Antitumor Activity of Hexane and Ethyl Acetate Extracts of 
Tragia involucrata

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ABSTRACT

The practice of traditional medicines in treatment of cancer is based on the knowledge and use of plant based medicine since ancient time. The study of folk medicinal practices revealed the use of Tragia involucrata (Euphorbiaceae) along with other medicinal plants to treat certain tumours in some parts of Kerala, India. The aim of the present study was to evaluate the antitumor potential of hexane and ethyl acetate extracts (HE and EAE) and to assess the antioxidant activity of EAE of T. involucrata. The mice were injected with 10^6 Ehrlich’s Ascites Carcinoma (EAC) cells subcutaneously and the extracts were administered intra-peritoneal (i.p.). In vitro antioxidant property of EAE was tested by 2, 2’-azinobis (3-ethylbenzo-thiozoline-6-sulphonic acid (ABTS)) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods. In vivo antioxidant potential of EAE was determined by nitric-oxide by Griess-reagent method, total antioxidant in serum by phosphomolybdenum method and super oxide dismutase by nitroblue tetrazolium (NBT) method. Administration of the extracts to mice challenged with EAC showed significant antitumour activity. The observed antitumor activity of the extracts was dose dependant. There was a significant increase in the percentage life span (ILS %) in extract treated mice. HE was not toxic to mice even at the dose of 2000 mg kg^{-1} body weight. EAE was toxic to animals when the dosage was increased beyond 150 mg kg^{-1}. EAE was showing even a significant in vitro as well as in vivo antioxidant activity. In vivo antioxidant activity was showing the reciprocal dose response. From the results it may be concluded that the plant possess antitumor activity as claimed in traditional practices.

Key words: Tragia involucrata, flavonoids, phenolic compounds, ascites tumor, toxicity

INTRODUCTION

Cancer is one of the leading causes of death in developed as well as in developing countries (Bagya et al., 2011). The demand for new anticancer agents is escalating due to the accumulation of carcinogenic and mutagenic agents in the environment. Although many synthetic anticancer agents are used in the treatment of cancer; side effects and emergence of synthetic drug resistant cancer cells among patients limits their purpose. The limitation of synthetic drugs highlighted the pivotal need of anticancer agents from natural products particularly medicinal plants (Kumar et al., 2007; Hafidh et al., 2009). Plant and plant derived products were the basis of many
traditional systems since ages and they are still providing the remedies for various ailments (Su et al., 2010). About 60% of the world population is dependant on traditional medicine. The use of traditional medicines derived from plants is common in developing and developed countries (Seth and Sharma, 2004). The great traditional knowledge and the rich biodiversity in India provide the basis for many effective anticancer agents. Selection of plants based on ethno medical knowledge and testing the selected plants efficacy as well as safety is one of the best approaches for the isolation of anticancer lead molecules from the medicinal plants (Thomas et al., 2002). The study of folk medicinal practices in Kerala revealed the ethno medical use of the aerial parts of Tragia involucrata along with other medicinal plants to treat certain tumours in few villages in Kerala, India (Mathew and Unnithan, 1992). This plant is known as antitumor agent in ancient system of medicines such as Ayurveda, Siddha and Unani. T. involucrata is a member of Euphorbiaceae family, widely found in the Indian subcontinent (Dhara et al., 2000). The efficacy of this plant is well known in Indian traditional medicine and it is used for treatment of eczema, wounds and headache (Samy et al., 1998). The anti-microbial, anti-inflammatory and antifertility activity of T. involucrata has been reported (Samy et al., 2006; Dhara et al., 2000; Perumalsamy et al., 2006; Joshi and Gopal, 2011). Recently, Joshi et al. (2011) have demonstrated the potent in vitro anticancer activity of T. involucrata extracts against cancer cells.

In the present study, to verify the medical claim further by in vivo studies; the hexane and ethyl acetate extracts of T. involucrata aerial parts were tested for their anti Erlich's Ascites Carcinoma (EAC) activity in mice. The toxicity as well as the in vivo and in vitro antioxidant properties of ethyl acetate extract was evaluated.

MATERIALS AND METHODS

Antitumor and antioxidant studies were carried out in the Department of Biochemistry, Davangere University/Kuvempu University, Davangere and Department of Biochemistry, Nitte University, Mangalore, respectively from December 2007 to September 2009.

Chemicals: 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2’-azinobis (3-ethylbenzo-thiazoline-6-sulphonic acid) diaminonium salt (ABTS), α-tocopherol were purchased from Sigma chemicals, St. Louis, USA. Gliclic acid monohydrate, dimethylsulphoxide (DMSO), riboflavin, nitroblue tetrazolium (NBT) and sulphamidazole were from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Folin-Ciocalteu's phenol reagent and sodium carbonate (Na₂CO₃) were from Merck (India) Ltd., India. All other chemicals were of analytical grade.

Plant material: The aerial parts of the plants were collected in the month of October and November 2004 from Bangalore and the herbarium was prepared from the aerial part of the plant and was identified as T. involucrata. Linn. by Dr. Geetha KR, curator, botanical Garden, Department of Botany, Gandhi Krishi Vijnana Kendara (GKVK), Bangalore and confirmed by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Herbarium was deposited in GKVK, Bangalore (Herbarium No. 3687).

Preparation of the extracts: One hundred grams of the dried powder of T. involucrata was extracted in a soxhlet extractor with hexane (500 mL) then methanol (500 mL). The mark was pressed and the expressed solvent was mixed with the main extract. The extract was concentrated to constant weight in a rotary shaker evaporator. The hexane fraction (1.78 g) was defatted and labelled as hexane extract {0.65g} (HE).
The concentrated methanol extract (2.64 g) was dissolved in 80% methanol (200 mL) with stirring and filtrated. The filtrate was extracted successively with n-hexane (500 mL X3), dichloromethane (500 mL X2), and ethyl acetate (500 mL X4). Ethyl acetate fraction was concentrated and the solvent was evaporated to dryness. The residue (0.29 g) was labelled as ethyl acetate extract (Jang et al., 2003). HE and EAE were dissolved in 1% tween 20 [in Phosphate Buffered Saline (PBS)] and propylene glycol: water (1:4), respectively for in vivo studies.

**Determination of total phenolic content:** The total phenolic compound in EAE was determined according to the method of Singleton and Rossi as modified by Coruh et al. (2007). The total phenolic content in EAE was expressed as gallic acid equivalent in mg g⁻¹ (GAE mg g⁻¹).

**Animals:** Swiss albino mice of either sex (8-10 weeks old) weighing 20-25 g, were used for the experiment. The animals were maintained under proper environmental conditions i.e., temperature 25±2°C and humidity 50±5% with a 12 h light and dark period. They were housed in polypropylene shoebox type cages with stainless steel grill top, bedded with rice husk. The animals were provided with pelleted diet (Gold Mohur, Lipton, India) and water *ad libitum*.

**Transplantation of tumors:** The ascitic fluid was drawn from EAC cells bearing mice using 18 gauge needles into a sterile syringe and tested for microbial contamination. The viability of the tumor cells were determined by trypan blue exclusion method and the viable cells were counted using hemocytometer. From the appropriately diluted (10×10⁶ cells mL⁻¹) stock ascitic fluid, 0.25 mL (2.5×10⁶ mL⁻¹ mice⁻¹) was injected subcutaneously (s.c) to right hind legs of mice to get the ascitic tumor.

**Experimental chemotherapy:** The study protocol was approved by the institutional ethics committee. The method of Kruczynski et al. (1998) as cited in Gopal et al. (2003) was used for experimental chemotherapy study with slight modification.

**Evaluation of anti-tumour activity: Life span:** Mortality was noted every day and the median life span was calculated as mentioned by Gopal et al. (2003).

**Tumour growth:** Treatment efficiency is assessed in terms of the compound’s effects on the tumour volumes of tumour bearing mice relative to the control and vehicle-treated mice. (Plowmann et al., 1997).

**Toxicity of EAE**

**Acute toxicity:** Acute toxicity was assessed on healthy Swiss albino mice of either sex (8-10 weeks old) weighing 20-25 g, in one i.p., administration. The drug dosage was fixed according to OECD/OCDE guidelines No. 420. The mice were observed continuously for 1 h for any gross behavioral changes and deaths intermittently for the next 6 h and then again 24 h after dosing. LD₅₀ of both extracts was calculated according to OECD guidelines. The behavior parameters observed were convulsion, hyperactivity, sedation, grooming, loss of righting reflex, increased or decreased respiration, food and water intake etc.

**Short-term toxicity:** To determine short-term (14 days) toxicity, groups of 8 mice each were administered, daily 60, 75 and 90 mg kg⁻¹, i.p., of EAE for 14 days. Control animals were treated
with vehicle in similar way. Body weight, food, water intake and general behaviour were monitored. After the treatment period, mice were sacrificed, blood was collected and important internal organs were removed, weighed and observed for pathological changes. Mice which received 150 mg kg⁻¹ extract, did not survive for more than 3 days. Therefore, for this dose, another group of 8 animals were given a single dose (150 mg kg⁻¹) and the animals were sacrificed 24 h after dosing, (along with the other 3 groups). Blood haematological parameters were determined (Jain, 1986). Serum Glutamate Pyruvate Transaminase (SGPT) and Glutamate Oxaloacetate Transaminase (SGOT) were determined by the method of Riteman and Frankel (1960), using commercial assay kits. Urea was estimated by enzymatic method (Tietz, 1987), using assay kit (Wipro Biomed, India). Calcium was estimated by o-cresolphthalein complex method (Tietz, 1987). Phosphorus was estimated by calorimetric method (Henry, 1974), using commercial reagents (Reagents Applications, Irk, San Diego, CA).

DPPH radical scavenging method: The assay was carried out in a 96 well micro titre plate using the method explained by Rao (1996).

Scavenging of ABTS radical cation: ABTS radical cation (ABTS⁺) scavenging activity was measured according to the method of Re et al. (1990).

In vivo antioxidant activity: Healthy mice (20±2 g) were divided into seven groups comprising of six animals in each group. Group I served as control and received 0.2 mL propylene glycol: water (1:4). Group II, III and IV received a single dose of EAE at the dose of 50, 70, and 90 mg kg⁻¹ body weight respectively as a single dose. Group V, VI and VII received EAE at 50, 70 and 90 mg kg⁻¹ body weight, respectively on alternative days for 8 days. All these treatments were given intraperitoneal. On day 9, the mice were anesthetized using diethyl ether and blood was collected from heart and kept at 37°C in the incubator for 30 min. Later, it was cold centrifuged at 2000 rpm for 15 min to get clear supernatant serum, which was used for the in vivo antioxidant assays.

Estimation of nitric-oxide by Griess-reagent method: Serum nitric oxide was measured according to the method of Bodis and Haregewin (1993).

Estimation of total antioxidant in serum by phosphomolybdenum method: A spectrophotometric method has been developed for the quantitative determination of antioxidant capacity (Prieto et al., 1999).

Estimation of super oxide dismutase (SOD) by nitroblue tetrazolium (NBT) method: SOD in the serum was estimated using the method followed by Winterbourn et al. (1975).

Statistical analysis: It was performed when required, using Student’s t-test and p-values less than 0.05 were considered significant (GraphPad Prism version 3). Data represented as Mean±SD.

RESULTS
Analysis of extracts: Phytochemical analysis and the TLC of the extracts showed the presence of steroid (R₅ value, 0.37) and terpenoids (R₅ value, 0.52 and 0.60) in HE and flavonoids in EAE (R₅ value, 0.88 and 0.95), respectively.
Total phenolic content in EAE: The total phenolic content in the EAE was expressed as gallic acid equivalent in mg g\(^{-1}\) of extract. EAE had the phenolic content of 71.6 mg g\(^{-1}\).

Anti tumour activity: The anti tumour activity of HE and EAE was evaluated using single dose and intermittent treatments over one week i.e. on days 1, 3, 5, 7, 9. HE and EAE were given i.p. as single dose significantly increased survival (p<0.001) of tumour bearing mice. Administration of HE at a dose 50 to 150 mg kg\(^{-1}\) body weight resulted in a significant tumour growth inhibition with an ILS\% from 7.65 to 86.22\% (Table 1). There was a reduction in the tumour volume in tumour bearing mice treated with either single (Fig. 1) or multiple schedules (Fig. 2) of HE. EAE had a significant antitumour activity and ILS\% on EAC bearing mice as shown in Table 2. EAE increased the life span (%) of experimental animals up to 104.54\%. Average of the tumour volume of control and treated mice with three concentrations of EAE administrations in single dose is shown in Fig. 3. Tumour volume was reduced up to 72\% at the dose of 90 mg kg\(^{-1}\) body weight. Even the multiple schedules of extract reduced tumour volume significantly. The extent of tumour volume reduction was highest at the dose of 90 mg kg\(^{-1}\) and found to be 76\% of that of control mice (Fig. 4).

Short term toxicity studies: Administration of EAE once at a dose of 60, 75, 90 mg kg\(^{-1}\) (i.p.) body weight did not show any abnormal behavioural changes. Mice which received 150 mg kg\(^{-1}\) body weight showed the toxic symptoms like inactiveness, dizziness, erection of hairs.

Table 1: Antitumor activity of T. inulicrata HE given (i.p.) against the sc implanted EAC

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>MTD (mg kg(^{-1}))</th>
<th>TIE</th>
<th>Dose (mg kg(^{-1}))</th>
<th>SGD</th>
<th>ILS%</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>&gt; 2000</td>
<td>13.33</td>
<td>50</td>
<td>-0.38</td>
<td>8.13</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>0.28</td>
<td>32.97</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150</td>
<td>0.04</td>
<td>86.22</td>
<td>9/10</td>
</tr>
<tr>
<td>D 1, 3, 5, 7, 9</td>
<td>&gt; 2000</td>
<td>13.33</td>
<td>50</td>
<td>-0.38</td>
<td>7.56</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>0.19</td>
<td>21.06</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150</td>
<td>0.19</td>
<td>86.22</td>
<td>9/10</td>
</tr>
</tbody>
</table>

Drugs are injected (i.p.) with in different schedules and % ILS\% are then determined. *MTD: Maximum tolerance dose. *TIE: Therapeutic index, SGD: (Td (drug-treated group)-Td (control group))/Td (control group), with Td being the time required for the tumour to double in volume; SGD>1 corresponds to minimal level of activity.

Fig. 1: In vivo antitumour activity of HE on Ehrlichs Ascitic Carcinoma. Tumors were generated by s.c. inoculation of EAC cells in Swiss albino mice. The compound was administered i.p., once (D1, Single dose) on the day after tumor inoculation. Average tumour volumes of treated (n = 10) and control (n = 10) are shown.
Table 2: Antitumour activity of *T. involucrata* EAE given ip against the sc implanted EAC

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>MTD (mg kg⁻¹)</th>
<th>TP</th>
<th>Dose (mg kg⁻¹)</th>
<th>SGD</th>
<th>ILS%</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>130</td>
<td>6.66</td>
<td>60</td>
<td>-0.04</td>
<td>18.18</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.04</td>
<td>35.00</td>
<td></td>
<td></td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.23</td>
<td>104.54</td>
<td></td>
<td></td>
<td>5/10</td>
</tr>
<tr>
<td>D1,3,5,7,9</td>
<td>130</td>
<td>6.66</td>
<td>60</td>
<td>0.14</td>
<td>52.27</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.09</td>
<td>4.54</td>
<td></td>
<td></td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.19</td>
<td>75.00</td>
<td></td>
<td></td>
<td>7/10</td>
</tr>
</tbody>
</table>

Drugs are injected ip with in different schedules and ILS% are determined. *MTD: Maximum tolerance dose. *TP: Therapeutic index. SGD: (Td (drug-treated group)/Td (control group)]/Td control group, with Td being the time required for the tumour to double in volume. SGD>1 corresponds to minimal level of activity.

Table 3: Effect of EAE on hematological and serum biochemical parameters in mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EAE (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
</tr>
<tr>
<td>Hb (g%)</td>
<td>14.90±1.62</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>38.80±2.30</td>
</tr>
<tr>
<td>RBC (10⁶/mm³)</td>
<td>8.62±0.60</td>
</tr>
<tr>
<td>Total WBC (10⁹/mm³)</td>
<td>1.50±0.02</td>
</tr>
<tr>
<td>MCHC (g dL⁻¹)</td>
<td>38.50±2.41</td>
</tr>
<tr>
<td>Platelets (10⁹/mm³)</td>
<td>454.00±4.3</td>
</tr>
<tr>
<td>SGOT (U L⁻¹)</td>
<td>538.00±2.01</td>
</tr>
<tr>
<td>SGPT (U L⁻¹)</td>
<td>88.00±1.23</td>
</tr>
<tr>
<td>Serum urea (mg dL⁻¹)</td>
<td>79.00±2.36</td>
</tr>
<tr>
<td>Serum calcium (mg dL⁻¹)</td>
<td>7.70±0.38</td>
</tr>
<tr>
<td>Serum phosphorous (mg dL⁻¹)</td>
<td>12.20±1.87</td>
</tr>
</tbody>
</table>

Repeated daily dose of the drug was administered (i.p) for 14 days in all groups except 150 mg kg⁻¹ where a single dose was administered. *Results are the Mean±SEM of three independent experiments. n = 8 in each group. Values marked with asterisk are significantly different from control. *p<0.05, **p<0.01, ***p<0.001 (Students t-test). PCV: Packed Cell Volume; MCHC: Mean cell hemoglobin concentration; SGOT, serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase

Fig. 2: *In vivo* antitumour activity of HE on Ehrlich’s Ascitic Carcinoma. Tumors were generated by s.c., inoculation of EAC cells in Swiss albino mice. The compound was administered i.p., five times: on the day after tumor inoculation (D1), on day D3, D5, D7, D9 and D11. Average tumour volumes of treated (n = 10) and control (n = 10) are shown

and hypothermia. There was a reduced food and water intake in the treated animals compared to control.
Fig. 3: In vivo antitumour activity of EAE on Ehrlich's Ascitic Carcinoma. Tumors were generated by s.c., inoculation of EAC cells in Swiss albino mice. The compound was administered i.p., once (D1, Single dose) on the day after tumor inoculation. Average tumour volumes of treated (n = 10) and control (n = 10) are shown.

Fig. 4: In vivo antitumour activity of EAE on Ehrlich's Ascitic Carcinoma. Tumors were generated by s.c., inoculation of EAC cells in Swiss albino mice. The compound was administered i.p., five times: on the day after tumor inoculation (D1), on day D3, D5, D7, D9 and D11. Average tumour volumes of treated (n = 10) and control (n = 10) are shown.

The repeated low doses (60, 75 and 90 mg kg\(^{-1}\)) or a single high dose (150 mg) of EAE did not show any significant haematological parameters (Table 3). Haemoglobin and RBC count remained unaltered at the dose of 75, 90, 150 mg kg\(^{-1}\). But there was a marginal increase in WBC count at 90 mg kg\(^{-1}\) body weight. There was an increase in the SGOT, SGPT and urea level in the mice treated with 150 mg kg\(^{-1}\) body weight of EAE along with other toxic symptoms while there were no toxicity signs observed when the dosage was 60-90 mg kg\(^{-1}\). This may be due to the non accumulation of drug in the mice and may be detoxified from the body (Thomas et al., 2002).

**In vitro antioxidant studies:** EAE showed significant antioxidant activity in both DPPH as well as in ABTS radical scavenging methods. The in vitro antioxidant activity showed the IC\(_{50}\) values of 0.85±0.012 and 6.46±0.371 µg mL\(^{-1}\) for ABTS and DPPH methods, respectively (Table 4).
Table 4: *In vitro* antioxidant activity of EAE of *T. involucrata*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method</th>
<th>IC_{50} value (µg mL^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>ABTS</td>
<td>11.25±0.49</td>
</tr>
<tr>
<td>EAE</td>
<td></td>
<td>0.85±0.01</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>DPPH</td>
<td>2.69±0.05</td>
</tr>
<tr>
<td>EAE</td>
<td></td>
<td>6.46±0.37</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SEM

Table 5: *In vivo* antioxidant activity of EAE of *T. involucrata*

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Dose (mg kg^{-1})</th>
<th>Total antioxidant (µg mL^{-1})</th>
<th>Nitric oxide (µg mL^{-1})</th>
<th>Superoxide dismutase (Units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>18.48±1.65</td>
<td>361.32±64.1</td>
<td>3.75±0.61</td>
</tr>
<tr>
<td>D1</td>
<td>50</td>
<td>79.87±3.04</td>
<td>795.97±67.0</td>
<td>16.62±1.10</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>24.48±0.98</td>
<td>357.36±84.3</td>
<td>5.36±0.87</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15.36±1.72*</td>
<td>86.40±16.3*</td>
<td>2.96±0.42**</td>
</tr>
<tr>
<td>D1, 3, 5, 7</td>
<td>50</td>
<td>60.76±14.3</td>
<td>640.10±127</td>
<td>20.97±0.85</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>21.17±4.34</td>
<td>635.01±194</td>
<td>9.83±0.43*</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>8.86±1.83*</td>
<td>260.40±58.2*</td>
<td>4.60±0.65**</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SEM. Values marked with asterisk are significantly different from control. *p<0.05, **p<0.01. (Students t-test)

**Effect of EAE on antioxidant enzymes:** The effect of EAE on the antioxidant enzymes is shown in Table 5. The level of SOD in the liver of control mice was found to be 3.75±0.61. After administration of EAE at the dose of 50 mg kg^{-1} increased levels of SOD by 79% and further there is a decrease in the SOD level by 32 and 17% as doses increases. The decrease in the *in vivo* antioxidant activity with the increase in plant extract can be explained in terms of the pro-oxidant activity of the phenolic compounds like flavonoids.

**DISCUSSION**

In the present study, HE and EAE of aerial parts of *T. involucrata* significantly reduced the tumour growth as well as increased the ILS% in tumour bearing mice. The extent of EAC tumour reduction was similar to the studies reported on different plant extracts in the recent past (Islam *et al.*, 2011; Onocha *et al.*, 2011; Bangou *et al.*, 2011). Prolongation of life span of animal has been well documented as criteria for judging the drug activity (Rajeshwar *et al.*, 2005). Ascites fluid is the direct nutritional source for tumour cells and a rapid increase in ascites fluid with tumour growth would be a mean to meet the nutritional requirement of tumour cells. The plant extracts increase the life span of EAC tumour bearing mice by depleting the nutritional fluid volume and delaying the cell division (Sur *et al.*, 1997). This hypothesis is in agreement with the studies of Zahan *et al.* (2011) who have reported 45% increase in life span of EAC bearing mice treated with the 10 mg kg^{-1} Alangium salviolium flower extract.

The phytochemical analysis of the extracts showed the presence of terpenoids and steroids in HE and flavonoids in EAE, respectively. Steroids and flavonoids are known to posses antitumor activities. Phytosterols alter membrane fluidity, activity of membrane bound enzymes and signal transduction resulting in the stimulation of apoptosis in tumour cells. They also have been shown to play a role in the activation of immune system function. Chemopreventive role of flavonoids is due to their influence on signal transduction in cell proliferation and angiogenesis (Jones and AbuMweis, 2000; Sodde *et al.*, 2011). The potent antitumor activity of HE and EAE may be due to these mechanisms as these extracts contain steroids, terpenoids and flavonoids.
As EAE was toxic to animals beyond 150 mg kg\(^{-1}\) body weight, it was subjected to toxicity studies. In short term toxicity study, EAE at higher dose level increased the urea and transaminase activity indicating its hepatorenal dysfunction and metabolism. The extract also restored the Haeoglobin and RBC count to normal levels. Short term toxicity studies indicated that at doses of 60, 75 and 90 mg kg\(^{-1}\) did not exhibit any adverse effect. Similar results were reported by Thomas et al. (2002).

There was a difference in the IC\(_{50}\) values between ABTS and DPPH methods of free radical scavenging activity. This is in agreement with the studies made by Dixit et al. (2005). According to them, the difference in values between the two methods is due to the action of antioxidants at different levels (Dixit et al., 2005).

Repeated administration of EAE for seven days or a single dose in experimental animals caused a significant increase in the level of total antioxidant, nitric oxide and SOD level in the serum. The antioxidant activity showed reciprocal dose dependence. Results of the in vivo antioxidant activity were contradictory to positive correlation between dose and in vivo antioxidant activity reported by Maruthappana and Shreeb (2010). However, the decrease in the in vivo antioxidant activity with the increase in plant extract can be explained in terms of the pro-oxidant activity of the phenolic compounds like flavonoids. These molecules have been shown to possess auto-oxidizing capacity to become free radicals and in turn produce the secondary free radicals by binding covalently to sulfhydryl groups or other essential groups. These secondary free radicals are responsible for the pro-oxidant activities. This process is dependant on the concentration of phenolics (Monks and Lau, 1992; Simons et al., 1990; Geetha et al., 2005). In our study also the decreased antioxidant activity with the increase EAE concentration may be due to the presence of pro-oxidants in the extracts. However, further exploration on the antioxidant activity and isolation of metabolites may be helpful in revealing the role of each component present in the plant extract.

CONCLUSIONS

The results of the present study demonstrate the potent anti tumour and antioxidant properties of T. involucrata. Data reported here provide a rational basis for the use of T. involucrata in the treatment of tumours in traditional medicine. The observed effect of this plant extracts may be due to the presence of steroids, terpenoids and flavonoids as evidenced by our in vivo model. Constituents of this plant may represent a new line of antitumor agents and serve as tool to fight against cancer. This plant needs further investigation for the isolation of its active constituents to reveal the mechanism of action.

ACKNOWLEDGMENT

This work was supported financially by University Grants Commission, New Delhi, India. Project No. 34-543/2008 (SR).

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