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Oroxylum indicum (Linn.) whole stem extract regulates expression of TNFα, IL6, NFkB, P38 MAPK and oxidative status in antitubercular therapy induced hepatotoxicity in Wistar rats

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Abstract

The objective of the present study is to assess the hepatoprotective activity of Oroxylum indicum Linn. (OI) whole stem extract on antitubercular drug induced liver toxicity in rats. Hepatotoxicity was induced by oral dosing of the combination of antitubercular drugs viz. AKT-4 for 90 days to Wistar rats. After assessing the hepatotoxicity in the animals, treatment with both aqueous and ethanolic whole stem extracts of Oroxylum indicum Linn. (OI) as well as standard marketed drug was done for 30 days. At the end of 30 days the hepatoprotective activity of OI was assessed using serum markers for liver dysfunction and expression of antioxidant enzymes in the liver tissue. Histopathological assessment and mRNA expression profiles for TNFα, IL6, NFkB, P38 MAPK was carried out to evaluate the protection of liver against antitubercular drugs by the extracts. Both the aqueous and ethanolic extracts of OI significantly (p<0.05) restored the serum enzyme levels. Also the various tissue antioxidant enzymes were also restored compared to normal group. Histopathological evaluations showed absence of any remarkable pathological and metabolic changes in the liver sections of treated groups. mRNA expression was significantly increased in disease induced group as compared to normal control. Treatment with extract significantly reduced cytokines and P38 MAPK mRNA expressions.

Introduction

Drug induced liver injury is responsible for 5% of all hospital admission and 50% of acute liver failure [1]. About 2.3 million new tuberculosis patients were identified in India in the year 2012 which was one 4^{th} of the worldwide tuberculosis population [2]. Introduction of Isoniazid (INH) was considered safe in 1963, The American Thoracic Society recommended INH for a year to tuberculin-positive persons as a chemoprophylaxis treatment regardless of age or duration [3]. A study conducted by United States Public Health Service revealed 1% incidence of hepatitis and 0.06% deaths from hepatitis are due to INH [4]. After introduction of Rifampicin (RMP), several studies inferred that hepatitis was more frequent and severe in patients receiving combination of INH and RMP than in those receiving INH alone [5]. The current treatment of tuberculosis is to start with a combination chemotherapy containing INH, RMP, and Pyrazinamide (PZA) with or without ethambutol for the initial 2 months followed by a continuation phase of 4–6 months of combination of INH and RMP [6]. It is known that anti tuberculosis treatment with INH, RMP and PZA induces hepatotoxicity and has intense adverse drug reactions [7]. Preventive therapy of latent tuberculosis with 2 months course of RMP and PZA has shown fatal and severe hepatotoxicity, than 6 month of INH therapy [8]. To reduce the incidence of hepatotoxicity due to exposure of these offending drugs in latent TB patients, recommendations for patient selection criteria, line of treatment and duration have been revised several times, but to the best of our knowledge no drug has been developed for prevention of hepatotoxicity [9]. Oroxylum indicum Linn. (OI) belongs to the family Bignoniaceae is widely used in Indian Traditional System of medicine for various purposes. The plant is distributed in Indian subcontinents. Different parts of the plant are used for asthma, cough, viral hepatitis, antianorexic, antirheumatic, antibronchitic, anthelmintic and anti-inflammatory [10]. Protection offered by Oroxylum indicum was reported in acetaminophen induced hepatic injury and modulation of liver function in rats at 500 mg/kg [11]. Hence 500 mg/kg dose was selected for the present

study. The present study was designed to evaluate the hepatoprotective effect of Oroxylumindicum whole stem, aqueous and ethanolic extracts against combination of INH, RMP, PZA and Ethambutol induced hepatotoxicity in experimental rat model.

Objective

The objective of the present study is to assess the hepatoprotective activity of Oroxylum indicum Linn. (OI) whole stem extract on antitubercular drug induced liver toxicity in rats.

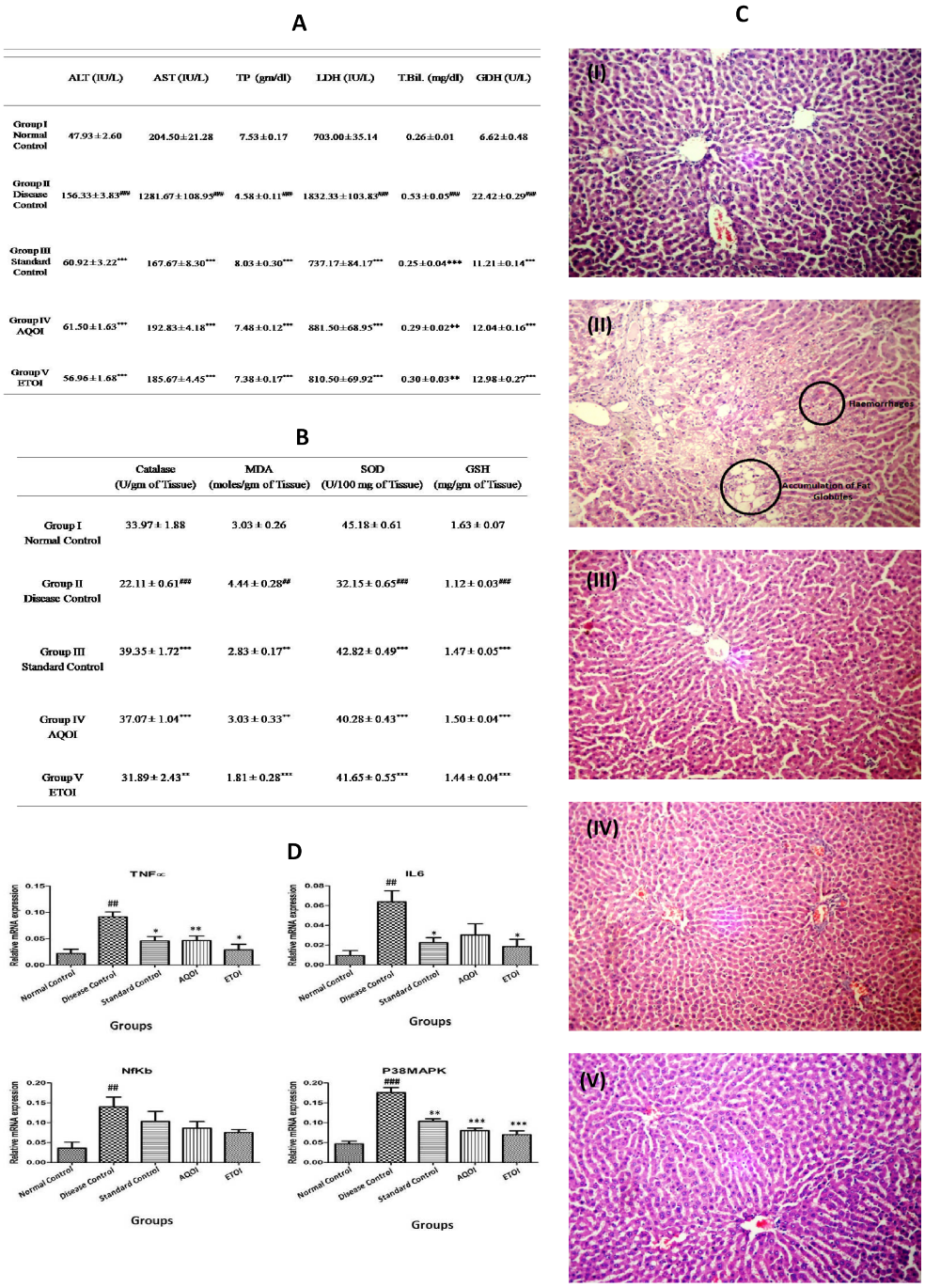

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Figure Legend

Figure 1.

(A) Effect of extracts of OI on serum biochemical parameters.

(B) Effect of Extracts of OI on Tissue Antioxidant Enzymes.

(C) Histopathological Observations I) Normal Control group shows normal histo-architecture, II) Disease Control group showed moderate to severe pathological

changes, III) Standard drug treated group showed absence of any remarkable pathological and metabolic changes, IV) & V) There was absence of any remarkable pathological and metabolic changes.

(D) mRNA expression levels. Values are expressed as mean ± SEM (n=6), Data was analysed using one way ANOVA followed by Tukey's multiple comparison post hoc test. Normal Control vs Disease Control: (#p<0.05, ##p<0.01, ###p<0.001). Disease Control vs Treated Groups: (*p<0.05, **p<0.01, ***p<0.001).

Results & Discussion

Effect of OI on biochemical parameters

As shown in figure 1A, After treatment for 30 days, disease control group showed significant elevated (p<0.001) levels of the biochemical parameters viz. ALT, AST, LDH and T Bil. when compared to normal control group. On the other hand, the animals treated with aqueous and ethanolic extracts of OI (500 mg/kg) as well as standard drug Silymarin (100 mg/kg) showed significant decrease (p<0.01, p<0.001) in the levels of the serum enzyme activity when compared with the disease control group. The TP level was significantly decreased (p<0.001) in the disease control group which is increased significantly (p<0.001) in the groups treated with extracts of OI and standard. Disease control group showed significant increased (p<0.001) value of GDH when compared to the normal control group. Treatment with standard drug and both the extracts of OI showed significant decrease (p<0.001) in the value of GDH.

Effect of OI on Tissue antioxidant enzymes

Effect on Catalase and SOD levels

As shown in figure 1B, there was significant decrease (p<0.001) in Catalase and SOD values in disease control group as compared to normal control group, The Group III treated with standard drug and Group IV and Group V treated with aqueous and ethanolic extracts of OI showed significant (p<0.01, p<0.001) increase in the Catalase and SOD values when compared with the disease control.

Effect on MDA level

The group III treated with standard drug and Group IV and Group V treated with extracts of OI showed significantly decreased (p<0.01, p<0.001) levels of MDA in liver tissue when compared to disease control.

Effect on Reduced Glutathione level

Disease control group showed significant decrease (p<0.001) in GSH level as compare to normal control group. Standard and test drugs treated groups showed significantly increased (p<0.001) values of GSH when compared with disease control after treatment.

Histopathology Evaluations

As shown in figure 1C, The microscopic examination of liver section from Normal Control group showed normal histo-architecture of hepatic parenchyma with normal cellular features (I). Whereas, Disease Control group showed moderate to severe pathological changes of hepatocytes with distorted hepatic cords along with the marked degenerative and necrobiotic changes with accumulation of fat globules inside the cytoplasm of hepatocytes along with haemorrhages and cellular swelling of hepatocytes (II). Standard drug treated group showed an absence of any remarkable pathological and metabolic change in all the sections of liver. Only minimal changes with focal cellular swelling of hepatocytes and focal congestion were evident in this group (III). Aqueous extract as well as ethanolic extract treated group showed focal cellular swelling of hepatocytes along with the slight congestion of hepatic artery and central vein. There was absence of any remarkable pathological and metabolic changes in all the sections of livers from this group when compared with the disease control group (IV, V).

Effect of OI on mRNA profiles

TNFα mRNA expression

As shown in figure 1D, as compared to normal control, TNFα mRNA level was significantly upregulated (p<0.01) in disease control group, indicating secretion of cytokines due to prolonged intervention of combinational anti-tubercular treatment. In treatment groups, ethanol extract showed significant down-regulation in TNFα mRNA expression (p<0.01), followed by standard and aqueous extract treatment group (p<0.05).

IL6 mRNA expression

As compared to normal control, in disease control group IL6 mRNA expression levels was significantly upregulated (p<0.01), indicating secretion of cytokines. In treatment groups, standard and ethanol extract showed significant reduction in TNFα mRNA expression (p<0.05). Aqueous extract suppressed IL6 mRNA expression levels but was not statistically significant.

NFkB mRNA expression

As compared to normal control, in disease control group NFkB gene was significantly upregulated (p<0.05), indicating appearance of inflammation. There was no significant reduction in NFkB mRNA expression in all the three treatment groups as compared to disease control. Ethanolic and aqueous extract showed relatively suppressed levels of NFkB mRNA expression as compared to standard treatment.

P38 MAPK mRNA expression

As compared to normal control, in disease control group P38 MAPK mRNA was highly upregulated (p<0.001), indicating appearance of cellular stress due to intervention of combinational anti-tubercular treatment. Both ethanolic and aqueous extract suppressed the P38 MAPK mRNA expression significantly (p<0.01) followed by standard treatment (p<0.05) when compared with disease control group.

World Health Organisation has revised its TB control programme and recommended and adapted Directly Observed Treatment Short Course (DOTS) strategy, as the most systematic and cost effective approach in developing and under developed countries (WHO). In the present study, hepatotoxicity induced in animals by combinational anti-tubercular drugs produced liver toxicity of different degrees of degenerative and necrobiotic changes along with alteration in the serum enzyme levels and antioxidant enzymes [12]. Hepatotoxicity increases when taken in the combination [13]. Increased lipid peroxidation and reduced SOD, Catalase and GSH suggested oxidative damage in hepatocytes. This caused damaged to the structural and functional integrity of the liver cells, significantly increased hepatocellular enzymes, as previously described [14]. In the present investigation the total recovery of liver injury was observed after treatment with aqueous and ethanolic extract of OI whole stem. Marked reduction in elevated circulating levels of hepatocellular enzymes, restored total protein levels may be due to the membrane stabilizing effect of the OI extracts. The results obtained for the serum biochemical parameters and tissue antioxidant enzymes are comparable with the standard marketed drug silymarin [15]. Glutamate dehydrogenase (GDH) is also another specific enzyme indicative of liver necrosis. Due to delayed release of GDH in liver toxicity, makes it more specific marker to study the liver necrosis [16]. After administration of both the extracts of OI, circulating levels of GDH reduced significantly in extract intervened groups as compared to disease control group. Depletion of the catalase, SOD and GSH suggests the damage to hepatic parenchymal cells due to depletion in the free radical scavenging capacity of the liver cells caused by oxidative stress [17]. We have observed the significant reduction in the catalase, SOD and GSH levels and significant increase in the LPO level in antitubercular drug treated group. Standard marketed drug silymarin significantly increased the levels of the antioxidant enzymes and results are in line with earlier findings [18]. The animals treated with standard drug and with aqueous and ethanolic extracts of the OI showed minimal pathological changes as compared to disease control and the results are in line with previous findings [15], these results clearly shows that OI extracts have protective effects towards toxic action of anitubercular drugs. P38 MAPK appears to play a major role in apoptosis, cytokine production and cytoskeletal reorganization. Previous studies suggested the role of P38 MAPK and NFkB transition factor as a key regulators of genes that involved in inflammation, immunity, wound healing, acute phase response and apoptosis [19] [20]. In the present study, role of P38 MAPK and NFkB in liver tissues from normal, disease control and OI extract treated animals were assessed. P38 MAPK signalling plays important role in regulating TNF-α in diseased liver tissue [21], and was significantly down regulated in OI extract treated animals. NFkB promotes liver regeneration by up-regulating IL-6 and other molecules like hepatocyte growth factors [22]. Though NFkB and IL-6 expressions were upregulated in liver tissue of disease control they were significantly down-regulated in OI extract treated liver tissue. It has been reported that inhibition of P38 MAPK and NFkB may be a useful target to treat pathophysiologic inflammation in liver injury [23]

[24]. Hepatocyte death is the major feature of liver injury. In response to hepatic injury certain intracellular processes are initiated to conserve the liver integrity. Inflammatory cytokines including TNFα and IL-6 are key mediators of these processes as they are involved in different cellular response such as activation of proliferation, survival and death. TNFα induces specific signalling pathways in hepatocytes leading to the activation of either pro-survival mediators or effectors of cell death [25]. Our study first time demonstrated that administration of OI stem extracts supressed P38 MAPK activity, NF-KB transcription factor and cytokine mRNA levels in tubercular drug induced hepatotoxicity and preventing further liver damage.

Conclusions

Given these promising findings, we suggest that OI, which is potentially safe and inexpensive for clinical use and may be considered as an effective supplement for the patients taking antitubercular medicaments.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201704000014.

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Ethics Statement

The experiment was conducted as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, with prior permission from the Institutional Animal Ethics Committee (IAEC).

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