Anticancer activity of *Jasminum angustifolium* Linn against Ehrlich ascites carcinoma cells bearing mice

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Abstract

**Objective:** Present investigations were carried out for evaluation of antitumor and in vitro antioxidant activity of ethanol and aqueous extracts of *Jasminum angustifolium* Linn.

**Methods:** For its antitumor activity, Ehrlich ascites carcinoma (EAC) induced Swiss albino mice were used and were divided into five groups with 6 animals each. The antitumor effect was assessed using viable tumour cell count, packed cell volume, body weight, mean survival time and percentage increase in life span. Apart from that, hematoxylographic and liver enzyme studies were noticed upon the ethanol and aqueous extracts of *Jasminum angustifolium* Linn administered at 500 mg/kg per day for 14 days, after 24 hours of tumor inoculation.

**Results:** Treatment with extracts significantly restored the altered parameters to normal when compared to cancer control group.

**Conclusion:** The results suggest that ethanol extract of *Jasminum angustifolium* Linn possess significant antitumor effects in EAC tumour bearing mice.

Introduction

Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacies due to the development of various side effects. This fostered our attempts to evaluate some plant products against cancer as they are less likely to cause serious side effects [1]. The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search for anticancer agents from plant resources is the selection of plants based on ethnomedical leads [2].

*Jasminum angustifolium* Linn (Oleaceae) is been used alone or in combination with other medicinal plants by the traditional systems of Siddha and Ayurvedic medicine for the treatment of various diseases. It was found from the tribes of south India that the plant has been used for the suppression of tumour like syndrome among their own population [1]. However, hepatoprotective [3] and antitumor activity has been reported against Dalton’s Ascitic Lymphoma [1] using ethanol and aqueous extracts. Hence, in the present study, based on the ethnomedical claims, we investigated the antitumor properties of the ethanol and aqueous extracts of *Jasminum angustifolium* Linn (EEJA and AEJA) against Ehrlich ascites carcinoma (EAC) induced tumor inoculation along with determining its in vitro antioxidant status.

Materials and Methods

**Plant material**

The whole plant of *Jasminum angustifolium* Linn was collected from Kanyakumari District which is the southernmost district of Tamil Nadu. The district lies at 77°E longitudes and 8°N latitudes. This plant was authenticated by Dr. Stephan, Department of Botany, The American College, Madurai, Tamil Nadu, India.

**Animals**

The male Swiss albino mice weighing 25 ± 5 g were selected for this study approved by the institutional animal ethical committee (Reg.No. KMCP/08/3-23). The mice were housed in clean polypropylene cages having 6 mice’s per cage and maintained under temperature controlled room (27 ± 2°C) with a photoperiod of 12 h light and 12 h dark cycle. The animals were fed with commercially available food pellet diet and water ad libitum.

1. Ehrlich ascites carcinoma (EAC) induced Swiss albino mice were used and were divided into five groups with 6 animals each.
2. Anticancer; Ehrlich ascites carcinoma; *Jasminum angustifolium* Linn; Mice
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4. Received: January 10, 2012  
   Accepted: April 18, 2012  
   Published online: June 5, 2012  
   DOI:10.5455/jeim.050612.or.031

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Preparation of the extracts

**Alcoholic extract**: a weighed quantity (500 g) of the air-dried powdered drug was taken and extracted with ethanol (90%) in a soxhlet extractor. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C (yield 38.45% w/w, dry weight basis). The ethanol extract was suspended in distilled water for experimental purpose.

**Aqueous extract**: the marc from the ethanol extract was macerated with chloroform water for 24 h to obtain the aqueous extract, then concentrated under reduced pressure (yield 11.36% w/w, dry weight basis) and dissolved in distilled water for experimental studies.

The ethanol extract (EEJA) and aqueous extracts (AEJA) were prepared and stored in air tight container for phytochemical study and isolation of active constituents.

**Induction of cancer using EAC cells**

Ehrlich ascites carcinoma cells were supplied by Amala Cancer Research Centre, Trissur, Kerala, India. The cells maintained in vivo in Swiss albino mice by intraperitoneal transplantation. The tumour cells were injected intraperitoneally (1 x 10⁶ cells per mouse) to all the mice in the four groups except the first group.

**Acute toxicity studies** [4]

Acute toxicity studies were carried out to study the acute toxic effects and to determine the minimum lethal doses of the drug extracts.

Swiss albino mice of either sex weighing 18-25 g were used for the study. The alcoholic and aqueous extracts were administrated orally to overnight fasted animals at doses of 30 mg/kg, 100 mg/kg, 300 mg/kg, 1000 mg/kg and 3000 mg/kg of body weight. After administration of the extracts, the animals were observed continuously for the first three hours, for any toxic manifestation like increased motor activity, salivation, acute convulsion, coma and death. Thereafter, observations were made at regular intervals for 24 hours. Further the animals were under investigation up to a period of one week.

**Treatment Protocol** [5]

Swiss albino mice were divided into five groups of six each. All the animals in four groups were injected with EAC cells (1 x 10⁶ cells per mouse) intraperitoneally, and the remaining one group is normal control group.

**Group 1** served as the normal control, and **group 2** served as the tumor control; groups 1 and 2 receives normal diet and water.

**Group 3** served as the positive control; treated with injection of 5-fluourouracil (5-FU) at 20 mg/kg body weight, intraperitoneally.

**Group 4 and group 5** served as the treatment controls; treated orally with 500 mg/kg of EEJA and AEJA, respectively.

Drug treatments were given 24 h after the inoculation, once daily for 14 days.

On day 15, all mice from each group were sacrificed; the blood was withdrawn from mice by retroorbital plexus method and subjected to hematological study.

The effect of EEJA and AEJA on tumour growth were examined by studying the following parameters such as viable tumour cell count, packed cell volume, body weight, mean survival time and percentage increase in life span (%ILS).

**Hematological study**

Hematological parameters such as Hemoglobin (Hb) content, WBC count, RBC count, Monocyte (%), Neutrophil (%) and Lymphocyte (%) were measured from the blood. Similarly serum enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total cholestrol (TC) and LDL-cholestrol were measured by using commercially available reagent kits.

**In vitro antioxidant studies** [6]

The antioxidant activities of EEJA and AEJA, and the standards were assessed using scavenging of DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), nitric oxide (NO), hydrogen peroxide (H₂O₂ reducing power), hydroxyl radical, and metal (Fe²⁺) chelating assay.

**Statistical analysis**

The results are expressed as mean ± SD. The evaluation of the data was done using one way ANOVA followed by Newman-Keul’s multiple comparison test; p < 0.05 implied significance.

**Results**

**Acute toxicity studies**

Ethanol and aqueous extracts of *J. angustifolium* Linn was administered separately up to 3000 mg/kg body weight; no toxic manifestation was recorded.

**In vitro antioxidant studies**

The EEJA exhibited potent in vitro antioxidant activity in, DPPH, nitric oxide, hydrogen peroxides, and hydroxyl radical scavenging methods. The IC₅₀ values found for the extract were comparable to those of the standard ascorbic acid used (Table 1). The results indicate that the antioxidant activity...
may be due to phytochemical content of the extracts.

The antioxidant activity is evident with the relatively potent response of the aqueous and ethanol extracts on comparison with ascorbic acid. DPPH level showed a varied response with IC\textsubscript{50} of 977.3 ± 39.4 and 583.3 ± 18 for EEJA and AEJA, respectively; this is roughly 4- and 2-times more than the IC\textsubscript{50} value ascorbic acid.

The hydroxyl radical level of both extracts has nearly the same responses with IC\textsubscript{50} 295.4 ± 29.3 and 271.7 ± 2.03 for EEJA and AEJA, respectively; IC\textsubscript{50} for ascorbic acid is 110.4 ± 1.7. ABTS and Fe\textsuperscript{2+} IC\textsubscript{50} levels of AEJA were similar to those of Ascorbic acid; however, EEJA has a double potency compared to ascorbic acid. NO levels in AEJA and EEJA groups were close and no difference was recorded between them; however, when individually compared with ascorbic acid the values are significant.

**In vivo antitumor studies**

Effect on tumor growth: In vivo antitumor studies

In vivo antitumor studies

Effect on tumor growth: in the EAC tumor control group, the average life span of animals was found to be 18.2 ± 1.3 days (Table 2). Oral administrations of EEJA and AEJA at the doses of 500 mg/kg increase the average life span to 39.4 ± 0.5 and 36.7 ± 0.7 days, respectively. These values were significant (p < 0.001) when compared with EAC control mice. However, the average life span of 5-FU treatment was found to be 37.5 ± 1 days indicating its potent antitumor nature.

The antitumor nature of EEJA and AEJA was indicated by the significant reduction in percent increase in body weights of animals treated with the extracts (p < 0.01 compared to EAC-tumor-bearing mice). It was also supported by the significant reduction in packed cell volume and viable tumor cell count in both the extracts treatment when compared to the EAC tumor control. Fourteen days of extracts treatment restored the %ILS values more or less similar to normal when compared with EAC bearing mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>EAC control</th>
<th>EAC + 5FU (20 mg/kg)</th>
<th>EAC + EEJA (500 mg/kg)</th>
<th>EAC + AEJA (500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>22.8 ± 1.3</td>
<td>28.9 ± 4.5</td>
<td>23.1 ± 0.8</td>
<td>23.4 ± 1.2***</td>
<td>22.3 ± 2.5***</td>
</tr>
<tr>
<td><strong>Mean survival (days)</strong></td>
<td>40</td>
<td>18.2 ± 1.3</td>
<td>37.5 ± 1.0</td>
<td>39.4 ± 0.5***</td>
<td>36.7 ± 0.7***</td>
</tr>
<tr>
<td><strong>ILS (%)</strong></td>
<td>100</td>
<td>-</td>
<td>98.7</td>
<td>98.4***</td>
<td>95.2***</td>
</tr>
<tr>
<td><strong>Packed cell volume (ml)</strong></td>
<td>-</td>
<td>1.87 ± 0.8</td>
<td>0.2 ± 0.51</td>
<td>0.2 ± 0.21***</td>
<td>0.3 ± 0.52**</td>
</tr>
<tr>
<td><strong>Viable tumor cell count</strong></td>
<td>-</td>
<td>15.6 ± 1.4</td>
<td>4.3 ± 0.5</td>
<td>5.6 ± 2.1***</td>
<td>4.8 ± 0.9***</td>
</tr>
</tbody>
</table>

**p < 0.01, ***p < 0.001 compared with EAC control group**
p < 0.01, ***p < 0.001 compared with EAC control group

**Table 4. Effect EEJA and AEJA on hematological parameters of EAC bearing mice**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>EAC control</th>
<th>EAC + 5-FU (20 mg/kg)</th>
<th>EAC + EEJA (500 mg/kg)</th>
<th>EAC + AEJA (500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.3 ± 0.4</td>
<td>9.6 ± 0.5</td>
<td>12.1 ± 0.2</td>
<td>12.2 ± 1.2***</td>
<td>11.5 ± 0.7**</td>
</tr>
<tr>
<td>RBC (x 10⁶ mm)</td>
<td>5.6 ± 0.2</td>
<td>3.2 ± 0.7</td>
<td>5.1 ± 0.5</td>
<td>4.9 ± 0.6***</td>
<td>5.1 ± 0.3***</td>
</tr>
<tr>
<td>WBC (x 10³ mm)</td>
<td>4.8 ± 0.3</td>
<td>12.7 ± 0.8</td>
<td>4.3 ± 0.03</td>
<td>4.1 ± 0.3**</td>
<td>4 ± 1.2***</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>2.1 ± 1.2</td>
<td>1.6 ± 0.02</td>
<td>1.9 ± 0.01</td>
<td>1.9 ± 0.4***</td>
<td>1.8 ± 0.9***</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>15.7 ± 2.6</td>
<td>58.9 ± 2.5</td>
<td>14.8 ± 0.4</td>
<td>15.4 ± 1.3**</td>
<td>16.7 ± 1.1**</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>83.6 ± 2.4</td>
<td>32.4 ± 3.4</td>
<td>79.5 ± 1.2</td>
<td>78.4 ± 2.1**</td>
<td>80.2 ± 1.5**</td>
</tr>
</tbody>
</table>

The inoculation of EAC cells caused significant (p < 0.01) decreases in ALP, TC and LDL levels which were brought back to normal upon the treatment with both extracts for 14 days. Similarly, significant (p < 0.01) increases in the levels of serum AST and ALT of tumor control animals were recorded when compared to the normal group. The treatment with EEJA and AEJA reversed these changes towards the normal levels (Table 4). Most of the values were found to be significant. The treatment with standard 5-FU also gave similar results.

**Discussion**

Plants have served as a good source of antitumor agents. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. A large number of plants possessing anticancer properties have been documented [7-9]. Whole plant of *Jasminum angustifolium* Linn was traditionally used in the treatment of tumors-like syndrome. The present investigation was carried out to evaluate the antitumor activity of the EEJA and AEJA in EAC tumor bearing mice.

The reliable criteria for judging the value of an anticancer drug is the prolongation of the life span of animals. In EAC-tumor-bearing mice, a regular rapid increase in ascitic tumor volume was observed [7]. The EAC bearing mice orally administered EEJA and AEJA at 500 mg/kg body weight showed significant change in the average life span compared to animals of the tumor control group. However, the percent increase in body weight, packed tumor cell volume, and number of viable tumor cells were found to be significantly less than the tumor control animals, indicating the anticancer nature of the extract. These results could indicate either a direct cytotoxic effect of EEJA on tumor cells as evidenced by the *in vitro* studies or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition. Hence, the observed antitumor nature of EEJA may be due to the cytotoxic properties.

The reversal of Hb content, RBC and differential count of WBC by the EEJA and AEJA treatment towards the values of the normal group clearly indicates that both extracts possessed protective action on the hemopoietic system. However, the elevation of WBC levels may be due to its adverse effect on the hemopoietic system [7].

The significant reversal of liver enzyme level in serum changes towards the normal by EEJA and AEJA treatment in most of the cases demonstrated the potent hepatoprotective and antioxidant nature of EEJA and AEJA. The antioxidant nature of EEJA and AEJA was also evident by the *in vitro* studies. Plants with high flavonoid content are known to possess strong antioxidant properties.
The observed antioxidant activity may be due to the flavanoid content of the extract [1]. Hepatocellular necrosis leads to high levels of AST and ALT, which are released from liver into the blood. ALP activity, on the other hand, is related to the functioning of hepatocytes. Increase in its activity is due to increased synthesis in the presence of increased biliary pressure [11]. Reduction in the levels of these towards the respective normal values in liver tissues is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by tumor inoculation. EEJA was found to be nontoxic and didn’t cause any death of mice up to 3 g/kg body weight, indicating the safety of the treatment. The hepatoprotective effects of chloroform and ethanol extracts of Jasminum angustifolium Linn against CCl4-induced liver damage were previously reported [3]. Similar results were also obtained in the present study. EEJA may cause firstly an antitumor effect and then influence biochemical parameters. The preliminary phytochemical studies of EEJA indicated the presence of several triterpenoids, saponins, flavanoids, tannins, glycosides, and so on. The observed antitumor, hepatoprotective and antioxidant activities may be due to the presence of any of these compounds in EEJA. In conclusion, the ethanol extract of Jasminum angustifolium Linn was effective in inhibiting the tumor growth in ascitic and solid tumor models. The biochemical and histological studies supported its antioxidant and hepatoprotective properties. The plant merits further investigation in an ascitic model at low doses and to elucidate its mechanism of action and isolation of its active constituents.

References