Research Article

HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF BACCAUREA RAMIFLORA AND MICROCOS PANICULATA AGAINST ALCOHOL AND PARACETAMOL INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Ayurveda an ancient Indian system of medicine claims to have remedy for disorders like jaundice and liver cirrhosis, which is inadequate with present allopathy. The present study was conducted to find out the hepatoprotective activity of ethanolic extracts of Baccaurea ramiflora and Microcos paniculata against alcohol and paracetamol induced liver damage in rats. Hepatotoxicity was induced by alcohol and paracetamol and the biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum alkaline phosphatase (sALP), serum bilirubin (SB) and histopathological changes in liver were studied along with silymarin as standard hepatoprotective agents. The phytochemical investigation of the extracts showed presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids. Ethyl alcohol (1.5ml/kg) and Paracetamol (750mg/kg) has enhanced the SGPT, SGOT, ALP and bilirubin level reduced. Treatment with ethanolic extracts of Baccaurea ramiflora and Microcos paniculata leaves (100mg/kg and 200mg/kg) has brought back the altered level of biochemical markers to the near normal levels almost comparable to the silymarin. The hepatoprotective activity was confirmed by histopathological examination of the liver tissue of control and treated animals. From the results it can be concluded that Baccaurea ramiflora and Microcos paniculata possesses hepatoprotective effect against alcohol and paracetamol-induced liver damage in rats.

Keywords: - Baccaurea ramiflora, Microcos paniculata, Ethyl alcohol, Silymarin, Paracetamol, Hepatoprotective.

INTRODUCTION

Liver plays a pivotal role in regulating the body’s internal chemical environment. It is involved in several important functions like metabolism, secretion and storage. It has a great capacity to detoxicate the toxic substances, synthesize physiologically vital principles.1 But unnecessary food habits, consuming of impure drinks may bring problems in functioning of the liver. Consuming more number of drugs also cause the liver damage, intake of alcohols, junk food etc are also questioning the functional ability of the liver by damaging the liver architecture. Damage of liver can be assessed by the elevated levels of serum enzymes like SGOT, SGPT and Bilirubin.2 Liver diseases are worldwide problem. Alcohol abuse can affect almost all organs of the body.3 However; the liver is particularly susceptible to injury because it is the site responsible for majority of ethanol oxidation.4 Chronic alcohol intake provokes susceptible hepatic changes consisting of steatosis (fatty liver), fibrosis, alcoholic hepatitis and cirrhosis. The alcoholic liver injury appears to be generated by the effect of alcohol metabolism and the toxic effect of acetaldehyde, which may be mediated by immune response to alcohol, or acetaldehyde altered proteins.5 Alcohol is the third leading cause of preventable mortality in India and also worldwide. Alcohol liver disease (ALD) is one of the major drinking related health problems and primary cause of liver disease.

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates.6,7 However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-4506 to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI).8 NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid.10 However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Silymarin is marketed as one of the standard hepatoprotective herbal formulation.

Management of liver diseases has become a critical concern in medical science. Very few drugs available in allopathy system of medicine are not free from side effects. So, there is an enormous scope for the herbs in the management of liver diseases. It is, therefore, necessary to search herbs available locally for treating hepatitis is continuing to reduce the cost of treatment.11,12,13

Baccaurea ramiflora (family: Euphorbiaceae) is native to Southeast Asia region and is found distributed in the sub-Himalayan tract, mainly from Nepal to Sikkim, Darjeeling hills,
Preparation of crude drug extracts

After collection of the plants, the leaves of both the plants were rinsed thoroughly in tap water and dried in shade for about 20 days under controlled temperature (25 ± 2 °C). Then the crude material was powdered, passed through a 40 mesh sieve and stored in a well closed container for further usage. Coarsely powdered material was powdered, passed through a 40 mesh sieve and stored in a well closed container for further usage.

Acute toxicity studies

The acute toxicity study for ethanolic extract of Baccara ramiflora and Microcos paniculata leaves were performed using albino rats as per OECD guidelines- 425 (OECD, 2008). The animals were fasted overnight before the experiment and maintained under standard conditions. All the extracts were administered orally in increasing dose and found safe up to dose of 2000mg/kg for all extracts.

Experimental animal and design

All rats were divided into 7 groups of 6 rats each:

Group I: Vehicle treated rats were kept on normal diet served as normal control and was orally given pure water for 15 days, and then intraperitoneally injected with 10ml/kg body weight isotonic 0.9% NaCl.

Group II: Rats orally received 30% alcohol (1.5 ml/rat / twice a day) served as toxic control for 15 days.

Group III: Rats orally received silymarin (25 mg/kg b. wt/day) served as standard and alcohol as group II, for 15 days.

Group IV: Rats orally received ethanolic extract Baccara ramiflora (EEBR1) (100 mg/kg b. wt / day) and alcohol as group II, for 15 days.

Group V: Rats orally received ethanolic extract of Baccara ramiflora (EEBR2) (200 mg/kg b. wt / day) and alcohol as group II, for 15 days.

Group VI: Rats orally received ethanolic extract of Microcos paniculata (EEMP1) (100 mg/kg b. wt / day) and alcohol as group II, for 15 days.

Group VII: Rats orally received ethanolic extract of Microcos paniculata (EEMP2) (200 mg/kg b. wt / day) and alcohol as group II, for 15 days.

Paracetamol- induced hepatotoxicity in rats

The experiment was conducted according to the modified procedures described previously. Paracetamol was dissolved in 0.5% CMC for oral administration.

All rats were divided into 7 groups of 6 rats each:

Group I: Vehicle treated rats were kept on normal diet served as normal control and was orally given pure water for 15 days,
and then intraperitoneally injected with 10ml/kg body weight isonic 0.9% NaCl.

**Group II:** Rats orally received paracetamol (750 mg/kg b. wt /day) for 15 days.

**Group III:** Rats orally received silymarin (25 mg/kg b. wt/day) served as standard and paracetamol as group II, for 15 days.

**Group IV:** Rats orally received ethanolic extract *Baccaurea ramiflora* (EEBR1) (100 mg/kg b. wt / day) and paracetamol as group II, for 15 days.

**Group V:** Rats orally received ethanolic extract of *Baccaurea ramiflora* (EEBR2) (200 mg/kg b. wt / day) and paracetamol as group II, for 15 days.

**Group VI:** Rats orally received ethanolic extract of *Microcos paniculata* (EEMP1) (100 mg/kg b. wt / day) and paracetamol as group II, for 15 days.

**Group VII:** Rats orally received ethanolic extract of *Microcos paniculata* (EEMP2) (200 mg/kg b. wt / day) and paracetamol as group II, for 15 days.

**Assessment of Liver Functions**

After the last dose-delivery, all rats were kept on starved condition for 24 hours, after that autopsied and blood was collected by cardiac puncture under ether anesthesia in heparinized tubes for biochemical investigation i.e. SGOT, SGPT, ALP and serum Bilirubin estimation of all the experimental rats. Blood was allowed to coagulate at 370C for 30 min and the serum was separated by centrifugation at 2500 rpm for 10 minutes. The liver of all the experimental animals were removed and washed with ice cold saline and processed immediately for histological investigation.

**Biochemical Estimation**

Serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), and serum bilirubin content were estimated by using commercially available kits from Span Diagnostic Ltd., Surat, India.

**Histopathological Study**

Liver tissue was dissected out and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 50 μ thickness processed in alcohol xylene series and were stained with alun haematoxylin and eosin (H-E) dye. The sections were examined microscopically for the evaluation of histopathological changes including cell necrosis, fatty change, hyaline regeneration and ballooning degeneration.

**Statistical analysis**

The statistical significance was evaluated using the student’s t-test. The values are expressed as mean ± SEM and p< 0.05 was considered significant in all comparisons.

### Table 1: Hepatoprotective Activity of *Baccaurea ramiflora* and *Microcos paniculata* in alcohol induced albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>93.4±2.008</td>
<td>69.05±2.595</td>
<td>74.57±2.607</td>
<td>0.285±0.0158</td>
</tr>
<tr>
<td>II</td>
<td>Toxic Control Alcohol</td>
<td>248.7±6.125</td>
<td>293.8±10.36</td>
<td>213.9±9.275</td>
<td>0.976±0.0323</td>
</tr>
<tr>
<td>III</td>
<td>Standard Silymarin (25mg/kg)</td>
<td>110.6±8.170</td>
<td>89.18±2.593</td>
<td>88.78±2.726</td>
<td>0.331±0.007***</td>
</tr>
<tr>
<td>IV</td>
<td>EEBR1 (100mg/kg)</td>
<td>170.5±5.229</td>
<td>190.1±6.261</td>
<td>181.8±5.141</td>
<td>0.60±0.008***</td>
</tr>
<tr>
<td>V</td>
<td>EEBR2 (200mg/kg)</td>
<td>144.3±5.364</td>
<td>124.8±4.086</td>
<td>104.7±6.873</td>
<td>0.34±0.008**</td>
</tr>
<tr>
<td>VI</td>
<td>EEMP1 (100mg/kg)</td>
<td>179.8±5.158</td>
<td>203.8±5.988</td>
<td>188.8±5.247</td>
<td>0.66±0.018**</td>
</tr>
<tr>
<td>VII</td>
<td>EEMP2 (200mg/kg)</td>
<td>157.7±5.172</td>
<td>139.8±4.672</td>
<td>124.4±3.983</td>
<td>0.44±0.01**</td>
</tr>
</tbody>
</table>

Values are given as Mean ± SEM (n=6 rats). * Significant at P<0.005 when alcohol treated compared with control group. **Significant at P<0.01 when alcohol treated compared with control group. ***Significant at P<0.001 when alcohol treated compared with control group.

### Table 2: Hepatoprotective Activity of *Baccaurea ramiflora* and *Microcos paniculata* in paracetamol induced albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>90.3±1.158</td>
<td>66.8±3.15</td>
<td>71.9±2.87</td>
<td>0.25±0.022</td>
</tr>
<tr>
<td>II</td>
<td>Toxic Control Paracetamol (750mg/kg)</td>
<td>244.5±10.93</td>
<td>292.6±5.98</td>
<td>211.5±6.46</td>
<td>0.95±0.012</td>
</tr>
<tr>
<td>III</td>
<td>Standard Silymarin (25mg/kg)</td>
<td>109.4±9.32</td>
<td>87.8±1.46</td>
<td>86.5±1.75</td>
<td>0.30±0.003***</td>
</tr>
<tr>
<td>IV</td>
<td>EEBR1 (100mg/kg)</td>
<td>168.4±7.38</td>
<td>187.9±5.13</td>
<td>178.1±5.45</td>
<td>0.57±0.003***</td>
</tr>
<tr>
<td>V</td>
<td>EEBR2 (200mg/kg)</td>
<td>142.3±7.72</td>
<td>123.4±7.10</td>
<td>102.9±5.48</td>
<td>0.31±0.004***</td>
</tr>
<tr>
<td>VI</td>
<td>EEMP1 (100mg/kg)</td>
<td>174.1±5.81</td>
<td>200.5±6.22</td>
<td>186.5±5.77</td>
<td>0.64±0.006**</td>
</tr>
<tr>
<td>VII</td>
<td>EEMP2 (200mg/kg)</td>
<td>156.2±4.73</td>
<td>137.8±4.12</td>
<td>122.3±8.11</td>
<td>0.41±0.005**</td>
</tr>
</tbody>
</table>

Values are given as Mean ± SEM (n=6 rats). * Significant at P<0.005 when paracetamol treated compared with control group. **Significant at P<0.01 when paracetamol treated compared with control group. ***Significant at P<0.001 when paracetamol treated compared with control group.

- **SGOT:** Serum glutamine oxaloacetate transaminase; **SGPT:** Serum glutamine pyruvate transaminase; **ALP:** Alkaline phosphatase; **EEBR:** Ethanolic extract of *Baccaurea ramiflora*; **EEMP:** Ethanolic extract of *Microcos paniculata*.
Graph 1. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on AST activity of alcohol-induced hepatotoxic albino rats

Graph 2. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on ALT activity of alcohol-induced hepatotoxic albino rats

Graph 3. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on ALP activity of alcohol-induced hepatotoxic albino rats

Graph 4. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Total Bilirubin activity of alcohol-induced hepatotoxic albino rats

Graph 5. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on AST activity of paracetamol-induced hepatotoxic albino rats

Graph 6. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on ALT activity of paracetamol-induced hepatotoxic albino rats
Graph 7. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on ALP activity of paracetamol-induced hepatotoxic albino rats

Graph 8. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Total Bilirubin activity of paracetamol-induced hepatotoxic albino rats

**Figure 1:** Histopathological changes of liver in alcohol induced hepatotoxicity
(a) Normal control (b) 30% ethyl alcohol 1.5 ml/rat (c) Silymarin 25mg/kg (d) EEBR 100mg/kg (e) EEBR 200 mg/kg (f) EEMP 100mg/kg (g) EEMP 200mg/kg
**Figure 2**: Histopathological changes of liver in paracetamol induced hepatotoxicity
(a) Normal control (b) Paracetamol 750mg/kg (c) Silymarin 25mg/kg (d) EEBR 100mg/kg (e) EEBR 200 mg/kg (f) EEMP 100mg/kg (g) EEMP 200mg/kg

**Table 3**: Histopathological observations of liver in alcohol-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>MICROSCOPY</th>
<th>NC</th>
<th>TC</th>
<th>STD</th>
<th>EEBR1</th>
<th>EEBR2</th>
<th>EEMP1</th>
<th>EEMP2</th>
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<tbody>
<tr>
<td>Apoptosis</td>
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<tr>
<td>Ballooning Hepatocytes</td>
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<td>+</td>
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<tr>
<td>Bile duct proliferation</td>
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<tr>
<td>Central vein congestion</td>
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<tr>
<td>Centrilobular necrosis</td>
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<td>Cirrhosis</td>
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<td>Degeneration</td>
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<td>Fibrosis</td>
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<td>Hepatocellular dysplasia</td>
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<td>Inflammation</td>
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<tr>
<td>Kupffer cell hyperplasia</td>
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<td>+</td>
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<td>+</td>
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<td>Necroinflammatory necrosis</td>
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<td>Portal truditis</td>
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<tr>
<td>Regenerative nodules</td>
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<td>-</td>
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<tr>
<td>Sinusoidal congestion</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Spotty necrosis</td>
<td>-</td>
<td>+</td>
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</table>
RESULTS
Phytochemical study
All the extracts subjected for phytochemical investigation revealed the presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids.

Acute toxicity studies
The extracts were found to be safe in the dose used did not show any signs and symptoms of toxicity and there was no mortality up to a dose of 2000 mg/kg, b.w. Hence 100 and 200 mg/kg b.w. p.o. were selected for the activity.

Effect of extracts on AST, ALT, ALP and total bilirubin
The results of hepatoprotective effect of extracts on alcohol and paracetamol intoxicated rats are shown in Table 1, 2. The elevated levels of serum AST, ALT, ALP and total bilirubin were significantly reduced in the animals’ groups treated with various extracts. Treatment with ethanolic extract showed highly significant activity (P<0.001) with maximum inhibition. So, the ethanol treated extract group was superior to the other extracts but not as effective as silymarin.

DISCUSSION
Hepatic cells appear to participate in a variety of enzymatic metabolic activities and both alcohol and paracetamol produced marked liver damage at the given doses as expected.23 Formation of reactive oxygen species (ROS) oxidative stress and hepatocellular injury have been implicated to alcoholic liver disease. It has been documented that Kupffer cells are the major sources of ROS during chronic alcohol consumption, and these are primed and activated for enhanced formation of pro-inflammatory factors.24

Additionally, alcohol-induced liver injury has been associated with increased amount of lipid peroxidation.25 It may thus be plausible that in our study, loss of membrane structure and integrity because of lipid peroxidation was accompanied with the elevated levels of marker enzymes like-AST, ALT, ALP and total bilirubin. Indeed, Baccaurea ramiflora and Microcos paniculata supplementation in our study, was potentially effective in blunting lipid peroxidation, suggesting that the extract possibly has antioxidant property to reduce ethanol-induced membrane lipid peroxidation and thereby to preserve membrane structure might be due to the presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids.26 Paracetamol in larger doses produces liver necrosis after undergoing bio-activation to a toxic electrophile, N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P-450 mono-oxygenase.27 NAPQI binds to macromolecules and cellular proteins, and also oxidizes lipids and alters homeostasis. The supplementation of Baccaurea ramiflora and Microcos paniculata extract brought down to elevated levels of AST, ALT, ALP and total bilirubin. These biochemical restorations may be due to the inhibitory effects on cytochrome P-450 or /and promotion of its glucuronidation.28 Histopathological analysis of the liver sections is in good agreement with biochemical changes.

Therefore, on the basis of our results, the possible mechanism of hepatoprotective effect through antioxidant activity of Baccaurea ramiflora and Microcos paniculata might be due to the presence of alkaloids, glycosides, tannins, saponins, proteins, flavonoids and other active constituents.

CONCLUSION
Our main aim was to identify Hepatoprotective formulation which could be safe with no interactions and useful in hepatoprotection. Biochemical examination revealed a dose dependent significant decrease in the levels of SGOT, SGPT, ALP, Total Bilirubin, an increase in the weight of liver of leaf extracted treated animals against alcohol and paracetamol provoked hepatotoxicity. Histopathological studies supported the findings by viewing mild hepatic degeneration with absence of necrosis in similarity with the normal control. In accordance with these results, it may be confirmed due to the presence of phytoconstituents such as flavonoids, alkaloids and glycosides which are present in the ethanolic extract could be considered as, responsible for the significant hepatoprotective activity. In conclusion, it can be said that the ethanolic extract of Baccaurea ramiflora and Microcos paniculata exhibited a hepatoprotective effect against alcohol and paracetamol induced hepatotoxicity. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant.
REFERENCES


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