Evaluation of Anti Tumor Activity of *Curcuma Amda Roxb*. Rhizome

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Abstract- **Aqueous and alcoholic extracts of *curcuma amda roxb.* rhizomes have been evaluated for its anti tumour activity in Swiss albino mice. Alcoholic extract exhibited more potent anti tumour activity than aqueous extract. *In-vitro* cytotoxicity studies were confirmed by brine shrimp bioassay method. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour induced mice after treatment with both of the extracts in ehrlich ascites carcinoma (EAC) model. The hematological parameters were also corrected by aqueous and ethanolic extracts in tumour induced mice. These observations are suggestive of the anti tumour effect of aqueous and alcoholic extracts of *Curcuma amda Roxb.* rhizomes in ehrlich ascites carcinoma.**

Keywords- **anti tumour activity, brine shrimp bioassay, *curcuma amda roxb.*, ehrlich ascites carcinoma.**

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I. INTRODUCTION

Cancer is a major cause of concern for public health service in most developed as well as under developed countries. The present study is focused on the evaluation of anti cancer activity of aqueous and alcoholic extracts of *curcuma amda roxb*. Rhizomes on primary stages of cancer. *Curcuma amda roxb*. is a species belongs to India and has not been worked for its possible anti cancer activity. On the basis of above facts, the present study has been under taken on the plant to carry out a preliminary investigation for its possible anticancer activity by *in-vitro* cytotoxic activity in the brine shrimp lethality bioassay method. Further *in-vivo* anti tumour activity was confirmed in the ehrlich ascites carcinoma model.

II. MATERIALS AND METHODS

Whole plant of *curcuma amda roxb*. was collected in and around **sheopur (M.P.)** district and authenticated by Dr. R.A.S. Chauhan, H.O.D., department of Botany, Ambah post graduation college, Ambah, Morena, M.P.

For alcoholic extraction, the crude drug was shade dried for thirty days and than coarsely powdered. The coarse powder was loaded in thimble made of whatman filter no.1 and extracted in the soxhlet extraction column with 1-2 L of 95% alcohol for 30-40 cycles for each batch. The extract so obtained was thick and syrupy with characteristic odour. The final extract was thick and was stored separately in vacuum.

For aqueous extraction, a dried coarse powdered crude drug was transferred to the round bottom flask and 1.5-2 L of distilled water was added to the round bottom flask and soaked for 24 hrs. this was then boiled for 4-5 hrs. the extract so For aqueous extraction, a dried coarse

powdered crude drug was transferred to the round bottom flask and 1.5-2 L of distilled water was added to the round bottom flask and soaked for 24 hrs. this was then boiled for 4-5 hrs. the extract so obtained was decanted in to beaker and then concentrated to $1/6^{th}$ of the total volume on water bath. This was preserved by adding few drops of chloroform and kept in the refrigerator.

III. *IN-VITRO* CYTOTOXICITY STUDIES

Brine shrimp (artemia salina) eggs were collected from the fisheries research station Banglore and stored in an airtight opaque container at room temperature. Drug samples of the concentration 10, 100, 1000 mcg/ml were prepared and transferred to vials of 7 ml capacity. Three replicate were

prepared for each dose level. Control vials were prepared by adding equal volumes of distilled water. Hatching of brine shrimp eggs was done by adding 2.5 L of artificial sea water to the special chamber. About 30 mg of the eggs were washed with water and then sprinkled in to the compartment which was darkened, aeration was provided in the compartments. After 48 hrs the photographic nauplli were collected by pipette from the lighted side and used for bioassay.

To carry out bioassay 48 hr. old nauplli were drawn in a pipette along with water and ten nauplli were transferred to each sample vial and artificial sea water was added to each vial and volume was made up to 5 ml. A drop of dry yeast suspension (3 mg in 5 ml sea water) was added to each vial as food for shrimps. The vials were maintained under illumination. After 24 hrs survivors were counted, by using 5X magnifying glass, and the percent deaths and Lc50 values were calculated by using provit method for aqueous and alcoholic extracts.

IV. ACUTE TOXICITY STUDY

Albino mice of either sex weighing 20-30 gm were selected. Drug samples were prepared by dissolving in water by heating and these were administered intraperitonialy. Three groups for each extract containing two animals in each group were used. Drugs were administered intraperitonialy after administration the animals were observed continuously for 1 hr, frequently for the next four hrs and then after 24 hrs.

V. *In-vivo* anticancer studies on mouse ehrlich ascites carcinoma

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Swiss albino mice from an inbred colony maintained under controlled conditions of light (12:12 hrs light:dark), temperature (25 ± 2^0c) and humidity were used. Six to eight weeks old female mice weighing 25±5 gm were used. All the animals were divided in to four groups (N=6), group 1 (tumour control), group 2(cisplatin), group 3(alcoholic extract of *curcuma amda roxb*.) and group four (aqueous extract of *curcuma amda roxb.)*. The doses selected for the extracts were about $1/10^{th}$ of the safe dose found in acute toxicity studies. Dose were administered on 10^{th} day of tumour cell inoculation, once daily by intraperitonialy route. The dose selected were 100mg/kg for group 3^{rd} and 4^{th} respectively. Both of the extracts were administered once a day, in the volume of 0.1 ml/10gm mouse through intraperitonial route. EAC was maintained and propagated intraperitonialy by serial transplantation in adult female swiss albino mice. The ascetic carcinoma bearing mice (donor) was taken 15 days after tumour transplantation.the ascetic fluid was drawn by using a 18 gauge needle in to sterile syringe and tumour viability was determined by trypan blue exclusion test and cells were counted using haemocytometer the ascetic fluid was suitably diluted in normal saline to get a concentration of 10cells/ml of tumour cells suspension. This was injected intraperitonialy to obtain ascetic tumour. The mice were weighed on the day of tumour inoculation and then for each three days. Treatment was started from 10^{th} day of tumour inoculation. Cisplatin (one dose) was injected on 10^{th} day intraperitonialy. Extracts were administered from 10^{th} day for five days intra peritonialy. Parameters monitored during the study were percentage increase in weight as compared to day 0 weights, median survival time(MST) and percentage increase in life span (% ILS), mean survival time(MEST), cell viability test (% survivors of malignant cells in ascetic fluid) and haematological parameters (total W.B.C. and differential leukocyte counts, total R.B.C. and IV. haemoglobin content). All experimental protocols were approved by institutional animal ethics committee, S.R.C.P. Banmore and statistical analysis result were analysed by oneway ANOVA by ORIGIN 60 computer package.

Results and discussion:

Both the extract showed considerable cytotoxic activity in the brine shrimp lethality bioassay. The alcoholic extract of *curcuma amda roxb*. Was found to be very potent. In *in-vivo cancer* model (ehrlich ascites carcinoma model). The plant extract significantly reversed the tumour induced changes in the parameter monitored.

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Table: summary of *invivo* anticancer anticancer studies on mouse ehrlich ascites carcinoma

Parameters	Groups		1st	2nd	3rd	4th
% increase in wt. as compared to day 0	Day 0		3.445±2.11	3.22±1.14	2.36±66	2.27±1.31
	Day 6		6.544±2.45	10.66±0.21	12.2±2.66	11.60±1.77
	Day 9		15.712±2.2	18.66±0.34	22.91±3.3	16.16±2.13
	Day 12		16.33±2.11	16.68±2.3	18.66±2.78	0.06±1.2
	Day 15		19.65±2.17	0.412±2.66	12.11±2.86	0.03±0.11
Survival time In tumour induced mice	Median survival time (days)	MST	16	22	17	19.6
		%T/C	-	140	110.2	120.24
		%ILS	-	46	11	19.98
	Mean survival time (days)	MST	16.10±1.4	22.77±0.8	19.71±2.13	17.91±0.55
		%T/C	-	150	123	112
		%ILS	-	48	24	17.67
Tumour living cell no. in ascitic fluid of tumour induced mice	No. of Tumour living cell (million cells/cm^3) (mean±SE)		19.38±0.17	8.66±0.64	11.52±1.10	10.75±0.62
	% decrease in tumour cells (compared to control)		-	56.44	42.75	45.56
Total and differential count of WBC in tumour bearing mice	Total no. of WBC cell/mm^3		31066±1190	10512±1066	201100±1128	25177±2019
	Differential count	Lymphocytes	40.4±1.16	72.2±1.66	52.4±7.2	67.2±0.55
		Neutrophils	56±1.12	20.3±0.72	42.1±0.11	28.1±0.11
		Monocytes	1.2±0.22	1.62±0.42	1.66±0.12	1.57±0.28
Total no. of RBC	Total no. (million /mm^3)		2.18±0.22	3.11±0.56	2.98±0.23	2.63±36
Haemoglobin (gm%)	Haemoglobin (gm%)		9.6±0.4	12.2±0.65	10.12±0.87	11.3±0.11

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