ORIGINAL ARTICLE

Relationship of Superoxide Dismutase and Glutathione Peroxidase Activities and Oxyresveratrol in Isoniazid Induced Hepatotoxicity in Experimental Model in Mice

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ABSTRACT

Objective: To evaluate the effect of glutathione peroxidase (GPx) and superoxide dismutase (SOD) in liver tissue in adult mice, orally as a supplementary agent for isoniazid-induced hepatotoxicity.

Study design: Experimental study

Place & Duration of study: Department of Pharmacology, Resource Lab and Department of Morbid Anatomy & Histopathology, University of Health Sciences, Lahore, Pakistan from 5th July 2019 to 5th January 2020.

Methodology: Adult male mice were divided into five groups with 7 mice in each group. Liver injury by isoniazid and its protection with oxyresveratrol was assessed by examining liver macroscopically and histological examination. We used liver homogenates for the antioxidant activity analyses. Liver samples have been tested with a few modifications for different antioxidant studies.

Results: Oxyresveratrol showed a non-significant elevation in serum glutathione levels [(1572±137.2 vs 1463±109.3) (1539±264.7 vs 1463±109.3)] respectively. However, combination therapy showed a significant increase in serum glutathione levels as compared to the INH group (1734±219.2 vs 1463±109.3). Oxyresveratrol treatment showed a significant raised serum SOD levels as rivalled to INH group (10.7 ± 0.53 vs 9.98±0.65). Treatment with oxyresveratrol and combination therapy significantly decreased pyknosis as compared to INH group (1.0±0.0 vs 1.57±0.53) (1.0±0.0 vs 1.57±0.53) and (1.0±0.0 vs 1.57±0.53) respectively. A substantial rise in parenchymal vessel congestion in the INH group as rivalled to control group (2.00±0.0 vs 1.0±0.0). Treatment with oxyresveratrol significantly decreased parenchymal vessels congestion (1.28±0.48 vs 2.00±0.0) as compared to the INH group. However, combination therapy generally showed a more significant decrease in the parenchymal vessel congestion (1.00±0.0 vs 2.00±0.0) as compared to the INH group.

Conclusion: We found a reduction in inflammation of the parenchyma and portal tract area, vascular congestion, and pyknosis may account for the anti-inflammatory activity of oxyresveratrol also, combination therapy alone significantly increased the GPx levels which confirmed their synergistic effects.

Keywords: Oxyresveratrol, Glutathione, Superoxide dismutase, Isoniazid (INH), Hepatotoxicity, LFT's, Hepatoprotection

INTRODUCTION

As far as we are well aware, there are two protective antioxidants in the species. The first is a non-enzypathymic composition of low-molecular wealth molecules that scavenge free radicals to reduce fluctuation in ROS levels: one is enzymatical, like superoxide dismutase (SOD), the other GLPx, etc.[1] Glutathione (GSH) is the primary cell-signalling regulator for the antioxidant and redox. By minimizing H_2O_2 and by escaping of reagent radicals for oxidation, GSH protects cells from oxidative injury.[2]

By scavengering free radicals, antioxidants play a protective role. Tobacco releases free radicals are known to lead to changes in the human antioxidant levels, and antioxidant-related damages in the blood are reflected.[3]

The rates of oxidation in the body are lowered by antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase. In phase 2 of the biotransformation of several substances, Glutathione plays a significant role. Variation in the expression of those enzymes indicates varying levels of antioxidant defense for each enzyme and regional variations in their distribution.[4]

Superoxide dismutase is a catalyst for the dismutation into hydrogen peroxide and molecular oxygen of two super oxides (O_2.-) anions. It protects the tissue from the harmful effects of superoxide radicals to a certain degree. Hydrogen peroxide (H_2O_2) hydrolyses CAT enzymes into the water and molecular oxygen. GPX catalyzes glutathione (GSH) reduction of hydro peroxide. Glutathione metabolism is one of the most important antioxidant defense mechanisms.[5]

The function of such enzymes is an important factor in the progress of the disease and can therefore be useful antioxidant therapy in combination treatment of chronic HCV. To our knowledge, no studies have been conducted in our population to examine the relationship between superoxide dismutase, glutathione peroxidase activities and oxyresveratrol in isoniazid induced hepatotoxicity in an experimental model in mice.

MATERIALS AND METHODS

This experimental research was performed in the Department of Pharmaceuticals, Resource Laboratory and Morbid Anatomy and Histopathology, Lahore, Pakistan, University of Health Science from 5th July 2019 to 5th January 2020 and comprised 35 mices.[6] It was simple random sampling using the lottery method. Mice were

given a number from 1 to 35 and were assigned randomly to five groups I, II, and III, using a 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 groups and so on. We used hepatic homogenates for the antioxidant activity studies. Liver samples were prepared for different antioxidant tests as defined with several amendments.

Preparation of experimental animals: Each of the mice had a controlled temperature (23±2°C), humidity (50±5%), and 12 hours each of light and obscurity cycles in the Experimental Research Laboratory for Universities of Hospital. The animals have been fed normal mouse food and ad libitum water. Animal body weight was first recorded and then regularly measured at alternate times.

Group I (Control): Mice were given normal saline orally for 30 days. **Group II (Isoniazid):** Mice were given isoniazid a dose of 100mg/kg bw orally for 30 days and **Group III (Isoniazid + Oxyresveratrol):** Mice were treated with Oxyresveratrol at a dose of (10mg/kg bw) orally, along with isoniazid at a dose of 100mg/kg bw orally for 30 days.

Euthanization: Animals were anesthetized on the 30th day of study with light ether. Blood samples were drawn by cardiac puncture in EDTA tubes. Mice were dissected for liver tissue that was placed in 20 % formalin. Some piece of liver tissue was kept at -80ºC for mRNA expression studies.

Determination of Glutathione Peroxidase and Super Oxide Dismutase: As the oxidative stress of tissue generally involves the Glutathione peroxidase and SOD system, the levels of serum Glutathione peroxidase and SOD were measured in each group by colorimetric method using Ransel and Ransod kits (Randox UK laboratory limited) by using the protocol given in the literature.

Determination of Glutathione Peroxidase: UV Method: This method was based on that of Paglia and Valentine. Cumene Hydro peroxide oxidation is catalysed by glutathione peroxidase (GPX) oxidation. Glutathione Reductases (GR) and NADPH rapidly transformed the oxidized Glutathione (GSSG) into a reduced form with a concomitant NADPH to NADP+ oxidation. The absorption decrease was estimated at 340 nm.

Sample preparation: Heparinized whole blood was used. Selected GPx in the run rest screen and carried out water blank as directed by Randox UK Laboratory Limited.

Assay of GPX activity: Ransel kits (cat. no. RS 505; Randox Labs., Chrumlin, Northern Ireland) determined GPX behaviour in both plasma and hemolysate. In a centrifugal Cobas-Bio analyzer we assessed activity. Cumene hydro peroxide as a substitute is needed for this test. The reagent concentrations recommended by the manufacturer were the final levels in the test. The sample volume was 5 L of four-fold diluted plasma or 5 L of five-fold diluted hemolysate. The total volume of the reaction was 265ml. Absorption at 340 nm has been recorded.

Determination of Super Oxide Dismutase: Assay Principle: The task of Superoxide Dismutase (SOD) in process of oxidative energy, hydrogen peroxide, and molecular oxygen, is to speed up the dispersal of toxic superoxide radical. To form a red formazan dye, the kit (Ransol) method uses xanthine and xanthine oxidase (XOD) to produce superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) Then the activity of superoxide dismutase is measured by the degree of inhibition of this reaction. One unit of SOD is the one that induces a 50 percent embarrassment of INT reduction rate under the assay conditions.

Sample Preparation: Using whole blood samples heparinized. With a 0.9 percent NaCl solution, erythrocytes were washed four times. At 3000 rpm, 0.5 ml of entire blood was centrifuged up to 10 minutes and thenceforth aspirated off the plasma. Afterwards, erythrocytes were splashed4 times after each wash by 3 ml of 0.9 percent NaCl solution centrifuging at 3000 rpm for 10 minutes. The washed centrifuged erythrocytes were then formed with cold redistilled water up to 2.0 ml, combined and left for 15 minutes to stand at +4°C. The lysate was diluted at 0.01 mol/l Phosphate Buffer pH 7.0 so the inhibition percentage decreased between 30% and 60%. A 25-fold dilution of lysate was prepared according to the protocol given by the commercially available kit.

All the data was entered and analyzed by using the Graph Pad version 5. One-way *ANOVA* was applied to observe the difference in groups. Post-Hoc Tukey test or student t-test was used to observe which group mean is different from others. AP-value $\leq$0.05was considered statistically significant.

RESULTS

Effect of INH, oxyresveratrol on serum glutathione peroxidases level showed that serum glutathione peroxidase levels were not raised significantly in INH group as rivalled to control group (1463±109.3 vs 1413±129.1). Oxyresveratrol and silymarin showed a non-significant elevation in serum glutathione levels [(1572±137.2 vs 1463±109.3) (1539±264.7 vs 1463±109.3)] respectively. However, combination therapy showed a significant increase in serum glutathione levels as compared to the INH group (1734±219.2 vs 1463±109.3).

Effect of INH, oxyresveratrol on serum super oxide dismutase showed that serum Super Oxide Dismutase levels were not raised significantly in INH group as rivalled to control group (9.67±0.73 vs 9.98±0.65). Oxyresveratrol treatment showed a significant rise in serum SOD levels as rivalled to the INH group (10.7±0.53 vs 9.98±0.65). Silymarin and combination therapy also significantly elevated the SOD levels as compared to the INH group (10.9±0.94 vs 9.98±0.65) and (11.4±1.00 vs 9.98±0.65) respectively.

Effect of INH, oxyresveratrol on pyknosis of hepatocytes showed that a significant increase in the pyknosis was observed in the INH group as compared to the control group (1.57±0.53 vs 1.0±0.0). Treatment with oxyresveratrol, silymarin, and combination therapy significantly decreased pyknosis as compared to the INH group (1.0±0.0 vs 1.57±0.53) (1.0±0.0 vs 1.57±0.53) and (1.0± 0.0 vs 1.57±0.53) respectively.

Effect of INH, oxyresveratrol on the parenchymal vessel congestion of the hepatocytes showed that a significant increase in parenchymal vessel congestion in the INH group as compared to the control group (2.00±0.0

vs 1.0±0.0). Treatment with oxyresveratrol significantly decreased parenchymal vessels congestion (1.28±0.48 vs 2.00±0.0) as compared to the INH group, whereas silymarin was unable to significantly decrease the parenchymal vessels congestion (1.57±0.53 vs 2.00±0.0) as compared to INH group. However, combination therapy generally showed a more significant decrease in the parenchymal vessels congestion (1.00±0.0 vs 2.00±0.0) as compared to the INH group (Figs. 1-4, Table 1)

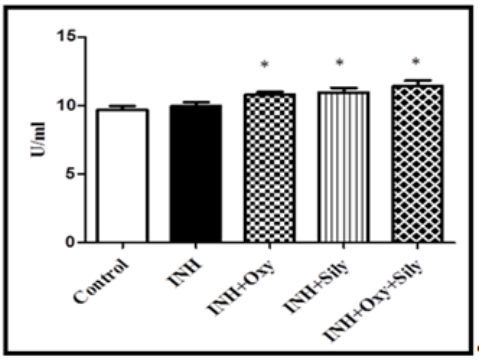

Fig. 1: Super oxide dismutase levels in all mice represent *p<0.05 that indicates significant difference between INH group with INH+Oxy, INH+Sily and combination therapy groups respectively

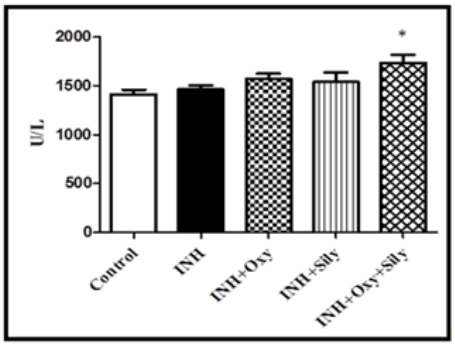

Fig.2: Serum glutathione levels in all mice showed *p<0.05 and indicates significant difference as compared to INH group

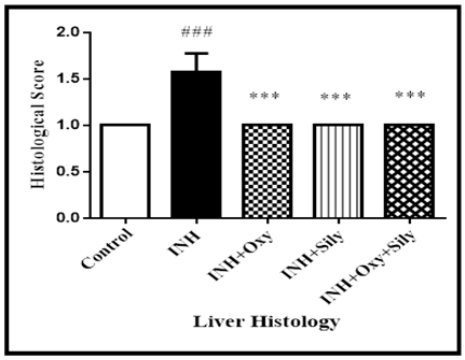

Fig. 3: Pyknosis in all mice showed ***p<0.001 and indicates significant difference as compared to INH group. ### shows p<0.001 and indicates significant difference as compared to control group

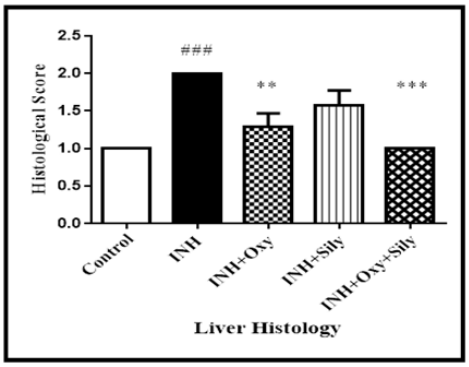

Fig. 4: Parenchymal vessels congestion in mice showed ***p<0.001 and indicates significant difference between combination therapy and INH group. ### shows p < 0.001 and indicates significant difference between INH and control group. ** shows p <0.01 and indicates significant difference between oxyresveratrol and INH group

Table 1: Results are presented as Mean SD of serum Bilirubin, ALT, ALP, AST, Glutathione peroxidase, and Superoxide Dismutase after 30 days of the experiment for all groups (n=7)

Serum	Control	INH	INH+OXY	INH+Sily	INH+Oxy+Sily
Bilirubin	0.65±0.17	0.65±0.07	0.68±0.16	0.70±0.08	0.72±0.11
ALT	120.1±42.2	361.1±64.4###	175.1±63.2***	147.1±50.3***	122.9±34.2***
ALP	66.14±8.41	222.4±43.2###	76.7±12.5***	66.5±12.0***	52.5±10.5***
AST	173.3±25.11	349.3±51.9###	225.1±42.9***	185.9±39.6***	178.9±32.4***
Glutathione Peroxidase	1413±129.1	1463±109.3	1572±137.2	1539±264.7	1734±219.2*
Superoxide Dismutase	9.67±0.73	9.98±0.65	10.78±0.53*	10.94±0.94*	11.42±1.0*

*P<0.05irepresentsiaicomparisoniofitreatmentigroupsiwithiINH. **iP<0.01 represents a comparison of treatment groups with INH. ***P<0.001 represents a 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 a significant comparison of oxyresveratrol

DISCUSSION

One of the most significant in the enzyme antioxidant protection system, SOD has been recorded.[7] To form hydrogen peroxide, scavenges superoxide anion and eliminates the toxicity resulting from this radical. Glutathione is a non-enzymatic, biological antioxidant that is another essential and most abundant tripeptide found in the liver. It eliminates free radical species such as peroxide hydrogen, radical super oxides, and retains the protein thiols membrane.[8] In the present study, SOD levels were found significantly increased in all treatment groups. It has been reported that oxyresveratrol possesses great antioxidant activity.[9] We also found a significant increase in glutathione peroxide levels in the combination therapy group as well. Glutathione helps the liver to clear the toxic levels of different drugs thus helping in detoxification and this is thought to be done via inhibition of Phase I detoxification.[10,11]

Resveratrol is a natural phytoalexin and known for its anti-inflammatory, antiviral, and antioxidant properties.[12,13] The protective role of resveratrol against several hepatic injuries due to oxidative damage has been reported by several studies.[14,15] Oxyresveratrol is an analog of resveratrol with an extra hydroxyl group. It is trans-2', 3,4', 5-Tetramethoxystilbene with a molecular formula of $C_{14}H_{12}O_4$. This extra hydroxyl group provides it with the extra power of anti-oxidant property than resveratrol.[16]

We found that oxyresveratrol significantly reduced the inflammation of the parenchyma and portal tract area, vascular congestion, and pyknosis. It reduced the hepatocyte inflammation more significantly as compared to silymarin. We also found that oxyresveratrol significantly increased the regeneration process. Previously, Chung et al[17] showed that oxyresveratrol reduced inflammation by inhibiting cyclo-oxygenase-2 and NOS enzymes.

We also evaluated the effects of oxyresveratrol on the markers of liver function test. Serum AST is commonly raised in acute liver damage, similar to ALT.[18,19] We found raised levels of ALT and AST which were indicative of liver damage caused by INH, in addition to histopathological findings. Both mitochondria and cytoplasm are rich in AST while the cytoplasm of hepatic cells is primarily the residing place of ALT.[20,21] Both the enzymes are considered as important markers for liver injury and are responsible for the catalysis of gluconeogenesis from noncarbohydrate sources. An increase in the serum concentrations of these two enzymes is indicative of a disruption of plasma membrane integrity, which ultimately leads to the escape of these enzymes into the blood.[22,23]

CONCLUSION

We found a reduction in inflammation of the parenchyma and portal tract area, vascular congestion, and pyknosis may account for the anti-inflammatory activity of oxyresveratrol. Also, combination therapy alone significantly increased the GPx levels which confirmed their synergistic effects. Moreover, our data showed that oxyresveratrol significantly reduced the elevated levels of serum ALT, ALP, and AST, which is indicative of a decrease in the severity of liver damage after treatment with oxyresveratrol hepatotoxicity by evaluating the activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) in liver tissue in adult mice.

REFERENCES

1. Lian Y, Zhao J, Xu P, Wang Y, Zhao J, Jia L, et al. Protective effects of metallothionein on isoniazid and rifampicin-induced hepatotoxicity in mice. PLoS One 2013;8(8):e72058.
2. Yuan L, Kaplowitz N. Glutathione in liver diseases and hepatotoxicity. Mol Aspects Med 2009;30(1–2):29–41.
3. Naga Sirisha C, Manohar R. Study of antioxidant enzymes superoxide dismutase and glutathione peroxidase levels in tobacco chewers and smokers: A pilot study. J Cancer Res Ther 2013;9(2):210.
4. de Hiragi CO, Miranda-Vilela AL, Rocha DMS, de Oliveira SF, Hatagima A, de Klautau-Guimarães MN. Superoxide dismutase, catalase, glutathione peroxidase and gluthatione s-transferases M1 and T1 gene polymorphisms in three brazilian population groups. Genet Mol Biol 2011; 34(1): 11–8.
5. Ms Y, Gulesci N, Bilgin R, Is K. Superoxide dismutase , glutathione peroxidase and catalase activities in patients with viral hepatitis C. Integr Mol Med 2020;7:1–3.
6. Peres W, Tuñón MJ, Collado PS, Herrmann S, Marroni N, González-Gallego J. The flavonoid quercetin ameliorates liver damage in rats with biliary obstruction. J Hepatol 2000;33(5):742–50.
7. Caverzan A, Casassola A, Brammer SP. Antioxidant responses of wheat plants under stress. Genet Mol Biol 2016;39(1):1–6.
8. Prakash J, Gupta SK, Kochupillai V, Singh N, Gupta YK, Joshi S. Chemopreventive activity ofWithania somnifera in experimentally induced fibrosarcoma tumours in Swiss albino mice. Phyther Res 2015;15(3):240–4.
9. Xu L, Liu C, Xiang W, Chen H, Qin X, Huang X. Advances in the Study of Oxyresveratrol. Int J Pharmacol 2014;10(1):44–54.
10. Hodges RE, Minich DM. Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application. J Nutr Metab 2015;2015:1–23.
11. Baer-Dubowska W, Szaefer H, Krajka-Kuzniak V. Inhibition of murine hepatic cytochrome P450 activities by natural and synthetic phenolic compounds. Xenobiotica. 1998 Jan 22;28(8):735–43.
12. Zhu X, Liu Q, Wang M, Liang M, Yang X, Xu X, et al. Activation of sirt1 by resveratrol inhibits tnf-α induced inflammation in fibroblasts. PLoS One 2011;6(11):e27081.
13. Dyson OF, Walker LR, Whitehouse A, Cook PP, Akula SM. Resveratrol Inhibits KSHV Reactivation by Lowering the Levels of Cellular EGR-1. PLoS One 2012;7(3):e33364.
14. Ara C, Kirimlioglu H, Karabulut AB, Coban S, Ay S, Harputluoglu M, et al. Protective effect of resveratrol against oxidative stress in cholestasis. J Surg Res 2005;127(2):112–7.
15. Farghali H, Černý D, Kameníková L, Martínek J, Hořínek A, Kmoníčková E, et al. Resveratrol attenuates lipopolysaccharide-induced hepatitis in d-galactosamine sensitized rats: Role of nitric oxide synthase 2 and heme oxygenase-1. Nitric Oxide 2009;21(3–4):216–25.
16. Galindo I, Hernáez B, Berná J, Fenoll J, Cenis JL, Escribano JM, et al. Comparative inhibitory activity of the stilbenes resveratrol and oxyresveratrol on African swine fever virus replication. Antiviral Res 2011;91(1):57–63.
17. Chung K-O, Kim B-Y, Lee M-H, Kim Y-R, Chung H-Y, Park J-H, et al. In-vitro and in-vivo anti-inflammatory effect of oxyresveratrol from Morus alba L. J Pharm Pharmacol 2017;55(12):1695–700.
18. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008;47(4):1363–70.
19. Nyblom H, Berggren U, Balldin J, Olsson R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol Alcohol 2004;39(4):336–9.
20. Zoppini G, Cacciatori V, Negri C, Stoico V, Lippi G, Targher G, et al. The aspartate aminotransferase-to-alanine aminotransferase ratio predicts all-cause and cardiovascular mortality in patients with type 2 diabetes. Medicine (Baltimore) 2016;95(43):e4821.
21. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. CMAJ 2005;172(3):367–79.
22. Wang TY, Libardo MDJ, Angeles-Boza AM, Pellois JP. Membrane Oxidation in Cell Delivery and Cell Killing Applications. ACS Chem Biol 2017;12(5):1170–82.
23. Ho WY, Yeap SK, Ho CL, Abdul Rahim R, Alitheen NB. Hepatoprotective activity of elephantopus scaber on alcohol-induced liver damage in mice. Evidence-based Complement Altern Med 2012;2012:1–8