Hepatoprotective Effect of Calotropis procera in Isoniazid and Rifampicin Induced Hepatotoxicity

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ABSTRACT

Objective: In this study anti-tubercular (Anti-TB) drugs (isoniazid [INH] and rifampicin [RMP]) induced liver toxicity has been studied for the hepatoprotective effect of hydroethanolic extract of Calotropis procera (CP) flowers in rats.

Materials and Methods: Animals were divided into four groups, group A was given normal saline (1 ml/kg), group B received INH (50 mg/kg) and RMP (100 mg/kg) group C received INH (50 mg/kg), RMP (100 mg/kg) and CP (150 mg/kg) orally for 14 days. Results: Biochemical markers of liver toxicity such as aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and bilirubin and tissue histology were done in all groups. Anti-TB drugs (INH 50 mg/kg and RMP 100 mg/kg) have enhanced the ALT, AST, ALP, bilirubin and histological changes in liver, whereas co-administration of anti-TB drugs with CP has reduced these levels within the normal range.

Conclusion: Findings of this study showed the hepatoprotective effect of CP against INH and RMP administration to reduce the liver damage for chronic treatment.

Keywords: Calotropis procera, hepatoprotective, isoniazid, rifampicin

INTRODUCTION

After human immune deficiency virus infection also known as acquired immune deficiency syndrome, tuberculosis (TB) has the highest mortality rate in the world.

A 2012 report of World Health Organization revealed that in 2011, 8.7 million people fell ill with TB and 1.4 million deceased from TB.¹ If active TB remained untreated or treatment is interrupted, two out of every three patients died, which deemed it necessary to carry out instantaneous, most suitable and uninterrupted treatment for this disease.² Hepatotoxicity is one of the most serious adverse drug reactions of anti-tubercular (anti-TB) treatment (ATT), which limits the use of these drugs.³

Anti-TB drugs induced hepatotoxicity is the condition in which liver enzymes aspartate aminotransferase (AST) and alanine transaminase (ALT) increased 3-5 times of normal levels, in severely damaged liver it can cause uncontrolled hepatitis, and 1-2% patients stop the treatment.⁴

A mortality rate of 5% is reported worldwide due to anti-TB drug-induced hepatotoxicity.⁵

One of the most important first-line anti-TB drug regimens included rifampicin (RMP), pyrazinamide and isoniazid (INH), which can induce hepatic injury. The co-administration of RMP and INH is known to increase the risk of hepatic injury.⁶ In slow metabolizers RMP can increase the hydrazine production induced by INH hydrolase when given concurrently with INH. This can explain the increased hepatotoxicity of these drugs in combination when compared to their individual administration.⁷

Hepatotoxicity caused by anti-TB drugs can affect the hepatocytes or vasculature and the biliary epithelium, but the exact underlying mechanism and contributing factors causing hepatic damage are not clearly known. Temporary and asymptomatic increased ALT levels may indicate minor and non-progressive damage to mitochondria, cell membranes, or other structures of hepatocytes.⁸ Infrequently these changes leads to inflammation, cell death, or major histopathological changes.⁹ On histopathological examination, focal hepatic necrosis maybe revealed with bridging in serious cases.¹⁰

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Various studies have been conducted to develop the most efficient way to eliminate or minimize the hepatotoxicity of anti-TB drugs, by using the natural botanical and/or synthetic drug products without interacting their therapeutic actions.11

It has been observed that the antioxidant action is the common mechanism of various herbal drugs.12,13 Natural plant products are extensively use in different disease conditions and serve as compounds of interest both in their natural form and for the synthetic derivatization.

The importance of natural products in current medicine has been acknowledged.

Approximately, more than 20 new drug products introduced world over between 2000 and 2005, are derivatives of natural products.14 It has been documented that natural products and related drugs are used to treat 87% of all categorized disease indications in humans.15 Among the herbal drugs, Calotropis procera (CP), which is a wild growing plant also possess flavonoids, alkaloids, cardiac glycoside, stanins, sterol, and triterpines. The flowers are reported to contain flavonoids, querectin-3-rutinoside, sterols, etc. Flavonoids are reported to possess anti-oxidant and hepatoprotective properties.16

CP, a wild growing plant belongs to Asclepiadaceae family.17 Its different parts exhibit antioxidant, analgesic, and anti-inflammatory properties.18 The current study was carried out to evaluate protective effects of the flowers of CP using anti-TB drugs induced hepatic toxicity model in rats.

MATERIALS AND METHODS

Research design and setting

This was an experimental study which was conducted in Dow International Medical College, Department of Pharmacology and Therapeutics in association with animal house and Dow Diagnostic Research and Reference Laboratory.

Animals

Adult Wistar rats (185-195 g) of either sex were obtained from the Charles's River Breeding Laboratory USA, cross breded in the Dow University Animal House.

All animals were acclimatized prior to the experiment in standard environmental conditions and given the rodent diet with water ad libitum for 1 week. Approval was obtained from the Institute of Basic Medical Sciences, Institutional Review Board, Funding Committee and finally from the Board of Advance Studies and Research of Dow University of Health Sciences.

Plant

The flowers of CP were collected in 2nd week of August from the vicinity of Dow University Ojha Campus Karachi. Dow College of Pharmacy, Pharmacognosy Department recognized and a specimen was deposited in the museum with No: DCP/H/00427 for herbarium.

Preparation of the flowers extract

The flowers were dried in the shade and were macerated with 70% ethanol and 30% water, and extract was dried out for concentrate at 40°C under vacuum by using a Rota evaporator, which gave 25% of the extract.

Drug

INH (100 mg/tablet) and RMP (150 mg/tablet) tablets were used. Tablets were dissolved in the distilled water, and doses were calculated according to the body weight.

Hepatoprotective activity

The method of Lenaerts et al.,19 was used in the study. Animals were divided into three groups of six animal of each and drugs were administered from the oral route with the help of stainless steel feeding needle for rodents for 2 weeks. Group A was treated as control group, which received normal saline solution 1 ml/kg. Group B was received INH 50 mg/kg and RMP 100 mg/kg for to induce hepatotoxicity. Group C received INH 50 mg/kg RMP 100 mg/kg and CP extract 150 mg/kg taken as anti-TB drugs plus CP treated group. All animals were sacrificed after giving deep anesthesia of chloroform. Blood was collected for the liver function tests after cardiac puncture and liver was removed for its histopathological examination, which was conducted at Dow Diagnostic and Research Laboratory.

Histology

Hematoxylin and eosin stain technique is used to stain the histopathology slides. The slides were examined under light microscope for general architecture of hepatic parenchyma (intact/disturbed), portal area (normal/disturbed), central vein (normal/disturb), hepatocytes (cytoplasm and nucleus status), sinusoids (normal/dilated), fibrosis (present/absent), necrosis (present/absent), cholestasis
(present/absent), any other findings (granulomas, etc.)20 Microscopic findings of inflammation were converted into numerical data using the Knodell score or histologic activity index (Table 1).21

### Biochemical parameters

The blood samples were obtained for the analysis of various biochemical parameters, which include the estimation of serum alanine ALT, AST, alkaline phosphatase (ALP), total bilirubin, and direct bilirubin levels.

### Statistical analysis

The data were expressed as mean ± standard error of the mean, (n = 6) and statistical analysis was performed by using the Statistical Package for Social Science program version 16 by IBM. Data were analyzed using one-way analysis of variance, followed by multiple comparisons using Dunnett’s procedure to compare all groups against Group-A. P < 0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

Anti-TB drugs, especially INH and RMP are used in combination, which is significantly hepatotoxic and it is documented in earlier studies.26,27 The mechanism of hepatotoxicity has been suggested by these two drugs is mediated via oxidative damage,28 the additive or synergistic hepatotoxic effect has been reported to the caused by monoacetyl hydrazine, hydrazine and other related compounds produced from hepatic biotransformation through enzyme induction;29 therefore, a regular weekly or biweekly monitoring of liver enzyme is required for initial 2 months,30 according to the guidelines of American thoracic society a rapid increase in liver enzyme like ALT is one of the most prominent indicator for development of hepatic injury.31,32 In order to manage the ATT induced hepatotoxicity it is recommended to hold the treatment when the hepatotoxicity is evident, until the liver enzymes normalize.33,34 Alternatively co-administration of appropriate hepatoprotective agents would prevent this hepatotoxicity.35 Literature survey revealed that, the CP is one of the many plants have the potential to be used for hepatoprotective effects against the drug or chemical-induced hepatotoxicity.36,37 CP is one of the plants used to protect the liver injury. Flavonoids are the active constituents of the flower of CP act as antioxidants against the reactive oxygen moieties by boosting the endogenous scavenging system of the body, these are also known to interfere various free-radical producing systems and also enhance the endogenous antioxidant function.38 Flavonoids are oxidized by radical, which makes a more stable and less reactive free radicals and made the radicals inactive.39

### Table 1: Knodell scoring system for portal inflammation

<table>
<thead>
<tr>
<th>Portal inflammation</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Mild, some or all portal areas</td>
<td>1</td>
</tr>
<tr>
<td>Moderate, some or all portal areas</td>
<td>2</td>
</tr>
<tr>
<td>Moderate/marked, all portal areas</td>
<td>3</td>
</tr>
<tr>
<td>Marked, all portal areas</td>
<td>4</td>
</tr>
</tbody>
</table>

### Table 2: Effects of CP floral extract on enzymes and histology of liver in anti-TB drug-induced hepatic toxicity in rats

<table>
<thead>
<tr>
<th></th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Direct bilirubin (mg/dl)</th>
<th>Microscopic finding</th>
<th>One-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A control</td>
<td>20.16±4.49</td>
<td>133.25±10.2</td>
<td>74.00±7.2</td>
<td>0.22±0.05</td>
<td>0.06±0.02</td>
<td>17.75±3.21</td>
<td>F=15.564, P&lt;0.01</td>
</tr>
<tr>
<td>(saline 1 ml/kg p. o. 2 weeks)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Group B anti-TB drugs</td>
<td>41.83±7.09</td>
<td>189.92±17.3</td>
<td>154.67±18.5</td>
<td>0.61±0.07</td>
<td>0.17±0.03</td>
<td>41.66±4.61</td>
<td></td>
</tr>
<tr>
<td>(INH 50 mg/kg+RMP 100 mg/kg p. o. 2 weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C treatment</td>
<td>13.83±2.72*</td>
<td>113.5±8.7*</td>
<td>83.33±11.3*</td>
<td>0.37±0.03**</td>
<td>0.11±0.02**</td>
<td>18.15±3.82*</td>
<td></td>
</tr>
<tr>
<td>(INH 50 mg/kg+RMP 100 mg/kg+ CP150 mg/kg p. o. 2 weeks)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*P<0.01, **P<0.05 versus anti-TB drugs. NS: Non-significant post-hoc t-test, INH: Isoniazid, RMP: Rifampicin, CP: Calotropis procera, ALT: Alanine transaminase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, ANOVA: Analysis of variance, anti-TB: Anti-tubercular.
causing less damage. Flavonoids are also found to inhibit xanthine oxidase activity, which is a source of free radicals leads to the oxidative injury to the tissues.

A further mechanism of flavonoids to decrease the number of immobilized leukocytes to endothelial wall causing derivation of oxygen-derived free radicals and cytotoxic oxidants and inflammatory mediator preventing the inflammatory condition.

Degranulation of neutrophils in plasma membrane by modulating Ca\(^{2+}\) channels can be hampered by flavonoids.

Various studies proved the successful use of rat’s models for INH and RMP induced hepatotoxicity. Therefore, same model has been used to determine the hepatoprotective activity of CP in the anti-TB drug-induced toxicity. INH and RMP were given daily for 14 days to produce liver toxicity at very high doses (INH 50 mg/kg, RMP 100 mg/kg) as compared with humans because rats metabolize drugs faster than humans and period of study shorter in comparison to the treatment of TB in humans. At day 15\(^{th}\) after the last dose administration hepatic injury was assessed by the measurement of liver enzymes (ALT, AST, ALP, and Bilirubin) and the presence of microscopic findings.

In the current study, rats were divided into three groups in which Group A received the normal saline as control and Group B received the anti-TB drugs, while Group C received the anti-TB drugs along with the CP extract.

On the comparison of Group A with Group B, there were significant increased \((P < 0.05)\) in the liver enzyme levels, and microscopic findings shown in Table 2, Figures 1 and 2, which were in agreement with previous studies, furthermore when Group B was compared with the CP treated Group C, the levels of enzymes and microscopic findings were significantly reduced and brought to the normal range shown in Table 2 and Figure 3. Therefore, these changes suggested that the extract of CP flowers possess hepatoprotective activity, which was further evident by the significant \((P < 0.01)\) reduction in inflammatory changes in liver tissues. These finding completely favor the previously conducted studies in which co-administration of CP showed hepatoprotective effect against hepatotoxic drugs and demands the further experiments on higher animals to prove its safe and effective use to reduce the liver damage with such toxic drugs for chronic treatment.

**REFERENCES**


Kamil and Imran-ul-Haque: Hepatoprotective effect of Calotropis procera


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