Effects of four Indian medicinal herbs on Isoniazid-, Rifampicin- and Pyrazinamide-induced hepatic injury and immunosuppression in guinea pigs

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Abstract

AIM: To evaluate and compare the hepatoprotective and immunomodulatory effects of *Curcuma longa* (CL), *Ocimum sanctum* (OS), *Tinospora cordifolia* (TC) and *Zizyphus mauritiana* (ZM) on liver injury and immunosuppression induced by Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PZA).

METHODS: Duncan Hartley guinea pigs, weighing 700-1050 g, were treated orally with 50 mg/kg of INH, 100 mg/kg of RIF and 300 mg/kg of PZA for 21-d. 200 mg/kg (bw) of each herb crude extract was administered to the herb control group and 2-h previous to INH + RIF + PZA (AKT) doses to the Herb + AKT groups. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin and Alkaline Phosphatase (ALP) were assessed on d 0 and 21 in all the groups. Phagocytic % (P%), Phagocytic Index (PI) and Chemotactic Index (CI) were also measured as immunologic parameters. Histological analysis was carried out to assess injury to the liver.

RESULTS: The AKT treated control group showed hepatotoxicity as judged by elevated serum AST 5-fold, ALT 4-fold, bilirubin 2-fold and ALP to normal levels in all four groups. All four herb + AKT groups showed normal to enhanced neutrophil function.

CONCLUSION: All four herbs showed hepatoprotective potential and prevented immunosuppression. CL and TC showed the highest hepatoprotective activity, while TC and ZM showed strong immunostimulatory activity.

INTRODUCTION

About one third of the world’s population has latent tuberculosis and roughly 9 million cases of active tuberculosis emerge annually, resulting in 2-3 million deaths. Most new cases occur in the most populated nations, such as India and China[1,2]. Combination chemotherapy containing INH, RIF, PZA, with or without ethambutol, for an initial 2 mo followed by a continuation phase of 4-6 mo of INH + RIF is the preferred regimen for successful treatment, which prevents acquired resistance and enhances efficacy[3]. Drug induced hepatotoxicity is a potentially serious adverse effect of antituberculosis or anti-Koch’s treatment (AKT) regimens containing INH, Rifampicin and Pyrazinamide[4]. A higher risk of hepatotoxicity has been reported in Indian patients (up to 11.5%) than in their Western counterpart (up to 4.3%)[5]. The only measure available for managing hepatotoxicity is stopping the offending agents, once there is evidence...
of liver damage, and reintroducing the agents after normalization of liver enzymes\(^6\)^7.

Preventive therapy for latent tuberculosis with a 2 mo course of RIF and PZA causes severe hepatotoxicity more often than does 6-mo of INH therapy or curative treatment for clinical tuberculosis\(^8\)^9. Similar high risk was observed with PZA and ethambutol or a fluoroquinolone when given to contacts of multi-drug resistant tuberculosis patients\(^10\).

_Tinospora cordifolia, Terminalia chebula, Emblica officinalis_ and garlic have been shown to offer hepatoprotection against hepatotoxic compounds, such as CCl\(_4\), and INH and RIF induced liver damage in rats. The antioxidative, membrane stabilizing and CYP2E1 and lipid peroxidase (LPO) inhibitory effects of the herbs might be important regarding hepatoprotective potential\(^11\)-\(^13\).

In view of the lack of definitive recommendation or alternative agents for treating latent or active tuberculosis, we thought it imperative to evaluate the ability of well known Indian herbs to offer hepatoprotection in a guinea pig model of AKT-induced hepatotoxicity. Liver histology, enzyme levels and the immune system of guinea pigs are nearer to humans than any other rodent species, hence we preferred guinea pig\(^14\)-\(^16\). Four Indian herbs that are inexpensive, easily available and have a well established place in traditional Indian medicine were selected. They have similar antioxidant, immunomodulatory, stress relieving and cellular protective potential.

_Curcuma longa L_

_C. longa_ is a plant that belongs to the family Zingiberaceae and is known as Haldi in Hindi and Turmeric in English. Its use has demonstrated a wide spectrum of therapeutic effects, such as anti-inflammatory, antioxidant, antimitagenic, antitumor, antifungal, antiviral, antibacterial, antispasmodic and hepatoprotective activities. Recently its potential utility in acquired immune deficiency syndrome (AIDS) was demonstrated\(^17\)^9.

_Ocimum sanctum L_

_O. sanctum_ is a plant that belongs to the family Labiatae and is known as Tulsi in Hindi and Holy Basil in English. It is known to have adaptogenic activity\(^18\) and has numerous pharmacological activities, such as hypoglycemic, antistress, immunomodulatory, analgesic, antipyretic, anti inflammatory, antileucocogenic, antihypertensive, CNS depressant, hepatoprotective, chemopreventive, radioprotective, antitumor and antibacterial activities\(^17\)-\(^20\).

_Tinospora cordifolia (wild) Miers ex Hook F and Thomas_

_T. cordifolia_ is a shrub that belongs to the family Menispermaceae. It is a large climbing shrub that grows throughout tropical India and is popularly known as Giloya in Hindi and _Tinospora_ in English. It is used in general debility, digestive disturbances, loss of appetite and fever in children. It is also an effective immunomostimulant\(^21\).

_Zizyphus mauritiana Lam_

_Z. mauritiana_ is a plant that belongs to the family Rhamnaceae. In India, it is commonly known as Ber and in English it is known as Indian berry. This plant is rich in biological active compounds, such as triterpenes, cyclopeptide alkaloids and flavonoids, which have been shown to have inhibitory effects on histamine release, cyclopeptide formation, and cyclooxygenase-1 and 2\(^22\)-\(^25\). It also has cytotoxic, immunological adjuvant and hepatoprotective activities\(^26\).

**MATERIALS AND METHODS**

**Animals**

Duncan Hartley guinea pigs were provided by SPAN Diagnostic Ltd. and were maintained in the animal house of the SPAN Research Centre under specific pathogen free laboratory conditions with free access to food and water. All the animals used were approved by the CPCSEA committee and its ethical rules were followed throughout the experimental procedures.

As hepatotoxicity is more common in old age\(^26\), we took adult (> 1 year old) guinea pigs weighing between 700-1050 gms (average wt. 925 gms).

**Preparation of the Guinea pig model for AKT induced hepatotoxicity**

For this study, a Macox-ZH Kid dispersible tablet containing RIF 100 mg, INH 50 mg and PZA 300 mg, manufactured by Macleods Pharmaceuticals Ltd., was used to induce hepatotoxicity in guinea pigs at a dose of 1-tablet/kg daily for 21 d. The tablets were dissolved in distilled water and given orally by force feeding.

**Herbs**

Turmeric powder was purchased from the local market and standardized by HPTLC, with curcumin (Bayir Chemical Bangalore) as the standard. A fine powder of shade dried leaves of Tulsi was purchased from a local pharmacy and standardized by HPTLC using ursolic acid content as a marker. Giloya powder of aerial roots was purchased from Atmamand Saraswati Pharmacy and standardized against tinosordin (Wockhardt Pharmacy) by HPTLC. Ber was purchased from a local market and seeds were ground to make a fine powder; standardization was done by HPTLC fingerprinting with saponins as the standard.

**Study groups**

Animals were divided into ten groups with four animals in each group. A force feeding method was adopted for feeding the animals. (1) **The Normal control group** was fed with Bengali gram powder for 21 d (CT). (2) **The AKT control group** was given 50 mg INH, 100 mg RIF and 300 mg PZA/kg bw for 21 d (AKT). (3) **C. longa, O sanctum, T. cordifolia and Z mauritiana** treated groups were fed with 200 mg/kg bw daily for 21 d with herbs (Cl, Os, Tc and Zm groups, respectively). (4) **Four herb + AKT treated groups** were fed with 200 mg/kg bw of the respective herb followed by Macox-ZH Kid 1-tab/kg bw at an interval of 2 h for 21 d (Cl + AKT, Os + AKT, Tc + AKT and Zm + AKT, respectively).

**Reagents**

The reagents for total bilirubin and alkaline phosphatase (ALP) [pNPP-AMP (IFCC), Kinetic assay] kit was
Table 1: Effects of AKT, Herb and Herb + AKT on Liver enzymes (mean ± SE, n = 4)

| Treatment  | AST  | ALT  | AST/ALT | ALP  |
|------------|------|------|---------|------|
| CT         | 47 ± 6.15 | 55 ± 7.86 | 0.92 ± 0.03 | 68.75 ± 2.69 |
| AKT        | 239.5 ± 7.75 | 61.75 ± 1.97 | 3.82 ± 0.23 | 125 ± 5.23 |
| CL         | 56.75 ± 4.77 | 51.25 ± 3.57 | 1.11 ± 0.09 | 54 ± 1.47 |
| CL + AKT   | 95.75 ± 7.69 | 48 ± 8.26 | 2.13 ± 0.28 | 62.5 ± 6.25 |
| OS         | 47 ± 2.16 | 57.4 ± 5.5 | 0.83 ± 0.03 | 61.5 ± 2.63 |
| OS + AKT   | 128.75 ± 10.87 | 64.5 ± 10.48 | 2.12 ± 0.31 | 54.25 ± 2.17 |
| TC         | 44.5 ± 4.29 | 49.5 ± 5.20 | 0.9 ± 0.03 | 66.25 ± 2.95 |
| TC + AKT   | 104 ± 6.79 | 62.75 ± 9.24 | 1.77 ± 0.31 | 60.75 ± 3.9 |
| ZM         | 43.75 ± 0.75 | 51.25 ± 2.02 | 0.85 ± 0.02 | 59.25 ± 1.55 |
| ZM + AKT   | 132 ± 25.88 | 62.75 ± 8.98 | 2.1 ± 0.35 | 61.25 ± 1.77 |

1P < 0.01, CT vs CL; 2P < 0.01, CT vs AKT; 3P < 0.01, AKT vs CL + AKT, OS + AKT, TC + AKT and ZM + AKT. No significant effect of any herb on liver enzymes.

Table 2: Effects of AKT, Herb and Herb + AKT on PMN function (mean ± SE, n = 4)

| Treatment  | P%  | P. Index | C. Index |
|------------|-----|----------|----------|
| CT         | 51.67 ± 1.68 | 2.0725 ± 0.05 | 1.8525 ± 0.04 |
| AKT        | 40.61 ± 1.28 | 0.61 ± 0.03 | 0.6925 ± 0.07 |
| CL         | 73.3475 ± 0.75 | 2.47 ± 0.14 | 1.83 ± 0.05 |
| CL + AKT   | 72.7525 ± 1.14 | 2.3425 ± 0.17 | 1.22 ± 0.03 |
| OS         | 58.3875 ± 1.42 | 2.46 ± 0.15 | 1.7975 ± 0.04 |
| OS + AKT   | 55.1325 ± 0.89 | 1.1875 ± 0.05 | 1.5425 ± 0.04 |
| TC         | 70.9925 ± 0.99 | 4.4 ± 0.09 | 1.8775 ± 0.02 |
| TC + AKT   | 70.9875 ± 0.97 | 4.4625 ± 0.16 | 1.8675 ± 0.04 |
| ZM         | 76.7675 ± 0.48 | 2.3925 ± 0.07 | 2.0425 ± 0.03 |
| ZM + AKT   | 75.1 ± 0.7 | 2.34 ± 0.10 | 2.0575 ± 0.05 |

1P < 0.01; 2P < 0.01, CT vs AKT, CL, OS, TC and ZM; 3P < 0.05, 4P < 0.001, AKT vs CL + AKT, OS + AKT, TC + AKT and ZM + AKT.

Biochemical assays
10 mL of blood was drawn by cardiac puncture from each animal in the above mentioned groups, before sacrificing the animals for liver biopsy by injecting pentobarbitone sodium 90 mg/kg (bw) ip[25], to study the biochemical and immune parameters.

Biochemical parameters
AST and ALT were measured by MDH-NADH Kinetic UV and LDH-NADH Kinetic UV, respectively. Total and direct bilirubin was measured by the Jendrassik and Grof[26] method. ALP was measured by pNPP-AMP (IFCC) Kinetic Assay[27].

Immune parameters (PMN functions)
Polymorphonuclear (PMN) cells were harvested by Boyum’s method[28] for carrying out Candida phagocytic assay by the Lehrer and Cline method[29] and the chemotactic assay was studied by the Chenoweth agarose gel method[30].

The phagocytic% and phagocytic index were determined as follows:

PHAGOCYTIC% = \(\frac{\text{No. of neutrophils demonstrating phagocytosis}}{\text{Total no. of neutrophils studied}}\) × 100

PHAGOCYTIC INDEX = \(\frac{\text{No. of Candida engulfed in 100 PMN cells}}{\text{Total cells involved in phagocytosis}}\)

Chemotaxis was calculated as follows:

Chemotactic index = \(\frac{\text{Distance traveled by neutrophil from central well to chemotactic well}}{\text{Distance traveled by neutrophil from central well to MEM well}}\)

Histology
After CO₂ euthanasia, representative blocks of liver were dissected out and fixed in 40 mg/L formaldehyde. Slides containing full cross sections of the major hepatic lobules were prepared. Paraffin wax blocks were preserved for future reference. Light microscopic examination of liver was done on sections stained with hematoxylin and eosin.

Statistical analysis
Data were analyzed by analysis of variance (ANOVA) followed by Tukey’s HSD procedure. The statistical program used was KY Plot for windows version 2.0 beta 15. The significance level was P ≤ 0.05.

RESULTS
In the present study, the hepatotoxicity guinea pig model was successfully produced by administration of INH 50 mg/kg (bw), RIF 100 mg/kg (bw) and PZA 300 mg/kg (bw) orally with force feeding for 21 d. A 5-fold rise in AST, 4-fold rise in AST/ALT ratio and 2-fold rise in ALP were taken as evidence of liver injury (Table 1). Histologically, focal areas of hepatocytic necrosis, triaditis and macro-microvesicular steatosis were observed. Along with hepatotoxicity, P%, PI and CI were lowered significantly suggesting concomitant immunosuppression (Table 2).

Liver enzymes
In 4 herb treated groups, after 21 d of herb treatment, there were no significant changes in levels of liver enzymes, except a statistically significant reduction (within range of normality) in ALP level seen in the CL treated group. Table 1 shows the effects of AKT and herb + AKT on liver enzymes, and the AKT group showed a more than 5-fold rise in AST (P ≤ 0.001), while in the CL+AKT and TC + AKT groups, the rise halted at 2-fold (P ≤ 0.001) and in the OS + AKT and ZM + AKT groups, the rise halted at 3-fold (P ≤ 0.001) when compared to the CT group. ALT group did not show any significant or consistent rise with AKT or herb + AKT.

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The AST/ALT ratio (normal range 0.7-1.4) significantly increased in all the 4 herb treated groups, after 21 d of herb treatment, while a statistically significant decline was observed in the ZM+AKT group. The ALT group did not show any significant or consistent rise with AKT or herb + AKT. An aspartate transaminase (AST; 2-fold rise in AST/ALT ratio and 2-fold rise in ALP) was measured by IFCC systems and an alanine aminotransaminase (ALT) kit was purchased from SPAN Diagnostic Ltd. An aspartate transaminase (AST) kit was purchased from BioSystems and an alanine transaminase (ALT) kit was purchased from Chemex, S.A. For liver-biopsy, haematoxylin and eosin were purchased from SPAN Diagnostic Ltd.

The phagocytic% and phagocytic index were determined as follows:

PHAGOCYTIC% = \(\frac{\text{No. of neutrophils demonstrating phagocytosis}}{\text{Total no. of neutrophils studied}}\) × 100

PHAGOCYTIC INDEX = \(\frac{\text{No. of Candida engulfed in 100 PMN cells}}{\text{Total cells involved in phagocytosis}}\)

Chemotaxis was calculated as follows:

Chemotactic index = \(\frac{\text{Distance traveled by neutrophil from central well to chemotactic well}}{\text{Distance traveled by neutrophil from central well to MEM well}}\)
limits with non significant changes (data not shown).

Liver histology

The liver histology of control and herb treated groups was found to be normal (Figure 1), that is, with normal architecture and without steatosis, inflammation, triaditis or necrosis. AKT group showed disrupted architecture, macro-microvesicular steatosis, mild inflammation, triaditis and scattered focal necrosis (Figure 2A). The CL + AKT group had normal liver architecture and occasional inflammatory cells with no triaditis or necrosis (Figure 2B). An even better result was seen in TC + AKT group where very mild microvesicular steatosis was the only abnormality (Figure 2C). The ZM + AKT group showed more microvesicular steatosis than the TC+AKT group liver histology (Figure 2D).

The OS + AKT treated group showed considerably less steatosis and inflammation than the AKT group. But, it was not as spectacular as the other herb+ AKT liver sections, suggesting partial protection at the cellular level (Figure 2E).

PMN functions

After 21 d of herb treatment, P% was enhanced with each of the 4 herbs (Table 2). CL, TC and ZM were equipotent (P ≤ 0.001). But, OS showed slightly weaker action (P ≤ 0.01). PI was raised significantly by TC only (P ≤ 0.001) while the increment observed with other herbs did not reach statistical significance. These observations suggest that although all 4 herbs recruit a greater number of neutrophils into phagocytic activity, the phagocytic capacity of individual neutrophils was enhanced by TC only. CL, OS, and TC showed no significant effect on CI; but ZM showed significant enhancement (P ≤ 0.001).

The effect of AKT on PMN function clearly showed significant reduction for all three parameters (i.e. P%, PI and CI) when compared to the CT group (P ≤ 0.001). The effects on PI and CI were more pronounced than on P%.

In herb + AKT treated groups, P% not only reverted to normal, but was found to match the enhanced levels seen in herb treated groups, suggesting that, in the presence of each of the 4 herbs, AKT could not exert an immunosuppressive effect. PI showed similar results, except in the OS + AKT group, where PI did reach a normal level, but was at a lower level than seen in OS treated group.

CI showed significant, but partial recovery in the CL + AKT and OS+ AKT groups (P ≤ 0.001), while the TC + AKT and ZM + AKT groups showed complete recovery (P ≤ 0.001).

DISCUSSION

Selection of an animal model is important if the results of the experiment are to be extrapolated to a human population. Looking at the comparative biology of test species for acetylation, deacetylation, debrisoquine oxidation, aryl hydrocarbon hydroxylase and β-glucuronidase activities, it is evident that the guinea pig mimics human biology more closely than any other rodent species. In the present study, a hepatotoxicity model of guinea pig was successfully produced by feeding 50 mg INH, 100 mg RIF and 300 mg PZA/kg (bw) orally for 21 d. This dose is somewhat higher than used by other workers, and much higher than the comparable human dose. But, this is justified by taking into account the fact that guinea pig liver is most resistant to injury among rodents and that rodents are fast metabolizers.

Oxidative stress, lipid peroxidation, choline deficiency leading to lowering of phospholipids protein synthesis with alteration in cell wall configuration, reduced glutathione level, activation of CYP2E1 are implicated in AKT induced hepatotoxicity and it is well known that many nontoxic herbs have opposite activities in the form of membrane stabilizing, antioxidative and CYP2E1 inhibitory effects. A review of the available literature suggests that a reduction in the lipid peroxide...
content of tissue and an increase in superoxide dismutase, catalase, glutathione, glutathione-S-transferase and glutathione peroxidase activities should help to maintain liver cell integrity and control the increased level of AST, ALT and ALP.

PMN function was markedly suppressed in our hepatotoxicity model though the effects of AKT on human PMN functions in vitro at therapeutic serum concentrations showed increased phagocytic activity [38]. Modulation of phagocytic activity by antibiotics is a most widely investigated and controversial area. RIF quenches superoxide anions and cyclones, which scavenge HOCI, while INH directly interferes with myeloperoxidase (MPO) and impairs the production of HOCl by the MPO-H2O2-halide system. The resultant effect would manifest as anti-inflammatory action by INH and decreased oxidative burst, as well as T and B lymphocyte function by RIF [39,40].

The available literature shows that rhinax [41], garlic [13], Liv-52 [32], Emblica officinalis [23] and Terminalia chebula [11] have demonstrated hepatoprotective activities in experimental animal models of AKT-induced hepatotoxicity in rat. N-acetylcysteine [13], tocopherol acetate and riboxine [43], have prevented morphological changes, suggesting that oxidative injury could be prevented by giving cellular antioxidant support in an animal model of INH + RIF hepatotoxicity.

The CL treated group showed no effects on AST, ALT or the AST/ALT ratio. S. ALP was significantly reduced (P ≤ 0.01). The significance of this finding is unclear. But, similar changes are seen in malnutrition and with antilipemic agents [44]. Curcumin is known to have anorexiant and antilipemic actions that may explain the findings in the present experiment. Liver histology was normal. P% was enhanced (P ≤ 0.001). But, PI and CI were not affected, suggesting that it could recruit more PMN in phagocytosis without enhancing the phagocytic and chemotactic activities. The available literature shows an effect of CL on neutrophil function in the presence of inflammation only, which indicates inhibition and modulation in the presence of different chemo attractants [45]. Looking at the effects of treatment with CL + AKT on liver enzymes, ALP was normalized and AST was kept at the two fold level, and the AST/ALT ratio was near two. More spectacular was the histological changes that were minimal when compared with that of the only AKT group. Only mild microvesicular steatosis and very little evidence of lobular inflammation were seen. Curcumin has free radical scavenging and hepatoprotective activities tested in vitro [46]. It suppresses the production of superoxide by macrophages, has a potent anti-inflammatory action that inhibits the production of tumor necrosis factor alpha (TNF-α), interleukin (IL) 1-β and the activation of NF-kB in human monocyte derived cells [47]. It also has a strong antioxidant property and it inhibits lipid peroxidation in rat liver microsomes, erythrocytes membrane and brain homogenates, by maintaining the activity of SOD, catalase and glutathione peroxidase at a higher level [48]. These properties clearly explain the hepatoprotective potential of CL that was seen clearly in the experimental results of this study. Curcumin has antiviral, antiprotozoal, antibacterial, anti-inflammatory, chemoprotective and antioxidant action, in addition to its hepatoprotective activity, without any known side effects or toxicity. We, therefore, think it imperative to devise a prospective human trial in immunocompromised, as well as latent tuberculosis patients, as an adjuvant to standard chemotherapy.

The OS treated group showed no significant change from the control group for all the parameters except an increase in P% (P ≤ 0.01). The OS + AKT treatment group, when compared with the AKT control group, showed partial recovery for liver enzymes and PMN function. The liver histology showed moderate microvesicular steatosis and inflammatory changes. Compared to the other herb treated group, the hepatoprotective effect of OS was weaker. Panda and Kar [49] have shown a significant decrease in hepatic lipid peroxidation and hepatic glucose-6-phosphate activation and a significant increase in SOD and superoxide catalase. These actions should have protected the liver more effectively. But, a high level of eugenol, which is hepatotoxic with glutathione-depleted liver [50], might have interfered as AKT is also known to deplete glutathione from liver tissue. OS has immunomodulatory action, which is probably dependent on a radical scavenging potential and dual effect on nitric oxide production [51].

The TC treated group showed insignificant changes in liver enzymes and a normal liver biopsy. P% was enhanced markedly when compared with the control group (P ≤ 0.001) and with other herb groups; i.e., ZM (P ≤ 0.05) and OS (P ≤ 0.001). PI was enhanced significantly compared to the control group (P ≤ 0.001). CI was not altered significantly. The AKT group showed all parameters suppressed. But, the TC + AKT treatment maintained the parameter to pretreatment levels. Liver biopsy showed minimal changes and was near normal. TC induces enzymes of drug metabolism and the antioxidant system and inhibits lipid peroxidation in mice. Cytochrome P450, NADP-Cytochrome (P sub 450) reductase, Glutathione-S-transferase, DT diaphorase, SOD and catalase are enhanced. These effects improve liver function, protect against toxic assaults and increase protein synthesis by the liver [52]. These activities can account for the observed hepatoprotective potential. It is a known immunostimulant. A compound polysaccharide in nature has been isolated from dry stem crude extract and has been shown to enhance the humoral immune response in mice. It has also been observed that treatment of mice with dried stem crude extract prevented cyclophosphamide-induced myelosuppression as well as immunosuppression [53]. Improved humoral immunity, enhanced macrophage and Kupffer cell function, enhanced neutrophil function, antioxidant activity and excellent hepatoprotective potential makes TC an ideal adjuvant agent to be evaluated in humans in clinical settings.

The ZM treated group showed normal liver enzymes and liver histology. P% (P ≤ 0.001) and CI (P ≤ 0.01) were enhanced significantly, while PI was not affected, suggesting immunostimulatory activity. The ZM + AKT treatment group showed significant reduction in AST when compared to the AKT group. ALP normalized completely. ALT showed a non significant rise. P%, PI
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and CI were not affected by ZM + AKT and the level remained similar to the ZM treated group only. These findings suggest that ZM has strong immunostimulatory activity that was not suppressed by AKT or hepatotoxicity. Looking at enzyme levels, the hepatoprotection afforded by ZM was comparable with OS. But the liver biopsy showed near normal histology with very little steatosis, suggesting higher efficacy. Antioxidant phytochemicals that would quench the reactive intermediates and radical species generated during oxidative stress, and constituents helping to keep normal levels of glutatione, might be responsible for its hepatoprotective activity. In traditional Kampo herbal formulae, Z. jujube (syn. mauritiana) is one of the 7 compounds that is used to maintain normal liver function, and when given prospectively to a large series of patients with cirrhosis of liver, it also prevented liver cancer. ZM enhances the activity of natural killer cells and hence may be called an immunopotentiator[30]. The active components and their activities resulting in hepatoprotection, immunopotentiation and chemoprevention may be investigated for a better understanding and application of the herb.

In the present study, it was observed that steatosis was the first change to occur, followed by evidence of triaditis and focal necrosis. Though all three drugs of AKT are hepatotoxic, INH metabolite hydrazine is implicated in inducing steatosis by altering the hepatic gene expression profile favoring production and intracellular transport of hepatic lipid over the removal of fatty acid metabolites. Peroxidation of accumulated lipids leads to formation of toxic reactive aldehyde byproducts and downstream effects, such as impaired membrane integrity, mitochondrial and sarcoplasmic reticulum dysfunction and altered calcium homeostasis[8]. CI, TC and ZM have markedly prevented steatosis and preserved the morphological integrity of liver, suggesting that they might have prevented the change in the hepatic gene expression profile, directly or indirectly, favoring steatosis in the first place followed by their property of supporting cellular antioxidant enzyme systems. This would imply that these herbs might be effective in many other clinical conditions where steatosis is the initial or ultimate liver pathology.

In conclusion, all four herbs were found to be effective in preventing hepatotoxicity partially with near similar efficacy, considering the profile of liver enzymes. Histologically, near normal morphology of liver was observed with CI, TC and ZM, suggesting their superior cellular protective potential even in the presence of drugs or toxins. OS showed partial protection probably due to its eugenol content. CI and TC showed higher hepatoprotective activity. PMN and macrophage function in human AKT induced hepatotoxicity cases must be done and its clinical significance must be decided. Human prospective trials with patients predisposed to develop hepatotoxicity and immunocompromised groups of tuberculosis patients, as well as patients with latent tuberculosis, are imperative to explore the possibility of improved outcome and preventing morbidity and mortality.
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