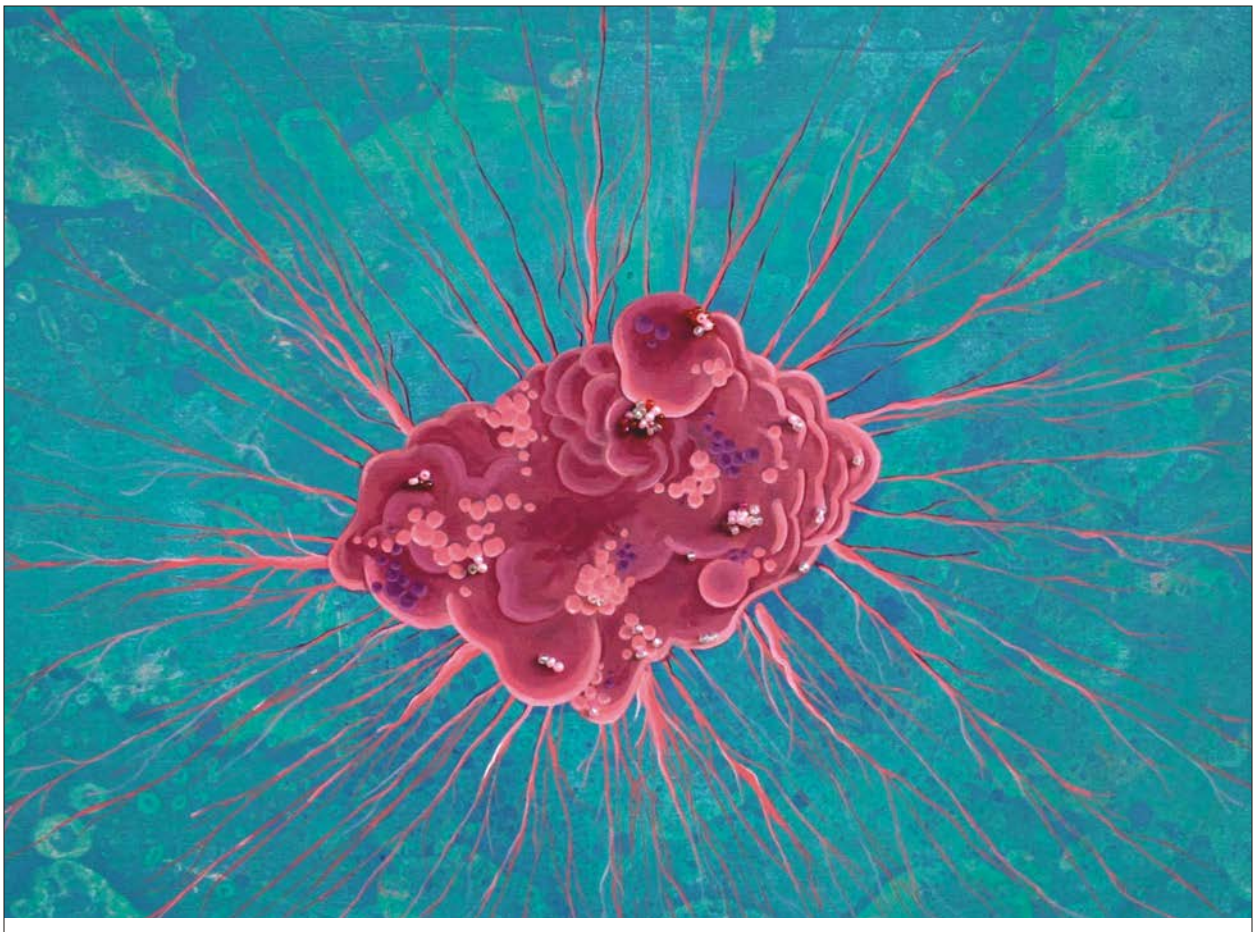

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Antitumour Activity of *Indigofera trita* on Ehrlich Ascites Carcinoma Induced Mice

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Abstract: The antitumour activity of ethanol extract of *Indigofera trita* L.f (EIT) was evaluated against Ehrlich Ascites Carcinoma (EAC) tumour model in Swiss albino mice on dose dependent manner. The activity was assessed using survival time, average increase in body weight, hematological parameters and solid tumour volume. Oral administration of EIT (200 and 400 mg kg^{-1}) increased the survival time and decreased the average body weight of the tumour bearing mice. After 14 days of inoculation, EIT was able to reverse the changes in the hematological parameters, protein and PCV consequent to tumour inoculation. Oral administration of EIT was effective in reducing solid tumour mass development induced by EAC cells. The results indicate that EIT possess significant antitumour activity on dose dependent manner.

Key words: *Indigofera trita*, ehrlich ascites carcinoma, life span, hematological parameters, solid tumor

INTRODUCTION

Cancer is one of the leading cause of mortality worldwide and the failure of conventional chemotherapy to effect major reduction in the mortality indicates that new approaches are critically needed. A large number of agents including natural and synthetic compounds have been identified as having some potential cancer chemotherapeutic value (Kellof, 2000). Many numbers of natural products have been studied for anticancer activity on various experimental models. This has been resulted in the availability of nearly 30 effective anticancer drugs (Ramakrishna *et al*., 1984).

Plants are playing an important role as a source of effective anticancer agents and it is significant that 60% of currently used anticancer agents are derived from natural sources, including plants, marine organism and micro-organism (Cragg *et al*., 2005; Newman *et al*., 2003). Plant-based medicine has definitely found a role in cancer treatment (chemotherapy) and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively (Kaufman *et al*., 1999). There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine

Indigofera trita L.f (Fabaceae), commonly known as Punalmurungai and Kattavuri, is an under shrub with wide distribution, mostly found in India, Ceylon, South Africa and North Australia. The entire plant has been used traditionally used for various ailments including tumours (Nadkarni, 1996; Kirtikar and Basu, 1993). Various species of the genus *Indigofera* have been used in the oriental

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traditional medicine for centuries. Previous studies have reported that ethanol extract of *Indigofera aspalathoides* was found to possess antitumour activity against transplantable tumors and carcinogen (Christina *et al*., 2003; Rajkapoor *et al*., 2004, 2005), a similar extract of *Indigofera oblongifolia* showed anticancer activity against FL-cells (Ali *et al*., 2001). Indirubin, an isolated active compound from *Indigofera tinctoria* was also found to be active against chronic myelocytic leukemia (Han, 1991). To date, there is no scientific evaluation has been carried out in *Indigofera trita* Linn. Based on these evidences we have selected *Indigofera trita* for the study. The aim of the present study is to evaluate the antitumour activity of ethanol extract of *Indigofera trita* (EIT) on Ehrlich Ascites Carcinoma (EAC) in mice.

MATERIALS AND METHODS

Plant Collection and Extraction

Entire plants of *Indigofera trita* was collected in and around the foothills of Shevaroys in Salem district, Tamilnadu, India, in the month of February 2004 and authenticated by Dr. R. Gopalan, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen (Voucher No. FIT.002) representing this collection has been retained in the Department of Pharmacognosy, Faculty of Pharmacy, Vinayaka Missions University, Salem, Tamilnadu, India.

The entire plants were shade dried and pulverized. The powder was treated with petroleum ether for dewaxing and removal of chlorophyll. Later, it was packed (250 g) in a Soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 mL ethanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a dessicator (yield, 11.25 g, 4.5% w/w) and suspended in 5% gum acacia for antitumour studies.

Animals

Swiss albino mice (20-25 g) were procured from Venkatershwara Enterprises, Bangalore, Karnataka, India and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature 25±2°C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*. The experiments were performed in accordance with the guidelines established by the European community for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee (IAEC).

Acute Toxicity Studies

The acute toxicity of the extract of *Indigofera trita* was evaluated in mice using the up and down procedure (OECD, 2001). This method was carried out in 15 animals, three animals per treatment group and widely different dose ranges, 1, 2, 3, 4 and 5 g kg^{-1}, respectively and observed 24 h. Based on the results the extract did not produce any mortality at the doses tested.

Cells

EAC cells were obtained through the courtesy of Amala Cancer Research Center, Thrissur, Kerala, India. They were maintained by weekly intraperitoneal inoculation of 10^6 cells $mouse^{-1}$ (Gothoskar and Ranadive, 1971).

Effect of EIT on Survival Time

Animals were inoculated with 2×10^6 cells $mouse^{-1}$ on day 0 and treatment with EIT started 24 h after inoculation, at doses of 200 and 400 mg kg^{-1} day^{-1}, p.o. The control group was treated with the same volume of 0.9% sodium chloride solution. All the treatments were given for 9 days. The Median Survival Time (MST) and average body weight changes of each group, consisting of 6 mice

were noted. The antitumour efficacy of EIT was compared with that of 5-fluorouracil (Dabur Pharmaceuticals, India; 5-FU, 20 mg kg^{-1} day^{-1}, i.p. for 9 days). The MST of the treated groups was compared with that of the control group using the following calculation.

$$\text{Increase in life span} = \frac{T - C}{C} \times 100$$

Where,
T = No. of days the treated animals survived
C = No. of days the control animals survived (Suffness and Douros, 1978)

Effect of EIT on Hematological Parameters

In order to detect the influence of EIT on hematological status of EAC bearing mice, a comparison was made among four groups (n = 5) of mice on the 14th day after inoculation. The groups comprised of (1) Tumor bearing mice (2) Tumor bearing mice treated with EIT (200 mg kg^{-1} day^{-1}, p.o. for 9 days) (3) Tumor bearing mice treated with EIT (400 mg kg^{-1} day^{-1}, p.o. for 9 days) and (4) Control mice (normal). Blood was drawn from each mouse by the retroorbital plexus method and the White Blood Cell count (WBC), Red Blood Cells (RBC) hemoglobin, protein and Packed Cell Volume (PCV) (D'Amour *et al*., 1965; Lowry *et al*., 1951; Docie, 1958) were determined.

Effect of EIT on Solid Tumor

Mice were divided into three groups (n = 6). Tumor cells (2×10^6 cells $mouse^{-1}$) were injected into the right hind limb of all the animals intramuscularly. The mice of group 1 were tumor control. Group 2 received EIT (200 mg kg^{-1} day^{-1}, p.o.) and group 3 received EIT (400 mg kg^{-1} day^{-1}, p.o.) for 5 alternative days. Tumor mass was measured from the 11th day of tumor induction. The measurement was carried out every 5th day for a period of 30 days. The volume of tumor mass was calculated using the formula $V = 4/3\ \pi r^2$, where r is the mean of r^1 and r^2 which are the two independent radii of the tumor mass (Ramnath *et al*., 2002).

Statistical Analysis

All values were expressed as mean±SEM. The data was statistically analyzed by Student's t-test. p-values< 0.05 were considered significant.

RESULTS AND DISCUSSION

The MST of the control group was 16±0.75 days, while it was 19±0.92, 28±0.76 and 31±0.41 days for the groups treated with EIT (200 and 400 mg kg^{-1}) and 5-FU (20 mg kg^{-1}), respectively. The increase in the life span of tumor bearing mice treated with EIT and 5-FU was found to be 18.75, 75 and 93.75%, respectively.

The average weight gain of tumor bearing mice was 13.3±0.61 g, whereas it was 8.3±0.84, 4.3±0.66 and 4±0.44 g for the groups treated with EIT (200 and 400 mg kg^{-1}) and 5-FU (20 mg kg^{-1}), respectively (Table 1).

Table 1: Effect of EIT on median survival time and average increase in body weight of EAC tumor bearing mice

Design of treatment	MST (in days)	Increase in life span T/C (%)	Average increase in body weight (g)
Tumor control	16±0.75	-	13.3±0.61
5-FU (20 mg kg^{-1}, i.p)	31±0.41*	93.75	4.0±0.44*
EIT (200 mg kg^{-1}, p.o)	19±0.92**	18.75	8.3±0.84 *
EIT (400 mg kg^{-1}, p.o)	28±0.76*	75.00	4.3±0.66*

N = 6 animals in each group, Values are expressed as mean±SEM, *: p<0.001; **: p<0.01 when compared with control

Hematological parameters of tumor bearing mice on the day 14 were showed significant changes when compared to normal mice. The total WBC count, protein and PCV were found to increase with a reduction in the hemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased while that of lymphocytes decreased (Table 2). At the same time interval, EIT (200 and 400 mg kg^{-1}) treatment could change those altered parameters significantly ($p<0.001$; $p<0.01$) near to normal. Maximum alteration occurred in the EIT treatment at the dose of 400 mg kg^{-1}.

There was significant reduction ($p<0.001$) in the tumor volume of mice treated with EIT on dose dependent manner. Tumor volume of control animals was increased by 14.2±0.38 mL, while it was 8.76±0.91 and 5.87±0.21 mL for the groups treated with EIT 200 and 400 mg kg^{-1}, respectively (Table 3).

The reliable criteria for judging the value of any anticancer drugs are prolongation of life span, inhibition of gain in average body weight and decrease of WBC from blood (Clarkson and Burchenal, 1965; Obiling and Guerin, 1954). The results of the present study show the antitumour effect of EIT against EAC in mice. A significant ($p<0.001$) enhancement of MST and decrement of gain in average body weight was observed.

The analysis of the hematological parameters showed minimum toxic effect in mice treated with EIT. After 14 days of transplantation, EIT was able to reverse the changes in the hematological parameters consequent to tumor inoculation.

The common problems encountered in cancer chemotherapy are myelosuppression and anaemia (Marklund *et al*., 1982; Price and Greenfield, 1958). Anaemia occurring in tumour bearing mice is mainly due to reduction in RBC or haemoglobin production and this may occur either due to iron deficiency or due to haemolytic or other myelopathic conditions (Hogland, 1982). Treatment with EIT brought back the haemoglobin content, RBC and WBC counts to near normal. This indicates that EIT have a protective effect on the haemopoietic system. Further, analysis of haemotological parameters showed minimum toxic effect in mice treated with EIT. In EAC bearing mice, haematological parameters were reversed to normal by EIT administration (9 days).

In EAC bearing mice, there was a regular and rapid increase in ascitic fluid volume. Ascitic fluid is the direct nutritional source for tumour growth, it meets the nutritional requirement of tumour cells (Feng *et al*., 2001). EIT treatment decreased the volume of solid tumour, viable cancer cell count and

Table 2: Effect of EIT on hematological parameters of EAC-bearing mice

Design of treatment	Hb (g %)	RBC 10^6 cells mm^{-3}	WBC 10^3 cells mm^{-3}	Protein (mg %)	PCV (mm)	Differential count (%)		
						Lymphocytes	Neutrophils	Monocytes
Normal	12.3±1.10	4.20±1.08	6.3±1.26	6.20±1.12	17.00±1.72	84±4.56	15±1.70	1±0
Tumor control	5.9±0.26	2.70±0.76	13.7±1.72	12.40±1.70	34.33±2.45	60±3.92	38±3.20	1±0
EIT (200 mg kg^{-1})	10.3±1.64	3.70±0.97	9.4±1.30	9.80±1.10***	29.00±2.70*	76±3.64	23±1.36**	1±0
EIT (400 mg kg^{-1})	11.5±0.72	4.05±1.16	8.1±1.10	8.12±0.90	24.00±2.16**	83±4.74	15±1.82	2±0

N = 5 animals in each group, Values are expressed as mean±SEM, *: $p<0.001$; **: $p<0.01$; ***: $p<0.05$ when compared with control

Table 3: Effect of EIT on solid tumor volume

Design of treatment	Solid tumor volume (mL)			
	15th day	20th day	25th day	30th day
Tumor control	7.99±0.23	9.63±0.26	11.13±0.41	14.62±0.38
EIT (200 mg kg^{-1})	6.56±0.28*	7.09±0.51*	8.13±0.68*	8.76±0.91*
EIT (400 mg kg^{-1})	5.27±0.37*	5.27±0.37*	5.66±0.19*	5.87±0.21*

N = 6 animals in each group, Values are expressed as mean±SEM, *: $p<0.001$when compared with control

increased the life span. It may conclude that EIT decrease the nutritional fluid volume and thereby arrest the tumour growth and increase the life span. There was reduction in solid tumour volume of mice treated with EIT ($p<0.001$).

All these data point to possibly developing the ethanol extract of *Indigofera trita* as a novel and potential agent in the cancer chemotherapy. Preliminary phytochemical screening indicated the presence of alkaloids and flavonoids in EIT. Flavonoids have been shown to possess antimutagenic and antimalignant effects (Brown, 1980; Hirano *et al*., 1989). Moreover, flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation (Weber *et al*., 1996) and angiogenesis (Fotis *et al*., 1997). The antitumour properties of the extract may be due to these compounds. The present study points the potential anticancer activity of *Indigofera trita* in a dose dependent manner.

CONCLUSIONS

All these observations clearly indicate the significant antitumour effect of ethanol extract of *Indigofera trita*. Further studies to characterize the active principles and elucidate the mechanism of action of EIT are in progress.

REFERENCES

Ali, N.A., W.D. Julich, C. Kusnick and U. Lindequist, 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol., 74: 173-179.

Brown, J.P., 1980. A review of the genetic effect of occurring flavonoids, anthraquinones and related compounds. Mutat. Res., 75: 243-277.

Christina, A.J., M. Alwin Jose, S.J. Heison Robert, R. Kothai, N. Chidambaranathan and P. Muthumani, 2003. Effect of *Indigofera aspalathoides* against Dalton's ascitic lymphoma. Fitoterapia, 74: 280-283.

Clarkson, B.D. and J.H. Burchenal, 1965. Preliminary screening of antineoplastic drugs. Prog. Clin. Cancer, 1: 625-629.

Cragg, G.M., D.G.I. Kingston and D.J. Newman, 2005. Anticancer Agents from Natural Products. FL: Brunner-Routledge Psychology Press; Taylor and Francis Group.

D'Amour, F.F., F.R. Blood and D.A. Belden, 1965. The Manual for Laboratory Work in Mammalian Physiology. The University of Chicago Press, Chicago, pp: 148-150.

Docie, J.V., 1958. Practical Haemotology. J and A Churchill Ltd., London, pp: 38-42.

Feng, Q., T. Kumangai, Y. Torii, Y. Nakamura, T. Osawa and K. Uchida, 2001. Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. Free Rad. Res., 35: 779-788.

Fotis, T., M.S. Pepper, E. Aktan and S. Breit, 1997. Flavonoids, dietary derived inhibitors of cell proliferation and *in vitro* angiogenesis. Cancer Res., 57: 2916-2918.

Gothoskar, S.V. and K.J. Ranadive, 1971. Anticancer screening of SAN-AB; An extract of marking nut, *Semicarpus anacardium*. Indian J. Exp. Biol., 9: 372-375.

Han, R., 1991. Highlights on the studies of anticancer drugs derived from plants in China. Stem Cells, 12: 53-63.

Hirano, T., K. Oka and M. Akiba, 1989. Antiproliferative effect of synthetic and naturally occurring flavonoids on tumour cells of human carcinoma cells lines. Res. Commun. Chem. Pathol. Pharmacol., 64: 69-78.

Hogland, H.C., 1982. Hematological complications of cancer chemotherapy. Seminars in Oncology, 9: 95-102.

Kaufman, P.B., J. Leland, C.S. Warber, A. James, D. Harry and L. Brielmann, 1999. Natural Products from Plants. CRC Press, London, pp: 1581-1589.

Kellof, G.J., 2000. Perspective on Cancer Chemoprevention Research and Drug Development. Adv. Cancer Res., 78: 199-334.

Kirtikar, K.R. and B.D. Basu, 1993. Indian Medicinal Plants. Int. Book Publisher, Dehradun, 1: 715-716.

Lowry, O.H., N.T. Rosenbrough and A.L. Farr, 1951. Protein measurement with Folin-Phenol reagent. J. Biol. Chem., 173: 265-275.

Marklund, S.L., N.G. Westman, E. Lundgren and G. Roos, 1982. Copper and zinc containing superoxide dismutase, manganese containing superoxide dismutase, catalase and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res., 42: 1955-1961.

Nadkarni, A.K., 1996. Indian Materia Medica. Popular Prakashan Pvt. Ltd., Bombay, 1: 683.

Newman, D.J., G.M. Cragg and K.M. Snader, 2003. Natural products as a source of new drugs over the period 1981-2002. J. Nat. Prod., 66: 1022-1037.

Obiling, C. and M. Guerin, 1954. The Role of Viruses in the Production of Cancer. Advances in Cancer Research 2. Academic Press, New York, pp: 406-410.

OECD (Organization of Economic Co-operation Development), 2001. The OECD Guideline for Testing of Chemical: 420 Acute Oral Toxicity. OECD, Paris, pp: 1-14.

Price, V.E., R.E. Greenfield, 1958. Anemia in cancer. Adv. Cancer Res., 5: 199-200.

Rajkapoor, B., B. Jayakar and N. Murugesh, 2004. Antitumour activity of *Indigofera aspalathoides* on Ehrlich ascites carcinoma in mice. Indian J. Pharmacol., 36: 38-40.

Rajkapoor, B., N. Murugesh, D. Chodan and D. Sakthisekaran, 2005. Chemoprevention of N-nitrosodiethylamine induced phenobarbitol promoted liver tumors in rat by extract of *Indigofera aspalathoides*. Biol. Pharm. Bull., 28: 364-366.

Ramakrishna, Y., A.I. Manohar P. Mamata and K.G. Shreekant, 1984. Plants and novel antitumour agents: A review. Indian Drugs, 21: 173-185.

Ramnath, V., G. Kuttan and R. Kuttan, 2002. Cytotoxic and antitumour activity of Abrin on transplanted tumours in mice. Indian J. Physiol. Pharmacol., 46: 69-77.

Suffness, M. and J. Douros, 1978. Methods in Cancer Research. Devita, V.T. (Ed.), Academic Press, New York, pp: 73-75.

Weber, G., F. Shen and Y.A. Yeh, 1996. Increased signal transduction activity and down regulation in human cancer cells. Anticancer Res., 16: 3271-3273.