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Research Article

IN VIVO AND *IN VITRO* ANTITUMOR ACTIVITY OF *JASMINUM SAMBAC* (Linn) AIT OLEACEAE FLOWER AGAINST DALTON'S ASCITES LYMPHOMA INDUCED SWISS ALBINO MICE

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ABSTRACT

The present study was aimed to evaluate the anticancer effect of *Jasminum sambac* against Daltons ascites lymphoma induced Swiss albino mice in *in vitro* and *in vivo* model. The tumor cell proliferation inhibitory activity of methanolic extract showed dose dependent in both HeLa and mouse fibroblast cells. At concentrations 25-400µg/ml, the percentage of cell inhibition concentration of normal and cancer cells was found to be 123.3 and 93.8 µg/ml respectively. The assessment of anticancer activity of *J.sambac* was evaluated by measuring the activity of hematological profiles, liver function marker and cancer marker enzymes. The methanolic extract at oral dose of 100mg/kg body weight exhibited a significant ($p< 0.05$) changes in the levels of hematological profiles, AST, ALT, ACP, ALT and LDH and cancer marker enzymes such as 5'Nucleotidase, β-D- Glucuronidase, γ-Glutamyl transferase as compared to DLA induced group. Thus it could be concluded that the methanolic extract of *J.sambac* possesses significant anticancer properties

Keywords: *Jasminum sambac*, Lymphoma, Hematological profiles, Anticancer properties

INTRODUCTION

Cancer is a abnormal types of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell[1]. Lymphoma is a disease of the lymphocytes (a type of white blood cell involved in immune responses) and the lymphatic system, which includes the spleen, thymus, and liver, as well as other lymphatic tissues. Dalton's ascites lymphoma is transplantable, poorly differentiated malignant tumor which appeared originally as lymphocytes in a mouse. It grows in both solid and ascitic forms[2].

A free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence[3]. Antioxidants are the chemical compounds which can delay the start or slow the rate of lipid oxidation reaction in food systems. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals[4].

Jasminum sambac Linn (Oleaceae) is commonly known as Jasmine. It is a well known glabrous twining shrub widely grown in gardens throughout India. The *J. sambac* flowers and leaves are largely used in folk medicine to prevent and treat breast cancer. Flowers of *J. sambac* are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding. It is widely used in the Ayurvedic, as an antiulcerative, anti cancer antileprotic, skin diseases and wound healing[5]. In view of the antioxidant and antitumor activities of *Jasminum sambac*, we tested antitumorogenic potential of *Jasminum sambac* in Dalton's ascites lymphoma promoted lymphatic cancer model mice.

MATERIALS AND METHODS

Collection of plant material

Jasminum sambac flowers were collected from Coimbatore district, Tamilnadu, India during the month of December, 2009. The plant was authenticated by Dr.G.V.S.Moorthy, Joint Director, Botanical survey of India, Tamilnadu Agricultural University, Coimbatore, India where the voucher specimen is (No.BSI/SRC/5/23/09-10/Tech-972).

Plant Extraction

The flowers were collected, shade dried and powdered. About 30g of the powdered material were extracted with 300ml of methanol in a water shaker for 72h. Repeatedly extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40-50°C. A light yellow powdered material was obtained and stored at 0-4°C. The yield of the extract material was about 15.14%.

Experimental animals

Female Swiss albino mice (6-7 weeks old) with an average weight of 20 to 25g were obtained from Small Animal's Breeding Centre of Kerala Agricultural University, Mannuthy, Thrissur. The animals were housed in large spacious cages, maintained at controlled condition of 12hr light/darkness, humidity, and temperature. They were fed with standard pellet diet (Hindustan Lever Ltd. Bangalore) and water ad libitum. The ethic guidelines for investigations using conscious animals were obeyed, and the procedures were approved by the Faculty Ethics Committee, Government of India.

Tumor cell proliferation inhibitory activity

To determine the proliferation inhibitory activity of the methanolic extract, MTT (3-(4, 5-dimethyl-thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide) assay was performed using HeLa cells[6].

In vivo antitumor activity of *Jasminum sambac*

The animals were divided into 4 groups containing 6 animals in each group. Group I served as control, group II served as DLA control (serial intraperitoneal (i.p) transplantation of $1x10^6$ tumor cells (0.25ml in phosphate buffered saline, pH 7.4) per animal), group III are treated with oral administration of *Jasminum sambac* at100mg/kg body weight for 14 days and group IV animals are treated with standard drug (5'fluorouracil) at 20mg/kg body weight. On 15th day these animals were sacrificed after an over night fast by decapitation.

Blood was collected in conventional way and used for the estimation of Red blood cell count (RBC) and White blood cell count (WBC)[7], Hemoglobin (Hb)[8], WBC differential counts. The remaining blood was centrifuged and serum was used for the estimation of liver function marker enzymes like AST and ALT[9], ACP and LDH[10], ALP[11], cancer markers such as 5'Nucleotidase[12], β-D-Glucuronidase[13], γ-Glutamyl transferase[14].

The liver was excised rinsed in ice-cold normal saline solution followed by cold 0.1M Tris-HCl (pH 7.4), blotted, dried and weighed. A 10% w/v homogenate was prepared in 0.1M Tris-HCl buffer and was used for the DNA and RNA[15]. Sections of liver and spleen organs were fixed with 10% formalin, embedded in paraffin sectioned at 5µm thick and stained with haematoxylin and eosin for histological analysis.

Statistical analysis

The values were represented as the Mean ± SD. The results were statistically analyzed using the statistical package (SPSS). One-way analysis of variance was employed for comparison among the six groups followed by Least Significant Difference (LSD). Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The methanolic extract of *Jasminum sambac* was tested for their *in vivo* and *in vitro* antitumor activity against DLA tumor bearing mice which are as follows.

Tumor cell proliferation inhibitory activity

Table 1 shows that the cytotoxic effect of MtJs was dose dependent in both the cells. At concentrations 25-400µg/ml, the percentage of cell inhibition concentration of normal and cancer cells was found to be 123.3 and 93.8 µg/ml respectively. This exhibited the flower extract showed maximum cytotoxicity against HeLa (the cancerous cells) and minimum cytotoxicity towards the mouse embryonic fibroblasts (normal cells). The *Rubia cordifolia* extract had the greatest activity with lowest IC_{50} values against HeLa and HEp-2 cell lines[16].

Table 1: Tumor cell proliferation inhibitory activities of methanolic extract of *Jasminum sambac* on NIH3T3 and HeLA cell lines

S.No	Concentration (µg/ml)	MTT assay (% of cell inhibition)		IC_{50} (µg/ml)	
		HeLa	NIH 3T3	HeLa	NIH3T3
1	25	16.99	5.36	-	-
2	50	32.35	28.24	-	-
3	100	60.02	49.80	93.8	123.3
4	200	90.54	72.81	-	-
5	400	98.17	95.56	-	-

In vivo* antitumor activity of *Jasminum sambac

The hematological parameters of tumor bearing mice were found to be significantly altered from normal group. WBC, neutrophils and eosinophils were found to be increased with a reduction of RBC, Hb and lymphocytes. At the same time interval MtJs and 5'fluorouracil could change those altered parameters to near normal (Table 2).

Table 2: Effect of *Jasminum sambac* on Hematological parameters in control and experimental mice

Parameters	Group I (Control)	Group II (DLA control)	Group III (DLA+ MtJs)	Group IV (DLA+ 5'fluorouracil)
WBC (T/mm^3)	8.64±0.06	20.24±0.16^a	14.17±0.03^b	10.52±0.21^c
RBC (m/mm^3)	10.11±.18	5.48±0.45^a	9.83±0.2 ^{b}	11.80±0.29^c
Hemoglobin(g/dl)	13.59±.50	7.67±0.47^a	11.07±0.48^b	12.88±0.30^c
Lymphocytes(%)	65.13±.53	24.06±0.10^a	32.76±0.33^b	60.41±0.41^c
Neutrophils (%)	30.50±.92	76.68±0.48^a	61.43±0.65^b	36.61±1.13^c
Eosinophils (%)	6.49±0.50	7.62±0.26^a	6.07±0.23^b	6.24±0.13^c

Values are expressed as mean ± SD (n=6). Statistical significance was done by one way ANOVA followed by LSD. a- Group II where compared with group I. b,c- Group III and IV compared with group II. The letters (a-c) represents statistically significant at $p < 0.05$.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia[17]. Anemia is found frequently occur in cancer patients[18]. The decrease in the levels of Hb, RBC, lymphocytes and increase in the levels of WBC, neutrophils in Dalton's ascitic carcinoma in swiss albino mice were reverted to normal on administration of *Holoptelea integrifolia and Bauhinia variegata*[19]. Similar trend was obtained in *Mucuna pruriens* against Ehrlich ascites carcinoma[20].

The increased level of eosinophils in EAC bearing mice. Treatment with *Hypericum hookerianum* and *Ichnocarpus frutescens* caused the reduction in eosinophil level[21]. Treatment with MtJs brought back the hemoglobin content, RBC, WBC and differential counts more or less to near normal levels. This indicates that flower extract possess protective action on the hemopoietic system. Treatment with flower extract brought back the hemoglobin content, RBC, WBC and differential counts more or less to near normal levels. This indicates that MtJs possess protective action on the hemopoietic system.

Liver function marker enzymes activities of serum are presented in table 3. Data pertaining to the levels of AST, ALT, ACP, ALP and LDH significant rise in the serum of DLA control mice when compared to normal control group (group I). All these parameters were restored to near normal levels in Group III and IV treated animals when compared to group II. It is well known that the elevation of ALT activity is repeatedly credited to hepatocellular damage. Also, the increase in ALP reflects a pathological alteration in biliary flow[22].

The significant increase in the levels of serum ALP, ACP and LDH in DEN induced animals was observed and it was significantly decreased by treatment with *Cassia fistula* leaf extract[23]. The increased activity of liver marker enzymes was brought back to near normal levels by the therapeutic efficacy of the drug.

Table 3: Effect of *Jasminum sambac* on liver function marker enzymes in serum of control and experimental mice

Groups	AST(µm/l)	ALT (µm/l)	ACP (µm/l)	ALP (µm/l)	LDH (µm/l)
Group I (Control)	81.09±0.21	34.09±0.34	48.09±0.21	77.64±1.18	92.98±1.01
Group II (DLA Control)	110.09±0.11^a	74.09±0.21^a	86.34±0.40^a	82.58±1.63^a	125.19±0.77^a
Group III (DLA + MtJs)	73.09±0.36^b	61.09±0.66^b	52.20±0.16^b	63.36±1.48^b	99.22±0.52^b
Group IV (DLA + 5'fluorouracil)	84.09±0.21^c	42.09±0.78^c	49.69±0.54^c	72.96±1.38^c	94.86±0.58^c

Values are expressed as mean ± SD (n=6). Statistical significance was done by one way ANOVA followed by LSD. a- Group II where compared with group I. b,c- Group III and IV compared with group II. The letters (a-c) represents statistically significant at $p < 0.05$.

Table 4 shows the activities of cancer marker enzymes (5'NT, β-D-Glu, γ-GT) in serum of control and experimental animals. The activity of cancer marker enzymes in the serum was significantly increased in ($p<0.05$) cancer bearing animals when compared with control mice. However, a promising reduction ($p<0.05$) to near normal levels was shown in animals treated with both MtJs and 5'fluorouracil.

Table 4: Effect of *Jasminum sambac* on 5'NT, β-D-Glucuronidase and γ- glutamyl transferase in serum of control and experimental mice

Groups	5'NT (µm/l)	β-D-Glucuronidase (µm/l)	γ-GT (µm/l)
Group I (Control)	5.19±0.20	34.12±0.20	2.18±0.05
Group II (DLA control)	9.13±0.14[a]	56.41±0.43[a]	5.54±0.06[a]
Group III (DLA + MtJs)	7.24±0.12[b]	40.56±0. 41[b]	2.76±0.14[b]
Group IV (DLA+5'fluorouracil)	5.74±0.26[c]	36.81±0.36[c]	3.30±0.09[c]

Values are expressed as mean ± SD (n=6). Statistical significance was done by one way ANOVA followed by LSD. a- Group II where compared with group I. b,c- Group III and IV compared with group II. The letters (a-c) represents statistically significant at $p < 0.05$.

Cancer markers enzymes are embedded in the hepatocyte plasma membrane, mainly in the canalicular domain, and its liberation into serum indicates damage to the cell and thus injury to the liver[24]. There was a significant increase in the levels of 5'NT in breast cancer induced animals and it was decreased by treatment with ethanolic extract of propolis and paclitaxel[25]. The present study are in substantiation with[26] who reported that significant increase in the levels of 5'NT and β-D-glucuronidase in oral carcinoma cells and it was decreased on treatment with plant extract. Gamma glutamyl transferase was raised in carcinoma bearing animals. Treatment with *Biophytum sensitivum* extract showed recoupment of this enzyme to near normal level[27].

DLA control group mice showed increased levels of DNA and RNA content compared to control group I. On the contrary, the MtJs and standard drug (group III and IV) mice significantly decreased ($p<0.05$), respectively, compared to DLA control animals (Table 5). The above result are in accordance with the study of[28] who reported the liver DNA and RNA levels were increased significantly in cancer induced animals were reverted to normal on treatment with blueberries.

Table 5: Effect of *Jasminum sambac* on DNA and RNA constituents in liver tissue of control and experimental mice

Groups	DNA	RNA
Group I (Control)	64.09±1.26	36.14±1.34
Group II (DLA Control)	97.09±1.32[a]	64.65±1.21[a]
Group III (DLA + MtJs)	77.09±1.12[b]	42.33±1.54[b]
Group IV (DLA + 5'fluorouracil)	69.09±1.22[c]	39.89±1.78[c]

Values are expressed as mean ± SD (n=6). Statistical significance was done by one way ANOVA followed by LSD. a- Group II where compared with group I. b,c- Group III and IV compared with group II. The letters (a-c) represents statistically significant at $p < 0.05$.

The increased DNA content may lead to increased transcription which might have resulted in elevated RNA content in tumor cell. Folate is important for normal DNA synthesis, repair, and converting homocysteine to methionine[29]. Therefore, increased demand of folate is postulated to be a result of increased hepatic levels of DNA and RNA and might indicate increased DNA and RNA synthesis and proliferation of cancer cells in response to growth stimulation.

CONCLUSION

All these observations clearly indicate a significant anticancer and cytotoxic effect of the extract of the flower of *Jasminum sambac*. Further studies to characterize the active principles and to elucidate the mechanism action are in progress.

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