AMELIORATIVE EFFICACY OF AQUEOUS EXTRACT OF *Andrographis paniculata* (Nees.) AGAINST ANTITUBERCULOSIS DRUG, RIFAMPICIN INDUCED HEPATOTOXICITY MALE ALBINO WISTAR RATS

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Abstract

Rifampicin is known to affect cytochrome P-450 in humans and animals and it is a macrolide antibiotic used for the treatment of tuberculosis. Rifampicin is an antituberculosis drug effectively cures tuberculosis but it will damage the liver. The present study was undertaken to scientifically prove the traditional use of *Andrographis paniculata* leaf extract against liver disorders. The ameliorative potential of *Andrographis paniculata* on liver damage was evaluated by rifampicin induced hepatotoxicity in rats. Male albino wistar rats were orally treated with *Andrographis paniculata* (100, 200 and 400 mg/kg body weight) or silymarin (25 mg/kg) daily to rifampicin (1g/kg, one day only) treated rats. Rifampicin induced liver damage and significantly increased the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Gamaglutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH), cholesterol and bilirubin and decreased the levels of protein in serum as compared with control group. Treatment with *Andrographis paniculata* or silymarin could significantly decrease the ALT, AST, ALP, GGT, cholesterol and bilirubin whereas protein levels in serum increased when compared with rifampicin alone treated rats. The probable mechanism of hepatoprotection by aqueous extract of *Andrographis paniculata* could be minimized the activities of liver marker enzymes.

INTRODUCTION

Tuberculosis is one of the fatal communicative diseases and is spread easily amongst people. Over one third of the world population is estimated to be infected with *Mycobacterium tuberculosis* and over 2 million people a year will die of the disease (1,2). WHO declared that the tuberculosis as “Global health emergency” (3). Tuberculosis is a leading public health problem worldwide, particularly in developing countries. About one third of world’s population has latent tuberculosis and approximately 9 million cases of active tuberculosis emerge annually resulting in 2-3 million deaths (4). Out of 1.86 billion people were living in developing countries, such as India and China (5). In the past decade, there has been a great increase in the use of complimentary treatments such as herbal remedies in the treatment of disease (6). Plants and plant products are part of the vegetarian diet and a number of them exhibit medicinal properties. Several Indian plants are also being used in Ayurvedic and Siddha medicines. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases (7,8). *Andrographis paniculata* Nees (Acanthaceae) is an erect herb found in the plains throughout India and Sri Lanka (9). In the traditional Indian medicine the whole plant of *A. paniculata* is extensively used in the treatment of dyspepsia, dysentry, malaria, respiratory infections, and as an antidote for snake-bite (10,11), antipyretic (12), antihepatotoxic (13), choleretic (14), immunostimulant (15) and anti-HIV (16) properties. Some clinical trials showed that it is effective in the treatment of the common cold (17) and pharyngothonsillitis (18). The purpose of this study was to evaluate the ameliorative efficacy of *Andrographis paniculata* extract on antituberculosis drug rifampicin induced hepatotoxicity.

MATERIALS AND METHODS

PROCUREMENT AND REARING OF EXPERIMENTAL ANIMALS

Adult male albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room
temperature (27 ± 2°C). The animals were randomized and separated into normal and experimental groups of body weight ranging from 170-200 g. The animals received a diet of standard pellets (Hindustan Lever Ltd., Bombay). Rats were provided free access to water ad libitum and food through the tenure of acclimatization to the environment for a minimum period of two weeks prior to commencement of experiment. The study was approved by the Institutional Animal Ethical Committee of Rajath Muthiah Medical College (160/1999/CPCSEA, Proposal No. 828), Annamalai University, Annamalainagar, Chidambaram.

**PREPARATION OF AQUEOUS EXTRACT**

The collected *Andrographis paniculata* leaves were air dried and powdered. The powdered *Andrographis paniculata* were kept in airtight containers in a deep freeze until the time of use. A sample containing 250 g was mixed with 1000 mL of distilled water and stirred magnetically overnight (12 h) at 37°C. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolved in normal physiological saline and used in the study. The yield of the extracts was approximately 14.8 g.

The suitable optimum dosage schedules were identified by administering the aqueous leaf extract of *Andrographis paniculata* at different dosages (100, 200, 400, 800 and 1600 mg/kg body weight) in a day daily for twenty eight days. The optimum dose was selected as 400 mg/kg body weight for twenty eight days.

**EXPERIMENTAL DESIGN**

The animals were divided into 7 groups of 6 rats each.

- **Group 1**: Control rats given physiological saline solution 10 mL/kg body wt.
- **Group 2**: Rats given rifampicin (1 g /kg body wt.) orally were using an intragastric tube.
- **Group 3**: Rats given rifampicin + *Andrographis paniculata* (100 mg/kg body wt.) administered orally using an intragastric tube.
- **Group 4**: Rats given rifampicin + *Andrographis paniculata* (200 mg/kg body wt.) administered orally using an intragastric tube.
- **Group 5**: Rats given rifampicin + *Andrographis paniculata* (400 mg/kg body wt.) administered orally using an intragastric tube.
- **Group 6**: Rats given rifampicin + silymarin (25 mg/kg body wt.) administered orally using an intragastric tube.
- **Group 7**: Rats given *Andrographis paniculata* (400 mg/kg body wt.) alone administered orally using an intragastric tube.

At the end of the experimental period in 24 h after last treatment the animals were killed by cervical decapitation. Blood was collected without anticoagulant for the separation of serum.

**Biochemical analysis**

Blood samples were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Serum biochemical parameter such as Transaminases (AST and ALT), ALP, GGT, LDH, Bilirubin, cholesterol and protein levels were estimated according to standard methods (19, 20, 21, 22, 23, 24, 25) respectively.

**Statistical analysis**

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan’s multiple range test (DMRT). The level of statistical significance was set at p ≤ 0.05(26).

**RESULTS**

**Hepatic serum marker enzymes**

The levels of serum AST, ALT, ALP and LDH were estimated in normal and experimental rats. Significant elevation in serum AST, ALT, ALP and LDH in rats treated with rifampicin when compared with the corresponding control rats. Oral administration of aqueous extract of *Andrographis paniculata* (100, 200 and 400 mg/kg body wt.) and silymarin to rifampicin induced hepatic damage rats caused a marked reduction in the activities of these enzymes. Extract alone administered rats did not shows any significant change (Table 1).

| Table 1. Serum hepatic marker enzyme activities in control and experimental groups |
|---------------------------------|-----------|-----------|-----------|-----------|
| Groups                          | AST (U/L) | ALT (U/L) | ALP (U/L) | LDH (U/L) |
| Control                         | 72.67±5.53 | 26.33±2.01 | 192.71±14.67 | 485.32±36.95 |
| Rifampicin (1g /kg)             | 158.37±12.05 | 118.76±9.04 | 357.38±27.21 | 1128.51±85.93 |
| *Andrographis paniculata* (100 mg/kg) | 141.28±10.76 | 89.67±6.83 | 302.69±23.04 | 932.80±71.03 |
| Rifampicin (1g /kg)+            | 102.20±7.78 | 64.29±4.90 | 254.26±19.36 | 705.40±53.71 |
| *Andrographis paniculata* (200 mg/kg) | 102.20±7.78 | 64.29±4.90 | 254.26±19.36 | 705.40±53.71 |
| Rifampicin (1g /kg)+            | 84.93±6.47 | 41.75±3.18 | 218.64±16.65 | 548.85±41.79 |
| *Andrographis paniculata* (400 mg/kg) | 84.93±6.47 | 41.75±3.18 | 218.64±16.65 | 548.85±41.79 |
| Silymarin (25 mg/kg)            | 89.28±6.79 | 55.87±4.26 | 230.80±7.57 | 587.04±44.70 |
| *Andrographis paniculata* (400 mg/kg) | 70.75±5.38 | 25.69±1.95 | 183.37±13.96 | 480.70±36.61 |

All the values are mean ± SD of six observations

Values which are not sharing common superscript differ significantly at 5% level (P < 0.05)

Duncan Multiple Range Test (DMRT)
Gammaglutamyl transpeptidase (GGT) Cholesterol, Bilirubin and Protein

The levels of GGT, cholesterol, bilirubin and protein were analyzed in normal and experimental rats. There was a significant decrease in protein levels whereas increase the level of GGT, cholesterol and bilirubin in rats treated with rifampicin when compared with the corresponding control rats. Oral administration of aqueous extract of *Andrographis paniculata* (100, 200 and 400 mg/kg body wt.) and silymarin to rifampicin induced hepatic damage rats caused a marked increase in the levels of protein and decreased the levels of GGT, cholesterol and bilirubin when compared with rifampicin alone treated rats. Extract alone administered rats did not show any significant change (Table 2).

**DISCUSSION**

Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India (27). Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine (28). Several studies were conducted in the field of drug discovery and development but due to the side effects of modern medicine, natural remedies are considered to be effective and safe alternate treatments for hepatotoxicity (29).

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are the source of great economic value all over the world. Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (30, 31). Serum AST, ALT, ALP and bilirubin are the most sensitive tests which are considered as the index for diagnosis of liver diseases (32). In the present investigation rats treated with rifampicin developed significant hepatic damage which was observed through a substantial increase in the concentration of AST, ALT, ALP, GGT, LDH, cholesterol and bilirubin whereas decrease the level of protein. Oral administration of rats with aqueous extracts of *A. paniculata* (100, 200 and 400 mg/kg body wt.) and silymarin after the challenge of rifampicin produced an alleviation of the hepatic injury to a considerable extent which was reflected by the ability of the aqueous extract *A. paniculata* to lower the elevated serum enzymes levels resulting from the administration of rifampicin alone. The increased levels of AST and ALT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (33). In view of this, the extract mediated reduction in levels of AST and ALT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by rifampicin. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (34). Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow (35). The use of ALP in chemical induced liver dysfunction has been investigated in this investigation. Rifampicin induced elevation of this enzymatic activity in serum is in line with high level of serum bilirubin content. The aqueous extract *A. paniculata* mediated suppression of the increased ALP activity with the concurrent depletion of raised bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction in rat liver during hepatic injury with rifampicin. GGT and ALP are membrane bound enzymes, which are released unequally depending on the pathological phenomenon. The elevation of serum GGT concentrations is regarded as one of the most sensitive indices of hepatic damage (36). LDH catalyses the conversion of lactate to pyruvate using NAD⁺ as coenzyme of NAD (37). The increase in LDH activity in serum may be due to leakage of the enzyme from the tissues into the blood on account of cellular injury. An elevation in the levels of the serum marker enzymes in generally regarded as one of the most sensitive index of the hepatic damage (38).

**Conclusion**

The results of the present study indicate that the administration of *Andrographis paniculata* extracts minimize the rifampicin induced hepatotoxicity in rats. Biochemically the high dose of *Andrographis paniculata* leaf extract showed better results as compared to low dose.

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**Table 2. Serum GGT, Bilirubin, Cholesterol and Protein levels in control and experimental groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>GGT (U/L)</th>
<th>Bilirubin (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
<th>Protein (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.32±1.24</td>
<td>0.582±0.042</td>
<td>93.44±7.11</td>
<td>8.93±0.67</td>
</tr>
<tr>
<td>Rifampicin (1g /kg)</td>
<td>34.19±2.60</td>
<td>2.355±0.179</td>
<td>206.42±15.72</td>
<td>6.24±0.47</td>
</tr>
<tr>
<td>Andrographis paniculata (100 mg/kg)</td>
<td>27.15±2.07</td>
<td>1.957±0.148</td>
<td>152.17±11.59</td>
<td>7.18±0.54</td>
</tr>
<tr>
<td>Rifampicin (1g /kg) + A. paniculata (200 mg/kg)</td>
<td>22.73±1.73</td>
<td>1.378±0.105</td>
<td>135.78±10.34</td>
<td>7.72±0.59</td>
</tr>
<tr>
<td>Rifampicin (1g /kg) + A. paniculata (400 mg/kg)</td>
<td>18.46±1.41</td>
<td>0.632±0.047</td>
<td>104.24±7.94</td>
<td>8.55±0.65</td>
</tr>
<tr>
<td>Rifampicin + Silymarin (25 mg/kg)</td>
<td>20.32±1.55</td>
<td>0.947±0.072</td>
<td>138.16±10.52</td>
<td>7.85±0.60</td>
</tr>
<tr>
<td>Andrographis paniculata (400 mg/kg) alone</td>
<td>15.10±1.15</td>
<td>0.577±0.045</td>
<td>90.17±6.86</td>
<td>8.96±0.69</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six observations.

Values which are not sharing common superscript differ significantly at 5% level (P < 0.05).

Duncan Multiple Range Test (DMRT)
The overall antihepatotoxic efficacy of *Andrographis paniculata* is probably due to counteraction of 25-desacetyl rifampin formed from rifampicin which is responsible for liver damage. Further pharmacological and isolation of active principles were underway to find out antihepatotoxic role of *Andrographis paniculata*.

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REFERENCES


33. Drotman R.B and Lowhorn, G.T, Serum enzymes as indicators of chemical induced liver damage. Drug Chemical Toxicology 1 (1978), 163–171.


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