HEPATOPROTECTIVE ACTIVITY OF HERBAL FORMULATION IN PARACETAMOL INDUCED TOXICITY IN ALBINO RATS

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ABSTRACT

Hepatotoxicity is an injury to the liver that is associated with impaired liver function caused by exposure to a drug or various other agents. The present study was carried out to evaluate the hepatoprotective activity of a formulation comprising of *Atrocarpus heterophyllus* Lam *Curcuma amada* Roxb and *Piper longum* Linn in wistar albino rats. The animals were divided in to six groups comprising of six rats each. Hepato toxicity was induced using paracetamol at a dose of 2g/kg bw orally as a single dose. Group 1 served as normal control. Group II was induced with paracetamol at a dose of 2g/kg bw orally as a single dose. Group III was induced with paracetamol and administered the formulation at a dose of 100mg/kg bw for 15 days orally. Group IV was induced with paracetamol and administered the formulation at a dose of 200mg/kg bw for 15 days orally. Group V was induced with paracetamol and administered Liv – 52 at a dose of 1ml/kg 3 times a day for 15 days. Group VI received formulation alone at a dose of 200/kg bw for 15 days. Serum enzyme markers Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT) were measured. Further that serum bilirubin, liver glycogen, membrane bound Na⁺ K⁺ - ATPase and lipid profile in both serum and tissue were analysed. Histopathology of liver tissue was performed. The results obtained were statistically significant (p<0.05) and were in par with that of the Liv – 52 treated group. All the biochemical changes observed in the hepatotoxicity induced rats were reversed in a dose dependent manner. The results obtained clearly depict the hepatoprotective potentials of the formulation.
KEY WORDS: Hepatoprotective, Na+ K+ ATPase, Atrocarpus heterophyllus, Curcuma amada, Piper longum, Paracetamol.

INTRODUCTION
Liver is the second largest organ in vertebrates and is involved in regulating the homeostasis in the body. The liver performs physiological functions in addition to which it protects the system from the hazards of harmful drugs and chemicals \(^1\). It plays an essential role in drug and xenobiotic metabolism. The detoxification process of endogenous and exogenous substances \(^2\) helps in the removal of substances from the portal circulation and makes it susceptible to a persistent attack by offending foreign (xenobiotic) compounds culminating in liver dysfunction, which is considered to be a serious health disorder. Drug induced hepatic injury is the most common reason cited for the withdrawal of an approved drug from the market \(^3\). Despite the tremendous strides in modern medicine, there are few drugs that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells \(^4\). Paracetamol is a well known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses. Hepatotoxicity induced by paracetamol is caused by the reaction metabolite N – acetyl – p – benzoquinone imine (NAPQI) which causes oxidative stress and glutathione (GSH) depletion. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P–450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity \(^5\). Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment \(^2\). The present study has been structured to evaluate the hepatoprotective potential of a formulation prepared from Atrocarpus heterophyllus LamCurcuma amada Roxb and Piper longum Linn against paracetamol induced hepatotoxicity in albino rats.

MATERIALS AND METHODS
Collection of Plant Material
The plants were collected from in and around Erode District, and identified with the help of Flora of Presidency of Madras and authenticated with the voucher specimen deposited at the Rapinat Herbarium, Dept of Botany, St Joseph’s College, Trichy.

Preparation of Aqueous Extract
The plant materials were shade dried and coarsely powdered. The powder was mixed
thoroughly with 6 times the volume of water and stirred continuously until the volume reduced to 1/3\textsuperscript{rd}. The extract was filtered with muslin cloth. The residue was re extracted. The filtrate was mixed and evaporated in a water bath till it reached a paste consistency. The extract was stored in refrigerator till further use. The aqueous extract of the plants \textit{Atrocarpus heterophyllus}: \textit{Curcuma amada}: \textit{Piper longum} was mixed in the ratio 2:2:1 and the formulation was used for the pre clinical screening.

**Experimental Models**

Wistar strains of Albino rats of both sexes weighing 150 – 200g were used for the study. Animals were housed in well ventilated cages in the CPCSEA approved animal house. The protocol was approved by the Institutional Animal Ethics Committee. They were fed with pelleted rat chow and water \textit{ad libitum}. They were acclimatised to the laboratory conditions for a week before starting the experiment.

**Experimental Design**

The animals were divided in to six groups comprising of six rats each. Hepatotoxicity was induced using paracetamol at a dose of 2g/kg bw orally as a single dose. Group 1 served as normal control. Group II was induced with paracetamol at a dose of 2g/kg bw orally as a single dose. Group III was induced with paracetamol and administered the formulation at a dose of 100mg/kg bw for 15 days orally. Group IV was induced with paracetamol and administered the formulation at a dose of 200mg/kg bw for 15 days orally. Group V was induced with paracetamol and administered Liv – 52 at a dose of 1ml/kg for 3 times a day for 15 days. Group VI received the formulation alone at a dose of 200mg bw for 15 days. At the end of the experimental period the animals were sacrificed. The blood, serum and liver tissue were used for the studies. All the biochemical parameters such as Aspartate Transaminase (AST)\textsuperscript{[6]}, Alanine Transaminase (ALT)\textsuperscript{[6]}, Alkaline Phosphatase (ALP)\textsuperscript{[6]}, Gamma Glutamyl Transferase (\( \gamma \)-GT)\textsuperscript{[7]}, Serum Bilirubin\textsuperscript{[8]}, Liver Glycogen\textsuperscript{[9]}, \( \text{Na}^+ \text{K}^+ \)- ATPase\textsuperscript{[10]}, Cholesterol\textsuperscript{[11]}, Triglycerides\textsuperscript{[12]}, Phospholipids\textsuperscript{[13]}, Free Fatty Acid\textsuperscript{[14]} were analysed.

**Histopathological Study**

Liver tissue was collected and was subjected to histological studies. The tissues were observed under light microscope for architectural changes in toxicity induced groups and the effect of the formulation in tissue repair\textsuperscript{[15]}. 
Statistical Analysis
The data of results obtained were subjected to statistical analysis and expressed as mean ± SEM. The data were statistically analysed by one – way analysis of variance (ANOVA) and p<0.05 was considered to be significant.

RESULTS
The group II animals showed an elevation in the levels of the marker enzyme which was restored to near normal in the plant drug treated groups. The group VI animals showed no change in the levels of the enzymes. The results are depicted in table 1.

Table 1: Activities of liver marker enzymes in hepatotoxic and formulation treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>GGT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>113.33±1.08</td>
<td>34.9±1.39</td>
<td>65±0.96</td>
<td>28.96±0.07</td>
</tr>
<tr>
<td>Group 2</td>
<td>216.66±1.32</td>
<td>63.62±0.98</td>
<td>151.25±0.81</td>
<td>40.53±1.03</td>
</tr>
<tr>
<td>Group 3</td>
<td>138.71±0.88</td>
<td>40.11±0.98</td>
<td>88.75±1.96</td>
<td>34.74±0.85</td>
</tr>
<tr>
<td>Group 4</td>
<td>108.12±0.96</td>
<td>35.31±0.93</td>
<td>67.3±0.84</td>
<td>28.95±1.96</td>
</tr>
<tr>
<td>Group 5</td>
<td>98.77±0.96</td>
<td>36.9±0.81</td>
<td>61.48±0.39</td>
<td>23.16±0.66</td>
</tr>
<tr>
<td>Group 6</td>
<td>103.32±1.13</td>
<td>27.07±1.42</td>
<td>61.25±0.77</td>
<td>27.38±1.93</td>
</tr>
</tbody>
</table>

Values are ± SEM, n=6
*p<0.05 statistically significant when compared with normal control.
**p<0.05 statistically significant when compared with paracetamol treated groups.

The biochemical parameters such as liver glycogen, bilirubin and Na⁺ K⁺ ATPase were analysed and the results are depicted in table 2. The glycogen and Na⁺ K⁺ ATPase Activity was lowered while the bilirubin levels were high in the group II animals, which were restored to normal on treatment with the formulation. The results were comparable to Liv – 52 treated groups.

Table 2: Analysis of the biochemical parameters in the experimental models.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver glycogen (mg/g of tissue)</th>
<th>Bilirubin (mg/dl)</th>
<th>Na⁺K⁺ ATPase (µg of pi liberated/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>38.31±0.85</td>
<td>1.01±0.06</td>
<td>43.28±0.51</td>
</tr>
<tr>
<td>Group 2</td>
<td>11.71±0.42</td>
<td>3.65±1.01⁺</td>
<td>19.34±0.70</td>
</tr>
<tr>
<td>Group 3</td>
<td>23.67±0.74</td>
<td>1.86±0.76</td>
<td>27.36±0.88</td>
</tr>
<tr>
<td>Group 4</td>
<td>36.48±0.57**</td>
<td>1.11±0.89**</td>
<td>40.11±0.97</td>
</tr>
<tr>
<td>Group 5</td>
<td>34.01±0.45</td>
<td>0.96±0.09</td>
<td>39.41±1.3**</td>
</tr>
<tr>
<td>Group 6</td>
<td>37.88±0.96**</td>
<td>1.02±0.19**</td>
<td>43.92±1.02**</td>
</tr>
</tbody>
</table>

Values are ± SEM, n=6
*p<0.05 statistically significant when compared with normal control
**p<0.05 statistically significant when compared with paracetamol treated groups.
Table 3: Level of Cholesterol, Phospholipids and Triglycerides, Fatty Acids in Hepatotoxic Rats and Formulation Treated Rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol (mg/dl)</td>
<td>Phospholipid (mg/dl)</td>
</tr>
<tr>
<td>Group 1</td>
<td>133.33±1.05</td>
<td>96.01±0.44</td>
</tr>
<tr>
<td>Group 2</td>
<td>249.18±0.96*</td>
<td>15.85±0.91*</td>
</tr>
<tr>
<td>Group 3</td>
<td>78.67±1.66</td>
<td>121.61±1.23</td>
</tr>
<tr>
<td>Group 4</td>
<td>124.17±1.01**</td>
<td>107.8±0.41**</td>
</tr>
<tr>
<td>Group 5</td>
<td>112.49±0.07**</td>
<td>115.25±0.59</td>
</tr>
<tr>
<td>Group 6</td>
<td>124.99±1.1</td>
<td>97.03±0.76**</td>
</tr>
</tbody>
</table>

Values are ± SEM, n=6

*p<0.05 statistically significant when compared with normal control

**p<0.05 statistically significant when compared with paracetamol treated groups.

The results depicted in table 3 indicate that cholesterol, Triglycerides and phospholipids in serum and tissue and free fatty acids in serum of the paracetamol treated groups were elevated. After the treatment with formulation a decrease in the tissue and serum cholesterol, triglycerides, phospholipids and free fatty acids was evident which was comparable with Liv – 52 treated groups.

**Histopathological Studies**

Histopathological examination of liver sections of the normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central veins (Fig 1) Massive fatty changes, necrosis, degeneration were observed in paracetamol induced animals (Fig 2). Distinct hepatic cells were observed in paracetamol induced and Liv – 52 animals (Fig 3). Normal lobular pattern, mild fatty changes were observed in group III animals (100mg/kg) (Fig 4). Normal lobular pattern, mild fatty changes were observed in group IV animals (200mg/kg) (Fig 5). Normal lobular arrangement were observed in formulation treated groups (200mg/kg) (Fig 6).
DISCUSSION

Paracetamol is widely used as analgesic and anti – pyretic and produces acute liver and renal damage at very larger doses. The hepatotoxicity of paracetamol has been attributed to the formation of toxic and highly reactive metabolite N– acetyl- para-benzoquinine imine (NAPQI) \(^{16}\).

AST and ALP are present in high concentration within the hepatocytes. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the serum increases. ALT is a sensitive indicator of the acute liver damage and elevation of this enzyme in non hepatic disease is unusual. ALT is more selectively a liver parenchymal enzyme than AST. The estimation of \(\gamma\) – GT levels is a valuable screening test with high predictive value for liver disease \(^{17}\), indicating the extent of liver damage.

In the present study the elevation of the enzymes in the serum may be due to the damage of membrane and the subsequent leakage of the enzymes in to serum. Treatment with the plant extract would have improved the membrane integrity, decreasing the enzymes levels in serum. Liver damage induced by paracetamol caused a significant elevation of cholesterol. The increased cholesterol damages the structural integrity of the liver because it is cytoplasmonic in location and released in to circulation after cellular damages \(^{18}\). Triglycerides are important simple lipids. It plays an important role in metabolism as an energy sources and transporter of dietary fat. Hepatotoxins have the capacity to impair the
phospholipids synthesis in liver mainly due to the inactivation of hepato microsomal glucose–6–phosphate dehydrogenase [19]. The paracetamol induced changes in the lipid profile was restored to normal on treatment with the formulation and Liv – 52, in the experimental models of the present study.

Liver is the vital organ which is involved in synthesis of glycogen. Liver glycogen is used to maintain blood glucose during fasting or exercise. Glycogen synthesis is promoted by activation of glycogen synthetase and increases concentration of glucose which enters liver cells via the hepatic portal vein [20].

Hence there is a significant reduction in the synthesis of glycogen in the disease control group of the present study. The plant extract activated the synthetic machinery of the hepatic tissue thus improving the glycogen stores of the animals. Calcium ions can decrease the lipid fluidity of hepatocyte plasma membrane by influencing membrane bound enzymes to alter the lipid composition. It has been reported that free radical enhanced calcium release from the sarcoplasmic reticulum inhibit sarcolemmal Na⁺ K⁺ ATPase possibly causing the activation of the Na⁺ Ca²⁺ exchange in the hepatic cell membrane [21]. The damage to the membrane integrity due to lipid peroxidation resulted in a decreased Na⁺ K⁺ ATPase activity which was restored to normal in plant treated groups. Bilirubin assay is a sensitive test to substantiate the functional integrity of the liver and severity of necrosis [22]. The reduction of the elevated bilirubin levels to normal levels indicated the restoration of functional integrity of the hepatocytes in the formulation treated groups.

CONCLUSION

The present study depicts the efficacy of the formulation in activating the protective systems of the animal there by restoring the normal functioning of the liver. It is clear from the study that the formulation possesses significant hepatoprotective potential.

REFERENCES


8 Malloy, HT, Evelyn, KA. The determination of bilirubin. *J.Biol Che*, 1937; 119, 481.


