Antitumor and antioxidant potential of *Tragia Plukenetii* R.Smith on Ehrlich ascites carcinoma in mice

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This investigation aims to evaluate the antitumour and antioxidant potential of the ethanol extract of *Tragia Plukenetii* R.Smith (ETP) on Ehrlich ascites carcinoma (EAC) tumor model. Tumor was induced in mice by intraperitoneal injection of EAC cells \(2 \times 10^6\) cells/mouse. Ethanol extract of *T. Plukenetii* (ETP) was administered to the experimental animals at the dose levels of 100, 200 and 300 mg/kg/day after 24 h of tumour inoculation. The antitumour effect of ETP was evaluated by assessing *in vitro* cytotoxicity, survival time, hematological and antioxidant parameters. Oral administration of ETP increased the survival time of the EAC bearing mice. The ETP brought back the altered levels of the hematological and antioxidant parameters in a dose dependent manner in EAC bearing mice. The results were comparable to that of the result obtained from the animals treated with the standard drug 5-fluorouracil (20 mg/kg.bw). Thus present study revealed that ETP possessed significant antitumor and antioxidant activity.

**Key words:** *Tragia Plukenetii*, Ehrlich ascites carcinoma, hematological parameters, antioxidant, tumour growth response.

**INTRODUCTION**

Drug discovery from the medicinal plants has played an important role in the treatment of cancer and indeed, most new applications of plant secondary metabolites and their derivatives over the last half-century have been made towards combating cancer (Newman et al., 2003). *Tragia Plukenetii* R.Smith (Euphorbiaceae) is an erect or climbing shrub distributed widely throughout India. The plant is traditionally used for the treatment of sore tongue and it also has diaphoretic action. This study was undertaken to evaluate the antitumor and antioxidant potential of the ethanol extract of *T. Plukenetii* (ETP) against the Ehrlich ascites carcinoma (EAC) tumor model.

**MATERIALS AND METHODS**

**Plant collection**

*T. plukenetii* R.Smith (Euphorbiaceae) was collected in and around Trichy District, Tamil Nadu. Plant material was identified with the help of "The Flora of the Tamil Nadu Carnatic". A voucher specimen [No: RHT 8279] was deposited in the Rapinat Herbarium, St.Joseph’s College, Trichy, Tamil Nadu.

**Preparation of ethanol extract**

The plant material was shade dried and pulverized. Alcohol extract of the coarsely powdered material was prepared employing Soxhlet method with 200 ml of ethanol for 7 h and the extract was concentrated to 5 g. The extract was dissolved in dimethylsulphoxide (<0.1%) before administration.

**Animals**

Adult Swiss male albino mice (20-25 g) were procured from Tamil-
nada Veterinary College, Chennai, Tamilnadu and used throughout the study. They were housed in microlan boxes in a controlled environment (temperature 25±2°C and 12 h dark and light cycle) with standard diet and water ad libitum. The study was conducted after obtaining institutional animal ethical committee clearance (Reg.No:790/03/ac/CPCSEA).

Cells
EAC cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur. They were maintained by weekly intraperitoneal inoculation of 10⁶ cells/mouse (Ramakrishna et al., 1984).

Effect of ETP on in vitro cytotoxicity
Short-term cytotoxicity was assessed by incubating 1x10⁶ EAC cells in 1 ml phosphate buffer saline with varying concentration of ETP at 37°C for 3 h. The viability of the cells was determined by the Tryphan blue exclusion method.

Effect of ETP on tumor growth response
Animals were inoculated with 2 x 10⁶ cells/mouse on day ‘0’ and treatment with ETP started 24 h after inoculation, at a dose of 300 mg/kg/day, p.o. The control group was treated with same volume of 0.9% sodium chloride solution. All the treatments were given for 14 days. The mean survival time (MST) of each group, consisting of 10 mice was noted. The antitumor efficacy of ETP was calculated using the following equation (Mazumder et al., 1997; Gupta et al., 2000):

\[ \text{MST} = \frac{\text{Day of First Death} + \text{Day of last death}}{2} \]

\[ \text{ILS} \% = \left[ \frac{\text{Mean survival time of treated group}}{\text{Mean survival time of control group}} - 1 \right] \times 100 \]

Antitumor activity
Male Swiss albino mice were divided into 6 groups (n = 6). All the groups were injected with EAC cells (0.2 ml of 2x10⁶ cells/mouse) intraperitoneally (Gupta et al., 2004) except Group I. This was taken as day Zero.

- Group I - Normal control.
- Group II - Disease Control, EAC cell line (2x10⁶ cell mouse).
- Group III - EAC cell line (2x10⁶ cells) treated with 100mg/kg p.o. of ETP.
- Group IV - EAC cell line (2x10⁶ cells) treated with 200mg/kg p.o. of ETP.
- Group V - EAC cell line (2x10⁶ cells) treated with 300mg/kg p.o. of ETP.
- Group VI - EAC cell line (2x10⁶ cells) treated with standard [5-fluorouracil (20 mg/kg i.p.)]

After 14 days of treatment, animals from each group were sacrificed by retro orbital plexus method to evaluate the antitumor and antioxidant potential of ETP (Kavimani and Manisenthil kumar, 2000).

Hematological studies
Hemoglobin content, red blood cells count (RBC) and white blood cells count (WBC) were measured from freely flowing tail vein blood from all the groups.

Antioxidant studies
The liver was excised out from the sacrificed animals rinsed in ice-cold normal saline followed by cold 0.15 M Tris-HCl (pH 7.4) and dried. A 10% (w/v) homogenate was prepared in 0.15 M Tris-HCl buffer, a portion was utilized for the estimation of lipid peroxidation and a second portion after precipitating proteins with TCA, was used for the estimation of glutathione (Ohkawa et al., 1979). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant that obtained was used for the estimation of super oxide dismutase (SOD) and catalase (Kakkas et al., 1984 and Aebi and Burgmeyer, 1983).

Statistical analysis
Values were recorded as mean ± S.E.M. The data were analyzed by student’s t test; P values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION
It is evident from Table 1 that the death rate of EAC cells increases with increase in the concentration of the ETP. ETP was found to be cytotoxic to EAC cells. The effect of ETP on the survival of tumor bearing mice is shown in Table 2. The MST for the control group was 17.42±0.17 days, whereas it was 31.33±0.13 days and 33.60±0.12 days for the groups treated with ETP (300 mg/kg/day p.o.) and 5-FU (20 mg/kg/day i.p.), respectively. The percentage increase in the lifespan of tumor bearing mice treated with ETP (300 mg/kg/day p.o.) and 5-FU (20 mg/kg/day i.p.) was found to be 82.52 and 92.88 respectively as compared to the disease control group. Hemato-

<table>
<thead>
<tr>
<th>Concentration of ETP (µg/ml)</th>
<th>No. of viable cells</th>
<th>Viable cells (%)</th>
<th>No. of dead cells</th>
<th>Dead cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>93</td>
<td>96.87</td>
<td>3</td>
<td>3.13</td>
</tr>
<tr>
<td>150</td>
<td>60</td>
<td>68.18</td>
<td>28</td>
<td>31.82</td>
</tr>
<tr>
<td>300</td>
<td>43</td>
<td>45.74</td>
<td>51</td>
<td>54.26</td>
</tr>
<tr>
<td>500</td>
<td>26</td>
<td>25.74</td>
<td>75</td>
<td>74.26</td>
</tr>
<tr>
<td>1000</td>
<td>15</td>
<td>15.79</td>
<td>80</td>
<td>84.21</td>
</tr>
</tbody>
</table>

| Table 1. In vitro cytotoxicity studies of Tragia Plukenetii R.Smith (ETP) on Ehrlich ascites carcinoma (EAC) cells. |
Table 2. Effect of *Tragia Plukenetii* R.Smith (ETP) treatment on the survival of Ehrlich ascites carcinoma (EAC) bearing mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Body weight (g)</th>
<th>MST (Days)</th>
<th>%ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II (Disease control)</td>
<td>28.1±0.14</td>
<td>17.42±0.17</td>
<td>-</td>
</tr>
<tr>
<td>Group III ETP (100 mg/Kg p.o.)</td>
<td>27.32±0.12*</td>
<td>22.51±0.16*</td>
<td>29.41</td>
</tr>
<tr>
<td>Group IV ETP (200 mg/Kg p.o.)</td>
<td>25.93±0.14**</td>
<td>25.13±0.17**</td>
<td>47.05</td>
</tr>
<tr>
<td>Group V ETP (300 mg/Kg p.o.)</td>
<td>24.7±0.16***</td>
<td>31.33±0.13***</td>
<td>82.52</td>
</tr>
<tr>
<td>Group VI 5-FU (20 mg/Kg i.p.)</td>
<td>22.81±0.17***</td>
<td>33.60±0.12***</td>
<td>92.88</td>
</tr>
</tbody>
</table>

MST = mean survival time; ILS = increased life span.
Statistically significant compared to Group II; *P<0.05. **P<0.01. ***P<0.001.

Table 3. Effect of *Tragia Plukenetii* R.Smith (ETP) on hematological parameters of Ehrlich ascites carcinoma (EAC) bearing mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hemoglobin (%)</th>
<th>RBC (1x10^6 cells/mm³)</th>
<th>WBC (1x10³ cells/mm³)</th>
<th>Packed cell volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal Control)</td>
<td>13.2±1.52</td>
<td>6.4±5.61</td>
<td>9.4±1.02</td>
<td>0.2±0.86</td>
</tr>
<tr>
<td>Group II (Disease Control)</td>
<td>10.3±1.39^AAA</td>
<td>2.9±9.36^AAA</td>
<td>18.0±3.46^AAA</td>
<td>2.7±0.80^AAA</td>
</tr>
<tr>
<td>Group III ETP (100mg/Kg p.o.)</td>
<td>11.1±1.05*</td>
<td>3.8±1.42*</td>
<td>15.9±0.97*</td>
<td>1.4±0.24*</td>
</tr>
<tr>
<td>Group IV ETP (200mg/Kg p.o.)</td>
<td>11.9±1.88**</td>
<td>5.0±2.98**</td>
<td>12.1±2.11**</td>
<td>0.9±0.77**</td>
</tr>
<tr>
<td>Group V ETP (300mg/Kg p.o.)</td>
<td>12.6±1.74***</td>
<td>5.7±2.57***</td>
<td>10.5±1.51***</td>
<td>0.3±0.65***</td>
</tr>
<tr>
<td>Group VI 5-FU (20mg/Kg i.p.)</td>
<td>13.0±0.95***</td>
<td>6.2±3.32***</td>
<td>11.1±0.88***</td>
<td>0.3±0.91***</td>
</tr>
</tbody>
</table>

RBC = Red blood cells; WBC = white blood cells.
Statistically significant compared to Group I; ^AAA P<0.001.
Statistically significant compared to Group II; *P<0.05. **P<0.01. ***P<0.001.

Table 4. Effect of *Tragia Plukenetii* R.Smith (ETP) on antioxidant parameters of Ehrlich ascites carcinoma (EAC) bearing mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO (nmol MDA/Mg protein)</th>
<th>Glutathione (mg/g wet tissue)</th>
<th>Catalase (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal Control)</td>
<td>0.96±0.031</td>
<td>2.35±1.53</td>
<td>26.4±0.023</td>
<td>4.49±3.74</td>
</tr>
<tr>
<td>Group II (Disease Control)</td>
<td>3.26±0.039^AAA</td>
<td>0.91±1.333^AAA</td>
<td>9.67±0.114^AAA</td>
<td>1.56±1.01^AAA</td>
</tr>
<tr>
<td>Group III ETP (100mg/Kg p.o.)</td>
<td>2.59±0.031*</td>
<td>1.28±2.056*</td>
<td>13.7±0.009*</td>
<td>2.28±3.39*</td>
</tr>
<tr>
<td>Group IV ETP (200mg/Kg p.o.)</td>
<td>2.42±0.075**</td>
<td>1.77±1.872**</td>
<td>19.4±0.055**</td>
<td>2.47±4.00**</td>
</tr>
<tr>
<td>Group V ETP (300mg/Kg p.o.)</td>
<td>1.33±0.015***</td>
<td>2.11±0.931***</td>
<td>21.3±0.063***</td>
<td>3.78±8.32***</td>
</tr>
<tr>
<td>Group VI 5-FU (20mg/Kg i.p.)</td>
<td>1.27±0.041***</td>
<td>2.09±0.977***</td>
<td>21.9±0.011***</td>
<td>3.62±3.17***</td>
</tr>
</tbody>
</table>

LPO = lipid peroxide; SOD = super oxide dismutase.
Statistically significant compared to Group I; ^AAA P<0.001.
Statistically significant compared to Group II; *P<0.05. **P<0.01. ***P<0.001.

The logical parameters of tumor bearing mice on day 14 showed significant changes when compared with normal control (Group I). The total WBC count and PCV were found to increase with a reduction in the hemoglobin content of RBC (Table 3). At the same time interval, ETP (300 mg/kg/day p.o.) treatment changed these altered parameters to near normal. Antioxidant parameters of tumor bearing mice were altered during diseased condition and brought back to normal level after the treatment with ETP (300 mg/kg/day p.o.) (Table 4). The antioxidant potential of ETP was evaluated by estimating the amount of parameters like super oxide dismutase (SOD), lipid peroxide (LPO), reduced glutathione, catalase from the liver tissues of EAC bearing mice. Generally, the result obtained at the dose level of 300 mg/kg.bw was highly significant and comparable to that of Standard drug.

The present study was planned to evaluate the anti-tumor potential and antioxidant status of ETP in EAC bearing mice. The *in vitro* cytotoxicity study revealed that the ETP is toxic to the EAC cells as there is an increase in the number of cells stained with tryphan blue dye with
the increase in concentration of ETP (Clarkson and Burchenal, 1965). The reliable criterion for assessing the value of any anticancer drug is the prolongation of the life span of animals. It is observed that ETP increased the life span of the EAC bearing mice by inhibiting the activity of the EAC cells. The hematological parameters revealed considerable changes leading to toxic effect in mice treated with ETP. After 14 days of inoculation, ETP was able to reverse these changes in hematological parameters and could reduce the toxic effects (Price et al., 1958; Hogland, 1982).

The implication of free radicals in tumor is well documented (Ravid and Korean, 2003; Feng et al., 2001). Lipid peroxide, an autocatalytic free radical chain propagation reaction, is known to be associated with pathological condition of a cell. The presence of tumor is known to affect many functions of the vital organs, especially the liver. This leads to an increase in the level of MDA (malondialdehyde), end product of lipid peroxide, in disease control group (Sinclair et al., 1990). It has been reported that a decrease in SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver of EAC bearing mice (Sun et al., 1989). Glutathione and catalase were also involved in the free radial scavenging activity. There is a reduction in the levels of the scavengers as a result of tumour growth in disease control animals. Treatment with ETP brought back the levels of these scavenges in a dose dependant manner and reduced the level of LPO. The findings were compared to that of the standard drug 5-flourouracil. The free radical hypothesis also supported the fact that the ETP possesses significant antitumour and antioxidant potential against EAC bearing mice.

REFERENCES


HEPATOPROTECTIVE ACTIVITY OF CAESALPINIA BONIQUELLA SEEDS ON PARACETAMOL INDUCED HEPATOTOXICITY IN MALE ALBINO RATS

ABSTRACT
Caesalpinia boniucella - seeds, an Ayurvedic drug source is studied for its hepatoprotective potentials and the results obtained are discussed. In the present work Hepatotoxicity is induced using Paracetamol. Animal model selected are male Albino rats. The alcohol extract of Caesalpinia boniucella seeds were prepared and administered at various dose levels (100, 150 and 300 mg/kg) to the induced rats. After 15 days the rats were sacrificed by cervical decapitation and the blood was collected to estimate the amount of Protein, Bilirubin, SGOT, SGPT and ALP. The liver homogenate was used to assess the activities of the membrane bound ATPases. The altered levels of the parameters were brought back to normal on treatment. From the results it is proved that the plant possess significant hepatoprotective activity at a dose level of 300 mg/kg body weight.

Key-words: Caesalpinia boniucella, Hepatoprotective, Paracetamol, Total protein, Bilirubin, SGOT, SGPT, ALP, Na+K+ ATPase, Ca+2 ATPase and Mg+2 ATPase.

INTRODUCTION
Liver diseases are a large public health problem in the world. Towards these pathologies which may appear with multiple and diversified causes and appearances, modern medicine does not find any curative treatments. Some oriental medicinal plants used in the treatment of hepatobiliary pathologies, show that some of them have a measurable hepatoprotective activity.

There is no rational therapy available for treating liver disorder and is still a challenge to the modern medicine. The modern medicines have little to offer for alleviation of hepatic ailments whereas most important representatives are of Phytoconstituents.

Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the populations for the treatment of liver diseases (jaundice, liver gallstones, hepatitis, etc). However, there weren’t enough scientific investigations on the hepatoprotective activities conferred to these plants.

Seeds contain caesalpins, bonducin, bonducellin and borgenin (vakerin). A novel spermidine alkaloid, caesalpinine A (C20H34O8N2) is also present. The drug exhibits astringent, anti-pyretic, antidiabetic and anti-fatigue effects in rats. It is used as a rejuvenator and there is no side effect reported on usage of this as medicine.

The present study deals with hepatoprotective potential of Caesalpinia boniucella seeds.

MATERIALS AND METHODS
Preparation of Extract
The plant material (seed) was collected from Rumi Herbals, Srirangam, Trichy-6. The seeds were identified and authenticated with RAPINAT Herbarium of St. Joseph’s college, Trichy. The seeds were air dried under shadlow for about 4 days and they were powdered in an electrical blender. About 100gm of the powder was extracted employing Soxhlet method with 200ml of ethanol for 7 hours and the extract was concentrated to 5gm.

Induction of Liver Toxicity by Paracetamol
The body weight of animals at the time of experimentation ranged from 150 – 200 g. The optimum dose of Paracetamol was found to be 1g / kg body weight. Paracetamol was dissolved in 1% saline. Approximately 1.0 mL (150mg) was administrated orally to the albino rats for 15-days to induce liver toxicity.
Table I: Hepatoprotective activity of *Caesalpinia bonducella* on Paracetamol induced hepatotoxicity in male albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>X''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
</tr>
<tr>
<td>(g/dL)</td>
<td>5.64±0.162</td>
<td>4.689±0.128</td>
<td>4.893±0.129</td>
<td>4.999±0.142</td>
<td>5.462±0.143</td>
<td>5.456±0.149</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>X''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
</tr>
<tr>
<td>(mg/dL)</td>
<td>1.0±0.141</td>
<td>1.983±0.172</td>
<td>1.776±0.163</td>
<td>1.252±0.159</td>
<td>1.033±0.142</td>
<td></td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>X''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
</tr>
<tr>
<td>1.009±0.144</td>
<td>1.835±0.79</td>
<td>6.9125±1.09</td>
<td>5.2315±1.09</td>
<td>2.326±0.93</td>
<td>1.902±0.85</td>
<td>1.914±0.81</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>X''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
</tr>
<tr>
<td>1.976±0.61</td>
<td>8.4972±1.74</td>
<td>6.3213±1.12</td>
<td>3.856±0.81</td>
<td>2.032±0.67</td>
<td>2.001±0.69</td>
<td></td>
</tr>
<tr>
<td>ALP (KU/L)</td>
<td>X''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
<td>Y''</td>
</tr>
<tr>
<td>4.4±0.393</td>
<td>10.56±0.787</td>
<td>7.32±0.516</td>
<td>5.13±0.492</td>
<td>4.71±0.452</td>
<td>4.59±0.413</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant variation

X as compared to Group I: Y as compared to Group II; *P<0.05, **P<0.01, ***P<0.001.

The results obtained at the dose level of 300 mg/Kg b.w are highly significant and comparable to that of standard drug (Silymarin).

**Experimental Design:**

Control animals received only normal food and water *ad libitum*. Whereas hepatotoxic control group was treated with Paracetamol in saline (1 g/Kg) orally for 15 days. Standard group received silymarin, a commercially available hepatoprotective drug at a dose level of 2.5 mg/Kg bodyweight P.O. daily for 15 days. Treatment groups were administered with test drug at dose levels of 100, 150 and 300 mg/Kg orally for 15 days, using oral gague. After 15 days blood was collected by cervical decapitation in clean dry test tubes. Liver was dissected out from the animals. The blood was centrifuged at 3000 rpm for 5 minutes to separate the serum and used for estimation of total protein, total bilirubin, SGOT, SGPT and ALP.

Liver homogenate was used for estimation of Na⁺/K⁺ ATPase, Ca⁺⁺ ATPase and Mg⁺⁺ ATPase. Values are expressed as Mean ± SD for 6 rats in each group.

**Group I:** Control

**Group II:** Animals treated with Paracetamol dissolved in normal saline at a dosage of 1g/Kg body weight for 15 days.

**Group III:** Animals treated as group 2 along with seed extract (100 mg/kg b.w) for 15 days.

**Group IV:** Animals treated as group 2 along with seed extract (150 mg/kg b.w) for 15 days.

**Group V:** Animals treated as group 2 along with seed extract (300 mg/kg b.w) for 15 days.

**Group VI:** (Standard group)

Animals treated as group 2 along with Silymarin (2.5 mg/kg b.w) for 15 days.

**Statistical Analysis**

The mean value ± SEM were calculated for each parameter. Results were subjected to statistical analysis using Student’s *t* test.

**RESULTS**

As seen in Table-1 Paracetamol decreased total proteins and increased Serum Bilirubin, SGOT, SGPT and ALP. Treatment with *Caesalpinia bonducella* seed extract increased Total Proteins and reduced serum Bilirubin, SGOT, SGPT and ALP.
### Table II: Hepatoprotective activity of *Caesalpinia bonduc* on Paracetamol induced hepatotoxicity in male albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺/K⁺ ATPase</td>
<td>X⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>0.0052</td>
<td>0.0053</td>
</tr>
<tr>
<td></td>
<td>±0.0003</td>
<td>±0.0002</td>
<td>±0.0002</td>
<td>±0.0002</td>
<td>±0.0002</td>
<td>±0.0003</td>
</tr>
<tr>
<td>Ca²⁺ ATPase</td>
<td>X⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>0.0054</td>
<td>0.0054</td>
</tr>
<tr>
<td></td>
<td>±0.00027</td>
<td>±0.00021</td>
<td>±0.00023</td>
<td>±0.00023</td>
<td>±0.00023</td>
<td>±0.00025</td>
</tr>
<tr>
<td>Mg²⁺ ATPase</td>
<td>X⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>Y⁺⁺⁺⁺</td>
<td>0.0066</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td>±0.00038</td>
<td>±0.00028</td>
<td>±0.00033</td>
<td>±0.00033</td>
<td>±0.00036</td>
<td>±0.00038</td>
</tr>
</tbody>
</table>

Statistically significant variation; X as compared to group I; Y as compared to group II; *P*<0.05, **P*<0.01, ***P*<0.001.

The results obtained at the dose level of 300 mg/kg.b.w are highly significant and comparable to that of standard drug (Silymarin).

As seen in Table - 2 Paracetamol reduces Na⁺/K⁺ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase. Treatment with *Caesalpinia bonduc* seed extract elevated the reduced levels of Na⁺/K⁺ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase enzymes ‡ significantly.

*Caesalpinia bonduc* seed extracts at the dose level of 300g/Kg body weight exhibited significant hepatoprotective activity comparable to that of Silymarin.

**DISCUSSION**

In the present study hepatoprotective action of a plant drug substance in an animal model is assessed using biochemical parameters. Paracetamol (in over dose), a hepatotoxicant used to produce acute liver injury in rats. Paracetamol gets converted into N-acetyl-p-benzoquinoneimine (NAPQI)⁴ in liver by action of cytochrome P-450 and alters the functional integrity of hepatic mitochondria leading to Liver damage. When hepatic cell membrane is damaged, the enzymes SGOT, SGPT and ALP⁵ which are normally located in the cytosol, leak into circulation from hepatocytes. As a result, serum levels of SGPT, SGOT and ALP increases. Similarly Paracetamol decreases the membrane bound enzymes viz., Na⁺/K⁺ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase.⁶ Hyperbilirubinemia, seen in liver injury, can result from impaired hepatic uptake of unconjugated Bilirubin. Paracetamol induced liver injury results in decreased serum total protein level and an elevated level of SGPT, SGOT,ALP⁴ and Bilirubin and reduced level of membrane bound enzymes and total proteins. Treatment with *Caesalpinia bonduc* seed extract showed dose dependent reduction in the elevated levels of SGPT, SGOT, ALP and Bilirubin and increased membrane bound enzymes and total proteins. *Caesalpinia bonduc* seed extract, control the damaging effect of Paracetamol on hepatocyte membrane. These biochemical restorations may be due to inhibitory effect of *Caesalpinia bonduc* seed extract on cytochrome P-450⁴.⁷ To sum up present findings suggest that the alcoholic seed extract of *Caesalpinia bonduc* can protect liver against the paracetamol induced toxicity.

**REFERENCES**
