Evaluation of the hepatoprotective activity of various extracts of *Dyschoriste littoralis* Nees on paracetamol-induced hepatotoxicity rats

Ravi Teja P D\(^1\)\(^2\), Balakrishnan K\(^2\), Kottai Muthu A\(^*\)\(^1\)

\(^1\)Department of Pharmacy, Annamalai University, Annamalainagar-608 002, Tamilnadu, India
\(^2\)Seshachala College of Pharmacy, Puttur, Chittoor, Andhra Pradesh, India

**Article History:**
Received on: 25.07.2019
Revised on: 18.10.2019
Accepted on: 25.10.2019

**Keywords:**
D. littoralis, Paracetamol, hepatic markers, acute toxicity, hepatotoxicity

**ABSTRACT**

The current investigation was to examine the hepatoprotective potential of *D. littoralis* Nees. (family Acanthaceae) On paracetamol-induced hepatotoxicity in rats. The aerial parts of *D. littoralis* Nees. The powder was concentrated with various solvents (PE, EA, and methanol) through Soxhlet concentrates and different crude concentrates utilized for hepatoprotective activity. Hepatotoxicity was induced by paracetamol (2g/kg b.wt.) on the 5\(^{th}\) day of the investigational period and given orally. Paracetamol-induced rats to exhibit elevated activities of liver enzymes such as SGOT, SGPT, ALP, gamma-glutamyltranspeptidase (GGT), creatinine, urea, total bilirubin, total cholesterol & triglycerides in serum. Furthermore, Oral administration of the ethyl acetate concentrates of *D. littoralis* (200 mg/ kg b.wt.) given rats were significant reduction the level of SGOT, SGPT, ALP, gamma-glutamyltranspeptidase (GGT) creatinine, urea, total bilirubin, total cholesterol & triglycerides when compared to other concentrates. They also significantly elevated the concentration of complete protein and albumin when compared to other concentrates. Thus, results suggested that ethyl acetate concentrates of *D. littoralis* could afford better hepatoprotective activity against paracetamol-induced hepatotoxicity rats.

\(^*\)Corresponding Author
Name: Kottai Muthu A
Phone: +919443171712
Email: akottaimuthu@gmail.com

ISSN: 0975-7538
DOI: [https://doi.org/10.26452/ijrps.v11i1.1820](https://doi.org/10.26452/ijrps.v11i1.1820)

© 2020 | All rights reserved.

**INTRODUCTION**

Synthetic drug-induced hepatic toxicity is a substantial clinical issue in the worldwide. Most of the drug-induced hepatic toxicity and acute liver failures occur due to either unplanned high doses of paracetamol. (Jaeschke et al., 2014). Paracetamol acts as an antipyretic and analgesic agent used in the clinical field. During beneficial treatments, paracetamol is metabolized by glucuronidation or sulfation by the cytochrome p450 structure into the reactive metabolite NAPQI. Under normal conditions, NAPQI is rapidly changed to harmless GSH. While a higher amount of paracetamol, elevated concentration of NAPQI reacts with hepatic proteins and leads to liver injury (Woolbright and Jaeschke, 2017; Lancaster et al., 2015). Paracetamol-induced liver damage can be considered in animal models, and most mechanisms are translatable to humans. (Woolbright and Jaeschke, 2017). Paracetamol-induced liver toxicity has been a most important problem for many years, and various strategies have been investigated, together with utilizing the natural bioactive compounds with hepatoprotective activity (Eugenio-Pérez et al., 2016; Ekpenyong et al., 2015).

*Dyschoriste littoralis* Nees. (family Acanthaceae) Leaves were used for the treatment of wounds...
Dyschoriste genus were generally known as snake herb. *D. littoralis* used as a treatment of pain, fever, and inflammation. *D. littoralis* was used for the treatment of severe coughs along with administered ginger. *D. littoralis* leaves were used for asthma in the form of cigarettes (Awan et al., 2014) and *D. littoralis* has used the treatment of diarrhea and dysentery. (Sharma and Alagarsamy, 2013). *D. littoralis* is was used for the treatment of severe coughs along with administered ginger. *D. littoralis* is leaves were used for asthma in the form of cigarettes (Awan et al., 2014) and *D. littoralis* is has used the treatment of diarrhea and dysentery. (Sharma and Alagarsamy, 2013). *D. littoralis* is have various activities like antimicrobial (Sharma and Alagarsamy, 2013; Henry and Winkelman, 1974) and wound healing. (Subha et al., 2017). Still, no literature available on the hepatoprotective and antioxidant activity of *D. littoralis*. Thus, the study to assess hepatoprotective activities of *D. littoralis* in paracetamol induced rats.

**MATERIALS AND METHODS**

**Chemicals**

Paracetamol was purchased from Sigma-Aldrich, the USA utilized in the experiment. All other chemicals and reagents were used AR grade.

**Gathering and Identification of Plant materials**

The aerial parts of *D. littoralis* Nees. (Acanthaceae) were gathered from Tirunelveli District, Tamil Nadu, India. Plant identification was made from Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu (Voucher No: 25834). The *D. littoralis* were desiccated under shadowy, segregated, crushed through the grinder. (Satheeshkumar et al., 2011).

**Preparation of Plant Concentrates**

The pulverized materials were progressively concentrated with PE (40-60°C) through hot constant percolation method in Soxhlet equipment (Harborne, 1984) for twenty-four hours. At that moment, the marc was used to EA (76-78°C) for twenty-four hours & then mark was subjected to methanol for twenty-four hours. The concentrates were concentrated through the rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired (Shaji selvin,C.D., Kottai Muthu,A., 2010).

**Animals**

Male Wister rats of 17-18 weeks age, weighing 160-185g, were collected from the Central Animal House, MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana, India. The animals were set aside in cages, 2 per cage, with twelve: twelve hours light and dark cycle at 25 ± 2 °C. The rats were maintained on their particular diets and water ad libitum. The Ethical Committee approved the animal Ethical Committee’s clearance of MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana (CPCSEA/COP/05/ 21-01-2019).

**Experimental design**

**Acute toxicity test**

Albino Wistar rats were separated into six groups, and each group contains six animals (n = 6). Rats fasted for four hours with free access to water only. The various concentrates of *D. littoralis* suspended in normal saline: 0.5% CMC was administered orally at a dose of 5 mg/kg at first, and mortality was noted for three days. The mortality was observed in 5/6 or 6/6 animals, and then the dose administered was measured as a toxic dose. However, mortality was noted in less than 4 rats. Out of 6 rats, then the same treatment was repeated to confirm the toxic effect. If mortality was not observed, the procedure was repeated for higher doses i.e., 2000mg/kg.

**Hepatoprotective activity**

Animals were separated into six groups, and each group contains six animals.

**Group I**

Animals served as Control group received vehicle (0.5% CMC) for 7 days.

**Group II**

Animals served as a negative control, received 7 days only 1ml vehicle and paracetamol 2g/kg b.wt. given on the 5th-day by orally.

**Group III**

Animals received Pet. Ether concentrates of *D. littoralis* 200mg/kg b.wt, orally for seven days. On 5th day onwards paracetamol 2g/kg b.wt. administered by orally.

**Group IV**

Animals received ethyl acetate concentrates of *D. littoralis* 200mg/kg b.wt, orally for seven days. On 5th day onwards paracetamol 2g/kg b.wt. administered by orally.

**Group V**

Animals received methanolic concentrates of *D. littoralis* 200mg/kg b.wt, orally for seven days. On 5th day onwards paracetamol 2g/kg b.wt. administered by orally.

**Group VI**

Animals received 25mg/kg b.wt of Silymarin by oral for seven days and on 5th day onwards paracetamol 2g/kg b.wt. administered by orally.

Groups III, IV, and V rats were orally fed with the various concentrates of *D. littoralis* (PE, EA, and
methanol), and Group VI rats were fed with silymarin. Both the *D. littoralis* concentrates and silymarin were suspended in 0.5% CMC individually and fed to the particular rats through oral intubation. Next day of experiments 8th day, all the animals were sacrificed through cervical decapitation (Sivakrishnan and KottaiMuthu, 2014). Blood was collected in test tubes in dry condition and allowed to coagulate at ambient temperature for 30 min. The serum was removed through centrifugation at 2000 rpm for 10 min. The removed serum was utilized for the examination of liver enzymes.

**Liver marker enzymes**

(Reitman and Frankel, 1957) method was used to determine the SGOT and SGPT and (Kind and King, 1954) method were to determine ALP. (Lowry et al., 1951) method was to determine the total protein (TP) levels, and (Patton and Crouch, 1977) method were used to determination of Urea. The (Jaffe, 1886) method was used to determination of creatinine and (Henry and Winkelman, 1974) method was utilized for the estimation of TC. (Mallay and Evelyn, 1937) method was used for the determination of total bilirubin (TB), and (Foster and Dunn, 1973) method was used for determination of TG.

**Statistical Analysis**

ANOVA conducted the statistical investigation, and groups were compared through Duncan’s Multiple Range Test (DMRT) using SPSS Software Package, version 10.0. Results were expressed as means ± standard deviation for six rats in each group. A value of P ≤ 0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

**Acute Toxicity**

The results of the critical toxicity study revealed that LD50 values of PE, EA, and methanolic concentrates of *D. littoralis* were better and actually showed the protection of concentrates. Administration of PE, EA, and methanolic foci of *D. littoralis* did not change any autonomic or behavioral response in rats. The 0% mortality for a various concentrates of *D. littoralis* was recorded at 2000mg/kg. Overall results revealed that the LD50 value of 2000mg/kg. Hence the therapeutic dose was considered as 1/10th (200mg/kg.wt.) of the lethal dose for hepatoprotective activity.

The activity of various concentrates of *D. littoralis* on average liver weight changes in rats

Table 1 appeared the activity of various foci of aerial parts of *D. littoralis* on average liver weight changes in normal and paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed increased liver weight. The administration of ethyl acetate concentrates of aerial parts of *D. littoralis* attenuated the liver weight reduced, and silymarin treated group restored liver weight.

**Effect of various concentrates of *D. littoralis* on liver enzymes and other parameters in paracetamol-induced hepatotoxicity in Wistar rats**

Table 2 shows the effect of various concentrates of *D. littoralis* on hepatic marker enzymes in the serum from normal and paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed increased activities of SGPT(176.34±8.43), SGOT(190.45±7.65), ALP(278.23±10.22) and GGT(1.96±0.14). Administration of PE concentrates of *D. littoralis* showed considerably SGPT(172.12±6.52), SGOT(185.48±4.34), ALP(273.65±6.87) and GGT(1.89±0.12) in group III rats. Administration of EA concentrates of *D. littoralis* showed considerably SGPT(89.26±4.76), SGOT(115.63±4.58), ALP(208.34±6.21) and GGT(1.48±0.05) in group IV rats. Administration of methanol concentrates of *D. littoralis* showed considerably SGPT(136.12±9.65), SGOT(169.65±4.23), ALP(248.24±8.38) and GGT(1.64±0.12) in group V rats. The administration of EA concentrates on *D. littoralis* attenuated the hepatic marker enzymes, and silymarin restored enzyme activities to normal values.

Table 3 summarized the effect of various concentrates of *D. littoralis* on Creatinine, Urea, and Total Bilirubin in the serum from normal and paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed increased the level of Creatinine, Urea, and Total Bilirubin when compared to a control group of rats. Treatment of EA concentrates on *D. littoralis* and a significant reduction in the Creatinine, Urea, and Total Bilirubin and silymarin restored Creatinine, Urea, and Total Bilirubin to normal values.

Table 4 showed the effect of various concentrates of *D. littoralis* on TG and TC in the serum from normal and paracetamol-induced hepatotoxic rats. The hepatotoxic control group showed increased the level of TG as 80.16±3.48 and TC as 192.54±8.65 that of group I rats. Oral administration of PE concentrates showed considerable TG and TC levels in group III rats as 75.56±3.12 and 181.32±5.52. Oral administration of ethyl acetate concentrates showed...
Table 1: Effect of various concentrates of *D.littoralis* on average liver weight changes in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Final Liver Weight (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.49±0.34</td>
</tr>
<tr>
<td>Group II</td>
<td>6.21±0.40a**</td>
</tr>
<tr>
<td>Group III</td>
<td>5.87±0.12b*</td>
</tr>
<tr>
<td>Group IV</td>
<td>4.76±0.62b**</td>
</tr>
<tr>
<td>Group V</td>
<td>5.17±0.18b*</td>
</tr>
<tr>
<td>Group VI</td>
<td>4.60±0.15b**</td>
</tr>
</tbody>
</table>

# Data are articulated as mean±SEM, *n* = six rats each group.
P values, *P<0.05; **P<0.01; ns= not significant; compared to Paracetamol group. One way ANOVA followed by Dunnett’s test. → Group II compared to Group I; b→ Group II compared to Group III, IV, V, and VI.

Table 2: Effect of various concentrates of *D.littoralis* on serum enzymes SGPT, SGOT, ALP and GGT in Paracetamol induced hepatotoxicity on Wistar rats

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT (IU.L(^{-1}))</th>
<th>SGOT (IU.L(^{-1}))</th>
<th>ALP (IU.L(^{-1}))</th>
<th>GGT (IU.L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>65.89±2.76</td>
<td>93.56±2.43</td>
<td>197.23±3.18</td>
<td>1.29±0.22</td>
</tr>
<tr>
<td>Group II</td>
<td>176.34±8.43a**</td>
<td>190.45±7.65a**</td>
<td>278.23±10.22a**</td>
<td>1.96±0.14a**</td>
</tr>
<tr>
<td>Group III</td>
<td>172.12±6.52b**</td>
<td>185.48±4.34b**</td>
<td>273.65±6.87b**</td>
<td>1.89±0.12 b**</td>
</tr>
<tr>
<td>Group IV</td>
<td>89.26±4.76b**</td>
<td>115.63±4.58b***</td>
<td>208.34±6.21b**</td>
<td>1.48±0.05b*</td>
</tr>
<tr>
<td>Group V</td>
<td>136.12±9.65b**</td>
<td>169.65±4.23b**</td>
<td>248.24±8.38b**</td>
<td>1.64±0.12 b**</td>
</tr>
<tr>
<td>Group VI</td>
<td>78.45±3.89b**</td>
<td>107.89±3.88b**</td>
<td>195.67±4.54b**</td>
<td>1.39±0.08b**</td>
</tr>
</tbody>
</table>

# Statistical information and particulars of group I-VI was similar as in Table 1

Table 3: Effect of various concentrates of *D.littoralis* on Creatinine, Urea, and Total Bilirubin in Paracetamol induced hepatotoxicity on Wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (millimol(^{-1}))</th>
<th>Urea (millimole(^{-1}))</th>
<th>Total Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>53.25±1.76</td>
<td>7.62±0.18</td>
<td>1.31±0.16</td>
</tr>
<tr>
<td>Group II</td>
<td>76.54±3.25a**</td>
<td>19.12±2.14a**</td>
<td>4.36±1.32a**</td>
</tr>
<tr>
<td>Group III</td>
<td>72.65±2.14b*</td>
<td>17.83±0.54b**</td>
<td>4.16±0.34b*</td>
</tr>
<tr>
<td>Group IV</td>
<td>58.87±1.45b**</td>
<td>8.95±0.28b**</td>
<td>1.45±0.14b**</td>
</tr>
<tr>
<td>Group V</td>
<td>66.56±2.23b*</td>
<td>11.83±0.76b**</td>
<td>2.67±0.22b*</td>
</tr>
<tr>
<td>Group VI</td>
<td>56.28±1.88b*</td>
<td>.86±0.42b**</td>
<td>1.33±0.20b**</td>
</tr>
</tbody>
</table>

# Statistical information and particulars of group I-VI was similar as in Table 1

Table 4: Effect of various concentrates of *D.littoralis* on TG and TC in paracetamol-induced hepatotoxicity on Wistar rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>52.65±2.34</td>
<td>97.63±2.36</td>
</tr>
<tr>
<td>Group II</td>
<td>80.16±3.48a**</td>
<td>192.54±8.65a**</td>
</tr>
<tr>
<td>Group III</td>
<td>75.56±3.12b*</td>
<td>181.32±5.52b**</td>
</tr>
<tr>
<td>Group IV</td>
<td>55.44±2.45b**</td>
<td>109.62±4.23b**</td>
</tr>
<tr>
<td>Group V</td>
<td>67.55±2.86b*</td>
<td>165.43±5.22b**</td>
</tr>
<tr>
<td>Group VI</td>
<td>53.42±2.34b***</td>
<td>99.76±3.72b***</td>
</tr>
</tbody>
</table>

# Statistical information and particulars of group I-VI was similar as in Table 1
Table 5: Effect of various concentrates of *D.littoralis* on serum (TP and ALB) in paracetamol-induced hepatotoxicity on Wistar rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TP (mg/dl)</th>
<th>ALB (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>7.76±0.24</td>
<td>6.56±0.10</td>
</tr>
<tr>
<td>Group II</td>
<td>5.12±0.18a**</td>
<td>5.06±0.12a**</td>
</tr>
<tr>
<td>Group III</td>
<td>5.24±0.30 b**</td>
<td>5.17±0.12b*</td>
</tr>
<tr>
<td>Group IV</td>
<td>6.95±0.24b*</td>
<td>5.94±0.13b**</td>
</tr>
<tr>
<td>Group V</td>
<td>5.83±0.11a**</td>
<td>5.43±0.10b*</td>
</tr>
<tr>
<td>Group VI</td>
<td>7.22±0.18b**</td>
<td>6.12±0.10b**</td>
</tr>
</tbody>
</table>

# Statistical information and particulars of group I-VI was similar as in Table 1

A marked reduction in TG and TC levels in group IV rats as 55.44±2.45 and 109.62±4.23. Oral administration of methanol concentrates showed reduced the level of TG and TC in group V rats as 67.55±2.86 and 165.43±5.22. The administration of EA focuses on *D.littoralis*, and silymarin treated a group of significant rat reduction in the level of TG and TC as compared to PE and methanol concentrates. The various concentrates of *D.littoralis* on TP and albumin in the serum from normal and paracetamol-induced hepatotoxic rats were depicted in Table 5. The hepatotoxic control group showed a reduction of TP as 5.12±0.18 and albumin as 5.06±0.12. Oral administration of PE concentrates showed considerable TP and ALB levels in group III rats as 5.24±0.30 and 5.17±0.12. Oral administration of ethyl acetate concentrates showed a marked reduction in TP and ALB levels in group IV rats as 6.95±0.24 and 5.94±0.13. Oral administration of methanol concentrates showed reduced the level of TP and ALB in group V rats as 5.83±0.11 and 5.43±0.10. The administration of EA concentrates on *D.littoralis*, and silymarin treated rats have significantly elevated the level of TP and albumin as compared to PE and methanol concentrates.

In the present study shown that a lethal dose of various concentrates of *D.littoralis* was showed the safety of concentrates. Administration of multiple foci of *D.littoralis* in rats did not change any autonomic or behavioral reaction. There was no mortality of various concentrates of *D.littoralis* was recorded at 2000mg/kg. Therefore we have calculated and selected for 200 mg/kg b.wt. *D.littoralis* concentrates were taken for further investigation.

The liver is profoundly affected primarily by toxic agents. Hence, the liver marker enzymes are very sensitive markers of toxicity and are of great importance in the assessment of hepatic damage. ([Faras and Elsawaf, 2017](#)). Activities of SGPT, SGOT, ALP, GGT, and the level of serum bilirubin are mostly utilized as the most common SGPT, SGOT, ALP, GGT to examine the liver damage. Paracetamol-induced liver damage is considered and also used for the toxic agent of liver toxicity. ([Remien et al., 2014](#)). This study, the important elevation of SGPT, SGOT, ALP, GGT, and bilirubin level in rats treated with paracetamol, are suggestive of cellular leakage and defeat of functional integrity of the liver cell membrane. ([Sabi et al., 2014](#)). This is an agreement with previous studies; we found that the higher dose of paracetamol might be toxic to the hepatocytes ([Kuriakose and Kurup, 2010](#); [Kanchana and Sadiq, 2011](#)). Administration of EA concentrates on *D.littoralis* in this study suppresses the elevated serum SGPT, SGOT, ALP, GGT, and the bilirubin concentration. Recovery toward normalization of the enzymes following *D.littoralis* treatment suggested that excellent hepatoprotective property and has a specific role in conserving the structural integrity of the hepatocellular membrane, thus inhibiting enzyme leakage into the bloodstream, in addition to restoring of liver injury caused by paracetamol. This activity is in agreement with the generally established sight that serum levels of transaminases come again to standard with the healing of hepatic parenchyma and the renewal of hepatocytes ([Ahmed and Khater, 2001](#); [Pawlikowska-Pawlega et al., 2007](#)).
CONCLUSIONS

The current study showed that EA concentrates on *D. littoralis* protects hepatocytes against paracetamol-induced hepatic tissue damage by reducing the activities of hepatic enzymes. From our results, ethyl acetate concentrates of *D. littoralis* had a better hepatoprotective effect compared to that of two foci in paracetamol-induced in rats.

REFERENCES


