In vitro Antioxidant and Anti-neoplastic Activities of Ocimum sanctum Leaves in Ehrlich Ascites Carcinoma Bearing Mice

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ABSTRACT

The present study was designed to investigate the antioxidant and anti neoplastic activities of Ocimum sanctum Linn. (OS) leaves. Antioxidant potential of the OS extract was evaluated in vitro by DPPH (1, 1diphenyl-2-picylhydrazyl) and NO (Nitric Oxide) scavenging assay and reducing power assay method. OS extract showed prominent scavenging activity in all the methods with IC_{50} value of 16.39±0.31 and 16.20±0.53 µg mL^{-1} for DPPH and NO assay method, respectively. In reducing power assay, OS extract also showed significant (p<0.001) activity. In addition, total phenolic and flavonoid content and total antioxidant capacity were also determined. The anti neoplastic effect of the leaves of OS against Ehrlich Ascites Carcinoma (EAC) in mice at the doses of 50 mg kg^{-1} body weight intraperitoneally. Significant (p<0.001) increases of survival times 33±1.81 days for crude extract of the (50 mg kg^{-1}) treated tumor bearing mice were confirmed with respect to the control group (20±0.12 days). Hematological studies reveal that the Haemoglobin (Hb) content was decreased in EAC treated mice whereas restoration to near normal levels was observed in extract treated animals. There was a significant (p<0.001) decrease in RBC count and increase in WBC counts in extract/fraction treated animals when compared to EAC treated animals. From the result it was showed that the extract has significant antioxidant as well as anti neoplastic activity.

Key words: Anticancer, ehrlich ascites carcinoma, free radical, Ocimum sanctum, phenolic content

INTRODUCTION

The morbidity and mortality of cancer, the second leading cause of death worldwide next to cardiovascular diseases, reaches a high plateau and is characterized by uncontrolled cellular growth, local tissue invasion and distant metastases (Dashora et al., 2010). Free radical, one of the
major cause for the conversion of normal cell to cancerous cells, are generated as consequences of a number of endogeneous metabolic processes involving redox enzymes and bioenergetic electron transfer and exposure to a plethora of exogeneous chemicals (Rajkumar et al., 2011). In normal metabolic condition, oxidants and antioxidants levels are maintained in balance within humans for sustaining optimal physiological conditions (Temple, 2000). However, overproduction of free radical and Reactive Oxygen Species (ROS) would assault on important biological molecules such as DNA, protein or lipid leading to many degenerative diseases, such as cancer, Alzheimers, arthritis and ischemic reperfusion (Suja et al., 2004). More and more evidence suggests that this potentially cancer-inducing oxidative damage might be prevented or limited by antioxidant. Antioxidant may mediate their effect by directly reacting with ROS, quenching them or chelating the catalytic metal ions (Sun et al., 2002). It has been shown that antioxidant rich diets can reduce oxidative damage to DNA, thus preventing a critical step in the onset of carcinogenesis and the impact of antioxidants on mutagenesis and carcinogenesis has been well established (Zhang et al., 2008; Meyskens and Szabo, 2005). Moreover, the rate of increase of cancer incidence and lack of anticancer drugs has forced scientists to pharmacological and chemical investigation of anticancer agents from medicinal plants (Koduru et al., 2006). The worldwide upsurge of the herbal preparation and medicinal plants with its isolated active compounds has provided one of the most importance sources for pharmaceutical industry for lead compound. Furthermore, over a 100 new products are in clinical development, particularly as anti-cancer agents and anit-infectives (Hafidh et al., 2009). Although, the mechanism of interaction between phytochemicals and cancer cells has been studied extensively and augmented the interest of pharmacological evaluation of various plants used in Bangladeshi traditional systems of medicine (Kumar et al., 2007).

_Ocimum sanctum_ (OS) commonly known as holy basil (English), or Tulasi (local language) is a herbaceous sacred plant found throughout Bangladesh. The leaves of OS contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene (Shishodia et al., 2003). Fresh leaves and stem of OS extract yielded some phenolic (Yanpallewar et al., 2004) and flavonoids (Gupta et al., 2002; Nair et al., 1982). OS also contains a number of sesquiterpenes and monoterpenes (Pandey and Madhuri, 2010). Essential oils of Tulsi have antibacterial (Singh et al., 2005; Mishra and Mishra, 2011) with emphasis on anti-tuberculosis (Farivar et al., 2006), antifungal (Geeta et al., 2001) and antiviral properties (Farida et al., 1997). Its extracts have marked insecticidal activity against mosquitoes (Kamaraj et al., 2008; Vinayagam et al., 2008). OS extracts play an important role in the management of immunological disorders including allergies and asthma (Singh and Agarwal, 1991) and psychiatric disorder like depression and anxiety (Chatterjee et al., 2011). As Tulsi has a positive effect over blood pressure (Rai et al., 1997) and also is a detoxicant, its regular use prevents heart attacks (Sharma et al., 2001). Hepatoprotective activity of _Ocimum sanctum_ leaf extract against paracetamol induced hepatic damage in rats has been reported (Gupta et al., 2006). The alcoholic extract of leaves of OS has a modulatory influence on carcinogen metabolizing enzymes which are important in detoxification of carcinogens and mutagens (Pandey and Madhuri, 2006). The OS fixed oil has been also evaluated for its antioxidant (Relm et al., 2000) analgesic, anti-inflammatory (Singh and Majundar, 1995) and antipyretic (Singh et al., 2007) activities. Literature reviews indicated that no studies combining the antioxidant and anti-neoplastic activities of the leaves of OS have so far been undertaken. Taking this in view and as part of our ongoing search on Bangladeshi medicinal plants (Rahman et al., 2011) the present study aimed at evaluating the antioxidant and anti-neoplastic activities of the methanolic extract of _Ocimum sanctum_ leaves.
MATERIALS AND METHODS

Plant materials: The leaves of the OS were collected from the adjoining area of Rajshahi University Campus, Bangladesh during February 2008 and were identified by Taxonomist, Department of Botany and University of Rajshahi, Bangladesh where a voucher specimen number (Voucher No. 192) has been deposited.

Chemicals: Ammonium molybdate, Folin-ciocalteu phenol reagent, sodium chloride, propylene glycol, trypsin blue, methyl violet, sodium sulphate, methylene blue and Bleomycin were purchased from Merck Limited, Mumbai, India. 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and potassium ferric cyanide were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used were of highest analytical grade.

Preparation of extracts: The leaves of the OS were dried in an oven at 37°C and then powdered with a mechanical grinder, passing through sieve No. 40 and stored in an air tight container. The dried powdered material (1.0 kg) was refluxed with MeOH for 3 h. The total filtrate was concentrated to dryness, in vacuo at 40°C to render the MeOH extract (100 g).

Preliminary phytochemical investigation: The extract/fractions was subjected to qualitative chemical investigation for the identification of different phytocomponents like Alkaloids, glycoside, Amino acids, Tannins, Phenolic compound, Triterpenoids, Steroids, Sterols, Saponins, Flavonoids (Yarnalkar, 1991).

Animal: Sixty Swiss albino mice (25-30 g) of both sexes were divided into five groups (n = 12) and used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water ad libitum. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into four different groups, each consisting of twelve animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

Acute toxicity: The acute oral toxicity of plant in male Swiss albino mice was studied as per reported method (Lorke, 1983).

In vitro antioxidant activity
The amount of phenolic compounds and flavonoids: The total phenolic and flavonoid content of methanolic extract of OS were determined using Folin-Ciocalteu reagent (Yu et al., 2002) and aluminium chloride colorimetric method (Chang et al., 2002), respectively. The content of total phenolics in the extract of OS was calculated from regression equation of the calibration curve: \( y = 0.013x + 0.127, r^2 = 0.988 \) and is expressed as Glicic Acid Equivalents (GAE) and the flavonoid contents of the extract in terms of quercetin equivalent the standard curve equation: \( y = 0.009x-0.036 \)

Determination of total antioxidant capacity: The antioxidant activity of the MeOH extract were evaluated by the phosphomolybdenum method according to the procedure of
Prieto et al. (1999). The assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

**Free radical scavenging activity measured by 1, 1-diphenyl-2-pieryl-hydrazyl (DPPH):** The free radical scavenging activity of MeOH extract, based on the scavenging activity of the stable 1, 1-diphenyl-2-pierylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). The percentage inhibition activity was calculated from:

\[
\frac{(A_0 - A_t)}{A_0} \times 100
\]

where, \(A_0\) is the absorbance of the control and \(A_t\) is the absorbance of the extract/standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg mL\(^{-1}\)) versus (%) inhibition.

**Nitric oxide radical scavenging assay:** The procedure is based on the method where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent (Sreejayan and Rao, 1997).

**Reducing power activity:** The reducing power of OS was determined according to the method previously described (Oyaizu, 1983). Increased absorbance of the reaction mixture indicated increased reducing power.

**In vivo anti-neoplastic activity**

**Transplantation of tumor:** Ehrlich Ascites Carcinoma (EAC) cells were obtained from Indian Institute of Chemical Biology (IICB), Calcutta, India. The EAC cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation of \(2 \times 10^6\) cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7-8 of tumor bearing) of the tumor cells. Each animal received 0.1 mL of tumor cell suspension containing \(2 \times 10^6\) tumor cells intraperitoneally.

**Treatment schedule:** Sixty Swiss albino mice were divided into five groups (n = 12) and given food and water ad libitum. All the animals in each groups except Group-I received EAC cells (\(2 \times 10^6\) cells/mouse i.p.). This was taken as day 0. Group-I served as normal saline control (5 mL kg\(^{-1}\) i.p.) and Group-II served as EAC control. The 24 h after EAC transplantation, Group-III and Group-IV received crude extract of OS leaves at a dose of 50 mg kg\(^{-1}\) i.p. for nine consecutive days, respectively. Group-V received reference drug Bleomycin (0.3 mg kg\(^{-1}\) i.p.) for nine consecutive days (Rana and Khanam, 2002). Twenty-four hours of last dose and 18 h of fasting, 6 animals of each group were sacrificed by cervical dislocation to measure antitumor and hematological parameters and the rest were kept with food and water ad libitum to check percentage increase in life span of the tumor host. The antitumor activity of the OS leaves extract was measured in EAC animals with respect to the following parameters.
Determination of tumor volume and weight: 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 weight immediately.

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 Neubauers counting chamber and the numbers of cells in the 64 small squares were counted.

Viable/nonviable tumor cell count: The viability and nonviability of the cell were checked by trypan blue assay. The cells were 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 dye were nonviable. These viable and nonviable cells were counted.

\[
\text{Cell count} = \frac{(\text{Number of cells} \times \text{Dilution factor})}{(\text{Area} \times \text{Thickness of liquid film})}
\]

Determination of median survival time and percentage increase in life span: The mortality was monitored by recording percentage increase in life span (%ILS) and Median Survival Time (MST) (Sur and Ganguly, 1994).

Hematological parameters: Collected blood was used for the estimation of Hemoglobin (Hb) content, Red Blood Cell (RBC) and White Blood Cell Count (WBC) (Armour et al., 1985).

Statistical analysis: All data are expressed as Mean± SEM (n = 6 mice per groups). Statistical significance (p) calculated by Student’s t test and computed using GraphPadPrism 4 (Graphpad). p<0.001 and p<0.05 were considered to be statistically significant

RESULTS
Phytochemical screening: The phytoconstituents were identified by various chemical tests which showed the presence of Alkaloids, glycoside, Amino acids, Tanins, Phenolic compound, Triterpenoids, Sterols, Saponins and Flavonoids whereas steroids are absent in crude extract of OS.

Acute toxicity studies: The acute toxicity studies mainly aims at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. The extract of OS was safe up to a dose of 4 g kg⁻¹ (p.o.) body weight which agrees with the previous study (Rahman et al., 2011). Behavior of the animals was closely observed for the first 3 h then at an interval of every 4 h during the next 48 h. OS extract did not cause mortality in mice during 48 h observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

Total phenolic and flavonoid contents: The total phenols and flavonoids content was found to be 39.74±0.05 mg g⁻¹ plant extract (in GAE) and 11.53±0.72 mg g⁻¹ plant extract (in quercetin equivalent), respectively, in crude extract of OS; presented in Table 1.
**Total antioxidant capacity:** Percentage yield of methanol extract of OS and its total antioxidant capacity are given in Table 1. Total antioxidant capacity of OS leaves extract is expressed as the number of equivalents of ascorbic acid and was found to be 279.3±0.05 mg g⁻¹ equivalent of ascorbic acid.

**DPPH radical scavenging activity:** The percentage (%) scavenging of DPPH radical was found to be concentration dependent i.e. concentration of the extract between 5-80 μg mL⁻¹ greatly increasing the inhibition activity (Fig. 1). The IC₅₀ value of the extract of OS was 16.39±0.31 μg mL⁻¹ while ascorbic acid showed the value of 12.30±0.11 μg mL⁻¹.

**Nitric oxide (NO) scavenging activity:** The percentage inhibition of nitric oxide production was illustrated in Fig. 2 and it is observed that scavenging of nitric oxide by the extract is also concentration dependent and statistically significant (p<0.001). Crude extract of OS (IC₅₀ value 18.23±0.33 μg mL⁻¹) showed little bit higher activity than the standard ascorbic acid (IC₅₀ value 18.23±0.15 μg mL⁻¹).

**Reducing power ability:** For the measurement of the reductive ability, we investigated the Fe³⁺ to Fe²⁺ transformation in the presence of crude extract of OS. Like the antioxidant activity, the reducing power of OS extract increased with increasing concentration of the sample and effect was statistically significant (p<0.001). Figure 3 shows the reductive capabilities of the OS compared with quercetin, gallic acid and ascorbic acid.

Table 1: Yield, total amount of plant phenolic compounds, flavonoids and total antioxidant capacity of methanolic extract of *Ocimum sanctum* leaves

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Total phenols mg g⁻¹ plant extract (in GAE)*</th>
<th>Total flavonoids mg g⁻¹ plant extract (in QA)*</th>
<th>Total antioxidant capacity mg g⁻¹ extract (in ASC)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS extract</td>
<td>10.00</td>
<td>39.74±0.05</td>
<td>11.53±0.72</td>
<td>279.3±0.05</td>
</tr>
</tbody>
</table>

*Gallic acid equivalents (GAE, mg g⁻¹ of each extract) for the total phenolic content. †Quercetin equivalents (mg g⁻¹ of each extract) for the total flavonoid content. ‡Ascorbic acid equivalents (mg g⁻¹ of each extract) for the total antioxidant capacity. The GAE, QA and ASC values are expressed as Mean±SEM of triplicate experiments.

![Graph](image.png)

Fig. 1: Free radical scavenging activity of different concentrations of crude extract of *Ocimum sanctum* and ascorbic acid by DPPH radicals
Fig. 2: Percentage inhibition of nitric oxide radical by different concentrations of crude extract of *Ocimum sanctum* and ascorbic acid

Fig. 3: Reducing power of crude extract of *Ocimum sanctum* and standards (quercetin, ascorbic acid and gallic acid) by spectrophotometric detection of Fe³⁺ to Fe²⁺ transformation

**Tumor growth and survival parameters:** Antitumor activity of OS extract against EAC tumor bearing mice was assessed by the parameters such as tumor volume, tumor weight, cell count (viable and non-viable), mean survival time and %increase of life span. The results are shown in Table 2. The tumor volume, tumor weight and viable cell count were found to be significantly (p<0.001) increased and non-viable cell count was significantly (p<0.001) low in EAC control animals when compared with normal control animals. Administration of OS crude extract at a dose of 50 mg kg⁻¹ b.wt. significantly (p<0.05) decreased the tumor volume, tumor weight and viable cell count. Furthermore, the median survival time was increase to 33±1.81 (%ILS = 66.66) on administration of OS crude extract. Finally, the change in body weight of the animals (data not shown) suggests the tumor growth inhibiting property of *Ocimum sanctum* leaves. All these results clearly indicate that the OS crude extract has a remarkable capacity to inhibit the growth of solid tumor induced by EAC cell line when compared with the standard Bleomycin.

**Hematological parameters:** Hematological parameters (Table 3) of tumor bearing mice on 14 day 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. At the same time interval on crude extract at a dose of 50 mg kg⁻¹ restored all the altered hematological parameters to almost near normal.
Table 2: Effect of the crude extract of Ocimum sanctum leaves on tumor volume, tumor weight, mean survival time (MST), percentage increase life span (%LS), viable and non-viable tumor cell count in EAC bearing mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EAC control</th>
<th>Crude extract</th>
<th>Bleomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume (mL)</td>
<td>3.70±0.21</td>
<td>0.72±0.22</td>
<td>0.55±0.21*</td>
</tr>
<tr>
<td>Tumor weight (g)</td>
<td>3.11±0.07</td>
<td>0.92±0.01*</td>
<td>0.82±0.12*</td>
</tr>
<tr>
<td>MST (days)</td>
<td>20.00±0.12</td>
<td>33.00±1.81</td>
<td>37.00±0.57</td>
</tr>
<tr>
<td>%LS</td>
<td>0.00</td>
<td>66.66</td>
<td>94.44</td>
</tr>
<tr>
<td>Viable cell (&gt;10⁶ cell mL⁻¹)</td>
<td>3.90±0.22</td>
<td>0.08±0.02*</td>
<td>0.02±0.15*</td>
</tr>
<tr>
<td>Non-viable cell (&gt;10⁶ cell mL⁻¹)</td>
<td>0.80±0.24</td>
<td>0.48±0.06*</td>
<td>0.27±0.05*</td>
</tr>
<tr>
<td>Total cell (&gt;10⁶ cell mL⁻¹)</td>
<td>4.70±0.15</td>
<td>0.56±0.15*</td>
<td>0.30±0.25*</td>
</tr>
<tr>
<td>Viable (%)</td>
<td>82.97</td>
<td>15.00</td>
<td>13.15</td>
</tr>
<tr>
<td>Non-viable (%)</td>
<td>17.03</td>
<td>85.71</td>
<td>90.00</td>
</tr>
</tbody>
</table>

Values represent the Mean±SEM (n = 6 mice per group), *p<0.05 statistically significant when compared with EAC control group.

Table 3: Effect of the crude extract of Ocimum sanctum leaves on hematological parameter in EAC bearing mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBC (&gt;10⁶ cell mL⁻¹)</th>
<th>WBC (&gt;10⁶ cell mL⁻¹)</th>
<th>Hemoglobin (g %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.40±0.33</td>
<td>11.00±1.16</td>
<td>11.60±0.05</td>
</tr>
<tr>
<td>EAC control</td>
<td>2.52±0.21*</td>
<td>22.00±0.56</td>
<td>5.54±1.80*</td>
</tr>
<tr>
<td>Crude extract (10 mg kg⁻¹)</td>
<td>3.80±1.06*</td>
<td>15.01±1.05*</td>
<td>7.30±0.00***</td>
</tr>
<tr>
<td>Bleomycin (0.3 mg kg⁻¹)</td>
<td>4.07±0.12*</td>
<td>12.05±0.83*</td>
<td>9.59±1.03*</td>
</tr>
</tbody>
</table>

Values represent the Mean±SEM (n = 6 mice per group), *p<0.001 statistically significant when compared with control group, **p<0.05 statistically significant when compared with EAC control group.

DISCUSSION

To determine the efficacy of natural antioxidants either as pure compounds or as plant extract, a great number of in vitro methods have been developed in which antioxidant compounds act by several mechanisms. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the compounds having antioxidant property and is successfully used to quantify vitamin E in seeds (Prieto et al., 1999). DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Nakayama, 1994) and is usually used as a substrate to evaluate the antioxidant activity of a compound (Chang et al., 2002). Based on the data obtained from this study, DPPH radical scavenging activity of OS crude extract (IC₅₀ 16.30±0.31 µg mL⁻¹) was slightly higher than the standard (IC₅₀ 12.30±0.11 µg mL⁻¹). It was revealed that OS extract did show the proton donating ability and could serve as free radical inhibitor or scavenger. In fact, the radical scavenging capability of phenolic compounds are due to their hydrogen donating ability/number of hydroxyl groups present which in turn is closely related both to the chemical structure and spatial conformation, that can modify the reactivity of the molecules (Gorelik et al., 2008). In the present study this possibility is supported by the estimation of total polyphenols and flavonoids (Gajula et al., 2009) which was found to be present in high concentration in the Ocimum sp. extracts. Present study also supported to the previous study (Ramesh and Satakopan, 2010) on producing the antioxidant effect of the hydroalcoholic extract of Ocimum sanctum against cadmium induced toxicity in rats.

A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh et al., 1999). Moreover, it has been reported that the phenol and polyphenolic compound (flavonoids) constituents of the plant possess antioxidant properties mainly
due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential (Hesham et al., 2002; Hsu, 2006). OS extract showed significant and prominent antioxidant activity. Our results suggest that the antioxidant activity of OS extract might be attributed to the phenolic and flavonoids which detected by phytochemical analysis in our study and also previously reported (Yanpallewar et al., 2004; Gupta et al., 2002; Nair et al., 1982).

In EAC tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. Ascetic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Prasad and Giri, 1994). Treatment with OS crude extract reduced the intraperitoneal tumor burden, thereby reducing the tumor volume, tumor weight and viable tumor cell count and increased the life span of the tumor bearing mice. The steadfast criteria for judging the potency of any anticancer drug are prolongation of life span of animals (Clarkson and Burchenal, 1965). It can therefore be inferred that crude extract increased the life span of EAC bearing mice may be due to decrease the nutritional fluid volume and delay the cell division (Sur et al., 1997). This hypothesis is strongly supported by the previous study, where in Aristochloia indica increase the life span 47% at a dose of 50 mg kg$^{-1}$ body weight (Rana and Khanam, 2002). Moreover, NO plays an important role in maintaining the tumor cells with blood flow, nutrients and oxygen supply. In established tumor macrocirculation, the increase NO load maintains tumor blood flow by increased vascular permeability in tumor, by dilation of arteriolar vessels and also by decreased eukocyte-endothelial interactions (Fukumura et al., 1997). The tumor angiogenesis and growth was repressed when the NO production were blocked. Our present study notes that OS extract have a prominent NO scavenging activity and may play a considerable role to suppress the tumor growth. The observation is supportive to the previous study wherein amentoflavone, (a biflavonoid) from Biophytum sensitivum had shown to inhibit the production of NO in B16F-10 melanoma cells, TAMs and peritoneal macrophages and also polyphenols such as rutin posses antiangiogenic property by inhibiting NO production in tumor cell lines and activated macrophage in vitro (Guruvarayoorappan and Kuttan, 2007, 2008).

Reduction in viable cell count and increased non viable cell count towards normal in tumor host suggest antitumor effect against EAC cell in mice. In this study, crude extract increase the non viable cell count upto 70.48% at a dose of 10 mg kg$^{-1}$ which agree with Khatune et al. (2003) and suggested that crude extract have direct relationship with tumor cells as these tumor cells are absorbed the anticancer drug by direct absorption in peritoneal cavity and this anticancer agent lysis the cells by direct cytotoxic mechanism (Kennedy et al., 2001). Anemia and myelosuppression have been frequently observed in ascites carcinoma (Hogland, 1982). Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number (Gupta et al., 2007). Treatment with crude extract brought back the hemoglobin content, RBC and WBC count more or less to normal levels, thus supporting its haematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Preliminary phytochemical study indicated the presence of alkaloid, tannins, phenolic and flavonoid compounds and glycosides in crude extract of Ocimum sanctum. A number of scientific reports indicate certain terpenoids, steroids and phenolic compounds have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis (Sreelatha et al., 2011). Furthermore, flavonoids such as quercetin, kaempferol and their glycosides
have been shown to possess antimitogenic and antimalignant effect (Potsis et al., 1997). Regarding the effective compounds of Ocimum sanctum, Anandjiwala et al. (2006) quantified four major constituents such as eugenol, luteolin, ursolic acid and oleanolic acid in this plant by High Pressure Liquid Chromatography (HPLC). These compounds are known to have multibiological activities. For instance, eugenol possesses anticancer (Yoo et al., 2005) and antiinflammatory (Sharma et al., 1994) activities and luteolin also has anticancer (Kim et al., 2005) and antiinflammatory (Chowdhury et al., 2002) activities. Thus, it can be thought that OS extract definitely exhibited antitumor activity through the combination of these antitumor phytochemicals in the present study.

CONCLUSION

In present study, it was accomplished that crude extract of Ocimum sanctum have persuasive antioxidant activity as well as significantly reduced tumor growth, viability of tumor cells, normalized the hematological profiles, raising life span as compared with those of EAC control mice. Now our next aim is to explore the isolation and characterization of lead compound liable for aforementioned activity from this plant.

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