Chemoprotective Activity of Ethanolic Extract of *Pseudarthria Viscida* Linn Against *N*-Nitroso Diethylamine Induced Liver Carcinogenesis in Rats

M. Vijayabaskaran, S. Gomathi*, R. Shanmuga Sundaram and R. Sambathkumar

Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Kumarapalayam – 638183

**Abstract**
Liver cancer is one of the leading causes of cancer deaths worldwide. This idea has prompted us to evaluate the hepatoprotective effect of ethanolic extract of *Pseudarthria v***

**Keywords:** *Pseudarthria* *viscida*, Liver cancer, Biochemical Parameters, Antioxidant

**Correspondence**
Author: S. Gomathi
Email: gomathiswaminathan03@gmail.com

**Introduction**
Hepatocellular carcinoma (HCC) is the most frequent primary malignancy of the liver and accounts for as many as one million deaths worldwide in a year. In some parts of the world, it is the most common form of internal malignancy and the most common cause of death from cancer [1, 2]. *N*-nitrosodiethylamine (NDEA) is a potent carcinogenic dialkylnitrosoamine used to induce liver cancer in animal models [3-6]. It is found in a wide variety of foods such as cheese, soybeans, smoked, salted and dried fish, cured meat and alcoholic beverages and producing reproducible hepatocellular carcinoma after repeated administration [7]. NDEA became metabolically active by the action of cytochrome P450 enzymes to produce reactive electrophiles, which increase oxidative stress leading to cytotoxicity, mutagenicity and carcinogenicity. Oxidative stress is considered as critical mechanism contributing to NDEA induced hepatotoxicity [8]. The recent approach of chemoprevention serves as an attractive alternative to control malignancy [9]. The plant *Pseudarthria v***

**Experimental**
Collection and authentication of the plant material
The plant *Pseudarthria v***

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Extraction

The whole plant of *Pseudarthria viscida* were dried under shade with occasional shifting and then powdered with a mechanical grinder. The powder was passed through sieve No. 42 and stored in an airtight container for further use. The dried powder material of whole plant (1.5 kg) was defatted with petroleum ether (60-80°C) by hot continuous extraction method in a soxhlet apparatus for 72 h. The defatted powder material was further extracted with ethanol (95% v/v) for 72 h by using soxhlet apparatus. The extract was made solvent free by distillation process and the resulting semisolid mass was vacuum dried to yield a solid residue. The extract was subjected to preliminary phytochemical studies [15, 16].

Acute oral toxicity study

The acute toxicity study was carried on swiss albino mice as per the guidelines No: 423 given by the organization for economic co-operations and development [17].

*N – Nitrosodiethylamine induced hepatocellular carcinoma*

Wistar albino rats were divided into 4 groups (n = 6). All the groups received a single intraperitoneal injection of NDEA (200 mg/kg, body weight) followed by weekly subcutaneous injection of carbon tetrachloride (3 ml/kg, body weight) for 8 weeks, except group 1 (normal). From the first day, normal saline 5 ml/kg and propylene glycol 5 ml/kg were administered to normal and NDEA control group (group 2) respectively for 8 weeks on alternate days, orally. EEPV at different doses (100 and 200 mg/kg body weight) were administered on alternate days orally to groups 3 and 4 respectively, for 8 weeks [18].

Sample collection

After administration of last dose, the animals were fasted overnight and the next day the blood was collected from the animal through retro orbital puncture for the determination of hematological parameters and serum was separated for the estimation of biochemical parameters. The rats were sacrificed by cervical dislocation and isolated the liver and kidney. The relative liver weight was calculated as the percentage ratio of liver weight to the body weight. A small portion of the tissue was fixed in formalin for histopathological examination.

Estimation of haematological parameters

The haematological parameters like Haemoglobin, total RBC count, total WBC count were estimated by standard procedures [19-21].

In vivo Anti-oxidant studies

The *in vivo* antioxidant enzyme studies like Superoxide Dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH) and glutathione peroxidase (GPx), LPO, Protein estimation were carried out as per the procedure [22-27].

Estimation of marker enzymes in the serum

The marker enzymes in the serum like SGOT, SGPT, ALP, Total and direct bilirubin, Urea, Creatinine [28-33] were estimated by standard procedures.

Histopathology

The animals used in the curative study were sacrificed and liver tissue was examined grossly. A small portion of liver tissue of each animal was fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax to obtain 5-6µm thick hematoxylin and eosin stained sections.
**Statistical Analysis**

All the data obtained were presented as mean ± SEM. The results were analyzed statistically by one-way ANOVA followed by Tukey’s multiple comparison tests using PRISM Instat software.

**Results and Discussion**

**Phytochemical studies**

Ethanolic extract of whole plant of *Pseudarthria viscida* was found to be 15.2 % w/w. The qualitative analysis of the EEPV reveals that the presence of flavonoids, tannins and phenolic compounds, saponins, triterpenoids, proteins & amino acids.

**Toxicological studies**

The acute oral toxicity method showed that there was no mortality up to the dose level of 2000 mg/kg b. wt. of animals. All the animals were found to be normal and there were no gross behavioral changes at the end of observation period (14 days). From these results, the maximum tolerated dose was found to be 2000 mg/kg b. wt. From this 1/20th and 1/10th of the maximum dose of the extract tested for acute toxicity were selected for evaluation of chemopreventive effect of EEPV i.e., 100 mg/kg and 200 mg/kg.

**Pharmacological screening**

The results of the present study indicated that the ethanolic extract of *Pseudarthria viscida* possesses potential hepatoprotective activity against NDEA induced hepatocellular carcinoma [34].

**Effect of EEPV on body weight**

The body weight of normal rats showed 165.12 ± 2.17 g which was significantly increased to 260.11 ± 2.31 g in rats following NDEA treatment. In NDEA +EEPV treated rats at the doses of 100, 200 mg/kg treated rats, the final body weights became 226.57 ± 7.14 and 229.04 ± 3.61 respectively. However, administration of 100 and 200 mg/kg of EEPV significantly reduced the relative body weight (Table 1).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>165.12 ± 2.17</td>
</tr>
<tr>
<td>NDEA Control 200 mg/kg</td>
<td>260.11 ± 2.31</td>
</tr>
<tr>
<td>NDEA+EPPV100 mg/kg</td>
<td>226.57 ± 7.14</td>
</tr>
<tr>
<td>NDEA +EPPV 200 mg/kg</td>
<td>229.04 ± 3.61**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. P < 0.001 as compared with normal group.

**Effect of EEPV on Haematological parameters**

The total WBC count was observed that increased with a reduction of Hb content of RBC. The total number of RBC showed a modest change. In differential count of WBC, the percentage of neutrophils increased while the lymphocyte count decreased. At the same time interval on EEPV (200 mg/kg) treatment restored all the altered hematological parameters to almost near normal. EEPV (100 mg/kg) treatment also recovered these altered depleted parameters towards normal, though EEPV (200 mg/kg) treatment was found to be comparatively more effective. Usually in cancer chemotherapy, the major problems that are being encountered are of myelosuppression and anemia. The anemia encountered in tumor bearing rats are mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. Administration with EEPV brought
back the hemoglobin content, RBC and WBC cell count near to normal levels. This indicates that EEPV have the preventive action in haemopoietic system (Table 2).

### Table 2 Effect of ethanol extract of whole plant of *Pseudarthria viscida* on haematological parameters of control and experimental group of rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Hb (gm %)</th>
<th>RBC (x10⁶ cells/mm³)</th>
<th>WBC (x10³ cells/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15.37 ± 0.68</td>
<td>10.14 ± 1.08</td>
<td>4.93 ± 0.38</td>
</tr>
<tr>
<td>NDEA Control 200 mg/kg</td>
<td>7.21 ± 0.39</td>
<td>6.94 ± 1.33</td>
<td>7.21 ± 1.39</td>
</tr>
<tr>
<td>NDEA+EEPV 100 mg/kg</td>
<td>9.47 ± 0.88</td>
<td>8.14 ± 1.36*</td>
<td>5.68 ± 1.24</td>
</tr>
<tr>
<td>NDEA+EEPV 200 mg/kg</td>
<td>10.41 ± 1.91*</td>
<td>9.18 ± 1.26</td>
<td>3.78 ± 0.43*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.001 as compared with normal group.

### Effect of EEPV on marker enzymes

The levels of serum marker enzymes SGOT, SGPT, ALP, TBL, DBL, Creatinine and Urea were significantly increased in NDEA treated rats when compared with the normal group (P < 0.01). Treatment with EEPV at the doses of 100 and 200 mg/kg showed decreased activity of serum marker enzymes in NDEA induced hepatocellular carcinoma compared to that of control group (P < 0.001) (Table 3).

### Effect of EEPV on LPO, Antioxidant enzymes

In hepatocellular carcinoma, there is disequilibrium between oxidant and antioxidant balance, which is tilted towards the oxidant side. Reactive oxygen species (ROS) are believed to cause genetic oxidation and damage to DNA and other macromolecules. Our data shows that the administration of NDEA induced a significant decrease in SOD, CAT, GPx and GSH activity in kidney and liver as compared to control. In EEPV treated animals there is a significant increase in the activities of SOD, CAT, GPx and GSH when compared with tumor bearing animals. This improvement may have resulted from changing the tissue redox system by scavenging the free radicals and improving the antioxidant status in the kidney and liver during NDEA hepatotoxicity. The hepatic LPO levels were found to be significantly increased in the NDEA treated rats. Administration of EEPV showed significant reduction in LPO at doses 100, 200 mg/kg, respectively (Table 4).

### Table 3 Effect of ethanol extract of whole plant of *Pseudarthria viscida* on marker enzymes in the serum of control and experimental group of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT U/L</th>
<th>SGPT U/L</th>
<th>ALP U/L</th>
<th>TOTAL BILIRUBIN &lt;1 mg/dl</th>
<th>DIRECT BILIRUBIN &lt;1 mg/dl</th>
<th>CREATININE mg/dl</th>
<th>UREA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>134.61±5.19</td>
<td>86.24±4.22</td>
<td>246.10±11.70</td>
<td>0.72±0.06</td>
<td>0.17±0.01</td>
<td>0.61±0.04</td>
<td>38.76±2.09</td>
</tr>
<tr>
<td>NDEA Control 200 mg/kg</td>
<td>416.87±11.91</td>
<td>236.71±8.10</td>
<td>486.71±12.15</td>
<td>1.29±0.02</td>
<td>0.27±0.03</td>
<td>0.95±0.01</td>
<td>46.86±2.43</td>
</tr>
<tr>
<td>NDEA+EEPV 100 mg/kg</td>
<td>389.36±11.02</td>
<td>215.64±9.02</td>
<td>371.61±8.19</td>
<td>1.16±0.25</td>
<td>0.23±0.00</td>
<td>0.89±0.06*</td>
<td>29.32±2.81</td>
</tr>
<tr>
<td>NDEA+EEPV 200 mg/kg</td>
<td>192.31±9.15</td>
<td>164.23±5.69*</td>
<td>288.14±14.10</td>
<td>1.02±0.28</td>
<td>0.19±0.01</td>
<td>0.72±0.20</td>
<td>34.04±2.08*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.01 as compared with normal group. * P < 0.001 as compared with control group

### Histopathological Observations

The histological examinations basically support the results obtained from serum enzyme and tumor marker assays. Figure 1 A shows the normal architecture (group I) and cells cytoplasm of hepatic cells with granulated cytoplasm, central vein, small uniform nuclei and nucleolus. Group II NDEA treated rats showed loss of architecture and neoplastic cells arranged in lobules separated by fibrous septa with inflammatory collection and small bile duct proliferation. Neoplastic cells were smaller than normal cells with granular cytoplasm and larger hyperchromatic
nuclei and tumor cells also contain intracytoplasmic violaceous, hyaline globules that represent proteins produced by
the tumor cells (Figure 1B). Architecture of liver sections of EEPV cotreated (100 mg/kg) group III rats showed
normal architecture with some hepatocytes and an isocaryosis minimal inflammatory cell infiltration around the portal
triads (Figure 1C), whereas EEPV cotreated (200 mg/kg) group IV rats showed normal architecture with few
neoplastically transformed cells and hepatocytes (Figure 1D).

Table 4 Effect of ethanol extract of whole plant of *Pseudarthria viscida* on antioxidant studies on liver and kidney of
control and experimental group of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD</th>
<th>CAT</th>
<th>GSH</th>
<th>GPx</th>
<th>LPO</th>
<th>Protein Estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.22±</td>
<td>8.78±</td>
<td>44.54±</td>
<td>61.54±</td>
<td>3.11±</td>
<td>3.72±</td>
</tr>
<tr>
<td>NDEA Control 200 mg/kg</td>
<td>4.38±</td>
<td>4.42±</td>
<td>18.48±</td>
<td>39.26±</td>
<td>1.12±</td>
<td>1.56±</td>
</tr>
<tr>
<td>NDEA+EEPV 100 mg/kg</td>
<td>4.46±</td>
<td>6.67±</td>
<td>23.16±</td>
<td>45.00±</td>
<td>1.98±</td>
<td>2.26±</td>
</tr>
<tr>
<td>NDEA+EEPV 200 mg/kg</td>
<td>4.73±</td>
<td>7.96±</td>
<td>38.26±</td>
<td>58.42±</td>
<td>2.86±</td>
<td>3.06±</td>
</tr>
</tbody>
</table>

Values are mean±SEM. P < 0.01 as compared with normal group.* P < 0.001 as compared with control group.

![Figure 1](image-url)

**Figure 1** Histopathology of liver tissues NDEA treated animals

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- Section shows hepatic tissue with foci showing periportal inflammation and congestion of central vein and few sinusoids.
- (B) Section shows hepatic tissue with most of the hepatocytes showing steatosis with foci showing periportal inflammatory infiltrate. Foci show dilatation of central vein with inflammation extending into lobules.
- (C) Section showing few neoplastically transformed cells and hepatocytes maintaining near normal liver architecture.
- (D) Section shows hepatic tissue with most of the hepatocytes showing steatosis with mild periportal inflammatory infiltrate. Also seen are dilatation of central vein and sinusoids.

**Conclusion**

In conclusion, analysis of the expression profile is a useful tool to provide new evidences and produce new research targets in the hepatocarcinogenesis field. In the present study, a rat model of liver cancer was established. The present study focused the attention on the global molecular events that occurred in NDEA treated rats (and probably represent the earliest ones that start the multistep process of hepatocarcinogenesis). Additional information may be mined from this and similar studies to provide clues to many areas including the very important search for diagnostic markers, therapy targets and prognosis prediction markers. From these observations it can be concluded that EEPV may suppress the formation of NDEA induced hepatocarcinogenesis in rats by alleviating lipid peroxidation through scavenging of free radicals, or by enhancing the activity of antioxidants, which then detoxify free radicals. Thus, the present investigation highlights the tumor suppressive effect of EEPV in N-nitrosodiethylamine induced hepatocarcinogenesis which may involve the free radical scavenging mechanism. The precise molecular mechanism of EEPV against NDEA induced liver cancer is under way.

**References**

[17] OECD-423, Guidance document on acute oral toxicity testing classification schemes to cover the transition period until full implementation of the globally harmonized classification system (GHS), 2001, 18-21.

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