HEPATOPROTECTIVE ACTIVITY OF DIFFERENT LEAF EXTRACTS OF TECOMARIA CAPENSIS IN RATS

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of ethyl acetate and ethanolic extracts of Tecomaria capensis against paracetamol and CCl₄ induced liver damage in rats. The ethyl acetate and ethanolic extracts of Tecomaria capensis were administered orally (in paracetamol induced method) and intraperitoneally (in CCl₄ induced method) to the animals with hepatotoxicity induced by paracetamol and CCl₄. Silymarin was given as reference standard. All the test drugs were administered by suspending in DMSO solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol and CCl₄ in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT), aspartate aminotransferase (AST) or Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the ethyl acetate and ethanolic extract of Tecomaria capensis possesses hepatoprotective activity against paracetamol and CCl₄ induced hepatotoxicity in rats.

Keywords: Tecomaria capensis, Paracetamol, CCl₄, Hepatoprotective and hepatotoxicity.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. But it is continuously and variably exposed to environmental toxins, abused by poor drug habits, alcohol and prescribed and over-the-counter drugs which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease. Thus liver diseases are some of the fatal diseases in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for disorders. But there are not much drugs available for the treatment of liver disorders.

Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl₄) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts. Tecomaria capensis is an ever green plant in warm climate areas but loses its b.wt. (High dose) respectively and a single dose of CCl₄, Group VI, VII received crude ethyl acetate extract of Tecomaria capensis at an intraperitoneal dose of 100mg/kg b.wt. (Low dose) and 200mg/kg b.wt. (High dose) respectively and a single dose of CCl₄.

Paracetamol induced method

A total of 42 animals were equally divided into 7 groups of each. Group – I served as control received neither the plant extract nor paracetamol, Group – II served as paracetamol control administered with paracetamol (3gm/kg) as single dose, Group – III served as the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for disorders. But there are not much drugs available for the treatment of liver disorders. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

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reference control, received Silymarin (25mg/kg) once daily, Group – IV, V received Tecomaria capensis ethanolic extract low dose (100 mg/kg) and high dose (200 mg/kg) respectively once daily for 3 days, Group – VI, VII received Tecomaria capensis ethyl acetate extract low dose (100 mg/kg) and high dose (200 mg/kg) respectively once daily. Group III - VII received paracetamol (3g/kg) as single dose thirty minutes after the administration of Tecomaria capensis extracts and silymarin.

Assessment of hepatoprotective activity

Rats were treated as per the treatment protocol. Body weights of these rats were monitored sequentially in control and experimental animals for a period of 21 days for CCl4 induced method and 3 days for paracetamol induced method.

Biochemical estimation: Rats were sacrificed 1 hour after administration on day 21. The blood was collected by retro orbital artery bleeding. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. SGOT, SGPT, ALP and Total Bilirubin levels were estimated from the serum.

Histopathological studies: The livers were excised quickly and fixed in 10% formalin and stained with haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of hepatotoxicity if any.

Statistics: The results were expressed as Mean ± SEM. Statistical analysis was carried out by using two tailed ‘t’ test.

RESULTS

The results of hepatoprotective activities of crude ethylacetate and ethanolic extracts of this plant at a dose of 100 mg/kg b.wt. and 200mg/kg b.wt. on rats intoxicated with carbon tetrachloride and paracetamol were illustrated in the table 1 and table 2 respectively. The tables also showed the comparison of effects among the untreated (control) and carbon tetrachloride treated (negative control) group with the drug treated group of rats. The results were represented as Mean ± Standard Error of Mean (M±SEM).

Carbon tetrachloride group significantly increased the serum level of SGOT (143.26%), SGPT (93.7%), Total Bilirubin (91.62%) and ALP (200.03%) as shown in table 1. The ‘t’ value obtained exceeded the limit of significance. That is in all cases, p < 0.0001.

Treatment Groups (Table 1):

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (mg/dl)</th>
<th>SGPT (IU/dl)</th>
<th>ALP (IU/dl)</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Direct</th>
<th>Indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49.33±1.73</td>
<td>65.0±1.43</td>
<td>28.3±1.71</td>
<td>0.8±0.115</td>
<td>0.3±0.58</td>
<td>0.41±0.095</td>
</tr>
<tr>
<td>Negative control</td>
<td>120.0±2.73</td>
<td>127.5±1.25</td>
<td>85.0±1.06</td>
<td>1.53±0.115</td>
<td>0.7±0.152</td>
<td>0.8±0.106</td>
</tr>
<tr>
<td>Positive control</td>
<td>56.33±2.33</td>
<td>75.50±1.18</td>
<td>38.83±1.42</td>
<td>0.7±0.115</td>
<td>0.4±0.106</td>
<td>0.4±0.073</td>
</tr>
<tr>
<td>Ethylacetate low dose</td>
<td>86.0±0.35*</td>
<td>108.67±1.4*</td>
<td>76.17±1.35*</td>
<td>1.66±0.154**</td>
<td>0.6±0.097***</td>
<td>1.0±0.106**</td>
</tr>
<tr>
<td>Ethylacetate high dose</td>
<td>80.0±0.20*</td>
<td>94.83±1.62*</td>
<td>55.67±1.38*</td>
<td>1.2±0.106***</td>
<td>0.7±0.154***</td>
<td>0.5±0.101</td>
</tr>
<tr>
<td>Ethanol low dose</td>
<td>75.50±1.26*</td>
<td>100.0±1.67*</td>
<td>64.17±1.74*</td>
<td>1.3±0.158***</td>
<td>0.5±0.115</td>
<td>0.8±0.139***</td>
</tr>
<tr>
<td>Ethanol high dose</td>
<td>62.17±0.83*</td>
<td>82.33±0.88*</td>
<td>44.83±1.82*</td>
<td>1.1±0.095</td>
<td>0.6±0.097***</td>
<td>0.5±0.097*</td>
</tr>
</tbody>
</table>

* p<0.0001; ** p<0.01; *** p<0.05 when compared with control

Paracetamol group significantly increased the serum level of SGOT (132.59%), SGPT (83.33%), Bilirubin (59.68%) and ALP (172.72%) as shown in table 2. The ‘t’ value obtained exceeded the limit of significance. That is in all cases, p < 0.0001.

Treatment Groups (Table 2):

Positive control (silymarin) Groups (G3): There was significant increase in total Bilirubin (1.5±0.106 mg/dl), accompanied by significant decrease in level of SGOT (56.33 ± 2.33 mg/dl) and also significant decrease in ALP (38.83 ± 1.42 IU/dl), SGPT (75.50 ± 1.18 IU/dl), as compared to the negative control.

Ethyl acetate low dose Groups (G4): There was significant increase in total Bilirubin (1.66 ± 0.154 mg/dl), accompanied by significant decrease in level of SGOT (80.0 ± 2.0 mg/dl) and also significant decrease in ALP (76.17 ± 1.35 IU/dl), SGPT (108.67 ± 2.14 IU/dl), as compared to the negative control.

Ethylacetate high dose (G5): There was significant increase in total Bilirubin (1.2±0.106 mg/dl), accompanied by significant decrease in level of SGOT (90.0 ± 2.0 mg/dl) and also significant decrease in ALP (55.67 ± 1.38 IU/dl), SGPT (94.83 ± 1.62 IU/dl), as compared to the negative control.

Ethanol low dose (G6): There was significant decrease in total Bilirubin (1.317±0.158 mg/dl), accompanied by significant decrease in level of SGOT (75.50 ± 1.26 mg/dl) and also significant decrease in ALP (64.17 ± 1.74 IU/dl), SGPT (100.0 ± 1.67 IU/dl), as compared to the negative control.

Ethanol high dose (G7): There was significant decrease in total Bilirubin (1.1±0.095 mg/dl), accompanied by significant decrease in level of SGOT (62.17 ± 0.83 mg/dl) and also significant decrease in ALP (44.83 ± 1.32 IU/dl), SGPT (92.33 ± 0.88 IU/dl), as compared to the negative control.

Histopathological studies results

Hepatocytes of the ethyl acetate extracts pretreated group, showed moderate inflammation with moderate cell death and their lobular architecture was normal. Hepatocytes of the ethanolic extracts pretreated group showed minimal inflammation with minimal cell death and their lobular architecture was normal.
Table 2: Effects of ethyl acetate and ethanolic extracts of *Tecomaria capensis* plant on various biochemical parameters in rats with paracetamol induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT</th>
<th>SGPT</th>
<th>ALP</th>
<th>Total bilirubin</th>
<th>Direct</th>
<th>Indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.67±1.91</td>
<td>72.0±1.39</td>
<td>33.0±1.53</td>
<td>0.95±0.099</td>
<td>0.667±0.067</td>
<td>0.7±0.106</td>
</tr>
<tr>
<td>Negative control</td>
<td>124.83±2.52</td>
<td>132.0±1.10</td>
<td>90.0±1.29</td>
<td>1.517±0.168</td>
<td>1.2±0.137</td>
<td>1.033±0.109</td>
</tr>
<tr>
<td>Positive control</td>
<td>63.17±2.20</td>
<td>79.67±1.69</td>
<td>41.67±1.80</td>
<td>1.633±0.206</td>
<td>0.583±0.114</td>
<td>0.817±0.095</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>89.83±3.60*</td>
<td>114.50±2.05*</td>
<td>79.67±1.43*</td>
<td>1.8±0.110**</td>
<td>0.833±0.088</td>
<td>1.100±0.106***</td>
</tr>
<tr>
<td>Low dose</td>
<td>84.33±1.61*</td>
<td>98.83±1.45*</td>
<td>59.0±1.88*</td>
<td>1.417±0.130***</td>
<td>0.883±0.135</td>
<td>0.7±0.106</td>
</tr>
<tr>
<td>Ethanol</td>
<td>80.50±1.48*</td>
<td>104.33±1.87*</td>
<td>68.17±1.89*</td>
<td>1.550±0.123**</td>
<td>0.717±0.135</td>
<td>1.033±0.150</td>
</tr>
<tr>
<td>High dose</td>
<td>66.83±1.08*</td>
<td>86.83±1.66*</td>
<td>48.0±1.73*</td>
<td>1.4±0.106***</td>
<td>0.817±0.101</td>
<td>0.667±0.088</td>
</tr>
</tbody>
</table>

*p<0.0001; **p<0.01; ***p<0.05 when compared with control

Histopathological results of ethyl acetate and ethanolic extracts of *Tecomaria capensis* plant

Hepatocytes of the normal control group showed a normal lobular architecture of the liver.

Hepatocytes of the silymarin treated group showed minimal inflammation with minimal cell death and their lobular architecture was normal.

Hepatocytes of the CCl₄ treated group showed maximum inflammation with maximum cell death.

Hepatocytes of the Paracetamol treated group showed maximum inflammation with maximum cell death.
Liver damage induced by CCl₄ is commonly used model for the screening of hepatoprotective drugs. The rise in serum levels of AST, ALT and cholesterol has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages. When rats were treated with carbon tetrachloride, it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical (CCl₃) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation. This results in changes of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver injury. Treatment with ethyl acetate and ethanolic extract recovered the injured liver towards normal, so it is having hepatoprotective effect.

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzoinemine (NAPQI), which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses. 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. Normally, AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic diseases is unusual. ALT is more selectively a liver parenchyma enzyme than AST. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present in higher concentrations in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. The elevated level of these entire marker enzymes observed in the group II, paracetamol treated rats in this present study corresponded to the extensive liver damage induced by toxin. The reduced concentrations of ALT, AST and ALP as a result of plant extract administration observed, during the present study, might probably be due in part to the presence of flavonoids. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes,
carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthones. Treatment with ethyl acetate and ethanolic extract recovered the injured liver towards normal, so it is having hepatoprotective effect. Comparing these two extracts of *Tecomaria capensis* ethanolic extract is showing more significant hepatoprotective action than ethyl acetate extract.

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