Antitumor Activity and in vivo Antioxidant Status of Mucuna pruriens (Fabaceae) Seeds against Ehrlich Ascites Carcinoma in Swiss Albino Mice

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Received May 1, 2005; Revised June 27, 2005; Accepted June 28, 2005

This paper is available online at http://ijpt.iums.ac.ir

ABSTRACT
The aim of the present study is to evaluate the antitumor effect and antioxidant role of Mucuna pruriens (Family: Fabaceae) against EAC bearing Swiss albino mice. The effect of methanol extract of Mucuna pruriens (MEMP) on tumor growth and host's survival time was studied by the following parameters: tumor volume, packed cell volume, viable and non-viable cell count and life span of the host. MEMP was administered at a 125 and 250mg/kg b.w. once a day for 14 days, after 24 h of tumor inoculation. Decrease in tumor volume, packed cell volume, and viable cell count were observed in MEMP treated animals when compared to EAC treated animals. Treatment with MEMP at a dose of 125 and 250mg/kg increased the mean survival time to 29.5 ± 0.55 and 34 ± 0.2 days respectively. The extract also decreased the body weight of the EAC tumor bearing mice. Hematological studies reveal that the Hb content was decreased in EAC treated mouse, whereas restoration to near normal levels was observed in extract treated animals. There was a significant decrease in RBC count and increase in WBC counts in extract treated animals when compared to EAC treated animals. The study was also extended to estimate the liver biochemical parameters such as LPO, GSH, and antioxidant enzymes like SOD, CAT etc. Treatment with MEMP decreased the levels of lipid peroxidation and increased the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The results suggest that the methanol extract of Mucuna pruriens seeds exhibits significant antitumor and antioxidant effects in EAC bearing mice.

Keywords: Mucuna pruriens seeds, Ehrlich ascites carcinoma, Anticancer activity, in vivo antioxidant activity

Oxygen free radicals are formed in tissue cells by many endogenous and exogenous causes such as metabolism, chemicals, and ionizing radiation [1]. Oxygen free radicals may attack lipids and DNA giving rise to a large number of damaged products [2]. Iron is known to be involved in the generation of reactive oxygen species (ROS) and in the formation of highly toxic hydroxyl radicals from other active oxygen species such as hydrogen peroxide [2-4]. The enhanced generation of ROS in vivo could be quite deleterious, since they are involved in mutagenesis, apoptosis, ageing, and carcinogenesis [4]. Free radicals also cause DNA strand breaks and chromosome deletions and rearrangements. Further, activated oxygen species most likely play an important role in tumor promotion and progression [5]. For these reasons, the search for antioxidants as cancer chemopreventive agents is a continued process. Various epidemiological, experimental, and metabolic studies have show that nutrition plays an important causative role in the initiation, promotion, and progression stages of several types of human cancers [6, 7]. In addition to substances that pose cancer risk, the human diet also contains vegetables, fruits, and beverages, which not only provide essential vitamins and minerals, but include important chemopreventive agents capable of protecting against some forms of human cancer [6-8]. Many cancer chemopreventive agents possess antioxidant potential [8]. Such chemopreventive agents are known as anticarcinogens, and ideally they should be non-toxic.

Mucuna pruriens (Fabaceae) is an established herbal drug in the Ayurvedic system of medicine used for the management of male infertility, nervous disorders and also as an aphrodisiac [9]. Its English name is cowhage and in Hindi it is known as Kinanchh or Kewach. It is an annual herbaceous twinning plant, found all over India, especially in the tropics [10]. Eighty percent of the total alcohol extract of the seeds shows the presence of 5-indolic compounds, especially tryptamine and 5-
Table 1. Antitumor activity of methanol extract of Mucuna pruriens (MEMP) seeds on tumor volume, packed cell volume, cell count, median survival time (MST) and increase in life (%ILS)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Tumor Volume (mL)</th>
<th>Packed Cell Volume (mL)</th>
<th>Tumor cell count ($2 \times 10^6$ cells/mouse)</th>
<th>Median survival time (days)</th>
<th>% increased life span (%ILS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (0.9% NaCl w/v)</td>
<td>-</td>
<td>3.1 ± 0.20</td>
<td>0.6 ± 0.11</td>
<td>8.9 ± 0.25</td>
<td>22.1 ± 0.05</td>
<td>-</td>
</tr>
<tr>
<td>EAC (2 × 10^6 cells/mouse)</td>
<td>-</td>
<td>2.2 ± 0.11^*</td>
<td>0.6 ± 0.11</td>
<td>4.1 ± 0.22</td>
<td>29.5 ± 0.35^*</td>
<td>33.48</td>
</tr>
<tr>
<td>EAC + MEMP</td>
<td>125</td>
<td>2.2 ± 0.17^*</td>
<td>0.6 ± 0.11</td>
<td>2.3 ± 0.25</td>
<td>34 ± 0.2^*</td>
<td>53.84</td>
</tr>
<tr>
<td>EAC + MEMP + 5-FU</td>
<td>250</td>
<td>1.5 ± 0.15^*</td>
<td>0.3 ± 0.14</td>
<td>0.9 ± 0.13</td>
<td>37.7 ± 0.10^*</td>
<td>70.58</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n = 8.

*p < 0.05 Statistically significant when compared with EAC control group.
saline solution). Food and water were withheld 18 h before sacrificing the animals. On day 15, half of the animals (n = 4) in each cage were killed and the remaining animals were kept to observe the life span of the hosts. 5-Fluorouracil (5-FU) at a dose level of 20 mg/kg body weight was used as standard.

The anti-tumor activity of the methanol extract of Mucuna pruriens (MEMP) was measured in EAC animals with respect to the following parameters:

- **Tumor volume.** The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.
- **Tumor cell count.** The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.
- **Viable/non-viable tumor cell count.** The cells were then stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and nonviable cells were counted.

\[
\text{Cell count} = \frac{\text{No of cells} \times \text{Dilution}}{\text{Area} \times \text{Thickness of liquid film}}
\]

- **Percentage increase life span (% ILS).** The effect of MEMP on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and percentage increase in life span (%ILS) was calculated.

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

\[
\text{Mean survival} = \left( \frac{\text{Day of 1st death} + \text{Day of last death}}{2} \right)
\]

- **Body weight.** Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.

**Acute Toxicity Study**

Albino male mice of 10 animals per group and weighing 20-25 g were administered graded doses (250-4000 mg/kg body weight, i.p.) of the methanol extract of Mucuna pruriens (MEMP). After administration of the MEMP the mice were observed for toxic effects after 48 h treatment. The toxicological effects were observed in terms of mortality and expressed as LD50. The number of animals dying during the period was noted [20]. The LD50 of the extract was calculated by the method of Litchfield and Wilcoxon [21].

**Hematological Parameters**

At the end of the experimental period, all mice were killed the next day after an overnight fast by decapitation. Blood was collected from freely flowing tail vein and used for the estimation Hemoglobin (Hb) content, red blood cell count (RBC) [22] and white blood cell count (WBC) [23]. WBC differential count was carried out from Leishman stained blood smears [24].

**Estimation of in vivo Antioxidants**

After collecting the blood samples, the mice were killed by cervical dislocation. The liver was excised, rinsed in ice-cold normal saline solution followed by cold 0.15 M Tris-HCl (pH 7.4), blotted dried and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-HCl buffer and was used for the estimation of lipid peroxidation (LPO) and reduced glutathione (GSH). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD), catalase (CAT) and total protein.

**Estimation of Lipid Peroxidation (LPO)**

The levels of Thiobarbituric acid reactive substances (TBARS) in the liver was measured by the method of Ohkawa et al. (1979) [25] as a marker for lipid peroxidation. A mixture of 0.4 mL of 10% liver homogenate, 1.5 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of 20% acetate buffer (pH 3.5) and 1.5 mL of 0.8% TBA solution was heated at 95°C for 1 h. After cooling, 5.0 mL of n-butanol-pyridine (15:1) was added, and the absorbance of the n-butanol-pyridine layer was measured at 532 nm.

**Estimation of Reduced GSH**

The tissue GSH was determined by the method of Beutler and Kelly [26]. Virtually all the non-protein sulfhydryl groups of tissues are in the form of reduced GSH. 0.2 mL of tissue homogenate was mixed with

<p>| Table 2. Antitumor activity of methanol extract of Mucuna pruriens (MEMP) seeds on hematological parameters. |
|--------------------------------------------------|-------------------------------------------------|--|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hb Content</th>
<th>RBC (cells/mL x 10^6)</th>
<th>WBC (cells/mL x 10^6)</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (0.9% NaCl w/v.)</td>
<td>13.5 ± 0.35</td>
<td>5.3 ± 0.12</td>
<td>7.8 ± 0.40</td>
<td>68 ± 1.3</td>
<td>27 ± 1.1</td>
<td>2.2 ± 1.5</td>
</tr>
<tr>
<td>EAC (2 x 10^6 cells/mouse)</td>
<td>10 ± 0.22</td>
<td>3.9 ± 0.80</td>
<td>15.4 ± 0.21</td>
<td>35 ± 1.5</td>
<td>65 ± 1.4</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>EAC + MEMP (125mg/kg)</td>
<td>11.4 ± 0.55</td>
<td>4.1 ± 0.35</td>
<td>12.1 ± 0.33</td>
<td>45 ± 1.1</td>
<td>52 ± 1.6</td>
<td>1.6 ± 1.2</td>
</tr>
<tr>
<td>EAC + MEMP (250mg/kg)</td>
<td>12.6 ± 0.50</td>
<td>4.6 ± 0.66</td>
<td>9.7 ± 0.08</td>
<td>54 ± 1.6</td>
<td>35 ± 1.0</td>
<td>1.7 ± 1.5</td>
</tr>
<tr>
<td>EAC + 5-FU (20mg/kg)</td>
<td>13.1 ± 0.37</td>
<td>4.9 ± 0.42</td>
<td>8.5 ± 0.04</td>
<td>63 ± 2</td>
<td>30 ± 0.1</td>
<td>1.7 ± 0.8</td>
</tr>
</tbody>
</table>

Values are mean S.E.M., n = 8.

*p < 0.01 Statistically significant when compared with normal group.

*p < 0.05 Statistically significant when compared with EAC control group.
Antitumor and in vivo antioxidant activity of *Mucuna pruriens*

1.8 mL of EDTA solution. To this 3.0 mL precipitating reagent (after precipitating proteins with TCA) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0 mL of the filtrate, 4.0 mL of 0.3-M disodium hydrogen phosphate solution and 1.0 mL of DTNB (5, 5-dithio bis 2-nitro benzoic acid) reagent were added and the absorbance read at 412 nm.

**Assay of SOD**

The activity of SOD in tissue was assayed by the method of Kakkar [27]. The assay mixture contained 1.2 mL sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1 mL phenazine methosulphate (186 mmol/L), 0.3 mL nitroblue tetrazolium (300 mmol/L), 0.2 mL NADH (780 mmol/L) and diluted enzyme preparation and water in a total volume of 3 mL. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0 mL of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 mL n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol.

**Assay of CAT**

Catalase was assayed according to the method of Maehly and Chance [28]. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 4°C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H$_2$O$_2$ and the enzyme extract. The specific activity of catalase is expressed in terms of units/mg protein. A unit is defined as the velocity constant per second.

**Estimation of Total Proteins**

The protein content of tissue homogenates was measured by the method of Lowry [29]. 0.5 mL of tissue homogenate was mixed with 0.5 mL of 10% TCA and centrifuged for 10 min. The precipitate obtained was dissolved in 1.0 mL of 0.1 N NaOH. From this an aliquot was taken for protein estimation. 0.1 mL of aliquot was mixed with 5.0 mL of alkaline copper reagent and allowed to stand at room temperature for 10 min. 0.5 mL of Folin’s phenol reagent was added and the blue color developed was read after 20 min at 640 nm.

**Statistical Analysis**

The experimental results were expressed as mean ± S.E.M. Data was assessed by ANOVA followed by the Students t-test, p-value < 0.05 was considered as statistically significant.

**RESULTS**

**Acute Toxicity**

LD$_{50}$ value of the methanol extract of *Mucuna pruriens* (MEMP) was evaluated in Swiss albino mice. The drug was administered intraperitoneally. As shown in the Fig 1, the LD$_{50}$ value of the MEMP was found to be 1333.52 mg/kg body weight.

Tumor growth response of MEMP on tumor and packed cell volume, viable and non-viable cell count and % increase in life span

Antitumor activity of MEMP against EAC tumor bearing mice was assessed by the parameters such as tumor volume, packed cell volume, cell count (viable and non-viable), mean survival time and % increase of life span. The results are shown in Table 1. The tumor volume, packed cell volume and viable cell count were found to be significantly (p < 0.01) increased and non-viable cell count was significantly (p < 0.01) low in EAC control animals when compared with normal control animals. Administration of MEMP at the dose of 125 and 250 mg/kg significantly (p < 0.05) decreased the tumor volume, packed cell volume and viable cell count. Non-viable cell count was significantly (p < 0.05) higher in MEMP treated animals when compared with EAC control animals. Furthermore, the median survival time was increased to 29.5 ± 0.55 (%ILS = 33.48) and 34 ± 0.2 (%ILS = 53.84) on administration of MEMP at 125 and 250 mg/kg respectively. Finally, the change in body weights of the animals suggests the tumor growth
inhibiting property of MEMP. All these results clearly indicate that the MEMP has a remarkable capacity to inhibit the growth of solid tumor induced by EAC cell line in a dose-dependent manner in experimental animals.

**Hematological Parameters**

Hematological parameters (Table 2) of tumor bearing mice on day 14 were found to be significantly altered compared to the normal group. The total WBC count was found to be increased with a reduction of Hb content of RBC. The total number of RBC showed a modest change. In differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval on MEMP 250 mg/kg treatment restored all the altered hematological parameters to almost near normal. MEMP 125 mg/kg treatment also recovered these altered depleted parameters towards normal though MEMP 250 mg treatment was found to be more effective.

**Antitumor Effect of MEMP on TBARS Levels**

ROS formed in cancer tissues results in lipid peroxidation and subsequently to increase in malondialdehyde (MDA) level. Fig 3 depicts the levels of TBARS in liver tissue of experimental animals. In the present study, the levels of MDA were significantly \( p < 0.01 \) increased in EAC control animals when compared with normal control animals. After treatment with MEMP at 125 mg and 250 mg/kg and 5-FU at 20 mg/kg significantly \( p < 0.05 \) reduced the MDA levels when compared with EAC control animals. Results were expressed as nmoles MDA/mg proteins/mL.

**Antitumor Effect of MEMP on Reduced GSH Levels**

Fig 4 illustrates the levels of reduced GSH in experimental groups. The levels of reduced GSH were significantly \( p < 0.01 \) decreased in EAC control group when compared with normal control group. The levels of reduced GSH were found to be increased on administration of MEMP at 125 mg and 250 mg/kg and 5-FU at 20 mg/kg when compared with EAC control group.

**Antitumor Effect of MEMP on SOD Levels**

Fig 5 shows the activity of SOD in liver tissue of experimental groups. There was a significant \( p < 0.01 \) reduction in the levels of liver SOD in EAC control animals. Administration of MEMP at 125 mg and 250 mg/kg and 5-FU 20 mg/kg increased the levels significantly \( p < 0.05 \) as compared with EAC control animals.

**Antitumor Effect of MEMP on CAT Levels**

Fig 6 demonstrates the activity of catalase (CAT) in liver tissue of experimental mice. A significant \( p < 0.01 \) reduction was observed in the activity of catalase in EAC control groups. Feeding with MEMP at 125 mg and 250 mg/kg and 5-FU 20 mg/kg increased the levels significantly \( p < 0.05 \) as compared with EAC control mice.

**DISCUSSION**

The present investigation was carried out to evaluate the antitumor activity and antioxidant status of methanol extract of *Mucuna pruriens* (MEMP) in EAC tumor bearing mice. The MEMP treated animals at the doses of 125 and 250 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count, and brought back the hematological parameters to more or less 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.

In EAC tumor bearing mice, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [30]. Treatment with MEMP inhibited the tumor volume, viable tumor cell count, and increased the life span.
of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals [31]. It may be concluded that MEMP by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC-bearing mice. Thus, MEMP has antitumor activity against EAC bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [32, 33]. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [34]. Treatment with MEMP brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicates that MEMP possess protective action on the hemopoietic system.

Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals [35], which is accepted as an indicator of lipid peroxidation [36]. MDA, the end product of lipid peroxidation, was reported to be higher in cancer tissues than in non-diseased organ [37]. Our findings indicate that TBARS levels in the tested cancerous tissues are higher than those in normal tissues. These results are in agreement with the published data [38, 39]. This emphasizes the reduction in free radical yield and the subsequent decrease in harm and damage to the cell membrane and decrease in MDA production.

Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage to macromolecules, for example, lipid peroxidation in vivo [40]. It was also reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially the liver, even when the site of the tumor does not interfere directly with organ function [41]. In our study, GSH levels in experimental mice were found to be significantly lower than that in the EAC control mice.

SOD, CAT, and glutathione peroxides are involved in the clearance of superoxide and hydrogen peroxide (H_{2}O_{2}). SOD catalyses the diminution of superoxide into H_{2}O_{2}, which has to be eliminated by glutathione peroxidase and/or catalase [42]. Further, it has been reported that a decrease in SOD activity in EAC bearing mice may be due to loss of Mn^{2+} containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver [43]. A small amount of catalase (CAT) in tumor cells was reported [43]. The inhibition of SOD and CAT activities as a result of tumor growth were also reported [44]. Similar findings were observed in our present study in EAC bearing mice. The administration of MEMP at two different doses significantly increased the SOD and CAT levels in a dose dependent manner. It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells [45] and antitumor activity in experimental animals [46]. Antitumor activity of these antioxidants is either through induction of apoptosis [47] or by inhibition of neovascularization [48]. The implication of free radicals in tumors is well documented [49, 50]. The free radical hypothesis supported the fact that the antioxidants effectively inhibit the tumor, and the observed properties may be attributed to the antioxidant and antitumor principles present in the plant extract.

In conclusion, the present study demonstrates that the methanol extract of Mucuna pruriens (MEMP) increased the life span of EAC tumor bearing mice and decreased lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. All these parameters suggest that the methanol extract of Mucuna pruriens seeds exhibits potential antitumor and antioxidant activities.

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