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# Evaluation of *in vivo* anticancer activity of *plectranthus vettiveroides* against ehrlich ascites carcinoma in swiss albino mice

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## ABSTRACT

The present study was carried out to evaluate the anticancer property of ethanol extract of *Plectranthusvettiveroides* leaves against Ehrlich ascites carcinoma in Swiss albino mice. The leaves powder was subjected to continuous hot extraction using ethanol and cold maceration by water to get Ethanol and Aqueous extracts, respectively. Identification of the chemical constituents of plant extract was determined by standard procedures. The in vivo anticancer study was determined in mice using Ehrlich ascites carcinoma cell line. The extract's in vivo cytotoxic impact was determined by measuring mean survival time, haematological parameters, cell count, tumour weight, and antioxidant enzyme activities such as lipid peroxidase, reduced glutathione, superoxide dismutase, and catalase. The crude extract indicated the presence of several chemical groups such as alkaloids, saponins, steroids, flavonoids, and glycosides during phytochemical screening. The extract resulted in a substantial increase in life duration as well as a reduction in the number of cancer cells, tumour weight, and tumour volume. At doses of 200 and 400 mg/kg, the extract was found to have a protective impact on the hemopoietic system. Serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, and alkaline phosphatase levels were significantly reduced by the extract. Lipid peroxidation was avoided, and antioxidant enzymes were restored, thanks to the extract. The ethanol extract of Plectranthus vettiveroides demonstrated a substantial (p0.001) in vivo cytotoxic impact when compared to the tumour control group, according to the findings.

**Keywords:** *Plectranthus vettiveroides*, In vivo Cytotoxicity studies, EAC cell line.

## INTRODUCTION

Cancer is a multicellular illness marked by the uncontrolled proliferation of slightly transformed normal human cells. Cancer is a primary cause of mortality worldwide, posing a significant public health threat. Cancer is the leading cause of death in developed countries and the second leading cause of death in developing countries, and the burden of cancer is rising in developing countries as a result of population ageing and growth, as well as an increase in the adoption of cancer-related lifestyle choices such as smoking, physical inactivity, and "westernised" diets. According to estimates, the total number of new cancer cases will increase by approximately 25% per decade, reaching 24 million

new cases per year in 2050; the total number of deaths will increase from 6 million in 2000 to 10 million in 2020 to over 16 million in 2050; and there will be 17 million new cancer cases in 2050. [1-4].

*Plectranthus vettiveroides* is also known as coleus vettiveroides, coleus zeylanicus, plectranthuszeynanicus (Lamiaceae). The main phytochemical constituents of the genus Plectranthus are diterpenoids, essential oils and phenolics. About 140 diterpenoids were identified from the coloured leaf –glands of plectranthus species. The main constituents of essential oils of plectranthus are mono and sesquiterpenes. Flavonoides seem to be rare in plectranthus, only two flavonoides were identified ,4',7-dimethoxy -5,6-identified, vone in plectra thus ambigns and chrysosplevetin from p.marruboides. Traditionally it has been used as an antibacterial, deodorant, cooling agent and also used against eye burning head ache and fever. [5-8]. The present research was designed to carry out In vivo Anticancer Activity of *Plectranthus vettiveroides* against Ehrlich Ascites Carcinoma in Swiss Albino Mice

## MATERIALS AND METHODS

Plant Material and Extract Preparation Plectranthus vettiveroides leaves were obtained from ABS Botanical Garden in Salem District, Tamilnadu, India. Using the Soxhlet Apparatus, the dried powdered leaves of Plectranthus vettiveroides were extracted using Ethanol. By fully evaporating the solvent under vacuum, the extract was dried and concentrated. Phytochemical analysis of the crude extract indicated the presence of tannins, saponins, steroids, flavonoids, and glycosides, among other chemical groups [7],[8]. Animals In this investigation, male Swiss albino mice weighing 25-30g were utilised. In the animal home, all of the animals were kept at a constant temperature of 22-25oC. All of the animals were cared for according to globally established ethical criteria for laboratory animal care. Animals were provided normal diet for one week before to the tests to help them adjust to the laboratory settings. The institutional ethics committee approved all animal operations before they were carried out.

### Acute Toxicity Study

At dosages of 5mg/kg, 50mg/kg, 300mg/kg, 500mg/kg, and 2000mg/kg, ethanol extracts of Plectranthus vettiveroides leaves were tested for acute toxicity. According to the OECD 420 guideline, a dosage of 2000mg/kg caused harmful symptoms, thus it is regarded an LD50 cut off value by the OECD. Fixed dosage techniques were used to determine doses of 200mg/kg and 400mg/kg for pharmacological investigations [9].

### Tumor Cells

Amala Cancer Research Centre in Trissur, Kerala, India provided the Ehrlich ascites carcinoma (EAC) cells. Intraperitonial transplanting was used to keep the cells alive in Swiss albino mice. EAC cells were extracted from the peritoneal cavity of mice and injected intraperitoneally to generate an ascetic tumour. Designing Experiments The mice were split into five groups, each containing twelve mice. Except for the normal group, the entire animal was injected intraperitoneally with EAC cells (2X106 cells/mouse) as follows:

Group I : Normal (only sodium CMC Suspension (0.1%)) Group II : Control (Induced EAC cell (2 X106 ) with sodium CMC Suspension (0.1%)) Group III : Standard (Induced EAC cell (2 X106 ) with 5-fluorouracil 20mg/kg body weight) Group IV : EEPV (Induced EAC cell (2X106 ) with ethanol extract of *Plectranthus vettiveroides* 200mg/kg body weight with sodium CMC (0.1%)) Group V : EEPV (Induced EAC cell (2X106 ) with ethanol extract of *Plectranthus vettiveroides* 400mg/kg body weight with sodium CMC (0.1%)) All groups were given with respective drugs 24 h after the tumor inoculation, once daily for 14 days. Six mice from each group were killed after the last dosage and a 24-hour fast. The animals' blood was taken using a retro-orbital puncher under mild anaesthesia, and haematological parameters such red blood cells (RBC), white blood cells (WBC), differential count (DC), and haemoglobin (HB) were calculated using a cell analyzer. In a blood smear, a differential count of WBC was performed. The ascetic fluid was taken from the animals' peritoneal cavity, centrifuged, and split into two portions. The packed cell volume of one component was determined after centrifugation at 1,000 rpm for 10 minutes in a graduate centrifuge tube. Centrifugation was used to separate the cells in the other portion of the ascetic fluid, which were then stained with trypan blue (0.4 percent in normal saline). A total of viable and non-viable cells were counted. The remaining animals were kept for six weeks to determine their average life duration and body weight change. The increase in life span (ILS) was estimated as a percentage increase [10] [11].

### Tumor Growth Response

The effect of Plectranthus vettiveroides ethanol extracts on tumour development and host survival time was investigated by looking at tumour volume, packed cell volume, tumour cell count, viable tumour cell count, nonviable tumour cell count, median survival time, and increase in lifespan.

## Determination of Tumor Volume

The ascetic fluid was collected from the peritoneal cavity after the mice were dissected. The volume was measured in a graduated centrifuge tube and promptly weighed.

## Tumor Packed Cell Volume

The ascetic fluid was collected from the peritoneal cavity. The packed cell volume was measured by taking it in a graduated centrifuge tube and by centrifuging at 1000 rpm for 5 min.

## Tumor Viable Cell Count

The ascetic fluid was diluted 100 times in a WBC pipette. The diluted cell suspension was then dropped into the Neubauer counting chamber. Trypan blue dye (0.4 percent in normal saline) was then used to stain the cells. The cells that did not take up the dye were alive, whereas those that did were dead. In the 64 tiny squares, viable cells were counted.

## Percentage Increase Life Span

The effect of Ethanol Extracts of *Plectranthusvettiveroides* on tumor growth was monitored by recording the mortality daily for a period of six weeks and percentage increase in average life span was calculated. % ILS = (A / B) – 1 x 100 A- Life span of treated group B - Life span of controlled group ILS - Increase in average life span.

## Body Weight Analysis

Body weights of the experimental mice were recorded both in the treated and control groups at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period and calculated on 15th day.

**C = (a – b / a) x 100 a – Wt. of animal on day 0 b– Wt. of animal on day 15 C – % increase in body weight.**

## Hematological Parameters

The collected blood was immediately used for the estimation of HB content, RBC and WBC. WBC differential count was carried out from Leishman stained blood smears [12].

## Study of Biochemical Parameters

The serum from the remaining blood was used to calculate hepatoprotective measures such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate Pyruvate transaminase (SGPT), and alkaline phosphatase (ALP). Tissue lipid peroxidation levels (LPO), Glutathione peroxidase (GSH), Superoxide dismutase (SOD), and Catalase are antioxidant markers (CAT).

## Statistical Analysis

All the values were expressed as mean ± standard error of mean (S.E.M) and analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test.

# RESULTS AND DISCUSSION

In phytochemical screening the Ethanol Extracts of *Plectranthus vettiveroides* (EEPV) showed the presence of alkaloids, saponins, steroids, flavonoids and glycosides. The animals of the tumor control group inoculated with EAC survived for a period 17.65±1.51 days. The treatment with EST at 200 and 400 mg/kg body weight increased the average life span of animals by 26±1.0 and 31.65±6.01 days, respectively, which is comparable to the standard drug (5-FluoroUracil) at the dose of 20mg/kg with the survival period of 35.32±5.84 (Table. 1 & Figure. 2). The increases in life span at 200 and 400 mg/kg body weight were found to be significant. The extracts at the 400 mg/kg body weight dose was found to be more potent in inhibiting the proliferation of EAC with the percentage increases in life span of 75.38%. The average increase in body weight of the EAC tumor control group was found to be 12.46±0.31%. Treatment at the doses of 200 and 400 mg/kg significantly inhibited the average increase in body weight (5.6±0.52, 4.85±8.87) when compared to the tumor control ($p<0.001$) (Table 1 & Fig. 1). The tumor volume (Table 1), packed cell volume, viable tumor cell count (× 106 cells/ml) (Table 1) and total WBC (x10/mm) (Table. 2 & Figure 3) were found to decreases significantly in animal treated with the extract at almost all the doses tested when compared to EAC tumor control which indicating the antitumor nature of the ethanol extract of Scaevolataccada.

**Table 1: The Effect of Ethanol Extract of *Plectranthus vettiveroides* (EEPV) on Tumor Parameters**

| Parameter | Control | Standard (20 mg/kg) | EEPV (200 mg/kg) | EEPV (400 mg/kg) |
|---|---|---|---|---|
| Survival Time (Days) | 17.65±1.51 | 35.32±5.84*** | 26±1.0* | 31.65±6.01*** |
| % Increase Life span | - | 84.11** | 61.8** | 75.38** |
| Body Weight (g) | 12.46±0.31 | 1.9±0.49* | 5.6±0.52 | 4.85±8.87 |

| | | | | |
|---|---|---|---|---|
| Tumor volume (ml) | 10.22±0.84 | 1.72±0.39*** | 4.1±0.19*** | 3.52±0.39*** |
| Packed Cell Volume (mm) | 6.82±0.27 | 1.15±0.27*** | 4.32±0.27*** | 3.4±0.49*** |
| Viable cells(×106 cells/ ml) | 8.23±0.27 | 1.20±0.19*** | 2.97±0.09*** | 2.27±0.20*** |
| Non viable cells (×106 cells/ ml) | 0.31±0.05 | 3.58±0.11*** | 1.90±0.06*** | 2.33±0.08*** |

n=6 animals in each group, Values are represented as mean ± SEM of six animals.* P<0.05, **P<0.01 and ***P<0.001 between disease control and treated groups. (Analysed by ANOVA Tukey-Kramer multiple comparison test).

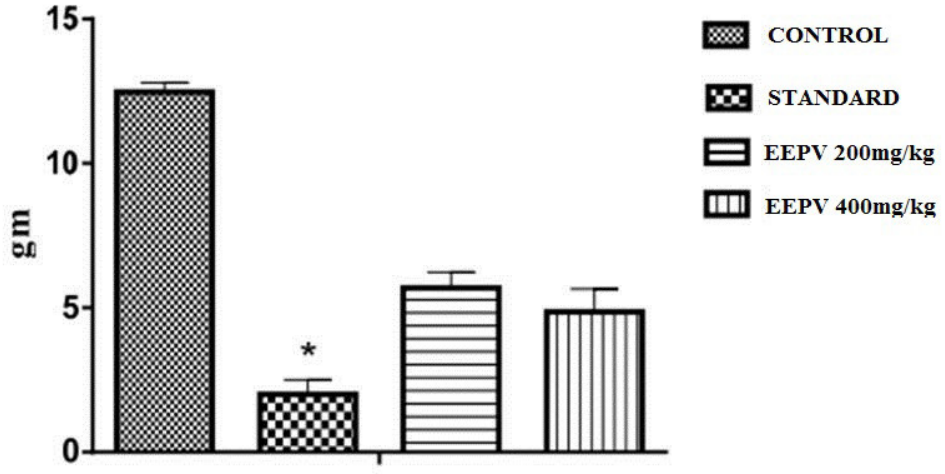


**Figure 1: The Effect of Ethanol Extract of Plectranthusvettiveroides (EEPV) on body weight in EAC bearing mice.**

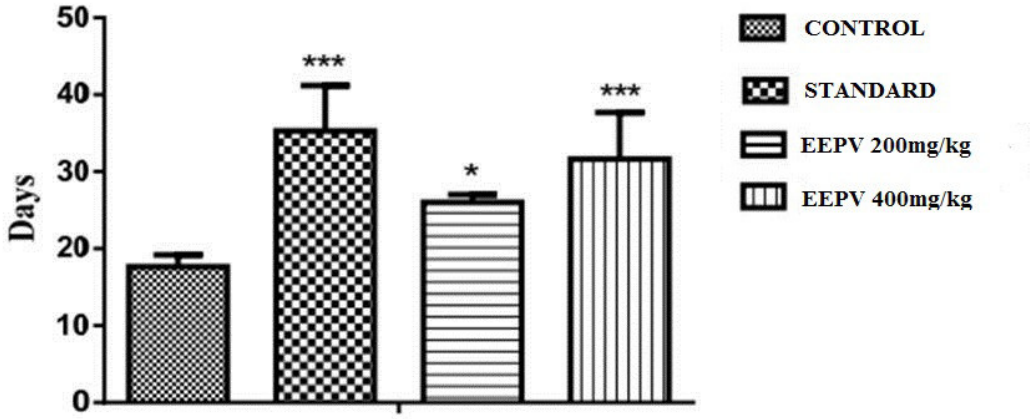


**Figure 2: The Effect of Ethanol Extract of *Plectranthus vettiveroides* (EEPV) on mean survival time in Ehrlich ascites carcinoma (EAC) bearing mice.**

Similarly, after EAC inoculation, RBC count, haemoglobin content, and lymphocyte count were observed to have considerably recovered to normal levels in the animals treated with the EST at both two dosages. The neutrophil count, which was higher in EAC tumour control mice, was shown to be substantially (p 0.001) reduced by the extracts at all dosages, returning to normal (Table 2& Figure 3). In all of these parameters, the conventional 5-FU therapy at 20 mg/kg body weight outperformed the extract treatment.

**Table 2: Effect of Ethanol Extract of *Plectranthus vettiveroides* (EEPV) on Hematological Parameters.**

| Hematological parameters | Normal | Control | Standard (20 mg/kg) | EEPV (200 mg/kg) | EEPV (400 mg/kg) |
|---|---|---|---|---|---|
| Hb (g %) | 14.12±1.24 | 4.7±0.8 | 12.21±1.0*** | 9.85±1.0* | 11.3±0.39*** |
| RBC (million/mm3) | 5.32±0.74 | 2.2±0.1 | 5.15±0.24*** | 3.15±0.10 | 4.2±0.42* |
| WBC(103cells/mm3) | 6.6±0.61 | 14.25±0.69 | 9.65±0.54*** | 12.2±0.41 | 11.4±0.61* |
| Lymphocytes | 70.0±0.39 | 32.3±0.69 | 66.25±0.49*** | 48.95±0.64*** | 57.92±0.64*** |
| Neutrophils | 31.32±0.91 | 35.92±0.24 | 29.6±0.19*** | 33.42±0.46* | 31.95±0.29*** |
| Monocytes | 2.02±0.14 | 4.3±0.19 | 28.6±0.19*** | 3.82±0.14 | 2.8±0.25*** |

n=6 animals in each group, Values are represented as mean ± SEM of six animals.*P<0.05, **P<0.01 and ***P<0.001 between disease control and treated groups. (Analysed by ANOVA Tukey-Kramer multiple comparison test).

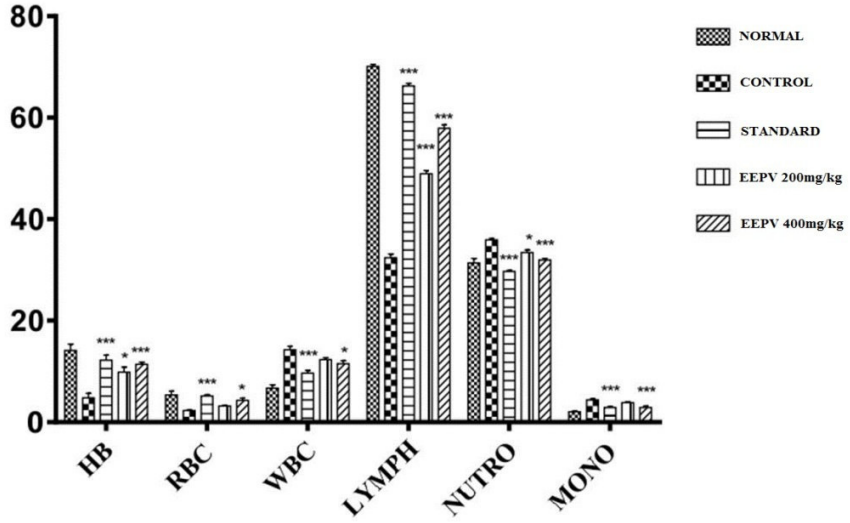


**Figure 3: The Effect of Ethanol Extract of Plectranthus vettiveroides(EEPV) on Hematological Parameters. HB: Hemoglobin, RBC: Red blood cells, WBC: White blood cells, LYMPH: Lymphocytes NUTRO: Neutrophils MONO: Monocytes.**

**Table 3: The Effect of Ethanol Extract of *Plectranthus vettiveroides* (EEPV) on Biochemical Parameters.**

| Design of treatment | Normal | Control | Standard (20 mg/kg) | EEPV (200 mg/kg) | EEPV (400 mg/kg) |
|---|---|---|---|---|---|
| Proteins (g %) | 8.15±0.46 | 11.47±0.16 | 7.27±0.29 | 9.85±0.04*** | 7.95±0.11* |
| SGPT (U/L) | 27.17±2.08 | 57.72±3.68*** | 32.94±1.09 | 43.53±1.86*** | 36.54±0.80* |
| SGOT (U/L) | 33.47±1.17 | 62.87±3.08*** | 40.15±1.35 | 45.76±2.28** | 37.72±2.12 |
| ALP (U/L) | 79.44±2.70 | 119.71±4.32*** | 81.30±1.47 | 99.41±2.58** | 86.44±4.25 |
| LPO (mol MDA/mg protein) | 0.75 ± 0.02 | 2.95± 0.038*** | 0.96± 0.040*** | 2.11 ± 0.074** | 1.02 ± 0.014*** |
| GSH (mol/ g. wet tissue) | 2.04 ± 1.52 | 0.60± 1.32 | 1.78± 0.96 | 1.46 ± 1.871 | 1.80 ± 0.930 |
| SOD (U/mg protein) | 4.18 ± 3.73 | 1.25 ± 1.00 | 3.31 ± 3.16 | 2.16 ± 4.00 | 3.47 ± 8.31 |
| CAT (U/mg protein) | 26.0 ± 0.022 | 9.36± 0.113 | 21.5± 0.010 | 19.0 ± 0.054 | 21.0 ± 0.062 |

n=6 animals in each group, Values are represented as mean ± SEM of six animals.*P<0.05, **P<0.01 and ***P<0.001 between disease control and treated groups. (Analyzed by ANOVA Tukey-Kramer multiple comparison tests).

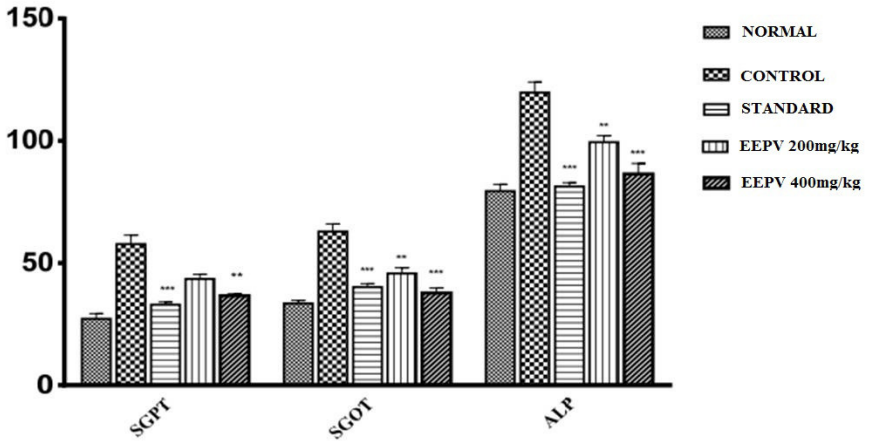


**Figure 4: The Effect of Ethanol Extract of Plectranthusvettiveroides(EEPV) on Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate transaminase and alkaline phosphatase of Ehrlich ascites carcinoma (EAC) bearing mice.**

When comparing the EAC bearing control group to the normal control group, the total WBC count was found to be substantially higher in the EAC bearing control group. When extract was given to EAC-bearing mice, the WBC count was substantially lower than in the control group. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP) levels were significantly reduced by the extract (Table 3 & Figure. 4). When compared to tumor-induced control animals, the extract avoided lipid peroxidation and restored the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, and glutathione-s-transferase in the liver. Tumor growth impacts a variety of haematological markers, and anticancer effectiveness is usually measured by restoring these alterations to normal, most notably in decreased WBC and increased RBC, Lymphocyte, and haemoglobin content when compared to the control group.

Natural products have long been thought to be valuable sources of possible chemotherapeutic medicines. Paclitaxel, vincristine, podophyllotoxin, and camptothecin, a natural product precursor produced from water soluble derivatives, are some of the novel chemotherapeutic drugs now accessible for usage. Natural goods are, without a doubt, a very significant source of therapeutic ingredients. While there are some innovative techniques to drug discovery, such as combinatorial chemistry and computer-based molecular modelling design, none of these can replace natural products in drug discovery and development [13-15]. The purpose of this study was to see if the ethanol extract of Plectranthus vettiveroides leaves extract has anticancer efficacy in mice with EAC tumours.

The extension of the life span of tumor-bearing animals is a valid criterion for assessing the efficacy of any anticancer medication. The frequent fast growth in the ascetic tumour volume causes a substantial increase in body weight in EAC tumour bearing mice [16]. The mice with EAC tumours that were given an ethanol extract of Plectranthusvettiveroides leaves extract orally exhibited a substantial improvement in life duration as well as a significant reduction in bodyweight gain compared to the EAC control mice. The tumour volume, packed cell volume, and viable tumour cell count all decreased significantly after treatment with the ethanol extract of Plectranthusvettiveroides leaves extract, demonstrating the anticancer nature of Plectranthus vettiveroides leaves extract. These findings suggest that Plectranthus vettiveroides leaf extract has a cytotoxic impact on tumour cells. Myelosuppression and anaemia are the most common side effects of cancer treatment [17]. Anemia in tumor-bearing mice is caused by a decrease in RBC count or haemoglobin percentage, which can be caused by iron shortage, as well as hemolytic or myelopathic diseases. In comparison to EAC mice, treatment with Plectranthusvettiveroides leaves extract repaired the haematological profiles. This suggests that the ethanol extract of Plectranthus vettiveroides leaves has hemopoietic system-protective properties.

## CONCLUSIONS

Based on the results, it can be inferred that the leaves of Plectranthus vettiveroides ethanol extract had significant anticancer effect against EAC-induced tumours in mice. It will take a lot of investigation to figure out which component is

responsible for the anticancer action and what the molecular mechanism is.

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