CHAPTER-4-

STUDIES ON ANTI-TUMOR ACTIVITY OF

C.FENESTRATUM EXTRACT
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4.4. DISCUSSION
4.1. INTRODUCTION

Cancer has been a leading cause of death in the developed countries. With changing standards of living and food habits and also due to availability of curative treatment for many infectious diseases, cancer is surpassing other illnesses as a principle cause of morbidity and mortality even in developing countries (Notani, 2001).

Three basic strategies are used in treatment of cancer: surgery, radiotherapy and chemotherapy, which may be employed alone or in combination with the other two methods. Systemic chemotherapy is mainly used for the treatment of the metastatic forms of neoplastic disease. Chemotherapeutic agents are classified into two major groups: synthetic and natural products. Despite enormous progress in the field of organic chemistry, currently 25% of all prescription drugs are derived from natural sources. This is more significant with regard to anti-cancer drugs in which more than 80% are plant-derived compounds (Aliabadi, 2003).

Antitumor agents can exert their cytotoxic effects in many ways but all of the mechanisms lead to one of the two distinguishable basic forms of cell death: apoptosis and necrosis. Necrosis is the consequence of an intensive physical or chemical insult, resulting in rapid degradation of the cell and the liberation of inflammatory mediators which deteriorate the surrounding tissues. On the other hand, apoptosis is the strictly regulated and programmed self-demolition of the cell; this is a general feature of multi-cellular organisms which is responsible for the elimination of damaged cells and plays a physiological role during embryonic development. This mode of death is characterized morphologically by cellular shrinkage, nuclear condensation and increased membrane permeability, and biochemically by the internucleosomal cleavage of DNA, leading to an oligonucleosomal “ladder”, phosphatidylserine externalization and proteolytic cleavage of a number of intracellular substrates (Cruchten and Broeck, 2002).

The different apoptosis signaling pathways induced by various anticancer drugs with distinct primary sub-cellular targets and mechanisms of action may converge on mitochondria to cause mitochondrial permeability transition (MPT), release apoptogenic factors from the mitochondrial intermembrane space (IMS) into the cytosol, activate a similar caspase proteolytic cascade that is amplified by a positive feedback loop involving the release of mitochondrial cytochrome c (Cyt C) and,
ultimately, trigger internucleosomal DNA fragmentation (Budihardjo et al., 1999; Nagata, 2000).

Combating cancer is of paramount importance today. Multidisciplinary scientific investigations are making the best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine. An alternative solution to western medicine embodied with severe side effects, is the use of medicinal plant preparations to arrest the insidious nature of the disease. Of the 92 anticancer drugs commercially available prior to 1983 in the United States, approved worldwide between 1983 and 1994, approximately 62% can be related to natural origins (Gordon and Newman, 2001). Ayurveda- a science of health and longevity has tried many herbal as well as Rasayana remedies with varying degree of success, but its main significance lies in its preventive approach. Hartwell has collected data, about 3000 plants, which possess anti-cancer properties and subsequently been used as potent anti-cancer drugs (Balachandran and Govindrajan, 2005). Among Indian Ayurvedic herbs, some 30 herbs have shown antitumor activities, and the number may rise as more and more herbs are studied (Ramakrishnan et.al., 1984). Many herbs have been evaluated in clinical studies and are currently being investigated phytocemia to understand their tumoricidal actions against various cancers (Premalatha and Rajgopal, 2005). The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search for anticancer agents from plant resources is the selection of plants based on ethnomedical leads (Spiridon, 2006).

Coscinium fenestratum Colebr. (Menispermaceae), commonly known as tree turmeric, grows widely in the Western Ghats (India) and Sri Lanka. The plant has been mainly used for treating diabetes mellitus in the traditional Ayurvedic and Siddha systems of medicine (Varier,1994). The stem contains berberine, ceryl alcohol, hentriacontane, sitosterol, palmitic acid, oleic acid and saponin, together with some resinous material. Isolation of tertiary alkaloids, berlambine, dihydroberlambine and noroxyhydrastinine from the roots has been reported (Datta et al., 1988).

Berberin is known to exhibit multiple pharmacological activities such as antimicrobial activity towards bacteria, fungi and viruses (Freile et al., 2003, Hayashi
et al., 2007), antimalarial (Iwasa et al., 1999), anti-inflammatory (Lee et al., 2007), antihypertension (Liu et al., 1999), reduce blood glucose level (Zhang et al., 2010) reduce blood lipid (Zhao et al., 2008) and antiproliferative (Letaiov et al., 2006) activity. It was also shown to inhibit the in vitro growth of a number of human cancer cell lines (Tungpradit et al., 2011).

In this study we sought to evaluate the cytotoxic effects of C.fenestratum extract on Hela cells, apoptotic induction on DLA cells, DNA fragmentation assay and cytochrome-c release assay along with in vivo tumor regression study.

4.2. MATERIALS AND METHODS

4.2.1. Preparation of the extract
Aqueous-ethanol extract of Coscinium fenestratum was prepared as described in section 2.2.1.

4.2.2. Cell lines
Ehrlich’s ascites carcinoma (EAC) and Dalton’s lymphoma ascites (DLA) cell lines were obtained from Cancer Institute, Adyar, Chennai and were maintained in mice by intraperitoneal inoculation of 1x 10^6 viable cells. Hela cells were obtained from Indian Institute of Technology (IIT), Chennai.

4.2.3. Animals
Female Swiss albino mice of 6 weeks old weighing 25 ± 2 g were used for the studies.

4.2.4. ANTI-PROLIFERATING ACTIVITY OF C.FENESTRATUM EXTRACT

4.2.4.1. Cytotoxic effect of C.fenestratum on human carcinoma cell
The cells used were Hela (carcinoma cell line) cells. Cells were maintained as monolayer cultures in DMEI medium supplemented with 10 % fetal calf serum and incubated at 37ºC in a humidified incubator at 5 % CO2. The cytotoxic effect of C.fenestratum extract against previously mentioned human tumor cell lines was determined by a rapid colorimetric assay, using 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) as described in section 2.2.23, and compared with untreated controls. This assay is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable tumor cells, into an insoluble
colored formazan product, which can be measured spectrophotometrically. Briefly, 100 µl of cells (5 × 10^4 cells /ml of media) were seeded in 24 microplates and incubated for 24 hours (37°C, 5% CO2 air humidified). Then 10 µl of prepared concentrations of extract (25 µg/ml-100 µg/ml) was added and microplates containing cells and extracts were incubated for another 72 hours in the same condition. Taxol (12 nM) was used as a positive control. Then the extract containing medium was aspirated and fresh medium containing 1mg/ml of MTT was added and again incubated for 4 hours at 37°C. MTT lysing solution (100 µl) was added to this and after 1 hour the extent of MTT reduction was measured colorimetrically at 570 nm.

4.2.5. **EFFECT OF C.FENESTRATUM EXTRACT ON INDUCTION OF APOPTOSIS IN TUMOR CELLS**

4.2.5.1. **Mitochondrial Apoptosis Detection by JC-1 staining method**

The JC-1 staining method provides a simple, fluorescent-based method for distinguishing between healthy and apoptotic cells by detecting the loss of the mitochondrial transmembrane potential (ΔΨ). Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur in the induction of apoptosis.

**Procedure:**

Hela cells were used for this study. The cells were cultured in 24 well plates as mentioned before. The cells are treated with and without *C.fenestratum* extract (50 µg/ml and 100 µg/ml) and incubated it for 24 hours. Then the cells are stained with JC-1 stain (JC-1 is prepared as a 1000x stock solution in DMSO (5 mg/ml)). For the staining of adherent cells it is diluted in medium to 5 µg/ml (with vortexing during the dilution to prevent the formation of precipitates); the JC-1 containing medium is added to the cells, followed by incubation for 5 min at 37°C and then observed directly under the fluorescent microscope. Taxol is used as positive control.

4.2.5.2. **Morphological analysis of apoptotic cells by DNA double staining method.**

For quantification of apoptosis by morphologic criteria, cells were stained with both acridine orange and propidium iodide, in a modification of a standard assay (Duke et
DLA cell were used for this study. The treated or untreated cells (1x10^6 cells/ml of media) were incubated for 0-8 hours at standard conditions (37°C and 5% CO_2). After incubation about 500µl of the cells were taken and centrifuged at 1500 rpm for 2-3 minutes. After centrifugation discard the supernatant and 60 µl of the pellets were taken. To this added 100 µg/ml of acridine orange (AO) and ethidium bromide (EB) at a ratio of 1:1. The suspension was immediately (fast uptake) examined by fluorescence microscopy. Acridine orange is taken up by both viable and nonviable cells and emits green fluorescence if intercalated into double stranded nucleic acid (DNA) or red fluorescence if bound to single stranded nucleic acid (RNA). Ethidium bromide is taken up only by nonviable cells and emits red fluorescence by intercalation into DNA.

Counted minimum of 250 total cells, recording the number of each of the following four cellular states: (1) Live cells with normal nuclei (bright green chromatin with organized structure); (2) Live cells with apoptotic nuclei (bright green chromatin which is highly condensed or fragmented and membrane blubbing); (3) Dead cells with normal nuclei (bright orange chromatin with organized structure); (4) Dead cells with apoptotic nuclei (bright orange chromatin which is highly condensed or fragmented along with and membrane blubbing).

The percentage of dead cells and cells with apoptotic nuclei was calculated as follows:

\[
\% \text{ dead cells} = \frac{\text{Dead cells} + \text{Dead apoptotic cells}}{\text{Total number of cells counted}} \times 100
\]

\[
\% \text{ apoptotic cells} = \frac{\text{Live apoptotic cells} + \text{Dead apoptotic cells}}{\text{Total number of cells counted}} \times 100
\]

4.2.5.3. DNA fragmentation assay

For the DNA fragmentation assay Hela cells were used. The cells were plated in a 24 well plate and the extract and the positive control taxol were added to the plate and incubate it for 72 hours. The cells were harvested and centrifuged for 10 minutes at 2000 rpm. The pellets were then suspended in 1 ml PBS and mixed well and again
subjected to centrifugation at 2500 rpm for 5 minutes. The DNA lysing solution was then added to the pellet and incubated it for 15 minutes. After incubation it was subjected to centrifugation for 5 minutes at 1500 rpm and the supernatant was separated. To this added equal volume of phenol chloroform isoamyl alcohol, mixed well and incubated for 5 minutes. The mixture was then centrifuged for 10 minutes at 1200 rpm and the upper layer was separated and added double volume of ethanol and 1/10th volume of potassium acetate. Mixed by inversion and kept overnight at -20°C. Then centrifuged it at 1500 rpm for 15 minutes and then washed with 70% ethanol and again centrifuged for 15 minutes at 1200 rpm. Discard the ethanol and air dried. Then add 50 µl of TE buffer (tris HCl 10mm & EDTA 1mm). After 10 minutes 4µl of RNase (20mg/ml) was added to this and incubated for 1 hour. Then add 4µl of protease (20mg/ml) followed by 1 hour incubation. Then the DNA was subjected to agarose gel electrophoresis at 80V for 1 hour and the laddering was observed.

4.2.5.4. Detection of Cytochrome-C release and caspase assay.

Preparation of Mitochondrial and Cytosolic Fractions and Total Cell Lysates - Next, we studied the release of cytochrome-C and the pro-apoptotic factors 3 and 9 triggered by the presence of *C.fenestratum* extract. For the determination of Cyt-C and caspase activity, HeLa cells were treated with 2000 µg/mL *C.fenestratum* extract for 48 hours and cytosolic fractions were obtained. Briefly, cells were washed twice with ice-cold PBS, pH 7.4, which was followed by centrifugation at 200 x g for 5 minutes. The cell pellet was then resuspended in 600 µL extraction buffer containing 200 mmol/L mannitol, 68 mmol/L sucrose, 50 mmol/L PIPES-KOH, pH 7.4, 50 mmol/L KCl, 5 mmol/L EDTA, 2 mmol/L MgCl₂, and 1 mmol/L dithiothreitol and protease inhibitors. Cell suspensions were then incubated with 100 µg/ml digitonin, and homogenates were centrifuged at 12,000 x g for 10 minutes at 4°C. The supernatant (cytosolic fraction) as well as pellet (enriched for nuclei and mitochondria) were recovered and stored at −80°C until analysis by gel electrophoresis. Protein concentration was measured using the Bradford reagent, and samples were denatured in standard buffer. Purity of the mitochondrial and cytosolic fractions was determined by probing with antibodies to cytochrome-C oxidase subunit IV and β-actin respectively.
Western Blot Analysis—Total cell extracts (20–60 µg) were resolved by electrophoresis on a 10–12% SDS-polyacrylamide gel and transferred to Immun-Blot TM polyvinylidene difluoride membranes (0.2 µM, Bio-Rad). Membranes were blocked with 5% nonfat milk in 0.1% TBS-T and subsequently probed with antibodies directed against cytochrome-C and subunit of pro-caspase-3 and 9. After washing, membranes were incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies. Proteins were visualized with the enhanced chemiluminescence (ECL) detection reagents. Membranes were reprobed with antibodies to β-actin to control for equal loading of protein.

4.2.6. ANTITUMOR ACTIVITY

4.2.6.1. Solid tumor model- Curative effect
Animals were divided into two groups of six animals each. Viable DLA cell (1x10^6 in 0.1ml PBS) were transplanted subcutaneously into the right hind limb of mice. After 15 days, animals with tumor size around 1.1± 0.1 cm^3 were divided into two groups of six animals in each group. C.fenestratum extract (250, mg/kg bw, p.o) was administered for 7 consecutive days. The group received only the cell line served as the control. The tumor development on animals in each group was determined by measuring the diameter of tumor growth in two perpendicular planes using vernier calipers twice a week for 4 weeks. The tumor volume was calculated using the formula 4/3πr1^2r2 where, r1 is the minor radius and r2 is the major radius.

4.3. RESULTS

4.3.1. Cytotoxic effect of C.fenestratum extract
MTT method was used to demonstrate the cytotoxic activity of C.fenestratum extract in vitro. Here taxol was used as a positive control. Data presented in fig.4.1 clearly showed that C.fenestratum extract had a marked inhibitory effect on HeLa cells and is concentration dependent. The percentage of survival was lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Moreover at a concentration of 100 µg/ml, the extract exhibited toxicity to the tumor cells than that of the positive control taxol, which clearly indicates its antitumor activity.
4.3.2. Effect on Mitochondrial Apoptosis

JC-1 (5, 5’, 6, 6´-tetrachloro-1, 1´, 3, 3´-tetraethylbenzimidazol-carbocyanine iodide) is a lipophilic fluorescent cation that incorporates into the mitochondrial membrane, where it can form aggregates due to the physiological membrane potential of mitochondria. This aggregation changes the fluorescence properties of JC-1 leading to a shift from green to orange fluorescence. In healthy cells, the negative charge established by the intact mitochondrial membrane potential allows the lipophilic dye, bearing a delocalized positive charge, to enter the mitochondrial matrix where it accumulates. With the increasing of the concentration of JC-1, it aggregates and become fluorescent red.

![Graph showing cell survival rates](image)

**Fig.4.1.** Effect of *C.fenestratum* extract on cell viability determined MTT assay in human carcinoma cells (HeLa).

When the mitochondrial potential collapses in apoptotic cells, JC-1 just exists as monomers and do not accumulate within the mitochondria. When dispersed in this manner, JC-1 remains in the cytoplasm in a green fluorescent monomeric form. It was observed from the present experiment that the cells treated with different concentrations of *C.fenestratum* extract exhibits intense green fluorescence when compared with that of the control non-treated cells which appears to be red in color.
(Fig. 4.2). From this it was clear that the cells become apoptotic after treatment with the extract hence the JC-1 stain cannot enter in to the cells and become green in color. The intensity of green fluorescence is much less in the taxol treated cells when compared to that of the extract treated cells.
Fig. 4.2. Effect of *C. fenestratum* extract on mitochondrial apoptosis detected by JC-1 staining method.
4.3.3. Detection of apoptotic morphology by DNA double staining method.

Treatment of DLA cells with *C.fenestratum* extract resulted in significant morphological changes, indicating that the cytotoxic action of *C.fenestratum* extract was due to its ability to induce apoptosis (Fig. 4.3). From the data presented in table 4.1 it was found that the extract induced apoptosis in time and concentration dependent manner.

![Photographic representation of C.fenestratum extract induced apoptosis in tumor cell line (DLA) detected by double staining method.](image)

(A) Live apoptotic cells, (B) Dead apoptotic cells.

**Fig.4.3.** Photographic representation of *C.fenestratum* extract induced apoptosis in tumor cell line (DLA) detected by double staining method. (A) Live apoptotic cells, (B) Dead apoptotic cells.
Table 4.1. Effect of *C. fenestratum* extract on apoptotic morphology.

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<th>Treatments</th>
<th>% dead cells</th>
<th>% apoptotic cells</th>
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<tr>
<td></td>
<td>0 hour</td>
<td>2nd hour</td>
</tr>
<tr>
<td>Control</td>
<td>1.4</td>
<td>3</td>
</tr>
<tr>
<td><em>C. fenestratum</em> (50µg/ml)</td>
<td>3.4</td>
<td>8</td>
</tr>
<tr>
<td><em>C. fenestratum</em> (100µg/ml)</td>
<td>6.2</td>
<td>11</td>
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- Treatments % dead cells | % apoptotic cells

- Control

- *C. fenestratum* (50µg/ml)

- *C. fenestratum* (100µg/ml)
4.3.4. DNA fragmentation assay

In order to detect DNA fragmentation, DNA samples were isolated from HeLa cells after 72 hours of incubation with the extract and separated by agarose gel electrophoresis. As shown in Fig. 4.4, after incubation of cells with the extract, oligonucleosome ladder pattern was displayed and became gradually more pronounced as concentration increased to 100µg/ml. The data indicated that cellular DNA was cleaved into multiple fragments upon apoptosis induced by *C. fenestratum* extract.

![DNA fragmentation induced by *C. fenestratum* extract in HeLa cells.](image)

**Fig. 4.4.** DNA fragmentation induced by *C. fenestratum* extract in HeLa cells. Lane.1-100bp marker, Lane.2-DMSO, Lane.3-Taxol (12 Nm), Lane.4-*C. fenestratum* extract (50µg/ml), Lane.5- *C. fenestratum* extract (100µg/ml).
4.3.5. Induction of Cytochrome-C release and caspase activation.

From the data presented in figure 4.5, it was found that *C. fenestratum* triggered the release of mitochondrial Cyt-C in HeLa cells. Because the release of Cyt-C from the mitochondrial IMS (intermembrane space) into the cytosol may be a limiting factor in caspase-9 activation and represents a central coordinating step in apoptosis (Liu et al., 1996; Li et al., 1997), the extract can activate the caspase proteolytic cascade. As compared to the untreated controls, the 15-kDa bands of cytosolic Cyt-C became increasingly visible 48 hours after treatments (Fig. 4.5).

The data presented in figure 4.6 confirmed that the release of Cyt-C coincided with the activation of pro-caspase-9. It was reported that release of Cyt-C coincided with the processing and activation of pro-caspase-9 event that is known to occur in a cytosolic complex termed the apoptosome, which consists of cytochrome c, procaspase-9, and the adaptor protein, apoptotic protease-activating factor-1 (Sun et al., 2005). Apoptosis induction by the extract is also determined by caspase-3 activation, which is the key downstream effector caspase proteolytically activated by the initiator caspase-9, and the result clearly indicated the activation of caspase-3 by the extract in HeLa cells.

4.3.6. Effect of *C. fenestratum* extract on tumor regression

The extract was also found to be highly effective against developed solid tumor (Fig. 4.7). Treatment with the extract at doses of 250 mg/kg body weight for 7 consecutive days after tumor development, showed regression in tumor volume as compared to the control animals.

4.4. DISCUSSION

Combating cancer is of paramount importance today. Multidisciplinary scientific investigations are making the best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine. An alternative solution to western medicine embodied with severe side effects is the use of medicinal plant preparations to arrest the insidious nature of the disease.
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Fig. 4.5. Inductions of Cyt-C release by *C. fenestratum* extract (200 µg/ml) in HeLa cells *in vitro*. Tumor cells were incubated for 48 hours in the presence or absence *C. fenestratum* extract and bands (arrows) of cytosolic Cyt-c released from the mitochondrial IMS were detected by Western blot analysis. Coimmunodetection of β-actin bands was performed on the same membranes to confirm equal protein loading in each lane.

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1. Control (DMSO); 2. Taxol (25 nM); 3. *C. fenestratum* (200 µg/ml)

Fig. 4.6. *C. fenestratum* extract (200 µg/ml) induced activation of caspase 3 and 9 in HeLa cells. The HeLa cells were treated with the extract for 48 hrs and western blot analysis were carried out. β-Actin was used as a control for equal loading of protein.
Cytotoxic compounds are one of the most important classes of drugs used for cancer treatment. There have been several researches to get new cytotoxic agents. In this regard compounds such as colchicine, *Vinca* alkaloids and paclitaxel isolated from medicinal plants showed considerable promises. The present study is mainly deals with the anti cancer activity of the ethanolic extract of *C.fenestratum* both under *in vitro* and *in vivo* conditions.

MTT assay was used to evaluate cytotoxicity based on metabolic reduction of MTT. The purpose of the study was to determine whether this extract has cytotoxic effect against cancer cells. MTT based cytotoxic assay was carried out using human carcinoma (HeLa) cells. The ethanolic extract exhibited significant growth inhibition of the tumor cell lines.
Recently, it was shown that one of the mechanisms of action of several antineoplastic drugs is by induction of apoptosis (Maldonado et al., 1996; Melendez-Zajgla et al., 1996). Apoptosis is a regulated cell death used by multicellular organisms to dispose redundant cells. It is morphologically and biochemically characterized by cell shrinkage, cell membrane blebbing, nuclear chromatin condensation, and nonrandom DNA fragmentation. This type of death is altered in many pathological states and is indispensable for elimination of “sick” or not normal cells in organisms (Rathmell and Thompson, 2002).

From the present experiment it was clear that the extract induces apoptosis in tumor cell lines, more over it also alter the membrane potential of mitochondria which inturn lead to the release of cytochrome-C, which is an indication of caspase activation. The caspase-cascade system plays essential roles in the apoptosis. Caspases are cysteineaspartic protease in a family of cysteine proteases. Caspases exist as inactive proenzymes known as procaspases that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small subunits that dimerize to form the active enzyme. Sequential activation of caspases played a central role in the execution phase of cell apoptosis (Launay et al., 2002, Fan et al., 2005). So we further examined the activation of caspase 9 and 3 which in turn down regulate the apoptotic pathways and it was observerd that $C.fenestratum$ extract induces activation of these caspases in tumor cell lines indicative of its apoptotic activity.

An apoptosis-induction capacity rather than necrosis induction is accepted as a key feature of a potential antitumor drug. The morphological changes were detected by AO/EB double staining with fluorescence microscopy. Typical markers, including cellular shrinkage, nuclear condensation and an increased membrane blebbing, were observed after extract treatment. In parallel with the morphological changes, the other important features of apoptosis i.e., fragmentation of nuclear DNA were also detected.

Tumor volume is one of the important criteria for direct or indirect anticancer activity. The in vivo experiment revealed tumor regression potential of the extract as shown by the small size of tumor volume in animals bearing solid tumors. These results suggest that the $C.fenestratum$ extract contain compounds that may modulate tumorigenesis at different stages or may act at the same stage. Berberin is one of the major constituent of $C.fenestratum$ extract. It was reported the the berberin has a
strong anti proliferative activity (Tungpradi et al., 2011). Hence the presence of berberin may be one of the reason for the anti tumor activity of the extract. It was observed that tumor cells produced more peroxides when they proliferate actively. This rise in peroxides indicated the occurrence of intensification of oxygen free radical production (Navarro et al., 1997). Cells which are equipped with enzymatic antioxidant mechanisms play an important role in the elimination of free radicals. High levels (up to 0.05 μmol/h per 10^4 cells) of H2O2 are constitutively released from a wide variety of human tumors (Szatrowski and Nathan, 1991). Our previous study has clearly shown that *C.fenestratum* extract has a strong antioxidant and anti inflammatory activity. Hence it can be assumed that the significant antioxidant and anti-inflammatory activity of the extract may also contribute to its significant antitumor and anticancer property. Hence *C.fenestratum* extract possibly provides additive or synergistic effect in the prevention and treatment of cancer.

Taken together, these data indicate that the *C.fenestratum* extract exhibit a combination of anticancer activities both under *invivo* and *in vitro* conditions. Overall, it is suggested that *C.fenestratum* extract can be regarded as promising starting structures for the development of future anticancer agents.