Antitumor Activity of Flavonoids against Ehrlich Ascites Carcinoma-induced Mice

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ABSTRACT

Flavonoids are phenolic compounds widely present in plants and foods of plant origin. Methylhesperidine and chrysin were evaluated for in vivo antitumor activity against Ehrlich ascites carcinoma (EAC)-bearing Swiss albino mice. The present study deals with the effect of methyl hesperidine and chrysin on the growth of transplantable murine tumor, life span of EAC-bearing hosts, hematological profile, and biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum creatinine levels. Test compounds were administered at the dose of 20 mg/kg body weight per day for 14 days after 24 h of tumor inoculation. After the last dose and 18 h fasting, the blood samples were collected from tail vein of all the mice. Both flavonoids produced a significant ($P < 0.05$) decrease in tumor volume, packed cell volume and viable tumor cell count, and they prolonged the life span of EAC-bearing mice. Compared to control mice, hematological profile is more or less normal levels in flavonoids-treated mice. Selected flavonoids significantly ($P < 0.05$) decreased the levels of serum creatinine levels. The results indicate that methylhesperidine and chrysin exhibited significant in vivo antitumor activity in EAC-bearing mice.

Keywords: EAC mice, tumor growth, mean survival time, anti tumor activity
Abbreviations: CAT, catalase; DMSO, dimethyl sulfoxide; EAC, Ehrlich ascites carcinoma; ILS, increased life span; MST, mean survival time; RBC, red blood cell; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SOD, super oxide dismutase; WBC, white blood cell

INTRODUCTION

India is a rich source of many medicinal plant derived natural products such as flavonoids, alkaloids, terpenes (Stevenson and Lowe 2009; Hounsome et al. 2010), which have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxicity and cancer chemo-protective properties (Roja and Heble 1994). Flavonoids are nearly ubiquitous in plants. They are rich in seeds, citrus fruits, olive oil, tea, and red wine. Flavonoids, like methylhesperidine and chrysin, have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors, precursors of toxic substances, and pigments and light screens (Carroll et al. 1998). They inhibit many enzymes like kinases, lipoxigenases and cyclooxygenases, phospholipase C, cyclic nucleotide phosphodiesterase, reverse transcriptase, RNA and DNA polymerases (Middleton et al. 2000). Flavonoids have been reported to possess a number of biological activities and are well known for their antioxidant properties (Rajnarayana et al. 2001). There are a number of reports on different natural products derived from plants indicate that they exert multiple biological effects due to their anti oxidant and free radical scavenging abilities of the flavonoids. These natural compounds were reported to produce protective effects against tumors, heart disease and different diseases (DeFeudis et al. 2003). Based on the above facts the present work has been carried out to evaluate the in vivo antitumor activity of methylhesperidine and chrysin against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.

MATERIALS AND METHODS

Flavonoids

The flavonoids methylhesperidine and chrysin were procured from Sigma-Aldrich Chemicals Ltd. (Germany). The stock solutions (100 mg/ml) of flavonoids were prepared using 1% DMSO and further diluted with water to obtain the required solutions (20 mg/kg) which were to be administered in the study. 5-fluorouracil (99% pure, a kind gift from Vimta laboratories ltd, Hyderabad, India) was used as a standard drug which was procured from the local market.

Animals

The study was carried out using Swiss albino male mice weighing 20 ± 2 g. They were obtained from the National Institute of Nutrition, Hyderabad, and Aandhra Pradesh. The mice were grouped peritonial (i.p.) inoculation of 2 × 10⁶ cells administered to other mice at the Pharmacology Research Lab, University College of

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**Antitumor activity using EAC mice**

Swiss albino mice were more than divided into 4 groups (n=8). All the groups were injected i.p. with EAC cells (0.3 ml of 2 × 10⁶ cells/mouse). This was day zero. The EAC-control group [group 1] animals did not receive any drug. Standard drug 5-flourouracil (Kavimani and Manisenthil kumar 2000), methylhesperidine and chrysin (20 mg/kg per day) were administered i.p. to the mice in groups 2, 3 and 4 respectively for 14 days. All mice were weighed on the day of tumor inoculation and every day before treatment. After a last dose and 18-h fasting, blood samples were collected on the day of tumor inoculation and every day before treatment. The tumor growth response was measured by studying MST and ILS. Most tumor-induced animals died after 4 weeks. So we extended our study up to 5 weeks or 35 days. The duration of the study was thus 35 days. Results were expressed as the mean ± standard deviation. Statistical evaluation was done by ANOVA followed by Newman-Keul’s test and the difference was considered statistically significant at P < 0.05. Graphpad prism software (Version 7, Lajolla, USA) was used for statistical analysis.

**Tumor growth response**

The tumor growth response of flavonoids was assessed by a change in body weight, ascites tumor volume, packed cell volume, viable and non viable tumor cell count, MST and %ILS. MST of each group was monitored by recording the mortality daily for 5 weeks and %ILS was calculated using following equation (Mazumder et al. 1997).

\[
\text{MST} = \frac{(\text{Day of first death} + \text{Day of last death})}{2}
\]

\[
\text{ILS} (%) = \left[\frac{(\text{Mean survival time of treated group} / \text{mean survival time of control group}) - 1}\right] \times 100
\]

**Hematological profile**

The blood samples were send to the physiology lab for the estimation of hemoglobin content, RBC and WBC count and Differential leukocyte count using Neubauer slide (Wintrobe et al. 1958; D’Armour et al. 1965).

**Biochemical parameters**

After the collection of blood samples SGOT, SGPT, serum creatinine levels were estimated in the biochemistry laboratory using standard SGOT, SGPT, serum creatinine kits (Marketed products of Dr. Reddy’s Laboratories Ltd., Hyderabad, India).

**RESULTS**

The results of the present study indicate that methylhesperidine and chrysin showed significant antitumor activity in EAC-bearing mice. The effect of flavonoids at a dose of 20 mg/kg on MST, %ILS, tumor volume, packed cell volume, and tumor cell count (viable and non viable) are shown in Table 1.

**Effect on mean survival time (MST)**

In the EAC control group, the MST was 14.5 ± 0.16 days, while it increased to 21.5 ± 2.27 days with methyl hesperidine, where as standard drug (5-flourouracil)-treated group had MST of 29 ± 3.47 days. No change was observed in MST with chrysin when compare with control mice (Table 1).

**Effect on tumor growth**

Treatment with methyl hesperidine and chrysin significantly (P < 0.01) reduced the packed cell volume and the viable tumor cell count as compared to that of the EAC control group. Furthermore non viable tumor cell count was significantly (P < 0.05) increased by methyl hesperidine (Table 1).

**Effect on hematological parameters**

Hematological parameters of flavonoids treated mice on day 14 showed some changes when compared with the EAC mice (Table 2). RBC, neutrophil and platelet count increased with methyl hesperidine. The differential count of WBC showed that the neutrophil, platelet count increased while the RBC and hemoglobin decreased.

**Effect on SGOT, SGPT, and Serum creatinine**

Some changes found in the SGOT, SGPT and serum creatinine levels of flavonoids treated mice when compared with EAC mice (Table 3). Treatment with methyl hesperidine significantly increased the SGOT levels. The serum creatinine levels were found to decrease significantly when compared with the EAC-control group. There are no significant changes were observed in SGPT levels.

**Table 1** Effect of the flavonoids on mean survival time, %ILS, tumor volume, packed cell volume and viable and non viable tumor cell count of EAC-bearing mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EAC control (2 × 10⁶ cells/ml/mouse)</th>
<th>Std (5-Fu) (20 mg/kg + EAC)</th>
<th>Methyl hesperidine (20 mg/kg + EAC)</th>
<th>Chrysin (20 mg/kg + EAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>33.61 ± 1.44</td>
<td>27.78 ± 1.41</td>
<td>30.83 ± 2.17</td>
<td>32.63 ± 0.44</td>
</tr>
<tr>
<td>Mean survival time (days)</td>
<td>14.5 ± 0.16</td>
<td>29 ± 3.47 ***</td>
<td>21.5 ± 2.27 **</td>
<td>14.5 ± 0.12</td>
</tr>
<tr>
<td>Increased life span (%)</td>
<td>--</td>
<td>100</td>
<td>48.3</td>
<td>0</td>
</tr>
<tr>
<td>Tumor Volume (ml)</td>
<td>13 ± 0.70</td>
<td>3.6 ± 0.23 ***</td>
<td>10 ± 0.70</td>
<td>10.38 ± 0.68</td>
</tr>
<tr>
<td>Packed cell volume (ml)</td>
<td>2.63 ± 0.23</td>
<td>0.13 ± 0.12 ***</td>
<td>1.5 ± 0.20 ***</td>
<td>1.08 ± 0.14***</td>
</tr>
<tr>
<td>Viable tumor cells (X 10⁷ cells / ml)</td>
<td>6.64 ± 0.02</td>
<td>0.11 ± 0.11 ***</td>
<td>6.16 ± 0.02**</td>
<td>5.44 ± 0.18***</td>
</tr>
<tr>
<td>Non via tumor cell (X 10⁷ cells / ml)</td>
<td>0.06 ± 0.018</td>
<td>0.02 ± 0.022</td>
<td>0.12 ± 0.11**</td>
<td>0.11 ± 0.004</td>
</tr>
</tbody>
</table>

*Data are reported as mean ± SEM (n=8). ***P < 0.001, EAC standard group compared with control group. **P < 0.05 flavonoids treated group compared with EAC control group.

**Table 2** Effect of the flavonoids on hematological parameters of EAC-bearing mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EAC control (2 × 10⁶ cells/ml/mouse)</th>
<th>Std (5-Fu) (20 mg/kg + EAC)</th>
<th>Methyl hesperidine (20 mg/kg + EAC)</th>
<th>Chrysin (20 mg/kg + EAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g %)</td>
<td>13.87 ± 1.14</td>
<td>12.62 ± 0.88</td>
<td>12 ± 1.07</td>
<td>13.7 ± 1.31</td>
</tr>
<tr>
<td>RBC (ml/cm)</td>
<td>7.39 ± 0.79</td>
<td>7.38 ± 0.61</td>
<td>7.91 ± 0.73</td>
<td>7.70 ± 1.22</td>
</tr>
<tr>
<td>WBC (T/cm)</td>
<td>30.47 ± 13.29</td>
<td>23.55 ± 4.92</td>
<td>40.55 ± 16.71</td>
<td>31.31 ± 2.05</td>
</tr>
<tr>
<td>Nutrophils (%)</td>
<td>38 ± 3.1</td>
<td>31.5 ± 7.12</td>
<td>48.25 ± 11.59</td>
<td>31.25 ± 4.49</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>62 ± 3.1</td>
<td>68.5 ± 7.12</td>
<td>51.75 ± 11.59</td>
<td>68.75 ± 4.49</td>
</tr>
<tr>
<td>Platelet count X 10⁵/ml</td>
<td>12.19 ± 3.16</td>
<td>9.70 ± 1.64</td>
<td>31.84 ± 19.49</td>
<td>12.75 ± 1.51</td>
</tr>
</tbody>
</table>

*Data are reported as mean ± SEM (n=8).
DISCUSSION

Many components isolated from plants have been approved to be potent anti-cancer agents. Plant-derived polyphenolic compounds are promising nutraceuticals for control of various disorders and cancer. These compounds may be the future developing anticancer drugs with no side effect and low cost for people all around the world. The much lower risk of colon, prostate and breast cancers in Asians, who consume more vegetables, fruits and tea than populations in the western hemisphere, raises the role of flavonoids components as protective factors against carcinogenesis (Rand et al. 2009).

The observations of the past studies were saying that the methanol extract of Caesalpinia bonducuella leaves treated animals significantly inhibited the tumor volume, packed cell volume, tumor cell count, and brought back to normal levels. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as antioxidant enzymes such as SOD and CAT in tumor-bearing mice to near normal levels. The pharmacological activity of MeCB is due to the presence of flavonoids (Gupta et al. 2004).

In another previous study the effect of various natural flavonoids, cinnamic acid derivatives, and a series of synthetic flavonoids on cell proliferation was evaluated in vitro in a panel of established human and murine tumor cell lines. The in vitro activity of different natural flavonoids, cinnamic acid derivatives, and a series of synthetic flavonoids in established cancer cell lines was studied. Analysis of each group of compounds indicated that apigenin, caffeic acid n-buty1 ester, and 2-nitroflavone possess the most potent antiproliferative activities (Mariano et al. 2006). LYG-202 significantly decreases tumor growth in mice inoculated with S180 sarcoma cells, compared with the control group. Meanwhile, the viabilities of various kinds of tumor cells were inhibited by LYG-202 with IC_{50} values in the range of 4.80 to 27.70 μM (Zeng et al. 2009).

In other study, the polyphenolic extract (PPE) of leaves of Ichnocarpus frutescens was evaluated for antitumor activity in vivo. A murine Ehrlich ascites carcinoma (EAC) model was used to assess PPE antitumor activity in vivo. Results of in vivo study showed a significant decrease in tumor volume, viable tumor cell count and a significant increase in the mean survival time in the PPE treated group compared to untreated one: the life span of PPE treated animals increased by 53.41% (50 mg PPE/kg) and 73.95% (100 mg PPE/kg) (Kumarapppan and Subhash 2007).

Santoshkumar et al. (2007) reported that the methanolic extract of H. hookerianum Wight and Arnott stem (MEHH) exhibited potent in vitro cytotoxic activity against various cancer cell lines. The results indicate that administration of the extract not only increased the survival of animals with ascites tumor, decreased the body weight induced by the tumor burden, and reduced packed cell volume and viable tissue cell count, but also altered many hematological parameters changed during tumor progression, indicating the potent antitumor nature of the extract. Among the three doses tested, the 200 mg/kg body weight dose was found to be the most potent.

Flavones, flavonols, isoprenoid-substituted flavonoids, benzophenones, xanthones, anthraquinones, phenylbuta-zones, stilbene glucosides, coumarin derivatives, hydroxyketones, stylylchromones, dihydroisoxazole and isoxazole derivatives showed low to moderate tumor-spe-

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