Research Article

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 viscida* (PV) Linn against N-Nitrosodiethylamine (NDEA) induced liver cancer in rats. Wistar albino rats were administered with PV extract (100 and 200 mg/kg b.w; p.o.) on alternate days for 120 days. Various *in vivo* biochemical parameters like lipid peroxidation, superoxide dismutase and catalase were evaluated to determine the hepatoprotective and antioxidant activity of PV. NDEA significantly increased LPO and decreased the endogenous antioxidant enzymes (SOD and CAT). The PV extract significantly restored the antioxidant enzyme level in the liver and exhibited significant dose dependant protective effect against NDEA induced liver toxicity, which can be mainly attributed to the antioxidant potential of the extract.

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 viscida* Linn (family: Fabaceae) is useful in vitiated conditions of pitta and vata, cough, bronchitis, asthma, tuberculosis, helminthiasis, dyspepsia, inflammation, cardiopathy, haemorrhoids, gout, hyperthermia and general debility. The plant has shown to possess antifungal, antioxidant, anti-tumor, anti-hypertensive and antidiarrhoeal activities [10-14]. In this investigation we evaluated the efficacy of *Pseudarthria viscida* on NDEA persuaded hepatocarcinogenesis in rats.

Experimental

Collection and authentication of the plant material

The plant *Pseudarthria viscida* was collected from Kolli Hills, Namakkal district of Tamil Nadu state, India and authenticated by Dr. G.V.S. Murthy, Botanical survey of India, Southern Circle, TNAU campus, Coimbatore, Tamil Nadu (No. BSI/SC/5/23/06-07/Tech-166) and the voucher specimen has been preserved in our laboratory for future reference.

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 upto 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 $^{1}/_{20}{}^{th}$ and $^{1}/_{10}{}^{th}$ 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 1 Effect of ethanol extract of whole plant of *Pseudarthria viscida* on body weight of control and experimental group of rats

Animals	Body weight (g)
Normal	165.12 ± 2.17
NDEA Control 200 mg/kg	260.11 ± 2.31
NDEA+EEPV100 mg/kg	226.57 ± 7.14
NDEA +EEPV 200 mg/kg	229.04 ± 3.61**

Values are mean ± SEM. P < 0.001 as compared with normal group.
** P < 0.0001 as compared with control 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.

GROUPS	Haematological parameters		
	Hb (gm %)	RBC ($x10^6$cells/mm^3)	WBC ($x10^3$cells/mm^3)
Normal	15.37 ± 0.68	10.14 ± 1.08	4.93 ± 0.38
NDEA Control 200 mg/kg	7.21 ± 0.39	6.94 ± 1.33	7.21 ± 1.39
NDEA+EEPV 100 mg/kg	9.47 ± 0.88	8.14 ± 1.36*	5.68 ± 1.24
NDEA+EEPV 200 mg/kg	10.41 ± 1.91*	9.18 ± 1.26	3.78 ± 0.43*
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

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

Groups	SOD		CAT		GSH		GPx		LPO		Protien Estimation	
	K	L	K	L	K	L	K	L	K	L	K	L
Normal	5.22± 0.12	8.78± 0.15	44.54± 0.51	61.54± 1.34	3.11± 0.10	3.72± 0.11	3.18± 0.08	4.06± 0.09	0.22± 0.04	0.12± 0.01	0.20± 0.00	0.22± 0.00
NDEA Control 200 mg/kg	4.38± 0.11	4.42± 0.10	18.48± 0.61	39.26± 1.36	1.12± 0.01	1.56± 0.08	2.75± 0.10	2.29± 0.06	0.24± 0.04*	0.20± 0.01*	0.16± 0.00*	0.19± 0.00*
NDEA+EEPV 100 mg/kg	4.46± 0.01	6.67± 0.07	23.16± 0.60	45.00± 0.62	1.98± 0.01*	2.26± 0.12	2.76± 0.01*	2.74± 0.08	0.23± 0.05	0.15± 0.02	0.15± 0.05	0.17± 0.03*
NDEA+EEPV 200 mg/kg	4.73± 0.11	7.96± 0.09*	38.26± 0.49	58.42± 0.40*	2.86± 0.11	3.06± 0.12	2.81± 0.07	3.04± 0.06	0.17± 0.01	0.16± 0.02*	0.19± 0.03	0.20± 0.02

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

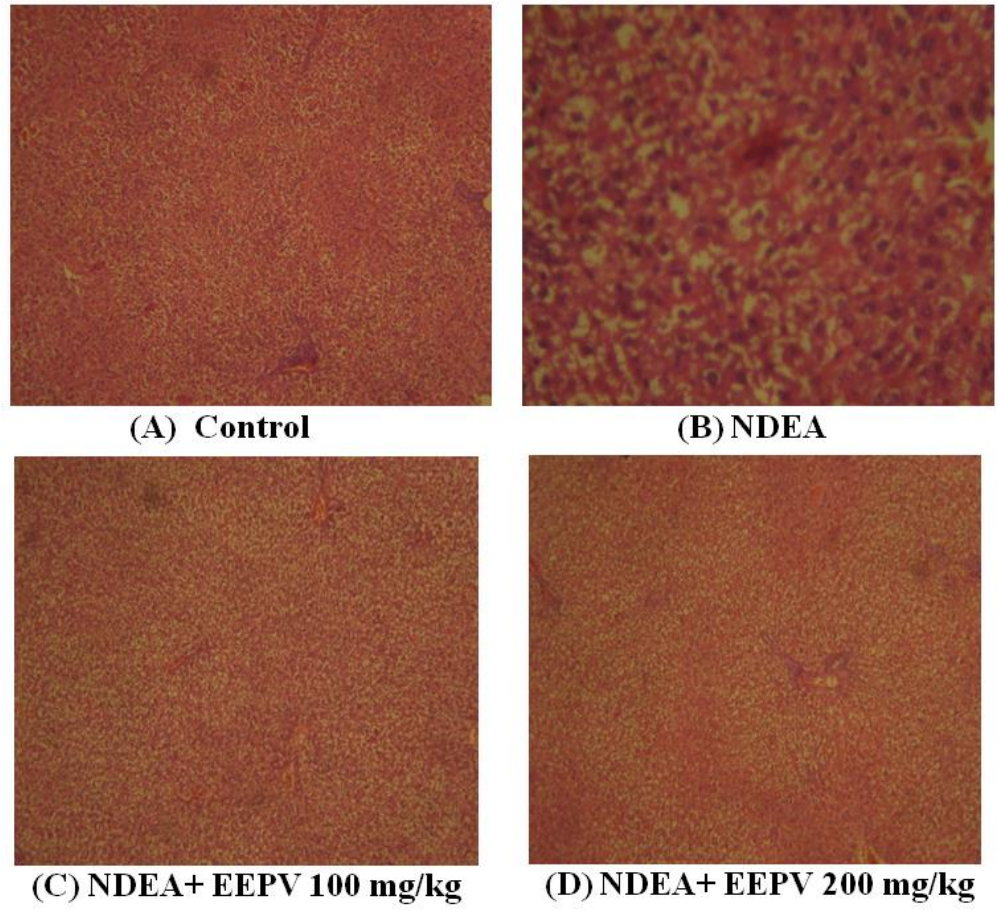

Figure 1 Histopathology of liver tissues NDEA treated animals

- 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.

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