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Edible Sword Bean Extract Induces Apoptosis in Cancer Cells In Vitro and Inhibits Ascites and Solid Tumor Development In Vivo

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ABSTRACT

Antitumor potential of edible sword bean (*Canavalia gladiata* (L.)) extract has been evaluated against Daltons lymphoma ascites (DLA) using in vitro and in vivo studies. Methanolic extraction was carried out and in vitro studies were performed against both DLA and A549, lung cancer cell lines. The results revealed that sword bean extract inhibited cell growth and induced apoptosis as evidenced by cytotoxic assay, Hoechst 33342 staining and acridine orange/ethidium bromide dual staining. In vivo studies performed on DLA induced solid as well as ascitic tumors models showed administration of sword bean extract (10 mg/kg B.wt.) could significantly inhibit ascitic and solid tumor development in mice. Therefore, our overall results revealed that *C. gladiata* treatment could significantly inhibit tumor development and induce apoptosis in tumor cells.

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Introduction

Cancer is one of the major health problem around the globe and its second largest disease in terms of mortality after heart disease . The lifestyle and environment of an individual being main factors for the development of approximately 90–95% of cancer cases (1). Worldwide approximately 9.6 million deaths were recorded and 18.1 million adults were diagnosed with cancer in 2018 as per the report of International Agency for Research on Cancer (2). Cancer is mainly caused by the disruptions in normal cellular function and uncontrolled proliferation of genetically altered cells (3).

Plants have been used to prevent and treat various diseases for thousands of years. Plants produce a wide variety of bioactive compounds that apparently have no direct role in plants' growth but these compounds have showed attractive therapeutic effect on various diseases including cancer. On considering the safety aspect of the new drug molecule, natural plant-based therapeutics serve as an attractive alternative compared to traditional therapeutic regimen such as chemotherapy and radiotherapy for cancer treatment. In United States, approximately 50–60% of cancer patients utilize different plant derived agents exclusively with traditional cancer therapy (4). Likewise, plants based foods had been good sources for treatment of diseases in Asian countries.

Sword bean (*Canavalia gladiata*) is an underutilized tropical perennial legume consumed in Asian countries. Sword bean seeds have the potential to be utilized in the manufacturing of processed foods due to their high protein and carbohydrate content (5). Canavalin, a Vicilin-class (7S) storage protein has been reported in sword bean which possess wide range of properties, especially in food processing industry (6). *Bacillus subtilis* fermented sword beans exhibited the presence of gallic acid, methyl gallate and ellagic acid and has shown to increase free radical scavenging and hyaluronidase inhibitory activity (7). Sword beans are excellent sources of gallotannins and their gallotannin-rich extracts can be utilized as natural antioxidant and antibacterial agents with potential health benefits as well as application in food industry (8). Lectins isolated from sword beans exhibited similarities with Concanavalin A in aminoacid composition and sequence and has been shown to have mitogenic activity in splenocytes and enhances the activity of spleen natural killer (NK) cells against YAC-1 cells (9). Sword beans serve as rich sources serine protease inhibitors (SBI-1, -2 and -3) of Bowman-Brik protease inhibitor family which have

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wide range of applications especially in the carcinogenesis inhibition (10). Some of the major beneficial phytochemicals that has been reported in sword beans include canavanine, canavalioside, acylated flavanol glycosides (gladiatoside A1, A2, A3, B1, B2, B3, C1 and C2), robinin, kaempferol 3-O-beta D-galactopyranosyl-7-O-alpha-L-rhamnopyranoside, kaika saponon III and polyamines (sym-homospermidine and canavalimine) (11, 12). Antioxidative potential of *C. gladiata* (L.) seeds extract was already demonstrated in *in vitro*. The sword bean seed extract can inhibit lipid peroxidation through multiple mechanisms including inactivation of scavenging free radicals, reactive oxygen species and chelation of pro oxidative transition metals (13). The antitumor potential of sword bean extract has not been explored against *in vivo* or *in vitro* tumor models till date. The present study was designed to investigate the cytotoxic and antitumor effect of edible sword bean extract against Daltons lymphoma ascites (DLA) induced ascites and solid tumors in BALB/c mice models.

Materials and Methods

Cell Lines

Daltons lymphoma Ascites (DLA) cell lines were used for the study. The cells were obtained from Amala Cancer Research Centre, Thrissur and were maintained in the peritoneal cavity of Balb/c mice.

Lung cancer cells, A549 were purchased from National Center for Cell Sciences, Pune. The cells were maintained in Duelbecco minimum essential medium (DMEM) supplemented with fetal bovine serum (10%) and antibiotics.

Animals

Balb/c mice (Male; 20–25 g) were used for the study. The animals were maintained in the animal house facility of Regional Cancer Centre, Thiruvananthapuram, Kerala under controlled conditions of temperature (25 °C), humidity (55%) and 12:12 h light/dark cycle. The animals were fed with standard pellet diet (Sai Durga Feeds) and water *ad libitum*. The experiments involving animals were performed after obtaining approval from Institutional Animal Ethics Committee (IAEC), Regional Cancer Centre, Thiruvananthapuram.

Plant Materials and Extraction

Edible sword beans (*Canavalia gladiata*) were collected from Horticorp vegetable and fruit market, Thiruvananthapuram. Fruits were authenticated from the Department of Botany, University of Kerala, Thiruvananthapuram. A voucher specimen was deposited in the Laboratory of Immunopharmacology and Experimental Therapeutics, Division of Cancer Research, Regional Cancer Centre, Thiruvananthapuram.

The beans were shade dried, pulverized and the powdered material was subjected to soxhlet extraction using methanol. The extract was concentrated using rotary evaporator. The yield of the extract was 18% (w/v).

For *in vitro* studies, the extract was suspended in DMSO and diluted further to the required concentration using DMEM supplemented with 10% fetal bovine serum and antibiotics. For *in vivo* experiments, the extract was suspended in gum acacia (0.01%) and administered intraperioneally (i.p.) for 10 consecutive days.

GC/MS Analysis

GC/MS analysis of the sword bean extract was performed using a Thermo GC-Trace Ultra VER:5.0 (Germany). MS DSQ II electron ionization mode with a mass range of m/z 50–650 and ionization energy of 70 eV was used for MS detection. GC was performed in a splitless mode and helium was used as the carrier gas at a flow rate of 1.0 ml/min. Sample volume of 1 µl was used for the detection. Retention time of the compound was determined by comparing the retention time of standards from National Institute of Standards and Technology and Wiley 9.0 library was used for the identification of major and essential compounds from the sample.

Cytotoxicity Assay Using Trypan Blue Dye Exclusion Assay

Viable DLA cells was aspirated from the peritoneal cavity of the animals and washed with phosphate buffered saline (PBS, pH 7.2). The DLA cells (1×10^6 cells/ml) were incubated with sword bean extract at an escalating concentration of 100, 200 and 400 µg/ml, respectively for 3 h. After the incubation trypan blue (0.04% in PBS; 0.1 ml) was added and the total number of live and dead cells were manually counted using a hemocytometer. The viable cell percentage was calculated using the equation 1 – (Number of blue non-viable cells/number of total cells) × 100 (14).

MTT Assay

A549 cells were harvested during their log phase and seeded (1×10^6 cells) in flat bottom microtiter plate and incubated overnight at 37 °C and 5% CO_2. The cells were treated with escalating concentration of sword bean extract (100, 200, 400 and 500 µg/ml, respectively) and further incubated for 48 h at 37 °C and 5% CO_2. Four hours before the termination of the incubation period MTT (0.1 ml; 5 mg/ml) was added and after the incubation, the cells were centrifuged and lysed with DMSO (0.1 ml). The absorbance of the color developed was taken at 570 nm using an ELISA plate reader (BioTek, USA). The percentage growth inhibition was calculated using the equation 100 – (Absorbance of treated cells/ absorbance of the control cells) × 100.

Detection of In Vitro Apoptosis Using Acridine Orange/Ethidium Bromide (AO/EtBr) Staining Assay and Hoechst 33342 Staining Assay

Freshly aspirated DLA cells (1×10^5 cells) were washed with phosphate buffered saline (pH 7.2) and incubated with sword bean extract (400 µg/ml) for 3 h at 37 °C and 5% CO_2. The cell suspension were then mixed with AO/ EtBr dye (1 µl; 100 µg/ml) on clean microscopic slides and then observed under fluorescent microscope (Olympus BX43) using the FITC filter.

In an another experiment, DLA cells (1×10^5 cells) were washed with phosphate buffered saline (pH 7.2) and incubated with sword bean extract (400 µg/ml) for 3 h at 37 °C and 5% CO_2. The cell suspension were then mixed with Hoechst 33342 stain (5 µl; 1 mg/ml) and incubated for 30 min, at 37 °C and 5% CO_2. The cells were then observed under fluorescent microscope (Olympus BX43) using the blue filter.

In Vivo DLA Induced Ascites Tumor Study

Male Balb/c mice (20–25 g) were randomly divided in to four groups ($n = 6$/group). Group I served as normal untreated group. Group II, III and IV animals were injected with DLA cells (1×10^6 cells). Group II served as tumor control whereas group III and IV were administered with cyclophosphamide (standard drug; 10 mg/kg B.wt.) and sword bean extract (10 mg/ kg B.wt.), respectively for 10 consecutive days starting from the same day of tumor induction (Figure 1a). Body weight was recorded every third day till day 15. Blood was collected by tail vein bleeding (on day, 10 and 15). Three animals from each group were euthanized by cervical dislocation on day 10 and day 15. Peritoneal fluid was aspirated and used for

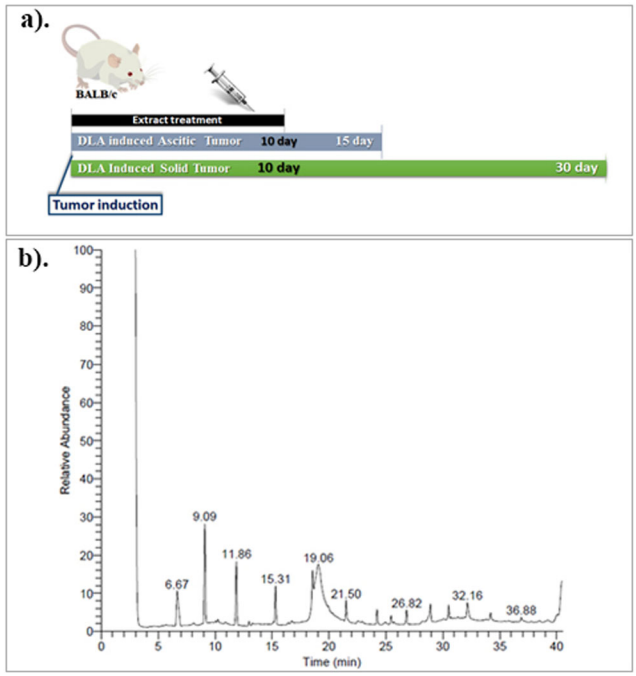

Figure 1. (a) Schematic representations of in vivo DLA induced ascitic and solid tumor models. BALB/c mice were used in the experiment each group contains six ($n = 6$) animals. Treatment start 1 h, after tumor cell inoculation. At the end of experiment mice were sacrificed and various parameters were assessed (b) gas chromatography-mass spectrometry analysis of bioactive compounds from sword bean methanolic extracts.

biochemical estimation and DNA fragmentation assay. The liver was dissected out, washed with physiological saline (pH 7.2) and a portion of the liver was subjected for histopathological analysis.

Quantification of Intracellular Glutathione (GSH)

DLA cells (1×10^5 cells) were collected by aspirating the fluid from peritoneal cavity of the animals (as mentioned above) were subjected to cell lysis by sonication. The lysed cells were then subjected to quantification of intracellular glutathione (GSH) (15).

Blood Hematology and Biochemical Analysis

Blood samples collected from the experimental animals were subjected to total WBC count and quantification of Hemoglobin content was also performed (16). The serum obtained after centrifugation was used for quantifying the ALP and NO levels (17).

DNA Fragmentation Assay

The DLA cells aspirated from the experimental animals were incubated with cell lysis buffer and subjected to isolation of genomic DNA using DNA isolation kit (Zymo

Table 1. GC-MS analysis of the sword bean extract.

S. no.	Retention time	Compound name	Molecular formula	Molecular weight	Peak area	%
1	766	2,2′-dihydroperoxy 2,2,2′,2′-tetrapropyl ether	$C_{14}H_{30}O_5$	278	1.94	23.32
2	704	1,3-butanedione, 2-methyl-1-phenyl-(CAS)	$C_{11}H_{12}O_2$	176	1.94	6.23
3	767	4-homocubyl acetate	$C_{11}H_{12}O_2$	176	1.94	3.77
4	573	1-3-(2-hydroxypyridyl)-2-ethoxyacrylonitrile	$C_{10}H_{10}N_2O_2$	190	1.94	3.63
5	533	1(3H)-Isobenzofuranone,3-ethoxy-(CAS)	$C_{10}H_{10}O_3$	178	1.94	3.06
6	512	1-acetoxy-3,7-methano-bicyclo [3,3,1]nonane	$C_{12}H_{18}O_2$	194	1.94	2.82
7	443	Butanoic acid, 3-phenyl-2-propenyl ester (CAS)	$C_{13}H_{16}O_2$	204	1.94	2.82
8	497	1H-Inden-1-ol,2,3-dihydro-(CAS)	$C_9H_{10}O$	134	1.94	2.49
9	417	1,2,3,4,5-pentamethylbenzene	$C_{11}H_{15}D$	148	1.94	2.30
10	945	1-butanol,4-butoxy-(CAS)	$C_8H_{18}O_2$	146	1.94	2.03

Research, USA). The isolated DNA was loaded in agarose gel (1%) and subjected to electrophoresis followed by ethidium bromide staining.

In Vivo *DLA Induced Solid Tumor Study*

Solid tumor was induced in Balb/c mice by injecting DLA cells (1×10^6 cells) into the right hind limb of the animals. The animals were divided into three groups ($n = 6$/group). Group I served as tumor control whereas group II and III were administered with sword bean extract (10 mg/kg B.wt.) and cyclophosphamide (standard drug; 10 mg/kg B.wt.), respectively for 10 consecutive days starting from the same day of tumor induction. Tumor volume was measured using vernier caliper every third day for 30 day and tumor volume was calculated using the equation $4/3\pi {r_1}^2 r_2$, where r_1 and r_2 are the radius of the tumors at two different planes. On day 30, the animals were euthanized by cervical dislocation and the tumor mass was dissected out and washed with physiological saline (pH 7.2). A portion of the tumor mass was fixed in formalin (5%) and subjected to histopathological analysis. The remaining portion of the tumor mass was homogenized and the tumor cells were lysed by sonication and subjected for intracellular glutathione quantification as mentioned above.

Statistical Analysis

All the values were expressed as mean ± SD. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad InStat Version 3.0 (GraphPad Software, USA). $P < 0.05$ were considered to be statistically significant.

Results

GC/MS Analysis of Sword Bean Extract

The GC/MS chromatogram of sword bean extract is shown in Figure 1b. The major components identified from the sword bean extract were 2, 2′-dihydroperoxy 2, 2, 2′, 2′-tetrapropyl ether (23.32%); 1, 3-butanedione, 2-methyl-1-phenyl-(CAS) (6.23%); 4-homocubyl acetate (3.77); 1-3-(2-hydroxypyridyl)-2-ethoxyacrylonitrile (3.63%); 1-(3H)-Isobenzofuranone, 3-ethoxy-(CAS) (3.06%) (Table 1).

Cytotoxic Effect of Sword Bean Extract Tumor Cell Lines

The results obtained from this study showed clearly decreased the levels of DLA cell viability in sword bean extract treated cells is shown in Figure 2a. The IC_{50} value was found to be 181 ± 0.62 µg/ml. The MTT assay on A549 cells using sword bean extract showed percentage inhibition of cell growth by 41%, 48%, 52% and 63% on treatment at a concentration of 100, 200, 400 and 500 µg/ml, respectively (Figure 2b). Sword bean extract inhibited the proliferation of A549 cell lines in a dose-dependent manner with IC_{50} value of 278 ± 0.27 µg/ml, respectively.

Effect of Sword Bean Extract on In Vitro Apoptosis Induction in DLA Cells

The morphological evaluation of the DLA cells treated with sword bean extract were determined by Hoechst 33342 staining and acridine orange/ethidium bromide dual staining (Figure 2c and d). Hoechst staining results clearly revealed the presence of condensed apoptotic nuclei on treatment with sword bean extract at a concentration of 400 µg/ml compared with untreated tumor control cells. Similarly, acridine orange/ethidium bromide dual staining also confirmed that treatment with sword bean extract could induce apoptosis in DLA cells by disturbing cell morphology and cell membrane integrity in a concentration-dependent manner (Figure 2d).

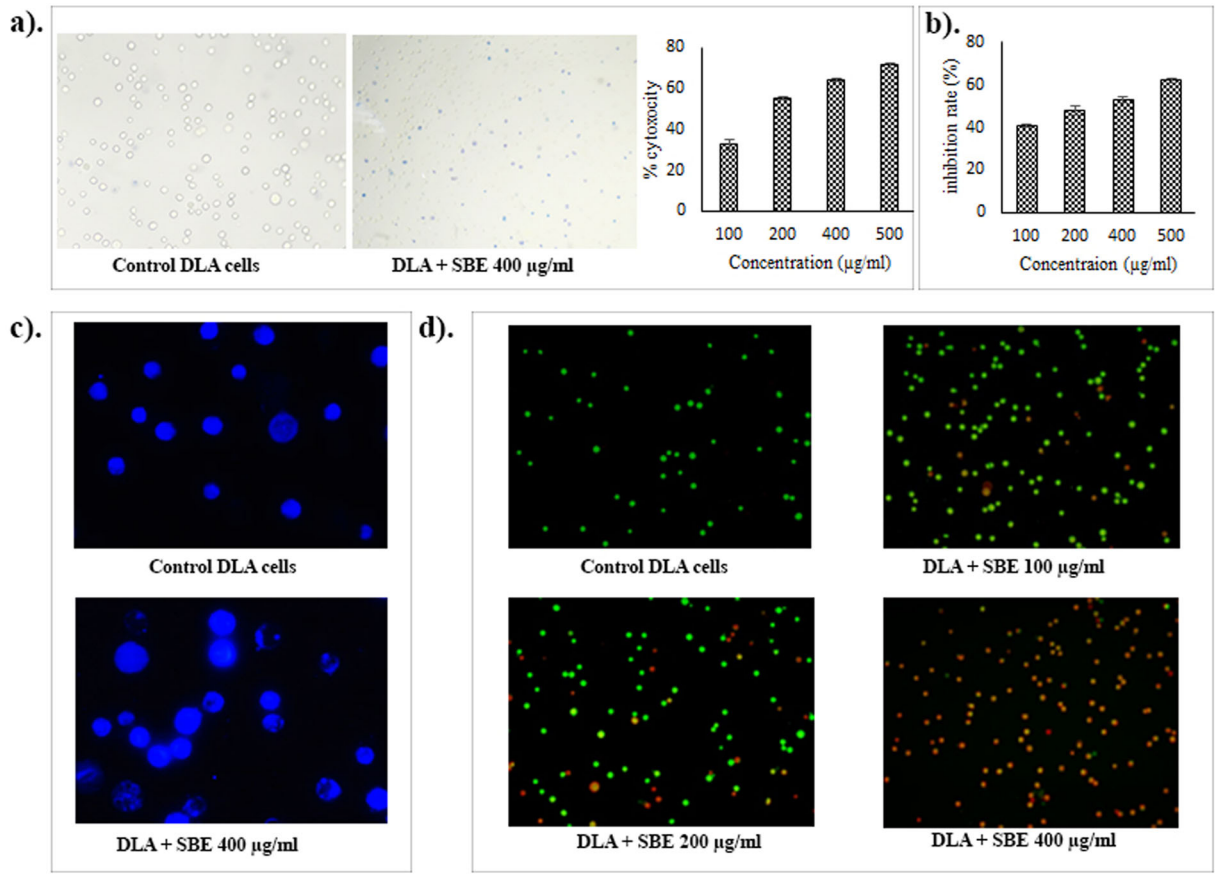

Figure 2. In vitro cytotoxicity and apoptosis studies on cancer cell lines using sword bean extract (SBE) (a) cytotoxic effect of SBE on the viability of DLA cell lines using Trypan blue exclusion assay, (b) cytotoxic effect of SBE on A549 cell lines by MTT assay, (c) Hoechst staining: fluorescent microscopic images (objective 20×) of control DLA cells and DLA + SBE (400 µg/ml), (d) acridine orange/Etbr staining: fluorescent microscopic images (objective 10×) of control DLA cells, DLA + SBE (100 µg/ml), DLA + SBE (200 µg/ml) and DLA + SBE (400 µg/ml).

Effect of Sword Bean Extract on In Vivo *Ascites Tumor Reduction*

Treatment with sword bean extract significantly decreased the volume of ascites fluid (2.67 ± 0.57 ml) and the development of ascites tumor in experimental animals compared to untreated tumor control animals (6.67 ± 1.15 ml). The results of sword bean extract effect on *in vivo* ascites tumor reduction is shown in Figure 3a. Similarly, the body weight of the tumor bearing animals after sword bean extract (27.6 ± 0.56 g) was found to be significantly decreased compared to tumor bearing control animals (32.0 ± 3.4 g). As expected, the standard drug treated animals were shows significant reduction in tumor volume (1.66 ± 0.15 ml) as well as body weight (24.6 ± 1.14 g) on the same day (Figure 3b).

Histopathological analysis of liver from normal, DLA tumor control, standard drug cyclophosphamide and sword bean treated animals were carried out after experimental period (after 15th day) as shown in Figure 3c. This result revealed that normal untreated animals shows normal liver morphology but in case of tumor control groups shows loss and altered morphology of hepatic cells. Sword bean extract treatment retained normal liver architecture compared to DLA tumor control groups. The treatment with standard drug cyclophosphamide also showed normal hepatic architecture.

Effect of Sword Bean Extract on Intracellular Glutathione (GSH)

The intracellular GSH level on 10th (9.47 ± 0.40) and 15th day was significantly increased in the tumor control groups and the maximum GSH level found on the 15th day (12.34 ± 0.20) of tumor progression. The GSH levels of sword bean extract treated animals were significantly decreased compared to tumor control animal groups on the same day (7.80 ± 0.11). Also, standard drug cyclophosphamide treatment exhibited significant reduction of GSH levels when compared to control groups. The results of sword bean extract on

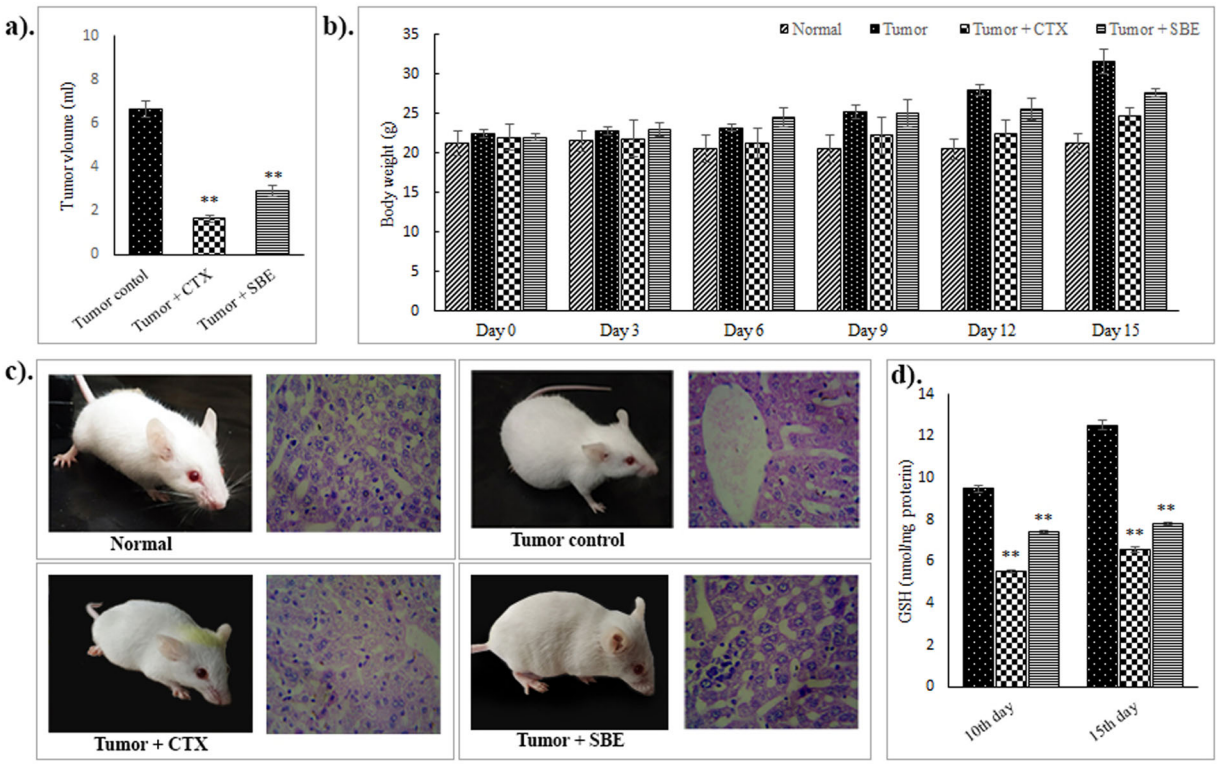

Figure 3. *In vivo* ascites tumor development study. (a) Effect of SBE on the ascites tumor volume. (b) Effect of SBE on body weight of DLA induced ascitic tumor animals. (c) Histopathological changes of liver sections in the tumor bearing animals (day 15 after DLA injection). (d) Intracellular glutathione (GSH) levels in DLA cells during tumor progression.

the levels of intracellular glutathione is presented in Figure 3d.

Effect of Sword Bean Extract on Blood Hematology and Biochemical Parameters

Hemoglobin levels of tumor control group on day 15 were found to be altered from the normal group. There was a significant decrease (12.32 ± 0.41) in Hb levels of the DLA tumor control (untreated) mice on 15th day when compared to normal mice (14.82 ± 0.47). Sword bean extract treatment improved the Hb content nearly similar to normal levels on 15th day. Treatment with sword bean extract significantly increased the hemoglobin level (13.63 ± 0.39) compared to DLA tumor bearing control animals. Standard drug (cyclophosphamide) treatment also significantly increased the hemoglobin level (14.14 ± 0.21) when compared to the tumor control group (Figure 4a).

DLA control groups resulted in a significant increase in the level of total WBC on 10th and 15th day, respectively when compared with the normal animal (Figure 4b). On 15th day, total WBC levels in the DLA tumor control (untreated) mice were increased (17,130 ± 702) compared to those in with normal mice (12,433 ± 152). Interestingly, administration of sword bean extract led to the significant reduction (14,650 ± 738) in the WBC counts from the values of the tumor control mice on the same day. In contrast, the standard drug cyclophosphamide treatment also showed significant reduction (11,350 ± 507) in the WBC levels when compared to the tumor control mice.

The effects of the sword bean extract treatments on serum nitric oxide level is shown in Figure 4c. Nitric oxide level in the tumor control groups were found to be maximum on the 15th day of tumor progression. In sword bean extract treated animals the NO levels significantly decreased compared to tumor control animal groups on the same day (9.84 ± 0.05). Moreover, the standard drug cyclophosphamide treatment also exhibited significant reduction of serum NO levels when compared to tumor control group.

Treatments effect of sword bean extract on serum alkaline phosphatase (ALP) levels is shown in Figure 4d. The serum ALP levels was significantly increased in DLA tumor control groups (72.13 ± 1.49). After administration of sword bean extract the serum ALP levels of the tumor bearing animals were significantly reduced as compared to the tumor control groups (41.79 ± 3.28). Likewise, standard drug cyclophosphamide treatment

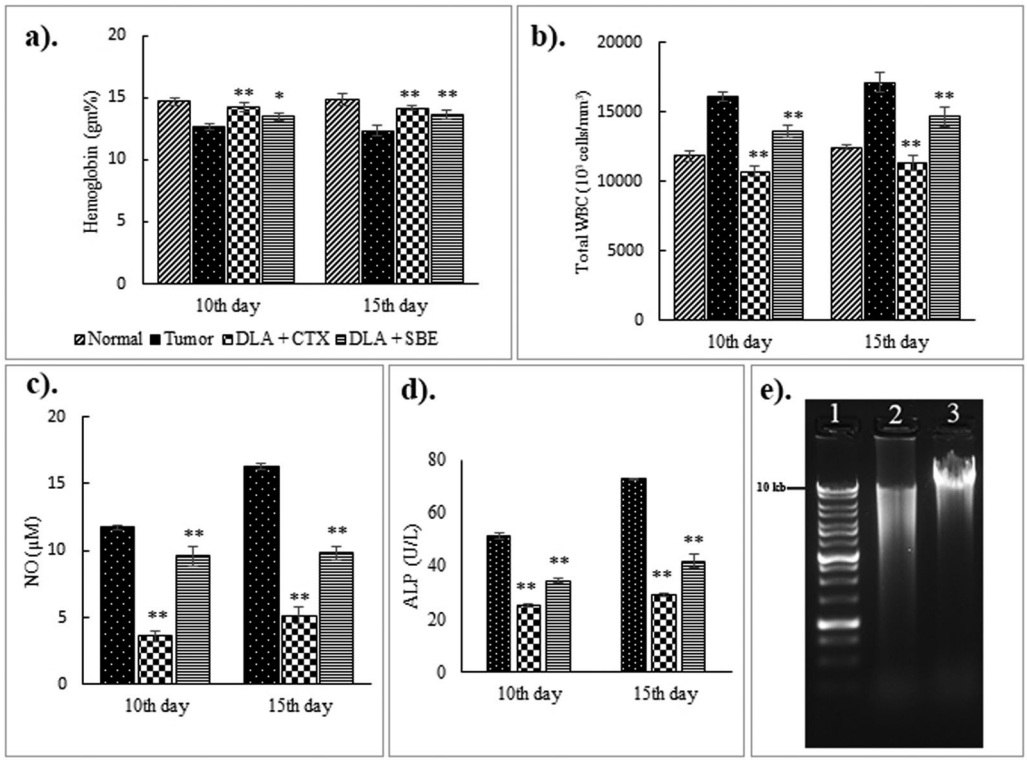

Figure 4. Effect of SBE on hematological parameters (a) hemoglobin, (b) total WBC levels during tumor development, (c) serum NO levels during tumor development, (d) serum ALP levels during tumor development, (e) DNA fragmentation assay; Lane 1: DNA marker, Lane 2: DLA + SBE and Lane 3: DLA ascitic tumor control.

also showed significant reduction (29.11 ± 0.57) in the serum ALP levels when compared to the tumor control mice.

Effect of Sword Bean Extract on DNA Fragmentation

DNA fragmentation analysis was performed to find out apoptosis induction activity of the sword bean extract on the DLA induced ascitic tumor mice. The genomic DNA isolated from sword bean extract treated groups showed DNA damage as evidenced by shearing of DNA when compared to tumor control groups wherein DNA was observed as a single band. These result indicated that sword bean extract treatment has the capability to induce apoptosis in DLA cells (Figure 4e).

Effect of Sword Bean Extract on In Vivo Solid Tumor Reduction

In order to study the antitumor effect of sword bean extract, the solid tumor mice models were induced by intramuscular injection of DLA cells into the right hind limb of mice. DLA induced solid tumor bearing mice were treated with sword bean extract for a period of 10 consecutive days. The effect of sword bean extract on DLA induced solid tumor is shown in Figure 5a. There was significant reduction in tumor volume of sword bean extract treated group. On 30th day the mean tumor volume of the control animals was 933 ± 54 mm^3, which was significantly higher compared to sword bean extract treated animals (341 ± 34 mm^3). Further, the standard drug cyclophosphamide treatment also exhibited significant reduction in tumor volume (227.46 ± 21 mm^3) when compared to tumor control group.

The effect of sword bean extract on cellular GSH level of DLA induced solid tumor mice is shown in Figure 5b. Treatment with sword bean extract (22.63 ± 0.49) significantly decreased the cellular GSH level of solid tumor bearing animals when compared to tumor control animal groups (28.39 ± 0.28) on 30th day of the experiment. Additionally, the standard drug cyclophosphamide treatment also exhibited significant reduction (16.71 ± 0.48) of cellular GSH levels when compared to tumor control group.

The histopathological observation of DLA induced solid tumor section of tumor control, cyclophosphamide and sword bean extract treated animals is shown in Figure 5c. DLA induced solid tumor control showed infiltrating neoplasm composed of cells

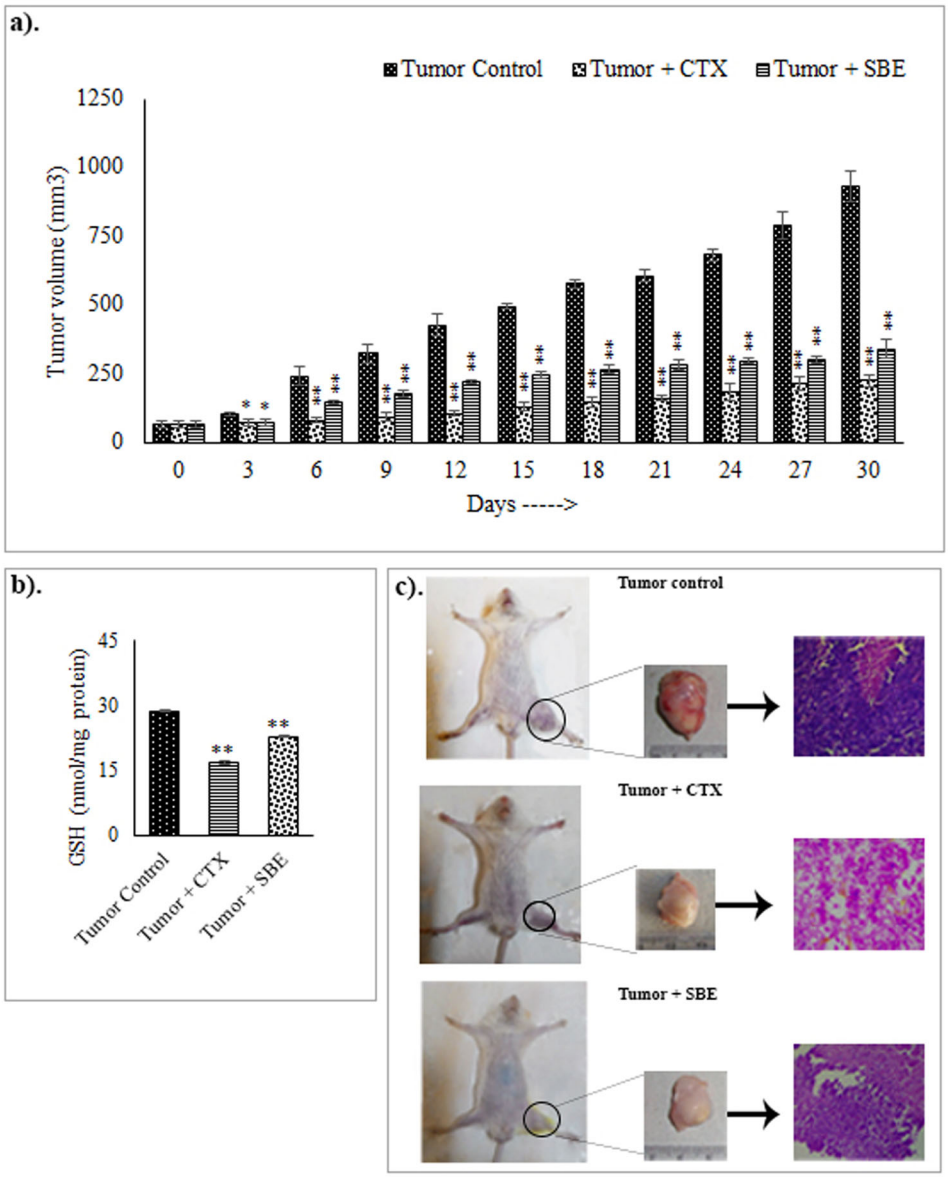

Figure 5. DLA induced solid tumor study; (a) effect of SBE on DLA induced solid tumor development on the indicated days after the inoculation with DLA cells. Treated animals received 10 doses of SBE (10 mg/kg B. wt.). (b) Cellular GSH levels of SBE treated and tumor control groups. (c) Tumor mass and tumor histopathology (day 30).

arranged in sheets and higher mitosis rate. In case of cyclophosphamide and sword bean extract treated groups showed lesser number of mitosis rate and reduced infiltrating neoplastic cells. Thus the results highlight the protective effect of sword bean extract against DLA induced solid tumor.

Discussion

Cancer, one of the largest life threatening disease and its incidence and mortality are rapidly growing worldwide (2). It is essential to development of highly effective, specific therapeutic agent for treatment and prevention of cancer. Currently different types of therapeutic formulations are available either chemical or naturally synthesized but majority of them are facing many challenges. Emerging interest toward drugs derived from natural products and its potential outcomes had led to considerable increase in drug discovery and play a dominant role in pharmaceutical care (18).

Sword bean (*Canavalia gladiata* (L.)) is a one of the very underutilized fabaceae family legume with lot of medicinal applications. *C. gladiata* (L.) possess rich source of alkaloids, flavonoids, phenolics and polysaccharides. The dried sword bean is composed of starch (35–45%) and protein (22–29%), with a good balance of amino acids (19). The present study investigated the cytotoxic and antitumor effect of sword bean

extract against DLA induced ascitic and solid tumor models. In the initial phase of the study, we evaluated the cytotoxic activity of sword bean extract carried out using trypan blue (20) and MTT assay against DLA and A549 cell lines, respectively. It was observed that sword bean extract inhibited the proliferation of both DLA and A549 cell lines in a dose-dependent manner with IC_{50} value of $181 \pm 0.62\,\mu g$ and $278 \pm 0.27\,\mu g/ml$, respectively. Most of the clinically used chemotherapeutic agent possess significant cytotoxic activity *in vitro*. The results of present study showed that sword bean extract have cytotoxicity against DLA and A549 cell lines. Also, acridine orange/ethidium bromide staining and Hoechst staining were performed to find out the apoptotic effect of sword bean extract on DLA cells. These *in vitro* study results revealed that sword bean extract has potential to inhibit cell growth and induce the apoptosis in DLA cells. *In vitro* results indicated the partial significant antitumor activity of sword bean extract against DLA induced ascitic and solid tumor. Thus, for further investigation efficacy of sword bean extract was studied in DLA induced ascitic and solid tumor in mice model.

In the present study, DLA tumor bearing mice showed increasing body weight due to the accumulation of ascitic fluid in the peritoneal cavity along with abnormal peritoneal enlargement in tumor control mice. Treatment with sword bean extract reduced the tumor volume, body weight and also increased the lifespan compared to tumor control mice. In DLA tumor bearing animals elevated levels of WBC and reduced level of Hb were observed which when treatment with sword bean extract restored the normal level of WBC and hemoglobin level, thus this result showed the hematopoietic protecting activity of sword bean extract.

The effect of administration of sword bean extract was tested against DLA induced solid tumor bearing animals. The solid tumor was induced by intramuscular injection of DLA cells into the right hind limb of mice. The tumor mass of solid tumor and is mostly epithelial driven (21). We observed that significant reduction of tumor development in sword bean extract treated groups when compared to tumor control animals. Also it was observed that elevated levels of cellular GSH and serum ALP in DLA tumor control which was significantly reduced in sword bean extract treated animals. These results strongly prove the antitumor capacity of sword bean extract against DLA induced solid tumor.

Glutathione (GSH), is the most profuse natural non-protein thiol tripeptide found within almost all cells in eukaryotes (22). It is involved in cell proliferation against free radicals, and also relevant in regulating carcinogenic mechanisms such as tumor cell proliferation and its metabolism (23–25). The increased GSH levels and resistance to chemotherapeutic agents have been observed in doxorubicin, melphalan, and arsenic (26). However, GSH depletion is a common feature not only of apoptosis but also of for other types of cell death, including necrosis and autophagy. Thus, GSH depletion based therapeutic strategy improve the efficacy of cancer treatment (27). Treatment with sword bean extract significantly reduced intracellular GSH levels in extract treated when compared to untreated tumor control animals.

Nitric oxide is a free radical which plays multiple role in intracellular and extra cellular signaling mechanisms. This highly reactive molecule mediate generation of reactive nitrogen species, DNA damage through the formation of carcinogenic nitrosamines and inhibition of DNA damage repair system (28, 29). Similarly, NO have effect on further stages of cancer progression. Thus, it can be considered as tumor initiating agent (30). Similarly, the results of the present study indicate the regulation of NO levels and its potential link with the antitumor activity of sword bean extract in DLA induced ascitic tumor animals. Alkaline phosphatase is an important prognostic factor for several cancers (31). Serum ALP levels are frequently elevated in cancer patients (32). Concomitant with the above literature elevated levels of serum ALP in DLA induced ascitic tumor animals was also observed. Treatment with sword bean extract showed the protective effect by decreasing the ALP to normal levels.

GC/MS is one of the best technique to identify the constituents of plant extract based on retention time and peak area. Retention time, molecular formula and peak area were used for the conformation of phytochemical compounds (33). GC/MS analysis of sword bean extract revealed the presence of numerous phytochemical such as 2,2′-dihydro peroxy 2, 2, 2′, 2′ tetrapropyl ether, 1, 3-butanedione, 2-methyl-1-phenyl-(CAS), 4-homocubyl acetate, 1-3-(2-hydroxypyridyl)-2-ethoxyacrylonitryl, 1-(3H)-isobenzofuranone, 3-ethoxy-(CAS), 1-acetoxy-3, seven methano-bicyclo [3, 3, 1] nonane, butanoic acid, 3-phenyl-2- propenyl ester (CAS), 1H-inden-1-ol, 2, 3-dihydro-(CAS), 1, 2, 3, 4, 5-pentamethyl benzene, 1-butanol, 4-butoxy-(CAS). This study helped to find out the lead phytochemical present in sword bean extract, further better

investigation has to be addressed to find out exact biocompounds which are involved in the apoptosis induction.

In conclusion, experimental findings suggest that treatment with sword bean extract was effective for inducing cytotoxicity *in vitro* and inhibiting tumor progression in *in vivo* BALB/c tumor models. Therefore, sword bean extract may be used as a natural antitumor agent to inhibit tumor progression. Further investigations are in progress to identify the underlying molecular targets responsible for antitumor activity and to reveal the exact molecular mechanism behind its therapeutic action.

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Disclosure statement

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