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Evaluation of Anti-tumor and anti-oxidant Activity of *Acalypha fruticosa* in Ehrlich's Ascites Carcinoma Bearing Swiss Albino Mice

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ABSTRACT

The methanol extract of the leaves of *Acalypha fruticosa* was evaluated for its anti-tumor activity against Ehrlich's Ascites Carcinoma (EAC) bearing Swiss albino mice. The defatted methanol extract of *Acalypha fruticosa* (MEAF) exhibited significant anti-tumor effect at the dose 250 and 500 mg kg^{-1} in mice. The MEAF was administrated for 14 days after 24 h of tumor inoculation. The effects of MEAF on tumor was assessed by the change in the body weight, ascitic tumor volume, mean survival time, increased life span (ILS), viable and nonviable tumor cell count and hematological profiles. Further, the effect of MEAF on lipid peroxidation (LPO), glutathione content (GSH), antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) activities were measured from hepatic tissues. The MEAF showed remarkable decrease in tumor volume and viable cell count, and prolonged the life span of EAC tumor bearing mice. Hematological profiles converted to more or less normal in extract treated mice. The LPO level significantly increased (1.41 n moles MDA) and GSH, SOD and CAT levels were significantly decreased in EAC control. After administration of MEAF at the dose 500 mg/kg to EAC bearing mice significantly decreased the LPO while it's increased the GSH (2.15 mg / g / wet tissue) and SOD level (4.47 U/mg of protein of tissue) as compared to that of EAC control group. The result indicates that the MEAF exhibited significant antioxidant and anti-tumor activity.

Key words: *Acalypha fruticosa*, Hematological profiles, Biomarker enzymes, Anti-tumor activity

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INTRODUCTION

Acalypha fruticosa Forsk (Family: Euphorbiaceae) commonly known as Sinnimaram (Tamil) and Chinniaka (Telugu), is widely used in traditional medicine for the treatment of various ailments. The plant is widely distributed in Deccan Pennisula, Ceylon, Peru, tropical Africa and South India. In traditional system of medicine the young twigs and leaves of the plant are prescribed in the treatment of dyspepsia, colic, diarrohea and in cholera [1]. The plant extract was also found to possess anti-microbial property [2]. In folklore remedy the plant was used in the treatment of cancer among the tribal population in Kolli Hills, South India. However, fewer reports are available with respect to the pharmacological properties of the plant. Hence, the present study was undertaken to evaluate the effect of the defatted methanol extract of *Acalypha fruticosa* (MEAF) for its antioxidant status and anti-tumor activity in standard animal models.

MATERIALS AND METHODS

Plant material

The plant *Acalypha fruticosa* (Family: Euphorbiaceae) was collected in the month of April 2007 from the Kolli Hills, TamilNadu, India. The plant material was taxonomically identified by the Botanical survey of India, Shibpur, Howrah, Kolkata, India and the voucher specimen GMS-7 was retained in our laboratory for future reference. The methanol was removed under reduced pressure and semi solid mass was obtained (yield 14.25 %). The extract was then stored in a vacuum dissector for further use.

Animals

Swiss albino mice of either sex weighing between (18-22 g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. All procedures described were reviewed and approved by the university animal's ethical committee.

Treatment schedule

Swiss albino mice were divided into 5 groups (n = 12) and given food and water *ad libitum*. All the groups were injected with EAC cells ($2x10^6$ cells/mouse) intraperitoneally except normal group. This was taken as day zero. On the first day normal saline (0.9 %, w/v, NaCl) 5 ml / kg / mouse/day and EAC control (Propylene glycol 5 ml/kg/day/mouse) were administered in groups 1 and 2 respectively. MEAF at the doses of (250 and 500 mg/kg/mouse/ day) and standard drug 5-Flurouracil (20 mg / kg) were subsequently administered in groups 3, 4 and 5 respectively, for 14 days intraperitoneally. On 15th day, after the last dose and 18 hrs fasting six mice were sacrificed from each group for the study of anti-tumor activity, hematological and biochemical parameters. The rest of the animal groups were kept to check the increase in the lifespan of the tumor bearing hosts. The effect of MEAF on tumor growth and host's survival

time were examined by studying the following parameters like tumor volume, tumor cell count, mean survival time, increase in lifespan of EAC bearing mice. The other biochemical parameters such as hemoglobin content [3], RBC and WBC count [4] and differential leukocyte count [5] was also estimated from the peripheral blood of normal, EAC control and MEAF treated groups. The homogenate was processed for estimation of lipid peroxidation [6], GSH [7], SOD [8] and CAT [9].

Statistical Analysis

Total variation present in a set of data was performed by using one way analysis of variance ANOVA and the results are expressed as mean ± S.E.M.

RESULTS & DISCUSSION

Effect on tumor volume and survival time

The MEAF was evaluated for its anti-tumor activity in EAC bearing mice and the results are tabulated in table 1- 5. The methanol extract exhibited significant anti-tumor activity at the tested doses of 250 and 500 mg/kg against EAC bearing mice.

The effects of MEAF (250 and 500 mg/kg) at the doses were studied on survival time, increase in life span (ILS), tumor volume, viable and non-viable cell count were shown in Table 1. In the EAC bearing mice control group the median survival time was 21.0 ± 0.91 days. Whereas it was increased 26.0 ± 0.18 (250 mg / kg), 30.0 ± 0.58 (250 mg/kg) 40 ± 0.85 (20 mg/kg days respectively) with MEAF and standard drug 5-Fluoruracil treated group.

The tumor volume of the EAC control group was 4.5 ± 0.07 ml. Treatment with MEAF at the dose of 250 and 500 mg / kg reduced tumor volume to 3.1 ± 0.07, 1.7 ± 0.03 ($p < 0.01$) and 0.5 ± 0.01 ($p < 0.01$) respectively as compared to that of EAC control group.

The effect of MEAF on hematological studies

As shown in table 1 and 2, the hemoglobin content in the EAC control mice was significantly decreased to 11.33 ± 1.5 (g %) in comparison with normal mice 13. 83 ± 1.1 (g %). Treatment of EAC bearing mice with MEAF at the dose of 250 and 500 mg / kg increased the hemoglobin content to 12.30 ± 0.95 and 12.80 ± 0.81 (g %) respectively. Moderate change in the RBC count was observed for the extract treated mice. The total WBC count was significantly higher in the MEAF treated group as compared to that of normal. In differential leukocyte count, the percentage of neutrophils was increased while the lymphocyte count was decreased in the extracts treated groups. In EAC bearing mice there was a decrease in bone marrow cell count and it was found to be more or less normal in MEAF treated groups in a dose dependent manner.

Effect of the lipid peroxidation and glutathione content

The levels of lipid peroxidation in liver tissue were significantly increased in EAC control group (1.41± 0.012 n moles of MDA / mg of protein) as compared to the normal group (0.98 ± 0.05 n moles of MDA / mg of protein). The GSH content in liver tissues of normal mice was found to be 2.43 ± 0.11 mg / g / wet tissue. Inoculation of EAC drastically decreased the GSH content to 1.67 ± 0.10 mg / g wet tissue, representing 31 % inhibition ($p < 0.01$) in EAC control group when compared with normal group.

The level of lipid peroxidation were decreased (1.21 ± 0.02, and 1.02 ± 0.03 n moles of MDA / mg of protein) and glutathione content (1.89 ± 0.03 and 2.15 ± 0.02 μg/mg of tissues) were increased by the administration of MEAF treated groups at the doses of 250 and 500 mg/kg respectively, as compared with the EAC control ($p<0.01$). The above said deleterious effects are controlled by the administration of MEAF, 3.4. Superoxide dismutase and catalase activity.

SOD level in the liver of EAC bearing mice was significantly decreased to 3.17 ±. 0.21 Unit/mg of protein, in comparison with normal mice 4.92 ± 0.4 Unit/mg of protein. The administration of MEAF were significantly increased the SOD levels of 3.79 ± 0.34 and 4.42 ± 0.42 Unit/mg of protein in tissues ($p<0.01$) at the doses of 250 and 500 mg/kg respectively.

The CAT level in the EAC control group was 1.19 ± 0. 8 U/g/ wet tissue. This is a significant decrease of 58.6 % ($p < 0.001$) in comparison with normal group 26.7 ± 0.5 U /g/ wet tissue. Treatment with MEAF at the dose of 250 and 500 mg / kg increased CAT levels to 1.61± 0.02 and 2.17 ± 1.4 (U/g / tissue) as compared to that EAC mice.

The present study was carried out to evaluate the effect of MEAF on EAC bearing mice. The MEAF were showed significant anti-tumor activity against the transplantable murine tumour. The reliable criteria for judging the value of any anticancer drug are the prolongation of life span of animal's [10]. The ascitic fluid is the direct nutritional source to tumors cells and the rapid increase in ascitic fluid with tumor growth could possibly by a means to meet more nutritional requirements of tumor cells [11]. The treatment with MEAF inhibited the tumor volume, viable cell count and enhancement in survival time of EAC bearing mice. The finding of result was enhanced survival time with respect to EAC control group and thereby suggests the anti-tumour effect of MEAF against EAC cell line.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [12, 13]. 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 [14]. Bone marrow serves as major source of the all blood cells. The majority of all the cell type involved in the immune system is produced from hemopoietic stem cells of bone marrow. It also provides microenvironment for antigen dependent differentiation of B cells. Different cytokines are important for renewal a

hematopoetic stem cells and their differentiation into different functionally mature blood cell types. Conventional therapy of cancer always produce side effect and most important being myleosuppression which at time produce life threatening consequence. Treatment with MEAF tumor bearing mice could bring back the hemoglobin content, RBC and WBC count more or less to normal values. This indicates that MEAF have protective action on heamopoietic system.

Table 1. Effect of methanol extract of *Acalypha fruticosa* on tumor volume, packed cell volume, viable and non-viable tumor cell count of EAC bearing mice

Parameters	EAC Control (2 X 10^6 cells/ mouse/ml)	MEAF (125 mg/kg) +EAC	MEAF (250 mg/kg) +EAC	MEAF (500 mg/kg) +EAC	Standard 5 - flourouracil (20 mg/kg) +EAC
Body weight (g)	26.22 ± 0.12	23.34 ± 0.17	22.52 ± 0.13	21.55 ± 0.13	20.23 ± 0.19
Tumor volume (ml)	4.41 ± 0.07	3.73 ± 0.03	2.72 ± 0.03	1.44 ± 0.01	-
Packed cell volume (ml)	2.31 ± 0.06	1.22 ± 0.05	0.96 ± 0.02	0.27 ± 0.01	-
Viable tumor cell count x 10^7cells/ml	11.22 ± 0.07	9.33 ± 0.06	5.51 ± 0.04	1.71 ± 0.06	-
Nonviable tumor cell count x 10^7cells/ml	0.34 ± 0.02	0.67 ± 0.07	0.82 ± 0.06	1.34 ± 0.09	-

(Values are mean ± SEM). Number of mice in each group (n=6)
P < 0.01, Experimental groups was compared with EAC control.

Table 2. Effect of the methanol extract of *Acalypha fruticosa* on survival time on EAC bearing mice

Groups	Experiment	Median survival (days)	Life span (%)	Increase of life span
1	Normal control (Normal saline 5 ml /kg b.w.)	-	-	-
2	EAC control (2x10^6 cells) + Propylene glycol (5 ml / kg b.w.)	21 ± 0.77	100	-
3	MEAF (125 mg/kg)+EAC (2x10^6 cells)	25 ± 0.30	125	25
4	MEAF (250 mg /kg)+EAC (2x10^6 cells)	28 ± 0.25	133.3	33.3
5	MEAF (500 mg /kg)+EAC (2x10^6 cells)	34 ± 0.32	161.9	61.9
6	5-Flurouracil (20 mg/kg)+EAC	31 ± 0.21	147.6	47.6

(Values are mean ± SEM). Number of mice in each group (n=6)
P < 0.01, Experimental groups was compared with control

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Table 3. Effects of methanol extract of *Acalypha fruticosa* on hematological parameters of EAC treated mice

Parameters	Normal Saline (0.5 ml/kg)	EAC ($2x10^6$ cells) Control + (Vehicles)	EAC ($2x10^6$ cells)+ MEAF 125 mg/kg	EAC ($2x10^6$ cells)+ MEAF 250 mg/kg	EAC ($2x10^6$ cells) + MEAF 500 mg/kg	EAC ($2x10^6$ cells) + Standard
Hemoglobin (g %)	13.8 ±1.10	11.3 ± 0.39 [b]	10.6 ±1.04	11.7 ±1.03	12.4 ±1.62	11.6 ±1.62
Total RBC (cells/ml $x10^9$)	6.4 ±0.54	4.5 ± 0.45	4.4 ± 0.32 [b]	5.6 ± 0.53 [b]	6.1 ± 0.68	5.7 ± 0.54
Total WBC (cells/ml $x10^6$)	6.7 ±0.58	18.9 ± 1.67 [b]	15.4 ± 1.34	11.6 ± 0.77	7.1 ± 0.70	8.4 ± 0.53
Cells/ femur 1 $x10^6$/ml	18.9 ±1.68	14.9 ±1.47 [b]	15.8 ±1.45 [a]	16.5 ±1.45 [a]	17.4± 1.48	16.7 ± 1.22
Cells/ spleen $2x10^6$/ml	16.7 ±1.88	28.4 ±1.47 [b]	24.95± 2.27 [b]	20.5± 1.70 [b]	14.4± 1.42	19.7± 1.27

Values are mean ± SEM (n = 6). EAC control group compared with normal group [b] p<0.05. Experimental groups were compared with EAC control. [a] p < 0.01, [b] p < 0.05.

Table 4. Effect of methanol extract of* Acalypha fruticosa *on differential counts of white blood cells in EAC bearing mice

Design of Experiment	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Normal saline (5ml/kg)	17.5 ± 1.25	0.6 ± 0.01	80.1 ± 2.31	1.8 ± 0.15
EAC ($2x10^6$ cells) + Propylene glycol (5 ml / kg)	66.6 ± 0.01	1.5 ± 2.48[b]	32.2 ± 0.07[b]	0.9 ±0.03[b]
EAC ($2x10^6$ cells) + MEAF 125mg/kg	54.2 ± 3.44[b]	1.1 ± 0.03[a]	43.7 ± 2.48[b]	1.0 ± 0.05[a]
EAC ($2x10^6$ cells) + MEAF 25m0g/kg	43.9 ± 2.57[b]	0.6 ± 0.02[a]	54.3 ± 2.22[b]	1.2 ± 0.03[a]
EAC ($2x10^6$ cells) + MEAF 500mg/kg	37.4 ± 2.34[b]	0.6 ± 0.03[a]	60.8 ± 2.81[b]	1.2 ± 0.09[a]
EAC ($2x10^6$ cells) + Standard drug (5-Flurouracil 20mg/kg)	45.3 ± 4.33[b]	0.7 ± 0.02[a]	52.7 ± 2.33[b]	1.3 ± 0.05

Values are mean ±SEM (n=6). EAC control group was compared with normal group [b] p<0.05.Experimental groups were compared with EAC control. [a] p<0.01, [b] p<0.05

Table 5. Effect of different doses of methanol extract of the *Acalypha fruticosa* on different biochemical parameters in liver in EAC bearing mice

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Parameters	Normal Saline (0.5 ml/kg)	EAC ($2x10^6$ cells) Control + MEAF (Vehicles)	EAC ($2x10^6$ cells)+ MEAF 125 mg/kg	EAC ($2x10^6$ cells)+ MEAF 250 mg/kg	EAC ($2x10^6$ cells) + MEAF 500 mg/kg
Lipid peroxidation (n moles MDA/ g of tissue)	0.97 ± 0.03	1.45 ± 0.03[b]	1.37 ± 0.02[a]	1.29 ± 0.01	1.19 ± 0.01[a]
GSH (mg/g of tissue)	2.36 ± 0.03	1.69 ± 0.12[b]	2.86 ± 0.17[a]	2.14 ± 0.21[a]	2.29 ± 0.03[b]
SOD (Units / mg Protein)	4.38 ± 0.43	3.29 ± 0.27[b]	3.59 ± 0.22[b]	3.96 ± 0.33[a]	4.22 ± 0.01[a]
Catalase (Units / mg tissues)	2.59 ± 1.91	1.63 ± 0.11[b]	1.78 ± 0.11[b]	1.97 ± 1.17[a]	2.14 ± 0.01[b]

Values are mean ±SEM (n=6). EAC control group was compared with normal group [b] $p<0.05$.Experimental groups were compared with EAC control. [a] $p<0.01$, [b] $p<0.05$

The presence of tumor in the human body or in experimental animals is known to affect many functions of the vital organ such as liver and kidney. Especially in liver even when the site of the tumor does not interfere directly with organ function [15]. It has been also demonstrated that tumor-bearing animals can experience a systemic change of enzymatic and non-enzymatic antioxidants in organs distinct from tumor sites [16, 17]. Taking these facts into consideration, the antioxidant related biomarker enzymes such as SOD and CAT and associated lipid peroxidation and GSH were estimated in the liver tissue of EAC and treated animal groups. In the present study, the EAC bearing mice showed significant deleterious effects on both free radicals scavenging systems like glutathione content and antioxidant enzymes such as SOD and CAT.

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocytes survival and membrane fluidity [18]. Increase in the levels of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defence mechanism to prevent the formation of excess free radicals. Malonaldehyde (MDA) is the end product of lipid peroxidation was reported to be higher in cancer tissues than in non-diseased organ [19]. Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids can induce lipid peroxidation *in-vivo* [20]. In the present study indicates that the elevated levels of lipid peroxidation was observed in the liver of EAC bearing mice. However, the deleterious effects of reactive oxygen species are protected by MEAF administration. It may be due to the antioxidant properties of different active phamacophore, which is responsible for the above said activity.

Reduced glutathione (GSH) is one of the most abundant tripeptide non-enzymatic biological antioxidants present in the liver. GSH is also presumed to be an important endogenous defence against the peroxidative distraction of cellular membranes. Its functions are concerned with the removal of free oxygen species such as hydrogen peroxide, superoxide radicals, alkoxy radicals and also maintenance of membrane protein thiols and as a substrate for glutathione peroxidase and GST [21]. It is also potent inhibitor of neoplastic process plays an important role in endogenous antioxidant system that is found particularly in high concentration in liver and it is known to have key function in protective process [22]. Tissue GSH concentration reflects the potential for detoxification. The present study indicates that the EAC control group produced elevation in the levels of lipid peroxidation and depletion in GSH content. With reference to this, the active role of GSH against cellular lipid peroxidation has been well recognized. The treatment with MEAF significantly reduced the elevated levels of lipid peroxidation, while increased the levels of glutathione content, it may be due to the antioxidant and free radical quenching of the active constituents.

Cells are also equipped with enzymatic antioxidant mechanisms that play an important role in the elimination of free radicals. SOD is a ubiquitous chain breaking antioxidant and is found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays an important protective role against reactive oxygen species induced by oxidative damage. SOD catalyses the diminution of superoxide into H_2O_2, which has to be eliminated by glutathione peroxidase and or catalase [23]. The SOD and CAT were play an important role in the elimination of reactive oxygen species derived from the redox process of xenobiotics in liver tissues. In correlation, it has been reported that EAC bearing mice showed decreased levels of SOD activity and this may be due to loss of Mn^{++} SOD activity in liver [24]. Inhibition of catalase activity in tumor cell lines was reported by Marklund *et al* [25]. Sun *et al* observed diminished levels of SOD and CAT activity as a result of tumor growth. Similar findings were observed in the present investigation with EAC control mice. It may be due the loss of CAT and SOD activity results in oxygen intolerance and triggers a numbers of deleterious reactions. In this study catalase and SOD were appreciably elevated by administration of MEAF, suggesting that it can restore SOD enzymes and /or activate enzymes activity in host. It means that the MEAF can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of hepatic antioxidant enzymes.

CONCLUSION

The present investigation indicates that the leaves of the *Acalypha fruticosa* exhibited significant antioxidant property and anti-tumor activity. However, further study needed in particular, to investigate the mechanisms by which the phytoconstituents of the extract can be induces its beneficial effects against transplantable murine tumor.

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