22.8***	117.7 ± 33.8***

* P < 0.05 represents comparison of treatment groups with INH.
** P < 0.01 represents comparison of treatment groups with INH.
*** P < 0.001 represents comparison of treatment groups with INH.
\# P < 0.05 represents comparison of INH with control.
\#\# P < 0.01 represents comparison of INH with control.
\#\#\# P < 0.001 represents comparison of INH with control.
^^^ P< 0.001 represents significant comparison of oxyresveratrol and silymarin

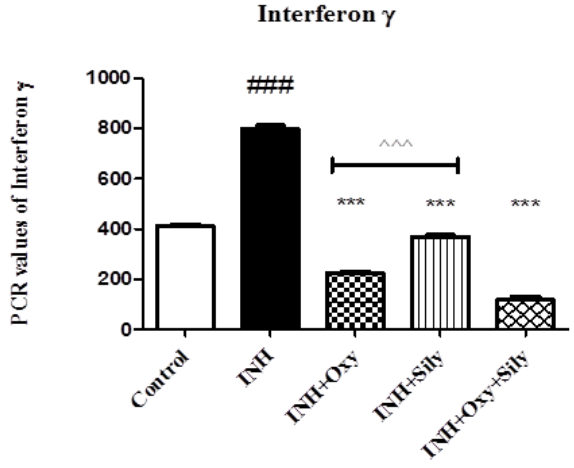

Figure No.1: Mean ± SD of IFN-γ expression levels in mice groups (n=7) * shows p < 0.001 and indicates significant difference as compared to INH group. ### shows p < 0.001 and indicates significant difference as compared to control group, while ^^^ shows significant difference between INH+Oxy and INH+Sily groups.**

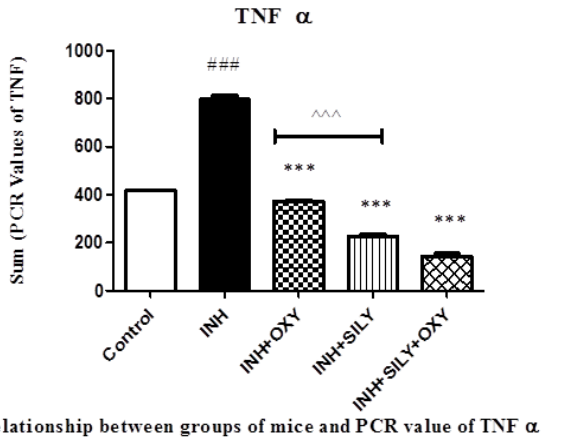

Figure No.2: Mean ± SD of TNF-α expression levels in mice groups (n=7) * shows p < 0.001 and indicates significant difference as compared to INH group. ### shows p < 0.001 and indicates significant difference as compared to control group, while ^^^ shows significant difference between INH+Oxy and INH+Sily groups**

DISCUSSION

TNF-α is a pleiotropic cytokine which triggers cellular responses, such as proliferation, the development of inflammatory mediators, and cell death.12 TNF-a plays a dichotomic function in the liver, not only acting as mediator of cell death, but also induce proliferation and regeneration of the hepatocytic cells. Macrophages and other cell forms, such As a product of lymphoid, mast, endothelial and neuronal cell, are primarily used in TNF-α. TNF-α is generated. The development of TNF-α is increased in response to inflammation and bacterial products.[14]

Even though TNF-α can function as a powerful activator both pro-inflammatory and pro-apoptotic pathways, such signalling paths interact at many levels in a complex network and one pathway activity also depends on another pathway's inactivation, which indicates that cells are able to point the TNF-α mediated signal to the proper answer.

In our study, we found significantly raised expression levels of TNF-α in the INH group. These raised levels were significantly reduced by oxyresveratrol. A similar study reported a decrease in gene expressions of TNF-α in Guinea pigs by Ascorbic acid.[15]

IFN-γ also exerts pleiotropic effects including antiviral and bactericidal activities, activation of macrophages and NK cells, and up-regulation of MHC class II expression on macrophages. It is produced mainly by NK cells and Th1 cells[16] and already has been reported to be involved in various kinds of liver injury models.[16,17] Enhanced IFN-γ expression is thought to induce inflammatory responses, leading to parenchymal cell damage in the liver. In the present study, we observed that the gene expression of IFN-γ was significantly enhanced in the livers of mice treated with INH. Treatment with oxyresveratrol alone as well as in combination with silymarin significantly attenuated IFN-γexpression levels. Attenuation of expression levels of TNF-α and IFN-γ by oxyresveratrol in our study might have led to the amelioration of isoniazid-induced liver injury. Also, oxyresveratrol more significantly decreases the IFN-γ as compared to silymarin. A study carried out in the US also showed that silymarininhibited the proliferation and secretion of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-2 (IL-2) by PBMC stimulation with anti-CD3.[18]

CONCLUSION

The results of the present study indicate that oxyresveratrol possesses a protective effect against the isoniazid induced hepatic-toxicity. These hepato-protective effects might have been the result of immunomodulatory and anti-inflammatory activities of oxyresveratrol. The decrease in the expression levels of TNF-α and IFN-γ may explain the immunomodulatory activity of oxyresveratrol, whereas, reduction in inflammation of the parenchyma and portal tract area, vascular congestion, and pyknosis may account for the anti-inflammatory activity of oxyresveratrol.

Acknowledgment: The authors of this manuscript acknowledge the staff of Sigma Research Solutions & Development Consultancy (SMC-Pvt) LTD Mr. Sadam Baloch for their technical help in the manuscript.

Author's Contribution:

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Final Approval of version:	Mohammad Abid

Conflict of Interest: The study has no conflict of interest to declare by any author.

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