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Anticancer activity of Methanolic leaves extract of *Avicennia officinalis* on Ehrlich ascitis Carcinoma cell lines in Rodents

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Abstract: Cancer is a disease charecterised by abnormal or uncontrolled growth, invasion and metastasis of cells. This may be caused due to effects of carcinogens, radiations and also may be due to errors in DNA replication. The methanolic leaf extract of *Avicennia officinalis*[MEAO] at the dose of 200mg/kg and 400mg/kg was studied against cytotoxic effect for management of cancer, when tested on mean survival time and haematological parameters. The Ehrlich ascitic carcinoma cell lines were used for inducing cancer in mice. The MST of EAC transplanted mice was shown an increase from 14 days to 17-22 days in extract treated group when compared with control. The extract reverses the haematological changes induced by the cell lines. The extract posses cytotoxic effect in dose dependant manner and was statistically analysed by graph pad prism and was found to be highly significant. ($p<0.01$**)
Keywords: Avicennia officinalis, Erlisch ascites carcinoma cells, mean survival time(MST), haematological parameters.

INTRODUCTION(1)

Avicennia officinalis is an ever green tree found sporadically on the banks of river and rarely found near the sea.Fruits are plastered on to boils and tumours, poultice of unripe seed stop inflammation roots used for its aphrodisiac, bark is used to treat skin problems especially scabies, resin for snake bite and contraceptive by women, seed for ulcers. This plant contains pentacyclic triterpenoids such as lupeol, betulin,betulinaldehyde,betulinicacid, beta-sitosterol and Iridoid glucosides having c-11 carboxylic acid group were also present and Other compounds present are flavanoids ,alkaloid, steroids, tannins, wax esters are the most considerable compounds.

MATERIAL AND METHODS

COLLECTION, AUTHENTIFICATION AND EXTRACTION

Leaves of MEAO were collected locally from kolli hills, Tamilnadu, India. The plant material was taxonomically authentified by Dr. Jayaraman, Botonist, Chennai, Tamilnadu. Leaves were shade dried, powered and extracted with ethanol in a soxhlet apparatus. The MEAO leaves were distillated, evaporated to dryness and gummy mass dissolved in ethanol (SISCO). The LD_{50} value of MEAO was found to be > 4 g/kg body weight of mice.

TUMOR CELLS

Ehrlich Ascites Carcinoma (EAC) cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur, Kerala. EAC cells were maintained by weekly intraperitoneal (i.p) inoculation of 10^6 cells / mouse.

ANIMALS

Male albino swiss mice weighing 20±3gms were procured from animal house, department of pharmacology for the present investigation. The animals were given standard laboratory diet (Saidurga foods Pvt Ltd, Bangalore) and water *ad libitum*. The experiment protocol was approved by the Institutional Animal Ethics Committee IAEC Ref. No.290/CPCSEA/2009/-PH/pcol-08.

TUMOR GROWTH RESPONSE (2)

The effect of MEAO on tumor growth and host survival was estimated by evaluating cell count and percentage increase in lifespan (ILS) of the tumor hosts and drug treated groups. For calculating the survival time, four groups of mice were used. Among group 1 is control , Group 2 &3 were 200 & 400mg/kg of MEAO treated and group 4 was standard drug treated group(5- flourouracil20mg/kg body wt). On day '0', $1X10^6$ cells/ mouse were inoculated i.p., and treatment with the corresponding drug schedule was started 24h after innoculation. The control group was treated with 0.9% Nacl. Mean survival time (MST) for each group was noted and results were compared with control group.

ILS= MST of treated group / MST of control group x 100-100

The viable tumor cell count (trypan blue test) was carried out with a hemocytometer.

HEMATOLOGICAL STUDIES

As per MST study, similar group treated wth corresponding schedule and on 10^{th} day animals were sacrificed by decapitation and blood was collected to analyse Red blood cell count (3) ,White blood cell count (4) Heamoglobin, parameter.The ascitic fluid was collected and smeared by using giemsa stain for cytological studies.

STATISTICAL ANALYSIS

Values are expressed as mean ± S.E.M. Statistical analysis was carried out by One-way ANOVA followed by Dunnett-t test, haematological parameters were analysed by tukey multiple comparison test and differences were considered statistically significant at $p< 0.01$.

Table: 1 Effect of MEAO ON MEAN SURVIVAL TIME

S.NO	TREATMENT	MEAN SURVIVAL TIME	INCREASE IN LIFE SPAN(%)
1	CONTROL	13.85±0.28	-
2	5 FLURO URACIL	26±0.44	87.72
3	MEAO 200mg	17.66±0.33	27.50
4	MEAO 400mg	22±0.5	58.84

TABLE:2 EFFECT OF*MEAO* HEMATOLOGICAL PARAMETERS

Treatment	HB(GM%)	RBC 10^6cells/cu.mm	WBC 10^3ls/cu.mm	Differential count (%)		
				Lymphocytes	Neutrophils	Monocytes
Normal	12.6±0.38	10.3± 0.27	5.7± 0.18	69.1±0.9	20.8±0.29	2± 0
Tumour control	5.71± 0.26	6.0 ± 0.22	15.0± 0.17	55.21 ± 1.10	37.0± 0.29	1 ± 0
MEAO (200MGkg)	6.81± 0.34	6.61± 0.15	12.2± 0.18	58.7 ± 1.02	34.3 ± 0.6	1 ± 0
MEAO400MG	8.71± 0.21	7.41± 0.43	10.31± 0.33	62.1 ± 0.65	28.3 ± 0.3	1 ± 0
5FU	10.8± 0.21	8.8 ± 0.26	8.0 ± 0.28	62.5 ± 1.25	24.9± 0.24	1 ± 0

RESULTS

The effect of MEAO on the survival time of EAC bearing mice have been summarized in the table:1. The mean survival time for the EAC control group is 22.33 days, while it is 44. 33 for 5-flourouracil (20 mg/kg) treated group, 29.32 for 200mg/kg and 33.66 for MEAO(400 mg/kg) treated group respectively.Table 1 shows significant increase of life span in dose dependent manner when compare with respect to tumor control group.

Hematological response of MEAO treated EAC bearing mice is presented in table:2. There is an increase in haemoglobin , RBC and lymphocytes when compared to tumor control group and decrease in WBC, cell volume, proteins, neutrophils and no change in monocyte.

CONCLUSION

The reliable criteria for judging the value of any anti cancer drug are prolongation of life span and decrease of WBC from blood. The results of present study clearly demonstrate the tumor inhibitory activity of MEAO against EAC strain as with evident of inhibition in body weight gain and increase in life span (MST) and also brought back heamatological parameters to more or less to normal levels.

Usually in cancer chemotherapy, the major problem that are being encountered are of myelosupression and anaemia(5) but results clearly shown that MEAO (400mg/kg) brought back haemoglobin content close to normal. Based on the cytotoxicity results the extract posses potent cytotoxic activity.

The pharmacological relevance of pentacyclic triterpenes has increased during the last two decades demonstrating multi-target properties such as wound healing, anti-inflammatory, anti-bacterial, antiviral,hepatoprotective and anti-tumoral effects(6,7,8,9,10). In the plant , pentacyclic triterpenes may possess anti tumor activity. Hence further research will be proceded to establish the exact mechanism of action of MEAO .

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