

## EVALUATION OF HEPATOPROTECTIVE EFFECT OF *PSEUDARTHRIA VISCIDA* LINN AGAINST DMBA INDUCED LIVER DAMAGE IN WISTAR RATS

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### ABSTRACT

The whole plant of *Pesudarthria viscida* Linn is used for the treatment of various hepatic disorders. In the present study, the effect of ethanolic extract of *Pesudarthria viscida* Linn (EEPV) on 7,12-dimethyl benz[ $\alpha$ ]anthracene (DMBA) induced liver damage in Wistar albino rats were studied. A daily dose of 100 mg/kg and 200 mg/kg of the extract was administered to the experimental animals for 90 days. After the induction of the tumour the treatment period was started and continued up to 150 days. The results showed that significant hepatoprotective effect ( $P < 0.01$  and  $P < 0.001$ ) was achieved against DMBA induced liver damage, by oral administration of EEPV as evident from decreased levels of serum enzymes in the treated groups, compared to that of control. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Thus the present study provides a scientific foundation for the traditional usage of this plant in the management of liver diseases.

**KEYWORDS:** *Pesudarthria viscida*, DMBA, hepatoprotective activity, histopathological studies.

### INTRODUCTION

The liver as a vital organ in the body is primarily responsible for the metabolism of endogenous and exogenous agents. It plays an important role in drug elimination and detoxification and liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anaemia and medications.<sup>[1]</sup> Despite the fact that hepatic problems are

responsible for a significant number of liver transplantations and deaths recorded worldwide, available pharmacotherapeutic options for liver diseases are very limited and there is a great demand for the development of new effective drugs.<sup>[2]</sup>

The great susceptibility of liver to damage by chemical agents is presumably a consequence of its primary role in the metabolism of xenobiotics.<sup>[3]</sup> Hepatotoxicity is characterized by liver enzyme elevations and/or the presence of signs or symptoms signifying such injury, including nausea, vomiting, jaundice or lower extremity edema.<sup>[4]</sup> 7,12-Dimethylbenzanthracene (DMBA), a polycyclic aromatic hydrocarbon (PAH) in which the methyl substitution greatly enhances carcinogenicity and toxicity, is produced during the incomplete combustion of carbon-containing compounds, and is predominantly found in tobacco smoke and motor vehicle exhaust emissions. Exposure to PAHs, including DMBA, can lead to toxicological changes in the liver including oxidative stress and production of carcinogenic metabolites.<sup>[5]</sup>

Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments.<sup>[6]</sup> Plants have played a remarkable role in healthcare since the ancient times. Traditional plant based medicines still exert a great deal of importance to people living in developing countries and also leads to the discovery of new drug candidates.<sup>[7]</sup>

The plant *Pseudarthria viscida* Linn (Fabaceae) is a perennial shrub.<sup>[8]</sup> The roots contain a flavonol called leucopelargonidin. The plant was reported for anti-diabetic, anti-inflammatory<sup>[3]</sup> anti-pyretic & anti-nociceptive, antidiarrhoeal and antitumour activities.<sup>[9-11]</sup> Antioxidant is a compound that inhibits or delays the oxidation process by blocking the initiation or propagation of oxidizing chain reaction. Antioxidants can interface with the oxidations by reacting with free radicals chelating metals and also by acting as oxygen scavenger.<sup>[12]</sup> Antioxidants are of interest for the treatment of many kinds of cellular degeneration.

Especially India being a wealthy land of medicinal herbs and since only 5% of the plant kingdom has been investigated, it becomes essential to explore the medicinal value of herbs. Since antioxidant principles may be used to combat various human diseases and this work aims at evaluating the antioxidant potential of the plant. Already so many drugs are available

for this activity, but all the drugs are symptomatic relief only that do not alter the total mechanism of such diseases. Many works had been done on this plant except hepatoprotective activity. We therefore, examined the effect of fractionation of the extract for hepatoprotective activity against DMBA in rats.

## EXPERIMENTAL

The plant was collected from Kolli hills, Namakkal District, Tamil Nadu. The plant was authenticated by Botanical survey of India, Southern Circle, Coimbatore and a voucher specimen was deposited (No.BSI/SC//23/06-07/tech-166). The whole plant of *Pseudarthria viscida* Linn was dried under shade, powdered and passed through 42 mesh sieve and stored in an air tight container. The powdered plant material was extracted with petroleum ether (60-80°C) and ethanol (95% v/v) successively in a soxhlet apparatus. The solvents were then evaporated under reduced pressure to obtain dry extract. Preliminary phytochemical studies were also performed for the extract.

## ANIMALS

Male Wistar albino rats (150-250 gm) of 5-8 weeks old were housed in cages and fed with standard laboratory feed (Hindustan lever foods, Bangalore, India) and water *ad libitum*. This experiment compiled with the guidelines of our laboratory for animal experimentation. The animal ethics committee of the institute cleared the experimental protocol.

## CHEMICALS

Dimethylbenz[ $\alpha$ ]anthracene, 1-chloro-2, 4-dinitrobenzenzene (CDNB) and bovine serum albumin (BSA) were purchased from sigma chemicals (USA). Thiobarbituric acid and nitrobluetetrazolium chloride (NBT) were obtained from Loba chemie (Mumbai, India). 5, 5'-dithio bis nitro benzoic acid (DTNB) was purchased from SISCO research laboratory (Mumbai).

## ACUTE TOXICITY STUDIES

Acute toxicity studies were carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Male Wistar albino rats weighing 200-250 g were fasted overnight, provided only water after that EEPV extract was administered to the respective groups orally at the dose of 300mg/kg and observed for 21 days. If mortality was observed in 2 or 3 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal then the same dose was repeated for further higher dose

such as 400, 500 and 1000 mg/kg b. wt. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsions and mortality for 72 hours.

## METHODS

All the animals were divided into four groups namely G1, G2, G3 and G4 of six each. G1 was kept as normal control; G2 was treated as negative control, which was administered with DMBA at a dose of 25 mg/kg/d for 90 days. G3 was treated with DMBA + ethanol extract 100 mg/kg/d and G4 was treated with DMBA + ethanol extract 200 mg/kg/d. Animals were kept 90 days (12 weeks) for the development of tumour, from the 91<sup>st</sup> day onwards treatment period Started and continued up to 150 days. After the experimental period was over, the animals were fasted for overnight. They were then sacrificed by anaesthetized ether. Blood samples were collected from all animals by puncturing retro orbital plexus. The liver and kidney were rapidly excised and rinsed in ice cold saline, weighed and homogenate was prepared for various estimations. A small portion of liver was fixed in 10% neutral buffered formalin for histopathological examination.

## BIOCHEMICAL ANALYSIS

The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the various biochemical parameters namely, aspartate amino transaminase (AST) and alanine amino transaminase (ALT) were estimated by a UV kinetic method based on the reference method of international federation of clinical chemistry in which both AST and ALT were assayed based on enzyme coupled system; where keto acid formed by the amino transferase reacts in a system using NADH. The coenzyme is oxidised to NAD and the decrease in absorbance at 340 nm is measured. For AST maleate dehydrogenase is used to reduce oxaloacetate to maleate whereas for ALT the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALP) was estimated by hydrolysis of p-nitro phenol phosphate by alkaline phosphatase to give p-nitro phenol which gives strong yellow colour in alkaline solution. The increase in absorbance due to its formation is directly measured photo metrically at 400 nm and is directly proportional to ALP activity; while total bilirubin (TB) and direct bilirubin (DB) involves the reaction of bilirubin with diazotised sulphanilic acid to form an azo compound the colour of which is measured at 546 nm. Urea was done by urease GLDH: (glutamate dehydrogenase) enzymatic UV test which was

measured at 340 nm. Creatinine forms a coloured complex with picrate in alkaline medium. The rate of formation of the complex was measured at 492 nm.<sup>[13-15]</sup>

After the collection of blood samples the rats were sacrificed, kidney and livers were excised rinsed in ice-cold normal saline followed by 0.15 M Tris-HCl (PH 7.4) blotted dry and weighed. Protein in the tissue homogenate was estimated with bovine serum albumin as standard<sup>[16]</sup> these proteins are producing a violet colour complex with copper ions in an alkali solution. The absorbance of the coloured complex is directly proportional to protein in the sample, measured at 740 nm. A 10% homogenate was prepared in 0.15 M Tris-HCl buffer and processed for estimation of lipid peroxidation (LPO).<sup>[17]</sup> A part of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for the estimation of glutathione.<sup>[18]</sup> Glutathione in the tissue reacts with 5' 5'dithiobis 2- nitrobenzoic acid to form a yellow colour complex which is measured at 412 nm. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of Superoxide dismutase (SOD)<sup>[19]</sup> and catalase (CAT). SOD activity was determined by NBT reduction method and CAT was measured by the rate of decomposition of hydrogen peroxide at 240 nm.

## HISTOPATHOLOGICAL STUDIES

Animals from control and treated groups were used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 hours, then embedded in paraffin using conversion methods and cut into 5 µm thick section and stained using haematoxylin-eosin dye and finally mounted in di-phenylxylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

## STATISTICAL ANALYSIS

The result of each parameter was reported as Mean  $\pm$  SEM. The reduction in biochemical parameters was calculated by considering the difference in biochemical parameter between hepatotoxin treated and control group to determine significant inter group differences of all treated groups with control group. Statistical significance was analyzed employing one-way ANOVA method. Values at  $p < 0.001$  were considered significant.

## RESULTS AND DISCUSSION

### Effect of EEPV on serum biochemical parameters

Table 1 shows that the values of AST, ALT, ALP, TB, DB, urea and Creatinine were significantly higher in animals which received DMBA alone than the normal control animal (Group I). On the other hand, the groups which received both EEPV and DMBA (Group III and IV), the values of the above biochemical parameters were near normal compared to the Group I animals. Among the group III and IV animals, there was no significant difference between Group I and Group IV. From this it is evident that, the Group IV received an optimum dose of (i.e. 200 mg / kg) EEPV against DMBA induced hepatotoxicity.

**Table 1: Effect of EEPV on serum biochemical parameters in DMBA induced Hepatocarcinogenesis in wistar rats.**

Parameters	Control	DMBA only	DMBA + EEPV 100 mg/kg	DMBA + EEPV 200mg/kg
AST (IU/L)	151.08±27.50	185.25±8.31	159.62±22.22	153.08±26.41
ALT (IU/L)	49.22±5.79	67.15±13.13	58.03± 13.02	54.23±10.69
ALP (IU/L)	86.22±9.42	124.98±40.17	98.33±23.39	94.15±18.87
TB (mg/dl)	0.44±0.24	0.66±0.33	0.45±0.25	0.52±0.28
DB (mg/dl)	0.12±0.02	0.26 ± 0.02	0.10±0.00	0.10±0.00
Urea (mg/dl)	15.75 ± 2.60	23.62 ± 7.86	19.32 ± 2.81	16.4 ± 2.08
Creatinine(mg/dl)	0.69 ± 0.10	1.13 ± 0.22	0.90 ± 0.29	0.92 ± 0.20

Values are presented as mean ± S.E.M of six rats in each groups II, III, IV rats were administered with single oral dose of DMBA (25mg/kg), after 90 days, the groups III and IV were administered orally 100 and 200 mg/kg EEPV respectively, in the form of aqueous suspension daily once a day. \*\* P<0.01 as compared with group I. \*\*\*P<0.001 as compared with group II.

### Effect of EEPV on non-enzymatic and enzymatic antioxidants

The effects of non-enzymatic and enzymatic antioxidants were summarized in Table 2. Increased LPO correlates with the depletion of reduced glutathione (GSH) and glutathione peroxidase (GP<sub>X</sub>) and this effect is expected at a prior step to alteration of poly unsaturated fatty acids. The administration of DMBA also depletes the glutathione levels. However the depleted GSH and GP<sub>X</sub> were significantly increased by EEPV (200 mg/kg). These enzymes were provided the first defense mechanism against oxygen toxicity by catalysing the dismutation of super oxide anion to H<sub>2</sub>O<sub>2</sub> and decomposition of hydrogen peroxide to water and molecular oxygen. EEPV treatment restored the depleted levels of SOD. Lipid

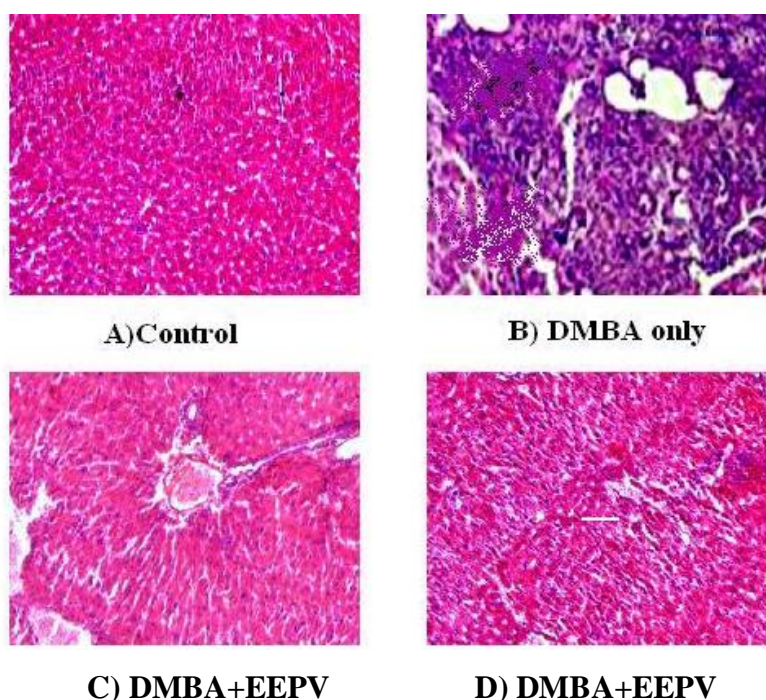
peroxidation plays an important role in hepatic carcinogenesis. The present study indicates that the increased levels of malondialdehyde (MDA) in the rats of the DMBA treated group could be ascribed to the excessive generation of free radicals. EEPV treatment (200 mg/kg) elevated the hepatic and renal LPO values.

**Table 2 Effect of EEPV extract on enzymatic and non-enzymatic anti-oxidants parameters in DMBA induced Hepatocarcinogenesis in Wistar rats.**

Parameters		Control	DMBA only	DMBA+EEPV 100mg/kg	DMBA+EEPV 200mg/kg
SOD U/mg protein	K	0.0966 ± 0.0974	0.0179 ± 0.0041	0.0345 ± 0.0026	0.0387 ± 0.004
	L	0.0413 ± 0.0042	0.0123 ± 0.0094	0.0119 ± 0.00007	0.0210 ± 0.0038
CAT U/mg protein	K	0.2790 ± 0.0026	0.1293 ± 0.0073	0.0883 ± 0.0034	0.1065 ± 0.0041
	L	0.1508 ± 0.0228	0.1339 ± 0.0101	0.1249 ± 0.00061	0.1287 ± 0.0074
GSH mcg/mg protein	K	0.3427 ± 0.0318	0.1955 ± 0.0283	0.1985 ± 0.0364	0.2315 ± 0.0119
	L	0.2792 ± 0.0093	0.1411 ± 0.0127	0.1759 ± 0.0166	0.2519 ± 0.113
GP <sub>x</sub> mcg/mg protein	K	0.1078 ± 0.0047	0.0054 ± 0.0006	0.0180 ± 0.0019	0.0642 ± 0.0014
	L	0.0935 ± 0.0024	0.0278 ± 0.0018	0.0295 ± 0.00016	0.0369 ± 0.0028
LPO nmoles/mg protein	K	0.2186 ± 0.0436	0.2414 ± 0.0446	0.2340 ± 0.0500	0.1572 ± 0.0104
	L	0.1170 ± 0.0160	0.2011 ± 0.0112	0.1497 ± 0.0256	0.1514 ± 0.0256
Protein estimation mg/dl	K	0.1995 ± 0.048	0.1597 ± 0.0033	0.1854 ± 0.0057	0.1892 ± 0.0032
	L	0.2243 ± 0.0073	0.1883 ± 0.0032	0.1923 ± 0.0034	0.1957 ± 0.0024

Values are presented as mean ± S.E.M of six rats in each groups II, III, IV rats were administered with single oral dose of DMBA (25mg/kg), after 90 days, the groups III and IV were administered orally 100 and 200 mg/kg EEPV respectively, in the form of aqueous suspension daily once a day. \*\* P<0.01 as compared with group I. \*\*\*P<0.001 as compared with group II.



*Histopathology*

**Figure 1: Representative photomicrographs of histopathological changes showing effect of the test material (EEPV) on rats intoxicated with DMBA.**

Histopathological examination of liver section of Group I showed normal architecture with distinct hepatic cells, sinusoidal spaces and central vein (A). Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in DMBA intoxicated liver sections of Group II (B). The liver section of Group III showed the recovery against the DMBA induced damage as compared to control (C). The liver section of Group IV showed less intoxication showed less vacuole formation and absence of necrosis and overall less visible changes observed as compared to control group (D).

**CONCLUSION**

In conclusion, we have found that EEPV counteracts lipid peroxidation induced by increased reactive oxygen species generation during DMBA induced Hepatocarcinogenesis and promote the enzymatic and non-enzymatic antioxidant defense system. This prevention might be due to the company of flavonoids present in the extract. Further studies are in progress in our laboratory to isolate and characterize the active compounds.

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