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# ANTICANCER ACTIVITY OF PETROLEUM ETHER EXTRACT OF *ABRUS PRECATORIUS* ON EHRLICH ASCITIS CARCINOMA IN MICE

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## ABSTRACT

This investigation aims to evaluate the Anti tumour potential of the petroleum ether extract of *Abrus precatorius Linn* (PEEAP) on Ehrlich Ascitis Carcinoma (EAC) tumour model. Tumour was induced in mice by intraperitoneal injection of EAC cells (1 $\times 10^6$cells/mouse). PEEAP was administered to the experimental animals at the dose levels of 250,500 and 1000 mg/kg per day after 24 hrs of tumour inoculation. The anti tumour effect of PEEAP was evaluated by assessing and body weight, survival time, haematological parameters. Oral administration of PEEAP increased the survival time of the EAC bearing mice. The PEEAP brought back the alter levels of the haematological parameters in a dose dependent manner in EAC bearing mice. The results were comparable to that of the result obtained from the animals treated with the standard drug 5-flurouracil (20mg/kg .b.w). Thus present study revealed that PEEAP possessed significant anti tumour activity.

## KEY WORDS

*Abrus precatorius*, Ehrlich Ascitis Carcinoma, Hematological parameters, Tumour growth response.

## INTRODUCTION

Drug discovery from the medicinal plants has played an important role in the treatment of cancer and indeed, most new clinical applications of plants secondary metabolites and their derivatives over the last half- century have been made towards combating cancer[1]. *Abrus precatorius* (Fabaceae) is a woody climber distributed widely throughout India [2, 3]. The plant is traditionally used for the treatment of sore tongue and it also has diaphoretic action. This study was undertaken to evaluate the anti tumour potential of the petroleum ether extract of *Abrus precatorius Linn* (PEEAP) against the Ehrlich Ascitis Carcinoma (EAC) tumour model.

## MATERIALS AND METHODS

***(i) Plant collection:***

*Abrus precatorius* seeds were collected in and around Chennai in the month of June 2009 and authenticated by Dr. Jayaraman, Botanical Survey of India, Chennai, Tamil Nadu, India.

***(ii) Preparation of Petroleum ether Extract:***

The plant material was shade dried and pulverized. Petroleum Ether extract of the coarsely powdered material was prepared employing Maceration with 2,500 ml of Petroleum ether (RFCL Limited) for 72 h and the extract was concentrated to 8gm.The extract was dissolved in Carboxy methyl cellulose (2%) (Loba chemie Pvt. Ltd) before administration.

***(iii) Animals:***

Adult Swiss male albino mice (20-25gm) were procured from animal house, Department of pharmacology, Vels University, and used throughout the study. They were housed in micro lane boxes in a controlled environment (temperature 25±2ºC and 12 h dark and light cycle) with standard diet and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee clearance. (290/CPCSEA/2009-PH/PCOL-07).

***(iv) Cancer cell line:***

EAC cells were obtained through the courtesy of Amala Cancer Research Center, Thrissur. They were maintained by weekly intraperitoneal inoculation of $10^6$ cells/mouse.[4]

***(v) Effect of PEEAP on tumour growth response:***

Animals were inoculated with $2\times10^6$ cells / mouse on day '0' and treatment with PEEAP started 24 h after inoculation, at a dose of 250,500,1000mg/kg/day, p.o. All the treatments were given for 10 days. The mean survival time (MST) of each group, consisting of 6 mice was noted. The anti tumour efficacy of PEEAP was compared with that of 5-fluorouracil 20 mg/kg/day, i.p for 10 days). Mean survival time and increased life span (%ILS) was calculated using the following equation.[5, 6]

MST= (Day of first death+day of last death)/2

ILS (%) = [(Mean survival time of treated group / mean survival time of control group)-1] ×100

***(vi) Anti-tumour activity:***

Male Swiss albino mice were divided into 6 groups (n=6).All the groups were injected with EAC cells (0.2ml of $2\times10^6$ cells /mouse) intraperitoneal[7]. This was taken as day zero.
Group I- Disease control, EAC cell line ($2\times10^6$cell mouse).
Group II - EAC cell line ($2\times10^6$cells) treated with 250 mg /kg p.o. of PEEAP.
Group III -EAC cell line ($2\times10^6$cells) treated with 500 mg /kg p.o. of PEEAP.
Group IV -EAC cell line ($2\times10^6$cells) treated with 1000 mg /kg p.o. of PEEAP.
Group V - EAC cell line ($2\times10^6$cells) treated with standard [5-Flurouracil (20 mg/kg i.p)]
After 10 days of treatment, animals from each group were sacrificed by retro orbital plexus method to evaluate the anti tumour potential of PEEAP.

***(vii) Haematological studies***:

Haemoglobin content, Red blood cells count (RBC), White blood cells count (WBC) and Packed cell volume (PCV) were measured from freely flowing tail vein blood from all the groups.[8, 9]

***(viii) Statistical analysis:***

All the values were expressed as mean ± SEM. The data were statistically analyzed by one-way ANOVA followed by Dunnett's test. The data of haematological parameters were analyzed using ANOVA followed by Dunnet T-test. P values < 0.05 were considered significant.

## RESULTS

Preliminiary phytochemical screening of PEEAP was carried out for the detection of phytoconstituents using standard chemical tests. Flavonoids, Alkaloids, tannins, phenolic compounds, saponins, triterpenoids detected in PEEAP. Oral administration of PEEAP increased the mean survival time of EAC bearing mice. In the EAC control group the mean survival time was 18.16 ± 0.4773, while it increased to 20.5±0.6009 (250mg/kg), 20.33±0.4944 (500mg/kg) and 21.166±0.6009 (1000mg/kg) respectively in PEEAP treated groups. The group treated with standard 5-FU (20mg/kg) showed 24.5±0.4282 for the same. The percentage increase in survivals, it was found to be 11.01%, 11.94%, 16.51% respectively as compared to control group which are represented in Table-1. The EAC bearing mice treated with the PEEAP showed excellent increase in life span when compared to control. Treatment with PEEAP at the doses of 250, 500 and 1000mg/kg reduced the body weight[10, 11], PCV[12, 13] and viable tumour cell count as compared to that of EAC control group and increased the haemoglobin content and RBC count towards normal level. Administration of PEEAP at the doses of 250, 500 and 1000mg/kg in EAC bearing mice reduced the WBC count as compared with the control. Treatment with PEEAP at different doses changed these altered parameters towards normal levels Table-2.

**Table-1**
***Effect of PEEAP treatment on the survival of tumour-bearing mice***

| Treatment | MST (d) | Increase in Life Span (%) | Body weight |
|---|---|---|---|
| Tumour control | 18.16±0.4773 | ------ | 37.45 ± 0.86 |
| 5-FU (20 mg /kg, i.p.) | 24.5±0.4282 | 34.9 | 25.15 ± 0.65 |
| PEEAP (250 mg/kg, p.o.) | 20.16±0.6009 **# | 11.01 | 27.5 ± 0.60**## |
| PEEAP (500 mg/kg, p.o.) | 20.33±0.4944 **# | 11.94 | 30.5 ± 0.44**## |
| PEEAP (1000 mg/kg, p.o.) | 21.166±0.6009 **## | 16.51 | 29.66 ± 0.45**## |

*Control $V_s$ T **P<0.01, *P<0.05; Standard $V_s$ T ##P<0.01, # P<0.05*
*Data was analysed by one way ANOVA followed by Dunnet's't' test, n=6*

**Table-2**

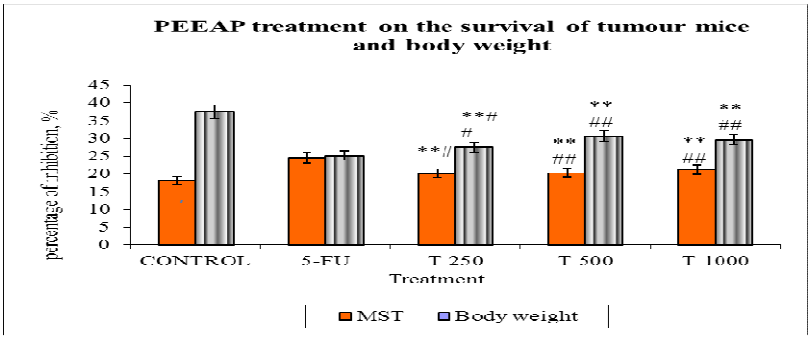
***Effect of PEEAP extract on hematological parameters of cancer (EAC) bearing mice***

| Treatment | Hb (g%) | RBC(million/mm$^3$) | WBC(10$^3$cells/mm$^3$) | PCV (mm) |
|---|---|---|---|---|
| Tumour bearing | 6.3166±0.1740 | 3.95±0.1088 | 18.3±0.0695 | 21.81±0.454 |
| 5-FU(20mg/kg,i.p.) | 11.865±0.0838 | 3.55±0.119 | 11.343±0.060 | 31.63±0.245 |
| PEEAP(250mg/kg) | 12.23±0.1406** | 12.183±0.1537**## | 4.041±0.1003**## | 38.11±0.192**## |
| PEEAP(500mg/kg) | 11.83±0.1116** | 11.833±0.116**## | 14.23±0.049** | 32.86±0.619**## |
| PEEAP(1000mg/Kg) | 9.35±0.1360**## | 9.35±0.1360**## | 13.08±0.087**## | 28.73±0.1944**## |

*Control V$_s$ T **P<0.01, *P<0.05; Standard V$_s$ T ##P<0.01, # P<0.05*
*Data was analysed by one way ANOVA followed by Dunnet's't' test, n=6*

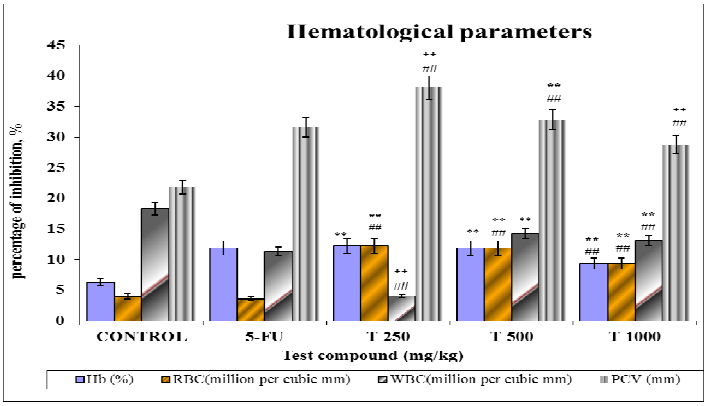
**Graph-1**

***Effect of PEEAP treatment on the survival of tumour-bearing mice***




**Graph-2**

***Effect of PEEAP extract on hematological parameters of cancer (EAC) bearing mice***



***CYTOLOGICAL STUDIES – EAC***

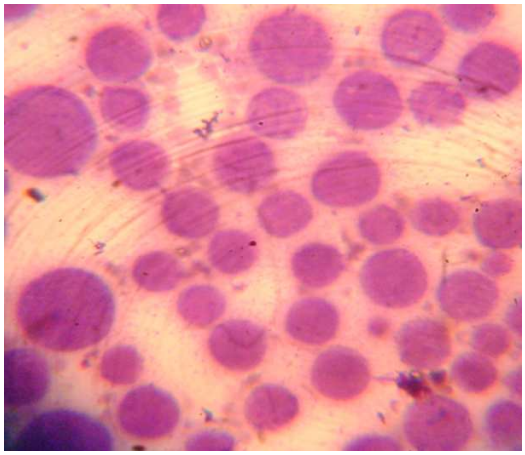


Fig 1

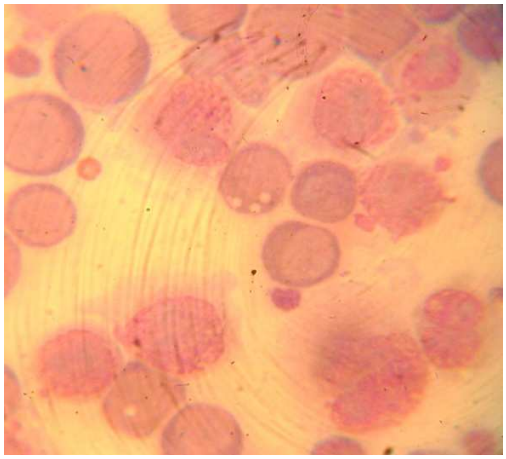


Fig 2

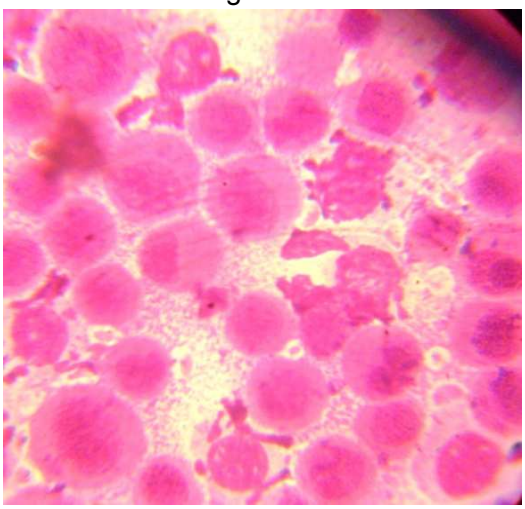


Fig 3

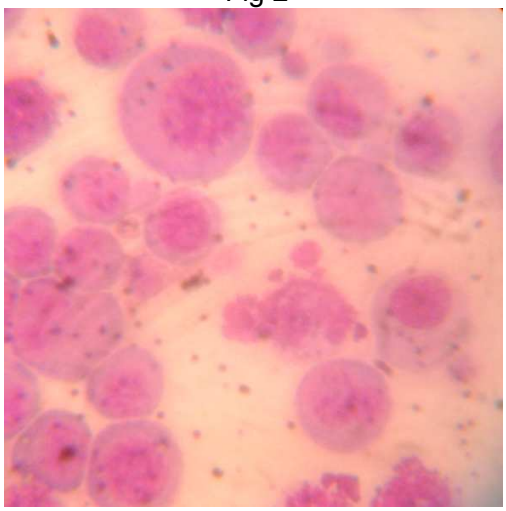


Fig 4

Fig 1 Tumour Control
The cells are larger in size and showed binucleation

Fig 2. PEEAP (250mg/kg)
The cells showed high n/c ratio, plasmocytoid feature with varying degree of degeneration and cytoplasmic vacuolation which is characteristic of immunoblast.

Fig 3. PEEAP (500mg/kg)
The cells showed, plasmocytoid feature with varying degree of degeneration and cytoplasmic vacuolation which is characteristic of immunoblast.

Fig 4, PEEAP (1000 mg/kg)
The cells showed high, plasmocytoid feature with varying degree of degeneration and cytoplasmic vacuolation which is characteristic of immunoblast.

## DISCUSSION

Flavonoids have been shown to possess antimalignant effects[14, 15]. Further more, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation [16]and angiogenesis.[17] Quercetin a well-known bioflavonoid is commonly found in human diet. This flavonoid along with Genistein gained much attention during the last few years as a potential anticancer drug[18]. The PEEAP is rich in flavonoids, alkaloids and terpenoids. The cytotoxic and antitumour properties of the extract may be due to these compounds. The present study points to the potential anticancer activity of *Abrus precatorius*. Further studies to isolate, characterize the active principles and elucidate the mechanism of the action of *Abrus precatorius* are in progress. The reliable criteria for judging the value of any anticancer drug are prolongation of lifespan and decrease of tumour volume and viable tumour cell count and increase of non-viable tumour cell count. The results of the present study show an antitumour effect of PEEAP against EAC cells in Swiss albino mice. The present study reveals that PEEAP treatment at the dose level of 250, 500 and 1000 mg/kg b.w are quite effective against Ehrlich ascites carcinoma cells, depicting increase in life span by about 11.01%, 11.94% and 16.51% respectively as compared with control. This substantiates its antitumour properties. Herbal medicine derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine.

Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and anticancer activity. There has been growing interest in the analysis of certain flavonoids, triterpenoids and steroids due to their potential benefits to human health. One of their main properties in this regard is their antioxidant activity, which enables them to attenuate the development of tumour and inflammatory disease. Antioxidant plays an important role in inhibiting and scavenging radicals, thus providing protection to human against infection and degenerative diseases.

Plants have been prime source of highly effective conventional drugs for the treatment of many forms of cancer, and while the actual compounds isolated from the plant frequently may not serve as the drugs, they provides lead for the development of potential novel agents. Myelosupression is a frequent and major complication of cancer chemotherapy. Compared to the EAC control animals, PEEAP treatment showed significant tumour inhibition resulted in appreciable improvement in hemoglobin content and RBC count. These observations assume great significance as anemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis and thereby limiting the use of drugs.

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