RESEARCH COMMUNICATION

Antitumor and Cytotoxic Activities of Methanol Extract of *Indigofera linnaei* Ali.

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Abstract

Methanol extract of *Indigofera linnaei* (MEIL) was investigated for antitumor, cytotoxic and antioxidant activities against transplantable tumors and human cancer cell lines. *In vitro* cytotoxicity was evaluated in HeLa, Hep-2, HepG-2, MCF-7, HT-29, Vero and NIH 3T3 cells by MTT assay and *in vivo* antitumor activity with Ehrlich ascites carcinoma (EAC) and Dalton's ascites lymphoma (DLA) tumor-bearing mice. Activity was measured by monitoring the mean survival time, effect on hematological parameters, antioxidant enzyme levels and solid tumor volume. The extract exhibited strong *in vitro* cytotoxicity against all the tested cancer cell lines, but it was found to be safe with normal cells. MEIL at the dose of 200 and 400 mg/kg, significantly increase the mean survival time (P<0.001), exerted a protective effect on the hemopoietic system, demonstrated *in vivo* antioxidant activity and significantly reduce solid tumor volume (P<0.01). These results show a significant antitumor and cytotoxic effect of MEIL against EAC, DLA and human cancer cell lines and support the ethnomedical use of *Indigofera linnaei*.

Keywords: *Indigofera linnaei* - antitumor activity - in vitro cytotoxicity

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Introduction

Over the past few years, cancer has remained a major cause of death and the number of individuals affected with cancer is continuing to expand. Hence, a major portion of the current pharmacological research is devoted to anticancer drug design customized to fit new molecular targets (Xia et al., 2004). Due to enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities (Kim et al., 2005; Indap et al., 2006). The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search of anticancer agents from plant sources is the selection of plants based on ethnomedical leads (Kintzios, 2006).

Indigofera linnaei Ali (Syn: I*ndigofera enneaphylla* Linn) is belonging to the family Fabaceae and is a reputed indigenous medicine. It is a small trailing, much branched annual or biennial herb, distributed throughout India. The juice of the plant is used as antiscorbutic and diuretic and for burns and epilepsy. It has long been used by tribes and native medical practitioners to treat rheumatism, arthritis, inflammation, tumor and liver diseases (Anonymous, 1992). Literature review revealed that three nitropropanoyl esters of glucose namely,1,2,6-tri-O-(3-nitropropanoyl)-β-D-glucopyranose,2,3,4,6-tetra-O-(3-nitropropanoyl)-α-D-glucopyranose and 3,4,6-tri-O-(3-nitropropanoyl)-α-D-glucopyranose were isolated from the aerial parts of *Indigofera linnaei* (Majak et al., 1992). The plant exhibit wound healing activity in rats (Hemalatha et al., 2001). A new isoflavone namely 7,8-methylenedioxy-4'-methoxyisoflavone was isolated from the entire plant of *Indigofera linnaei* (Rajendraprasad and Chakradhar 2004). Except these studies, so far, no other investigations on the biological activities of *I. linnaei* have been carried out. Hence, in the present study, based on the ethnomedical claims, we investigated the antitumor properties of the methanol extract of *Indigofera linnaei I. linnaei* (MEIL) against Ehrlich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLA) tumor models along with its effects on antioxidant status.

Materials and Methods

Chemicals

5-Fluorouracil (5-FU) was obtained from Dabur Pharmaceutical Ltd (New Delhi, India). Trypan blue, thiobarbituric acid, trichloroacetic acid, ethylenediaminetetracetic acid (EDTA), RPMI-1640 media and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltertazolium bromide (MTT) were procured from HiMedia (Mumbai, India). Dimethyl sulfoxide and methanol were obtained from Loba Chemie (Mumbai, India). All other chemicals used were of analytical grade.

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Plant material and Extraction

Entire plants of *I. linnaei* were collected from the foothills of Yercaud in the month of November 2008. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference (Voucher No. P. Ch. IL 002). The plant material was shade dried, pulverized and extracted (500 g) with 80% methanol at room temperature for 72 h. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (40 ºC to 50 ºC) in a rotary evaporator. The extract was a dark yellowish brown solid weighing 50.2 g (yield, 10.4 %) and was preserved in a vacuum d esiccator until further use.

Preliminary Phytochemical Screening

The extract was screened for the presence of various phytochemical constituents employing standard screening tests (Wagner et al., 1984). Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc., was used.

Tumor cells and inoculation

Normal Mouse Embryonic Fibroblast (NIH 3T3), Normal African Green Monkey Kidney Cells (Vero), Human Cervical Cancer cells (HeLA), Human Laryngeal Epithelial Carcinoma (HeP-2), Human Liver Cancer cells (HepG2), Human Breast Cancer cells (MCF-7) and Human Colon Cancer cells (HT-29) were obtained from National Centre of Cell Sciences (Pune, India). The cultures were maintained in Dulbecco's modified eagles medium (DMEM) containing 10 % inactivated calf serum and were grown in 25cm^2 tissue culture flasks (Tarson Products Ltd, Kolkatta, India) until confluent and used for cytotoxic assays. EAC and DLA cells were supplied by Amala Cancer Research Centre (Trissur, Kerala, India). The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation. Tumor cells aspirated from the peritoneal cavity of mice were washed with normal saline and were used for further studies.

Preparation of suspensions and solutions

For cytotoxicity assays, the extract was dissolved in dimethyl sulfoxide (DMSO) and the volume made up to 10 ml to obtain a 1000 μg/ml stock solution. Serial two-fold dilutions were made using DMSO to get lower concentration. MEIL was suspended in distilled water using sodium carboxy methyl cellulose (CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter to study *in vivo* antitumor activity.

Short term cytotoxic activity

Short term cytotoxicity assay of MEIL was determined by using trypan blue dye exclusion method (Sunila and Kuttan, 2004). EAC and DLA cells were cultured in peritoneal cavity of healthy albino mice by injecting a suspension of tumor cells (1 x 10^6 cells/ml) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of the mice on day 15 and washed with normal saline and centrifuged for 15 min at 1500 rpm in a cooling centrifuge. The pellet was resuspended with normal saline and the process was repeated until to get a clear supernatant. Finally the cells were suspended in a known quantity of normal saline and the cell count was adjusted to 1 x 10^6 cells/ml. Then, 0.1 ml of this cell suspension was distributed in to Eppendrof tubes and exposed to 0.1 ml of various concentrations of MEIL (500 – 31.25 μg/ml) and incubated at 37 ºC for 3 h. After 3 h, the trypan blue dye exclusion test was performed to determine the percentage cytotoxicity and the GI$_{50}$ was calculated.

In vitro cytotoxicity studies on human cancer cell lines

Stock cells of Normal Mouse Embryonic Fibroblast (NIH 3T3), Normal African Green Monkey Kidney Cells (Vero), Human Cervical Cancer cells (HeLA), Human Laryngeal Epithelial Carcinoma (HeP-2), Human Liver Cancer cells (HepG2), Human Breast Cancer cells (MCF-7) and Human Colon Cancer cells (HT-29) were cultured in RPMI-1640 and DMEM supplemented with 10 % calf serum, penicillin (100 IU/ml) and streptomycin (100 μg/ml) in a humidified atmosphere of 5 % CO_2 at 37 ºC until confluent. The cells were dissociated with 0.2 % trypsin and 0.02% EDTA in PBS. The cytotoxic assay was carried out by adding 0.1 ml of cell suspension containing 10,000 cells to each well of a 96-well microtitre plate (Tarson, Kolkatta, India) and fresh medium containing various concentrations of extract was added at 24 h after seeding. Control cells were incubated without the extract and with DMSO. The microtitre plates were incubated at 37 ºC in a humidified atmosphere with 5 % CO_2 for a period of 72 h. The percentage cytotoxicity and GI$_{50}$ were determined by the standard MTT assay method (Vijayan et al., 2003).

Animals

Healthy male Swiss albino mice weighing 25 ± 2g were obtained from Venkateshwara Enterprises, Banglaore, India. The mice were grouped and housed in polypropylene cages and maintained under standard conditions (25 ± 2 ºC) with 12 h dark/light cycle. The animals were fed with standard animal pellet diet and water ad libitum. The experiment protocols received clearance from the Institutional Animal Ethical Committee (IAEC) and CPCSEA, Chennai, India (Proposal No: SVCP/IAEC/Ph.D/01/2008-09 dt 24.12.2008).

Acute toxicity studies

The oral acute toxicity study of MEIL was carried out in Swiss albino mice using the OECD guidelines (Acute Oral Toxicity 423; OECD, 2001). The animals received MEIL starting at 2 g/kg orally by gavage and were observed for toxic symptoms and mortality continuously for first 4 h after dosing. Finally, the number of survivors was noted after 24 h and these animals were then maintained for further 13 days with observations made daily.

Antitumor studies

Antitumor activity of MEIL was evaluated by the procedure described by Senthil Kumar et al., 2007.

Effect of MEIL on survival time

Healthy Swiss albino mice were divided into eight groups (I-VIII) each group consisting of six animals. Group I – IV were inoculated with EAC cells (1 x 10^6 cells/mouse) and Group V – VIII were inoculated with DLA cells (1 x 10^6 cells/mouse) on day '0' and treatment with MEIL started 24 h after inoculation. Group I and V served as tumor control which received the vehicle (CMC, 0.3 %). Group II and VI were treated with the standard drug (5-FU, 20 mg/kg) by intraperitoneal route. Group III, IV, VII and VIII received the plant extract at the dose of 200 mg/kg and 400 mg/kg by oral route, respectively. All the treatments were given for nine days. The median survival time and percentage increase in life span was calculated:

% increase in life span = [T-C/C] x 100

Where, T= No. of days the treated animals survived

C= No. of days the control animals survived.

Effect of MEIL on hematological parameters

In order to detect the influence of MEIL on hematological status of tumor bearing animals, a comparison was made among eight groups (n=5 animals per group) of mice on the 14th day after inoculation. Group I and V served as normal control which received the vehicle (CMC, 0.3 %). Group II and VI served as tumor control for respective cell lines. Group III, IV, VII and VIII were treated with the plant extract at the dose of 200 mg/kg and 400 mg/kg by oral route for nine days. Blood was drawn from each mouse by retro orbital plexus method after anaesthetized slightly with anesthetic ether. The hematological parameters like total red blood cell (RBC), white blood cells (WBC), lymphocytes (LYM), platelet (PLT), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW) and MID cells (less frequently occurring and rate cells correlating to monocytes, eosinophils, basophils etc.,) were determined using a blood automatic analyzer (Celldyn, Abbot Inc. USA).

After blood collection, animals were sacrificed by cervical dislocation. The liver from each mouse was excised and rinsed in ice cold normal saline solution. A 10 %w/v liver homogenate was prepared in ice cold 10 % KCl solution and was centrifuged for 15 min at 4 °C. The supernatant, thus obtained was used for the estimation of lipid peroxidation (LPO) (Devasagayam and Tarachand, 1987), catalase (CAT) (Sinha, 1972), superoxide dismutase (SOD) (Marklund and Marklund, 1974), glutathione peroxidase (GPx) (Rotruk et al., 1973) and glutathione S-transferase (GST) (Habig et al., 1974).

Effect of MEIL on solid tumor volume

Mice were divided into six groups and each group consisting of six animals. Group I - III were injected EAC cells (2 x 10^6 cells/mouse) into the right hind limb of the animals intramuscularly. In the same way, the animals from Group IV - VI were injected DLA cells (2 x 106 cells/mouse) into the right hind limb, intramuscularly. Group I and IV served as tumor control. Group II, III, V and VI were treated with MEIL at the dose of 200 mg/kg and 400 mg/kg by oral route, respectively, for five alternative days. From the 15th day onwards, tumor diameter was measured every fifth day and recorded up to 30 days by using vernier calipers. The tumor volume was calculated by using the formula $V = 4/3\ \pi r^2$, where 'r' is the mean of r^1 and r^2 which are the two independent radii of the tumor mass.

Statistical Analysis

All the values were expressed as mean ± SEM. The data were statistically analyzed by one-way ANOVA, followed by Tukey multiple comparison test and data for solid tumor volume were analyzed by Dunnett test. P values <0.05 were considered significant.

Results

The preliminary phytochemical screening revealed that the extract contains glycosides, alkaloids, saponins, flavonoids, phenolic compounds and terpenoids.

The GI_{50} of MEIL was found to be 52.12 μg/ml for EAC cells and 62.82 μg/ml for DLA cells. In MTT assay, the percentage cytotoxicity progressively increased in a concentration dependent manner. The GI_{50} of MEIL was found to be less than 100 μg/ml against all the human cancer cell lines used. However the GI_{50} values against the normal mouse embryonic fibroblast (NIH 3T3) and normal African green monkey kidney cells (Vero) were found to be very high when compared to that of cancer cell lines. This indicated that MEIL possess selective cytotoxicity against the cancerous cell lines, but is safer towards the normal cells (Table 1).In acute toxicity studies, animals treated with MEIL did not show any toxic symptoms or mortality when dosed up to 2000 mg/kg body weight by oral route. This indicated that the extract was found to be safe at the tested dose level. Hence 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of this dose were selected for the *in vivo* studies.

In vivo, there was a significant (P<0.001) increase in mean survival time and percentage increase in life span on EAC (55 and 93% at 200 and 400mg/kg, resepctively) and DLA tumor bearing mice (54 and 76%) in a dose dependent manner. The results were almost comparable to that of 5-FU, astandard drug (91 and 99%, respectively). In both tumor models, the extract significantly (P<0.01) reduces the tumor volume in dose dependent manner when compared to tumor control groups.

Table 1. *In Vitro* Cytotoxicity Studies of Methanol Extract of *Indigofera linnaei* against Human Cancer Cell Lines by MTT Assay Method

Cell lines studied	GI_{50} (μg/ml)*
NIH 3T3 (Normal Mouse Embryonic Fibroblast)	244.4
Vero (Normal African Green Monkey Kidney)	270.3
HeLa (Human Cervical Cancer)	74.8
Hep-2 (Human Laryngeal Epithelial Carcinoma)	78.7
HepG2 (Human Liver Cancer)	57.4
MCF-7 (Human Breast Cancer)	75.9
HT-29 (Human Colon Cancer)	89.7

*Average of three determinations, three replicates; GI50, Drug concentration inhibiting 50% cellular growth following 72 h of drug exposure

Table 2. Effect of Methanol Extract of Indigofera linnaei on Antioxidant Enzyme Levels of EAC tumor Bearing Mice

Treat	LPO	SOD	CAT	GPx	GST
Normal	5.7±0.4	0.41±0.01	32.2±2.0	15.6±1.0	0.17±0.01
Tumor	26.9±1.5[a]	0.11±0.01[a]	14.2±1.3[a]	45.2±1.3[a]	0.35±0.01[a]
200 mg	16.7±1.1[a,d]	0.25±0.01[a,d]	25.7±1.3[c,d]	24.9±0.6[a,d]	0.25±0.01[a,d]
400 mg	13.5±1.1[a,d]	0.34±0.02[b,d]	28.1±1.3[d]	20.1±0.6[c,d]	0.19±0.01[d]

N=5; Data are means ± SEMs; [a]P<0.001; [b]P<0.01; [c]P<0.05 vs Normal; dP<0.001 vs Tumor control; data were analyzed by Tukey-Kramer multiple comparison test; LPO, μ moles of MDA/min/mg protein; SOD, units/min/mg protein; CAT, μ mole of H_2O_2 consumed/min/mg protein; GPx, μ moles of GSH oxidized/min/mg protein; GST, μ moles of CDNB conjugation formed/min/mg protein

Hematological parameters of both EAC and DLA tumor bearing mice on day 14 were found to be significantly altered from normal group. There was a significant decrease in hemoglobin, RBC and lymphocytes in tumor bearing animals, accompanied by an increase in WBC, hematocrit (HCT), MID cells and protein. At the same treatment interval, MEIL at the dose of 200 and 400 mg/kg changed these altered parameters significantly to near normal.

The levels of lipid peroxidation in liver tissue were significantly increased in EAC (see Table 2)and DLA tumor control groups as compared to the normal group (P<0.001). After administration of MEIL at different doses to EAC and DLA tumor bearing mice, the levels of lipid peroxidation were significantly (P<0.001) reduced as compared to tumor control groups. Inoculation with the tumor cells drastically increased the GST and GPx content in both tumor control groups as compared with normal group. Administration of MEIL at the tested doses decreased GST and GPx levels as compared with the tumor control group (P<0.001).

The levels of superoxide dismutase (SOD) in the livers of the EAC and DLA tumor bearing mice decreased significantly (P<0.001) when compared with normal group. After administration of MEIL at the tested doses, increased levels of SOD as compared with the tumor control groups were observed (P<0.001). The catalase (CAT) levels in EAC and DLA tumor control group decreased as compared with normal group. Treatment with MEIL increased catalase levels as compared to that of tumor control groups.

Discussion

Cancer is a disease of misguided cells that have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is the second largest cause of death in the world. Of all the available anticancer drugs during 1940-2002, 40% were natural products or natural product derived, with another 8% being natural product mimics (Newman et al., 2003). The greatest recent impact of plant derived drugs is observed in the area of anticancer research, where compounds such as taxol, vincristine, vinblastine and camptothecin have dramatically improved the effectiveness of the chemotherapy against some of the dreadful cancers (Rates, 2001). Hence, there is a great potential for the development of anticancer drugs from the essentially untapped reservoir of the plant kingdom. A large number of plants possessing anticancer properties have been documented (Kim et al., 2005; Akoi et al., 2005; Gupta et al., 2004; Shimizu et al., 2004). Plants belonging to the genus *Indigofera* and several of their constituents have shown potent anticancer properties in many models based on the studies conducted throughout the world (Han, 1999; Awadh Ali et al., 2001; Christina et al., 2003; Rajkapoor et al., 2004, 2005, 2007; Senthilkumar et al., 2007; Vieira et al., 2007; Alex et al., 2010). Based on these observations, in the present study, the MEIL was evaluated for its *in vitro* cytotoxicity and *in vivo* antitumor properties along with its antioxidant potential.

The reliable criteria for judging the value of any anticancer drug is the prolongation of life span, the disappearance of leukemic cells from the blood and reduction of solid tumor volume (Oberling and GueRin, 1954; Marklund et al., 1982). Transplantable tumor cells such as EAC and DLA are rapidly growing cancer cells with aggressive behavior (Segura et al., 2000). The tumor implantation includes a local inflammatory reaction, with increasing vascular permeability, which results in an intense ascetic fluid accumulation (Fecchio et al., 1990). The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells (Shimizu et al., 2004). Our results show an increase in life span accompanied by a reduction in WBC count in MEIL treated mice. The plant extract also inhibited the accumulation of ascitic fluid in the peritoneal cavity of the tumor bearing animals. These results clearly demonstrated the antitumor effect of MEIL on EAC and DLA tumor cells.

The most common problems encountered in cancer chemotherapy are myelosuppression and anemia (Marklund et al., 1982; Price, 1958). Anemia is found frequently in cancer patients (De Vita, 1993). Similar results were observed in the present study in animals of the EAC and DLA tumor control group. This is mainly due to reduction in RBC or hemoglobin production and this may occur either due to the iron deficiency or to hemolytic or other myelopathic conditions (Hoagland, 1982). Treatment with MEIL brought back the hemoglobin content, RBC and WBC counts near to normal. This indicates that the extract have a protective effect on the hemopoietic system.

Excessive production of the free radicals resulted in oxidative stress, which leads to damage to macromolecules such as lipids, and can induce lipid peroxidation in vivo (Fenninger and Mider, 1954). Increased lipid peroxidation causes degeneration of tissues. Lipid peroxidase formed in the primary site to be transferred through the circulation and provokes damage by propagating the process of lipid peroxidation (Yagi, 1991). Malondialdehyde, the end product of lipid peroxidation has been reported to be higher in carcinomatous tissue than in non diseased organ (Fenninger and Mider, 1954). Glutathione, a potent inhibitor of neoplastic process, plays an important role as an endogenous antioxidant system that is found particularly in high concentrations in liver and is known to have key functions in the protective process.

The free radical scavenging system, superoxide dismutase and catalase are present in all oxygen-metabolising cells and their function is to provide a defense against the potentially damaging reactive of superoxide and hydrogen peroxide (Sinclair et al., 1990). Decrease in SOD activity in tumor bearing animals, which might be due to loss of Mn-SOD activity in tumor cells and loss of mitochondria, leading to a decrease in total SOD activity in the liver. Inhibition of SOD and catalase activities as a result of tumor growth was also reported (Marklund et al., 1982; Sun et al., 1989). Similar findings were obtained in the present investigation with EAC and DLA tumor bearing mice. Treatment with MEIL in different dose levels significantly increased the SOD and catalase levels in a dose dependent manner.

Plant derived extracts containing antioxidant principles such as flavonoids, phenolic compounds and tannins showed cytotoxicity towards tumor cells (Marklund et al., 1982) and antitumor activity in experimental animals (Li and Oberley, 1997). Antitumor activity of these antioxidants is either through induction of apoptosis or by inhibition of angiogenesis (Ruby et al., 1995; Ming et al., 1998). The involvement of free radicals in tumors is well documented (Putul et al., 2000; Ravid and Koren, 2003). The lowering of lipid peroxidation, GST, GPx and increase in levels of SOD and catalase in MEIL treated group indicates its potential as an inhibitor of tumor induced intracellular oxidative stress.

In EAC and DLA tumor bearing animals, there was a regular and rapid increase in ascetic fluid volume. Ascitic fluid is the direct nutritional source for tumor growth and it meets the nutritional requirements of tumor cells (Shimizu, 2004). MEIL treatment decreased the volume of solid tumor and increases the life span. Hence it may concluded that MEIL, by a direct cytotoxic effect or by decreasing the nutritional fluid volume and arresting the tumor cell growth. The present study revealed that the extract was cytotoxic towards EAC and DLA cell lines and it was also found to be potent cytotoxic against human cancer cell lines.

The cytotoxic potency of the extract was confirmed by the in vitro cytotoxic assay methods against animal cancer cells lines and human cancer cell lines. The extract exhibit potent cytotoxicity against all the tested cancer cell lines (GI_{50}<100 μg/ml). At the same time, the GI_{50} for the normal cell lines were found to be very high when compared to cancer cells, which indicated that the extract is having potent cytotoxicity against the cancer cells, but it is safe for normal cells.

Preliminary phytochemical studies indicated the presence of flavonoids, saponins, tannins, phenols and triterpenoids in MEIL. Many such compounds are known to possess potent antitumor properties (Kintzios, 2006). The extract of Indigofera linnaei is rich in flavonoids and saponins. Flavonoids have been found to possess antimutagenic and antimalignant effect (Brown, 1980; Hirano et al., 1989). Moreover, they have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and inhibition of neovascularization (Weber et al., 1996; Fotis et al., 1977). Saponins have been found beneficial targeted on inhibition of tumor angiogenesis by suppressing its inducer in the epithelial cells of blood vessels and then on adhering, invasion and metastasis of tumor cells. They also exhibit the antitumor effect by cell cycle arrest and apoptosis (Man et al., 2010). Plants from the Indigofera genus contains Indirubin, is a purple 3.2'-bis indole. It binds to and subsequently inhibits cyclin-dependent kinases (CDKs), glycogen synthase kinase 3 (GSK3) and the aryl hydrocarbon receptor and thus suppress the growth of various cell types through cell cycle arrest (Ding, 1987; Han, 1994; Knockaert et al., 2004; Sugihara et al., 2004). Antitumor and cytotoxic properties of the extract may be due to these phytochemical constituents.

In conclusion, the present study demonstrates the potent cytotoxic and antitumor properties of methanol extract of *Indigofera linnaei*. Further studies to characterize the active principles and to elucidate the mechanism of action are in progress.

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