Int J Pharm Bio Sci 2014 Oct; 5(4): (P) 119 - 125

Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTITUMOR PROPERTIES OF RHIZOME OF *ACORUS CALAMUS* L USING DALTON'S ASCITES LYMPHOMA BEARING SWISS ALBINO MICE.

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ABSTRACT

Herbal concoctions have recently been investigated for various chronic and complicated ailments as effective therapeutics. The Ayurvedic literature of India is essentially focussed for their biologically active constituents in whole for efficacy. In this study, a preliminary analysis of the rhizomes of *Acorus calamus* L, has been investigated as a potent antitumour agent against Daltons Ascites Lymphoma in mice by evaluating the tumour growth, toxicity and haematological parameters. The methanolic extract of *Acorus calamus* (MEAC) was observed to restore the hematological parameters as compared with the DAL bearing mice in a dose dependant manner indicating a significant antitumour activity, thus indicating A. calamus to be a potent ethnomedicine.

KEY WORDS: ***Acorus calamus*, Daltons ascites lymphoma, methanol, extract, rhizome.**

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INTRODUCTION

It has been opined based on global statistics that over 20 million new cancer cases and more than 17 million cancer deaths are probable to occur by the year 2050. Anticancer therapies have largely involved the use of chemical and drugs as a part of chemotherapy against various types of cancer either singly or in combination with surgery and / or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacy due to the development of various side effects. Herbal concoction, on the other hand, has been widely used since times immemorial as a part of Indian Ayurveda or Chinese folklore medicines. This fostered our attention towards some curative natural products as they are less likely to cause serious side effects. Among the many Indian species quoted to be useful in different types of cancer [1], *Acorus calamus*. L, belonging to the family Acoraceae, is a versatile medicinal plant and is a unique source of various types of compounds rhizome forming plant found in marshy land having diverse biological activities and morphologically resembles a grass-like, , shallow water and pond edge on the northern temperate, subtropical and warm regions of the Indian subcontinent [2]. *A. calamus* has been known for its beneficial and medicinal value in Asia for a long time; in different systems, it is used in different ways. In Ayurvedic medicine, it is an important herb and is valued as a “rejuvenator” for the brain and nervous system and as a remedy for digestive disorders. The leaves, roots, rhizomes and stem of *A. calamus* have been used as medicine for the last 2000 years. The rhizomes of sweet flag are used for numerous medicinal purposes. Dried and powdered rhizome has a spicy flavour and is used as a substitute for ginger, cinnamon and nutmeg for its odour [4], it was used by ancient Greeks and was included in the traditional remedies of many other European cultures. The rhizomes are considered to possess anti-spasmodic, carminative and antihelmintic, aromatic, expectorant, nauseant, nervine, sedative, stimulant properties and also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and glandular and abdominal tumors [4]. As the potential of Indian flora in particular have been a veritable source for therapeutic agent, a preliminary attempt was made to find out the anticancer potential of rhizome of *A. calamus* against breast cancer in this study.

MATERIALS AND METHODS

Plant Material

The rhizome of *Acorus calamus*, was collected from Alappuzha district of Kerala, India. Identification of the plant material was done in the Department of Botany, Sanadhana Dharma College, Alappuzha and a voucher specimen is preserved as herbarium and submitted to the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. Fresh rhizomes used for extraction were shade dried and powdered using a mechanical grinder. The powder was collected in two clean, air tight containers for further use.

Experimental Animals

Healthy Swiss albino mice, *Mus musculus* (20±5 gm) were used for the study. The animals were obtained from Amala Cancer Institute, Trissur, Kerala. Animals were kept in polypropylene cages with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided *ad libitum*. The animals were acclimatized to laboratory conditions for about one week before commencement of the experiment. The experiments were performed after the approval from the institution of Animal Ethical Committee and in accordance with the recommendation for the proper care and use of the laboratory animals.

Acute Toxicity Study

Healthy Swiss albino mice (Group I – IV), starved overnight, were orally fed with MEAC in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 gm/kg body weight, while group V (untreated) served as control. The animals were under continuous observation for two

hours for changes in behavior, autonomic profiles and effects were observed intermittently for 72 h for death. IC_{10} and IC_{50} values of the extract were tested for acute toxicity and invivo experiments.

Treatment Protocol

Animals were divided into five groups each comprising of six animals. One group served as the control while the remaining four groups were injected with Dalton's ascites lymphoma (1×10^6 cells/ mouse) to induce tumor. The treatments were given intraperitonially at 24 h after the tumor inoculation and continued for 14 consecutive days.The body weights of all animals in all groups were noted daily. After the final dose, five animals of each group were sacrificed to study the tumor growth parameters (Mean survival time, Viable, Nonviable cell count, Tumor volume, Tumor weight and Tumor packed cell volume) and the rest were kept with to check with the percentage increase in the life span of the tumor in the host.

Hematological Studies

Blood was withdrawn by cardiac puncture from *A. calamus* treated and untreated DAL bearing mice. Different hematological parameters analysed were Red blood cell count (RBC), Hemoglobin (Hb), Mean Corpscular Hemoglobin (MCH), White blood cell count (WBC), Packed cell volume (PCV) and differential leucocyte count by standard procedures.

Statistical Analysis

Values were expressed as mean ± S.E.M. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's test using SAS (Version 9.1) software.

RESULTS AND DISCUSSION

Natural products discovered from medicinal plants have played an important role in the treatment of cancer. Many drugs that are used for cancer treatment are presently obtained from plant sources. DAL implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration and a progressive ascitic fluid formation [5]. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells. Our data showed that administration of MEAC significantly reduced the ascites and solid tumor burden without any side effects. MEAC treatment was found to enhance nonviable cell counts in peritoneal exudates and decrease the viable cell count. It might be due to the absorption of methanolic extract by viable cells which leads to lysis of cell through to the activation of macrophages or some cytokine production in peritoneal cavity. Viable cell count of the tumour bearing mice was significantly decreased while non- viable cell count were increased in the methanolic extract treated groups when compared with DAL treated group. Similar results were observed in Diospyros peregrine against DAL in rodents [6].

Acute toxicity study

The results of acute toxicity study of MEAC are presented in Table I. No mortality or change in body weight was observed in rats at a dose level of MEAC 50 mg/kg and 500 mg/kg body weight. Some clinical signs such as tremors, pilo erection and abdominal breathing were observed after the oral dosing of 1000 and 2000 mg/kg but no mortality or change in body weight was observed. These observations indicated that the calculated LD 50 value (Dixons likelihood method) for the oral doses of the MEAC was found to be more than 2000 mg/kg body weight, accordingly 100 and 200 mg/kg body weight were taken as low and high dose of MEAC for the experiment.

Effect on Tumor growth

The mean survival time increased to 25.67 ± 0.82 and 29.00 ± 0.63 on administration of 100 and 200 mg/kg of MEAC respectively when compared with DAL control group. When compared with the DAL control mice (97.67%),positive control (48.8%) and treated groups MEAC 100mg and 200mg showed a reduction in the percentage increase in life span (16.67% and 31.82%) whereas a lower

dose of 100mg showed a lower percentage increase in life span among treated groups. When compared with DAL control mice (21.33 ± 5.35 ml), all treated mice showed significant reduction in the tumor volume. Maximum reduction was found in positive control (5.42 ± 1.59; $P < 0.05$) followed by a higher MEAC dose of 200 mg.In case of ascites tumors, the measurement of the total amount of tumor cell material in the ascetic fluid can be done in terms of the total cell volume or packed cell volume (PCV). PCV may be considered comparable to that volume of solid tumors. In this regard the present study revealed that the lower dose of MEAC (100 mg) significantly reduced the tumor cell material compared to that of DAL control. In DAL control mice the tumor PCV was found to be 6.45 ± 0.89 ml which was reduced in positive control (3.67 ± 0.88 ml; $p < 0.05$) When compared with DAL control mice, the positive control group showed a significant reduction in viable cell count (5.04 ± 0.04). On administration of 200 mg doses of MEAC showed similar results (0.68 ± 0.04) whereas the 100 mg was found to be not significant. Between the treatment groups MEAC 100 mg showed similar results (4.07 ± 0.19; $p < 0.05$) whereas the higher dose (200 mg) was found to be not significant (3.6 ± 0.26). There was a significant ($p < 0.05$) increase in the level of non-viable cell count in positive control when compared with DAL control mice. This result was in par with observations of Chockalingam et al., 2013 on Aegle marmelos.

Body weight analysis

Body weight indicates health status of living beings. Changes in body weight are an important factor to monitor the health of an animal. Loss of body weight is frequently the first indicator of the onset of an adverse effect [7]. Although a gain in body weight was observed throughout the experimental period, tumour induction results revealed a decrease in body weight the treatment groups (positive control & MEAC 100 mg/kg) on comparision with the tumour bearing groups at the end of 11 days. At the end of 20^{th} day, when compared with DAL bearing mice (31.33± 1.03) all the treated groups showed a decrease in body weight (22.00 ± 1.26, 22.83 ± 0.177 and 22.17 ± 0.75).

Effect on Hematological parameters

Mean corpuscular hemoglobin (MCH) denotes the amount of hemoglobin per red blood cells. The non- significant effect of the extract on mean corpuscular Hemoglobin (Hb) indicates that MEAC does not affect the erythropoiesis, morphology, or osmotic fragility of the red blood. The level of RBC that has been observed to decline during the progression of tumour has been found to improve in mice treated with MEAC 100 and 200 mg (7.54 ± 0.66 and 7.84 ± 0.51). With regard to Hb content all the treatment groups showed significant increase when compared to DAL control (9.11 ± 0.35). The treatment with MEAC 100 mg and 200 mg to DAL bearing mice enhanced Hb content to 12.40 ± 1.20 and 12.9 ± 0.85 respectively at the 95% significance. Data pertaining to Mean Corpuscular Hb level, a slight elevation was recorded in all treatment groups. A significant ($p< 0.05$) result was shown in MEAC 100 mg treated animals (19.10 ± 1.31) with a higher dose of MEAC (200 mg) resulting in a near normal value (16.62 ± 0.65) with the standard drug (16.87 ± 0.55). Total WBC count was found to be increased in DAL control group (43.08 ± 3.15) when compared with normal control animals (10.12 ± 1.07). Administration of MEAC at the dose of 100 mg and 200 mg reduced the WBC count to 23.20±4.66 and 18.94 ± 1.99. With regard to packed cell volume, though the treatment with MEAC at 100 mg and 200 mg concentrations increased the packed cell volume to 32.46 ± 6.1and 33.01 ± 4.59 respectively when compared with that of DAL control (21.12 ± 6.7), the study was found to be statistically non significant. In a differential count of WBC, a significant ($p< 0.05$) decrease in monocyte, neutrophil and eosinophil and an increase in lymphocyte count in DAL control mice. Treatment with MEAC at different doses changed these altered parameters more or less to the normal values (Table 5).Evaluation of the haematological parameters the explain blood relating functions of a plant extract or its products [8]. Furthermore, such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are

translated from animal studies [9]. Myelosuppression and anaemia is the foremost difficulties encountered in the cancer chemotherapy. The anemia encountered in tumor bearing mice is mainly due to the reduction in RBC or Hemoglobin percentage and this may occur either due to deficiency due to hemolytic or myelopathic conditions similar results were observed in the present study. Treatment with MEAC brought back the hemoglobin and RBC count more or less to normal levels. This indicates that MEAC possess protective action in the hemopoietic system. Similar results were obtained in *Cucurbita maxima* against ethanolic extracts of *A. calamus* [10] and in *Jasminum sambac* flowers against DAL in Swiss albino mice [11]. Leukocytes are the first line of cellular defence that responds to infectious agents, tissue injury, or inflammatory process. In a differential count of WBC the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval MEAC treatment could change those altered parameters to near normal. These results are in accordance with the reports of Chitra et al., (2009) indicating the possibility of MEAC as a potential agent in the area of cancer chemotherapy.

Table 1
Clinical signs of toxicity observed during acute oral toxicity study of MEAC

Sl.No	Dose (mg/kg b.wt)	Latency	Symptoms
1	50	-	None
2	500	-	None
3	1000	-	Piloerection, abdominal breathing
4	1500	-	Tremor, Piloerection, abdominal breathing
5	2000	-	Tremor, Piloerection, abdominal breathing

Latency – Time of death after the dose.

Table 2
Effect of MEAC on tumor growth parameters

Parameters	Mean survival time (Days)	Increased life span (%)	Tumor volume (ml)	Tumor PCV (ml)	Viable cell count (10^6 cells/ml)	Non viable cell count (10^6 cells/ml)
Normal Control	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DAL Control	22.00± 0.63	97.67	21.33 ± 5.35a	6.45 ± 0.89a	12.30 ± 0.07	0.89 ± 0.06
Positive Control (5FU:10mg / kg)	32.67 ± 1.97	48.4	5.42 ± 1.59b	3.67 ± 0.88b	5.04 ± 0.04b	5.75 ± 0.07b
MEAC (100mg/kg)	25.67 ± 0.82	16.6	14.17 ± 5.23b	6.93 ± 0.84 NS	8.84 ± 0.06 NS	4.07 ± 0.19b
MEAC (200mg/kg)	29.00 ± 0.63	31.8	13.83 ± 3.43b	7.32 ± 0.49 NS	0.68 ± 0.04b	3.6 ± 0.26 NS

Values are expressed as mean ± S.E.M. (n = 6); Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test; [a]p < 0.05 Normal control vs DAL control, [b] p < 0.05 Treatment groups vs DAL control, NS – Non Significant.

Table 3
Effect of MEAC on body weight

Experimental group	Before Induction	After Induction	
		On 11th day	On 20th day
Normal control	21.00± 0.63	-	-
DAL control	22.00 ± 0.63	26.17 ± 0.75	31.33 ± 1.03
Positive control (5-FU)	21.50 ± 0.55	24.83 ± 0.75	22.00 ± 1.26
MEAC (100mg/kg)	22.00 ± 0.63	25.83 ± 0.75	22.83 ± 0.177
MEAC (200mg/kg)	21.17 ± 0.75	26.70 ± 0.16	22.17 ± 0.75

Values are expressed as mean ± S.E.M.

Table 4
Effect of MEAC on hematological parameters

Parameters	RBC ($x10^6$ cells / µl)	Hemoglobin (gm/dl)	MCH (pg)	Total WBC ($x10^3$ cells / µl)	PCV (%)
Normal control	8.73 ± 0.28	15.46 ± 0.40	15.92±0.69	10.12±1.07	46.1 ± 1.26
DAL control	4.09 ± 1.18a	9.11 ± 0.35a	16.22±0.87	43.08±3.15	21.12 ± 6.7
Positive control (5 –FU)	8.80 ± 0.18b	17.58 ± 0.92	16.87±0.55 NS	11.93±1.44 b	53.43 ± 3.5
MEAC (100 mg)	7.54 ± 0.66 b	12.40 ± 1.20 b	19.10±1.31 b	23.20 ± 4.66	32.46 ± 6.1NS
MEAC (200 mg)	7.84 ± 0.51 b	12.9 ± 0.85 b	16.62±0.65 NS	18.94±1.99 b	33.01 ± 4.59 NS

Values are expressed as mean ± S.E.M. (n = 6); Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test; [a]p < 0.05 Normal control vs DAL control, [b] p < 0.05 Treatment groups vs DAL control, NS – Non Significant

Table 5
WBC Differential Count

Parameters	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)	Eosinophil (%)
Normal control	86.50 ± 5.28	12.50 ± 1.22	5.50 ± 2.59	5.17 ± 0.98
DAL control	91.83 ± 3.19 a	3.00 ± 0.89	2.50 ± 2.07	1.83 ± 0.75
Positive control (5- FU :)	55.50 ± 6.28 b	13.17 ± 1.72	13.83 ± 1.33	4.83 ± 0.75 NS
MEAC (100 mg)	81.17 ± 3.19 b	7.67 ± 0.82 b	2.50 ± 1.38 b	4.67 ± 0.82 NS
MEAC (200 mg)	65.50 ± 3.94 b	5.17 ± 2.64	3.00 ± 0.14 b	2.00 ± 0.89 b

Values are expressed as mean ± S.E.M. (n = 6); Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test; [a]p < 0.05 Normal control vs DAL control, [b] p < 0.05 Treatment groups vs DAL control, NS – Non Significant

CONCLUSION

Our present study indicates *Acorus calamus* as a promising antitumour strategy against cancer for a malignant DAL carcinoma in induced Swiss albino mice. Additional investigations are required to understand the mechanism of the anti-tumour action of *Acorus calamus* in the other cancer cell lines studied here.

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