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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-picrylhydrazyl) 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±0.33 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 Heamoglobin (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 a 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 use 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 (Parida *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 (Kelm *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 (Habibur 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-chiocaltu phenol reagent, sodium chloride, propylene glycol, trypan 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 phytoconstituents like Alkaloids, glycoside, Amino acids, Tanins, 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 Galic 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-picryl-hydrazyl (DPPH): The free radical scavenging activity of MeOH extract, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001). The percentage inhibition activity was calculated from:

$$[(A_0-A_1)/A_0]\times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration ($\mu g\ mL^{-1}$) 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, 1986). 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 2×10^6 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 2×10^6 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 (2×10^6 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.*, 1965).

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 (Habibur Rahman *et al.*, 2011). Behavior of the animals was closely observed for the first 3h 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 IC_{50} 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 (IC_{50} value 16.20±0.33 μg mL^{-1}) showed little bit higher activity than the standard ascorbic acid (IC_{50} value 18.23±0.15 μg mL^{-1}).

Reducing power ability: For the measurement of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} 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, galic 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

Sample	Yield (%)	Total phenols mg g^{-1} plant extract (in GAE)[a]	Total flavonoids mg g^{-1} plant extract (in QA)[b]	Total antioxidant capacity mg g^{-1} extract (in ASC)[c]
OS extract	10.00	39.74±0.05	11.53±0.72	279.3±0.05

[a]Gallic acid equivalents (GAE, mg g^{-1} of each extract) for the total phenolic content. [b]Quercetin equivalents (mg g^{-1} of each extract) for the total flavonoid content. [c]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

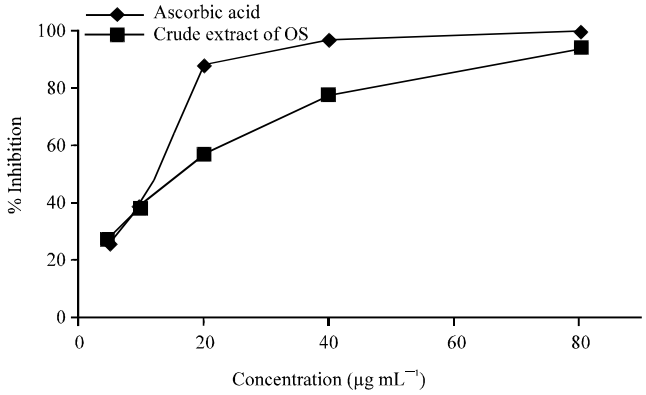

Fig. 1: Free radical scavenging activity of different concentrations of crude extract of *Ocimum sanctum* and ascorbic acid by DPPH radicals

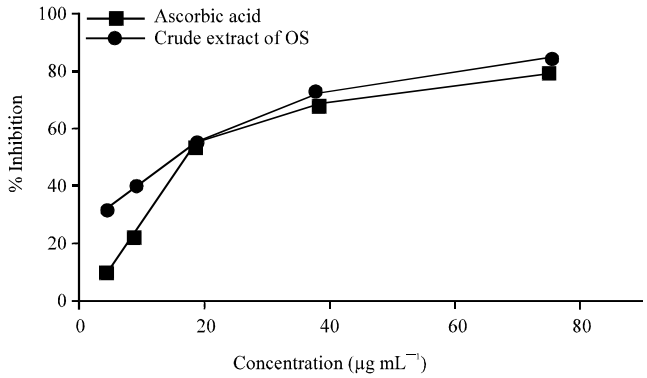

Fig. 2: Percentage inhibition of nitric oxide radical by different concentrations of crude extract of *Ocimum sanctum* and ascorbic acid

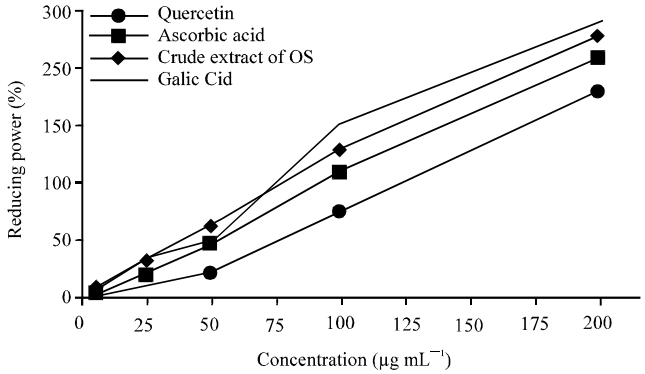

Fig. 3: Reducing power of crude extract of *Ocimum sanctum* and standards (quercetin, ascorbic acid and galic acid) by spectrophotometric detection of Fe^{3+} to Fe^{2+} transformation

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 (%ILS), viable and non-viable tumor cell count in EAC bearing mice

Parameter	EAC control	Crude extract	Bleomycin
Tumor volume (mL)	3.70±0.21	0.72±0.22*	0.55±0.21*
Tumor weight (g)	3.11±0.07	0.92±0.01*	0.82±0.12*
MST (days)	20.00±0.12	33.00±1.81	37.00±0.57
%ILS	0.00	66.66	94.44
Viable cell ($\times 10^7$ cell mL^{-1})	3.90±0.22	0.08±0.02*	0.03±0.15*
Non-viable cell ($\times 10^7$ cell mL^{-1})	0.80±0.24	0.48±0.05*	0.27±0.05*
Total cell ($\times 10^7$ cell mL^{-1})	4.70±0.15	0.56±0.15*	0.30±0.25*
Viable (%)	82.97	15.00	13.15
Non-viable (%)	17.03	85.71	90.00

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

Treatment	RBC ($\times 10^9$, cell mL^{-1})	WBC ($\times 10^6$, cell mL^{-1})	Hemoglobin (g %)
Normal control	5.40±0.33	11.00±1.16	11.60±0.05
EAC control	2.52±0.21*	22.00±0.50*	5.53±1.80*
Crude extract (10 mg kg^{-1})	3.80±1.05**	15.01±1.05**	7.30±0.09**
Bleomycin (0.3 mg kg^{-1})	4.97±0.12**	12.05±0.83**	9.50±1.03**

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 (IC_{50} 16.39± 0.31 μg mL^{-1}) was slightly higher than the standard (IC_{50} 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 intraperitonial 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 *Aristolochia 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 microcirculation, 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* (Guruvayoorappan 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 70.46% 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 antimutagenic and antimalignant effect (Fotsis *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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