

# Evaluation of anti-cancer potential of aqueous extract of *Pandanus odoratissimus* (Y.Kimura) Hatus. forma *ferreus*, by *in vivo* ascitic tumor model in swiss albino mice

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## ABSTRACT

**Background:** India is a rich source of medicinal plants and number of plant extracts are used against diseases in various systems of medicine such as ayurveda, unani and siddha where only a few of them were scientifically explored. **Objective:** The objective of the present study was undertaken to perform dose dependent anti-cancer effect of aqueous and methanolic extracts of *P. odoratissimus* roots and leaves whose scientific documentation for anti-tumor agent is lacking despite using traditionally. **Materials and Methods:** The anti-cancer activity of methanolic extract of *P. odoratissimus* (MEPO) and aqueous extract of *P. odoratissimus* (AEPO) were tested against Ehrlich ascites carcinoma induced liquid tumors in swiss albino mice. The degree of protection was determined by change in body weight (gm), tumour volume (ml), packed cell volume (ml), cell viability (%), hematological parameters (R.B.C, W.B.C and hemoglobin content), mean survival time (MST), % increase in lifespan (% ILS) and histopathological observation of part of peritoneal layer. **Results:** The treatment with AEPO 400mg/kg, p.o. in EAC treated mice reduced tumor volume, packed cell volume, body weight, cell viability and improved all hematological parameters, mean survival time and life span. Histopathological changes showed degenerative changes of tumor cells in peritoneal layer. The anti-cancer effects of AEPO 400mg/kg, p.o. are equally more with that of the standard drug cisplatin. **Conclusion:** The results suggested that aqueous extract of roots and leaves of *P. odoratissimus* possess *in vivo* anti-cancer activity comparable to cisplatin and this study scientifically validated the traditional use of this plant.

**Keywords:** Anticancer, *Pandanus odoratissimus*, Ehrlich ascites carcinoma.

## INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in indian traditional systems of medicine. *Pandanus odoratissimus* (Pandanaaceae) is one of such plants, distributed commonly throughout india. In ayurveda, unani and siddha the leaves are used for treating back ache, rheumatic diseases, epilepsy, wound healing, nervous disorders, loss of appetite, indigestion, constipation, diabetes, infertility, skin diseases,

urinary disorders and fever.<sup>[1]</sup> The plant is known to possess a broad spectrum of medicinal, pharmacological and therapeutic properties. Tribals believe that this herb is an effective remedy for wide range of illnesses.<sup>[2]</sup> Leaves of *Pandanus* plants contain alkaloids such as pandamine, pandamerilactones with pyrroline derived structures as major chemical constituent<sup>[3]</sup> and was found to possess anti-oxidant, anti-inflammatory and anti-diabetic activities.<sup>[4-6]</sup> In ayurveda paste of *P. odoratissimus* with sugar is used for treating cancers.<sup>[7]</sup> Active principles of methanolic extract of whole plant are 3-(4-(dimethylamino)cinnamoyl)-4-hydroxycoumarin, 3,3'-methylenebis(4-hydroxycoumarin), erythro-9,10-dihydroxyoctadecanoic acid, octadecanedioic acid and dihydroagathic acid.<sup>[8]</sup> Recently, the acute and subacute toxicological studies on methanolic extract of *P. odoratissimus* did not produced

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any significant changes in hematological, biochemical parameters and histopathology of vital internal organs.<sup>[9]</sup> The scientific evidence for supporting anti-tumor activity of *P. odoratissimus* is lacking, despite it is being used as potential anti-tumor agent in traditional system. Hence it was decided to illustrate the ethnobotanical use of this plant by examining dose dependent anticancer effects of methanolic and aqueous extracts from roots and leaves against Ehrlich ascites carcinoma induced liquid tumors in swiss albino mice.

## MATERIALS AND METHODS

### Chemicals

Cisplatin was obtained from sigma chemicals Ltd., India. All other chemicals and solvents were obtained from reachem laboratory and Sd fine chemicals mumbai, India and were of analytical grade with highest purity.

### Collection, authentication and extract preparation of *pandanus odoratissimus* (Y.Kimura) Hatus. forma *ferreus*

The plant *Pandanus odoratissimus* used for the present study was collected from the forest near punalur at kollam district, Kerala during mid winter season of 2012. The plant was identified, confirmed and authenticated by Dr. M. D. Rajanna, professor and head, department of botany (No. 3/proj/B-Garden), university of agricultural sciences, GKVK, bangalore, karnataka, India. A voucher specimen was deposited in department of botany GKVK for future reference.

The roots and leaves of the plant were shade dried, chopped into small pieces and powdered by a mechanical mixer. Coarse material of 500gm was extracted with two different solvents i.e., methanol (2.5L) and distilled water (2.5L) separately using soxhlet extraction apparatus. The solvents were evaporated using rotary vacuum evaporator (YamatoRE300, Japan) at 50°C and dried in dessicator.<sup>[10]</sup>

### Phytochemical analysis

The qualitative and the quantitative analysis of the plant's constituents were examined by the methods described by Trease and Evans, El-Olemmy and Harbone<sup>[11-13]</sup> and were later used for assessment of *in vivo* anti-cancer activity.

### Experimental animals

Swiss albino mice of 25–30g were procured from biogen, bangalore. They were maintained in the animal house of

Gautham college of pharmacy for experimental purpose. The study conducted was approved by the Institutional Animal Ethics Committee (IAEC) of Gautham college of pharmacy, bangalore (REF-IAEC/03/05/2011) according to prescribed guidelines of CPCSEA (Reg No: 491/01/c/CPCSEA), Govt. of India.

### Determination of acute toxicity (LD<sub>50</sub>)

Acute oral toxicity studies were performed according to OECD 423 (acute toxic class method) (Organization for Economic Co-operation and Development).<sup>[14]</sup> Swiss albino male mice (n = 6/each dose) selected through random sampling technique were employed in this study. The general behavior such as motor activity, tremors, convulsions, straub reaction, aggressiveness, pilo erection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin color were observed for the first hour and after 24 h of test drug administration.

### *In vivo* Anti-tumor activity

Ehrlich ascites carcinoma (EAC) cell lines used for the study was supplied by amala cancer research center, thirsur. Cell lines were aspirated aseptically from the mouse peritoneum of a fully grown tumor using syringe with 18 gauge needle, washed thrice with 0.9% saline and suspended in phosphate buffer saline and about 1×10<sup>6</sup> cells in 0.3ml of PBS was injected intraperitoneally into a new healthy mouse.<sup>[15]</sup>

### Treatment protocol

- Group I : Normal saline control (10ml/kg p.o)
- Group II : EAC control (10ml/kg i.p.)
- Group III : EAC (0.3ml) + cisplatin (25mg/kg i.p.)
- Group IV : EAC (0.3ml) + aqueous extract of *P.odoratissimus* (200mg/kg p.o.)
- Group V : EAC (0.3ml) + aqueous extract of *P.odoratissimus* (400mg/kg p.o.)
- Group VI : EAC (0.3ml) + methanolic extract of *P.odoratissimus* (200mg/kg p.o.)
- Group VII : EAC (0.3ml) + methanolic extract of *P.odoratissimus* (400mg/kg p.o.)

### Determination of anti-tumor activity

The anti-tumor potential of MEPO and AEPO were assessed for change in body weight, determination of survival time, % ILS, cell viability by trypan blue dye exclusion method, total ascites fluid volume, packed cell volume, hematological parameters and histopathological evaluation.<sup>[16-19]</sup> Formulae used for calculating above parameters are mentioned below.

$$\% \text{ decrease in body weight} = \frac{\left( \begin{array}{l} \text{gain in body weight} \\ \text{of control group} \\ - \text{gain in body weight} \\ \text{of treated group} \end{array} \right)}{\left( \begin{array}{l} \text{gain in body weight} \\ \text{of control group} \end{array} \right)} \times 100$$

$$\% \text{ viability} = (\text{no. of viable cells}) / (\text{no. of total cells}) \times 100$$

$$\% \text{ ILS} = \frac{\left( \begin{array}{l} \text{MST of treated group} \\ - \text{MST of control group} \end{array} \right) \times 100}{\left( \text{MST of control group} \right)}$$

### Statistical analysis

The values are expressed as mean  $\pm$  SEM. The data were analysed by one way ANOVA followed by Dunnett's test using Graph pad prism software.  $p$  value  $< 0.05$  was considered statistically significant.

## RESULTS

The results of phytochemical analysis showed the presence of alkaloids (1.2%), flavonoids (4.6%), glycosides (2.6%) and phenolic content (3.1%) in aqueous extract of *P.odoratissimus* and presence of alkaloids (1.7%), flavonoids (1.3%) and carbohydrates (2.8%) in methanolic extract of *P.odoratissimus*.

In both phase I and phase II procedures, none of the animals had shown any toxicity up on single administration of MEPO and AEPO (2000mg/kg p.o.). Thus, 1/10<sup>th</sup> and 1/5<sup>th</sup> doses (200, 400mg/kg p.o.) from maximum dose were selected to study the dose dependent response.

Treatment with AEPO at dose of 400mg/kg, p.o. showed the maximum decrease in body weight, tumor volume, packed cell volume, cell viability more than standard cisplatin and greater than other treatment groups. There was a significant reduction in mean body weight  $1.14 \pm 0.31$ , mean tumor volume  $1.25 \pm 0.30$ , mean packed cell volume  $0.75 \pm 0.17$  ( $p < 0.001$ ) and percentage cell viability 18%, in AEPO treatment group at a dose of 400mg/kg, p.o. over standard cisplatin and MEPO. Mean survival time in EAC control group was found to be 14 days which increased significantly to 23 days (62.06%) with AEPO 200mg/kg p.o. and to 31 days (113.79%) with AEPO 400mg/kg p.o., which showed maximum increase in the life span over standard cisplatin 26 days (79.31%) and different doses of MEPO on comparison with EAC

control animals. There was a gradual decrease in percentage of body weight in EAC inoculated animals treated with AEPO, cisplatin and MEPO respectively. The percentage cell viability was found to be 92% in EAC control group which was reduced gradually by different treatment groups like AEPO, MEPO and cisplatin, respectively.

Table 1 shows the effect of cisplatin, AEPO and MEPO on EAC inoculated mice for all the above parameters including hematology profile. AEPO 200, 400mg/kg p.o. showed better improvement in the hematological parameters and reverted to normal levels. The total WBC, RBC and hemoglobin count in EAC control group were found to be  $20.86 \pm 0.36$ ,  $3.21 \pm 0.058$  and  $7.63 \pm 0.08$ . There was a significant decrease in WBC count in EAC inoculated animals treated with AEPO ( $p < 0.001$ ), MEPO ( $p < 0.001$ ) and cisplatin ( $p < 0.001$ ) respectively. RBC count and hemoglobin content in AEPO treated animals showed significant increase ( $p < 0.001$ ) equally more than cisplatin ( $p < 0.001$ ). Whereas, MEPO at 400mg/kg p.o. ( $p < 0.01$ ) treated animals showed slight improvement in RBC and hemoglobin ( $p < 0.01$ ).

Histological section of peritoneum (Fig. 1) showed skeletal muscle with mesothelial proliferation (small arrows). The mesothelial proliferation consists of tumor cells having round to pleomorphic vesicular nucleus with prominent nucleoli and scant cytoplasm. Some of these tumor cells showed degenerative changes (large arrows) in AEPO at 400mg/kg p.o treated animals and cisplatin 25mg/kg i.p.

## DISCUSSION

Flavonoids have been reported to act as anti-cancer agents *via* regulation of signal transduction pathways of cell growth and proliferation, suppression of oncogenes and tumor formation, induction of apoptosis, modulation of enzyme activity related to detoxification, oxidation and reduction, stimulation of the immune system and DNA repair, and regulation of hormone metabolism.<sup>[20]</sup> Polyphenols have protective role in carcinogenesis, inflammation, atherosclerosis, thrombosis and have high antioxidant capacity.<sup>[21]</sup>

In ascitic model following the inoculation of EAC tumor cell lines a marked decrease in life span and increase in body weight of mice were observed. Ascites fluid is the direct nutritional source to tumor cells and faster increase in ascites fluid with tumor growth could possibly means to meet more nutritional requirement of tumor cells. A rapid increase in ascites tumor volume was noted in tumor bearing mice was an indication of increase in body weight.<sup>[22]</sup>

**Table 1. Effect of AEPO, MEPO and Cisplatin on following parameters.**

| Parameters                 | Normal control | EAC + Solvent 10 ml/kg i.p. | EAC i.p. + cisplatin 25 mg/kg i.p. | EAC i.p. + AEPO 200 mg/kg p.o. | EAC i.p.+ AEPO 400 mg/kg p.o. | EAC i.p. + MEPO 200 mg/kg p.o. | EAC i.p.+ MEPO 400 mg/kg p.o. |
|----------------------------|----------------|-----------------------------|------------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|
| Change in b. w (gm)        | 0.40 ± 0.11    | 10.43 ± 0.13                | 1.80 ± 0.23***                     | 2.39 ± 0.20***                 | 1.14 ± 0.31***                | 6.97 ± 0.35***                 | 5.33 ± 0.31***                |
| TV (ml)                    | –              | 10.15 ± 0.14                | 1.66 ± 0.24***                     | 2.13 ± 0.21***                 | 1.25 ± 0.30***                | 6.80 ± 0.35***                 | 5.20 ± 0.33***                |
| PCV (ml)                   | –              | 5.55 ± 0.31                 | 0.95 ± 0.12***                     | 1.03 ± 0.20***                 | 0.75 ± 0.17***                | 3.9 ± 0.21***                  | 3.06 ± 0.24***                |
| % Decrease in b.w          | –              | –                           | 82.69                              | 77.05                          | 89.00                         | 33.09                          | 48.89                         |
| % CV                       | –              | 92                          | 24                                 | 33                             | 18                            | 62                             | 45                            |
| MST (days)                 | –              | 14.50 ± 0.50                | 26 ± 1.00***                       | 23.50 ± 0.50**                 | 31 ± 1.00***                  | 16.50 ± 0.50 <sup>ns</sup>     | 17.50 ± 0.50 <sup>ns</sup>    |
| % ILS                      | –              | –                           | 79.31                              | 62.06                          | 113.79                        | 13.80                          | 20.68                         |
| WBC (x10 <sup>6</sup> /ml) | 7.26 ± 0.25    | 20.86 ± 0.36                | 10.20 ± 0.24***                    | 10.52 ± 0.23***                | 8.20 ± 0.08***                | 15.42 ± 0.11***                | 16.32 ± 0.13***               |
| RBC (x10 <sup>9</sup> /ml) | 5.56 ± 0.06    | 3.21 ± 0.058                | 5.18 ± 0.04***                     | 5.23 ± 0.05***                 | 5.58 ± 0.07***                | 3.36 ± 0.12 <sup>ns</sup>      | 3.80 ± 0.04**                 |
| Hb (gm %)                  | 12.53 ± 0.13   | 7.63 ± 0.08                 | 12.07 ± 0.12***                    | 11.38 ± 0.19***                | 12.19 ± 0.11***               | 7.68±0.07 <sup>ns</sup>        | 8.61 ± 0.12**                 |

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$  and ns represents not significant. All values were compared with EAC control. Where, AEPO = Aqueous extract of *P. odoratissimus*; MEPO = Methanolic extract of *P. odoratissimus*; b.w = body weight; TV = Tumor Volume; PCV = Packed Cell Volume; CV = Cell Viability; MST = Mean Survival Time, ILS = Increase in Life Span.

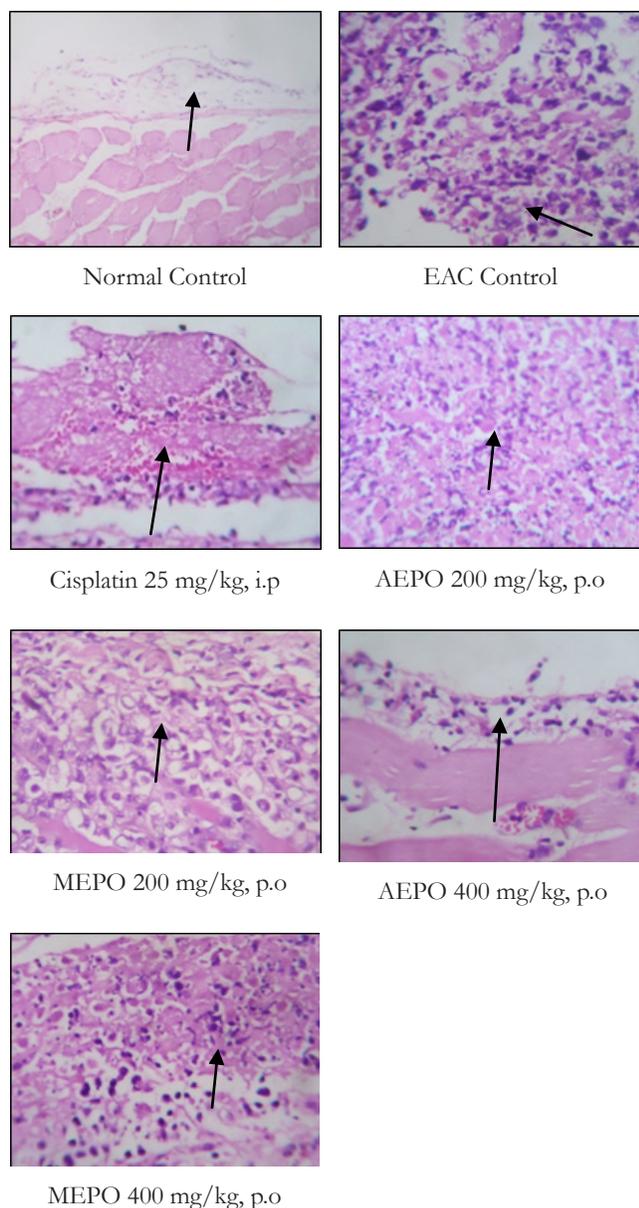
The reliable criteria for judging the efficacy of any anti-cancer drug is prolongation of lifespan of the animals and the decrease of leukemic cells from blood.<sup>[23]</sup> The EAC control group was marked by significant increase in packed cell volume, WBC and viable tumor cell count whereas RBC, hemoglobin, lymphocytes, neutrophils and monocytes showed pronounced decrease. It is understood that the significant rise in WBC in EAC induced group, might be a defensive mechanism against cancer cells. As the progression of cancer was brought under control by *P. odoratissimus*, the WBC count got reduced in treated groups. In cancer chemotherapy, the major problems are myelosuppression and anemia.<sup>[24]</sup> The anemia encountered in tumor bearing mice is 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.<sup>[25]</sup> Interestingly, the present study showed that anti-tumor activity of *P.odoratissimus* was associated with the remarkable restoration of various hematological parameters including RBC and hemoglobin content, which was comparable with the standard anti-cancer drug cisplatin.

The mesothelial proliferation consisting tumor cells showed degenerative changes in aqueous extract treated animals at a dose of 400mg/kg p.o. Control of the cell cycle is accomplished via the coordinated interaction of cyclins with their respective cyclin-dependent kinases

(CDKs) to form active complexes and drive cells into the next phase at the appropriate time. Disordering the cell cycle may result in genomic instability and apoptosis. Bcl-2 family proteins are important regulators of apoptosis. The family comprises both anti-apoptotic (e.g., Bcl-2) and pro-apoptotic proteins (e.g., Bax) with opposing biological functions.<sup>[26]</sup> Apoptosis in cells might happen through very complex functions. It has been suggested that apoptosis may happen by disruption mitochondrial function and induces lysosomal damage as the first target which leads to other cellular events including ROS production and oxidative damage<sup>[27]</sup> lysosomal damage, lipid peroxidation, DNA strand breaks, gene expression, chromosomal aberrations, inhibition of DNA repair processes and induction of apoptosis.<sup>[28]</sup>

## CONCLUSION

AEPO at the dose of 400mg/kg p.o. had shown significant prolongation of lifespan, reduction in tumor volume, packed cell volume, cell viability, improvement in the hematological parameters, changes in histopathological observation when compared to the rest of the groups. There by it can be concluded that AEPO at 400mg/kg p.o. possess better anti-cancer activity than rest of the dose and doses of other drug.



**Figure 1.** Histopathological study of peritoneum.

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