

Hesperidin alleviates antitubercular drug induced oxidative stress, inflammation and apoptosis in rat liver

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Abstract

Background: Drug induced hepatotoxicity is a serious health problem that leads to treatment interruption and change in the treatment regimen during the course of therapy. Isoniazid (H), Rifampicin(R) and pyrazinamide (Z) are the commonly used drugs for the treatment of tuberculosis but leads to undesirable side effects, such as hepatotoxicity. Hence the present study undertaken to investigate the hepatoprotective activity of Hesperidin (HDN) against Isoniazid, Rifampicin and Pyrazinamide induced hepatotoxicity.

Methods: Wistar albino rats were randomly divided into four groups of six animals each. Isoniazid, Rifampicin, Pyrazinamide were used to induce Hepatotoxicity, Hesperidin 200mg/kg was given one hour prior administration of antitubercular drugs (HRZ), Silymarin 100mg/kg was used as a standard drug daily for 50 days.

Results: Antitubercular drug induces hepatotoxicity was evident by increased in the levels of liver marker enzyme (AST, ALT, ALP & LDH) and also a significant rise in the level of LPO along with decline in the level of both enzymatic and non enzymatic antioxidant enzymes (SOD,CAT,GSH,VIT C and VIT E) . Moreover, antitubercular drug causes hepatic damage by inducing apoptotic death and inflammation in hepatic cells, manifested by an increase in the expression of Bax, caspase-3, caspase-9, NF- κ B, IL- 10, TNF- α and decrease in Bcl-2 expression. These results were further supported by the histopathological examination of liver. All these features of antitubercular drugs induced toxicity were reversed by the co-administration of Hesperidin.

Conclusion: Therefore, our study favors the view that Hesperidin may be a useful modulator in alleviating antitubercular drug induced oxidative stress, inflammation and apoptosis.

Keywords: Reactive oxygen species (ROS), Isoniazid, Rifampicin, Pyrazinamide, Hesperidin, Hepatotoxicity

1. Introduction

Tuberculosis (TB) is considered to be one of the common problems in undeveloped and developing countries. Despite the advances and the fact that nearly all cases can be cured, TB remains one of the world's biggest threats which killed 1.5 million people and 6 million new cases of TB were reported to WHO. Worldwide 9.6 million people are estimated to have fallen ill with TB, in India it is about 23% of world TB cases (WHO 2015) [1].

Isoniazid (H), Rifampicin(R), Pyrazinamide (Z), Streptomycin(S) and Ethambutol (E) are the first line antitubercular drugs that serve as a therapeutic agent for the treatment of TB. Among the effective anti tubercular drug combination, HRZ will induce significant hepatotoxicity thereby inducing persistent oxidative stress, cell injury, and apoptosis in humans [2-4].

Since oxidative stress and apoptosis are the major factors for Anti TB drug induced hepatotoxicity, there is no constrictive treatment to treat liver toxicity. Hence interest has been grown in identifying the hepatoprotective drug from the natural origin, because of their safety and efficacy and that can be supplemented with the hepatotoxic drugs. Hesperidin (3,5,7 – trihydroxyflavanone 7 – rhamnoglucoside) is a phytoflavone belong to the family of polyphenolic compounds present in citrus fruits, especially in the grape fruit, peel of bitter orange and sweet orange[5]. Hesperidin exerts numerous pharmacological functions such as anti-inflammatory, vascular protection [6], sedative, antinociceptive [7], antihypertensive [8], and anti-tumor properties [9]. Hesperidin was reported to have antioxidant property and possess satisfactory capability to neutralize free

radicals [10]. This antioxidant property may be the rationale behind these various pharmacological actions and they can be used as protective agents in a number of diseases. Therefore, the present study was designed to evaluate the hepatoprotective, antioxidant, antiapoptotic and anti-inflammatory effect of Hesperidin (HDN) against Isoniazid, Rifampicin and Pyrazinamide induced toxicity in rat liver.

2. Materials and Methods

2.1 Chemicals

Hesperidin, Isoniazid and Silymarin were purchased from Sigma- Aldrich, USA. Pyrazinamide (Sterling healthcare Ltd), Rifampicin from Lupin limited. Antibody for TNF- α , NF- κ B, IL-10 were purchased from Abcam Ltd (Cambridge, U.K) and for Bcl-2, Bax, caspase 3, and caspase 9 were purchased from cell signaling technology (Beverly, MA). All other chemicals and solvents used in the study were of analytical grade. Standard orogastric cannula was used for oral drug administration.

2.2 Experimental animals

Adult male wistar rats (150-200g) were obtained from the Central Animal House VMKVMC, Salem and were housed in standard polypropylene cages at normal atmospheric temperature and humidity with a 12 h light/dark cycle. The animals were acclimatized for 1 week before the study and had free access to standard laboratory feed and water *ad libitum*. Experimental design was approved by the Animal ethical committee VMKVMC (Approved No: IAEC/VMKVMC/04/2013 and CPCSEA guidelines were followed for animal handling and treatment.

2.2.1 Treatment regimens

The treatment regimen and selection of dose for Hesperidin [11], Silymarin [12] and anti-tubercular drugs [13] based on the previous literature. Fasted rats were randomly divided into 4 groups of 6 rats each such that the weight difference within and between group does not exceed $\pm 20\%$ of the average weight of the total rats.

Group I rats that served as control, were orally administered single daily dose of distilled water for 50 days.

Group II rats that served as the toxic control were orally administered single daily dose of H (27 mg/kg), R- (54 mg/kg), and Z (135mg/kg) for 50 days.

Groups III was co-treated daily with single oral dose of HDN at 200 mg/kg b.wt. 1 h before oral administration of HRZ for 50 days.

Groups IV served as a standard group co-treated daily with single oral dose of Silymarin 100mg/kg b.wt. 1 h before oral administration of HRZ (27, 54, 135 mg/kg b.wt.) for 50 days.

At the end of 50 days all the animals were fasted overnight, were sequentially anesthetized with inhaled diethyl ether for about 30–40 s. Then the blood was collected by retro orbital vane puncture and serum was separated by centrifugation at 3000 rpm for 15 min to determine the

biochemical parameters. After that the animals were sacrificed by cervical dislocation. For biochemical analysis, liver tissues were quickly removed and homogenized with appropriate buffer and centrifuged at 4°C and the supernatant was used in the assay of antioxidants and lipid peroxidation. For western blot studies and RT PCR studies liver was dissected, immediately frozen on dry ice or RNA later (sigma), and stored at -80°C . Sections of the liver lobe were collected in 10% formalin and stored for histopathology.

2.3 Biochemical estimations

2.3.1 Estimation of liver marker enzymes

The liver marker enzymes AST, ALT, ALP, and LDH were assayed by using commercial available Standard kit (Transasia Bio-Medicals, Bengaluru).

2.3.2 Estimation of oxidative stress markers

The level of tissue thiobarbituric acid reactive substances (TBARS/LPO) was measured using the method of Ohkawa et al. [14].

2.3.3 Estimation of Enzymatic and Non enzymatic antioxidants

Superoxide dismutase (SOD) was assayed by the method of Kakkar *et al.*, [15]. The activity of catalase (CAT) was determined by the method of Sinha [16]. Reduced glutathione (GSH) activity was assayed by the method of Rotruck et al. [17]. Ascorbic acid (Vitamin C) and -tocopherol (Vitamin E) content were estimated by the methods of Omaye et al [18] and Desai ID [19]. The total protein content was determined by the method of Lowry et al [20].

2.4 Histological examination

The liver was quickly removed after sacrificing the rats and fixed with 10% formalin solution. Histological sections were prepared, stained with hematoxylin and eosin and then examined under microscope.

2.5 Western blotting analysis of protein expression

Liver tissue sample was subjected to lysis in a sample buffer and the protein concentration of lysates was determined by Bradford protein estimation kit. Equal amounts (40 μg) of liver tissue proteins from each sample were subjected to 12% SDS-PAGE gels and transferred onto PVDF membranes. The membranes were then probed with the indicated primary antibodies (rabbit polyclonal antibody against Bax, Bcl₂, Caspase-3, Caspase 9, IL-10, TNF $-\alpha$ and NF- κ B) overnight at 4°C. Each membrane was washed and then incubated with horseradish peroxidase (HRP)-labeled anti-rabbit secondary antibodies for 1 h at room temperature. The protein bands were visualized by Enhanced chemiluminescent HRP substrate (ECL) kit (Amersham Bioscience) according to the manufacturer's instructions. The expression levels were quantified using the image J analysis. (Version 1.43, NIH, USA) for Windows. Blots were reported with β - actin antibody as a loading control.

2.6 Reverse Transcription PCR

Total RNA was isolated from liver tissue using Trizol reagent.(Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's recommendations. 0.1 µg of the total RNA was reverse transcribed using SuperScript First-Strand Synthesis System for RT-PCR. (Invitrogen), as per the manufacturer's recommendations. By use of first-strand cDNA as a template, PCR was carried out using the gene-specific upstream and downstream primers

(Table 1) for caspase-3, caspase-9, Bcl-2, Bax, TNF-α, NFκB, IL-10 and β-actin (as reference gene) were subjected to 35 cycles of PCR amplification with 30 s denaturation at 94 °C, 30 s annealing temperature at 55°C and 2 min extension at 72 °C. The PCR products were resolved by agarose gel electrophoresis (Biorad) on 1.5% agarose and visualized by ethidium bromide. β- Actin was used as an internal loading control. The intensity of individual bands was semi-quantitatively assessed using NIH Image.

Table: 1 Primers used in RT-PCR analysis

S. No	Genes	Forward primer	Reverse primer
1	Caspase-3	TCCTAGCGGATGGGTGCTAT	TCACGGCCTGGGATTTCAAG
2	Caspase-9	TGTTTCAGGCCCATATGATCG	GGACTCACGGCAGAAGTTCA
3	Bcl-2	GAAGTGGGGAGGATTGTGG	GGCAGGCATGTTGACTTCAC
4	Bax	CCCTTTTGCTTCAGGGTTTCAT	ACAGGGACATCAGTCGCTTC
5	NF-κB	ACTTCTCCTGAAAGCCGGTG	AGGAAGAGGTTTGGATGCCG
6	IL-10	TACGGCGCTGTCATCGATT	AAGGTTTCTCAAGGGGCTGG
7	TNF-α	CTGGGCAGGTCTACTTTGGG	CTGGAGGCCCCAGTTTGAAT
8	β- Actin	GAGCACAGAGCCTCGCCTTT	AGAGGCGTACAGGGATAGCA

2.7 Statistical analysis

All the data was expressed as mean±SD. Statistical significance between more than two groups was tested using one way ANOVA followed by Dunnet's multiple comparison test using SPSS.16. Differences were considered statistically significant at p<0.05.

3. Results

3.1 Effect of Hesperidin on serum biochemical parameters

Serum AST, ALT, ALP and LDH were assessed as a biochemical marker for hepatic damage Table 2. HRZ

administration results in the significant (p<0.001) elevation of serum AST, ALT, ALP and LDH level compared to control group.

In contrast, Hesperidin treatment significantly prevented the HRZ induced elevation of the AST (p<0.01), ALT (p<0.01), ALP (p<0.05) and LDH (p<0.05) levels when compared to HRZ treated group. Similar results were seen in Silymarin group.

Table 2: Effect of Hesperidin on serum biochemical parameters in control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
AST (IU/L)	7.55±0.38	11.62±0.18 ^{***}	9.39±0.13 ^{###}	8.51±0.81 ^{####}
ALT (IU/L)	38.36±1.63	46.64±1.15 ^{***}	41.86±0.78 ^{###}	40.57±0.84 ^{####}
ALP (IU/L)	202.97±12.64	296.82±10.15 ^{***}	260.02±4.30 [#]	211.84±11.87 ^{####}
LDH (IU/L)	1.64±0.13	4.55±0.48 ^{***}	3.25±0.27 [#]	2.31±0.37 ^{####}

Data are express as Mean±SD (n=6), one-way ANOVA followed by Dunnet's multiple comparison test. ^{***} p<0.001, ^{**} p<0.01, ^{*} p<0.05 significantly differ from control, ^{###} p<0.001, ^{##} p<0.01, [#] p<0.05 significantly differ from group II.

3.2 Effect of Hesperidin on hepatic oxidative stress biomarkers

Table 3 reveals that there was a strong enhancement in LPO in HRZ administration rats (group II) when compared to the control (p<0.001). In contrast cotreatment of

Hesperidin and HRZ significantly (p<0.001) restored the membrane integrity in the liver thereby suppress the LPO levels, compared to the HRZ group. Similar results were seen in Silymarin group. (p<0.001).

Table 3: Effect of Hesperidin on hepatic oxidative stress biomarkers and antioxidant enzyme activities

Tissue	Group I	Group II	Group III	Group IV
LPO (nmol/g Tissue)	71.60±8.83	182.35±9.39 ^{***}	131.70±9.61 ^{###}	91.37±1.38 ^{####}
SOD(units/mg protein/min)	12.38±0.83	3.28±0.27 ^{***}	7.28±0.92 ^{###}	11.41±0.79 ^{####}
CAT(Mm of H2O2 consumed/min/ mg protein)	66.44±3.27	38.82±1.93 ^{***}	54.23±3.62 ^{###}	62.90±1.75 ^{####}
GSH (µg /mg protein)	73.78±4.35	23.69±0.52 ^{***}	41.59±1.74 ^{###}	65.43±1.68 ^{####}
VIT C(mg/g of wet tissue)	2.08±0.05	0.67±0.06 ^{***}	1.38±0.08 ^{###}	1.93±0.06 ^{####}
VIT E(mg/g of wet tissue)	8.10±0.75	2.53±0.44 ^{***}	3.64±0.45 [#]	7.42±0.54 ^{####}

Data are express as Mean±SD(n=6), one-way ANOVA followed by Dunnet's multiple comparison test.

^{***} p<0.001, ^{**} p<0.01, ^{*} p<0.05 significantly differ from control, ^{###} p<0.001, ^{##} p<0.01, [#] p<0.05 significantly differ from group II.

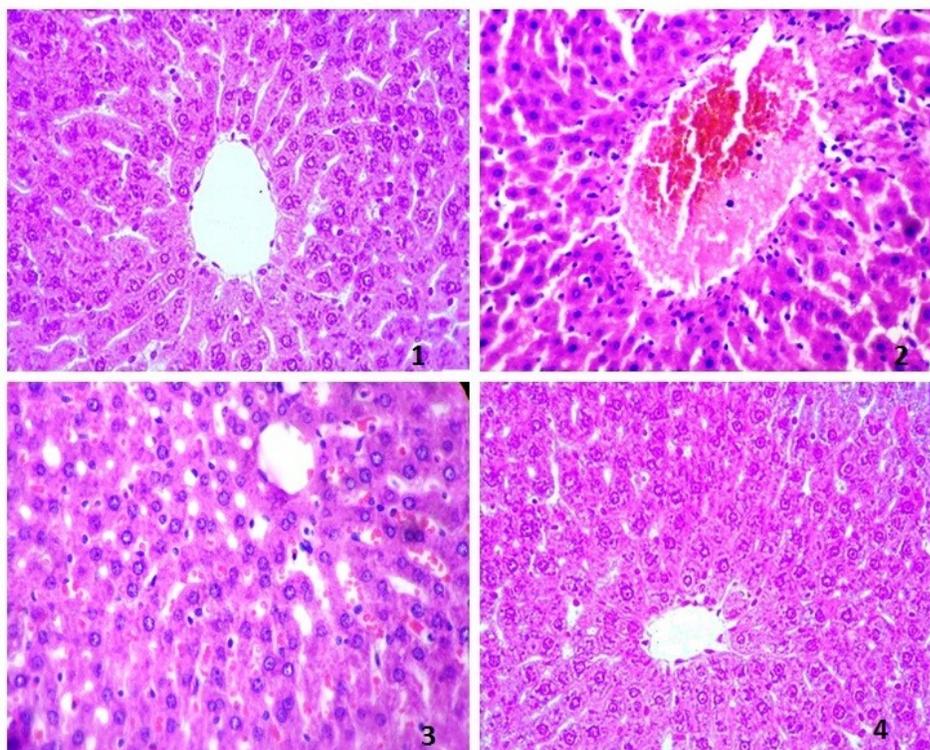
3.3 Effect of Hesperidin on hepatic antioxidant enzymes

Hepatic antioxidant enzyme SOD, CAT, GSH, VIT C and VIT E activities (**Table- 3**) were significantly decreased in HRZ treated rats ($p < 0.001$) when compared to the control rats. However, co-treatment of rats with Hesperidin substantially restored the hepatic antioxidant levels ($p < 0.001$) when compared to the HRZ treated rats. Silymarin similarly increase the antioxidant levels when compared to HRZ treated rats ($p < 0.001$).

3.4 Effect of Hesperidin on histopathological changes in liver

The histopathological examination of control liver sections showed normal architecture without any signs of hepatic damage (**Figure 1**). Antitubercular drugs exposure results in changes in liver architecture as indicated by congestion in the central vein, bridging necrosis, inflammation and sinusoidal widening (**Figure 2**). HRZ along with Hesperidin treatment showed normal hepatocyte with mild congestion (**Figure 3**). Standard drug Silymarin treated rats shows normal architecture (**Figure 4**).

Representative photomicrographs of liver histopathology (400 \times): Fig 1 shows normal histology. Fig 2 shows HRZ exposure causes congestion in the central vein, bridging necrosis, inflammation and sinusoidal widening. Fig 3 shows HRZ plus Hesperidin showed normal hepatocyte with mild congestion. Fig 4 silymarin treated rats shows normal architecture.

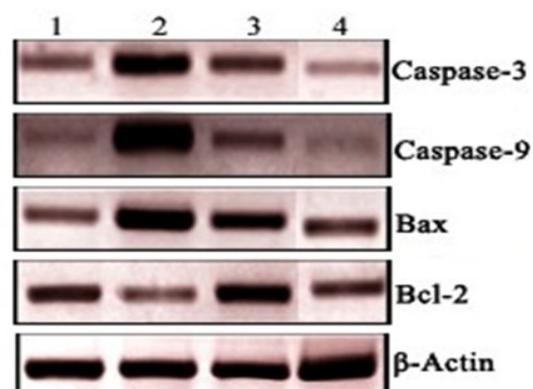


3.5 Effect of Hesperidin on Apoptotic Marker

3.5.1 Western blotting

The protein expression of Bax, Bcl₂, caspase 3 and caspase 9 were shown in **Figure 5**. Administration of HRZ decreased the protein expression of Bcl₂ and increased the expression of Bax. However treatment with Hesperidin attenuated the HRZ induced reduction in the Bcl₂ expression and increased expression of Bax. Administration of HRZ significantly increased the expression of caspase 3 and caspase 9. However co-administration of Hesperidin significantly ameliorated the HRZ induced expression of caspase 3 and caspase 9. Standard drug Silymarin also significantly reduces the HRZ induced caspase activation.

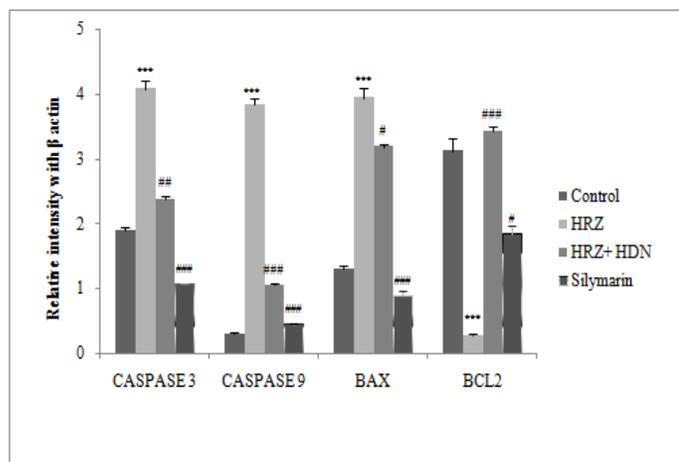
Figure 5: Protein expression of Caspase 3, Caspase 9, Bax, Bcl2.



3.5.2 RT-PCR Analysis

The mRNA expressions of Bcl-2, Bax, caspase 3 and caspase 9 are shown in **Figure 6**. The levels of Bax, caspase 3 and caspase 9 were markedly increased, and the level of Bcl-2 was markedly decreased in the HRZ administered group compared with the control group, which indicated that the liver was acutely injured. Their levels were significantly reversed in HDN treated group and Silymarin group.

Figure 6: mRNA expression of Caspase 3, Caspase 9, Bax, Bcl2

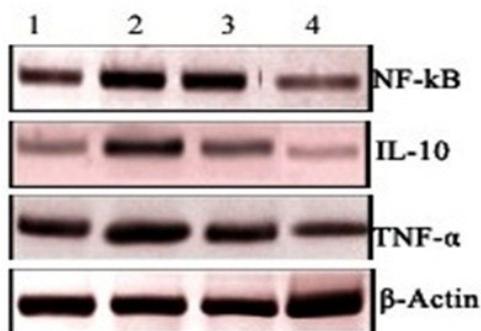


3.6 Effect of Hesperidin on inflammatory response in rat liver

3.6.1 Western blotting

Western blotting results indicated the protective effect of Hesperidin on the expression of inflammatory proteins like TNF- α , NF- κ B, IL-10 on liver tissues (**Figure 7**). These inflammatory proteins are significantly up regulated in HRZ administered group when compared to the control. However co administration of Hesperidin significantly down regulated these inflammatory proteins when compared to the HRZ administered rats. Standard drug Silymarin also significantly down regulated these proteins.

Figure 7: Protein expression of NF- κ B, IL-10, TNF- α .

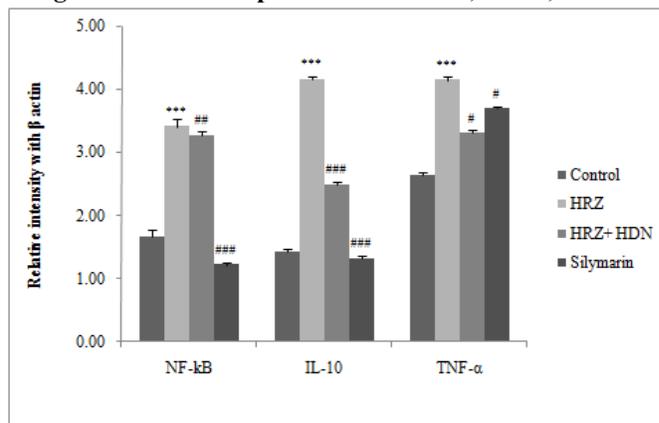


3.6.2 RT-PCR Analysis

Figure 8 shows the mRNA expression of the inflammatory proteins. The mRNA expressions of TNF- α , NF- κ B, IL-10 were significantly increased in HRZ administered group when compared to the control group.

Treatment with Hesperidin and Silymarin significantly attenuated the HRZ-induced expression of mRNA levels of inflammatory proteins.

Figure 8: mRNA expression of NF- κ B, IL-10, TNF- α .



4. Discussion

Liver is the central organ responsible for metabolism, detoxification and excretion of various endogenous and exogenously administered drugs/ toxic compounds; therefore it's more prone for damage. Drug-induced liver injury (DILI) is the most common ADRs and accounts for more than 50% of acute liver failure cases. As contrast to western countries danger of Hepatotoxicity is higher in India. Clinically useful drugs can cause hepatocellular damage through the activation of the parent compound to highly reactive/toxic substance and also by provoking the generation of oxygen derived free radicals [21].

Antitubercular drugs (HRZ) induced Hepatotoxicity is mediated through the production of toxic metabolite in its metabolic pathway which covalently binds to liver cell macromolecule and induces liver injury. During metabolism Isoniazid is converted into an active metabolites Acetyl hydrazine and hydrazine. These metabolites are oxidised by cytochrome P4502E1 (CYP2E1) in the liver, which results in the generation of reactive oxygen species (ROS) that will induce oxidative stress [22]. Rifampicin is a powerful inducer of drug metabolising enzyme; thereby enhance the production of hepatotoxic metabolite (hydrazine) of Isoniazid[23]. Pyrazinamide in combination with those drugs is also associated with an increased incidence of hepatotoxicity [24].

Research evidence suggested that the Antitubercular drugs causes hepatocellular damage through the induction of oxidative stress and consequence dysfunction of hepatic antioxidant defense system [25]. During oxidative insult free radicals (ROS) generated from the toxic metabolites initiate the peroxidative damage to hepatocellular membrane this might be the reason for increased formation of lipid peroxides (LPO) which was observed in our study. In our body there is always a dynamic balance between the free radical productions and antioxidant defence system, thereby protecting the body against oxidative damage [26]. In the

current study Antitubercular drugs decrease the level of enzymatic and non enzymatic antioxidant (SOD, CAT, GSH, VIT C & VIT E) this might be due to excessive production of oxidant species, so the antioxidant are not able to neutralize them, which result in the depletion or failure of intracellular antioxidant defense system.

Antitubercular drug induced hepatocellular damage is further confirmed by our histopathological examination which shows the presence of necrosis, inflammatory changes and apoptotic cells. These in turn lead to the elevation of liver marker enzymes in serum due to the leakage of enzymes from liver (ALT, AST, ALP, and LDH). Our results were in agreement with that of Gopi Sudheer Kumar *et al* and Mannan Hajimahmoodi *et al.*[27].

Treatment of antitubercular drugs disturbs the hepatocytes, by generating ROS and imparting oxidative stress in the tissue [28]. Furthermore, oxidative stress is closely associated with cell damage and apoptosis [29]. Increased in the level of ROS within mitochondria causes mitochondrial membrane depolarisation, which triggers caspase cascade and initiate apoptosis [30]. Studies show that Bax is the positive regulator which promote mitochondrial polarization and accelerate cell death, the Bcl₂ is the negative regulator of cell death by restores mitochondrial polarization [30,31]. In our study antitubercular drugs administration result in the up regulation of caspase 3 and caspase 9 which might be due to the migration of proapoptotic marker Bax to the mitochondria and inhibit the protective effect of Bcl₂. Therefore Bax is up regulated and Bcl₂ is down regulated by antitubercular drugs due to the activation of intrinsic apoptotic pathway by ROS.

On the other hand ROS triggers an inflammatory response through the activation of transcription factor NF- κ B[32]. The classical NF- κ B-activating pathway is induced by an immunity mediators, such as pro-inflammatory cytokines (TNF α) [33,34]. TNF- α which induces apoptosis via the TNF- α receptor 1 and an intracellular signaling cascade involving both activation of caspase-8 and caspase-3[35,36]. In our present study Antitubercular drugs increase the expression of NF- κ B and TNF- α , this might be due to the activation of extrinsic apoptotic pathway by the ROS. Furthermore, IL-10 with anti-inflammatory and immunomodulatory effects can attenuate the activation of TNF- α -induced NF- κ B pathway [37,38]. In our study IL-10 cytokine expression was increased by antitubercular drugs because of compensatory mechanism against the inflammation caused by TNF- α .

Oxidative stress is the main mechanism behind antitubercular drug induced Hepatotoxicity which leads to treatment interruption or change in treatment regimen during the course of TB therapy. Hence there is an urgent need to find out the hepatoprotective drug with an antioxidant property and having the capacity to neutralize the ROS

generated by reactive metabolite during metabolism of antitubercular drugs.

Hesperidin is a citrus flavanoid having antioxidant and free radical scavenging property, thereby activate the antioxidant defence system and suppress the oxidative stress. So the present study was aimed to evaluate the protective effect of Hesperidin against antitubercular drug induced oxidative stress, inflammation and apoptosis.

In our study, Supplement of Hesperidin along with antitubercular drug increased the enzymatic and non enzymatic antioxidants level, thereby it decreases the generation of lipid peroxidation by neutralizing the ROS. This antioxidant property of Hesperidin is well in accordance with the earlier report [10,39]. Hesperidin curtailing the generation of lipid peroxides and maintaining the membrane integrity of hepatocytes and protect the hepatocytes against toxic effects of antitubercular drugs. This might be the reason for decreased level of liver marker enzyme AST, ALT, ALP and LDH in the blood stream. Our results are further supported by our histopathological examination; Hesperidin supplement with antitubercular drugs shows the absence of histological lesions. This indicates the membrane stabilising property of Hesperidin. Few researchers also discovered the membrane stabilising property of Hesperidin against various drugs and toxin induced liver damage [10].

Hesperidin supplement with antitubercular drugs increased the expression of Bcl₂ and decreased expression of Bax, Caspase 3 and Caspase 9 which indicates that the Hesperidin stabilize the mitochondrial membrane from the oxidative damage thereby it prevent the activation of caspase cascade (intrinsic apoptotic pathway) and also restored the balance between Bax and Bcl₂. Reports shown that over expression of Bcl₂ inhibits the transcriptional activation of Bax thereby prevent the activation caspases and enhance the cell survival [40].

On other hand Hesperidin eliminated the oxidative stress by neutralising the ROS which result in the inactivation of NF- κ B pathway and subsequently suppress the release of proinflammatory cytokines TNF- α , this might be the reason for decreased expression of TNF- α and NF- κ B, which observed in our study. Hesperidin decreased the level of IL-10 due to attenuation the inflammatory stress and in turn secured the formation of anti-inflammatory cytokines (IL-10). Thus Hesperidin have protective effect against inflammation by suppressing the release of proinflammatory cytokines and oxidative stress responsive transcription factor there by inhibit the activation of caspase 3 and caspase 8 mediated apoptotic pathway (extrinsic pathway).

Finally, the present study confirmed that oxidative stress has been found to be an important mechanism in antitubercular drugs induced liver damage; treatment with hesperidin alleviates the changes induced by these drugs and protects the cell from oxidative damage, inflammation and apoptosis.

5. Conclusion

The results of our present study suggested that Hesperidin attenuates hepatic damage induced by Isoniazid, Rifampicin and Pyrazinamide by reducing oxidative stress, mitochondrial dysfunction and thereby ameliorating apoptosis. This finding may have important implications in the use of Hesperidin against antitubercular drug induced hepatotoxicity. However further research is need to validate Hesperidin as a new therapeutic agent supplemented with antitubercular drugs in the treatment of TB.

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Authors Contribution

S. Nathiya Msc, PhD (persuing), designed, researched and analysed the data and wrote the manuscript.

Dr. S Rajaram M.D, research guide contributed in designing the study and edited the manuscript.

Dr. Philips Abraham, M.sc PhD. contributed in designing, analysis and edited the manuscript. All authors read and approved the final manuscript.

Reference

- [1] World Health Organization (WHO), Global tuberculosis report WHO; 2015.
- [2] Tasduq SA, Peerzada K, Koul S, Bhat R, Johri RK. Biochemical manifestations of anti-tuberculosis drugs induced Hepatotoxicity and the effect of Silymarin. *Hepatol Res* 2005; 31:132-5.
- [3] British Medical Research Council (Hong Kong Chest Service). Controlled trial of 2, 4 and 6 months of pyrazinamide in 6 month, three times weekly regimens for smear positive pulmonary tuberculosis, including an assessment of a combined preparation of isoniazid, rifampin and pyrazinamide. *Ann Rev Respir Dis* 1991; 143: 700-6.
- [4] Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampicin. *Chest* 1991; 99:465-71.
- [5] Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: Chemistry, bioavailability and effects on health. *Nat Prod Rep* 2009; 26:1001-43.
- [6] Garg A, Garg S, Zaneveld LJ and Singla AK. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. *Phytother Res* 2001; 15(8): 655–69.
- [7] Loscalzo LM, Wasowski C, Paladini AC, Marder M. Opioid receptors are involved in the sedative and antinociceptive effects of hesperidin as well as in its potentiation with benzodiazepines. *Eur J Pharmacol* 2008, 580: 306-13.
- [8] Chanet A, Milenkovic D, Manach C, Mazur A, Morand C. Citrus fla-vanones: what is their role in cardiovascular protection. *J. Agric. Food Chem* 2012; 60: 8809–8822.
- [9] Kamaraj S, Anandakumar P, Jagan S, Ramakrishnan G, Devaki T. Modulatory effect of hesperidin on benzo (a) pyrene induced experimental lung carcinogenesis with reference to COX-2, MMP-2 and MMP-9. *European Journal of Pharmacology* 2010; 649: 320–327.
- [10] Cho J. Antioxidant and neuroprotective effects of hesperidin and its aglycone hesperetin. *Arch Pharm Res* 2006; 29:699-706.
- [11] Ahmad ST, Arjumand W, Nafees S, Seth A, Ali N, Rashid S, Sultana S. Hesperidin alleviates acetaminophen induced toxicity in Wistar rats by abrogation of oxidative stress, apoptosis and inflammation. *Toxicol. Lett* 2012; 208:149-161.
- [12] Basini Jyothi, S. Mohanalakshmi, Anitha k. Protective effect of *Mirabilis jalapa* leaves on Anti-tubercular drugs induced hepatotoxicity. *Asian J Pharm Clin Res* 2013; 6(suppl 3): 221-224.
- [13] Chandane RD, Jaju JB, Ghadlinge MS, Bhosale RR, Chandrakapure AR. Effect of honey on hepatotoxicity induced by antitubercular drugs in albino rats. *Int J Basic Clin Pharmacol* 2013; 2:177-81.
- [14] Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxide in animal tissue by TBA reaction. *Anal Biochem* 1979; 95:351-8.
- [15] Kakkar P, Das B and Viswanathan PN. A modified spectrophotometric assay of SOD. *Indian J Biochem Biophys* 1984; 21(2): 130-132.
- [16] Sinha AK. Colorimetric assay of catalase. *Anal. Biochem* 1972; 47: 389-394.
- [17] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 1973; 179: 588-590.
- [18] Omaye ST, Turnbull JD, Sauberlich, HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Methods Enzymol* 1979; 62: 1–11.
- [19] Desai ID. Vitamin E analysis methods for animal tissues. *Methods Enzymol* 1984; 105: 138-144.
- [20] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin's phenol reagent. *The Journal of Biological Chemistry* 1951; 193(1):265– 275.
- [21] Mary C, Kasturi S, Parames C S. Herbak (*Phyllanthus niruri*) protein isolate protects liver from nimesulide induced oxidative stress; *Pathophysiol* 2006; 13:95-102.
- [22] Tayal V, Kalra BS, Agarwal S, Khurana N, Gupta U. Hepatoprotective effect of tocopherol against isoniazid and rifampicin induced hepatotoxicity in albino rabbits. *Indian J Exp Biol* 2007; 45(12):1031-6.
- [23] Asduq SA, Kaiser P, Sharma SC, Johri RK. Potentiation of isoniazid-induced liver toxicity by rifampicin in a

- combinational therapy of antitubercular drugs (rifampicin, isoniazid and pyrazinamide) in Wistar rats: a toxicity profile study. *Hepatology* 2007; 37(10):845-53.
- [24] Talib Hussain T, Ramesh K Gupta, Sweety K, Mohd SK, Md Safaraj Khan, Mdarif, et al. Evaluation of antihepatotoxic potential of *Solanum xanthocarpum* fruit extract against antitubercular drugs induced hepatopathy in experimental rodents. *Asian Pac J Tropical Biomed* 2012; 2:454-460.
- [25] Attri S, Rana SV, Vaiphei K, Sodhi CP, Katyal R, Goel RC, Nain CK, Singh K. Isoniazid and rifampicin induced oxidative hepatic injury protection by N-acetylcysteine. *Hum. Exp. Toxicol* 2000; 19:517-522.
- [26] Young IS, Woodside JV. Antioxidants in health and disease. *J Clin Pathol* 2001; 54(3):176-86.
- [27] Mannan Hajimahmoodi, Ghazaleh Moghaddam, Seyed Mohsen Mousavi, Naficeh Sadeghi, Mohammad Reza Oveisi and Behrooz Jannat. Total Antioxidant Activity, and Hesperidin, Diosmin, Eriocitrin and Quercetin Contents of Various Lemon Juices. *Tropical Journal of Pharmaceutical Research* 2014; 13 (6): 951-956
- [28] Bhadauria S, Mishra R, Kanchan R, Tripathi C, Srivastava A, Tiwari A, et al. Isoniazid-induced apoptosis in HepG2 cells: generation of oxidative stress and Bcl-2 down-regulation. *Toxicol Mech Methods* 2010; 20(5):242-51.
- [29] Wu C-C, Bratton SB. Regulation of the intrinsic apoptosis pathway by reactive oxygen species. *Antioxidants & redox signalling* 2013; 19: 546-558.
- [30] Desagher S and Martinou JC. "Mitochondria as the central control point of apoptosis," *Trends in Cell Biology* 2000; 10(9): 369-377.
- [31] Budihardjo I, Oliver H, Lutter M, Luo X, and Wang X. "Biochemical pathways of caspase activation during apoptosis," *Annual Review of Cell and Developmental Biology* 1999; 15: 269-290.
- [32] Czaja MJ. Cell signalling in oxidative stress induced liver injury. *Semin. Liver Dis* 2007; 27: 378-389.
- [33] Martin MU and Wesche H, Summary and comparison of the signaling mechanisms of the Toll/interleukin-1 receptor family. *Biochim Biophys Acta* 2002; 1592(3): 265-80.
- [34] Devin A, Cook A, Lin Y, Rodriguez Y, Kelliher M and Liu Z. The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* 2000; 12(4): 419-29.
- [35] Tacke FT, Luedde and Trautwein C. Inflammatory pathways in liver homeostasis and liver injury. *Clin. Rev. Allergy. Immunol* 2009; 36:4-12.
- [36] Leifeld L, Nattermann J, Fielenbach M, Schmitz V, Sauerbruch T, Spengler U. Intrahepatic activation of caspases in human fulminant hepatic failure. *Liver Int* 2006; 26: 872-879.
- [37] Bourdi M, Masubuchi Y, Reilly TP, Amouzadeh HR, Martin JL, George JW, Shah AG, Pohl LR. Protection against acetaminophen-induced liver injury and lethality by interleukin 10: Role of inducible nitric oxide synthase. *Hepatology* 2002; 35: 289-298.
- [38] Dhingra S, Sharma A.K, Arora RC, Slezak J, Singal PK. IL-10 attenuates TNF-alpha-induced NF kappa B pathway activation and cardiomyocyte apoptosis. *Cardiovasc. Res* 2009; 82: 59-66.
- [39] Shiekh Tanveer Ahmada, Wani Arjumanda, Sana Nafeesa, Amlsh Sethb, Nemat Ali a, Summya Rashida, Sarwat Sultanaa. Hesperidin alleviates acetaminophen induced toxicity in wistar rats by abrogation of oxidative stress, apoptosis and inflammation. *Toxicology Letters* 2012; 208:149- 161.
- [40] Lucken-Ardjomande S and Martinou JC. Regulation of Bcl-2 proteins and of the permeability of the outer mitochondrial membrane. *CR Biol* 2005; 328: 616-631.