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

MANOKARAN KALAISELV1,2, RAJASEKARAN NARMADHA2, PARAMASIVAM RAVAGENTHAR2, GANESAN RAVIKUMAR2, DURAIASAMY GOMATHI2, DOMINIC SOPHIA2, CHINTHAMONY ARUL RAJ, CHANDRASEKAR UMA2 AND KALAIVANI.K1*

1Department of Biochemistry, Kongunadu Arts and Science College, 2Department of Biochemistry, Karpagam University, Coimbatore.
Email: drkalaivani.vani4@gmail.com

Received: 12 Feb 2011, Revised and Accepted: 18 May 2011

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 cell1. 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 forms2.

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 existence3. 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 radicals4.

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, preventing breast cancer and stopping uterine bleeding. It is widely used 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 radicals5.

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/S/25/09- 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 colourless 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 Animals 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 cells6. Experimental animals

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 were treated with standard drug (5fluorouracil) 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 ALT9, ACP and LDH10, ALP11, cancer markers such as 5’Nucleotidase12, β-D-Glucuronidase13, γ-Glutamyl transferase14.
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 RNA15. 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 *Rutia cordifolia* extract had the greatest activity with lowest IC50 values against HeLa and HeP-2 cell lines.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>MTT assay (% of cell inhibition)</th>
<th>WBC (T/mm³)</th>
<th>RBC (m/mm³)</th>
<th>Hemoglobin(g/dl)</th>
<th>Lymphocytes(%)</th>
<th>Neutrophils(%)</th>
<th>Eosinophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>HeLa 16.99</td>
<td>5.36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>HeLa 32.35</td>
<td>28.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>HeLa 60.02</td>
<td>49.80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>HeLa 90.54</td>
<td>72.81</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>HeLa 98.17</td>
<td>95.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**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>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (DLA control)</th>
<th>Group III (DLA + MtJs)</th>
<th>Group IV (DLA + 5'fluorouracil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (T/mm³)</td>
<td>8.0±0.06</td>
<td>20.2±0.16</td>
<td>14.17±0.03</td>
<td>10.52±0.21</td>
</tr>
<tr>
<td>RBC (m/mm³)</td>
<td>10.11±0.18</td>
<td>5.48±0.45</td>
<td>9.63±0.28</td>
<td>11.18±0.29</td>
</tr>
<tr>
<td>Hemoglobin(g/dl)</td>
<td>13.59±0.50</td>
<td>7.67±0.47</td>
<td>11.07±0.48</td>
<td>12.8±0.30</td>
</tr>
<tr>
<td>Lymphocytes(%)</td>
<td>65.13±0.53</td>
<td>24.06±0.10</td>
<td>32.76±0.33</td>
<td>60.41±0.41</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>30.5±0.92</td>
<td>7.68±0.48</td>
<td>61.43±0.65</td>
<td>36.61±1.13</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>6.49±0.50</td>
<td>7.6±0.26</td>
<td>6.07±0.23</td>
<td>6.24±0.13</td>
</tr>
</tbody>
</table>

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. Anemia is found frequently occur in cancer patients. The decrease in the levels of Hb, RBC, lymphocytes and increase in the levels of WBC, neutrophils 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 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.

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. The increased activity of liver marker enzymes was brought back to near normal levels by the therapeutic efficacy of the drug.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (µm/l)</th>
<th>ALT (µm/l)</th>
<th>ACP (µm/l)</th>
<th>ALP (µm/l)</th>
<th>LDH (µm/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>81.09±0.21</td>
<td>34.09±0.34</td>
<td>48.09±0.21</td>
<td>77.64±1.38</td>
<td>92.98±1.01</td>
</tr>
<tr>
<td>Group II (DLA Control)</td>
<td>110.09±0.11</td>
<td>74.09±0.21</td>
<td>86.34±0.40</td>
<td>82.58±1.63</td>
<td>125.19±0.77</td>
</tr>
<tr>
<td>Group III (DLA + MtJs)</td>
<td>73.09±0.36</td>
<td>61.09±0.66</td>
<td>52.20±1.16</td>
<td>63.36±1.48</td>
<td>99.22±0.52</td>
</tr>
<tr>
<td>Group IV (DLA + 5'fluorouracil)</td>
<td>84.09±0.21</td>
<td>42.09±0.78</td>
<td>49.69±0.54</td>
<td>72.96±1.38</td>
<td>94.86±0.58</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Groups</th>
<th>5′NT (μm/l)</th>
<th>β-D-Glucuronidase (μm/l)</th>
<th>γ-GT (μm/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>5.19±0.20</td>
<td>34.12±0.20</td>
<td>2.18±0.05</td>
</tr>
<tr>
<td>Group II (DLA control)</td>
<td>9.13±0.14</td>
<td>56.41±0.43</td>
<td>5.54±0.06</td>
</tr>
<tr>
<td>Group III (DLA + MtJs)</td>
<td>7.24±0.12</td>
<td>40.56±0.41</td>
<td>2.76±0.14</td>
</tr>
<tr>
<td>Group IV (DLA+5′fluorouracil)</td>
<td>5.74±0.26</td>
<td>36.81±0.36</td>
<td>3.3±0.09</td>
</tr>
</tbody>
</table>

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 canicular domain, and its liberation into serum indicates damage to the cell and thus injury to the liver24. 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 pachitai25. The present study are in substantiation with26 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 level27.

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 study28 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>64.09±1.26</td>
<td>36.14±1.34</td>
</tr>
<tr>
<td>Group II (DLA Control)</td>
<td>97.09±1.32</td>
<td>64.65±1.21</td>
</tr>
<tr>
<td>Group III (DLA + MtJs)</td>
<td>77.09±1.12</td>
<td>42.33±1.54</td>
</tr>
<tr>
<td>Group IV (DLA+5′fluorouracil)</td>
<td>69.09±1.22</td>
<td>39.09±1.78</td>
</tr>
</tbody>
</table>

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 methionine29. 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.

ACKNOWLEDGEMENT

We, the authors are thankful to our Secretary and Joint secretary of Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India for providing facilities and encouragement.

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