HEPATOPROTECTIVE EFFECT OF OXYRESVERATROL IN ISONIAZID INDUCED HEPATOTOXICITY IN EXPERIMENTAL MICE MODEL.

Mohammad Abid[1], Muhammad Yaqoob Shahani[2], Asma Hameed[3], Mehvash Sikandar[4], Zahida Anwar[5], Zubaida Anwar.[6]

Abstract

Introduction: The liver is the main organ for maintaining the body's metabolic homeostasis. The prescription for tuberculosis (TB) diagnosis is Isoniazid (INH), which is a frequent source of serious and often lethal acute chronic liver damage.**Objective**: To compare the thirty day hepatoprotective effect of oxyresveratrolformulation and silymarin as an oral supplementary agent for isoniazid induced hepatotoxicity in adult mice.**Study design:** Experimental study**Place of study:**Department of Pharmacology, Resource lab and Department of Morbid Anatomy and Histopathology, University of Health Sciences, Lahore, Pakistan.**Duration of study:**Six months from 1st January 2019 to 30th June 2019. **Methodology:** Adult male mice were divided into five groups with 7 mice in each group. Group I served as a control. Group II, III, IV and V were given isoniazid dissolved in distilled water (100mg body weight per day). Group III was treated with oxyresveratrol 10mg/kg bw/day orally while group IV was given silymarin at 125mg/kg bw/day. Group V was given silymarin 125mg/kg bw/day and oxyresveratrol (10mg/kg bw /day). Liver injury by isoniazid and its protection with oxyresveratrol was assessed by examining liver macroscopically and histological examination. **Results:** Oxyresveratrol significantly decreased hepatic and portal tract inflammation. Silymarin also decreased the inflammation in both areas significantly but inflammation was better treated by oxyresveratrol. Combination therapy also showed significant decrease in inflammation. Vascular congestion was reduced by oxyresveratrol alone and in combination with silymarin however silymarin alone did not significantly reduce the vascular congestion. Other histological parameters and serum LFTs were same for both drugs and in combination therapy that showed significant improvement and reversal of liver insult. **Conclusion:** Oxyresveratrol showed hepatoprotective effects against isoniazid induced hepatic damage and showed better protection than silymarin.

Keywords: Oxyresveratrol, silymarin, Isoniazid (INH), hepatotoxicity, LFT's, Hepatoprotection.

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INTRODUCTION

Isoniazid (INH) is first line anti-tuberculosis drug.[1] It was founded in 1952 and significantly contributed to reducing tuberculosis morbidity and death.[2] Tuberculosis (TB) currently is one of the leading cause of death globally by infecting one-third of population all over the world.[3] INH has proven its efficacy to be used as anti-tuberculous dug.[4] Hepatotoxicity is one of the major adverse effect encountered by isoniazid during anti-tuberculous therapy.[5] INH metabolism is responsible for INH-induced liver injury. Most common biochemical mechanism is metabolism of INH into reactive metabolites that bind and damage the macromolecules in hepatocytes. Acetylation of INH in liver produces acetyl INH which is furtheroxidized to either hydroxylamine which is a toxic metabolite or hydrolyzed into hydrazine which is also toxic and further hydrolyzed to another noxious compound i.e. acetyl hydrazine.[6] INH induced hepatotoxicity is diagnosed by raised serum liver function tests (LFT's) such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin).[7] Hepatotoxicity produced by INH is proven by observing inflammatory infiltrates, steatosis and hepatocytic ballooning.[8,9]

No research study has been carried out as yet on comparison of aqueous and ethanolic extract of oxyresveratrolin perspective of hepatoprotective effect. Our study was planned to compare the thirty day hepatoprotective effect of oxyresveratrol formulation and silymarin as an oral supplementary agent for isoniazidiinducedihepatotoxicityiinadultmiceby

estimating serum LFT's and observing the microscopic histopathological changes of liver.

MATERIAL AND METHODS

The Experimental study was conducted at department of Pharmacology, Resource lab and Department of Morbid Anatomy and Histopathology, University of Health Sciences, Lahore, Pakistan for the period of six months. 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 (Peres et al., 2000). It was simple random sampling using lottery method. Mice were given number from 1 to 35 and were assigned randomly to five groups I, II, III, IV and V using 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. First seven were assigned control group and so on.

Preparation of Experimental Animals

Each of the mice had a controlled temperature (23±2 ° C), humidity (50±5 percent), and 12 hours each of light and obscurity cycles initheiExperimentaliResearch 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.

Animal Groups:

Group I (Control)

Mice were given normal saline orally for 30 days.

Group II (Isoniazid)

Mice were given isoniazidiat a doseiof100mg/kg bw orally for 30 days (Jehangir et al., 2010).

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 (Mouihate et al., 2006).

Group IV (Isoniazid + Silymarin)

Mice were given Silymarinatiaidoseiof 125mg/kg bw orally, alongiwithiisoniazid at a dose of 100mg/kg bw orally for 30 days.

Group V (Isoniazid + Silymarin + Oxyresveratrol)

Mice in this group were given oral Silymarin and Oxyresveratrol for 30 days at the dose of 125mg/kg bw and 10mg/kg bw respectively. Isoniazid at dose of 100 mg/kg bw was also given orally for 30.

Euthanization

Animals were anesthetized on 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.

Estimation of Serum Total Billirubin

Colorimetric method has been identified to determine total bilirubin serum. Specific bilirubin responds in alcalin media to aibluei colore di complex i with diazotic sulfanilic acid. Total bilirubin is determined by the reaction with diazotic sulphanilic acid in the presence of caffeine that releases bilirubin bond albumin. At 578 NM the reaction was controlled. (Mendie et al., 2015).

Estimation of Serum Alanine Aminotransferase (ALT)

Serum ALT was determined according to IFCC method. ALT catalyzes the transition from alanine to oxoglutrate of the amino group by glutamate production into pyruvate. Then, in the presence of a reduced nicotinamide adenine dinucleotide (NADH), the lactate is reduced to lactate by dehydrogenase (LDH).The reaction is kinetically controlled at 340 nm, proportional to the action of ALT in your sample.

Estimation of Serum Alanine Phosphatase (ALP)

IFCC approach has been used to evaluate serum ALP; alkaline phosphatase catalyzes phosphate hydrolysis p-Nitrophenyl and p-Nitrophenole phosphate hydrolysis. This reaction is closely controlled at 405 nm (Schumann et al., 2011).

Estimation of Serum Aspartate Aminotransferase (AST)

Serum AST was determined by IFCC method. Apartate aminotransferase catalyzes the transfer by glutamate and oxaloacetate from aspartate to oxoglutarate of the amino group. The presence of decreased nicotinamide adenine dinucleotide (NADH), oxoloacetate is decreased to L-malate dehydrogenase (MDH). The reaction is kinetically controlled at 340 nm, proportions to the sample's behavior, by the reduction in absorption resulting from NADH oxidation in NAD+.

Liver Histology

After dissecting the mice, liver tissue was prepared for histopathological examination. The dehydration, separation from the tissues, impregnation and embedding measures in tissue processing is automated in all tissues of histology. Paraffin tissue blocks embedded have been developed. At least two tissue sectionsiof 4-6 µmithicknessiwerecutibyirotaryimicrotome from each block which were then stained with hematoxylin and eosin (skip et al., 2012) and Periodic acid-Schiff stains (bankroft and gamble, 2008). Periodic acid oxidizes the carbon to carbon bond forming aldehyde which reacts with fuschin acid (Schiff s reagent) which forms the megenta color with glycogen in the cell and basement membrane (bankroft and gamble, 2008). Slides were observed under light microscope using different magnifications.

Statistical Analysis

Allitheidataiwasienterediandanalysed by using the Graph Pad version 5.Datawas expressed as mean ± SD. Oneiway*ANOVA* wasiapplied to observe the difference in groups. Post Hoc Tukey test or student t-test was applied toiobserveiwhich group mean is different from other. A P-value ≤ 0.05 was considered statistical significant.

RESULTS

Effect of INH, Oxyresveratrol and Silymarin on architecture of liver

Results showed that there was no significant difference in the general architecture of liver in the INH group as compared to control group (1.0 ± 0.0 vs 1.0 ± 0.0). Treatment with Oxyresveratrol, silymarin, and combination therapy also did not show any effect on the architecture of liver [(1.0 ± 0.0 vs 1.0 ± 0.0), (1.0 ± 0.0 vs 1. 0 ± 0.0) and (1.0 ± 0.0 vs 1.0 ± 0.0), respectively].

Effect of INH, Oxyresveratrol and Silymarin on hepatocytes size

Results showed that there was no difference in hepatocytes size in the INH group as compared to control group (1.0 ± 0.0 vs 1.0 ± 0.0). Treatment with Oxyresveratrol, silymarin, and combination therapy also had no effect on the size of hepatocytes [(1.0 ± 0.0 vs 1.0 ± 0.0), (1.0 ± 0.0 vs 1.0 ± 0.0) and (1.0 ± 0.0 vs 1.0 ± 0.0), respectively].

Effect of INH, Oxyresveratrol and Silymarin on hepatocytes for ballooning degeneration

Results showed that there is no significant increase in ballooning degeneration of hepatocytes in the INH group as compared to control group (1.140 ± 0.37 vs 1.0 ± 0.0). Similarly, non-significant difference was observed in all treatment groups as compared to INH group [(1.0 ± 0.0 vs 1.140 ± 0.37), (1.0±0.0 vs 1.140 ± 0.37) and (1.0 ± 0.0 vs 1.140 ± 0.37), respectively].

Effect of INH, Oxyresveratrol and Silymarin on vacuolar degeneration of the hepatocytes.

Results showed that there was a significant increase in vacuolar degeneration in INH group as compared to control group (1.85 ± 0.37 vs 1.0 ± 0.0). Treatment with oxyresveratrol, silymarin and combination therapy non-significantly decreased vacuolar degeneration as compared to INH group [(1.42 ± 0.37 vs 1.85 ± 0.37), (1.57 ± 0.53 vs 1.85 ± 0.37) and (1.140 ± 0.37 vs 1.85 ± 0.37), respectively].

Effect of INH, Oxyresveratrol and Silymarin on apoptosis of hepatocytes

No significant change in the apoptosis of hepatocytes was found in the INH group as compared to control group (2.0 ± 0.0 vs 2.0 ± 0.0). Treatment with silymarin, oxyresveratrol, and combination therapy had no significant effect on apoptosis of hepatocytes [(2.0 ± 0.0 vs 2.0 ± 0.0), (2.0±0.0 vs 2.0 ± 0.0) and (2.0 ± 0.0 vs 2.0 ± 0.0), respectively].

Effect of INH, Oxyresveratrol and Silymarin on nuclear morphology i.e bi/multinucleation for regeneration.

Treatment with oxyresveratrol caused a significant increase in bi/multinucleation as compared to INH group (1.0 ± 0.0 vs1.0 ± 0.0). oxyresveratrol, silymarine and combination therapy significantly increased bi/multinucleation as compared to INH group (1.71 ± 0.48 vs 1.0 ± 0.0), (1.71 ± 0.48 vs 1.0 ± 0.0) and (2.0 ± 0.0) respectively.

Effect of INH, Oxyresveratrol and Silymarin on Pyknosis of hepatocytes

A significant increase in the pyknosis was observed in INH group as compared to control group (1.57 ± 0.53 vs 1.0 ± 0.0). Treatment with oxyresveratrol, silymarin 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.

Effect of INH, Oxyresveratrol and Silymarin on the parenchymal vessel congestion of the hepatocytes

Results showing significant increase in parenchymal vessels congestion in INH group as compared to control group (2.00 ± 0.0 vs 1.0 ± 0.0). Treatment with oxyresveratrolsignificantly decreased parenchymal vessels congestion (1.28 ± 0.48 vs 2.00 ± 0.0) as compared to 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 more significant decrease in the parenchymal vessels congestion (1.00 ± 0.0 vs 2.00 ± 0.0) as compared to INH group.

Effect of INH, Oxyresveratrol and Silymarin on portal tract

Portal tract inflammation was significantly high in INH group as compared to control group (2.26 ± 0.48 vs 1.00 ± 0.0). Treatment with oxyresveratrol significantly decreased portal tract inflammation (1.42 ± 0.53 vs 2.26 ± 0.48) as compared to INH group, whereas silymarin also significantly decreased the portal tract inflammation as compared to INH group (1.57 ± 0.53 vs 2.26 ± 0.48). However combination therapy generally resulted in more significant reduction in the portal tract inflammation as compared to INH group (1.14 ± 0.37 vs 2.26 ± 0.48)

Effect of INH, Oxyresveratrol and Silymarin on fatty changes

No difference in fatty changes was observed in INH group as compared to control group (1.0 ± 0.0) vs (1.0 ± 0.0). Treatment with Oxyresveratrol, silymarin and combination therapy also did not have any effect on fatty changes of mice liver [(1.0 ± 0.0 vs 1.0 ± 0.0), (1.0 ± 0.0 vs 1.0 ± 0.0) and (1.0 ± 0.0 vs 1.0 ± 0.0), respectively].

Effect of INH, Oxyresveratrol and Silymarin on hepatocyte inflammation

INH caused significant hepatocyte inflammation in INH group as compared to control group (2.42 ± 0.53 vs 1.00 ± 0.0). Treatment with oxyresveratrol significantly decreased hepatic cells inflammation as compared to INH group (1.42 ± 0.53 vs 2.42 ± 0.53), whereas silymarin also significantly reduced hepatic cells inflammation (1.57 ± 0.53 vs 2.42 ± 0.53) as compared to INH group. Combination therapy with oxyresveratrol and silymarin showed more significant reduction in the hepatic cells

inflammation as compared to INH group (1.26 ± 0.48 vs (2.42 ± 0.53).

Effect of INH, Silymarin, oxyresveratrol on serum bilirubin level

The following graph shows that there is no significant increase in the serum bilirubin levels in the INH group as compared to control group (0.65 ± 0.07 vs 0.65 ± 0.17)). Treatment with oxyresveratrol had no significant change on serum bilirubin levels along with other treatment groups (0.68 ± 0.16 vs 0.65 ± 0.07), (0.70 ± 0.08 vs 0.65 ± 0.07) and (0.72 ± 0.11vs 0.65 ± 0.07) respectively.

Effect of INH, Silymarin, and Oxyresveratrol on Serum ALT level

The following graph shows that serum ALT levels were raised significantly in INH group as compared to control group (361 ± 64.4 vs 120 ± 42.2). Treatment with oxyresveratrol significantly reduced the ALT levels as compared to INH group (175 ± 63.2 vs 361 ± 64.4). Treatment with silymarin also significantly reduced the ALT levels as compared to INH group (147 ± 50.3 vs 361 ± 64.4). While combination therapy showed high drop in ALT levels as compared to INH group (122 ± 34.2 vs 361 ± 64.4).

Effect of INH, Silymarin, Oxyresveratrol on Serum ALP level

The results indicate that serum ALP levels were raised significantly in INH group as compared to control group (224 ± 43.2 vs 66.1 ± 8.41). Treatment with oxyresveratrol significantly reduced the levels of ALP as compared to INH group (76.7 ± 12.5 vs 224 ± 43.2). Treatment with silymarin also significantly attenuated the ALP levels as compared to INH group (66.5 ± 12.0 vs 224 ± 43.2). The combination therapy showed higher drop in ALP levels as compared to INH group (52.7 ± 10.5 vs 224 ± 43.2).

Effect of INH, Silymarin, Oxyresveratrol on serum AST level

The results show that serum AST levels were raised significantly in INH group as compared to control group (349 ± 51.9 vs 173 ± 25.1). Treatment with oxyresveratrol significantly reduced the AST levels as compared to INH group (225 ± 42.9 vs 349 ± 51.9). Treatment with silymarin also significantly reduced the AST levels as compared to INH group (185 ± 39.6 vs 349 ± 51.9). While combination therapy showed significant drop in AST levels as compared to INH group (178 ± 32.4 vs 349 ± 51.9).

DISSCUSSION

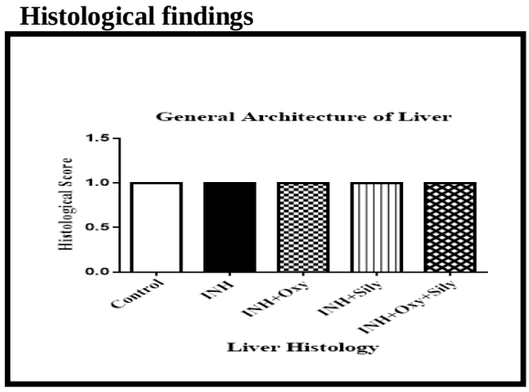
The central role of liver as principle drug metabolizing site has already been well documented. Since liver is correlated with drug detoxification, hepatic oxidative species are especially susceptible to injury.[10] Many studies have documented that oxidative stress is involved in the process of INH hepatotoxicity.[10] Consequently, it is vulnerable to injury due to toxic byproducts of various drugs which can cause damage to body's guardian, the liver. Drug-induced reactions are mostly idiosyncratic in nature while some have allergic component too and account for half of the cases of acute liver failure in the United States.[11] In the present study we induced hepatotoxicity by isoniazid. Isoniazid is metabolized in liver by acetylation with the help of hepatic N-acetyl transferase which yields acetyl-isoniazid. INH toxicity was observed during current study in the form of histopathological changes like vacuolar degeneration, apoptosis, inflammation of parenchyma and portal tract, ballooning degeneration and pyknosis. Our results of histopathological experiment were in line with the findings of previous studies which also showed inflammation of hepatocytes with ballooning degeneration, focal necrosis, minimal cholestasis, and fibrosis when INH was administered for 30 days.[12] Resveratrol is a natural phytoalexinand known for its anti-inflammatory, antiviral, and antioxidant properties.[13,14] The protective role of resveratrol against a number of hepatic injuries due to oxidative damage has been reported by several studies.[15]Oxyresveratrol is an analog of resveratrol with an extra hydroxyl group.

We studied the hepatoprotective effect of oxyresveratrol in isoniazid-induced hepatotoxic mice model alone and in combination with silymarin, a well-known drug for its hepatoprotective effects.[16] To the best of our knowledge no study has been conducted to see the hepatoprotective effect of oxyresveratrol and to compare it with silymarin. Current study therefore was designed to see hepatoprotective effect of oxyresveratrol on experimental modal of isoniazid induced hepatotoxicity in mice.

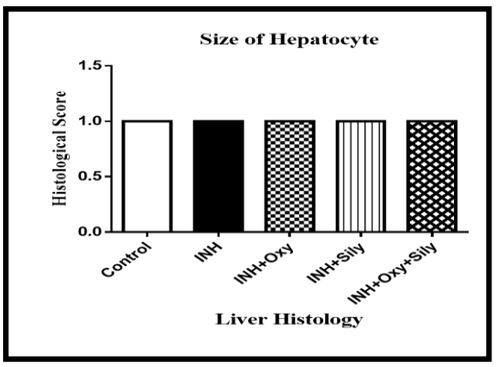
We found that oxyresveratrol significantly reduced the inflammation of the parenchyma and portal tract area, vascular congestion, and pyknosis. It reduced the hepatocyte inflammation more significantly as compare to silymarin.We also found that oxyresveratrol significantly increased the regeneration process. Previously, Chung et al.[17] showed that oxyresveratrol reduced inflammation by inhibiting cyclo-oxygenase-2 and iNOS 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] 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 cytoplasm of hepatic cells is primarily the residing place of ALT.[19] Both the enzymes are considered as important markers for liver injury and are responsible for catalysis of gluconeogenesis from noncarbohydrate sources. Increase in the serum concentrations of these two enzymes is indicative of disruption of plasma membrane integrity, which ultimately leads to escape of these enzymes into the blood circulation.[20] Our data showed that oxyresveratrol significantly reduced the elevated levels of serum ALT, ALP and AST, which is indicative of decrease in the severity of liver damage after treatment with oxyresveratrol.

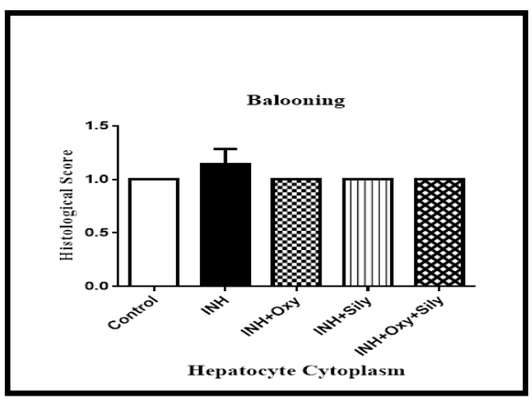
Histological findings



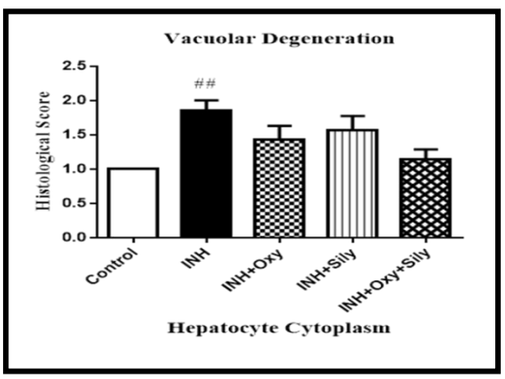
1. Graphical representation of mean ± SD of histopathological score of general architecture of liver in all groups (n=7)



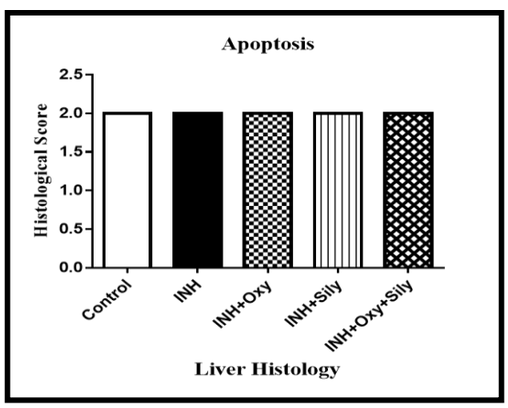
2. Graphical representation of mean ± SD of histopathological score of hepatocytes size in all groups (n=7)



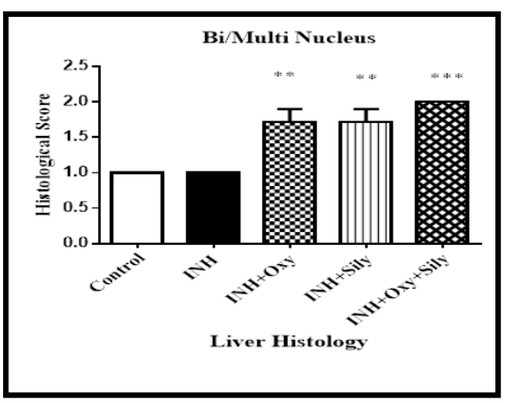
3. Graphical representation of mean ± SD of histopathological score of ballooning in all groups (n=7)



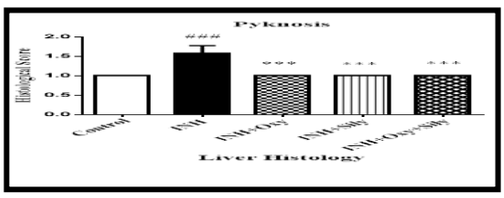
4. Graphical representation of mean ± SD of histopathological score of vacuolar degeneration in all



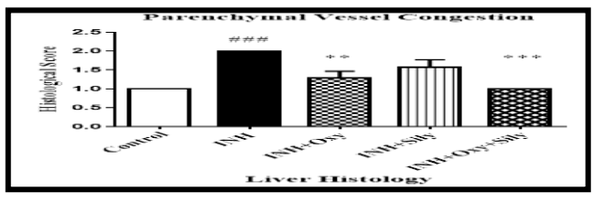
5. Graphical representation of mean ± SD of histopathological score of apoptosis in all groups (n=7).



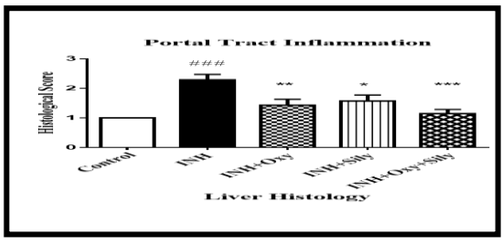
6. Graphical representation of mean ± SD of bi/multi-nucleation in all groups (n=7) * shows p < 0.001 and indicates significant difference between combination therapy and INH group. ** represents p < 0.01and indicates significant difference between treatment and INH groups.**



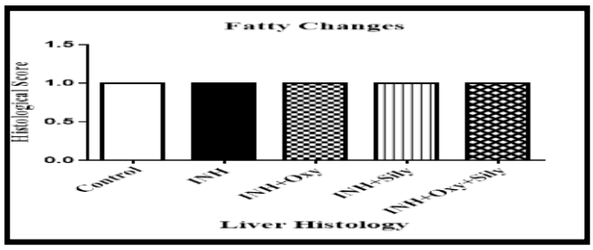
7. Graphical representation of mean ± SD of pyknosis in all mice groups (n=7) * shows p < 0.001 and indicates significant difference as compared to INH group. ### shows p < 0.001 and indicates significant difference as**



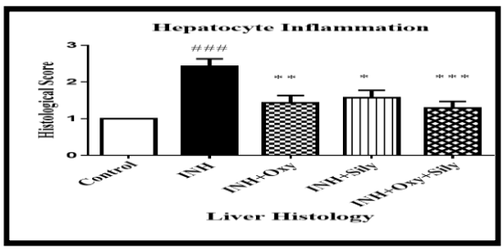
8. Graphical representation of mean ± SD of parenchymal vessels congestion in all groups (n=7) * shows p < 0.001 and indicates significant difference between combination therapy and INH group. ### shows p < 0.001 and indicates significant difference between INH and control group. ** shows p <0.01 and indicates significant difference between oxyresveratrol and INH group**



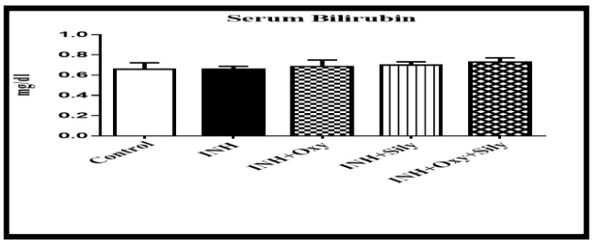
9. Graphical representation of mean ± SD of portal tract inflammation in mice hepatocytes with all groups (n=7). * shows p < 0.001 and indicates significant difference as compared to INH group. ### shows p < 0.001 and indicates significant difference as compared to control group. * shows p < 0.05 that**



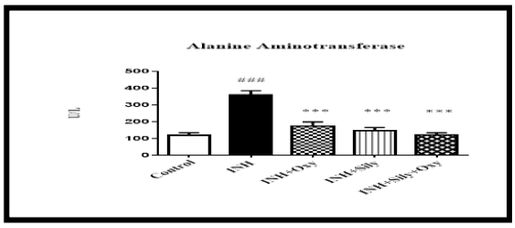
10. Graphical representation of mean ± SD of fatty changes in mice liver in all groups (n=7)



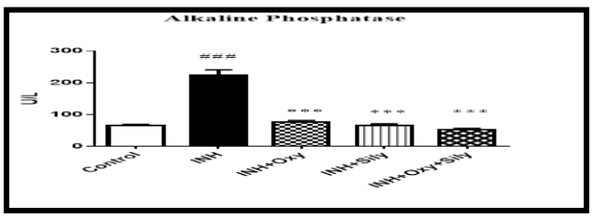
11. Graphical representation of mean ± SD of hepatocyte inflammation in all mice groups (n=7). * shows p < 0.001 and indicates significant difference between INH with combination therapy whereas, ** and * represent p < 0.01 and 0.05 that indicates significant difference between INH group with INH+Oxy and INH+Sily groups respectively. ### shows p < 0.001 and indicates significant**



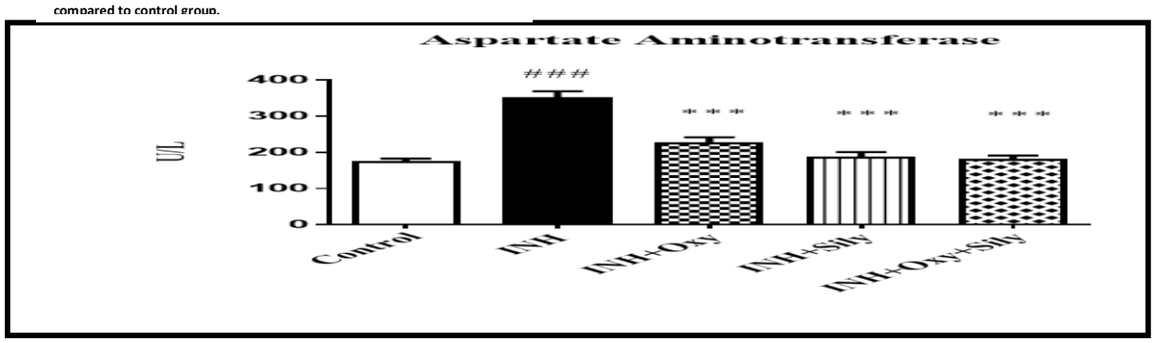
12. Graphical representation of mean ± SD of serum bilirubin levels in all mice groups (n=7)



13. Graphical representation of mean ± SD of ALT 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.**



14. Graphical representation of mean ± SD of ALP levels in mice groups (n=7) * shows p < 0.001 and indicates significant difference as compared INH group. ### shows p < 0.001 and indicates significant difference as compared to control group.**



15. Graphical representation of mean ± SD of AST 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.**

Table 01: Results are presented as Mean ± SD of all the histological parameters after 30 days of experimental protocol (n=7)

Histology	Control	INH	INH+OXY	INH+Sily	INH+Oxy+Sily
General Architecture	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Size of Hepatocyte	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Balooning	1.0 ± 0.0	1.14 ± 0.37	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
VacularDengeneration	1.0 ± 0.0	1.85 ±0.37±$^{\#\#}$	1.42 ± 0.37	1.57 ± 0.53	1.14 ± 0.37
Apoptosis	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Bi/Multi Nucleation	1.0 ± 0.0	1.0 ± 0.0	1.71 ± 0.48**	1.71 ± 0.48**	2.0 ± 0.0***
Pyknosis	1.0 ± 0.0	1.57 ± 0.53$^{\#\#\#}$	1.0 ± 0.0***	1.0 ± 0.0***	1.0 ± 0.0***
Parenchymal vessel congestion	1.0 0.0	2.0 ± 0.0$^{\#\#\#}$	1.28 ± 0.48**	1.57 ± 0.53	1.0 ± 0.0***
Portal Tract Inflammation	1.0 ± 0.0	2.28 ± .48 $^{\#\#\#}$	1.42 ± 0.53**	1.57 ± 0.53*	1.14 ± 0.37***
Fatty Changes	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Hepatocyte Inflammation	1.0 ± 0.0	2.42 ± 0.53$^{\#\#\#}$	1.42 ± 0.53**	1.57 ± 0.53*	1.28 ± 0.48***

Values given are mean ± S.D (n=7).
* 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 respresents significant comparison of oxyresveratrol and silymarin

CONCLUSION

The results of the present study indicate that oxyresveratrol possesses protective effect against the isoniazid induced hepatic-toxicity. This hepatoprotective effects might have been the result of immunomodulatory and anti-inflammatory activities of oxyresveratrol. Also we used combination therapy with oxyresveratrol and silymarin both to observe for their synergistic effects and it showed much better results in some parameters like in reducing portal tract inflammation, hepatocytes inflammation, vascular congestion and increasing binucleation for regeneration of hepatocytes. Also combination therapy alone significantly increased the GPx levels which confirmed their synergistic effects. Further studies are needed to see if higher dose and different routes of administration have more protective effects against isoniazid induced hepatotoxicity. To what extent these findings have clinical value also requires further studies.

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