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HAEMATOLOGICAL AND HISTOPATHOLOGICAL ALTERATIONS DUE TO COMBINED TOXICITY OF ISONIAZID AND RIFAMPICIN; AMELIORATION WITH *WITHANIA SOMNIFERA* AND VITAMIN-E IN WISTAR RATS

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ABSTRACT

The present study was designed to investigate the combined toxicity Isoniazid (ISN) and Rifampicin (RIF), which are widely used in treating tuberculosis; and protective effects of *Withania somnifera* and Vitamin E against the toxixcity induced ced by the drugs on haematological and histopathological alterations. The experimental study was conducted with healthy male Wistar rats (age 60-100 days, weighing 175-260mg) procured from National Institute of Nutrition, Hyderabad and the experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee. Animals were divided into four groups consisting of 6 in each group. 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 III-ISN@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 experiment was carried out for 21days and blood was collected 4 times at weekly intervals (0,7,14 and 21 days) for estimation of various haematological parameters and tissues were collected for histopathological studies. The hematological assays showed a significant reduction in packed cell volume, haemoglobin, total erythrocytes count and significant increase in total leucocytes count in group II in comparison to other groups. Histopathological changes were noticed significantly. In group II, liver showed severe sinusoidal congestion and mild congestion of central vein, portal tracks were enlarged and infiltrated with mononuclear cells, disorganization of hepatic cords and moderate to severe necrosis. Cardiac muscle fiber showed varied degree of injury, disruption and seperation of muscle bundles. In kidney, glomeruli revealed degeneration of tubules and haemorrhages in interstitial tubular spaces, disrupted tubules. Brain showed congestion and mononuclear cell infiltration surrounded by areas of fatty changes. The groups III and IV animals 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, Vitamin-E, Withania Somnifera, Histopathology, Haematology**

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INTRODUCTION

Isoniazid (ISN) and Rifampicin (RIF), Ethambutol and Pyrazinamide are the first line drugs used for treatment of tuberculosis and among them ISN and RIF are commonly used, as they are economic. Isoniazid is a prodrug activated by bacterial catalase-peroxidase (KatG) and kills actively growing tubercle bacilli[1] and Rifampicin has bactericidal activity against *M.tuberculosis* by inhibiting bacterial DNA-dependent RNA polymerase[2]. TB drug-induced reactions are a major problem, because of involvement of reactive metabolites[3and 4]. Rats have been successfully used as laboratory models to investigate isoniazid and rifampicin induced hepatotoxicity models[5,6 and 7]. Protective attempts to overcome the liver damage resulted by ISN and RIF is essential to continue the use of the above drugs indiscriminately to combat tuberculosis. Of the ameliorating agents, *Withania somnifera* (Indian ginseng, or winter cherry) has been an important herb in the Ayurvedic and used as an aphrodisiac, liver tonic, anti-inflammatory agent and antioxidant[8]. The most active component of vitamin E complex is *Alpha*-tocopherol and this organic substance are the most powerful antioxidant in the lipid (fat) phase [9and10].

MATERIALS AND METHODS

Male Wistar rats (age 60-100 days, 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. Animals were divided into four groups consisting of 6 in each group. The animals were provided *ad lib* water and mash feed (procured from NIN) Hyderabad throughout the period of the experiment. Isoniazid tablets (Macleods Pharmaceuticals, Andheri, Mumbai) and Rifampicin capsules (Lupin Ltd, Kartholi, Jammu & Kashmir) were dissolved in sterile distilled water and given orally. As per the body weight, *Withania somnifera* (Dabur Ayurvet Limited) at the rate of 1 percent and Vitamin E (Hi Media Laboratories, Mumbai) at the rate of 0.3 percent were incorporated in the feed for groups III and IV, respectively.

Group I : Control
Group II**:** Isoniazid @ 50mg/kg body wt + Rifampicin @100mg/kg body wt once daily orally for 21 days.
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 at weekly intervals (0, 7, 14 and 21 days) for 4 times in sterile vacutainers containing EDTA and in serum vacutainers after fasting the animals for 12hrs. The haematological results were also analyzed in automatic whole blood analyser (Humacount, Medsource Ozone Biomedicals Pvt. Ltd) and compared with manual estimations. 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 pieces of liver, heart, kidney and brain were collected and preserved in 10% neutral buffered formalin 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 and 12]. The stained sections were examined under light microscope for histological changes. The data of various haematological parameters were subjected to statistical analysis by one way ANOVA [13].

RESULTS

The calculated mean values of PCV (%), Hb (g %), TEC (10^6 /Cmm) and TLC (10^3 /Cmm) from 0 to 21 days in different groups varied from 38.03±1.75 to 42.75±0.18, 12.39±1.21 to 16.27±0.11, 5.72±0.91 to 8.40±0.12 and 14.45±0.13 to 20.53±2.49 respectively. The PCV (%), Hb (g %) and TEC (10^6 /Cmm) values in group II were lower in comparison to other groups at 0, 7, 14 and 21 days (Table I).The mean PCV (%), Hb (g %) and TEC (10^6 /Cmm values of group II showed a significant ($P<0.05$) reduction in comparison to other groups. The PCV (%), Hb (g %) and TEC (10^6 /Cmm) values of groups III and IV were higher than group II but lower than control group I. The mean PCV (%), Hb (g %) and TEC (10^6 /Cmm) values of groups III and IV differ significantly ($P<0.05$) from the control group I but they did not differ significantly ($P<0.05$) from each other. The calculated mean values of TLC (10^3 /Cmm) from 0 to 21 days in different groups varied from 14.45±0.13 to 20.53±2.49. The TLC values in group II were higher in comparison to other groups at 0, 7, 14 and 21 days (Table I).The TLC values of group II showed a significant ($P<0.05$) increase in comparison to other groups. The TLC values of groups III and IV were lower than a toxic group but higher than control group. The mean TLC values of groups III and IV differ significantly ($P<0.05$) from the control group, but they did not differ significantly ($P<0.05$) from each other. In the group II animals liver revealed mild hepatomegaly, rounded borders, congestion and petechiae in comparison to control groups and in heart pin point ecchymotic haemorrhages were observed. Petechiae were also noted in hearts of group III and IV animals. Kidneys of group II animals showed focal haemorrhages and group III and IV animals did not reveal any changes. In the group II animals, liver showed severe sinusoidal congestion and mild congestion of central vein, portal tracks were enlarged and infiltrated with mononuclear cells, disorganization of hepatic cords and moderate to severe necrosis. Group III and IV animals revealed mild infiltration of mononuclear cells in and around portal triads with mild sinusoidal congestion (Fig.1). Cardiac muscle fiber showed varied degree of injury, ranging from loss of striation to complete necrosis and fragmentation, disruption and separation of muscle bundles with infiltration of mononuclear cells (Fig.2). In group III and IV mild to moderate range of degeneration and disruption of muscle bundles was observed. In kidney, glomeruli revealed reduction in glomerular space, degeneration of tubules infiltration of mononuclear cells and haemorrhages in interstitial tubular spaces, disrupted tubules and focal areas of tubular atrophy were observed (Fig.3). Group III and IV animals revealed mild to moderate haemorrhages in the interstitial spaces and normal glomerular tufts. Brain showed congestion and mononuclear cell infiltration surrounded by areas of fatty changes (Fig.4). Group III and IV animals revealed decreased cellularity and mild congestion in and around the meninges.

TABLE I
Haematological findings in different Groups

PARAMETERS	DATE OF BLOOD COLLECTION	GROUPS			
		I	II	III	IV
Packed cell volume (%)	0 Day	42.9	43.61	43.22	42.73
	7th Day	42.41	38.84	37.27	39.28
	14th Day	43.21	36.08	39.16	38.73
	21st Day	42.51	33.61	41.51	40.27
	Mean±S.E	42.75±0.18^{c}	38.03±1.75^{a}	40.79±1.04^{b}	40.25±0.88^{b}
Haemoglobin (Hb g%)	0 Day	16.17	16.4	16.55	13.81
	7th Day	16.12	12.38	13.53	13.05
	14th Day	16.19	12.88	14.5	14.01
	21st Day	16.61	10.5	14.95	14.68
	Mean±S.E	16.27±0.11^{c}	12.39±1.21^{a}	14.88±0.62^{b}	14.63±0.79^{b}
Total erythrocytes counts (10^6 /Cmm)	0 Day	8.41	8.2	8.49	8.58
	7th Day	8.74	5.94	5.34	5.75
	14th Day	8.33	4.67	6.74	6.49
	21st Day	8.14	4.08	7.29	7.34
	Mean±S.E	8.40±0.12^{c}	5.72±0.91^{a}	6.96±0.65^{b}	6.99±0.58^{b}
Total leucocytes counts (10^3/Cmm)	0 Day	14.05	14.86	14.66	14.95
	7th Day	14.67	18.57	20.12	20.51
	14th Day	14.57	22.17	18.6	19.88
	21st Day	14.51	26.54	16.53	17.6
	Mean±S.E	14.45±0.13^{a}	20.53±2.49^{c}	17.47±1.19^{b}	18.23±1.26^{b}

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

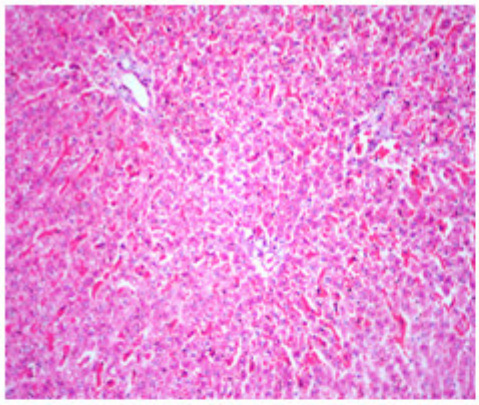

Figure 1
Photomicrograph of liver showing severe sinusoidal congestion and congestion of central vein with infiltration of mononuclear cells. H & E X 200 (Group 2)

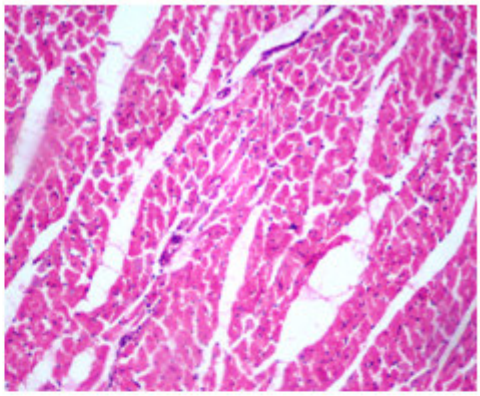

Figure 2
Photomicrograph of heart showing severe separation and disruption of cardiac muscle with infiltration of mononuclear cells. H & E X 200 (Group 2)

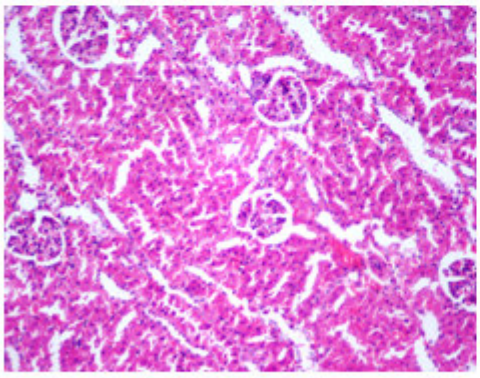

Figure 3
Photomicrograph of kidney showing reduction in glomerular spaces, haemorrhages in interstitial spaces with infiltration of mononuclear cells. H & E X 200 (Group 2)

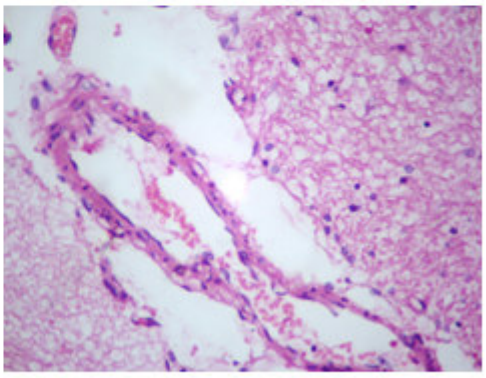

Figure 4
Photomicrograph of brain showing congestion and increased cellularity. H & E X 200 (Group 2)

DISCUSSIONS

The mean PCV, Hb and TEC values of group II showed a significant reduction in comparison to other groups, while the TLC value of group II showed significant increase in comparison to other groups. The reduction in haematocrit, haemoglobin and erythrocytes count may be due to the effect of antituberculosis drugs resulting in hepatic damage[14and 15], where as the elevation in the leukocytes count may be due to improved phagocytic activity of the blood cells leading to improved immune status[16].The mean PCV, Hb, TEC and TLC values of groups III and IV differed significantly from the control group but they did not differ significantly from each other [17,18 and 19].

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 inturn causing release of toxic metabolite.

INH metabolite hydrazine is implicated in inducing fatty change/steatosis by altering the hepatic gene expression profile favoring production and intracellular transport of hepatic lipid over the removal of fatty acid metabolites[20,21 and 22]. *Withania* fed group revealed moderate portal triaditis and mild fatty change while vitamin E fed group

showed mild fatty change and mild portal triaditis due to it's anti-inflammatory action[23] and [24] . In toxic group, heart muscle fibers showed varied degrees of damage and the lesions extended to myocardium, which might be due to toxic metabolite of the isoniazid[25]. Mild to moderate range of degeneration and disruption of muscle fibers was observed in ameliorative groups due to their anti-inflammatory and antioxidant properties[26]. In the toxic group, glomeruli revealed reduction in glomerular space vacuolation in few glomeruli, disruption and degeneration. Mild infiltration of mononuclear cells and severe haemorrhages in interstitial spaces were observed. Tubular disruption and focal areas of tubular atrophy were observed which might be due to isoniazid resulted renal tubular necrosis[27]. In toxic group, histological changes included congestion, haemorrhages, mononuclear cells infiltration in vessels[28] and [29], fatty change and increased cellularity in granular cell layer of cerebellum occurred due to toxic metabolites of the drugs used [30] and [31]. Ameliorative groups revealed mild to moderate changes were observed due to the antistress and antioxidant effects of *Withania somnifera* and vitamin E[32] and [33].

CONCLUSION

The present changes resulted from the antitubercular drugs in the biochemical changes might be attributed to the toxic metabolites of the isoniazid and rifampicin. The toxic, reactive metabolites of the drugs bind to cellular macromolecules and release or form toxic free radicals intern caused the tissue damage. The present study was concluded that Supplementation of *Withania somnifera* (1%) resulted in significant improvement in the biochemical 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 biochemical parameters which can be attributed to the antioxidant and anticholesterimic effects. 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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