Biosciences, Biotechnology Research Asia Vol. **5**(1), 389-394 (2008)

Anticancer activity of *Pandanus fascicularis* Lam. on Ehrlich ascites carcinoma (EAC) in mice

R. SENTHIL KUMAR[1]*, R. MANIVANNAN[1], A. BALASUBRAMANIAM[1], R. NATARAJAN[1] and B. RAJKAPOOR[2]

[1]Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengodu- 637 205 (India)
[2]Department of Pharmacology, St Johns Pharmacy College, No 6, 9th Cross, 2nd Main, Vijayanagar 2nd Stage, Bangalore - 560 104 (India)

(Received: November 26, 2007; Accepted: January 12, 2008)

ABSTRACT

Anticancer activity of ethanol extract of *Pandanus fascicularis* Lam. (EPF) was studied in mice. The anticancer activity of EPF was evaluated against Ehrlich Ascites Carcinoma (EAC) tumor model on dose dependent manner. The activity was assessed using survival time, average increase in body weight, hematological parameters and solid tumor volume. Oral administration of EPF increased the survival time and decreased the average body weight of the tumor bearing mice. After 14 days of inoculation, EPF is able to reverse the changes in the haemotological parameters, protein and PCV consequent to tumor inoculation. Oral administration of EPF was effective in reducing solid tumor mass development induced by EAC cells. The results shows EPF possess significant anticancer activity on dose dependent manner.

Key words: Ehrlich Ascites Carcinoma, Haematological parameters, *Pandanus fascicularis,* Life span, Solid tumor

INTRODUCTION

Cancer is 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[1]. Many numbers of natural products have been studied for anticancer activity on various experimental models. This has resulted in the availability of nearly 30 effective anticancer drugs[2].

Pandanus fasicularis Lam. (Syn. *P. odoratissimus*), a plant belonging to the family Pandanaceae, grows throughout India. The decoction is widely used by the tribes and native medical practitioners to treat various ailments including headache, rheumatism, various types of tumours and leprosy[3]. Literature survey reveals that the plant has strong antioxidant activity[4]. Preliminary phytochemical screening showed the presence of glycosides, alkaloids, phenolic compounds and flavonoids. The aim of the present study is to evaluate the anticancer activity of ethanol extract of *Pandanus fascicularis* Lam. (EPF) on Ehrlich Ascites Carcinoma (EAC) in mice.

MATERIAL AND METHODS

Plant material

The roots of *Pandanus fascicularis* were collected from Trichy district, during the month of February 2006. The plant material was identified

and authenticated by the Botanist, Botanical Survey of India, Coimbatore, Tamilnadu. A voucher specimen was kept in our laboratory for future reference. The plant material was shade dried and pulverized.

Preparation of the extract

The powdered plant material (500 g) was packed in a soxhlet apparatus and subjected to continuous hot percolation for 8h using 450 ml of ethanol (95 %v/v) as solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a dessicator (yield, 28.5 g, 5.7%w/w). The extract was suspended in 5 % gum acacia and used for further experiments.

Animals

Swiss albino mice (20-25g) 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 study was conducted after obtaining Institutional Animal Ethical Committee clearance.

Acute toxicity studies

Acute oral toxicity (AOT) of EPF was determined using Swiss albino mice. The animals were fasted for 3 h prior to the experiment and were administered with single dose of extracts dissolved in 5 % gum acacia and observed for mortality up to 48 h (short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 425. All the animals were also observed for long-term toxicity (14 days). The LD_{50}- of the test extract was calculated using 'AOT 425' software provided by Environmental Protection Agency, USA.

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[5].

Effect of EPF on survival time

Animals were inoculated with 1×10^6 cells/mouse on day '0' and treatment with EPF started 24h after inoculation, at doses of 250 and 500 mg/kg/day, p.o. The control group was treated with the same volume of 5 % gum acacia solution. All the treatments were given for nine days. The median survival time (MST) and average body weight changes of each group, consisting of 6 mice were noted. The anticancer efficacy of EPF was compared with that of 5-fluorouracil (Dabur Pharmaceuticals, India; 5-FU, 20 mg/kg/day, i.p. for 9 days). The MST of the treated groups was compared with that of the control group using the following calculation.

Increase in life span = [1-T / C] × 100

Where T = number of days the treated animals survived and C = number of days the control animals survived[6].

Effect of EPF on hematological parameters

In order to detect the influence of EPF 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 (I) Tumor bearing mice (II) Tumor bearing mice treated with EPF (250 mg/kg/day, p.o. for 9 days) (III) Tumor bearing mice treated with EPF (500 mg/kg/day, p.o. for 9 days) and (IV) 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) were determined[7-9].

Effect of EPF on solid tumor

Mice were divided into three groups (n = 6). Tumor cells (1×10^6 cells/mouse) were injected into the right hind limb of all the animals intramuscularly. The mice of group I were tumor control. Group II received EPF (250 mg/kg/day, p.o.) and group III received EPF (500 mg/kg/day, 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[10].

Statistical analysis

All values were expressed as mean ±

SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's '*t*' test. *P* values < 0.05 were considered to be statistically significant when compared to control.

RESULTS

The animals were observed for short term and long term toxicity. No mortality and behavioral changes was observed at the doses tested showing safety of the plant extract.

The effect of EPF on the survival of tumor bearing mice is shown in Table 1. The MST of the control group was 16 ± 0.75 days, whereas it was 23 ± 0.92, 28 ± 0.76 and 32 ± 0.48 days for the groups treated with EPF (250 and 500 mg/kg) and 5-FU (20 mg/kg) respectively. The increase in the life span of tumor bearing mice treated with EPF and 5-FU was found to be 43.75 %, 75 % and 100 % respectively.

The effect of EPF on the inhibition of average increase in body weight is shown in Table 1. The average weight gain of tumor bearing mice was 13.3 ± 0.61g, whereas it was 8.3 ± 0.84, 4.3 ± 0.66 and 4 ± 0.44 g for the groups treated with EPF (250 and 500 mg/kg) and 5-FU (20 mg/kg) respectively.

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

Design of treatment	MST (in days)	Increase in life spanT/C%	Average increase in body weight (g)
Tumor control	16 ± 0.75	-	13.3 ± 0.61
5-FU (20 mg/kg, i.p)	32 ± 0.41*	100	4.0 ± 0.44*
EPF (250 mg/kg, p.o)	23 ± 0.92**	43.75	8.3 ± 0.84 *
EPF (500 mg/kg, p.o)	28 ± 0.76*	75	4.3 ± 0.66*

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

Hematological parameters of tumor bearing mice on the day 14 were showed significant changes when compared to normal mice (Table 2). 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. At the same time interval, EPF (250 and 500 mg/kg) treatment could change these parameters near to normal. Maximum alteration occurred in the EPF treatment at the dose of 500 mg/kg.

There was reduction in the tumor volume of mice treated with EPF (250 and 500 mg/kg, p.o.). Tumor volume of control animals was 14.2 ± 0.38 ml whereas it was 8.76 ± 0.91 ml and 5.87 ± 0.4 ml for the groups treated with EPF 250 and 500 mg/kg respectively (Table 3).

DISCUSSION

The reliable criteria for judging the value of any anticancer drug are prolongation of life span, inhibition of gain in average body weight and decrease of WBC from blood[11,12]. The results of the present study showed an anticancer effect of EPF 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 EPF. After 14 days of transplantation, EPF was able to reverse the changes in the hematological parameters consequent to tumor inoculation.

The reduction of tumor volume of EPF treated mice shows dose dependent reduction, which was observed on 30th day. The maximum inhibition produced by EPF at the dose of 500 mg/kg. The decrease in the tumor volume may be due to the cytotoxic effect of EPF on EAC cells.

All these data point to the possibly developing ethanol extract of *Pandanus fascicularis* as a novel and potential agent in the cancer chemotherapy. Earlier phytochemical work revelas that the roots of *Pandanus fascicularis* contains phenolic compounds, lignan type compounds and benzofuran derivatives namely, 4-hydroxy-3-(2',3'-dihydroxy-3' methyl butyl)-benzoic acid methyl

Table 2: Effect of EPF on hematological parameters

Design of treatment Mono	Hb (gm %)	RBC10^6 10^6cells/mm^3	WBC10^3 10^6cells mm^3	Protein mg%	PCV (mm)	Differential Count (%) Lymph-ocytes	Neutro phils	cytes
Normal	12.3 ±1.1	4.2 ±1.08	6.3 ±1.26	6.2± 1.12	17±1.72	84±4.56	15±1.7	1±0
Tumor Control	5.9 ±0.26	2.7 ±0.76	13.7±1.72	12.4 ±1.7	34.33±2.45	60±3.92	38±3.2	1±0
EPF (250 mg/kg)	10.3 ±0.64^{NS}	3.7±0.97^{NS}	9.4 ±1.3^{NS}	9.8 ±1.1***	29±2.7*	76±3.64^{NS}	23±1.36**	1±0
EPF (500 mg/kg)	11.5 ±0.72^{NS}	4.05 ± 1.16^{NS}	8.1±1.1^{NS}	8.12±0.9^{NS}	24±2.16**	83 ±4.74^{NS}	15±1.82^{NS}	2± 0

N = 5 animals in each group
* P<0.001; **P<0.01; ***P<0.05 when compared with control
^{NS} – Not Significant when compared with normal
Values are expressed as mean ± SEM.

Table 3: Effect of EPF 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
EPF (250 mg/kg)	6.56 ± 0.28*	7.09 ± 0.51*	8.13 ± 0.68*	8.76 ± 0.91*
EPF (500 mg/kg)	5.27 ± 0.37*	5.27 ± 0.37*	5.66 ± 0.19*	5.87 ± 0.21*

N = 6 animals in each group
*P <0.001when compared with control.
Values are expressed as mean ± SEM.

ester, 3-hydroxy-2-isopropenyl-dihydrobenzofuran-5-carboxylic acid methyl ester and Pinoresinol(3,4-*bis*, 4-hydroxy-3-methoxy benzyl)tetrahydrofuran. Among these compounds Pinoresinol(3,4-*bis*, 4-hydroxy-3-methoxy benzyl)tetrahydrofuran showed strong antioxidant activity when BHA was used as a standard in thiocyanate method[4]. Preliminary phytochemical screening indicated the presence of alkaloids and flavonoids in EPF. Flavonoids have been shown to possess antimutagenic and antimalignant effects[13, 14]. Moreover, flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation[15] and angiogenesis[16]. The antitumor properties of the extract may be due to these compounds.

CONCLUSION

The present study points to the potential anticancer activity of *Pandanus fascicularis* in dose dependent manner. This plant contains natural products such as flavonoids, alkaloids and phenols etc, has received considerable attention due to its pharmacological properties including antioxidant activity. Further studies to characterize the active principles and elucidate the mechanism of the action of EPF are in progress.

ACKNOWLEDGEMENTS

The authors are thankful to the Chairman and Secretary Dr. M. Karunanidhi, Angammal Education Trust, for the support rendered.

REFERENCES

1. Kellof, G.J. “Perspective on cancer chemoprevention research and drug development”. *Adv. in Cancer Res,* **78**: 199-334 (2000).
2. Ramakrishna, Y., Manohar, A.I., Mamata, P., and Shreekant, K.G. “Plants and novel antitumor agents: A review”. *Indian Drugs,* **21**: 173-85 (1984).
3. Ananymous, *Wealth of India, Raw material*, *Vol. VII,* CSIR, New Delhi, 218- 220 (2001).
4. Ting Ting Jong and Shang Whang Chau. “Antioxidative activities of constituents isolated from *Pandanus odoratissimus”. Phytochemistry,* **49**: 2145-2148 (1998).
5. Gothoskar, S.V., and Ranadive, K.J. “Anticancer screening of SAN-AB; An extract of marking nut, *Semicarpus anacardium”. Indian J. Exp. Biol,* **9**: 372-375 (1971).
6. Suffness, M., and Douros, J. In: Devita, V.T. editor. *Methods in cancer research*, Academic Press, New York, 73-75 (1978).
7. D’Amour, F.F., Blood, F.R., and Belden, D.A. *The Manual for Laboratory Work in Mammalian Physiology*, The University of Chicago Press, Chicago, 148-150 (1965).
8. Lowry, O.H., Rosenbrough, N.T., and Farr, A.L. “Protein measurement with Folin- Phenol Reagent”. *J. Biol. Chem,* **173**: 265-275 (1951).
9. Docie, J.V. *Practical Haemotology,* J&A Churchill Ltd, London, 38-42 (1958).
10. Ramnath, V., Kuttan, G., and Kuttan, R. “Cytotoxic and antitumour activity of Abrin on trasnplanted tumours in mice”. *Indian J. Physiol. Pharmacol,* **46**: 69-77 (2002).
11. Clarkson, B.D., and Burchenal, J.H. “Preliminary screening of antineoplastic drugs”. *Prog. Clin. Cancer,* **1**: 625-629 (1965).
12. Obiling, C., and Guerin, M. The Role of Viruses in the Production of Cancer, Advances *in Cancer Research 2,* Academic Press, New York, 406-410 (1954).
13. Brown, J.P. “A review of the genetic effect of

occurring flavonoids, anthraquinones and related compounds". *Mutation Res,* **75**: 243-277 (1980).

14. Hirano, T., Oka, K., and Akiba, M. "Antiproliferative effect of synthetic and naturally occurring flavonoids on tumour cells of human carcinoma cells lines". *Res. Comm. Chem. Path. Pharmacol,* ***64***: 69-78 (1989).
15. Weber, G., Shen, F., and Yeh, Y.A. "Increased signal transduction activity and down regulation in human cancer cells". *Anticancer Res,* **16**: 3271-3273 (1996).
16. Fotis, T., Pepper, M.S., Aktan, E., and Breit, S. "Flavonoids, dietary derived inhibitors of cell proliferation and *in vitro* angiogenesis". *Cancer Res,* **57**: 2916 -2918 (1997).