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Combined isoniazid and rifampicin induced hepatotoxicity and its amelioration in wistar rats

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Abstract
This study was conducted to know Combined Isoniazid (ISN) and Rifampicin (RIF) induced Hepatotoxicity and emeliorative effects of Withania somnifera and Vitamin E against the toxixcity. Healthy male Wistar rats were divided into four groups consisting of 6 in each.. The experimental study was designed as follows: Group 1-Control, Group II-ISN@50mg/kg body wt + RIF@100mg/kg body wt once daily orally for 21 days, Group IIIISN@50mg/kg body wt + RIF@100mg/kg body wt once daily orally for 21 days + Withania somnifera@1000mg/kg feed daily for 21 days, Group IV-ISN@50mg/kg body wt + RIF@100mg/kg body wt once daily orally for 21 days + vitamin E@300mg/kg feed daily for 21 days. The blood was collected for estimation of serological parameters and tissues were collected for histopathological studies. The serum AST, ALT and GGT activities were increased significantly in group II. Enhanced lipid peroxidation (TBARS and GSH) in tissues was observed indicating oxidative damage. Histopathological changes were noticed significantly in liver of group II rats. The groups III and IV showed significant improvement in all parameters and tissue changes in comparison to group II by the ameliorative effects of Withania somnifera and vitamin E.

Keywords: Isoniazid, rifampicin, hepatotoxicity, withania somnifera and vitamin e.

Introduction
Isoniazid (ISN) and Rifampicin (RIF) are the major drugs used for treatment of tuberculosis (TB). Isoniazid kills actively growing tubercle bacilli [1] and Rifampicin has bactericidal activity against *M. tuberculosis* [2]. Drug-induced reactions are a major problem, because of involvement of reactive metabolites [3]. Rats have been successfully used as laboratory models to investigate isoniazid and rifampicin induced hepatotoxicity models [4-6]. Protective attempts to overcome the liver damage resulted by ISN and RIF is essential to continue the use of above drugs indiscriminately to combat tuberculosis. Of the ameliorating agents, *Withania somnifera* (Indian ginseng, or winter cherry) is used as an aphrodisiac, liver tonic, anti-inflammatory agent and antioxidant [7]. Vitamin E complex is the most powerful antioxidant in the lipid (fat) phase [8]. Hence the present study under taken to know the combination effect of two anti-oxidants agents against the hepatotoxicity induced by ISN and RIF.

Materials and Methods
Male Wistar rats, weighing 175-260mg were procured from National Center for Laboratory Animal Sciences, National Institute of Nutrition (NIN), Hyderabad for the present experimental study and the experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (Approval No. 6/2008). Animals were divided into four groups consisting of 6 in each and the experimental design as follows.

Group I: Control

Group II: Isoniazid @ 50mg/kg body wt + Rifampicin @100mg/kg body wt once daily orally for 21days.

Group III: Isoniazid @ 50mg/kg body wt + Rifampicin @100mg/kg body wt once daily orally for 21 days + *Withania somnifera* @1000mg/kg feed daily for 21 days.

Group IV: Isoniazid @ 50mg/kg body wt + Rifampicin @ 100mg/kg body wt once daily orally for 21 days + vitamin E @ 300mg/kg feed daily for 21 days.

Whole blood was collected from retro-orbital venous plexus in sterile serum vacutainers after fasting the animals for 12hrs for the estimation of ALT, AST, GGT. All the animals were observed for exhibition of clinical symptoms if any and at the end of the experiment all were euthanized by CO_2 and a systematic and detailed necropsy was conducted on all the animals. The gross pathological changes were noted and 0.5 grams liver issue was collected for estimation of anti-oxidant profile like TBARS [9] and GSH [10]. Liver was collected and preserved for histopathological examination. The preserved tissues in 10% neutral buffered formalin were trimmed for uniform size and shape and kept overnight for washing. The tissues were dehydrated in ascending grades of alcohol and cleared in xylol and further transferred to paraffin for embedding. Paraffin blocks were prepared and 5 µ size sections were cut by Rotary microtome (Lieca, Germany). The sections were lifted on precoated clean, greese free slides and kept overnight for drying in the incubator at 37^0C. Sections were stained by routine Haematoxylin and Eosin staining method described by [11, 12]. The stained sections were examined under light microscope for histological changes. The data of various parameters were subjected to statistical analysis by one way ANOVA described by [13].

Result and Discussion

In the present study, group II (combination of ISN+RIF) showed a significant (P<0.05) increase in the activity of AST, ALT, GGT and TABRS and significant (P<0.05) decrease in the GSH concentration when comparison to other groups at different time intervals. The *Withania somnifera* (G III) and vitamin E (G IV) fed groups showed significant improvement in all the parameters when compare to other groups (TABLE No.1) these results are well supported by marked alteration in the histopathology of liver sample. The higher concentration of TBARS in the toxic group (II) indicated the increased lipid peroxidation. Lipid peroxidation is a complex and natural deleterious process, might be associated with cellular damage. The TBARS values in groups III and IV were lower than toxic group and higher than the control group. The concentration of GSH in toxic group (II) showed a significant (P<0.05) lower in comparison to other groups, while treatment groups (III and IV) showed significant higher than toxic group but lower than control group. Increased level of TBARS (a marker for oxidative stress), reduction in the GSH concentration is indication for increased oxidative stress in ISN+RIF treatment group [14]. These results suggest that oxidative stress and lipid peroxidation are involved in isoniazid plus rifampicin-induced hepatoxicity. These results are further supported by elevated levels of liver enzymes like AST, ALT and GGT at different time intervals in the group (II). The concentrations of AST, ALT, GGT, TBARS and GSH in the ameliorative groups differed significantly from the control group but did not differ from each other which might be due to their antioxidant effects. The present findings are in agreement with earlier report [15, 16].

Histological sections of liver in toxic group revealed enlarged portal tracts and infiltrated with mononuclear cells predominately lymphocytes (Fig.1). Lymphocytes were scattered in few areas within the liver lobule. Severe sinusoidal congestion (Fig.2) and mild congestion of central vein was noted. Severe fatty changes (Fig.3) were recorded in the periportal region. Moderate swelling and ballooning of hepatocytes tending to hydropic degeneration and individual hepatocytes revealed moderate to severe necrosis (Fig.4). The treatment group III and IV animals revealed mild infiltration of mononuclear cells in and around portal triads with mild sinusoidal congestion. Structural details were intact (Fig.5, 6) and mild fatty changes were evident. The histological changes resulted might be attributed to the toxic metabolite of isoniazid which is released after acetylation. Acetylhydrazine is a reactive metabolite, which binds to and damages cellular macromolecules in the liver. Rifampicin is an inducer of microsomal metabolizing enzymes which enhance the metabolism of isoniazid in turn causing release of toxic metabolite. INH metabolite hydrazine is implicated in inducing fatty change/steatosis by altering the hepatic gene expression profile favouring production and intracellular transport of hepatic lipid over the removal of fatty acid metabolites. The present findings are in accordance with the earlier report [17- 20].

Table 1: Mean±S.E values of AST, ALT, GGT, TBARS and GSH in different groups.

Groups	AST(IU/L)	ALT(IU/L)	GGT(IU/L)	TBARS (n moles/gm of protein)	GSH (mg/gm of protein)
I	41.26±0.17 ^{a}	14.09±0.31 ^{a}	1.39±0.11 ^{a}	75.90±1.20 ^{a}	27.85±0.43 ^{c}
II	89.70±16.60^d	40.60±10.65^c	3.68±0.82 ^{c}	126.18±7.16 ^{c}	11.69±0.23 ^{a}
III	65.03±8.44 ^{b}	20.24±2.75 ^{b}	2.76±0.25^b	96.09±1.18 ^{b}	19.95±0.13 ^{b}
IV	74.68±11.97^c	24.55±4.93 ^{b}	2.63±1.76 ^{b}	98.06±1.75± ^{b}	19.87±.0.20 ^{b}

Means bearing common superscripts did not differ significantly (P<0.05)

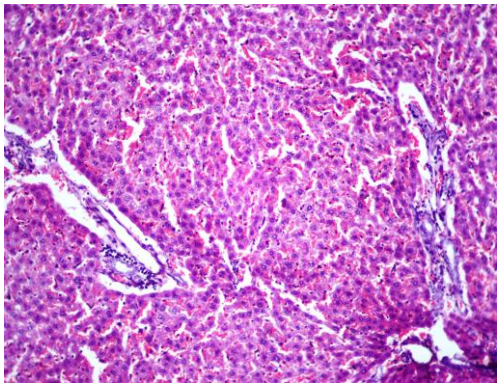

Fig 1: Liver showing sinusoidal congestion & enlarged portal tracts with infiltration of mononuclear cells predominately lymphocytes in group II. HE × 200

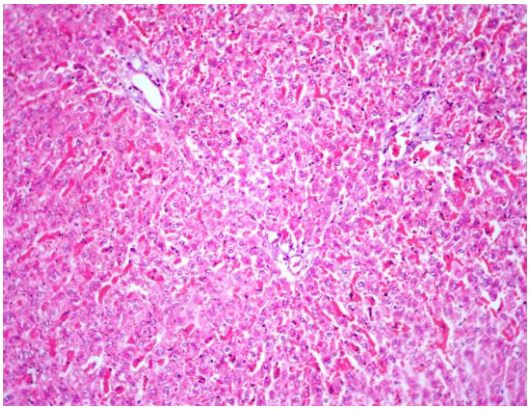

Fig 2: Liver showing severe sinusoidal congestion and mild congestion of central vein in group II. HE × 200

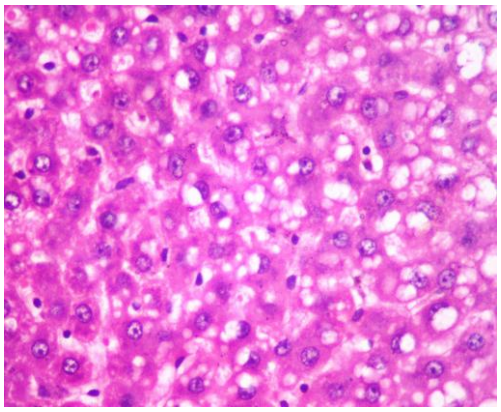

Fig 3: Liver showing severe fatty change in periportal areas in group II. HE x 400.

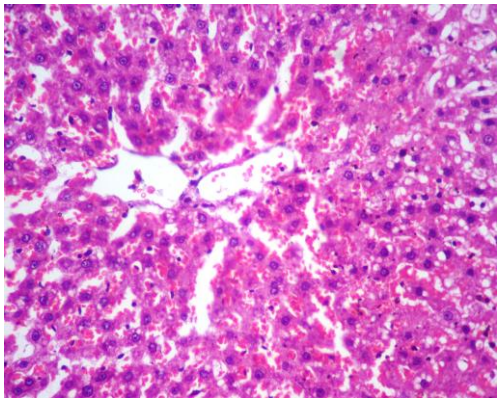

Fig 4: Liver showing sinusoidal congestion, fatty changes and necrosis of Hepatocytes in group II. HE × 200

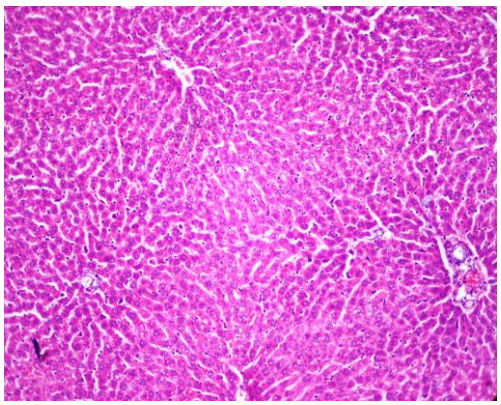

Fig 5: Liver showing mild sinusoidal congestion, mild infiltration in portal tract and mild fatty changes in group III. HE × 200

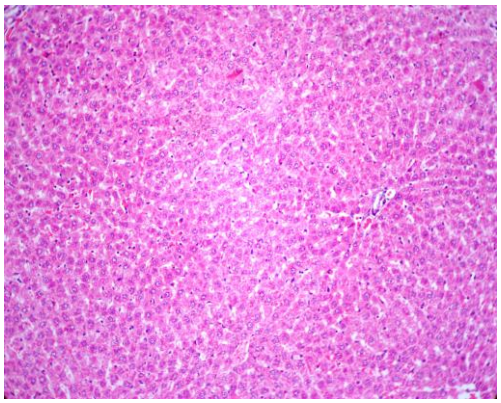

Fig 6: Liver showing mild sinusoidal congestion, mild infiltration of mononuclear cells in portal tract with intact structural details in group IV. HE × 200

Conclusion

The oxidative stress might be attributed to the toxic metabolites of the isoniazid and rifampicin. The gross and the histological changes in the study might be due to the toxic reactive metabolites of the drugs, which bind to cellular macromolecules and release or form toxic free radicals that inturn caused the tissue damage. Supplementation of *Withania somnifera* (1%) resulted in significant improvement in the parameters might be due to the antioxidant, antistress, liver tonic and anti-inflammatory properties. Supplementation of vitamin E (0.3%) resulted in significant improvement in the parameters which can be attributed to the antioxidant and anti-dyslipidaemic effects. Elevation of tissue TBARS and reduction of GSH in the study might be due to increased lipid peroxidation due to toxic reactive metabolites of the drugs. Therefore, the present study indicated that *Withania somnifera* @ 1000mg/kg feed and vitamin E @300mg/kg feed were effective in counteracting the toxic effects of the antitubercular drugs but not up to the required levels. Keeping this in view, further studies can be advocated using different dose rates and different routes of administration to overcome the affects of antitubercular drugs.

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References

1. Santos LC. Review: The Molecular Basis of Resistance in Mycobaterium tuberculosis Open Journal of Medical Microbiology. 2012; 2:24-36
2. Ruben C, Hartkoorn Claudia Sala, Sophie J, Magnet Jeffrey M, Chen Florence Pojer, Stewart T. Cole Sigma Factor F Does Not Prevent Rifampin Inhibition of RNA Polymerase or Cause Rifampin Tolerance in *Mycobacterium tuberculosis* J Bacteriol. 2010; 192(20):5472-5479.
3. Hartman T, Weinrick B, Jacobs WR Jr. Nat Commun. Mycobacterium tuberculosis is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. Vilcheze C. 2013; 4:1881
4. Aashish Pandit, Tarun Sachdeva, Pallavi Bafna. Drug-Induced Hepatotoxicity: A Review Journal of Applied Pharmaceutical Science. 2012; 02(05):233-243
5. Tostmann A, Boeree MJ, Aarnoutse RE, Lange WCM, Vander Ven AJ, Dekhuijzen R. Anti-tuberculosis drug-induced hepatotoxicity: Concise up-to-date review. J Gastroenterol Hepatol. 2008; 23:192-202.
6. Rana SV, Attri S, Vaiphei K, Pal R, Attri A. Role of N-acetylcysteine in rifampicin-induced hepatic injury of young rats. World J Gastroenterol. 2006; 12(2):287-291.
7. Mohammad Shahriar Md, Ismail Hossain, Farzana Anwar Sharmin, Sadika Akhter Md, Aminul Haque and Mohiuddin Ahmed Bhuiyan. *In Vitro* Antioxidant and Free Radical Scavenging activity of *Withania somnifera* Root. Iosr Journal of Pharmacy. 2013; 3(2):38-47.
8. Ali Riza Soylu, Nurettin Aydogdu, Umit Nusret Basaran, Semsi Altaner, Orhan Tarcin, Nursal Gedik *et al*. Antioxidants vitamin E and C attenuate hepatic fibrosis in biliary-obstructed rats. World J Gastroenterol. 2006; 12(42):6835-6841.
9. Balasubramanian KA, Manohar M, Mathan VI. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. Biochimica et Biophysica Acta. 1988; 962:51-58.

10. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S transferase in rat lung and liver. Biochem. Biophys. Acta, 1979; 582:67.
11. Culling CFA. Handbook of Histopathological technique. London Butterworth and Co. (Publishers) Ltd, 1957.
12. Clayden EC. Practical section cutting and staining. J and Churchill Limited, 4th Edition, 1962.
13. Snedecor GW, Cochran WG. Statistical methods, 8th edition, IOWA State University Press, Amer, IOWA, USA, 1994.
14. Naheed S, Hassan, Sahar M, Awad. Reverse Effect of Vitamin E on Oxidative Stress, Derivatives and Conductivity Changes of Hemoglobin Induced by Exposure to Cadmium. J Appl Sci Res 2007; 3(6):437-443.
15. Tariq MA, Ayesha Z, Naheed B. Remedial antioxidants action of withania somnifera on restraint stress induced oxidative damage. The FASEB Journal. 2008; 22:611-612.
16. Rana SV, Attri S, Vaiphei K, Pal R, Attri A. Role of N-acetylcysteine in rifampicin-induced hepatic injury of young rats. World J Gastroenterol. 2006; 12(2):287-291.
17. Tasduq SA, Peerzada K, Koul S, Bhat R, Johri RK. Biochemical manifestation of anti-tuberculosis drugs induced hepatotoxicity and the effect of Silymarin. Hepatol Res. 2005; 31:132-135.
18. WU Run-hua, ZENG Yi-ming, CHEN Xiao-yang. Intermittent hypoxia and isoniazid plus rifampicin affect hepatic ultrastructure in mice. Chin Med J. 2011; 124(23):4034-4038
19. Jeyakumar R, Rajesh R, Meena B, Rajaprabhu D, Ganesan B, Buddhan S *et al*. Antihepatotoxic effect of *Picrorhiza kurroa* on mitochondrial defense system in antitubercular drugs (isoniazid and rifampicin)-induced hepatitis in rats. Journal of Medicinal Plants Research 2008; 2(1):017-019.
20. Tasduq 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. Hepatol Res. 2007; 37(10):845-853.