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EVALUATION OF ANTICANCER POTENTIALS OF *TECOMA STANS* (L). JUSS.EX. KUNTH AGAINST EAC CELL LINES

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ABSTRACT

The work was planned to investigate the antitumor potentials of ***Tecoma stans*** (L). Juss.ex. Kunth on EAC cell lines. *In-Vitro* cytotoxic assay such us Tryphan blue and MTT assays were carried out against the tested cell lines. *In-vivo* studies Ehrlich Ascites Carcinoma cell lines at dose level 1X10^{6}cells /mouse was injected to the experimental animals and administered with the water extract of ***Tecoma stans*** (L). Juss.ex. Kunth (WETS) at varying doses (100,200 & 300mg/kg. bw). The antitumor effect of WETS was accessed by studying, increase in life span, Tumor growth response, hematological and biochemical parameters. The data of the results revealed that the effect of the test drug was comparable to the standard drug 5-Flurouracil (20mg/kg.bw). The results obtained in the present study suggested that the WETS possess significant anticancer activity against the tested cancer cell line.

Keywords: Ehrlich Ascites Carcinoma cell lines (EAC), Water extract of Tecoma *stans* (L). Juss.ex. Kunth (WETS), Increase in life span (ILS), Tumor growth response, GLycoproteins.

INTRODUCTION

Uncontrolled growth, tissue invasion and distinct metastasis are the characteristic features of cancer cells [1]. Newer methodologies are required for its control and treatment and it is the leading health problems in all over the world [2]. Chemotherapy provides some relief to cancer patients, but they are highly toxic, and minimal quantity of injected drug only can reach the cancerous tissue and may damage the normal system especially bone marrow, epithelial tissue, reticulo-endothelial system and gonads [3]. Recent times plants and plant derived compounds showed promising anticancer efficacy against various cancer cell lines and experimental models [4]. 60 % of FDA approved anticancer drugs are from the plant origin. Hence in the preset study a Common ***Bignoniaceae*** member botanically identified as ***Tecoma stans*** (L). Juss.ex. Kunth. Was selected and its water extract was subjected to anticancer evaluation against EAC cell lines employing various *In-Vitro* and *In-Vivo* protocols.

MATERIALS AND METHODS

Plant collection and extraction: The aerial parts of ***Tecoma stans*** (L). Juss.ex. Kunth. was collected from in and around Trichy, Tamilnadu, selected plant material was identified using Flora of presidency of Madras [5]and authenticated with the help of voucher specimen deposited in the Rapinant Herbarium St. Jospeh's College, Trichy. Water extract was the selected plant drug was prepared using hot extraction method.

Cells: EAC (Ehrlich Ascites carcinoma) cell lines were obtained from maintained at the dose of 1X10^{6}cells/mouse by intraperitoneal inoculation at once in a week [6].

In-vitro cytotoxicity

The cytotoxic effect of the water extract of the test drug was Scrrened against EAC cell lines using trypan blue dye exclusion method [7] and MTT assay [8].

Effect of WETS on survival time

The Swiss albino mice were divided into five groups of six animals each and injected with EAC cell lines at the dose level of 1x10^{6}cells. Treatment with WETS at different concentrations (100, 200, 300 mg/kg bw p.o.) administrated orally group II, III, IV respectively for 14 days. Group V animals were treated with standard drug 5-Flurouracil (20mg/kg bw) for 14 days. Group I animals administered with normal saline. The percentage of increase in lifespan (ILS) and mean survival time (MST) was calculated using standard calculation [9].

Experimental design

Animals were divided into six groups of six mice (both sex) each.

Group-I- Normal Control

Group-II- EAC (1X10^{6}cell/mouse) (Disease Control)

Group-III - EAC (1X10^{6}cells) treated with 100mg/kg bw. of WETS

Group-IV - EAC (1X10^{6}cells) treated with 200mg /kg bw. of WETS

Group-V - EAC (1X10^{6}cells) treated with 300mg /kg bw. of WETS

Group VI - EAC (1X10^{6}cells) treated with Standard drug 5-Fluorouracil (20mg/kg.bw.).

WETS - Water Extract of ***Tecoma stans*** **(L). Juss.ex. Kunth.**

After 24 h of tumor inoculation the selected plant drug (**WETS**) was orally administrated for 14 days. After the experimental period the ascites fluid was collected and were sacrificed by cervical decapitation, blood was collected and liver tissue was dissected out and washed in ice cold saline.

Tumor growth response

Tumor growth response was assessed using Ascites fluid by measuring Tumor volume, Tumor cell count, and Viable and non-viable cell counts [10].

Hematological parameters

The whole blood was collected and tested for enumeration Erythrocytes (RBC) Hemoglobin and Leucocytes (WBC) [11].

Biochemical parameters

Various biochemical parameters were analaysed in serum and hepatic tissue . Serum protein12, Hepatic glycogen [13], Hepatic Nucleic Acid Contents like DNA [14] and RNA [15], serum hepatic marker enzymes such as AST [16], ALT [16], ALP [16] & LDH [17], Hepatic Glycoproteins such as protein bound hexsoes [18], Hexosamine [19] & Fucose [20] and antioxidant parameters such as LPO [21], GSH[22], GPX[23], SOD [24] and CAT [25].

Statistical Analysis

The experimental data were expressed as mean ± SEM. Statistical analysis was performed using ANOVA followed and P values <0.05 were considered as highly significant.

RESULTS

Cytotoxic Effect of WETS on EAC cells (Trypan Blue Method)

Treatment with various concentration of water extracts of *Tecoma stans* (L). Juss.ex. Kunth. against EAC cell lines showed increased cell death(Table 1). Maximum cytotoxicity was observed in 1000 µl/ml concentration and the viability of cell was decreased with increase in concentration of WETS.

Cytotoxicity effect of WETS on EAC cell lines (MTT ASSAY)

The IC_{50} (87.38µg/ml) values was obtained in the cytotoxic assay against on EAC cell lines were shown in (Table 2). The 100 µg/ml concentration produced 57.22 % cytotoxicity where as 25 µg/ml showed 21.48% cytotoxicity.

Effect of WETS on MST

In WETS administration to the tumor bearing animal increased life span upto 27.84%, 51.96, & 60.71% (100,200 &300mg/kg bw.) respectively in plant drug treated group when compared to ILS of Disease control animals. (Table 3).

Effect of WETS on Tumor response

On treatment with WETS of EAC bearing animals significantly reduced the tumor growth response (Table 4) which is evident from decrease in tumor volume, viable cell count and increase in non-viable cell count.

Effect of WETS on Hematological parameters

The decreased levels of Hemoglobin, RBC and elevated levels WBC counts were reverted back to normal (Table 5) after the drug administration of the test drug (WETS).

Effect of WETS on Biochemical parameters

In EAC inoculation to the experimental animals the elevated levels of serum protein (Table 6), hepatic nucleic acid contents (DNA and RNA) (Table 7) serum hepatic marker enzymes (ALT, AST, ALP, LDH) (Table 8) and hepatic glycoprotein levels (Hexose, Hexosamine and Fucose)(Table 9) were restored to normalcy on treatment with the test drug under study.

Table 1: Cytotoxic Effect of WETS on EAC Cells (Trypan Blue Method)

Concentration of WETS (µg/ml)	Viable cells (%)	Dead cells (%)
Control	95.80	4.20
50	70.21	29.79
100	60.15	39.85
500	40.15	59.85
1000	23.26	76.74

Table 2: Cytotoxic Effect of EETS on EAC Cells (MTT ASSAY)

Concentration of WETS (µg/ml)	OD1	OD2	OD3	Average	%
Control	0.567	0.480	0.490	0.512	-
25	0.438	0.412	0.356	0.402	21.48
50	0.416	0.395	0.389	0.400	21.85
75	0.326	0.331	0.313	0.323	36.91
100	0.229	0.211	0.219	0.219	57.22

IC_{50} value = 87.38 microgms/mL

Table 3: Effect WETS on MST and ILS

Particulars	Mean Survival Time (days)	Increase in Life Span (ILS) (%)
GroupI	19.32±2.13	_
Group II	24.15±2.67	27.84
Group III	29.36±2.26	51.96
Group IV	31.05±1.75*	60.71
Group V	34.2±2.26*	70.01

Values: Mean±S.E.M., n=6, *Statistically significant at the level of p<0.05 when comparing Group II

Table 4: Effect of WETS on tumor growth

Group	Tumor volume (mL)	viable cells	Non-viable cells
Group II	4.21±0.26	20.42±0.57	1.16±0.18
Group III	3.85±0.46	18.51±0.65	2.90±0.70
Group IV	3.06±0.21	13.52±0.57	4.52±0.62
Group V	2.23±0.41*	10.97±0.31*	5.93±0.71*
Group VI	0.80±0.32*	8.15±0.14*	6.90±0.21*

Values: Mean±S.E.M., n=6, *Statistically significant at the level of p<0.05 when comparing Group II

Table 5: Effect of WETS on Hematological parameters

Particulars	Hb content (g/dl)	RBC count	WBC count
Group I	14.85±1.06	4.6±0.28	6.21±1.19
Group II	9.15±1.34*	3.1±0.14*	9.32±0.87*
Group III	13.7±0.70	3.47±0.21	8.17±0.91
Group IV	14.4±0.65	4.01±0.45	7.46±1.01
Group V	14.9±0.16**	4.26±0.51**	6.23±0.87**
Group VI	15.2±0.35**	4.6±0.13**	6.15±0.37**

Values are mean±S.E.M, n=6,*Statistically significant at the level of p<0.05 when comparing Group I,** Statistically significant at the level of p<0.05 when comparing Group II

Table 6: Effect of WETS on Serum and Tissue Biochemical Parameters

Groups	Serum protein (mg/dL)	Liver glycogen (mg/g of Tissue)
Group I	5.97±0.68	9.95±2.23
Group II	3.35±0.07*	5.26±2.61*
Group III	3.81±0.25	4.86±2.37
Group IV	4.96±0.36	7.17±2.82
Group V	6.39±0.65**	8.39±2.69**
Group VI	6.95±2.33**	9.10±2.23**

Values are expressed as mean±S.E, (n=6), *Statistically significant at the level of p<0.05 when comparing Group I, ** Statistically significant at the level of p<0.05 when comparing Group II

Table 7: Effect of WETS on Hepatic nucleic acid contents

Groups	DNA (mg/g tissue)	RNA (mg/g tissue)
Group I	14.16±1.77	13.13±0.95
Group II	26.66±2.88*	26.90±4.85*
Group III	22.16±2.88	21.63±1.55
Group IV	19.91±2.69	17.14±1.39
Group V	16.50±2.50**	15.80±0.40**
Group VI	15.43±0.74**	13.50±0.47**

Values are expressed as Mean± SE (n=6)

*Statistically significant at the level of p<0.05 when comparing Group I; ** Statistically significant at the level of p<0.05 when comparing Group II

Table 8: Effect of WETS on serum hepatic marker enzymes

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)
Group I	18.43±4.10	24.94±4.23	88.91±1.29	663.21±9.52
Group II	63.06±4.21*	84.83±3.92*	166.91±2.31*	1317.34±11.65*
Group III	55.61±2.16	71.45±3.20	134.91±1.29	987.62±10.91
Group IV	49.43±2.10	66.27±1.21	109.52±4.26	864.32±13.45
Group V	35.52±1.61**	45.19±1.84**	98.16±2.13**	779.61±11.52**
Group VI	26.1±4.7**	39.6±1.47**	93.56±1.36**	717.65±8.11**

Values are expressed as Mean± SE (n=6), *Statistically significant at the level of p<0.05 when comparing Group I, ** Statistically significant at the level of p<0.05 when comparing Group II

Table 9: Effect of WETS on Hepatic Glycoproteins

Groups	Hexose (mg/g of defatted tissue)	Hexosamine (mg/g of defatted tissue)	Fucose (mg/g of defatted tissue)
Group I	14.386±0.427	10.583±2.02	16.785±1.26
Group II	24.293±0.323*	21.05±1.96*	27.24±1.40*
Group III	21.25±1.06	18.57±1.20	23.32±1.11
Group VI	19.25±2.32	16.49±1.66	20.92±1.85
Group V	16.32±1.74**	13.62±1.71**	17.46±1.31**
Group VI	13.12±0.23**	11.51±1.21**	16.13±1.10**

Values are expressed as Mean± SE (n=6), *Statistically significant at the level of p<0.05 when comparing Group I, ** Statistically significant at the level of p<0.05 when comparing Group II

Table 10: Effect of WETS on Enzymatic and Eon-enzymatic Antioxidant

Groups	LPO nmol MDA·mg^{-1} (protein)	GPX U·mg^{-1} (protein)	GSH mg.g^{-1} (wet tissue)	SOD U·mg^{-1} (protein)	Catalase U·mg^{-1} (protein)
Group I	0.97±0.58	3.05±0.49	14.76±2.18	5.71±0.018	24.166±2.323
Group II	3.57±1.05*	1.21±0.84*	4.14±1.71*	2.29±0.12*	11.606±5.575*
Group III	2.96±0.22	1.75±0.49	5.10±1.48	2.99±0.01	14.414±2.871
Group VI	1.73±0.40	2.05±0.49	8.36±1.76	3.16±0.01	15.612±2.33
Group V	1.31±0.31**	2.90±0.98**	10.79±1.06**	4.09±0.05**	17.162±5.196**
Group VI	1.17±0.36**	2.74±0.04**	12.61±0.17**	5.33±0.12**	22.47±1.57**

Values are expressed as Mean± SE (n=6), *Statistically significant at the level of p<0.05 when comparing Group I, ** Statistically significant at the level of p<0.05 when comparing Group II

Effect of WETS on Enzymatic & Non-Enzymatic Antioxidant system

EAC inoculation resulted in the marked elevation of LPO and reduced levels of non-enzymatic (GSH) and enzymatic antioxidants (GPX, SOD and catalase). The tested drug administration effectively restored the antioxidant system.

DISCUSSION

Cancer, the second largest killing disorder in the world and described by uncontrolled cellular proliferation and metastases. Cancer is the disease associated with modern life style, for which modern medicine has little to offer [26]. Now day's researchers are focusing their research towards complementary and alternative medicine such as phyto medicine. Traditional plants might have provide useful sources for developing new anticancer drugs and could be a good alternative to existing lines of cancer therapies [27]. In the present study attempts was made to arrive a multifaceted anticancer drug from a common plant source botanically equated as ***Tecoma stans*** (L). Juss.ex. Kunth. Belonging to the family ***Bignoniaceae,*** the water extract of the selected plant drug was tested for its anticancer potential against EAC cell lines.

Plant extracts (WETS) was screened for its anticancer activity against EAC cell lines in Trypan blue dye exclusion method. 76.74% cytotoxicity was observed at the concentration 1000 µg/mL. The extract may produced substantial injury to the membrane and might have enhanced the apoptotic pathway in EAC cells.

Cytotoxicity of the selected planted drug was also studied employing MTT assay. After 24 hrs treatment with WETS showed the maximum cytotoxicity 57.22% (Table 2) was found at the dose level of 100 µg/mL and the IC50 value was found to be 87.38 µg/ml. The plant drug might have disturbed the mitochondrial assembly which resulted in the increased cytotoxicity of EAC cell lines [28].

The major decisive factor for assessing the potential of antitumor drug is the persistence increase in lifespan of cancer bearing animal [29]. The test drug (WETS) increased the life span of cancerous animal's upto 60.71%.

EAC inoculation to the experimental animal increased the ascites fluid formation. The ascites fluid is the major source of nutrition for the rapidly growing cancer cells [29]. Treatment with WETS resulted in decreased the ascitic fluid formation (Table 4). The tested drug might have provided the cytotoxicity or inhibited the vascular permeability and cellular migration.

The anemia occurs in cancer is mainly due to decreased absorption of iron or increased hemolysis [30]. In EAC inoculation to cancer bearing animal resulted in the decreased Hemoglobin and RBC and the elevated levels WBC is due to increased immune response. Administration of WETS reverted back the Hb content, Erythrocytes and Leucocytes counts to near normalcy indicating the efficacy of the test drug to protect the hemopoietic system.

The inoculation of EAC cell line caused the formation of secondary carcinoma in the hepatic tissue resulted in the disfunction of the liver which in turn produced decreased synthesis of the serum protein and glycogen. Administration of the tested drug WETS significantly increased the serum protein and tissue glycogen levels. The tested drug might have inhibited or prevented the secondary carcinoma and metastasis of EAC cells.

In EAC inoculated animals showed increase amount of nucleic acid contents (DNA and RNA). This may be due to increased activity of cellular proliferation and increased activity of polymerase enzymes [31]. Administration of WETS to the cancer bearing animals, the levels of nucleic acids contents were reverted back to normal. The tested drug might have blocked the proliferation of tumor cells at the early stages of the cell cycle and inhibited the polymerase enzyme activity. The test drugs may also inhibit the pentose phosphate pathway which provides the precursors for the synthesis of nucleic acids.

The elevated levels of AST, ALT and ALP found in Tumor bearing animals are attribute to secondary carcinoma and metastasis [32]. The activities of liver marker enzymes are correlated to the degree of malignancy and can be used as an indicator for diagnosis and prognosis of cancer. Treatment with WETS reverted back the elevated levels of serum hepatic marker enzymes to near normalcy, this could be due to the inhibition of tissue necrosis or cell injury or cancer cell growth resulting in altered membrane permeability leading to prevention of enzyme leakage.

LDH is anaerobic glycolytic enzyme and recognized as an important marker for cancer cell growth and malignancy. The number of reports revealed cancer in associated with the elevated level of LDH 33. Test drug administration at various dose levels effectively restored the altered activities of this enzyme by blocking the metastasis of cancer cells and anaerobic glycolyitic pathway which is the major energy yielding pathway of cancer cells.

In malignant tumor the glycosidase enzyme activity were increased twice when compared to healthy cells. The elevated level of glycoprotein components such as Hexose, Hexosamine and Fucose of EAC bearing animals are due to the increased glycosidase activity, leakage of distributed membrane compound either disintegrating or dying neoplastic cells or as a consequent of shedding of plasma membrane [34]. On treatment with WETS the glycoprotein contents were restored to near normal levels. This reduced glycoprotein levels indicated that the test drug might have cytostabilising property, malignancy suppresses activity and metastases and tumor growth inhibiting properties.

EAC inoculation tot the experimental caused the oxidative stress which facilitated the increased production of free radicals damage the lipids molecules and can induce lipid peroxidation [35**]**. After treatment the elevated levels of MDA were brought back to normal by increase in the test drug concentration. The test drug might have reduced the free radical formation resulting in a subsequent decrease in the membrane damage and LPO levels.

The decreased Glutathione level in liver tissue of tumor bearing animals is mainly due to oxidation of GSH to GSSH caused by increased peroxidation production. Added to this GSH level is further reduced due to the decreased activity of glutathione reductase. The administration of WETS to the experimental animals increased the glutathione content which favors the blockage of neoplastic process and increased the life span of the tumor bearing animals. Decreased activity of Glutathione peroxidase (GPX) of tumor bearing animals is primarily due to the lack of glutathione and increased production of hydroxyl radicals [41].

In oxygen metabolizing cells free radicals such as superoxide and hydrogen peroxide are effectively cleaved by SOD and CAT. A decreased activity of SOD in tumor inoculated animal is due to the disintegration of Mn+ in SOD system and loss of mitochondria [36]. A WETS treatment increased SOD level in the tumor bearing animals indicates its potentials to protect the mitochondria from oxidative stress.

Catalase (CAT) is a heme containing enzyme and key component in the management of oxidative stress. In Disease control group the levels of CAT were considerably reduced [37**].** This is because super oxides radicals present in the host tissue of tumor bearing animals converts the ferrous of the catalase into ferroxy or ferryl state and make the enzyme inactive. On test drug administration CAT levels increased due to scavenging of superoxide radicals this may be attributed to the increased activity of the catalase as the ferrous group is restored.

On treatment with different dose levels of WETS resulted in decreased lipid peroxidation and increased the activities of GSH, GPX, SOD and CAT indicated its potential as an inhibitor of tumor induced intracellular oxidative stress.

Thus the data of the results obtained in the *In-Vitro and In-vivo* studies conducted against EAC cell lines depicted that the WETS has the significant anticancer activity against tested cell lines. The data of the results were compared with that of the standard drug 5-fluorouracil (20mg/kg bw) and it was observed that the 300mg/Kg.bw. dose level of WETS is the most effective dose.

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