

Preventive Effect of *Tephrosia Purpurea* Against N,N-Diethylnitrosamine Induced Hepatocellular Carcinoma In Swiss Albino Mice

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Abstract

The aim of the present study is to divulge the preventive nature of *Tephrosia purpurea* Methanolic Extract (TpME) during DiEthylNitrosAmine (DENa) induced liver cancer in male Swiss albino mice. Anticancer activity of methanolic extract of *Tephrosia purpurea* was evaluated in DENa induced Swiss albino mice at the doses of 200 and 300 mg/kg body weight orally and both doses were administered for 14 days. After 24 h of the last dose, the mice were sacrificed and antitumor effect of TpME was assessed by evaluating liver serum marker enzyme and haematological parameters. Decrease in Aspartate transaminase, Alanine transaminase, Alkaline phosphatase, Bilirubin and Total protein count were observed in TpME treated animals when compared to DENa treated animals. Haematological profiles such as haemoglobin, red blood cell (RBC) and white blood cell (WBC) count reverted to normal level in TpME treated mice. A comparative histopathology study of liver from test group exhibited almost normal architecture, as compared to DENa treated group. The results demonstrated that *Tephrosia purpurea* methanolic extract at 300 mg/kg dose exhibits

significant antitumor activity than 200 mg/kg.

Keywords: Diethylnitrosamine, *Tephrosia purpurea*, Liver cancer, Haematology and carcinogenesis

1. Introduction

Cancer is a complex genetic disease that is caused primarily by environmental factors. The cancer-causing agents (carcinogens) can be present in food and water, in the air, and in chemicals and sunlight that people are exposed to. The cancer becomes the second major cause of death in the human after cardiovascular disease (Gruddy, 1991). Therefore, there is a continuing search for better control and preventive methods in order to reduce cancer mortality and related side effects. Many investigations are now being carried out to discover naturally occurring compounds, which can suppress or prevent the process of carcinogenesis (Wargovich., *et al* 1988 and Samaranayake *et al.*, 2000).

Medicinal plants play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. *Tephrosia purpurea* is a species of flowering plant in the pea family, Fabaceae, that has a pantropical distribution. It is used in the treatment of some major diseases. The antioxidant and anticancer activity of the extracts from medical plants and herbs was associated with their components of phenolic compounds; the major types of phenolic compounds included phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids (Cai *et al.*, 2004).

In view of this, we have evaluated the preventive effect of TpME against hepatocellular carcinoma in Swiss Albino Mice. The discovery and development of chemotherapeutics that are effective for the treatment and control of liver cancer is urgently needed for present hour.

2. Material and Methods

2.1 Animals

Male Swiss albino mice strain approximately 8 weeks young mice (weighing approximately 22-25g) were used in this study. The healthy animals were procured from United Institute of Pharmacy, Allahabad, Uttar Pradesh India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (temperature 27 ± 2 °C and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water *ad libitum*. The animals were acclimatization to the environment for 7days prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.2 Plant Materials and Preparation of Plant Extract

The *Tephrosia purpurea* plant was collected from Yamuna river bank, Allahabad District, in Uttar Pradesh, India. The collected plant materials were washed, sliced and completely dried in

a hot-air oven at 37 °C. The dried materials was ground into make a fine powder and used for extraction. Two hundred grams (200g) of the powered plants were extracted with methanol (80%) using “Soxhlet Apparatus” for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytocomponents. For experiments 200mg/kg and 300mg/kg body weight of TpME were used. This effective dose was selected based on dose dependent studies of TpME carried out in our laboratory.

2.3 Experimental Design

Body weights of the animals were recorded and they were divided into 6 groups. Each group have 6 animals and the details as follow.

Group 1 (Positive Control)

Normal control mice will be fed with standard diet and served as a control, which received saline.

Group 2 (Negative Control)

Mice induced with hepatocellular carcinoma by providing DENA 175 µl /kg/days for a week through intra peritoneal injection

Group 3

Mice induced with hepatocellular carcinoma by providing DENA 175 µl /kg/day for 7 days through intra peritoneal injection with methanolic extract of *Tephrosia purpurea* oral gavage daily at the dose of 200 mg/kg for 14 days.

Group 4

Mice induced with hepatocellular carcinoma by providing DENA 175 µl /kg/day for 7 days through intra peritoneal injection with methanolic extract of *Tephrosia purpurea* oral gavage daily at 300 mg/kg body weight for 14 days.

Group 5

Alone methanolic extract of *Tephrosia purpurea* oral gavage daily at a different dose of 200 mg/kg body weight for 14 days.

Group 6

Alone methanolic extract of *Tephrosia purpurea* oral gavage daily at a different dose of 300 mg/kg body weight for 14 days.

2.4 Collection of Samples and Hemato & Biochemical Assay

On completion of the experimental period, animals were anaesthetized with diethyl ether (2ml/kg). The blood was collected with and without EDTA as anticoagulant. Blood, plasma for the estimation Hemoglobin (Armour *et al.*, (1965) content, Red blood cell count (Wintrobe *et al.*, (1961) and White blood cells (Dacie and Lewis., (1958) and serum were separated for the

estimation of various biochemical parameters such as Bilirubin, Aspartate amino transaminase (IFCC method., 1986), Alanine amino transaminase (IFCC method., 1986) and Alkaline phosphatase (King and Armstrong., 1934) and Total Protein (Lowry *et al.*, 1951) were determined.

2.5 Histopathology Studies

The liver were excised from the experimental animals of each group after collecting the blood sample and washed with normal saline. Initially, the materials were fixed in 10% neutral formalin solution. Sections were cut using microtome techniques after paraffin embedding. The sections were possessed in alcohol xylene series and were stained with hematoxylin and eosin. The different sections were examined microscopically for the evaluation of histopathological changes.

2.6 Statistical Methods

All values are expressed as mean $\pm$ SEM. Statistical significance was determined by two-way analysis of variance (ANOVA) and the difference were considered significant at $p < 0.05$ level.

3. Results

Recently, the use of some herbs has attracted a great deal of attention as one of the alternative cancer therapies from the point of less toxicity and cost benefits. Therefore, an attempt has been made to evaluate the anticancer activity of methanolic extract of *Tephrosia purpurea*.

3.1 Effect of Methanolic Extract of *Tephrosia Purpurea* on Hematological Parameters

The hematological parameters of tumor bearing mice were found to be significantly differences compared to the extracts treated groups. In tumor bearing mice it was found that increased in WBC count (15.1 ± 0.21) and decreased in HB content (7.78 ± 0.04) with RBC count ($2.88 \pm 0.10 \times 10^6$). The methanolic extract treated group at dose of 200 mg/kg and 300 mg/kg body weight restored all the altered hematological parameters to almost near normal (Table 1). All these results suggest the anticancer nature of the extract, the dose of 300 mg/kg body weight was found to be effective potential than 200 mg/kg body weight.

Table 1. Effect of methanolic extract of *Tephrosia purpurea* on the hematological parameters in Swiss albino mice

Design of Treatment	Hemoglobin (gm %)	WBC (10^3 cells/mm ³)	RBC (10^6 cells/mm ³)
Normal	13.45 ± 0.09	8.8 ± 0.07	5.23 ± 0.03
Tumor Control DENA (175ul/kg)	7.78 ± 0.04	15.1 ± 0.21	2.88 ± 0.10
DENA (175ul/kg) + TPME 200 mg/kg	$9.38 \pm 0.21^*$	$13.12 \pm 0.15^*$	$3.39 \pm 0.17^*$
DENA (175ul/kg) + TPME 300 mg/kg	$11.02 \pm 0.04^*$	$10.31 \pm 0.08^*$	$4.63 \pm 0.06^*$
TPME 200 mg/kg	$13.11 \pm 0.06^*$	$8.67 \pm 0.10^*$	$5.22 \pm 0.02^*$

TPME 300 mg/kg	13.17 ± 0.04*	8.73 ± 0.04*	5.29 ± 0.03*
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Values are expressed as mean S.E.M., (n = 6)

*P < 0.05 as compared to tumor control group (Two-way ANOVA).

3.2 Effect of Methanolic Extract of *Tephrosia Purpurea* on Liver Enzyme and Histopathology Studies

The clinical liver parameters of tumor bearing mice were found to be significantly differences compared to the extracts treated groups. In tumor bearing mice it was found that increased in Bilirubin, ALT, AST, ALP and Total Protein. The methanolic extract treated group at dose of 200 mg/kg and 300 mg/kg body weight restored all the altered Clinical liver enzymes are almost near normal (**Table 2**). All these results suggest the anticancer nature of the extract, the dose of 300 mg/kg body weight was found to be potential activity than 200 mg/kg body weight. Histopathology study of liver in each group of animals given Fig-1.

Table 2. Effect of methanolic extract of *Tephrosia purpurea* on the Clinical parameters in Swiss albino mice

Treatments	Bilirubin (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	T. Protein (g/dl)
Normal	0.77 ± 0.02	37.3 ± 0.33	32.67 ± 0.21	74.33 ± 0.49	7.16 ± 0.10
Tumor Control DENA (175ul/kg)	1.93 ± 0.04	100.17 ± 0.70	91.5 ± 0.22	188.33 ± 1.42	9.98 ± 0.18
DENA (175ul/kg) + TPME 200 mg/kg	1.75 ± 0.03*	86.17 ± 0.40*	74.5 ± 0.22*	150 ± 0.51*	9.13 ± 0.09*
DENA (175ul/kg) + TPME 300 mg/kg	1.48 ± 0.03*	62.5 ± 0.22*	59.50 ± 0.22*	135.5 ± 1.78*	8.32 ± 0.06*
TPME 200 mg/kg	0.78 ± 0.02*	37 ± 0.26*	31.67 ± 0.21*	72.17 ± 0.30*	7.17 ± 0.05*
TPME 300 mg/kg	0.80 ± 0.02*	34 ± 0.37*	28.83 ± 0.17*	62.5 ± 0.22*	7.48 ± 0.08*

Values are expressed as mean S.E.M., (n = 6)

*P < 0.05 as compared to tumor control group (Two-way ANOVA).

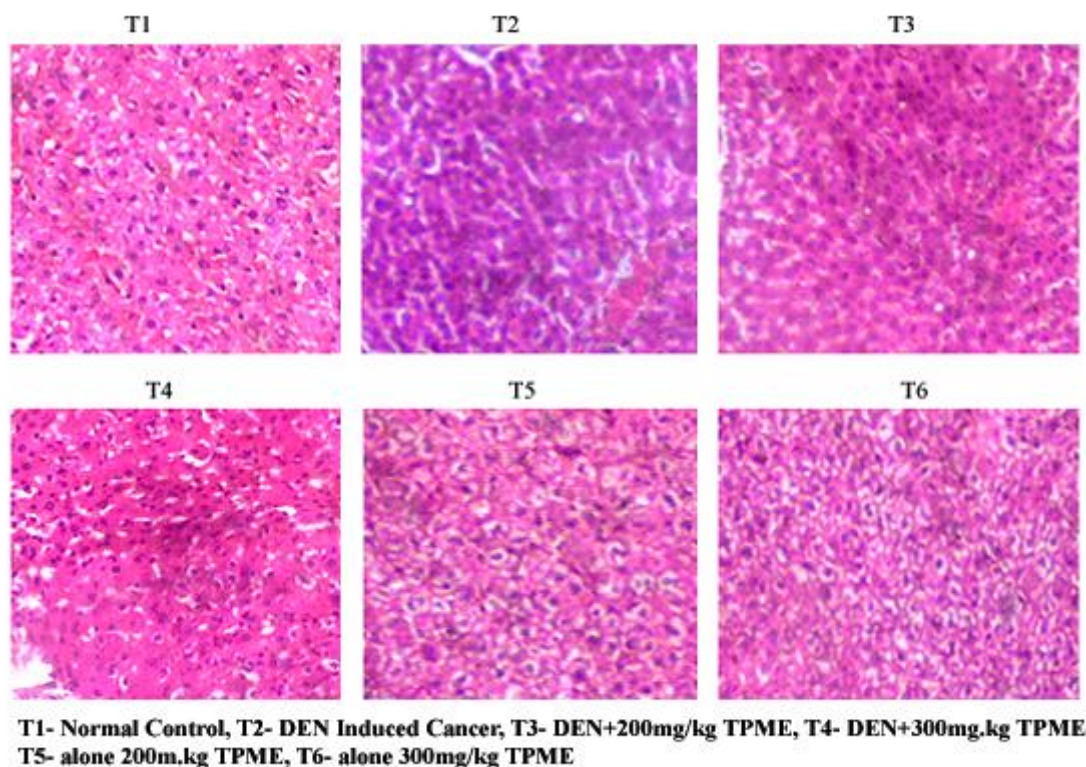


Figure 1. photomicrograph of liver (10x) (T1) control group showing normal architecture of liver; T2 animal treated with DENA showing cancer cell developed; (T3) animal treated with DENA+200mg/kg of TPME showing less cancer cell compared to T2; (T4) animal treated with DENA+300mg/kg of TPME showing very less cancer cell compared to cancer control T2; (T5&T6) animal treated with alone 200mg/kg & 300mg/kg of TPME showing normal architecture of liver compared to T1.

4. Discussion

It is well known that liver cancer is one of the most important cancers in the world, resulting in more than 1 million patients and over 260,000 deaths per year (Liu *et al.*, 2006). Therefore, the chemoprevention and treatment of liver cancer is very important. Liver is the major site of metabolism of ingested components; it is highly susceptible to carcinogenic effect. Natural products have long been used to prevent and treat many diseases, including cancer and thus they are good candidates for the development of anti-cancer drugs. The large population use ayurvedic medicine worldwide, there is urgent need for additional, carefully conducted, high quality intensive research to evaluate its efficacy and to develop this discipline to meet ever-new challenges of modern medicine in the field cancer research. Different in vivo and in vitro screening models are available for anticancer activity (Vishal and Thangavel, 2011).

The result of present study showed anticancer effects of TPME leaves against DENA in Swiss albino mice. A significant enhancement of TPME of haemoglobin, red blood cells and decrement of liver marker enzymes and WBC. The proposed study revealed that the fractions

obtained from TPME plant potent in inhibiting the cancer growth.

Hepatocellular carcinoma is the most frequent primary malignancy of the liver. DENA widely used for studying the mechanism of Hepatocellular carcinoma in animal experimental model (Stelzner, 2006). *Tephrosia Purpurea* are rich in flavonoid content and the cytotoxic might be due to the presence of flavonoids in the fractions utilized in this study as flavonoids have been shown to possess antimutagenic and antimalignant effect (Hirano *et al.*, 1989; Brown, 1980). The flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis (Osman *et al.*, 2011; Fotis *et al.*, 1997; Weber *et al.*, 1996). Our results suggests that the fractions of extracts obtained in from TPME have good cytotoxic activity against DENA induced liver cancer, which may be attributed to the secondary metabolites and antioxidant of the fractions.

5. Conclusion

The methanolic extracts of *Tephrosia purpurea* showed good potent anticancer activity against n,n-diethylnitrosamine induced hepatocellular carcinoma in Swiss albino. Further studies are in progress in our laboratory in screening of phytochemicals and antioxidant and investigation of molecular mechanisms and responsible for the anticancer activity of this plant. These results will help in the efficient cancer treatments and that would be more beneficial to our social and economic aspects of world.

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